STUDIES IN THE PATHOLOGY OF TUMOURS.

Anatomical, Experimental, Serological, and Historical.

by

Archibald Leitch, M.B., Ch.B. (Glasgow)

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

L I S T O F P A P E R S.

ADENOMYOMA OF THE UTERUS AND OF NEIGHBOURING STRUCTURES.

A Statement of the theory of migration.

Incorporating papers on

Adenomyoma of the Uterus (in collaboration with S.J.Cameron) The Lancet 9th July 1904

and

Migratory Adenomyomata of the Uterus

Proc.Royal Soc.Med.(Gyn.Sect.) July 1914.

ADENOMYOMA OF THE UTERUS AND OF THE NEIGHBOURING STRUCTURES.

Though a few scattered references to myomatous tumours of the uterus containing glandular elements may be found by the industrious searcher through previous literature, yet it was not until the publication of Von Recklinghausen's book in 1896 (Die Adenomyome und Cystadenome der Uterus and Tubenwandung) that any considerable attention was directed to this distinct and interesting group of tumours.

In our own country the first paper on the subject, by Dr. S.J. Cameron and myself, appeared in 1904 (The Lancet, 9th July, 1904). In an edition of Bland-Sutton's text-book (Tumours Innocent and Malignant) which had been published earlier in the year there was a sceptical reference to gland-bearing myomata as having been described by Continental observers. The irony of the situation was that the first case which we recorded should have been one of Bland-Sutton's patients. The uterus, removed under a diagnosis of fibroid, was found to contain gland tubules surrounded by a mantle of small cells embedded in the thickness of the thickened muscular wall. The Pathologist of the Chelsea Women's Hospital reported "Carcinoma of the body of the Uterus" but the macroscopic appearances of the tumour were so like those of a rather diffuse myoma that Cameron and I had doubts about the pathologist's diagnosis and on examining the section we came to the conclusion that the gland tubules though infiltrating practically the whole thickness of the uterine wall were much too regular and well-developed to be considered malignant. We had not at that time access to the literature of the subject but F.E.Taylor acting on our provisional diagnosis of "Adeniferous myoma" searched out many of the previous German

communications for us including one by Cullen in a Festschrift to Von Recklinghausen. Within a few months we had collected four cases, the full particulars of which we knew, and a search through the collections of histological specimens in the Cancer Hospital and the Chelsea Hospital for Women revealed eleven more which were diagnosed either as fibromyoma or as adenocarcinoma. In the years that have intervened since then I have seen so many uterine adenomyomata that I consider these tumours to be of fairly common occurrence. At the time when we published our paper Von Recklinghausen's theory of origin, namely that uterine and tubal adenomyomata arose from remnants of the Wolffian duct, was generally accepted, but we endeavoured to show that they arose from the uterine mucosa even though, as in one of our cases, the direct connection between the two was not demonstrated. Cullen had stated that in some of his cases he was able to establish the connection. We independently took up that position and though perhaps our short paper had little or no influence in upholding the theory of the mucosal origin of adenomyomata yet it is interesting to reflect that the weight of opinion veered round to it afterwards and was generally accepted in English speaking countries.

It is practically impossible to diagnose the condition clinically and hysterectomy is usually performed for signs and symptoms interpreted by the Surgeon as being those of fibromyomata, fibrosis uteri, subinvolution, chronic metritis, or even carcinoma of the corpus uteri. Even on macroscopic examination of a specimen there are no definite characteristics that enable one to diagnose the lesion with certainty, and it is only by routine histological examination of sections taken through the thickness of the uterine wall that we discover the true

nature of the lesion. In the commonest type of adenomyoma there is a general thickening of the uterine wall, rather like a diffuse myoma without capsulation, and there is no sharp demarcation of the endometrium from the musculature. The latter is really the only feature that excites suspicion and so little is this evident in many a case that I frequently discard my specimens after taking a portion for microscopic examination and am only aware later that I have been dealing with an adenomyoma. There is another appearance which, when it is found, enables us to anticipate the microscopical evidence - namely, the presence of numerous small cystic cavities in a diffuse thickening of the wall of the uterus. The diffuse thickening does not as a rule implicate the whole uterus but only a part of it. Sometimes, but much less commonly, we meet with more localised growths which in rare cases are encapsuled. I have seen subsereus almost pedunculated growths, removed by myomectomy, looking exactly similar to subsereus fibromyomata, which on microscopic examination proved to be adenomyomata.

Occasionally the tumour appears to be a myomatous polypus growing into the uterine cavity or even projecting through the external os: an instance of this variety was the fourth case recorded in the paper by Cameron and myself.

On microscopic examination we find gland tubules, perfectly well formed, with a wall ever one cell thick, and often containing blood corpuscles and epithelial cell debris, lying in richly cellular areas, the cells of such areas being absolutely identical with those in the normal endometrial mucosa. Indeed, it is impossible to distinguish such a microscopic field from one of the normal endometrium.

These islets, surrounded by fibromuscular tissue which has undergone hyperplasia, are distributed throughout the uterine wall. Serial sections in most cases demonstrate a connection between them and a direct continuity with the endometrium (Fig.1)

Sometimes the tubules are unaccompanied by strema cells and abut directly on the muscle: at other times the tubules are in relatively small proportion.

Generally the invasion by the endometrium takes place over a wide area, but sometimes the position of entry is more restricted and the connection between gland islets and endometrium is difficult to establish: indeed, the original connection may be wholly severed as in the third case reported by Cameron and myself. Of course a single deep prolongation of endometrium into the muscle (see Fig 2.) which is not an uncommon occurrence especially in those cases known as fibrosis uteri, is not sufficient to justify the name of adenomyoma, but such penetrations, both in the body and in the cervix show us how these growths start and also demonstrate the liability of the uterine mucosa to proliferate into chinks in the musculature. The outstanding features of the adenomyoma are thus the infiltration of the uterine wall by the <u>two</u> constituents of the uterine mucosa - the tubules and the cellular strema. It is not a destructive infiltration nor lymphatic permeation such as we get in carcinoma, but a "worming" of endometrial tubules among the loose tissue between muscle bundles. There is no round cell infiltration. The fibromyomatous constituents are probably due to the irritation produced by the infiltrating tubules and this reaction may be out of all proportion to the amount of glandular epithelium present: it may

be allied to the physiological activities or necessities of this specialised epithelium for it has been proved beyond doubt that such displaced endometrial elements function like the normal endometrium during menstruation and during pregnancy. The frequent formation of small cysts is probably due to the contraction of the fibrous tissue around the tubules. As already remarked, we sometimes find intramural adenomyomata in which it is impossible to demonstrate any endometrial connection, and it was mainly on the observation of such tumours that Von Recklinghausen built his theory of a genesis from Wolffian duct remains. His position was argued with a great deal of ingenuity: he accepted wholeheartedly the views then held by embryologists regarding the developement of the mesonephros and he found in his tumours fancied resemblances to glomeruli and other structures which no one would now recognise: but most of the arguments he put forward could with equal force be applied to explain the occurrence of ordinary fibroid tumours. His experience of ademonyomata of the uterus was unusual because he had seen very few of the common diffuse type, in which the endometrial connection is perfectly demonstrable, and a large number of more marginal tumours in which a variable amount of muscle intervened between them and the endometrium. Later on as tumours of identical appearances were found in situations where Wolffian duct remains could not reasonably be located, the idea was mooted by Iwanoff and strongly advocated by Meyer and others (See Lockyer's Fibroids and Allied Tumours, 1918) that adenomyomata of the uterus were derived from the endothelium of the peritoneum. The absence of a demonstrable connection between endometrium and tumour in many cases is responsible for all these theories which at once explain too much and too little. Their improbability impresses me.

The pathogenesis of tumours has been dominated far too much by theories of embryology. It certainly would be absurd to deny the occurrence of embryonic nests in the neighbourhood of the uterus and adnexa but that these rests have a greater liability to give rise to tumour formations than normal tissues, normally situated, as Cohnheim and his supporters held, is very problematical. The cells composing such rests manifestly age just the same as normal cells and the embryonic potentialities for growth depart with their youth. These potentialities of embryonic cells are not unlimited: their predestined end is set before them. Immured apart from their normal position they behave as their relatives. When Rip Van Winkle awoke from his long sleep amongst the hills he was no longer endowed with the exuberant vitality of boyhood but he was an old and a decrepit man.

The polypoid variety of adenomyoma is readily explained by the efforts of the uterus to extrude a tumour lying in the submucosa. This is widely accepted in the case of myomatous polypi. In the latter case the tumour is not derived from the mucosa, which contains no muscular elements, but from the muscular tissue of the uterine wall. Submucous, or even definitely more intramural at first, it is extruded into the cavity by the uterine contractions pushing the endometrium in front of it and thinning it out. Similarly a localised hypertrophy of muscle occurring round infiltrating endometrial tubules might be extruded into the uterine cavity and would constitute the polypoid variety of adenomyoma (fig.3). This phenomenon of extrusion of tumours originating in the uterine wall forms the basis of the theory I put forward (See Proc.Roy.Soc.Med.July 1914), to explain the

Fig. 1.

Drawing of adenomyoma of uterus (diffuse type) Shows the islets of tubules and cellular stroma among the muscle bundles and the direct connection with the endometrium. Drawn from actual section.

Fig. 2.

Microphotograph of wall of uterus in a case of "fibrosis uteri" showing the proliferation of tubules from the endometrium (right-hand top corner) into crevices between the muscle bundles.

To explain the origin of adenomyomata

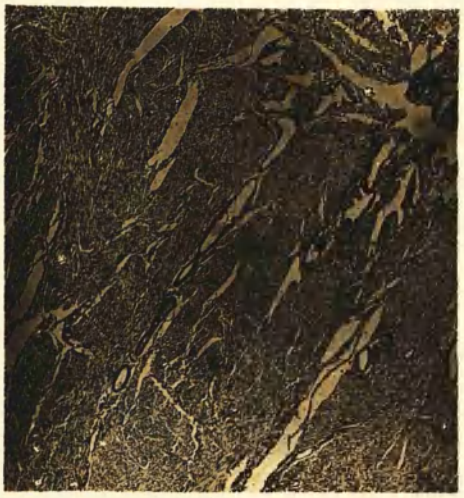


Fig. 3.

Microphotograph of an adenomyomatous polypus lying in the uterine cavity. The presence of muscular tissue serves to separate this tumour from the more common adenomatous polypus.

To illustrate the extrusion of adenomyomata.

the occurrence of adenomyomata in situations where definite connection with the endometrium could not be demonstrated. Extrusion can take place in two directions (1) into the uterine cavity and (2) away from the cavity. The latter is the more common occurrence. Perhaps the majority of myomatous uteri that one sees exhibit subserous tumours which sometimes are actually pedunculated, and no one denies that such tumours originated in the body of the musculature and were pushed out to the periphery by muscular contractions of the uterus. I hold that a similar occurrence takes place with adenomyomata and that the endometrial connection may be broken in the gradual process of extrusion. Further, pure myomatous tumours which have become subserous and pedunculated may form secondary connections with neighbouring structures and their original attachment to the uterus may be wholly severed. There are numerous observations to support this. Similarly with adenomyomata. I think that this theory will explain the occurrence of adenomyomata in any of the situations outside the uterus in which they have been found. (In view of a criticism that was brought forward when I proposed this explanation, namely that adenomyomata had been found in various parts of the small intestine and even in the Stomach it is necessary to say that the adenomyomata I am discussing are a separate class of tumours composed not only of muscle and gland tubules but also of a tissue identical with the extraglandular stroma of the endometrium, and in that respect differ from these small nodules found in the muscular coat of the small intestines, formed of gland tubules only, and known usually as myoadenomata. The latter are unmistakeably inclusions (from the intestinal epithelium). But the separation of adenomy-

omatous tissue from the endometrium is not merely owing to the mechanical efforts at expulsion on the part of the uterine muscle. There is an active migration of the tubules and their proper stroma. When endometrial tubules penetrate between the superficial chinks of the adjacent muscles, as seen in figure 2, they take part in the usual cyclical changes of the endometrium. In menstruation they and their stroma cells become engorged: the swelling further opens up the potential crevices between the muscle bundles; and in the stage of re-proliferation they advance still further into the wall. They may reach the limits of the uterus and progress beyond them. Though the original connection be broken these cells carry on their function. In all my own cases of extra-uterine adenomyomata these aberrant tumours were actually found to be excessively tender during menstruation. Numerous observers have testified to the same fact. Histological observations have shewn menstrual changes in many of the aberrant adenomyomata. Decidual cells even have been found in such tumours. Now supposing an adenomyoma reaches the peritoneal surface of the uterus it may form an adhesion with a neighbouring organ not merely as a fibroid tumour might do but principally because it actually menstruates. Thus its peritoneal coat may be ruptured and adhesions more readily be formed. The tumour having adhered, the tubules will go on infiltrating the new ground, and we can imagine the extreme case in which the connection with the uterus has become completely broken off, and the tumour is found in a tissue in which no one can otherwise account for its presence. This extreme case is not hypothetical. I have a case that, to my mind, definitely proves my theory of migration.

@ @ @ @ @ @

Apart from those actually in the uterus, with or without definite mucosal connection, adenomyomata have been found in the Fallopian tubes, in the round ligament, in the broad ligament, in the ovarian ligament, in the rectovaginal septum, in the pedicles of ventro-suspended uteri, in abdominal scars, and in the ovary. The theory of migration should stand the test of these cases. The theory of Wolffian or Müllerian duct remains certainly cannot account for the occurrence of adenomyomata in all these situations. Cullen, who is the cheif supporter of the endometrial origin of most adenomyomata, confesses his inability to explain these extrauterine tumours on that basis. Meyer's theory, supported by Pick, Aschoff and others, that these tumours are derived from the serosa by metaplasia of the endothelium into epithelium is the one that has been almost universally adopted. It necessitates the supposition that such tumours arise by ~~from~~ an inflammatory process which not only brings about the profound changes in the characteristics of endothelial cells but also stimulates the connective tissue to produce fibroblasts of a type identical with the cells of the normal endometrial stroma. Lockyer who has collected every case of adenomyoma reported and has steeped himself in the views of every writer and speaker on the subject is an enthusiastic supporter of the serosal theory of origin. According to him this change of serosal endothelium "has been proved by repeated investigations carried out by reliable observers, and, moreover, it has been conclusively shown that the connective tissue which surrounds the endothelial inclusions can be excited to a hyperplasia which causes it to assume the characteristic histological features of the stroma of the uterine musosa". It may be remarked that we have to imagine such

a wonderful metaplasia of endothelium that a columnar cell epithelium is produced, that this epithelium forms itself into perfectly formed gland tubules identical in every respect with those of the uterine mucosa, that these gland tubules incite the connective tissue to produce a new kind of fibroblast in large numbers around them so as to simulate exactly the structure of normal endometrium, and, not stopping at this extraordinary mimicry, this metamorphosed structure proceeds to adopt the physiological functions of the endometrium. Further we are forced to imagine that the serosal endothelium behaves in this fashion only in the neighbourhood of the female reproductive organs, for it is only there that adenomyomata have been found. The hypothesis is too staggering for me. The cells of the stroma of the endometrium have none of the properties of fibroblasts.

The migratory theory quite easily explains the occurrence of adenomyomata in any of the situations in which they have been found. Take for example an adenomyoma in a ventrofixed uterus. Such cases have been recorded by Meyer (Zeit.f.Geb.XLIV,1903) and Klages (ibid., LXX,S 858). (The latter even records that the tumour was painful during menstruation). The suspension of the uterus was performed for some pathological condition of that organ: a new connection was established between the uterus and the abdominal wall: the migrating tubules could easily penetrate this band: suspension bands not infrequently thin out and break, and in the extreme case the tumour would be transferred to the uterine wall.

The tubal adenomyomata are not essentially different from those of the uterus proper. They are not uncommon. Generally they are found close up to the point of entrance of the tube into the body of

the uterus. Sometimes little adenomyomatous nodules are found more distally. It is said that because the tubal mucosa does not contain glands like those of the corporeal endometrium it cannot be the source of these adenomyomatous nodules, though all admit that it originates from exactly the same embryonic tissue as the uterine mucosa. The serosal origin is again invoked. But in many conditions of a chronic inflammatory nature, the normal stellate folding of the epithelium is so much increased that pouching actually takes place and prolongations of the epithelium occur into crevices in the muscle coat. There is the same liability to infiltration as in the body of the uterus.

Shortly after the appearance of our first paper in which we committed ourselves unreservedly to the mucosal theory of origin of uterine adenomyomata Pick sent us a section of an adenomyoma of the vagina, and at the time we had to confess that there might be exceptions. Of recent years a goodly numberof such growths have been recorded. Lockyer has collected 47 cases from the literature. They are more properly considered tumours of the rectovaginal septum. I had two cases within a few months. They are therefore not uncommon and as they may on occasion form tumours on the rectal wall they are important to the surgeon from the point of view of diagnosis of cancer of the rectum. If the real nature is not appreciated by the Pathologist he may agree in regarding the rectal tumour as an adenocarcinoma of the rectum. In both cases the tumour was excessively painful during menstruation. In one there was a distinct tumour in the anterior rectal wall very hard on palpation but without ulceration of the mucous membrane. This hardness was continuous right up to the uterus. The uterus, and part of the rectovaginal septum including

an oval piece of rectal mucosa on one side and vaginal on the other, was removed. The few septal adenomyomata that were recorded by this time were considered by the authors to have originated from the serosa of Douglas's pouch. I made sections from the posterior wall of the uterus, cervix, and vagina, and found an unusually extensive proliferation of endometrial tubules into the musculature. There was a congregation of adenomyomatous islets towards the serous covering of the uterus and between the two, scattered more sparsely, small adenomyomatous islets. Such islets were traced down into the cervix, in the wall of the posterior fornix, and in the rectovaginal wall. In the latter situation there was an unusual amount of fibrous tissue reaction, so much indeed that it looked like a diffuse fibroma, and the tubules were few in number. These were found however under the vaginal epithelium and under the submucosa of the rectum. Individual section did not show the whole process but from the hundreds that were examined it was possible to determine that the tubules found in the septum were continuous with those in the uterine submucosa. (Fig.4). In the other case the hardness of the cervix, together with the presence of a hard ill-defined prolongation from the back of the cervix along the course of the left sacrouterine ligament, led the Surgeon to think he was dealing with a carcinoma of the endocervix spreading along the sacro-uterine lymphatics, and the uterus, part of the vagina, and the left sacro-uterine ligament were removed. As I do not think that carcinoma of the uterus ever spreads in that direction, I suggested that it might be a migrating adenomyoma, before the section was cut. So it proved. The cervical endometrium had invaded the whole of the musculature of the cervix very deeply, and right throughout it there were small tense cysts. Unfortunately a continuous section was

not taken right into the sacrouterine ligament and so I cannot definitely and indubitably demonstrate a continuity of the process but a transverse section across the latter portion showed the typical appearance of adenomyoma with tubules imbedded in cellular stroma (fig.5). Unlike the other case it did not show actual invasion of the rectal wall but it was getting on towards it.

I have had two cases also of adenomyoma of the broad ligament. In one of these, from a patient of 64, the infantile uterus contained a pea-sized intramural myoma of the ordinary type and several walnut sized subserous fibroids. One of these lay between the layers of the broad ligament towards the tube and had a thin pedicular attachment to the lateral wall of the uterus. As no suspicion of adenomyoma entered my mind I took a section from this last tumour and the rest of the specimen was destroyed. Sections showed it to be a typical adenomyoma. I wish to emphasise the fact that the tumour was connected definitely with the uterus. In the second case, the surgeon removed from the base of the right broad ligament a tumour of the size of a Tangerine orange which he informs me was attached to the uterus by a cord of fibrous tissue. The section showed typical adenomyona (fig.6).

In a case of adenomyoma of the ovary reported by Semmelink and de Josselin de Jong (Monat.f.Geb.u.Gyn,xxii,p 244) the ovary was found to be adherent to an adenomyoma on the posterior wall of the uterus. Doubtless the ovary had formed an adhesion with the uterine tumour and had subsequently been infiltrated though the authors regarded their case as a proof that the serosa was a source of the tumour.

Fig. 4.

Drawing composed from 3 adjacent blocks in a case of adenomyoma of the recto vaginal septum, showing the excessive proliferation of the endometrium outwards into the musculature, the presence of a few tubules in the wall beyond this, more tubules in the septum (under the vaginal mucous membrane and under the rectal mucosa) and much fibrous tissue reaction around the few tubules near the rectum. The rectum is represented by a short black line to this side. (Neighbouring sections show tubules intervening between those of the endometrium and the few here seen above the posterior fornix)

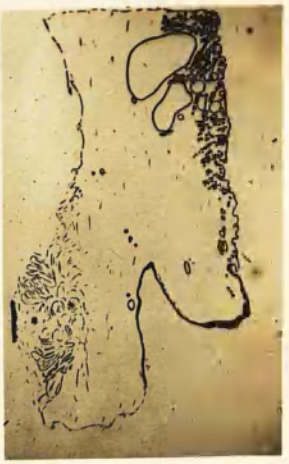


Fig. 5.

Microphotograph of a transverse section through the utero sacral ligament showing an adenomyomatous islet.

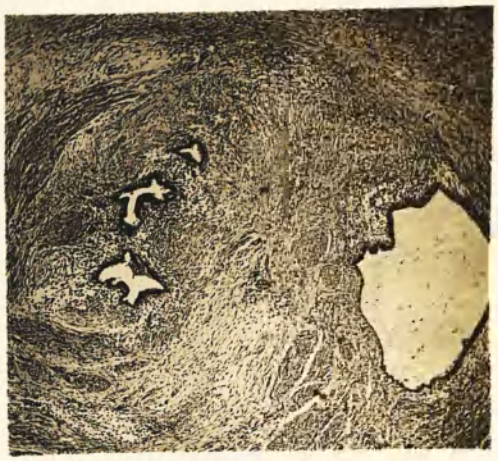
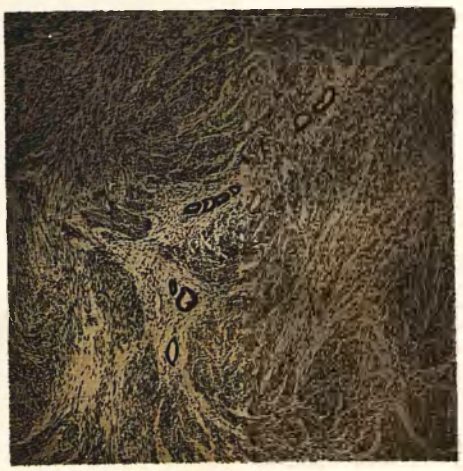


Fig 6.

Microphotograph of section of an adenomyoma of the broad ligament.



If my theory were correct I argued that we might expect to come across an adenomyoma in a situation normally out of connection with the uterus or reproductive organs to which place it had been transferred and had broken its connection. In 1912 I was lucky enough to meet with such an extreme case and I put it forward as demonstrating the infiltrating and migratory properties of adenomyoma of the uterus. The particulars of the case are as follows:-

> The patient was a lady aged 33, under the care of my colleague Mr. Cecil Rowntree. She complained of epigastric pain brought on by any slight exertion. She had long been of a very constipated habit and had been employing rectal irrigation for four months. The menstrual history was unimportant. She complained of pain in the back and had been ~~kaaxing~~ losing flesh Enemata were returned in two or three distinct portions. X-ray examination showed marked visceroptosis. Her symptoms were held to point to ptosis of the sigmoid colon, and in July,1912 laparotomy was performed under this diagnosis. Mr. Rowntree found a very long sigmoid loop lying in Douglas's pouch, the left ovary enlarged and prolapsed, and adhesions between the left broad ligament and the mesosigmoid. The broad ligament was divided and the ovary removed. The sigmoid loop was pulled up out of the pouch of Douglas with slight difficulty. On replacing it there was noticed on its antimesenteric border a small puckered area, half an inch in diameter, at the midpoint (the most dependent part) of the loop. Underneath this area there was a very hard nodule in the bowel wall of the size of a Barcelona nut. It seemed from its appearance and the sensation it gave to the finger to be perfectly typical of carcinoma, and such was the opinion of Mr. W.Ernest Miles, who happened to be in the Nursing Home at the time. Inspection of the posterior wall of the uterus showed a localised thickening or placque of growth under the peritoneum at the junction of the cervix and corpus. On allowing the sigmoid to take up its previous position it was found that the two came in contact (Fig.7) The placque, flat, circular, hardly raised from the surface, a few millimetres in thickness, was considered to be an implantation of cancer from the sigmoid. Under such circumstances a radical operation had to be postponed until the patient's relatives were informed. The uterine placque was removed, and when I examined it I found it to be an adenomyoma. Permission was given for a local resection of the sigmoid growth, but at the operation before proceeding with the resection Mr. Rowntree opened into the bowel beyond the margins of the growth and found that the epithelial layer was quite intact. A small portion of bowel was therefore removed and end-to-end anastomosis performed. The specimen showed a small puckered area on the serous surface with slight haemorrhage; cutting through this we found a white

Fig 7.

Diagram of adenomyoma of sigmoid colon adherent to adenomyoma on posterior wall of uterus

Fig. 8.

Microphotograph of wall of sigmoid colon showing infiltration of wall from the outside by an adenomyoma. The distorsion of the musculature will be noticed.

Fig 9.

Higher power microphotograph of same showing tubules in the muscular layers and in the submucosa of the sigmoid colon.

fibrous growth with rather indefinite margins apparently extending right up to the mucous layer but not involving it. On microscopic examination it is evident (figs. 8 & 9) that the outermost part is composed of fibrous tissue in which are found gland tubules surrounded for the most part by a small amount of cellular stroma. These tubules spread out into the muscular coat, which is much distorted; they reached right up to the submucous coat, and some sections show them actually in the mucous layer, from the tubules of which they can be distinguished the be absence of goblet cells and mucinous material. The raggedness of the little puckered area on the serous coat and the presence of haemorrhagic areas in that situation show that it has lately been adherent to something else and that the adhesion had been broken.

The sequence of events almost certainly is as follows:-

An adenomyoma having started from the endometrium migrated, and was extruded through the wall posteriorly; owing to haemorrhage occurring during a menstrual period the serous surface was broken; the loaded and dependent sigmoid colon became adherent to this; the glandular constituents infiltrated the wall of the sigmoid; the adhesion became attenuated by movements of the intestine and at operation the slender adhesion was broken leaving a discoid portion in the uterus and an abraded area in the colon. Supposing a greater length of time had elapsed the scar of the sigmoid might have healed over and the evidence of connection between the two would have been quite lost. As it is, it serves to show how the tumours infiltrate and migrate, and we need invoke the theory of rest-cell origin to explain the occurrence of such tumours in situations at a distance from the endometrium.

When I had reported this case Mayo Robson sent me notes of a case and sections of a tumour from the wall of the sigmoid colon - a typical adenomyoma. The case had been diagnosed as ulcer of the sigmoid or adhesions from local peritonitis.

He had found at operation an adhesion between the bowel and the Fallopian tube and ovary. When the adhesions were detached there was found a well-marked cicatrix in the sigmoid six inches from the rectum. The bowel was not constricted and the finger could be passed through on ingagination. As no permission had been obtained for enterectomy the abdomen was closed. No relief resulting from the breaking down of adhesions, the abdomen was again opened seven weeks later and the bowel cicatrix was resected. The tumour was about the size of a filbert and was situated on the antimesenteric border of the sigmoid.

Clara Stewart (Journal of Obst. and Gyn. 1914) reported an adenomyoma of the rectum similar in many respects to the sigmoid tumour I had recorded. Recently she reported to the Pathological Society of Great Britain and Ireland the discovery of a typical adenomyoma in the wall of the ileum in a case in which there were evidences of old adhesions to the pelvic organs. (see Journ. of Path. and Bact. Vol.xxiii p.235), and regarded it as evidence in favour of the theory of migration.

The question is left open as to whether adenomyomata should be considered true tumours or not. The myomatous part is adventitous. By some the condition is called "Adenomyositis". It is not necessary to invoke an inflammatory factor in the pathogenesis of the condition and the evidence for it is very slender. The essential feature is an infiltration of tissues by the constituents of the endometrium. "Infiltrative adenomatosis" might be a better designation.

MALIGNANT CUTICULAR OEDEMA.

("Peau d'Orange" and "Brawny Arm")

Investigation of the pathogenesis of the condition.

Incorporating a paper on Peau d'Orange in acute Mammary Carcinoma : its cause and diagnostic value contributed to The Lancet, 18th Sept, 1909.

MALIGNANT CUTICULAR OEDEMA

There are two conditions, the one rare and the other comparatively common, in which this hitherto undescribed lesion may be found. The former of these is what has been termed "acute carcinoma" of the breast, the latter is the so-called "brawny arm" in advanced mammary carcinoma. The skin over the affected breast in the acute carcinoma, and, the skin of the swollen arm in the advanced cancer, both show a remarkable regular superficial pitting and an apparent thickening of the epithelium. These appearances are due, as I shall show, to a blockage of the lymphatic vessels of the cutis vera by cancer cells with a consequent oedema of the corium, causing a bulging outwards of the epidermis except at those places where it is moored down by the deep insertion of the hair follicles into the cutis vera. From various observations, which I need not here discuss, I have concluded that there is no plexus of lymphatic channels in the cutis vera, but that there is an arborisation there of the perpendicular lymphatics which run down to a plexus situated on or under the deep fasciae. It is a peculiar fact, the explanation of which I do not know, that in ordinary dropsical conditions the cutis vera is not distended. I have therefore used the term "cuticular oedema" to express the condition to be described. I have only found it where the lymphatics are extensively blocked by cancer cells. It may exist in elephantiasis but I have not had an opportunity of examining that condition.

(1) The Peau d'orange of Acute Mammary Carcinoma.

Acute mammary carcinoma is seldom met with, or at any rate is rarely diagnosed. During a period of eighteen years and amongst

many hundreds of cases of breast tumours I have only encountered the condition ten times. Most of these cases were wrongly diagnosed by the clinicians, the rapidity of the process and the age of the patient leading them to think that they had to deal with an infective condition. In such cases the duration of the disease from beginning to end may be measured in weeks. Though it is always of importance to recognise any cancer promptly and to operate promptly and as extensively as possible, no matter how small or how slow in growing it may be, yet in these cases of acute mammary carcinoma prompt recognition and immediate and extensive operation are absolutely essential if the remotest hopes of its cure are to be entertained. Few of the surgical or pathological text-books refer to this particular well-defined class of breast cancers, and of the few text-books that do mention it, only one to my knowledge, adopting the explanation of the skin condition which I pointed out in 1909, gives the real cause of the peau d'orange.

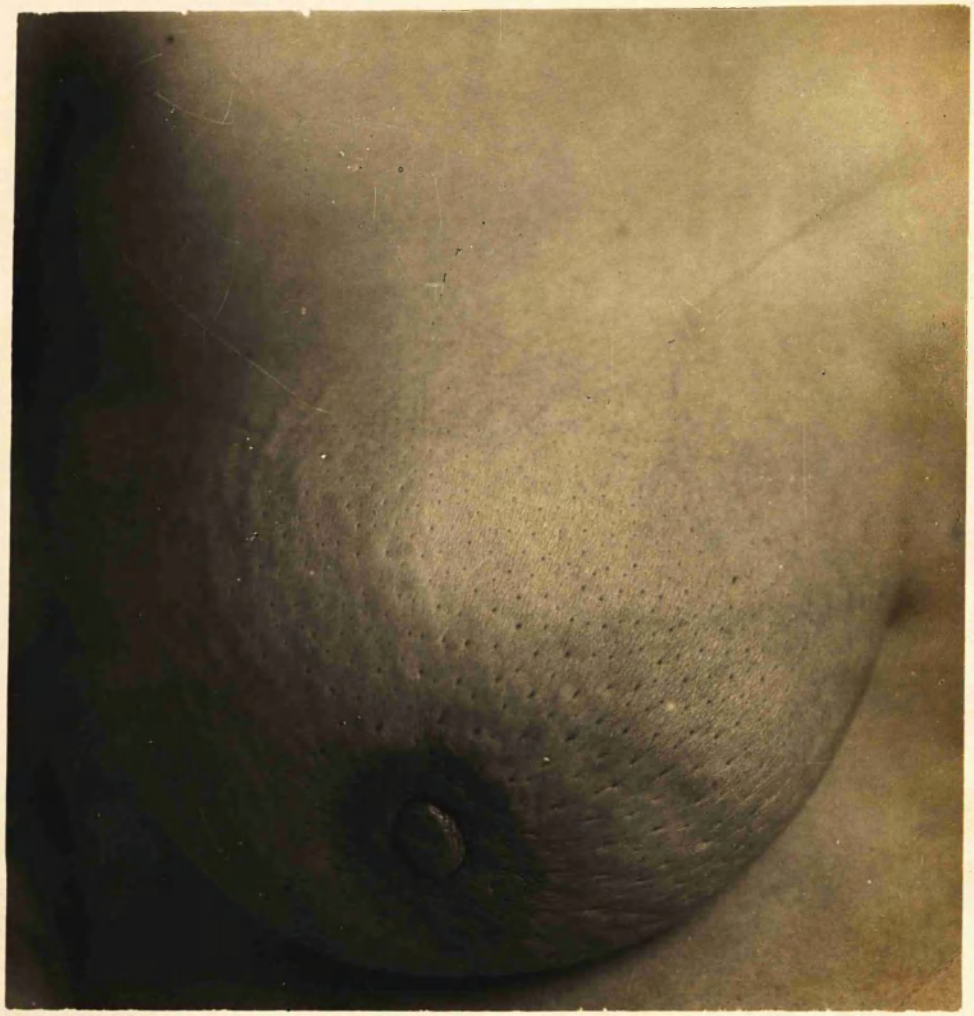
Clinical characters.- Acute carcinoma presents itself as a diffuse swelling of the breast in which the normal shape of the organ is retained; in fact, in comparing the two breasts one would be inclined to say that from an artistic point of view the affected breast is the better modelled. There is no flattening to be observed, no puckering even on digital movement, no asymmetrical bulging, no appearance as if of a healed scar. The nipple may not be appreciably indrawn nor give any sero-sanguineous discharge. There may or may not be a slight blush on the skin, especially in the lower part of the breast, but at any rate there is neither heightened temperature nor tenderness. Palpation of the breast shows that the swelling is diffuse and that there is no localised hardness, perhaps no hardness at all.

The disease may exist during pregnancy or lactation, and in these cases is liable to escape detection, or it may be found at any age, though generally it occurs in women who have not reached the period at which the occurrence of cancer is most common. Thus it follows the broad law, to which there are exceptions, that the younger the age of the patient the more rapid the growth. There are two points in the diagnosis of the condition that are of great value - the diffuse swelling like a hypertrophy and the occurrence of peau d'orange - and the conjunction of these two signs is pathognomonic. To the latter attention may be more fully directed. The name peau d'orange is descriptive enough to one who has seen it, but to those who have not noticed the condition it may not convey much impression, for orange skin is very variable and the irregular pitting and puckering of the latter might very well describe the fairly common appearance of the skin in cancer of the breast when it is affected by contraction of a growth underneath. In acute carcinoma, on the other hand, the skin has minute pits very regularly about a quarter of an inch apart giving the appearance as if the skin had been dabbed with a blunt pin.

The accompanying photograph gives a better idea than any description can of the characteristic appearance of the skin. (Fig. 1)

The usually accepted opinion is that the pitting is caused by the contraction of the fibrous tissue in the tumour acting through the suspensory ligaments of Astley Cooper. Fig.2 shows one of these "ligaments" of trabeculae running to the corium through the intervening adipose tissue. It will be referred to later. Against this view there are many objections. The Astley Cooper ligaments are not so uniform in their arrangements, nor are they so closely set in relation to each other as to produce by their contraction such

Figure 1.



MALIGNANT CUTICULAR OEDEMA. Peau d'Orange in a breast which is the seat of a carcinoma of the acute type.

regular pitting. Again, any contraction from below would not produce depressions, the depth of which is as great as, if not greater than, their breadth. We should expect when we free the skin by dissecting under it that there would be diminution in the depth of the pits, if they were due to contraction from below. But the chief argument against accepting the general view is that the condition is found only in very rapidly growing, rapidly expanding tumours, in which the fibrous tissue is at a minimum. Contraction in less acute tumours produces a much rougher puckering.

Microscopic appearances of acute carcinoma.- Sections taken from the tumour shows large areas of spheroidal cells closely packed together and generally well preserved, though sometimes exhibiting central disintegrations. The appearance is sometimes called "medullary" carcinoma. The strema is usually relatively small in amount. Portions may be chosen from the growth which show only proliferation within the ducts and acini, but this proliferation is always a marked feature and differs from that of mastitis, in that there is no loss of nuclear staining, whilst the cells tend to form numerous daughter lumina. At such parts as these, the elastic tissue sheath of the ducts is sometimes intact, at other parts it seems to be undergoing solution. This proliferation is found everywhere throughout the gland; the epithelium of the whole organ seems to be malignant. In addition, as we get nearer the edge of the growth, we find extensive lymphatic blocking by masses of cancer cells. In fig.2 the trabecula running from the mammary gland to the dermis through the intervening fat carries in its lymphatics epithelial cells in direct continuity with those of the growth.

Appearance of the skin.- When the skin is cut through it appears to be enormously thickened - from the normal 0.5-1 millimetre over the breast to 8 millimetres or more. It is dense and white and appears like ordinary squamous epithelium much thickened, and it retains this appearance in alcohol. But the microscope shows that the epithelium as itself is thinner than usual. The normal papillae are to a great extent obliterated or are only irregularly maintained. At some parts the papillary layers and elastic tissue are quite absent, at others they are dense, whilst intermediate conditions are found. Where they are absent the change seems to be associated with the presence of collections of lymphocytes and some plasma cells directly under the epithelium. The condition then resembles what has been described by Ribbert, Victor Bonney, and others as tissue prepared for cancerous invasion by the surface epithelium. It may, however, here be noted that the surface epithelium is not proliferating, almost certainly never would do so and it is probable that the so-called "precancerous" conditions are brought about by the same mechanism.

The whole apparent thickening of the skin is due to the condition of the corium. The connective tissue appears to be in a sodden condition, the fibrous strands are widely separated from each other, and arterioles are dilated, and their walls are thickened. The lymph channels are especially marked. Towards the deeper parts of the corium it would seem as if practically every lymphatic channel were filled with cancer cells; some are filled right up to their origin immediately under the epithelium, others as they approach the surface are seen to be empty and often dilated.

Figure 2.

Figure 3.

Fig. 2. A projection drawing of a section of the skin of the breast in the condition of peau d'orange. The strand of fibrous tissue passing up to the corium through the fat is strewn with masses of cancer cells in lymphatics. The black dots in the corium represent minute collections of cancer cells.

Fig. 3. A projection drawing on a larger scale of the corium at the site of one of the skin pits shown in figure 1. All the lymph vessels are widely dilated. Many of them contain cancer cells. Perilymphatic collections are common.

Perilymphatic collections of lymphocytes, with a few plasma cells amongst them, are marked features, and these perilymphatic infiltrations seem to follow a definite plan; they converge towards collecting lymphatic trunks below the corium. It is probable that the lymphatics in the trabeculæ represent the trunk and the smaller vessels in the cutis the branches of a lymphatic tree. The cancer cells have permeated the lymphatics in the opposite direction to the course of the lymph stream. In the centre of some of these perilymphatic infiltrations we may see small masses of cancer cells in well defined lymphatic spaces; in some the channels are quite empty, whilst, on the other hand, we may find cancer cells in lymphatic channels, and even empty channels without any perilymphatic infiltrations. It is evident from this that the "round cell" infiltration is not due to a reaction to the cancer cells as such; it may be present where these are absent or absent where these are present. In the former condition it precedes the lymphatic permeation. Such infiltrations seem to me to be the direct result of complete blockage of the channels, seeing that where we find them it is possible to trace the empty channel back to a place where the epithelial cells are. When they are absent either a round and empty or a filled lymphatic channel it is to my mind an evidence that collateral branches carry on the small amount of lymph circulation that is necessary as long as the surface epithelium can live. Small areas of round cell infiltration can also be seen round definite arterioles, but they are much less marked.

The depressions of the surface that give the appearance of orange skin are the exaggerated pits of the hair follicles. (Fig.3.) The erector pilae has its fibres separated like the rest of the corium. The thickening of the skin is thus due to changes in the corium. These changes are the results of lymphatic permeation and consequent lymph stasis. The corium is expanded and the overlying epithelium is raised above its normal level by the pressure except at those places where it is bound down by the insertion of a hair follicle deep in the corium. There is no disease of the breast at all likely to produce this appearance other than cancer. Thus even in the absence of other signs a diagnosis of acute mammary cancer from this alone would be practically certain.

(2) Brawny Arm.

In cancer of the breast oedema of the arm on the affected side is a fairly frequent late phenomenon. It occurs also in cases where the breast has been removed for carcinoma and there is recurrence in the axilla. Sometimes, too, it is found after complete removal of the mammary tumour together with thorough clearance of the axillary contents in cases that have survived for several years with apparently no malignant tissue in the axilla. The oedema is obviously due to a blockage of the lymphatic trunks presumably in the axilla for practically all the lymph drainage of the arm passes through that locality. Now there are two distinct varieties of this oedema of the arm though the second may later be added to the first. The first is an oedema of a simple uncomplicated nature. The arm is swollen, it may be intensely swollen;

frequently the hand is extremely oedematous. The skin is almost translucent. But the distinguishing character of this variety is that the skin, anywhere in the arm, pits easily on pressure: it is soft and yielding. If we cut into such an arm we shall find that the skin is not at all increased in thickness. The condition is caused by fibrous contraction round the axillary lymphatic trunks. The second variety to which the name "Brawny Arm" is more properly given has different characters. The arm is swollen just as much as in the first but the skin over the whole or part of it is hard and resistant to the touch, no longer translucent except perhaps on the back of the hand or lower part of the arm, and it does not pit on pressure. In extreme cases the skin shows what appear to be flat superficial nodules but usually it gives a uniform dull board-like appearance. It shows numerous little superficial pits similar to the peau d'orange already described in acute mammary carcinoma especially on the outside of the arm. If we cut into such an arm we find that the skin is apparently much thickened. In some instances we may see this "brawny" condition in the upper part of the arm and the more simple oedema in the forearm and hand. The recognition of the causes of oedema of the arm in breast cancer is of practical importance on account of the surgical measures proposed for its relief. Even in slight degrees it is very uncomfortable; in advanced states it is extremely distressing. Some surgeons amputate the arm at the shoulder. In all cases so treated that I have been able to follow the relief is merely temporary; the wound at the shoulder becomes filled with a fungating mass of growth, and the pain is as great as before. To get rid of the oedema

some Surgeons adopt the operation of Kondoleon in which an endeavour is made to establish a connection between the more superficial plexus of lymphatics and those deeper in the intermuscular septa. Handley introduced the rather ingenious operation of lymphangiophlasty; he inserted three or four strands of stout silk twist above the level of the wrist threaded on long probes which he pushed up in the subcutaneous tissues to the axillary and scapular regions, expecting thus to supply artificial drains for the collected fluid. The results in some cases which I saw were very striking; certainly much of the surplus fluid was drained off; but the relief was merely temporary and the condition after a time was as bad as before. A.R.Ferguson showed that round the silk threads a dense fibrosis occurred rendering the artificial channels impermeable to the collecting fluid without.

In the examination of brawny arm I have taken long continuous strips from the shoulder to the wrist carrying the knife down to the bones. Such strips after fixation in formalin were divided into numbered blocks, the position of which was recorded in a diagram of the whole strip. Sections of these blocks showed actual permeation of the lymphatic channels of the arm, not only of the plexus in and below the deep fascia, but also of the superficial arborisations and of the intermuscular and interfascicular lymphatics. Every lymphatic channel that one could recognize seemed to be dilated and to contain uninterrupted cords of cancer cells. Kondoleon's operation could not therefore be of the slightest use in this condition for the deep lymphatics were blocked as well as the superficial, though perhaps not so extensively, Many muscle fibres were necrosing or had already quite degenerated. But the surprising

Figure 4.

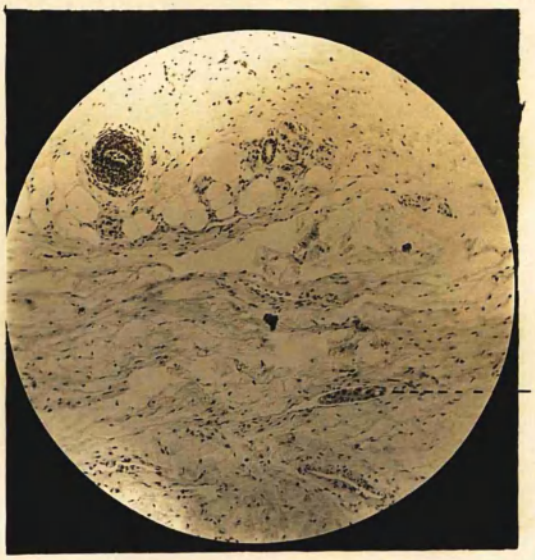
Figure 5.

Fig. 4. Projection drawing of a section through the skin and deeper structures on the outer side of the arm two inches above the elbow, showing spread of cancer in the deep plexus (DP), some masses in the superficial layer of the superficial fascia (SF), and infiltration of the corium. The black dots represent small collections of cancer cells.

Fig. 5. Larger scale drawing of a section of the same arm taken one inch above the wrist posteriorly. The main masses of cancer cells are lying in the deep lymphatic plexus in the substance of the deep fascia. A few isolated masses consisting of a few cells are to be found in the corium (marked with dotted squares, C,D,E.). The cells in A and B are in the lymphatics connecting the deep plexus with the corium.

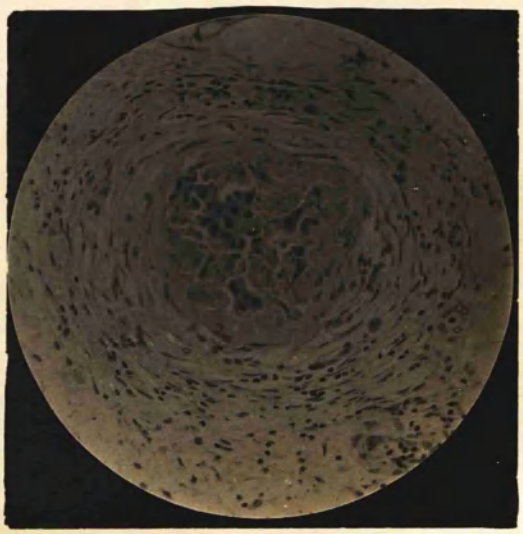
point was the extent of the lymphatic permeation in the superficial plexus situated in and beneath the deep fascia. In six cases masses of cancer cells were easily detected in this plexus as far down as the back of the wrist. Lagging a little behind this was the infiltration of the corium; that is to say, the deeper spread was always more extensive that the more superficial spread. (Fig 5+6) As the strips lay in the formalin one could detect even with the naked eye the thickened white cutis with minute nodules within it especially in the upper part and sometimes and small nodule in the fibrous tissue amidst the superficial fat, especially in the superficial fascia. (Fig 4) The thickening was seemed to be similar histologically to that described above in peau d'orange; the epithelium was not hypertrophied, the corium was oedematous, and the cuticular lymphatics were blocked by cancer cells. Some dilated and empty lymphatics in the cutis showed around them lymphocytes and plasma cells. Not infrequently little cancer cells masses could be found in lymphatics right up to the papillae. The involvement of the arborisatious could often be traced from the deep plexus right up through the perpendicular stalk. Sometimes the continuity between the two was broken by a perilymphatic fibrosis in the midst of which could still be detected the remains of malignant epithelial cells. (Fig. 7) The minute pits seen on the skin in this condition corresponded with those seen in the breast in acute mammary carcinoma. They represented the points of insertion of the hair follicles. Both conditions are due to the same cause - cuticular oedema brought about by occlusion of the lymphatics of the corium. Further confirmation of this idea can be obtained experimentally. If we inject intracutaneously by a fine hypodermic

Figure 6.



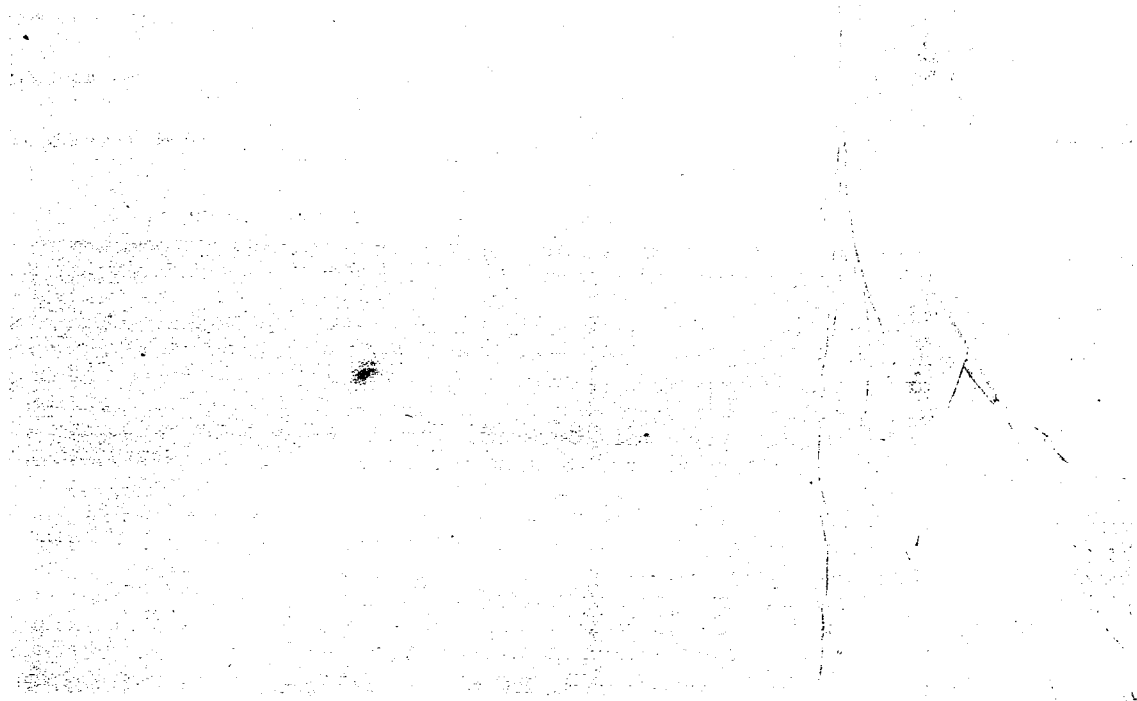
MALIGNANT CUTICULAR OEDEMA. A small mass of cancer cells in a lymphatic vessel (A) in the cutis vera an inch above the wrist posteriorly. This photograph is taken from the area marked E in Figure 5.

Figure 7.



Fibrosis round degenerating cancer cells in a lymphatic vessel of the upper arm. The vessel ran from the deep plexus to the cutis vera.

needle any fluid substance, such as cocaine solution, we produce locally the same appearance as in peau d'orange . The localised oedema thus induced takes an appreciable time to disappear: with serum the time is longer than with watery solutions of drugs. If we try to disperse the local swelling by pressure we notice that it does not sensibly increase its area, and if we inject blood corpuscles belonging to a group that is lysed in the patients serum we find that there is no increase in the diameter of the ecchymosis during absorption. All these facts go to show that the lymphatics in the cutis vera do not freely communicate with one another, but are merely upgrowths from a deeper plexus. Finally they demonstrate the fact that in a cutieular oedema extending over a considerable area there must be a blockage of the lymphatics of the corium, or of the radicles from them, as extensive as the area of such oedema.



A CASE OF MALIGNANT HAEMANGIOMA.

A MALIGNANT ANGIOMA

True angiomata, as distinguished from certain errors of vascular developement with which they are often confused, are generally of a more or less locally infiltrative character. This infiltration may be very extensive. Supposing they infiltrate the wall of a vein, as they sometimes do, they may proliferate along its wall or into its lumen and form local tumours, as Mallory (Principles of Pathological Histology, 1914) has pointed out, which may be mistaken for metastatic growths. That angiomata, giving, for the most part, a histological picture which we associate with benignity, can actually metastasise to form secondary nodules without intervening continuity between them, is a position that is now admitted owing to the remarkable cases reported by Borrmann and Shennan. I have to add a third case to the list of generalising haemangiomata. I wish to avoid the term "metastases" as applied to the secondary localisations because there is no proof that these originated from cells or cell masses derived from the primary growth: they seem to be independent vascular proliferations, secondary in point of time, brought about perhaps by some general condition - a cacoethes of vascular endothelium.

The details of my case are as follows:-

CLINICAL COURSE

Miss E.E. aged 38, was admitted to hospital on 25th April, 1913 with a diffuse swelling of the right breast.

The previous history and the family history were unimportant. In December 1912 she noticed that the right breast was larger than the left and the nipple not so

prominent. Occasional shooting pains were felt in the right breast but these were not at all acute; they were chiefly noticed during her menstrual periods. There had been no discharge from the nipple. On examination there was found considerable, uniform enlargement of the right breast with evident retraction of the nipple. There was no dimpling nor discolouration of the skin. On palpation the breast was diffusely thickened, the enlargement being somewhat granular and envolving the whole of the mammary glands. No definite tumour could be made out. The impression obtained was that of a diffuse chronic mastitis. There were no lymphatic glands palpable in the axilla. "If the breast is palpated after it has remained undisturbed for some time it feels very firm but on gentle pressure this firmness disappears and it becomes soft and the individual lobules can be felt. The left breast has its lobules nodular but not to the same extent as in the right".

On April 30th, Cecil Rowntree made a submammary incision, and, reflecting the mammary glands upwards, removed a portion from the lower and outer quadrant. The incised surface was chocolate in colour with a few red bleeding points. The cut surface looked very much like an angioma but there was surprisingly little oozing. The breast and wound were separately sutured. The wound had quite healed and the patient was discharged on May 17th, but the appearance of the breast remained as before operation.

She was seen from time to time at the Out-patient Department and as the enlargement of the breast was slowly increasing and beginning to distress the patient she was again taken into hospital and on the 28th July 1913 the complete breast was removed. The patient was discharged on the 16th August with the wound soundly healed.

On the 2nd March 1914 there was noticed for the first time, although fortnightly examinations had been made since the last operation, a small cystic swelling about half an inch in diameter to the left of the scar near its middle.

On the 5th of November 1914, the patient was readmitted complaining mainly of a lump in front of the left thigh which caused lameness and was accompanied by pain shooting up to the outer side of the thigh in walking. She declared that this lump in the thigh had "always been there" but only recently had increased in size. It was larger when she stood for a time. The scar along the outer border of the pectoralis muscle was healthy, but mesial to it there was a soft boggy swelling that disappeared almost entirely on firm pressure. At the upper and lower ends of the scar there were two bullae of a dark bluish colour which also could be compressed to a level with the skin. Another raised dark spot was found above

the hyoid bone, and still another on the left side over the ribs. There were many purpuric spots in the skin of the back. In front of the left femur in its middle third was a soft compressible swelling apparently among the muscles and unattached to the bone. The patient was anaemic in appearance and complained of menorrhagia and metrorrhagia; this she said had existed before the operation and she was liable to have slight bleeding if she stood for a time, but the complaint cleared up after the operation and had returned four months ago.

On 13th November 1914, the tumours of the breast and thigh were excised; the former were directly subcutaneous and slightly haemorrhagic round the periphery: the latter were was inextricably attached to the quadriceps fibres and had no defined limits. Both wounds healed per primam.

On the first of January 1915 a swelling was noticed below Paupart's ligament on the left side spreading outwards to the great trochanter, and pushing the femoral vessels in front of it; there was eversion of the leg and movement was so painful that X-ray examination was impossible.

On the 17th February 1915, after twelve hours vomiting and dyspnoea the patient died.

@ @ @ @ @

From an examination of the portion of pinkish tissue removed from the Mammary gland for diagnosis I reported that the condition was a haemangioma, more of the capillary than of the cavernous type. The whole of the piece chosen for histological examination showed a uniform appearance: here and there one could detect compressed acini of the breast surrounded by a tissue composed of many irregular communicating channels, mostly filled with blood cells, bounded by a single layer of vascular endothelium. The supporting structure of the endothelium consisted of fine, very loose, oedematous strands with few nuclei, recalling pretty much the histological picture of the strema in hydatidiform mole. As I was not aware of any previous record of a diffuse angioma of the mammary gland I persuaded the Surgeon to remove the whole breast. The simple uncomplicated

pattern of the angioma afforded no grounds whatever for supposing that the condition was other than an ordinary benigh haemangioma. (See fig.5)

Mammary Gland. The operation specimen of 28th July, the complete breast, showed that the greater part of the mammary gland, measuring 9 cm. in diameter by 3.5 cm. in ~~diameter~~ thickness, was of a scarlet colour with white fibrous streaks throughout, the colour being accentuated to a dark red apparently by small haemorrhages into the tissue or by blood in dilated vessels. The tumour condition was of an irregular ill-defined outline: nowhere was there a trace of a capsule or of a condensation of mammary tissue at the boundary: but on all sides it seemed to be lost insensibly in the fat and surrounding tissues. The cutis vera of the areola showed telangiectasis continuous with the deeper reddish gland but for the most part the tumour was separated from the overlying skin by subcutaneous fat and the layer of fascia covering the gland (fig.4)

Numerous histological preparations, stained by various methods, were obtained from the tumour. For the most part the sections revealed nothing more than the appearances already ascertained from the diagnostic section. The greater part was of the same simple type seen in fig.3. Here and there, there were areas of haemorrhage with long spindle cells going out from the margin into the clot: these cells actually formed short canals and were regarded as being of endothelial origin, though it could not be said beyond doubt that they were not fibroblasts. The purely angiomatous areas were partly separated from each other by the more or less normal collagenous fibrous stroma of the breast, but even there, adjacent to the

tortuous mature blood vessels, there were little irregular bunches or intercommunicating capillaries. An endeavour was made to trace a direct connection between the mature vessels and the capillary bunches by serial sections, but the latter were so widely connected with similar neighbours that it was impossible to arrive at a decided conclusion. Certainly the maturer blood vessels, especially the veins, showed unusual irregularity of their internal coat, the endothelium dipping down between clefts of the inner muscular layer. At some portions the texture of the tumour proper was of a more open kind, the spaces being wider and the endothelial-covered tissue more compressed, so that the appearance given was that of the extreme cavernous type of angioma. It thus approached the condition seen in fig. 9 which is a microphotograph of a recurrent nodule. Again the other extreme was encountered, the ~~middle~~ endothelial cells being more crowded together and irregularly arranged with but little supporting strema in comparison. This higher degree of endothelial cellularity, although I have since found it in other angiomas in which there was no suspicion of malignancy, caused me to think that this particular tumour might not be quite benign in its nature. The extreme margins of the tumour showed an infiltration of the adipose tissue. Between individual fat cells (See figs 7 and 8) tumour cells, small spindles or round, with round uniformly staining nuclei could be seen forming definite capillaries with blood corpuscles within them. It was often impossible to differentiate between the marginal nuclei of fat cells and this endothelial cell infiltration. Sometimes the blood corpuscles seem to intervene between fat cells without the accompaniments of endothelial cells, sometimes there were small masses of endothelial

cells without canalisation and without blood corpuscles. Possibly two processes were at work at the same time in adjacent parts, and infiltration of the fat by endothelial cells with subsequent canalisation, and an escape of blood between the fat cells from an inmature or ~~form~~ capillary. This infiltration of the fat coupled with the irregularity and higher cellularity noted above, both of which can be found in some simple angiomata as I have since discovered, confirmed the suspicion that the tumour was not benign and I requested the Surgeon to keep a close watch in the subsequent progress of the case. The recurrent nodules in the neighbourhood of the breast scar were thus detected soon after they appeared. The recurrent nodules in the neighbourhood of the breast scar showed two widely different histological appearances even in close proximity to each other. At one part of the nodule the texture was very loose. Fig. 9 shews the condition. Running throughout the section were trabeculae of fine widely spaced fibres covered with a single layer of flat endothelium. Papillary processes of the same structure cut in section were dotted over the field, some minute and some large. The stroma of these papillary processes was always small in amount with a tendency of the fine fibres to congregate to the margins leaving the centre empty save for an occasional strand. The cavernous spaces between these processes were filled with blood most of which was lost in the preparation of the sections. The appearance so far did not differ from that of a simple cavernous angioma but in close association with that open structure was the picture shown in fig. 10 which is taken from a microscopic field almost next to that already seen in the preceding figure. Here though the stroma was still

small in amount the endothelium had proliferated to a marked extent, being irregularly heaped up in several layers, the cells crowded together, losing their flattened shape and assuming a short irregular spindle form. Further the cells stained more deeply, the nuclei appearing darker by reason of their closer association, and the small amount of cytoplasm reacting more to the contrast stains. This character seemed definitely to remove the tumour from the benign class of neoplasms. Still, it is worth noting that, exuberant though the endothelial proliferation was, the blood channels were wide open and irregular spaces: there was not the slightest sign of any whorling arrangement of the cells such as is held to be a characteristic of endotheliomata. The portion of tissue removed from the thigh showed neither of the two characters seen in the breast nodule. It was merely a plexiform arrangement of mature veins and venules in a moderately dense fibrous tissue stroma. The small recurrent nodules removed from the neck gave the structure of a simple capillary angioma.

(Just at this time Shennan's paper on a metastasising angioma appeared.) The patient was retained in Hospital on the chance that widespread metastases might occur. The postmortem examination was performed on 18th February 1915 during my absence on military service by my colleague Dr. Paine to whom I am indebted for the notes and for preserving certain organs which I examined on my return to the laboratory in 1919. The following is an extract from the postmortem record:

> "Emaciation not extreme. In the right submaxillary region there was a small angiomatous patch about the size of a barley seed in the skin. There was a dark purple spot

beneath the skin on the top of each shoulder. Above the linear scar of the right breast and to its outer margin there was a purple patch of the size of the thumb nail. The right leg was everted, the knee flexed, and a fullness was noticable in front of the thigh just below Poupart's ligament. The limb was otherwise slightly thicker than the other leg owing to oedema.

On reflecting the skin from the neck, thorax, and abdomen, the under surface was seen to contain numerous scattered angiomatous tumours varying in size from a pin head to a large shot. These were especially marked and grouped in the left breast the area of which was mapped out by aggregations of bright red spots. The tumours for the most part were sharply defined, though irregularly rounded on surface and margins. They were fewer in number all over the subcutaneous tissues of the abdomen but though reflected with the skin were not visible from its surface. No growth could be detected in the neighbourhood of the scar.

Lungs. On the surface of the right lung there were several small angiomata, bright red and standing out from the surface as if they had developed in the pleura. Other nodules of a dark purple, larger in extent, were detected in the lung substances in all the lobes. The pleural cavity contained several ounces of clear bloodstained serum. The left lung was firmly adherent to the chest wall laterally and to the diaphragm. Angiomata were absent over the surface though present in the substance.

(The condition of the breast and the structures in the neck, thyroid, larynx, and phanynx, was not noted, the specimens having been put aside for subsequent examination. No further note was added)

Omentum and Mesentery. The omentum contained about half-a-dozen small angiomata the bright red colour of which contrasted strongly with the yellow fatty tissue. They were raised and discrete. The mesentery was studded with small shotty purple tumours mainly situated along the branches of the mesenteric vessels. The tumours were chiefly distributed over the visceral rather than the parietal layer of peritoneum.

Lymphatic glands. The retroperitoneal lymph glands in front of the spinal column were moderately enlarged, reddish brown in colour and probably infiltrated with growth.

Liver. The liver was not enlarged. Its colour was normal though its surface was covered with patechia-like patches, like a purpuric eruption. These angiomatous patches were confined to the capsule. One large growth fairly well circumscribed, the size of a walnut, was found in the substance of the left lobe; it was of a yellowish white colour streaked with red. The gall bladder was not affected.

Spleen. Over the surface there were similar small purple areas as in the liver. The size of the organ was normal.

No nodules were found in the kidneys, adrenals, pancreas, stomach or intestines.

Pelvic organs. Both ovaries were enlarged to the size of a hen's egg and apparently contained numerous haemorragic cysts. The uterus was of small size and contained a small fibroid protruding from the fundus. Along the right brim of the pelvis the external iliac glands were enlarged, firm and reddish in colour.
Thigh. The femoral vessels were stretched over a fluctuating tumour bounded by the psoas muscle in front and laterally by the tensor fasciae femoris. Behind the psoas there was a large cavity filled with blood and necrotic tissue bounded by necrotic haemorrhagic tissue. Into this cavity protruded from below the jagged end of the shaft of the femur. The remains of the head of the femur were found in the acetabulum but the upper part of the shaft and the great trochanter had been destroyed by secondary growth.

Museum specimens were made of the left mammary gland, the right lung, part of the liver containing the secondary nodule, the spleen, and the uterus and adnexa. These were available to me for examination. As far as these specimens are concerned it only remains to add that the left mammary gland was mostly free of angiomatous growths only one portion at the extreme margin (fig.2) showing actual infiltration, although several small angiomata had formed in the fat above and below the gland. The superficial angiomata noted as projecting from the surface of the pleura at the postmortem examination have, perhaps in the course of fixation, become flattened haemorrhagic areas and the nodules in the lung substance are irregular owing to diffusion of the blood into the surrounding alveoli. The liver nodule appears to be of a firm texture, whitish for the for the most part with red blotches and streaks (fig.3) In the capsule are many flattened haemorrhagic areas. Similar areas are found in the spleen but none in the splenic substance. The right ovary (Fig.4) is enlarged and shows numerous dark red areas: the left ovary contains merely a dermoid cyst of the ordinary characters.

Histologically the liver nodule (Fig. 2) shows an angioma of a

surprisingly simple type. Nowhere is there any irregularity in the formation of the endothelial walls, nor is there any appearance to suggest its malignant nature. It is not so markedly cavernous as the usual hepatic angioma. The only unusual character about it is its solid appearance and its definitely whitish colour. Sections taken from the margin of the liver and spleen show subcapsular capillary angiomata. Two retroperitoneal glands which had been taken for examination revealed no angiomatous formation within them: they showed much destruction of blood corpuscles and marked phagocytosis of corpuscles by large endothelial cells. The ovary showed angioma of a mixed type resembling the condition found in the breast. The pulmonary nodules gave the most interesting histological picture of the lot (Fig.II)Here there had been excessive proliferation of the endothelium so that the blood spaces were relatively small in amount. The endothelial cells were of a uniform appearance containing a spherical nucleus that stained homogenously, and a moderately small amount of cytoplasm. Here we have a true tumour of endothelium which shows none of the characteristics of the "endotheliomata" which one sees in text-book illustrations. The diagnosis of a tumour as "endothelioma" generally seems to be a light-hearted way of escaping from the difficulty of calling an obscure malignant tumour either a carcinoma or a sarcoma, or it is the result of a mental process, akin to revelation, the workings of which cannot be communicated to the sceptical.

Numerous cases have been reported of widespread multiple angiomata of single organs, especially the liver, and of multiple angiomata in different organs in the same individual, but the only cases bearing any resemblance to mine, of a definitely malignant nature, are those of Borrmann and Shennan.

Borrmann (Beitr.z.path.Anat.u.z.allg.Path., Bd XL,p.372 1907) reported a case of a woman of 26 who had a subcutaneous hard walnut-sized purplish tumour over the right breast. It recurred four weeks after removal and then the breast and axillary glands were removed. Apparently no histological examination was made. Nearly a year afterwards recurrences were seen in the neighbourhood of the scar. These were again removed and subsequently recurrences were noticed in the right scapular region and later in the gluteal region. At postmortem, numerous angiomatous nodules were found on the pleural surface and in the substance of both lungs.

Shennan (Journ. of Path. and Bact. Vol.xix p.139,1914) recorded an angioma with numerous metastases, which he thought originated in the spleen, with numerous angiomata in the mediastinum, pericardium, heart muscle, lungs, liver, peritoneum, skin and bone marrow.

Both these authors regard the secondary tumours as blood-stream metastases. I could find no evidences of a metastasing process. It was possible to see in the fat over the left breast small isolated angiomatous patches: a blood vessel entering and one leaving the area could be made out with the naked eye. Several of these were cut out in toto and serial sections made from them. It was determined that the little angiomata arose in connection with small veins. Owing to the excessive twisting and turning of the blood

channels all round the vein it was exceedingly difficult to follow their ramifications. It seemed that several communications existed with the parent vein by means of fine capillaries. These latter opened sometimes immediately, sometimes after a short but tortuous course, into the communicating dilated vessels forming the nodule: In no case was there any growth within the wall of the parent veins as would be expected if there had been a blood metastasis. The multiplicity of angiomata in my opinion, is not to be explained by the detachment of cells from a parent tumour. In Borrmann's and Shennan's cases, as in my own, the striking feature of practically all the secondary nodules was their histologically benign character. If metastases had been formed we should have expected very aberrant tumour growths with a more or less retarded differentiation of structure. Wherever nodules were found they were in free communication with the blood stream as was shown by the fact that they contained the normal formed elements of blood in the usual condition, and further they retained these elements without allowing them to escape into the surrounding tissue, with the possible exception I have noted in the breast. The absence of anything approaching cell-containing thrombi or of proliferation within the lumen of the venules is against the idea of metastasis formation. The multiplicity of angiomata in many of the cases reported of particular organs without secondary nodules elsewhere would point to a vascular defect localised to that organ: and this angiomatous condition may be very widespread within the limits of the organ. I imagine that in the three cases which we shall agree to call "malignant angioma" we have to deal with multiple foci not originating simultaneously.

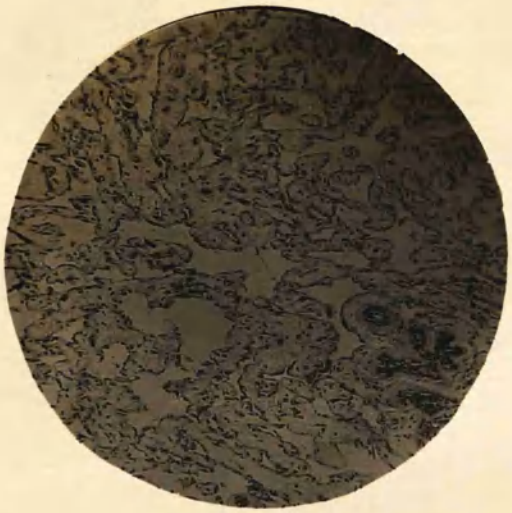


Fig 5.
Original section showing apparently simple angioma of mammary gland. Mammary acini at right margin

Fig 6
From the left breast
On the right half there is excessive proliferation of endothelial cells.

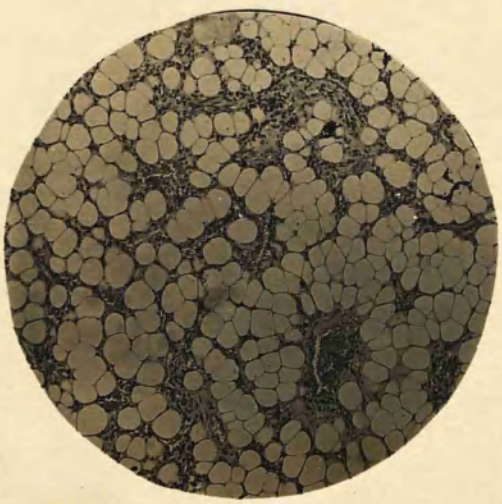


Fig 7.
Infiltration of fat at outer margin of breast tumour

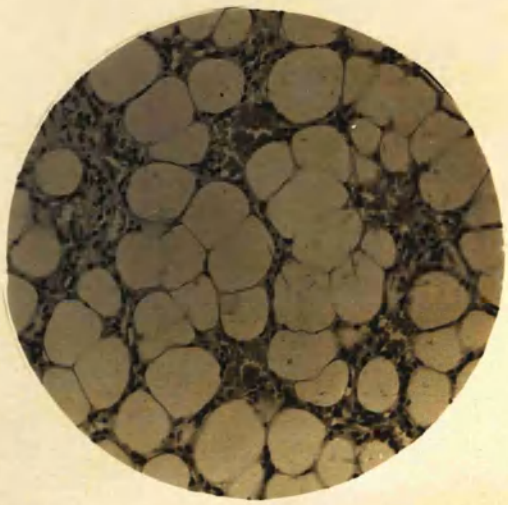


Fig 8.
Higher power view of same showing endothelial cell masses between fat cells and blood-vessel formation

Fig. 1.
Original tumour of breast (left)
Operation specimen

Fig. 2.
P.M. specimen of right breast

Fig. 3.
P.M. specimen
Nodule in liver and subcapsular
angiomatous patches

Fig. 4.
Angioma of ovary (split).

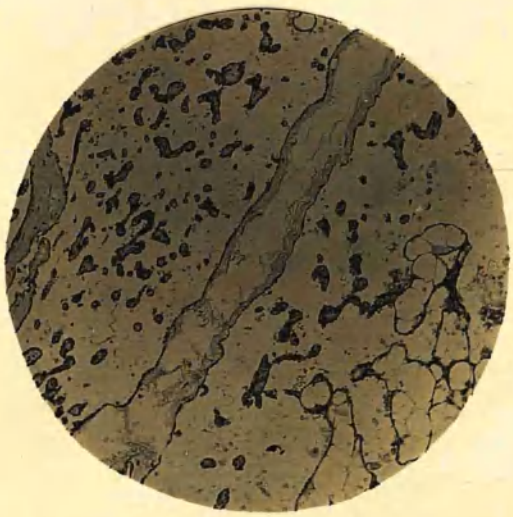


Fig 9.
Section of recurrent nodule after removal of breast. Open cavernous type

Fig 10.
Another part of the same section showing irregular endothelial cell proliferation

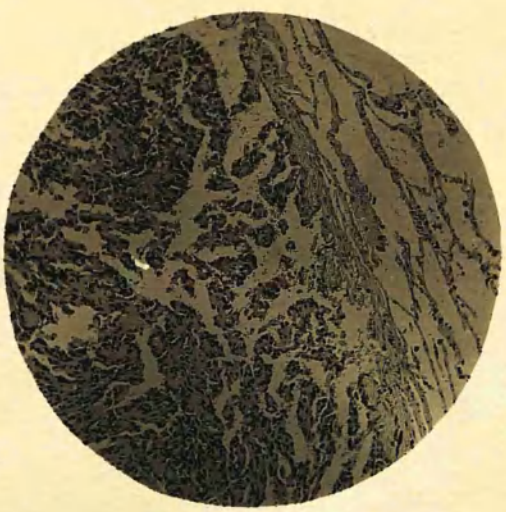


Fig 11.
Nodule in the lung.

Fig 12.
Nodule in liver.

CANCER of the UTERUS

Statistics and Surgical Pathology.

Incorporating papers on:

Cancer of Uterus etc. (Arch. of Middlesex Hosp. 1906)

Pathological Bases of Operative Treatment
(Proc. Roy. Soc. Med. 1911)

The Ureter in Uterine Cancer (J. of Obst. & Gyn. 1910)

CANCER of the UTERUS

ETIOLOGICAL CONSIDERATIONS.

Frequency

The uterus is the commonest situation in which cancer is found.

In 1906 I had the opportunity of reading and abstracting the clinical and postmortem of all cases of cancer of the uterus, vagina, and vulva, in the practice of the Middlesex Hospital from 1855 to 1904. There were in all 2347 cases of malignant disease of these organs, the uterus (cervix 2111 cases, corpus uteri 69) accounting for 2180 of these. As the total number of all cancers occurring in women during the same period was 5349 it will be seen that uterine cancers form a fraction over 40 per cent of the whole. Some of our English text books place cancer of the breast as first in order of frequency. Williams from statistics collected from the London Hospitals estimated cancer of the breast at 40 per cent and cancer of the uterus at 34 per cent. Orth put uterine cancer at 30 per cent of all female cancer. Some estimate as low as 14 per cent. The United States census of 1911 (quoted by Ewing in Neoplastic Diseases) showed a preponderance of cancer of the Stomach and liver over uterine cancer of nearly three to one, but Ewing regards the figures as misleading and is of opinion that cancer of the uterus is the commonest cancer in America.
The mortality statistics of the Registrar-General for England and Wales show that cancer of the uterus is the commonest in this Country.

It may be found that each particular animal species has its peculiar organ-propensity to cancer. In human beings the uterus of

the female is the organ most liable to the disease. It will be convenient to consider the cervix and the corpus uteri separately, for there are differences of various kinds between them.

CANCER of the CERVIX UTERI

Civil state and fertility.

The most arresting fact about cancer of the cervix, and one that has formed the basis of much speculation, is that it is so markedly a disease of married women. Of 1876 cases in my notes, cancer of the cervix occurred in 1790 married women (including amongst these, single women who had borne children) and in 86 (= 4.59 per cent) unmarried women: that is to say it was 19 times more common in the married than in the unmarried. Several gynaecologists of considerable experience have remarked that cancer of the cervix does not occur in virgins. Though the clinical notes of these 86 unmarried women are silent on this delicate point it is possible, without being probable, that some may have preserved intact their virginity. Ethically, celibacy can be considered neither moral nor unmoral: from the biological point of view it is normally wrong. If then women by the sacrifice of their virginity render themselves so extraordinarily more liable to cancer of the cervix in so doing it would seem at first sight an unjust reward of nature to those who obey her dictates.

Marriage, however, is not what nature demands; it is merely an incident: the prime physiological destiny of women is to bear children. How does this affect the question? My notes show that of 1552 married women with cervical cancer 1403 had given birth to one or more children, 22 had been pregnant but had never produced full-term

or living children, and 127 (9 per cent) were sterile: in other words women who more or less successfully carry out their biological destiny are 10 times more liable to cancer of the cervix than their barren sisters. The incidence of sterile marriages is generally reckoned as about 1 in 10, so that the disparity here is not to be wondered at, and this consideration puts in its proper perspective the idea put forward by some gynaecologists that the lacerations of the cervix incidental to childbirth are lesions unusually susceptible to malignant degeneration.

There is another point with regard to fertility to which attention has been directed. It is said that the average number of children borne by women who become the subject of cervical cancer is appreciably higher than the normal average. Williams, Köblanc, and Gasserow find that in uterine cancer the average is over 5. Unfortunately I made no notes on this point, but one of my colleagues found the average number of pregnancies amongst 400 parous women (with cervical cancer) to be 5.23, whilst amongst cancers not affecting the generative system the average was 5.67. It is possible to arrive at a mean fertility age in the general population, but the cases from which hospital statistics are compiled belong to the labouring classes where the fertility rate is high. There does not seem to me to be much in the point.

However, we regard it, there is no getting away from the fact that cancer of the cervix is overwhelmingly a disease of married life. As we cannot connect childbearing with its occurrence - and besides, the lower animals have a much higher fertility and yet do not develope uterine cancer save as an extreme rarity - we are left with two possible explanations. It may be, as I have said, the organ-

proclivity of the species, a hypothesis that entails the admission of a hereditary factor, or it may be that married women are liable to contract diseases of the cervix of a chronic character prone to eventuate in cancer.

Certainly in the female the cervical canal is the site of choice for chronic gonorrhoea, but on the other hand the prostate and the seminal vesicles in the male, where the infection lingers, are not commonly the seat of cancer. There are of course other chronic affections of the crevix.

Heredity

In the records of family histories of cancer cases at the Middlesex Hospital, as in most other hospitals in the past, special attention was paid to the presence or absence of a cancerous or phthisical taint. Among 1500 cases so recorded, 789 (= 52.6 per cent) were free from cancer or phthisis, 242 (= 16.13 per cent) gave a history of cancer, and 557 (= 37.13 per cent) one of phthisis, whilst 88 (= 5.9 per cent) had both cancer and phthisis amongst their relatives. Further 83 (= 5.5 per cent of the total) gave a family history of cancer of the uterus: the mother was affected in 44 (= 2.9 per cent), a sister in 27 (= 1.8 per cent), an aunt in 15 (= 1 per cent) and the maternal grandmother in 7 (= 0.41 per cent). It is obvious that hospital patients cannot tell with any degree of exactness the causes of death of their ancestors and relatives, and the figures given, though just as weighty as the majority of statistics dealing with family histories in disease, are slender bases on which to found arguments for or against the hereditary

transmission of liability to cancer. The connection between tuberculosis and cancer, is, to say the least, not obvious, and it will be seen from the figures that a phthisical history is more frequently given by patients than a cancerous history.

As cancer and tuberculosis are two of the commonest causes of death there can be few families indeed that are free from the one or the other. We certainly know that some tumours have a strong hereditary character, but in general there is no clear evidence of the hereditary transmission of cancer. I remember a women with cancer of the cervix whose mother, one aunt, and four out of five sisters, had cancer of the cervix also. From time to time one reads in the medical journals records of similar histories but they are probably no more than chance occurrences. It is these striking occurrences that remain in the memory more than the common experiences of life and tend to bias the judgment. A few years ago J.A.Murray published the results of breeding experiments with mice that afterwards developed malignant tumours: the incidence of cancer in the offspring as compared with unselected strains was particularly high, and seemed to be far beyond all chance. He also pointed out that in addition to the enhanced general liability to cancer there was an organ-liability, but as this conclusion was drawn from the fact that mammary cancer was the principal malignant disease found in the offspring it cannot be accepted seeing that the overwhelming majority of mouse cancers originate in the mammary glands. Still his observations give experimental proof of the hereditary element in cancer, and on cannot dismiss the question merely by invoking the workings of chance.

Conjugal Cancer. Amongst all the cases only 7 gave a history of the husband being the subject of Cancer,- representing not more than 0.5 per cent. In only one of these cases, however, was the cancer localised in the penis, and in that case the husband had died seven years before the onset of the patient's own symptoms. Now and again one sees reports in literature notes of a case of such conjugal cancer and usually the reporter over-emphasises the importance of the occurrence. Cancer of the cervix being such a common condition, we should expect a corresponding frequency of penile cancer if natural transplantation took place. The rarity of the conjunction of the two shows that it does not occur: it is merely a coincidence.

Age. The age of onset of the disease was estimated by subtracting the duration of the cardinal signs and symptoms - pelvic pain, haemorrhage, and vaginal discharge - from the age of the patient on admission. In those cases where a single symptom was separated by an inordinate length of time from the others, the single symptom was ignored in making this calculation. The age at onset was thus approximately determined in 1639 cases. The duration of symptoms was calculated not in months but in the approximate quarters and the age given by the patient on admission was taken as if an exact one on the date of admission, a source of fallacy which will render the average age at onset in the calculation inaccurate by an unknown fraction of a year. Nevertheless the number of cases considered will eliminate from the averages the errors of particular over - and under - estimations.

Another way of getting at the point, which I did not adopt, would have been to subtract the average duration of the disease from the age at death, but as all the cases did not die in hospital it would have meant that the numbers for calculation would have been much reduced.

The average age at onset is 44.63 years. The oldest patient had an onset age of 86.5 and the youngest 22. At the London Cancer Hospital there was a case of cancer of the cervix in a girl of 13 - an epithelioma. A case was reported of columnar cell carcinoma of the uterus in a girl of 2½ years (Proc. Roy. Soc. Med:,Path. Section 1914) but from an examination of the histological sections I am convinced that it was a teratoma. The average age at onset amongst the unmarried was 43.85, but the smallness of the number of cases (47) from which it was calculated renders the slightly earlier age of onset of no comparative importance.

By arranging the cases in their appropriate quinquennial period we find that the largest number occur from 40 to 44, the next largest between 35 and 39, and the third between 45 and 49. The following table gives the numbers and the percentages in the quinquennial periods.

Age.	No. of cases.	Percentage.
20-24	6	0.37
25-29	85	5.19
30-34	167	10.19
35-39	305	18.61
40-44	319	19.46
45.49	289	17'63
50-54	205	12.51
55-59	136	8.30

Table Continued

Age.	No. of Cases.	Percentage
60-64	89	5.43
65-69	23	1.40
70-74	11	0.67
75-79	3	0.18
80-84	0	0
85-89	1	0.06

It would seem therefore that more cases occur before the menopause than afterwards. This is contrary to the usual teaching. It is said that most cancers of the cervix occur after the menopause: that it is a disease of an organ ceasing to function. From particulars given in the clinical notes of the cases I was able to get exact information as to the cessation, or continuance of menstrual periods and I found that 51 per cent occurred before the menopause and 40 per cent after the menopause, whilst in the remaining 9 per cent the two phenomena were inseparably blended. It is quite true to say, as one always hears, that cervical cancer occurs mostly in the fifth decade but it is misleading to some extent. My figures show that 37 per cent occur between 40 and 50, but 38 per cent occur between 35 and 44. The maximum point of our curve (See accompanying Chart) differs markedly from a graph made up from the Registrar-General's mortality returns. He places the maximum incidence a decade later. I am quite unable to explain the difference. I have calculated the age at onset of the disease: he records the age at death: but the average duration of the disease (see subsequent paragraph) does not account for the discrepancy. The relatively small proportion of cases of cancer of the corpus which are included in the official returns would not so markedly alter the curve. The

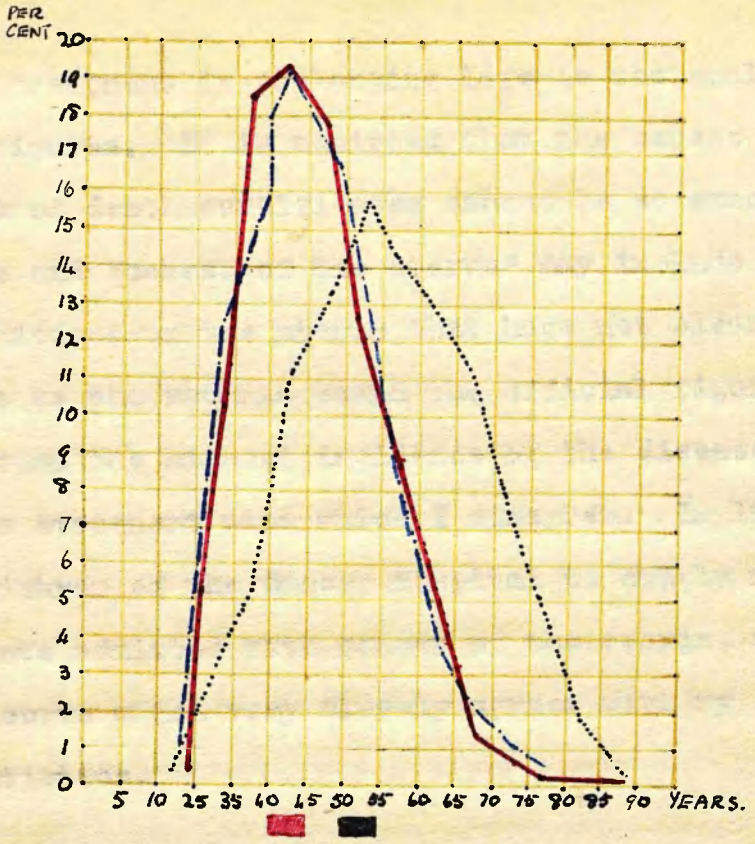


Chart showing the maximum age incidence of carcinoma of the cervix uteri.

The dotted black line represents a graph composed from the Registrar-General's returns of 1912 showing the percentages at various ages of women dying from uterine cancer, cervix and body.

The red line shows the percentages at age of onset according to my statistics.

The interrupted blue line is constructed from figures of cases at the Cancer Hospital in the same way .

The last two graphs closely correspond. The maximum incidence given by the Registrar-General's returns is a full decade later.

influence of operative treatment in prolonging life is too small to be felt in such large figures. If is admitted that the causes of death given in the bulk of death certificates cannot be so exact as in Hospital practice and "cancer of the uterus" may include fibroids and often conditions of the uterus that have not clearly been ascertained. But as the returns stand the official figures can be cited as proof that the highest incidence of the disease is after the menopause - a statement with which I disagree. In 1914 I got the surgical registrar of the Cancer Hospital to supply me with the ages of patients admitted with cancer of the cervix, and from this I drew up a curve which very closely agrees with my Middlesex Hospital Statistics.

Relation to Menstrual function and Pregnancy

A close investigation of the records was made to see if any etiological factor could be discovered in the menstrual history. Particulars of the age at which the menses started, the periodicity duration of flow, regularity, quantity of blood lost, and the age at which menstruation ceased, are given in my original paper. In no respect did they differ from the particulars that would be elicited in a series of non-cancerous cases.

From 947 cases where information was given of the onset of symptoms and the time of the last pregnancy or miscarriage it was estimated that 103 (= 10.9 per cent) bore a close relation in point of time to childbirth. More particularly the onset of symptoms of cancer of the cervix preceded an abortion or live birth in 17 cases

(= 1.8 per cent): the symptoms were present during the later stages of gestation, during the puerperium, or at the time of abortion in 32 cases (= 3.4 per cent): in 43 cases (= 4.5 per cent) the onset of symptoms dated from the puerperium: and in an additional 1.2 per cent (11 cases) less than six months had elapsed between birth or abortion and the commencement of the disease. It has been stated that pregnancy has a grave effect on, the progress of cervical cancer (Ewing) but whether this is so or not cannot be ascertained from my notes of the cases. Seeing that the disease occurs so frequently in the childbearing years a considerable proportion of the cases are bound to have a close relation in point of time to pregnancy. The pathological changes in the cervix may be expected to militate against fertilisation but they are not an absolute bar to conception. One woman who was an inpatient of the Middlesex Hospital suffering from inoperable cancer of the cervix was discharged: on returning to her home she resumed marital relations and was delivered with forceps of a live full-term child at another hospital about a year afterwards: she was readmitted to the Middlesex Hospital and died there.

Clinical details bearing on Etiology.

Amongst the mass of clinical details there is little information regarding previous predisposing diseases. Five cases are noted as having had a previous operation for cancer of the breast: no case apparently had both diseases at the same time. This is a point which has interested me considerably.

Given the facts that cancer of the cervix and cancer of the breast are so very common and that they occur at the same ages why is the conjunction of the two so very rare? I should imagine that the laws of chance would allow a not uncommon simultaneous occurrence. It is a question for the statisticians. Local diseases such as prolonged leucorrhoea (53 cases), "inflammation of the womb" (31 cases), prolapse of the uterus (16 cases), and fibroids (6 cases) are too scanty in the records to be of value. A great deal has been made of fibroids by Gynaecologists as predisposing to cancer of the cervix: the postmortem records of 915 cases of cancer of the cervix stated that fibroids were present in 54 cases (= 6 per cent) I doubt if a similar number of autopsies on women dying from other diseases would show a lesser proportion.

Duration.

By adding the duration of the disease before admission to the length of time that elapsed between admission and death, omitting those cases in which a surgical operation of any importance affected the natural evolution, and reckoning in approximate quarters, I obtained the approximate duration of the disease in 900 cases. From the appearance of the first symptom till the time of death the shortest duration was 3 months and the longest 7 years. The average duration is 1.74 years or 1 year and 9 months. It was found that the duration increases gradually with the age of the patient until it reaches a maximum between the ages of 60 and 64 (2.27 years), thence it diminishes quickly to its minimum during

the age period of 75 - 79.

CANCER of CORPUS UTERI.

Only 69 cases, including 11 sarcomas, were fully recorded. How rare it is in relation to cancer of the cervix is well-known.

Civil state and fertility. It has often been said that cancer of the body of the uterus, in contradistinction to cancer of the cervix, is predominantly a disease of unmarried women. My figures show that 47 were married and 11 single. Among the married 25 had borne children, 7 had produced only miscarriages, and 7 were barren - that is to say, a quarter were absolutely sterile. These are noticeable differences.

Heredity. 23 had a family history free from a cancerous or a phthisical taint (= 48 per cent), 16 had a history of phthisis (= 33 per cent), and 11 (= 23 per cent) had a history of cancer. Among the last one patient had a sister with cancer of the uterus.

Age at onset. The age at onset could be calculated from particulars given in 52 cases and the average is 51 years. Thus cancer of the corpus uteri occurs usually much later than cancer of the cervix. The average age for 6 cases of sarcoma of the uterus was 48.46 years. Of the carcinomata 12 were previous to, 2 were at the time of, and 44 were subsequent to, the menopause. In this it differs distinctly

from cancer of the cervix.

Duration.

All gynaecologists who have spoken on the subject and all the text books I have consulted agree in declaring that cancer of the body of the uterus is very much slower in its progress than cancer of the cervix. I have never seen any statistical bases for that opinion. Reckoning from the time of the appearance of the first symptom till death in 30 cases, including one sarcoma only where the approximate duration was 6 months, I find that the average duration is 1.83 years, not much longer than in the case of cancer of the cervix. Still 30 is a small number of cases on which to argue.

POST MORTEM EXAMINATIONS.

I shall briefly mention the principal facts elicited by a study of the reports of 915 postmortem examinations of cancer of the cervix and 33 of the uterine body in so far as they indicate the paths of dissemination of the disease, and then certain observations which I have made on surgical pathology will be considered in order to form a rational basis for the surgical treatment of uterine cancer.

Although the clinical notes recording the local appearances when the patient was first examined showed that ulceration of the growth had not then occurred in 26 of 802 cases (= 3.14 per cent),

nevertheless in only one case out of the 915 autopsies was ulceration absent. In 32 instances (= 3.5 per cent) there was no naked eye evidence of extension of the disease beyond the cervix uteri nor had any metastatic deposits occurred.

Invasion of the Vagina.

The vagina was recorded as being involved at postmortem examination in 826 cases and uninvolved in 21 (= 2.5 per cent). In 68 cases there was no note on the point. A spread so extensive as to involve the vulva is recorded in 23 cases (= 2.5 per cent)

Involvement of Bladder and Rectum.

In 404 cases (= 53.6 per cent) there was a vesicovaginal fistula, and in a further 204 cases the wall of the bladder was involved without perforation. Altogether the bladder was involved by the spread of the disease in 608, and was apparently unaffected in 77 (= 11.3 per cent), whilst in 230 cases there was no note on the point. In 151 cases there was an ulceration into the rectum (= 16.5 per cent): the rectal wall was involved without perforation in an additional 98, making a total of 249. Freedom from involvement is recorded in 48 instances (= 16.2 per cent) and in 618 cases there was no record either way. Double fistulae (i.e. vesicovaginal and rectovaginal) was found in 86 (= 9.4 per cent).

Parametrial invasion.

Spread from the uterus into the broad ligaments is noted specially in 120 cases (= 13.11 per cent) but as

these structures are so frequently matted to their surroundings by adhesions and peritonitis and therefore so impracticable of examination, the figures are to be regarded as an understatement. Such matting of the pelvic structures is recorded definitely as present in 221 cases (= 25 per cent).

Glandular and visceral metastases.

In general it is fairly easy to differentiate between local continuous spread of the disease and metastatic deposits but the estimation becomes more complicated when we have to deal with affected structures normally separated from, but in the process of this disease in continuity with, the originally affected organ. Not infrequently the omentum becomes adherent to the uterus, to the recto-vesical pouch, or to the pelvic contents generally, and the same applies to the intestine, so that there is a continuous dissemination and not what is reckoned as true secondary growth. Giving a fairly liberal interpretation to "metastases" I estimated that 187 cases (= 20.44 per cent) showed visceral or parietal secondary deposits, and that 351 showed deposits in the lymphatic glands (= 38.36 per cent): of the former, no lymphatic spread is recorded in 55 cases, so that the total number of cases with secondary deposits is 406 (= 45 per cent) of all postmortem cases. The frequency of involvement of the various organs was as follows:

Liver	122 cases	13.5 per cent.
Lungs	55 "	6 " "
Kidneys	23 "	2.6 " "
Adrenals	16 "	1.9 " "
Heart	9 "	1 " "
Spleen	9 "	1 " "

Pancreas	8	cases	0.9	per	cent
Gallbladder	5	"	0.6	"	"
Diaphragm	6	"	0.7	"	"
Abdominal wall	4	"	0.4	"	"
Thoracic wall	3	"	0.3	"	"
Subcutaneous	3	"	0.3	"	"
Thyroid gland	1	case	0.1	"	"
Oesophagus	1	"	0.1	"	"
Mamma	1	"	0.1	"	"
Bones	8	cases	0.9	"	"
Intestines	8	"	0.9	"	"
Pleurae	13	"	1.4	"	"
Peritoneum	45	"	5	"	"
Pericardium	1	case	0.1	"	"

With regard to the subcutaneous tissues above, one was a case of general carcinomatosis: in the second case the subcutaneous mass was in the axillary region: and in the third case the metastatic nodule was at the ankle. In addition to the 8 cases of intestinal metastases (distributed as follows: pylorus 3, duodenum 2, jejunum 2, and appendix 1), the adherent intestines were affected in 22 cases. The osseous deposits included 1 in the skull *, 1 in the ribs, 2 in the vertebral column, 1 in the tibia, and 2, which were probably due to a continuous spread, in the innominate bone: the sacrum was affected once.

Of the 351 cases of deposits in the lymphatic glands, the distribution

---

[* It is interesting to note that if a Skull containing a cancerous metastasis in the vault be macerated we get an appearance exactly like the prehistoric trephined skulls I have seen. I do not dispute the trephine origin of these defects in the latter skulls for sometimes one can plainly see the rough marks of the flint used for the purpose]

was as follows:- Axillary 1, supraclavicular 6, thoracic (i.e. mediastinal and bronchial) 17, higher abdominal (i.e. the mesenteric, portal, coeliac, and higher prevertebral chains) 31, lumbar 305, pelvic 250, inguinal 45. Owing to the matting together of the pelvic structures and the common postmortem method of removing the pelvic contents en masse, a precise description of the intra-pelvic glands is impossible, and my statistics on the point are undoubtedly an under-statement.

Other post-mortem conditions.

Out of the varied accompanying conditions of which I made a note in my original paper there is only one of any importance for our present purposes that I need mention, and that is, the condition of the kidneys. In no less that 692 instances (= 75 per cent) there was hydronephrosis of one or both kidneys with accompanying dilatation of the ureters. In 76 cases the dilated pelves contained pus.

We can briefly sum up the post-mortem findings in 33 autoposies of carcinoma of the corpus uteri. Glandular deposits were found in 15 (= 45 per cent), and visceral metastases in 16 (= 48.5 per cent). In the latter the distribution was as follows: liver 5, lungs 5, intestines 4, peritoneum 9, and spleen, pericardium, pleura, diaphragm, and ribs 1 each. Extending by continuity the malignant process involved the broad ligaments in 4 cases, the ovaries in 4, the Fallopian tubes in 1, the bladder in 3, and the rectum in 4. The vagina was involved in 3 cases: utero-vesical

fistula occurred once, and utero-rectal fistulae 6 times. The pelvic structures were matted together in 15 cases (= 45 per cent) and hydronephrosis occurred in 11 (= 33 per cent). Uterine fibroids co-existed in 4 cases (= 12 per cent).

SURGICAL PATHOLOGY OF CANCER OF THE UTERUS.

Less than twenty years ago a famous surgeon said that "no radical operation is worth performing when the disease extends beyond the limits of the uterus into the connective tissue about the cervix or into the utero-sacral or broad ligaments. In such cases prolonged immunity, to say nothing of the permanent benefit from an operation, is out of the question." We have travelled far in these years; the brilliant results that have attended advanced operations have quite changed the comparatively hopeless outlook. The surgery of cancer in general is advancing rapidly, and one would be rash to delimit its future scope. In a very short time what was considered the limit of operation has been surpassed, and what was formerly a "radical" operation becomes in the light of experience incomplete. Instead of appealing to results which may take years to accumulate, we can base our judgment of the probable completeness of any operative technique on the surer ground of pathological observation. The more the surgical pathology of cancer is appreciated, with a consequent adaptation of operative procedure, the better will the outlook become; but the first essential in advance is a more accurate knowledge of the pathological processes at work.

No one would deny the utility of earlier diagnosis of cancer of the uterus, and the educative measures proposed for that purpose; but if earlier diagnosis implies restricted operative measures, then there is danger of retrogression. How is it possible to ascertain when we have to deal with an early case?

Early with regard to time of duration may be late in respect to time of dissemination. The warning signs and symptoms that call attention to cancer of the uterus vary greatly in their severity, and we cannot calculate with any degree of exactness how long the lesion has existed. The evolution of some cancers is rapid, of others slow, and of others again rapid growth and slow growth may follow each other. The rate of growth of malignant tissue follows no laws that we know. The apparent size of a primary lesion visible to our eyes or demonstrable to palpation may be far from the actuality. Probably every surgeon has had the experience of removing small growths so thoroughly in relation to their size as to give every hope of success, only to find a speedy demonstration of failure, and, on the other hand, has met with large growths, treated in a relatively restricted manner, which have never recurred. Experience in post-mortem examination soon teaches us that a small focus of origin is by no means infrequently associated with a very extensive dissemination, and, on the contrary, that the malignant process may be locally confined to the site of its origin. In clinical examinations it follows that we cannot invariably, or even usually, say that a small original lesion denotes an early case, for beyond that lesion the cancer cells, unseen and impalpable, may be permeating the lymphatic

tracts. In cancer of the uterus, of the cervix, or of the body, another process may be at work - septic absorption - which may considerably complicate a judgment of the real extent of the cancer, leading one to conclude that the cancerous process is extensive when in reality it may be limited. I shall refer to this later.

The histological examination of a portion of the growth cannot give us sure information with regard to the rapidity of the process. I am aware that such a statement is at variance with the views of some pathologists. It is not uncommon to read in histological reports that the tumour is growing rapidly or slowly, or even that the case is considered to be an early one. Such an opinion takes no account of certain fallacies - namely, that a particular section of a certain portion at a particular time may exhibit considerable variation from the appearances found in another section from an adjacent part. It is based on two considerations, the relative amounts of connective-tissue stroma and epithelial cells, and on the numbers of mitotic figures found in the portion examined. In the experimental investigation of mouse cancer, where we can take all these factors into account, we find that the histological criteria on which such opinions are founded are quite untrustworthy. We actually find that some tumours with a relatively large preponderance of stroma over epithelial elements may have a speedier growth and a higher percentage of successful inoculations than a tumour of high epithelial cellularity. Of course, the opposite may obtain; there is no constant relationship between structure and degree of malignancy. It has also been shown that some slow-growing tumours of mice may

exhibit a surprising amount of mitotic figures.

Taking all such factors into consideration, the impossibility of determining from clinical or histological examination the exact extent of the disease in any given case, we may justifiably conclude that the only way of encompassing a radical extirpation, within reasonable limits of course, is to apply to every case a technique which is designed for the most advanced. Such a technique does not mean a haphazard removal of surrounding tissue, but an extirpation of definite danger zones' The proposition which I advance is that these danger zones are the structures which in the autopsies of advanced cases we actually find the most often affected; that is to say, the terminal picture gives the key to the partially-revealed processes during life.

One of the most striking and unexpected features revealed by postmortem statistics was that no less than 55 per cent. of cases were found to be free from any visceral or glandular metastasis. At a later date MacCormac analysed the records of over a hundred autopsies on cancer of the cervix performed by the special cancer research staff at the Middlesex Hospital and his figures agree in surprising detail with mine.

If we accept these as a true indication of the potential spread of the disease, and make allowances for the fact that in earlier stages the liability to metastasis must be lower, we can account for the effectiveness claimed for such restricted operations as vaginal hysterectomy or other measures that leave the pelvic glands untouched. (The fact that 3.5 per cent of cases on the postmortem table showed no

advance of the disease beyond the cervix, gives us an explanation of the fact that the older operation of amputation of the cervix sometimes succeeded in eradicating the disease). But though these figures may be accepted as fairly accurate for extra-pelvic metastases, it may be doubted if they give a true estimate of the frequency with which the pelvic lymphatic glands are involved. It must be borne in mind that in approximately a quarter of the cases that come to the post-mortem table the pelvic organs are so matted together, either from uterine and tubal septic processes or from perforation of the pouch of Douglas, that accurate descriptions of the state of the intra-pelvic structures is out of the question. The lymph glands lying between the external and internal iliac arteries/which are the main regional glands of the uterus would therefore probably escape examination in a great many cases. The pelvic contents in advanced cases with perforation, disorganization, and foetid debris, do not invite long and pains-taking examination, nor do our usual methods for their removal enable us to be exact on the point. Again, the utility of exact observation of the intra-pelvic glands has only recently been evident. If these glands have been enlarged and have escaped examination, it would be impossible to say whether they were the seat of deposit or the result of the septic processes. But, at any rate, the observations can be taken as fairly accurate of the dissemination beyond the brim of the pelvis, and up to that point glandular extirpation is practicable in the modern operation. When cancer gets beyond the wall of the uterus into the paravaginal tissues and broad ligaments it travels to the lateral walls by

by lymphatic tracts. Most of our anatomical text-books are very incomplete in their descriptions of these tracts. Poirier, Delamere, and Cuneo, the authors of the Standard work on lymphatics mention three tracts from the cervix; one directed outwards draining into the inter-iliac lymph glands, a second running obliquely outwards and backwards to glands on the various branches of the internal iliac, and a third descending at first and then ascending to the pararectal glands. The last set course through the utero-sacral ligaments, but it is questionable if they are really uterine lymphatics; they are not reckoned as such in our English text-books. And yet how commonly do we find in cases of cancer of the uterus the clinical note, "involvement of the sacro-uterine ligaments"! How many cases are denied operation on this ground alone! I have often been struck with the fact that where this was recorded in the clinical notes no apparent involvement of these folds could be found at post-mortem examination. The records to which I have alluded were made before anatomy of the lymphatic system was as well recognized as it now is, but, still, involvement of the lateral sacral glands into which these lymphatics drain would occasionally have been noticed; but there is no mention of such an occurrence. In this case the matting of the pelvic structures would not hide them. In MacCormac's paper they were found to be affected once only in 107 cases. Personally I have never seen them enlarged in uterine cancer. In operation specimens submitted by my colleagues this particular tract of tissue is sometimes removed because it is considered to be involved, but careful microscopic examination of various parts of it

always fails to demonstrate any cancer cells. I should not like to deny the presence of such a lymphatic tract, much less to say that "involvement of the sacro-uterine ligaments" is always a false observation; but, in view of the disagreement between clinical and post-mortem findings and the fear that some otherwise operable cases may be denied operations on that account, I think too much has been made of it. Lymphangitis from uterine septic trouble, closely following these sacro-uterine tracts and embracing the rectum, has been observed by Miles, and is considered by him to produce the so-called "syphilitic stricture of the rectum." This is probably the real explanation of the clinical finding.

The middle tract of Poirier draining into lymph glands on the branches of the internal iliac is omitted from most text-books. I have been unable to verify this tract by injection from the uterus, and in the numerous complete dissections I have made of pelves with cancer of the uterus I have failed to discover enlarged glands in the situation figured. Supposing the observation of Poirier and his collaborators is verified, it will mean that, if nothing is to be left to chance, probably the anterior division of the internal iliac would have to be cleaned out, if not removed at operation. At the present time we have no evidence that these lymphatic glands are frequently the seat of cancerous deposits.

The inter-iliac glands are important (fig. 2). They vary in number, usually three, and are supplied by lymphatics from both the cervix and the corpus. In some books they are figured as being supplied from the cervix only, the principal tract from the

body running over the end of the common iliac and turning up to the lumbar glands; but the two tracts are very intimately connected, and as a matter of fact we find the inter-iliac glands not infrequently affected in cancer of the uterine body, while, on the other hand, we find the lumbar glands affected in cancer of the cervix with no apparent involvement of the inter-iliac glands. The inter-iliac glands, as their name implies, lie between the external and internal iliac arteries, the uppermost being usually partly hidden by the ureter which passes over it. These glands in turn communicate by a connexion over - and sometimes by another under - the artery with another set of three or four which lie in close association with the outside of the common iliac artery (fig.8). The lowest gland of the latter is very apt to escape detection, as the artery may have to be displaced inwards to show its presence even when it is enlarged. This particular gland has never been described but I almost invariably find it in postmortem examinations: it is sometimes involved when the inter-iliac chain is the seat of cancer. It is important to recognise it for the surgeon finding the inter-iliac chain involved may remove the higher common iliac group and yet leave behind this intermediate gland which, if it happens to contain cancer cells, will defeat the purpose of the operation.

Enlarged glands do not necessarily indicate invasion by cancer, for we not infrequently fail to detect any epithelial cells in such cases, whilst, on the other hand, glands may be infected without being enlarged or even hardened; still, it may be taken as a

working rule that an enlarged gland is prima-facie evidence of cancerous deposit, and should be removed, whilst the routine extirpation of pelvic glands in cancer of the cervix obviates the risk of leaving behind a focus for recurrence. When the inter-iliac glands are definitely enlarged it will obviously be risky to stop short at their extirpation, and the higher set lying to the outer side of the common iliac, especially the gland which I have mentioned as being hidden behind the artery below its bifurcation, should be removed also. The most distal enlarged gland does not necessarily mark the limit of dissemination, any more than the visible surface margin of a growth denotes the extent of invasion. The lesson has been learnt and successfully applied in the surgery of breast cancer, and the same principle should be applied to cancer of the uterus; the area through which the common iliac and its divisions run is the "axilla" of the pelvis. Closely connected with the inter-iliac group of lymph glands, if not actually to be reckoned one of them, is what is normally a large gland lying between the external iliac vein and the obturator nerve. This gland can be injected from the cervix, and from it the obturator gland often becomes affected in cancer of the uterus. In a few advanced cases - approximately 5 per cent.- we find the inguinal glands to be enlarged, but probably in a very small number only is that enlargement due to cancerous infiltration. There is said to be a lymphatic tract from the uterus through the round ligament to the inguinal region, but though I have frequently prepared microscopic sections across the ligament, I have not been able to see invaded channels within it. That does not, of course, negative

Figure 2.

Figure 3.

Figure 4.

Fig 2. Lateral view of dissected pelvis to show position of invaded lymph glands. AAA, the interiliac glands, the uppermost being crossed by the ureter. The arrow D points to the communication between the highest interiliac gland and the lowest of the common iliac group, lying behind the artery. BBB glands below the external iliac vein communicating above with the interiliac and below with the inguinal groups. EEE lateral sacral glands — not involved. The large obturator gland has been removed.

Fig 3. Anterior view. A, highest interiliac gland. DDD, common iliac group, the lowest member of which has not been described in text-books. The artery has been displaced inwards to show it. EE, lateral sacral

Fig 4. F is an enlarged inguinal gland. B is the lowest intrapelvic gland of a chain communicating with the interiliac glands.

THE PELVIC GLANDS AFFECTED IN CANCER OF THE UTERUS.

such a route of dissemination, for there may be embolic spread to the inguinal gland. But it is possible that the inguinal glands may become involved from the chain of glands lying along the external iliac vein which communicate with the interiliac group (fig. 4). It must be remembered that lymphatics permeated by cancer-cells do not coincide with the channels mapped out by artificial injection. In their growth the cells block the lymphatics, destroy the lymph current, and make their way by the paths of least resistance through tributary channels, it may be, against the direction of the lymph flow.

Contained within the broad ligaments are the tracts from the uterus to the pelvic glands; they are numerous and extensively communicating. Parallel to the long axis of the uterus and close to it is a vertical channel of communication between the lymphatics of the corpus and those of the cervix, so that for all practical purposes in cancer of the uterus the lymphatic spread outside the walls of the organ is the same for both. It may be that it takes longer in general for cancer to penetrate the wall of the body than it does to pass beyond the confines of the cervix (I doubt it), but once it does so the course is the same for both; but it is open to question, especially when we take into account the dangers of wide implantation and embolic dissemination entailed by the preliminary diagnostic curetting usually practised, if cancer of the body should be treated by less extended operation than cancer of the cervix. The postmortem statistics already given afford no support to restricted operation in cancer of the corpus. The involvement of

the parametric tissues may only be revealed by the microscope, and even escape detection altogether.

Several authors have published the results of their microscopic investigations of the parametrial tissues in operation cases. In 17 out of 27 radical hysterectomies reported by Sampson there was microscopic evidence of invasion of the parametrium. Kundrat in 160 cases found 55 per cent. thus affected; Lamaris and Kermauner estimate the frequency at 57.5 per cent.; Schauta at 69 per cent.; Baisch at 50 per cent.; Schieb at 43 - 73 per cent.; Wertheim at 60 per cent. I have frequently found lymphatic infiltration in cases in which the gynaecologist failed to detect any abnormality by palpation, just as I have failed sometimes to obtain evidence of the spread of the disease when clinically the parametrial tissues were said to be hard. There is thus overwhelming evidence that more than half the cases of cancer of the cervix deemed to be operable contain cancer cells in the parametrium. Now with regard to the involvement of the interiliac lymphatic glands in operable cases, various estimations of the frequency have been given by Surgeons practising a radical operation. Berkeley and Bonney who have recently published the results in 100 of their operation cases found microscopic proof of gland involvement in 35. In one of their cases they removed involved glands as high up as the lower inch of the aorta and this case has not had a recurrence. Wertheim found affected glands in 35 per cent, and Bumm in a third of his cases; whilst on the other hand Kroemer maintained that in 66 per cent. of postmortem cases the pelvic glands were not invaded. Baisch estimates that only half the number of cases with parametrial

involvement will have invaded pelvic glands, and that about 15 per cent. of cases with invaded pelvic glands may show no evidence of parametrial involvement. This point is of considerable interest in the surgical pathology of cancer in general. Though frequently we can demonstrate the presence of cancer cells in the lymphatics of tissues lying between the primary focus and the affected lymphatic glands yet sometimes we fail to find them. Of course this may be due to the fact that only a portion of this intermediate tissue has been examined microscopically. Permeation of the lymphatics per continuationem is not infrequent but is by no means constant. The usual theory is that a process of lymphatic embolism occurs. Handley succeeded in demonstrating that in the intermediate zone the position of a previously permeated lymphatic may be taken by a minute fibrous nodule and he stated that this represented a reparative fibrosis, the surrounding fibrous tissue proliferating and killing the cancer cells by pressure. I frequently find such changes: indeed one may see the process actually at work, with a concentric mass of fibrous tissue around degenerating cancer cells, but I do not think it is a defensive process directed against living malignant cells for it is never evident at the ultimate tip of the permeating mass in the lymphatic channels. It seems to me that it is a fibrosis around a mass of cells already dead. It is extremely difficult to be sure of small numbers of degenerated cells in a lymphatic vessel. I have spent considerable time tracing out the paths of dissemination from various organs and in cancer of the rectum in particular I have been able to identify isolated cancer cells in lymphatic vessels with some degree of certainty. By their

characteristic reaction to mucicarmin they are unmistakeable. As a matter of fact one can detect degenerated mucous cell material by this staining method, and I find that the usual process of dissemination through the lymphatics is by isolated small collections of cells, and that a large number of such cells perish and degenerate during their progress through the lymphatics. In such circumstances, dealing with tumour cells arising from situations where the epithelium does not give a characteristic micro-chemical reaction, the failure to find involved lymphatic vessels is not to be wondered at.

Embolic involvement of the lymph glands must be admitted, but for practical purposes the tissues must be treated as if there were always continuous lymphatic spread within them. There are no means of determining at operation which of the two modes of dissemination is at work in a particular case, and even in embolic spread the emboli may still be in course of passage at the time of intervention. It follows, then, that in removing the dangerous structures glands and broad ligiments should be taken away in one continuous piece, for otherwise one runs the risk of producing artificial grafts of cells with the knife.

There is a point about the interiliac glands which is worth mention.

In examining these glands in cases of cancer of the cervix I have on two occasions come across a little mass of columnar epithelial cells arranged in single layer round a central lumen, set right in the middle of the lymphatic gland which in neither case contained

any secondary deposit from the cervical growth. Prof. Browning showed me a similar lymph gland removed from the abdominal pre-aortic region. Similar appearances have been noted by Borst, Meyer, and Scheib, and are interpreted by Borst as inclusions of the Wolffian duct and by Meyer as altered lymphatic endothelium. It may be said that if there is any epithelial structure found in or near the reproductive organs of women which cannot be traced directly to a normal epithelium, all German gynaecological pathologists refer it to Wolffian duct remains or to metamorphosed endothelium. The practical importance, however, of the finding, is that it may lead one, in the case of columnar cell carcinoma of the uterus, to think that there is a metastasis.

When the uterus is the seat of cancer the ureter may be said to pass through a danger zone in its course through the broad ligament. Its proximity to the lower part of the uterus and the upper part of the vagina makes it particularly liable to be affected when the malignant disease passes beyond the confines of the organ. The lymph vessels that come from the lower part of the uterus and from the vagina, anastomosing with each other and with those coming from the fundus, surround the ureter as they make their way to the lateral walls of the pelvis. In the parametrium, close to the ureter, there is what some have described as a lymph gland, often seen affected in cancer of the uterus. Poirier, Delamare and Cuneo insist that it is a junction of several lymph channels - the junta-cervical knot. At any rate it is a focus where the spreading cancer is apt to congregate to form a

perceptible nodule.

That the ureter is often affected we can demonstrate roughly in two ways - first, by post mortem examination, and second, by clinical evidence. Involvement of the ureters, shown by dilatation, hydronephrosis and all its sequelae, is, in fact, by far the most common post mortem finding. We are rather surpised if we do not meet with it in any case. As I have already stated, in three-quarters of the number of all cases dilatation of the ureters is sufficiently marked to merit record. I think this may be an understatement; slight degrees of dilatation would probably be unrecorded. It is generally considered that if one ureter only be affected the kidney on the opposite side will go on functioning with but little loss to the economy. And, actually, we frequently find, on post mortem examination, that, while the kidney on the side of the dilated ureter has atrophied to a considerable extent, the other kidney shows compensatory hypertrophy. But that is not the whole story. Even with unilateral hyroureter it is exceedingly rare to find the other kidney in a normal condition, apart from the hypertrophy. It exhibits all sorts of conditions. Nefedieff (Annales de l'Institut Pasteur 1901,XV,17) showed that when one ureter in rabbits was ligated a toxin was elaborated which produced nephritis of the unaffected kidney as well, and that when the serum of such an animal was injected into a normal rabbit it produced nephritis with albuminuria. The changes in the kidney in cancer of the uterus are probably the expressions of this

nephrotoxin. The clinical evidence of ureteral involvement is the uraemic condition that causes death; and death from uraemia is very common indeed.

The obstruction to the ureter is always found in the parametrium. Is the stricture of the lumen of the ureter extrinsic or intrinsic? I mean, is it due merely to some contracting influence around the ureter, or is it due to a distending growth within its wall? The general opinion seems to be that it is due to cancerous invasion of the surrounding tissue with consequent fibrous contraction, the ureteral wall itself escaping invasion. Such a condition does actually occur; how frequently I am unable to determine. I have also seen dilatation of the ureter above a stricture with no evidence of growth in the immediate neighbourhood. That may be due to a chronic inflammatory process following septic absorption from the ulcerating surface of the primary growth or to a fibrosis accompanying the infiltrating cancer.

Comyns Berkeley and Victor Bonney (British Med.Journal 1903,ii,961) in a paper on Wertheim's operation, say that "it is remarkable how resistant the ureteral sheath is to carcinomatous invasion, even when this structure is surrounded by growth." And, to judge from current practice, this immunity of the ureter is generally accepted, or at least unquestioned. The position these authors take up is that the ureter is highly resistant to direct infection and also to permeation because of its separate lymphatic system. I do not agree with these opinions. In the first place, what of the resistant sheath? The ureteral sheath is an anatomical

fiction. In the parametrium there may be said to be a covering but no sheath. The connective-tissue strands in the outermost part of the ureteral wall blend with those of the parametrium. Around the ureter the tissue is of an open texture, offering, in fact, a very favourable structure for the spread of cancer cells. In this loose tissue the ureter has a certain amount of play, but it cannot be dissected away from the parametrium without leaving ragged strands adhering to its wall. In this loose tissue run the blood-vessels, forming a rich plexus that supplies the wall. In the second place, the ureter is liable - in my opinion very liable - to be invaded by surrounding growth. Döderlein and Krönig give an illustration of a ureter which is very much invaded from the parametrium. It is an operative specimen. The microscopic examination of the constricted portions of ureters ~~found~~ found, post mortem will show that there is often definite invasion. Recently I examined the wall of a dilated ureter which showed an intramural involvement for almost 4 centimetres; the invading cancer could be traced from its site of entrance along through the wall to a point where it broke through the mucous membrane and entered the lumen. Quite apart from cases where there is visible dilatation of the ureter the ureteral wall may be invaded. Fig. 7 shows a part of a section of a ureter which, on naked eye examination, gave no hint of invasion. In this particular instance the ureter seemed fairly free in the parametrium, and I feel sure that if an operation had been attempted the ureter could have been dissected out without any difficulty; and yet the

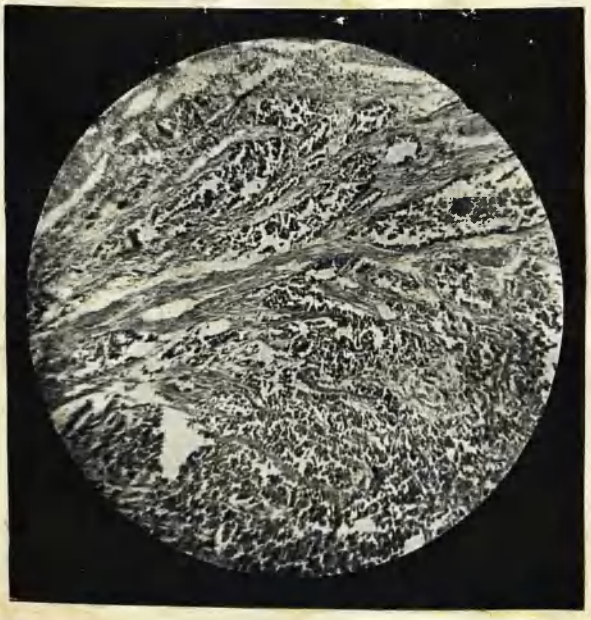
microscope shows extreme infiltration. Mackenrodt,(Zeit.f.Geb.u.Gyn. 1905 LIV,514) who estimates the frequency of involvement of the ureter at 1½ per cent. of cases, holds that the invasion can be recognized during operation. I know of no means whereby the ureter can be pronounced invaded or free except by prolonged microscopic examination. No one would attempt to say whether a tissue is free of cancer or not by palpation, and any estimate built on such opinion must be far short of the actuality. When there is a dilatation of the ureter there is evidence that the wall may be invaded, but in the absence of this sign only a laborious investigation of many cases by serial section could give us any indication as to the frequency of involvement. Would such an investigation be worth the trouble? The demonstration of a few cases of ureteral invasion without dilatation is quite sufficient to emphasize the point that when the parametrium is affected there is a danger of operative measures having their aim defeated if the parametrial portion of the ureter is left behind untouched.

The third point is the relation of the ureteral lymphatics to those of the parametrium. The continuity between them is denied. I am unaware of any anatomical work on the point; in fact, practically nothing has been done in working out the lymphatics of the ureter. In Bartels' text-book (Das Lymphgefässsystem 1909,p 233) there is a reference to the course of lymph vessels in the wall, but nothing is said regarding their continuity or otherwise with extramural channels. Whether this continuity exists or not is not of much practical importante. I have endeavoured to find evidence

of lymphatic permeation of the ureter in cancer, but there are many practical difficulties in the way. Figs.5 and 6 show a small cluster of cancer cells in a well-defined lymphatic vessel a little external to the submucous layer of the ureter, and the remainder of the wall perfectly free. The operation was performed by one of my colleagues and a portion of the ureter on the right side, though it might, without very much difficulty, have been dissected free, was removed along with the affected parametrial tissue. A large number of serial sections were taken from a part of this ureter and its surroundings, and a continuous lymphatic plug of epithelial cells was traced gradually approaching the lumen and then ceasing, but the series did not reveal the point of entrance. The observation, therefore, does not prove conclusively that the cells entered the wall in a lymph vessel. It may have happened that the neighbouring masses invaded the wall by direct extension, beyond the point at which the series commenced, and burst into a lymphatic vessel proper to the ureter; but for all practical purposes it does not matter whether the cells gained access to the wall in a definite pre-existing channel or by invasion through the loose tissue around the ureter. What is of importance to recognize is that, supposing the ureter to have been dissected out as cleanly as some imagine possible, the ends of an otherwise perfect operation would have been defeated. In a septic process a few bacteria could be left with impunity for the natural defences of the body to overcome. In malignant disease, on the other hand, a few cells left behind would suffice for recurrence.

5. Projection drawingof ureter and surrounding parametrium in an operation specimen of cancer of cervix. A,B,C, are three tracts of cancer invading the parametrium following the principal lymphatic paths. There is a minute nodule in the ureteral wall.

6. High power projection drawing of portion of the ureter wall indicated in last figure. Shows part of the mucous membrane of the ureter and a small mass of cancer cells in a lymphatic vessel.



7. Portion of wall of ureter in an advanced case of cancer of the cervix with involvement of the parametrium (P.M. case). There was no naked eye evidence of invasion nor was the ureter dilated.

Handley, it is true, holds that the body can sometimes deal successfully with cancer cells, killing them off by a natural reparative process, but this process is always too late; the growth outstrips it, and it is highly probable that the cells crushed out by fibrosis are dead already before the process starts.

Offergeld (Monats.f.Geb.1909,XXIX,181) could only find three cases where the ureter was definitely invaded in gynaecological literature. In a fourth case Krönig found cancer nests in the ureter wall. In a fifth case Wertheim demonstrated cancer cells in the wall of a ureter resected on account of carcinomatous parametrium. Döderlein (Hegar's Beitrage, 1904, IX,181) reported a similar occurrence. The fact that Offergeld's three cases were all from one clinique suggests the possibility of the rarity being due to want of opportunities for investigation.

The vascular supply of the ureters has an important bearing on the operative treatment. As Feitel (Zeit.f.Geb.u.Gyn.1901,XLVI, 256) and Sampson (Johns Hop.Hosp.Bull.1904,XV,39) have shown, the arterial supply is a rich one, branches being derived from the aorta immediately above the renals, from the renals, the ovarians, the aorta above the bifurcation, the common iliac on the left side, the internal iliac on the right side, the uterines and the vesicals. These branches anastomose in an open network around the ureter, and from this network the arterioles pierce the walls. Whether these intramural vessels anastomose in turn has not been determined. If the ureters are cut and reimplanted in the bladder - as the pathological observations would indicate - there is abundance of blood

supply assured for proper healing. If, on the other hand, the ureters are dissected from their surroundings in the parametrium two dangers are at once evident. The minor danger is ureteral necrosis due to the stripping off of the vascular plexus, and this actually occurs by no means infrequently, even in Wertheim's hands. The major danger is the risk of leaving attached to the dissected ureter cancer-bearing lymph vessels or tissue infiltrated with cancer cells. In such cases a recurrence could not clearly be traced to its real origin. It may be objected that the evidence here adduced as to the danger of ureteral involvement in cancer of the uterus is based upon post mortem and advanced operative cases. These are the only cases a pathologist can investigate, and I would urge that it is the terminal pictures that give us an insight into the hidden processes occurring during life; they are the evidences of dangers which, otherwise undemonstrable, would not be avoided by the surgeon.

When the cancer originates in the cervix the liability to involvement of the vagina is very great. In cases where there is a recurrence after operation it is almost invariably in the upper part of the vagina. In our post-mortem cases only a very small number (2.5 per cent.) escape. The involvement may be evident to the naked eye as sub-epithelial nodules or ulcers. Ulceration of the vagina, where it is in contact with a cancerous cervix, is fairly common, and it is usually cited as an example of contact transference or auto-inoculation. Such a process is always open to suspicion. The necrotic condition of the surface of a cervical tumour and the septic condition of the material are almost certain

obstacles to tumour grafting. The anastomosis is so rich between the lymphatics of the upper part of the vagina and those of the cervix that downward spread is quite common. Hence an apparently independent nodule or ulcer may be, or may have been, in direct continuity with the parent growth through the underlying lymphatics, though separated from it on the surface by unchanged epithelium. Even where there is no naked-eye evidence of involvement of the vagina we may have it revealed by the microscope. Thus in Wertheim's operation the vaginal cuff made to enclose the septic cervix serves also to remove a highly dangerous tissue, but the amount of vagina removed is not nearly enough to eradicate the disease. From the vagina, by continuity of tissue, the bladder and the rectum become involved, the former in approximately two-thirds of post-mortem cases with fistulae in 44 per cent., and the latter in one quarter of the cases with 16 per cent. of perforations. The timely removal of the greater part of the vagina would prevent the occurrence of these distressing and hopeless sequelae.

The points, then, on which the pathologist can insist as worthy of careful consideration by any gynaecological surgeon who would seek to treat cancer of the uterus on hopeful lines are the necessity of removing the various danger zones indicated, (1) the interiliac pelvic glands, (2) the parametrium, and (3) the greater part of the vagina together with the uterus containing the primary focus, and that in all cases, however small the primary focus may be, the maximum operation should be done. Whether the danger of involvement of the ureter be great or small is a point on which I cannot speak with

authority, and the question of resection of the ureters may be left open. When first I brought the other points before gynaecologists I was met with the answer that, if operations were so designed as to remove the three main danger zones indicated above, the consequent mortality would be very great. Experience has proved that such is not the case. The technical difficulties are gradually being overcome. One of the difficulties pointed out was that in a complete one-piece extirpation the considerable heamorrhage in the pelvis would prolong the operation unduly. Having seen a few experimental operations on advanced cancer of the uterus in which an endeavour to starve the growth was made by injecting melted paraffin into both internal iliac arteries I was struck with the fact that there was no sloughing of the bladder wall, nor, as far as one could determine, any change in that viscus, though the blood supply of the bladder, according to anatomical text-books is derived solely from various branches of the internal iliac artery. I therefore induced one of my surgical colleagues to make a point of ligaturing both internal iliac arteries at their commencement as the first step in radical operations on cancer of the uterus. By so doing he obtaine d a practically bloodless field of operation and was enabled to remove the tissues in one piece from the pelvic glands to the vagina and to save much time, with its consequent shock to the patient, thereby. In 30 cases so treated only one died as a result of the operation. The more precise the indications which studies in the surgical pathology of cancer can bring forward and the more thorough the surgeon's technique in adopting them, the less hopeless will be the outlook of the cancerous patient.

A CARCINOMA IN A RABBIT.

Attempts at experimental propagation of the tumour.

Incorporating a paper on the subject contributed to the Proceedings of the Royal Society of Medicine (Pathological Section), Vol V. 1911.

A CARCINOMA IN A RABBIT.

The propagation of malignant tumours and the attendant phenomena have been studied hitherto almost exclusively in mice and rats, and perhaps some of the conclusions drawn from experiments in these animals might be overturned or modified in their general application if a more suitable laboratory animal could be utilised. Dogs and Cats which we know are frequently the subjects of malignant tumours are unsuitable for experiment in this respect because it would be well nigh impossible to deal with large numbers. If a transplantable tumour could be propagated in guinea-pigs and rabbits the field would be open to experimental investigations, such as chemotherapeutic efforts, which are impossible in such small animals as rats and mice. Unfortunately, rabbits and guinea-pigs very rarely develope malignant tumours. A veterinarian, Mr. T.J.Ambrose, who has had extensive experience of postmortem examinations on rabbits and cavies, informed me that he had never seen any definitely malignant tumour in these two animals. Previous to my own case in 1911 I could find only two references in pathological literature to the finding of tumours in rabbits, and though we advertised in a fanciers' journal for several months, offering good prices for animals with tumours yet in only one instance did we obtain a true neoplasm. Breeders are accustomed to destroy or dispose of rabbits and guinea-pigs as soon as the breeding qualities of the animals deteriorate so that one seldom has an opportunity of seeing aged animals. If it were possible to make a large collection of old rabbits the chances of discovering malignant tumours would be considerably increased, because the general rule seems to obtain in the lower animals that cancer is a disease of

advanced life.

There is a famous example of uterine carcinoma in a rabbit. Lambert Lack (Journ. of Path. and Bact., 1900, VI, p 154), impressed by the theory that malignant tumours were the result of body cells displaced by from their normal surroundings cut open the ovaries of a rabbit, scraped the raw surfaces with a knife, and allowed the milky juice containing epithelial cells to diffuse through the peritoneal cavity. The animal remained in good health for nearly a year thereafter, but at the end of that time, becoming weaker and thinner and suffering from dyspnoea, it was killed. On examination he found in the mesentery numerous hard white nodules varying in size froma pinhead to a pea, small white densely hard patches in the liver, infiltration of the diaphragm with projecting masses on its pleural surface, nodules on the parietal pleura and a few in the lungs; the whole mediastinum was occupied by a mass of tumour-like tissue. The uterus was greatly thickened, the mucous membrane papilliform, and at one place in the wall there was a tumour as large as a cherry. Microscopical examination of the nodules from various parts showed alveolar spaces lined by one or more layers of columnar epithelial cells, or entirely filled up with cells that had lost their columnar shape. It was a typical columnar cell carcinoma. Lack regarded it as having "all the characteristics of an ovarian cancer", and considered it to have resulted from the mechanical dissociation of ovarian cells which he had produced. Though the uterine tumour does not appear to have been the largest of the nodules, yet the great thickening of the rest of the uterus and the papilliform arrangement of the mucous membrane, together with the absence of any noted change in the ovaries or other sites of columnar epithelium in the vicinity,

point to the uterus being the only probable source of a tumour with such histological characters.

Strangely enough, within the same year Shattock (Trans.Path. Soc.Lond.,1900,li,p.56) reported an undoubted case of spontaneous carcinoma of the uterus in a rabbit, and was able properly to orientate the hopes that otherwise might fairly reasonably have been entertained regarding such mechanical production of malignant change. In Shattock's case there were a certain number of nodules in the peritoneal cavity; the uterine cornua were enlarged and tortuous, and contained nodular swellings reaching a maximum diameter of 3 cm. The peritoneum over the swellings was smooth. There was scarcely any area of uterine mucosa between the actual tumours that was not obviously abnormal, the membrane being papillary and cystic, though the muscular wall at these situations was not invaded. The tumours had a villous or cystic surface, and involved the entire thickness of the muscular wall. The histological appearances were those of a columnar cell carcinoma, in many places of the villous or papilliform variety.

Boycott (Proc.Roy.Soc.Med.,1911,iv(Path.Sect.),p.225.) reported four cases of epithelial tumours in rabbits. All four were found within a few months "during the breeding season" in a batch of 140 to 150 females. The first showed a solid tumour 8 cm. by 2 cm. by 3 cm. in one cornu of the uterus. It was covered with cubical epithelium, and consisted of a loose connective tissue stroma with irregular epithelial growths, mostly solid masses of cells, but with a goodly number of alveoli lined with cubical epithelium. The muscular coat was deeply infiltrated on the mesometric side. The other cornu of the uterus contained five foetuses with abnormal placentation.

In the second case there was a single tumour mass measuring 2 cm. by 1.5 cm. Solid masses of epithelial cells and alveoli existed in equal proportions. Some of the latter were much distended and there was a considerable amount of intra-alveolar papillary growth. The third case also showed a single uterine mass, 6.5 cm. by 2 cm. by 1.5 cm., almost entirely alveolar, with a flattened lining epithelium and a number of intra-alveolar growths. It extended into the wall of the uterine cavity over the fundus, and spread along between the deep ends of the glands and the muscular coat. In the fourth case, one cornu contained five, the other six, small equidistant pedunculated tumours with small alveoli, some solid masses and many intra-alveolar growths. There was haemorrhage into the stroma at one place, and some necrosis both of connective tissue and epithelium. All the tumours agreed in having a delicate stroma with a free blood supply. In the distended alveoli there were no microscopic contents. These vessels were quite large, but had simple endothelial walls of only capillary thickness. They all appeared to have arisen mesometrically in the site of normal placentation, and Boycott suggests that they arose in sequence to abortions.

The history of my own case may therefore be of interest. We had in the laboratory for a few months a crossbred doe of the "Flemish Giant" type at least two and a half years old, but probably much older. In the first week of February 1911 she was mated to a buck and apparently became pregnant. She was therefore expected to litter in the first week in March, and actually did give the usual warning by plucking out the hair around the milk glands, a thing that usually happens one or two days before littering. She passed the calculated

time without anything happening. The distension of the abdomen slightly diminished, and on March 21 she was again mated to a Dutch buck. The swelling of the abdomen now increased more than previously. On April 19, two days before the expected littering, she again started to pluck out hair and prepare a nest, * but again she passed the period without anything happening, and the swelling decreased somewhat. We thought we could detect foetuses on abdominal examination.

On May 3 she was found in the morning with some blood in the cage, half a pint being in a dish. The rabbit was quite lively, but on examination we found blood coming from the vagina. The animal was chloroformed and the abdomen opened. The uterus showed three large tumours, one towards the extremity of the right cornu, one at the junction of the cornua, and a third on the left side (fig.1). Between the first two there were two smaller moniliform swellings of the uterine wall. The largest tumour measured 5 cm. long by 3.5 cm. in height. The peculiar condition of a portion of the serous covering led me to think I might have to deal with a malignant tumour, so the animal was killed after the uterus had been removed, and a search was made throughout the body for metastases. None was found. As experience afterwards proved, the killing of the rabbit was a costly mistake. I ought to have transplanted a portion of the uterine tumour to another situation, for preference under the skin,

---

* The fact that the rabbit on two occasions made timed preparations for expected litters which did not arrive, would seem to indicate that rabbits calculate the date of littering not from any specific internal stimulus, but by reckoning from the supposed time of conception, as human beings do. This is also borne out by observations on rabbits exceeding their term.

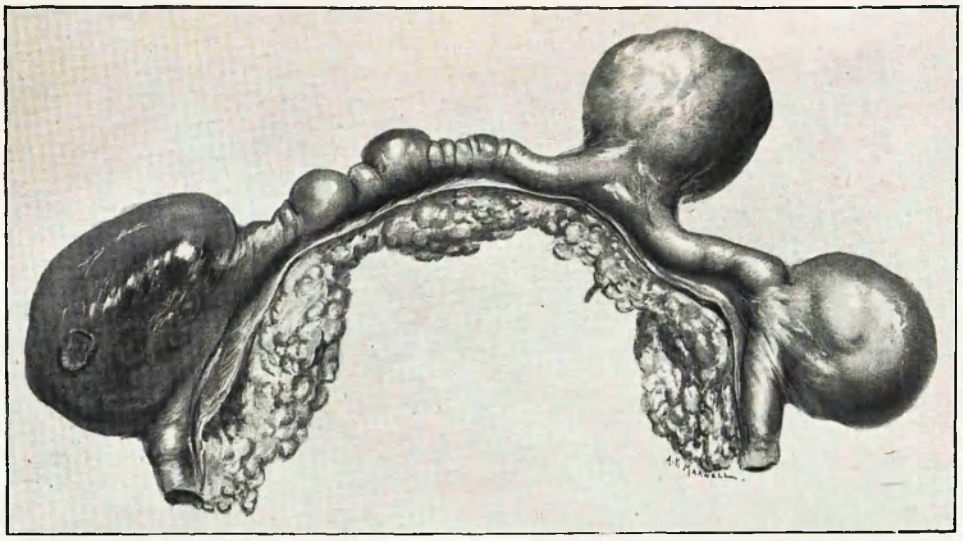


Figure 1

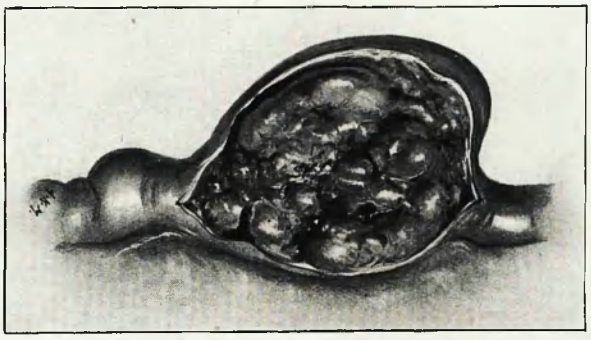


Figure 2

Fig. 1. (X $\frac{2}{3}$). Drawing of the uterus showing three large tumours. Tumour A, situated in the right cornu, shows a puckered area of infiltration of the serous coat. Tumour B is at the junction of the two uterine cornua. Tumour C is in the left cornu. Between A and B are two smaller swellings.

Fig. 2. (1/1). Uterus opened posteriorly to show tumour A.

in the same animal, and then when it grew to transfer it to a large series of other rabbits, but at the particular moment my mind was so occupied with the idea that the tumour would prove to be a chorionepithelioma - a tumour which has never been found in any animal other than man * - that I failed to realise the unique chance I was losing. As soon as the animal was dead I realised my mistake even if it had proved to be a chorionepithelioma. If we were always as wise five minutes before the event as we are five minutes afterwards this would be a land of supermen.

The uterus was opened over the largest tumour, and small portions, the size of hemp seeds, were removed from it and injected by means of a transplantation canula subcutaneously into both flanks of four rabbits, and into the right flank only of five other rabbits. The specimen was put into the ice-chest for two days, and then several larger portions of the same tumour were inserted beneath the deep fascia or into the flank muscles of ten rabbits. Thus nineteen rabbits, of various breeds, all we could procure, were injected with tumour material..

The uterine cornua are considerably thickened. The largest tumour (tumour A) in the right side, situated close to the extremity, bulges out the uterine wall anteriorly, and towards the fundus, and overhangs the uterus on both sides. The peritoneal covering, though slightly and irregularly grooved, is quite smooth and intact save for a small oval area less than 1 cm. in greatest diameter, where there is

---

* A chorionepithelioma was reported by Schumann as having been found in a Canada Porcupine (ERETHYSON DORSATUS) but the details of the case have never been published, and the diagnosis is questionable. (vide Journ. of Comp. Path. Dec 1915)

a depressed puckering with a raised margin. Internally the tumour is seen to be attached anteriorly and to the fundus (fig.2). It is a very irregular nodular growth with some ulceration and haemorrhage towards the centre of its surface. It is soft on section and shows an appearance like testicular substance. The central part is necrotic. The tumour infiltrates the muscular wall, and is continued through the serous coat at the externally puckered area (fig.1). The distended endometrium of the cavity is fairly smooth.

Tumour B, situated over the cornual junction, is spherical and pulls the uterus upwards. It is not so grooved as the former tumour, and its peritoneal coat is uniformly smooth and intact. Internally it is seen to be composed of two distinct growths which have a smooth surface. They are attached towards the fundus and the cornua open towards them. The vagina is normal.

Tumour C is in the middle of the left cornu. It is the smallest of the three. Its internal surface is smooth, and like the last it does not infiltrate the muscular wall. Sections through the smaller nodular thickenings and at various parts throughout the cornua show considerable papilliform proliferation of the endometrium, through which the cavity of the uterus winds tortuously. Here and there there are solid subendometrial growths.

The portion of tumour A growing into the uterine cavity is composed of simple tubules lined with a single layer of cubical epithelium set in a delicate and relatively abundant areolar stroma (fig.3). Throughout the stroma run numerous thin-walled capillaries. Alveoli with simple walls predominate. Some of the spaces contain cellular debris. There are other alveoli with complicated intra-

alveolar growths, and others, again, quite filled up with proliferating cells that tend to produce daughter lumina in the masses. The epithelium lining the simple spaces is cubical, though at parts more typically columnar. In the infiltrating portion of the tumour as it makes its way through the muscle we have no longer a delicate surrounding stroma, nor the regular glandular formation. Between the muscle-fibres the cell masses are very irregular and the shape of the cells distorted. Attempts at glandular formation are still seen. The muscular wall is quite broken up, and the epithelial masses come right up to and involve the serous coat (fig.4). In this part of the nuclei are not so large or round as in the free portion. In some of the masses there are areas of necrosis. Close to the serous coat there are many large blood spaces. At the edge of the tumour the unproliferated endometrium is reflected on to the surface of the tumour, separated from it at first by a thin open-meshed connective tissue, but gradually thinning out as it proceeds till finally it disappears and the tumour cells appear on the surface.

In the other two tumours simple single-layered tubules are scarcely to be found. Many branched spaces abound, lined usually with columnar epithelium often several layers thick. At parts the alveoli are practically solid masses, although traces of glandular formation are also seen. The endometrium reflected on to the tumours gradually fades away and becomes indistinguishable from the superficial cell masses. The stroma is at parts of the same fine areolar type as in tumour A, though at parts considerably denser, and is well supplied with thin-walled capillaries. The invasion of the muscular wall has not progressed so far as in the first tumour.

A section taken through the nodular swelling of the uterine wall close to tumour A shows a double cavity, both parts being lined by ciliated columnar epithelium. In the anterior part the endometrium is but slightly papilliform, in the posterior part it is much more exuberant. Between the two, extending from fundus to mesometrial attachment, is a mass of tissue consisting of a fine cellular stroma in which are set alveolar spaces lined by columnar or cubical epithelium with considerable infolding of the wall. All gradations are found between this and the ultimate small acini with no observable lumen. Anteriorly the endometrium lies directly over the growth posteriorly it is separated from it by a highly vascular connective tissue. Posteriorly to the second cavity there is a smaller mass of the same characters, and insinuating between the edge of this mass and the musculature of the wall can be seen a portion of the endometrium infiltrating among the muscle-fibres (fig.5 (1),). This gives us a possible clue to the way in which the tumour masses are formed. In the next nodular swelling the cavity is also double. The endometrium is more complicated, and the axis of tumour growth extends diagonally backwards and downwards between the two portions of the cavity (fig.5 (2),). The epithelial cells have formed themselves into solid alveoli and the stroma is very scanty. The contrast between the papilliform endometrium with its well-marked columnar cells and the densely cellular subjacent mass is very striking. Between this last swelling and tumour B there is extreme papillomatous development of the lining membrane, splitting up the cavity into a complicated pattern (fig.5 (3),), but there is no infiltrating growth. Between tumours B and C, and distally to C, there is the same proliferation

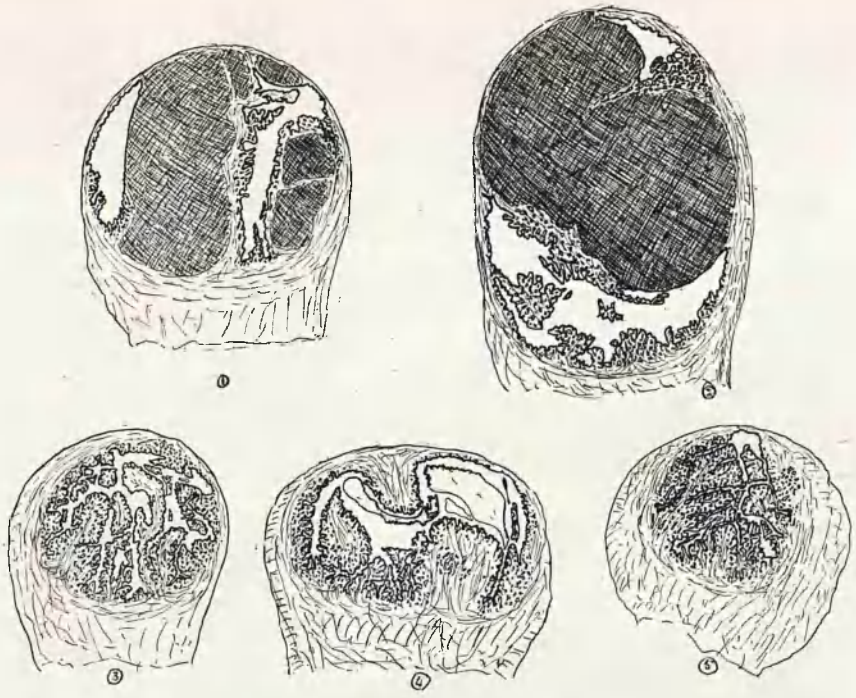


Figure 5.

1. Transverse section of the uterus between tumours A and B, close to A. Cavity double. Two separate neoplasms.

2. Transverse section of uterus between A and B, midway. Cavity divided into two by an infiltrating new growth.

3. Transverse section of uterus between tumours A and B, close to B. Vaulted papilliferous formation of endometrium.

4. Transverse section of uterus between tumours B and C. Papilliferous ingrowths of endometrium. No definite invasion of wall.

5. Transverse section of uterus distally to tumour C. Excessive papilliferous formation.

of endometrium (fig.5 (4 and 5),). At some parts the stroma of the papillae is myxomatous.

The animals into which portions of the tumour were inoculated were first examined on the tenth day, and thereafter three or four times a week (fig.6). Fourteen of the nineteen gave evidences of successful transplantation. Four of the nine inoculated with fresh tumour material on May 3 showed nodules; all those injected on May 5 with large amounts showed at first relatively larger nodules, but as there had been more laceration of tissue in the second series, and as the material injected was older, it is probable that the greater inflammatory reaction gave an exaggerated idea of the real size of the grafts. In the latter series, after this reaction subsided, steady diminution, apparently the result of absorption, was the invariable result, in some more rapid than in others. The rabbits ~~have now been~~ were observed over a period of five months. Some ~~have~~ died, one was killed owing to injuries it sustained in fighting, two ~~have~~ had portions of their tumours removed from them and transplanted into a second generation, and of the remainder only one nodule ~~has~~ survived with practically no variation in size over this period of time. Whether it is wholly replaced by fibrous tissue, or still contained living epithelium, I do not know. None of the tumours showed progressive increase. My experience in transplantation of diminishing tumours in mice has not encouraged me to attempt transplantation of these diminishing nodules in the rabbits. The sequence of events in the grafts would seem to be as follows; first, degeneration of the introduced stroma and of the more central parts of the parenchyma, and their replacement by connective tissue derived from the host, the epithelial

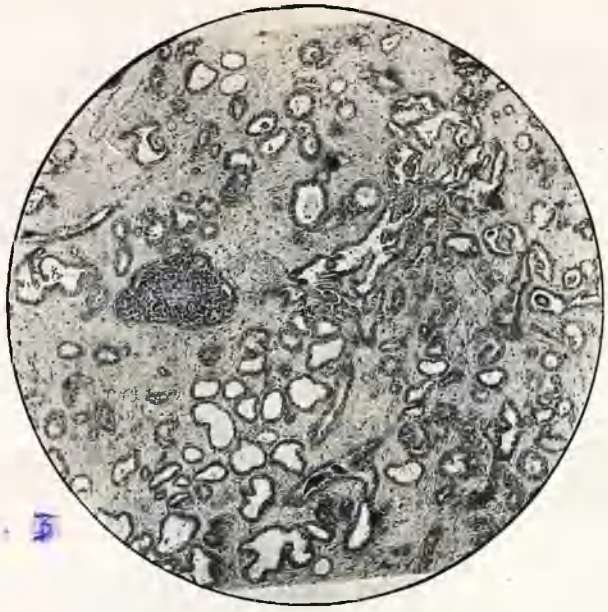
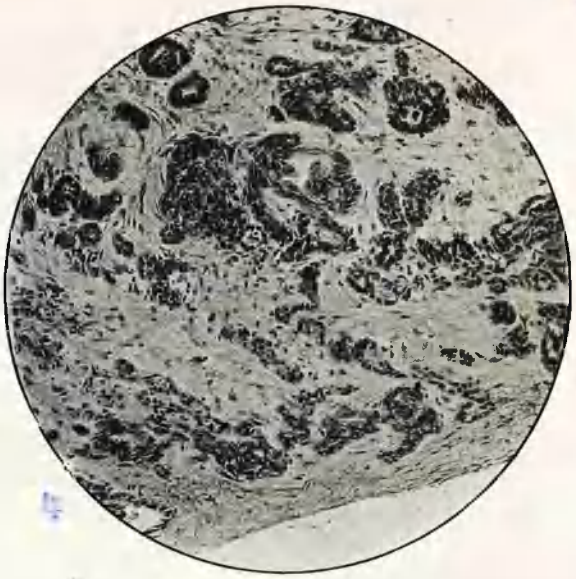
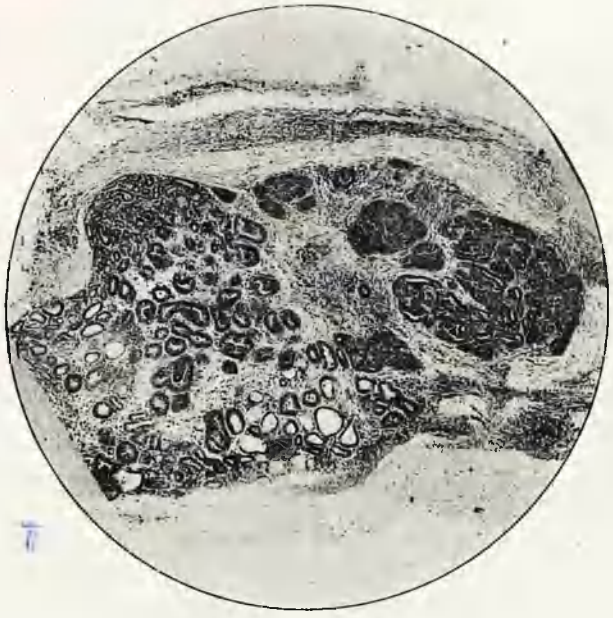


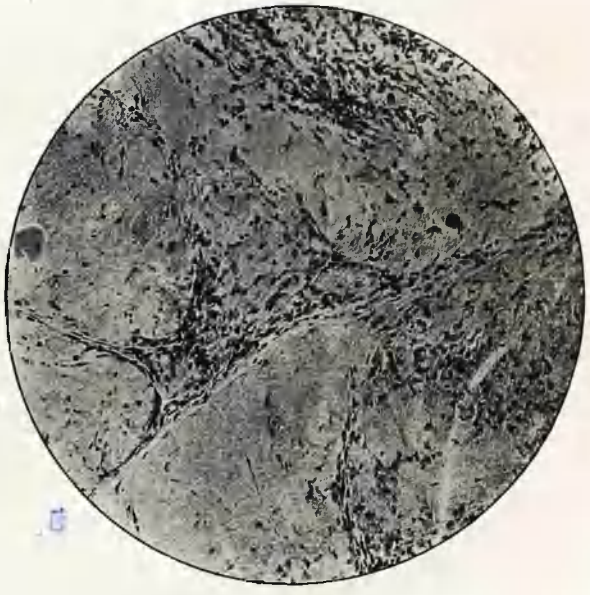
Fig. 3



4



7



8

Fig.3. Section of portion of the largest tumour. X 60.

Fig.4. Infiltration of muscular wall in tumour A. X 125

Fig.7. First generation of transplants. Age of graft twenty-two days. X 60.

Fig.8. First generation. Age of graft 24 days. X125.

elements towards the periphery surviving, and perhaps multiplying to a certain extent; second, an increase in the fibrous tissue encapsulation of the graft with maturing and densification of the new stroma; third, cystic formation and coalescence of neighbouring acini with desquamation of the epithelial cells, a feature that is more marked as the graft ages; and finally, a degeneration of the remaining epithelial cells and their complete replacement by overgrowth of the connective tissue.

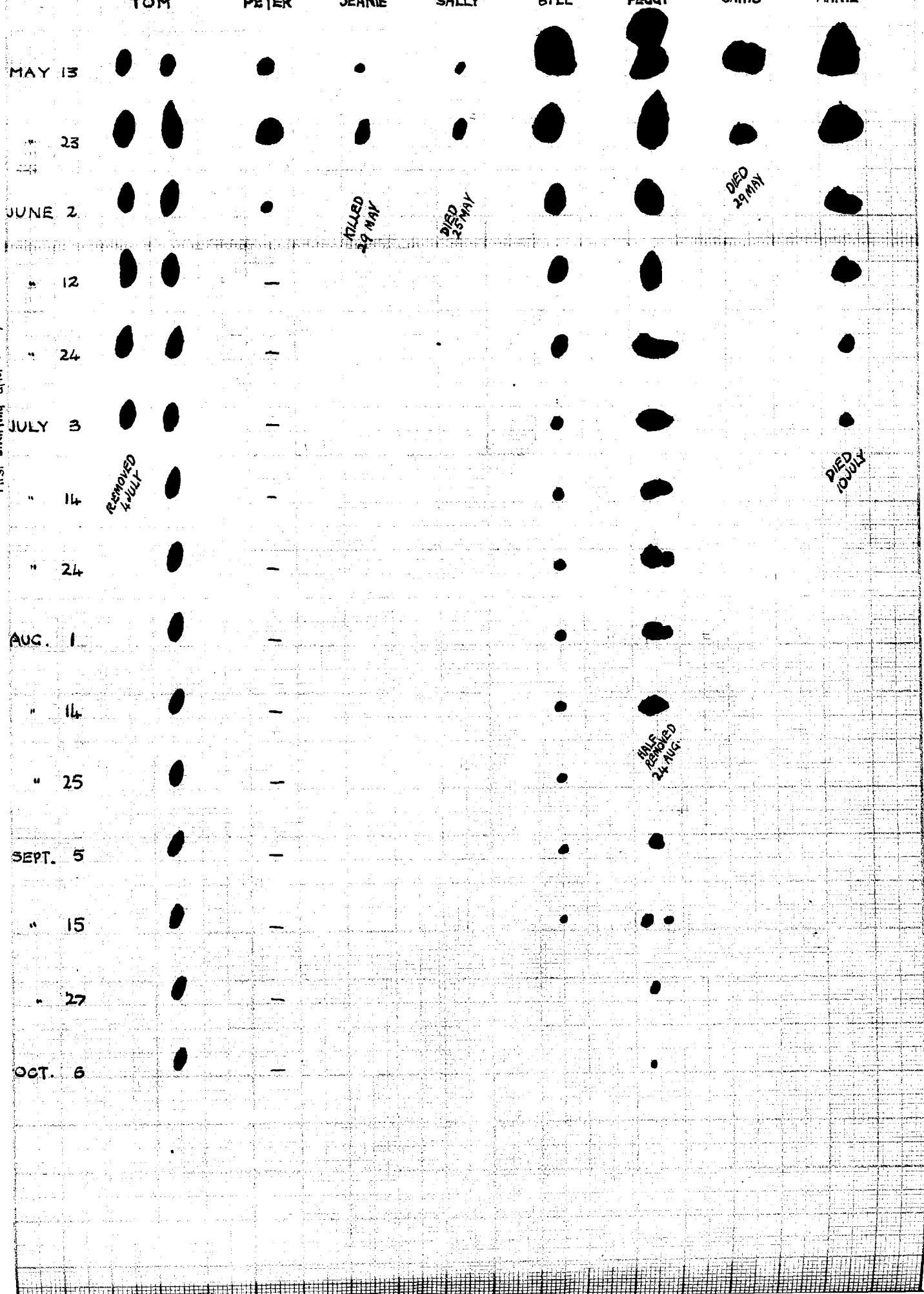
The nodules examined were as follows:-

(1) Young rabbit, "Sally." Injected on right flank with fresh tumour tissue. On the tenth day the nodule was about 2 mm. Ten days later it measured 5 mm. by 3 mm. The animal was found dead twenty-two days after injection. The graft is surrounded by a thin capsule of young fibrous tissue, in which there are several dense collections of small round cells (fig.7). In the centre of one of these collections there is an isolated alveolus filled up with degenerated epithelial cells. Fibroblastic tissue penetrates between the external alveoli at parts, and cuts them off from the others. Here and there the alveolar spaces are dilated and the lining cells consequently flattened out. The original stroma in the centre is degenerating; the protoplasm of the cells is ragged and granular. Most of the alveoli are well preserved and healthy-looking, though all show more or less desquamation of lining cells. Lymphocytes are invading some of the epithelial cells. No polymorphonuclears are seen. Karyokinetic figures were not with certainty recognised. This nodule had undoubtedly increased in size, and the increase was not due solely to the proliferation of the tissues of the host.

CHART

RABBIT CARCINOMA

First charting after 10 days.



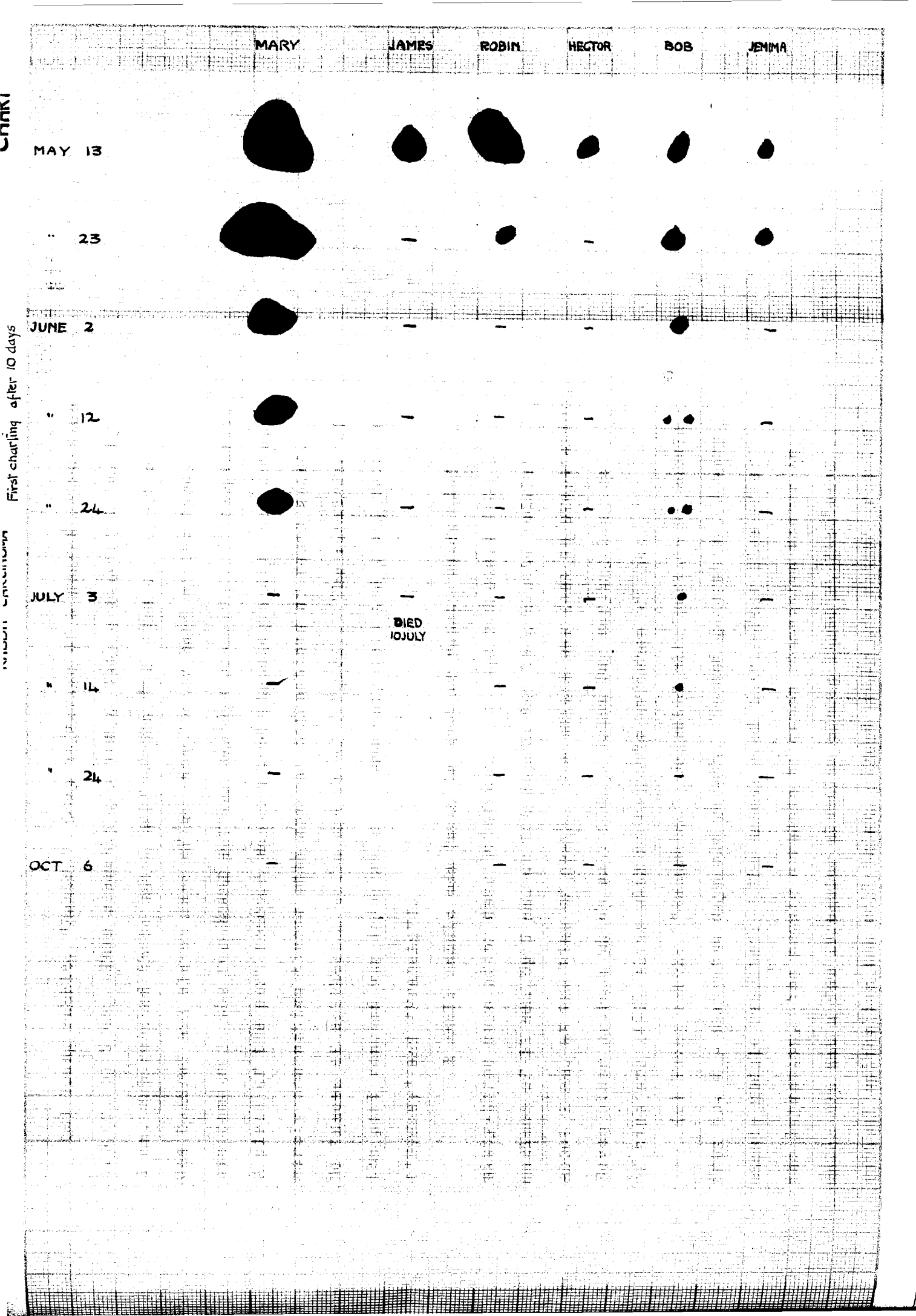
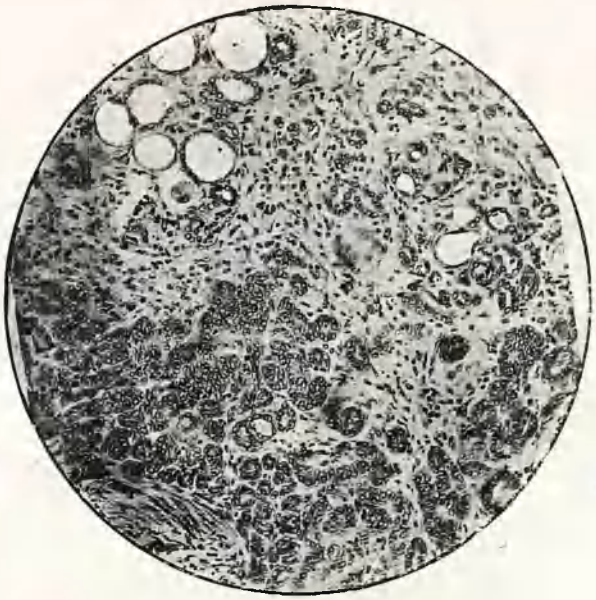


CHART
MARY
JAMES
ROBIN
HECTOR
BOB
JEMIMA
MAY 13
" 23
JUNE 2
" 12
" 24
JULY 3
DIED 10JULY
" 14
" 24
OCT 6
First charting after 10 days

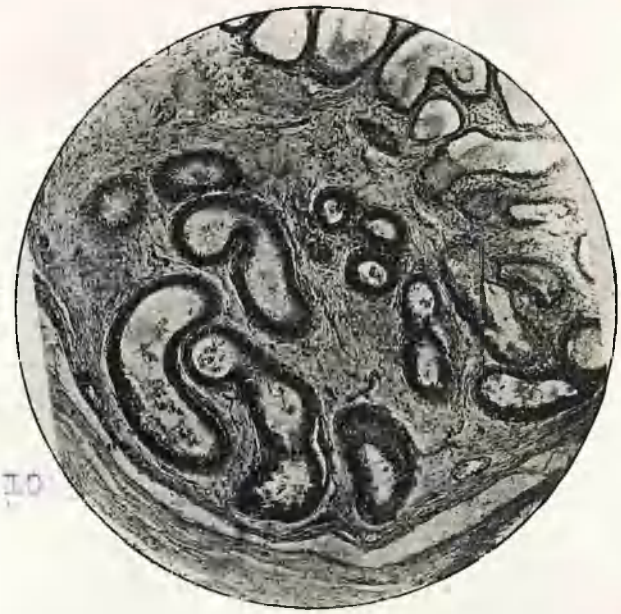
(2) Young rabbit, "Sahib." Deep injection of a pea-sized portion 1.5 cm. by 9 mm. This steadily diminished till a size of 5 mm. by 4 mm. was reached three and a half months from time of injection. One hundred and eleven days after inoculation half of the tumour was removed; the remaining portion has now practically disappeared. A piece of the part removed was examined histologically, but shows merely connective tissue with several collections of round cells. No traces of epithelial cells are found (fig.11). The remainder of the part removed was injected subcutaneously into two young rabbits ("Sydney" and "Francis").

A small nodule was found in another rabbit which died sixty-three days after deep injection. It had decreased from a maximum of 11 mm. by 9 mm. on the eighth day to a minute nodule about 1 mm. at the time of death. The nodule was lost in preparation. Seven other animals which exhibited nodules for varying lengths of time are still alive with no palpable evidence of them.

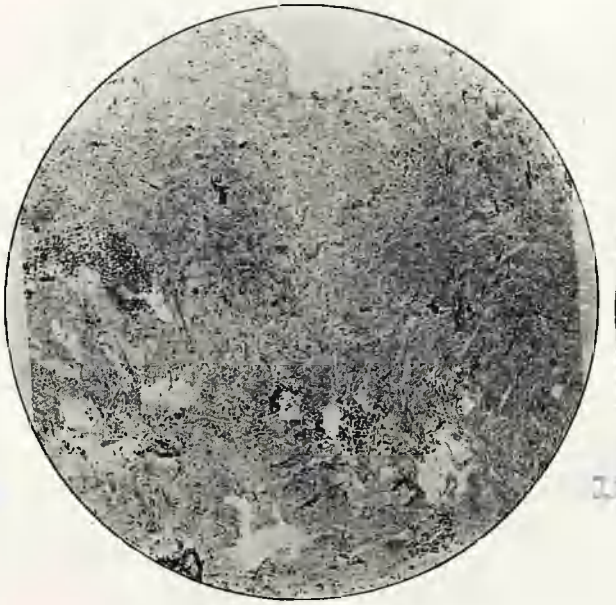
(6) Young rabbit, "Secundus." Injected with portion of nodule from No. 4 - sixty-two days old growth. Second generation. A very minute nodule resulted. The animal died thirty-four days after the injection. The nodule consists of dense connective tissue in which are set a few irregular alveolar spaces lined by flattened cubical epithelium one cell thick. These cells are rather granular and the nuclei are not always very clear. The internal margins of the cells are seldom well defined. In addition to these there are several other spaces from which the cells have almost entirely disappeared, and round these last are dense collections of black pigment. Other masses of black pigment are found in cells between the fibres (fig.12).



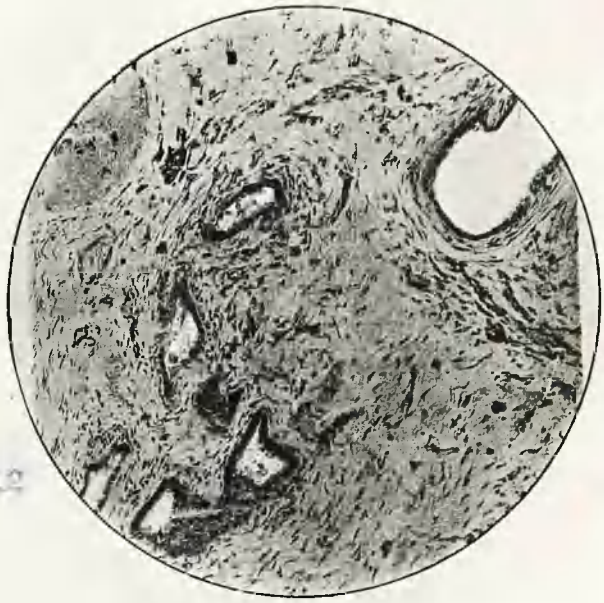
9



10



11



12

Fig. 9. First generation. Age of graft 26 days. X 125.

Fig. 10. First generation. Age of graft 62 days. X 60.

Fig. 11. First generation. Age of graft 111 days. X 60.

Fig. 12. Second generation. Age of graft 34 days. X 125.

(7) Young rabbit "Sydney," Injected with portion of tumour removed from No. 5 - one hundred and eleven days old growth. Second generation. Though the portion examined from the last generation showed no trace of epithelium, yet when this rabbit of the second generation died thirty-three days after inoculation there was found in the midst of a small fibrous nodule a cystic space a little over 1 mm. in diameter lined by flattened epithelium, in which space was a considerable amount of epithelial debris.

The other animal injected with the same material at the same time showed a very minute nodule thirty-three days afterwards, composed merely of connective tissue.

Boycott thinks that the onset of the condition in his cases was associated with pregnancy. They all occurred "during the breeding season." In his first case one-half of the uterus contained foetuses, with tumour formations in the other half, and all the tumours showed an attachment on the side of normal placentation. He thinks that if the foetuses came to a bad end the proliferated epithelium at the edges might grow over the depressed epithelium of the placental site, and by cutting off the latter allow it to grow and develope malignant infiltrating characters. He was unable to demonstrate any connexion between the neoplastic formation and the endometrium. In Shattock's case, as in my own, and probably also in Lack's, a very noticeable feature was the enormous papilliform proliferation of endometrium throughout the rest of the uterine cavity. Whether this general hyperplastic "endometritis" preceded or followed the conception is a point on which, of course, we have no information. It may quite as well have been the cause as the consequence of the foetuses coming to a bad end. But it would

seem more probable that if the sequence were as Boycott suggests the proliferation of endometrium would be confined to the vicinity of the tumour formations and not be generalised over the uterus as they were in our cases. In my own, for example, the endometrium more closely approximates the normal in the immediate vicinity of the tumour. Again, the attachment of the tumours in the present case was mostly anteriorly and in the fundus in the case of the larger, and irregular in the case of the smaller, nodules. I have not been able to trace a definite unmistakeable connection between the more or less normal endometrial epithelium and that forming the bulk of the tumours,- but neither have I been able to demonstrate an epithelial covering of the larger tumours distinct from them and continuous with that of the endometrium. It is in most cases impossible in adeno-carcinomata of a surface to demonstrate an unbroken epithelium from the purely simple to the undoubtedly malignant portion, though such a connexion did undoubtedly originally exist. In the smaller swellings there is a distinct endometrial covering often separated from the under-lying tumour mass by a well-marked vascular stroma. But some of my sections are, I think, capable of demonstrating a connexion between tumour and endometrium or at any rate of showing how it has arisen and been lost. From all sides of the uterus there are papilliferous ingrowths with their covering epithelium extensively folding as it proliferates. The cavity of the uterus becomes excessively complicated and the available space is filled up. An internal pressure exists, the wall is distended, and at the bases of the papillae the proliferating epithelium is forced inwards in the submucous coat or between the separated

fibres of the muscular wall. The delicate stroma of the undoubted tumour formations is probably continuous with that of the endometrium, in which case the tumours at one stage of their history might be comparable to adenomyomata of the uterus in the human subject, which condition, as Cameron and I pointed out (Lancet, 1904,ii,p.84) is midway between and has characters of both hyperplastic "endometritis" and adenocarcinoma. In the present case we pass insensibly from a condition of hyperplastic "endometritis" to infiltrating adenomatous formation. The growth of stroma lags behind the growth of gland tubules, and the growth of gland tubules lags behind the growth of individual gland cells. The same factor that started the papillomatous proliferation of endometrium is also the cause of the adenocarcinomatous formation.

As far as the history of the case goes it seems in some way to be related to pregnancy. In no part of the specimen could I detect remains of conception products nor any decidual cells in the wall or in the tumours. At no time had we any evidence of abortion having taken place. On the whole, Boycott's theory of formation cannot be ruled out, and it may quite well be that the general proliferation of epithelium throughout the uterus, which is not a normal condition in ordinary pregnancy, was the result of the stimulus of a superadded second conception. Whether the condition can be produced experimentally by interference with early pregnancies, as Boycott suggests, is a point on which I am not able to offer an opinion; the technical difficulties are great.

The particular portion of the tumour transplanted was histologically in that period of transition in which it is impossible

to say definitely whether it is simple or malignant. The failure of the grafts to go on increasing does not prove it to have been simple, nor do I think that if, on the contrary, they had increased it would necessarily have proved that we originally started with a tumour in a malignant condition. The failure to transplant is not an evidence of the benignancy of a tumour, nor is the transplantability a criterion of its malignancy. If I had transplanted the tumour into the animal itself and subsequently transferred pieces of that secondary tumour into a large series of other rabbits, preferably young rabbits, I probably would have had in some of them increasing growths capable of indefinite propagation and have been able by means of them to carry on investigations which are subject to severe limitations in laboratory animals so small as mice. As it is, we have only a fragmentary study in degeneration.

@ @ @ @ @ @ @

Previous to the publications of my observations on this tumour of the rabbit very few notes had appeared in literature. The cases of Lambert Lack, Shattock, and Boycott have already been considered. Wagner (Ueber multiple Tumorem im Uterus des Kaninchens: Zeit.f.Path. 1905, S 131) recorded a case in which the uterus contained five tumours, one of them reaching the size of a pigeon's egg. Although the musculature was apparently invaded the author regarded them all as simple adenomata.

Lubarsch (Centr. f.allgem. Path. u.path.Anat., 1905 p 342)

discovered an epithelioma of the Kidney whibh he endeavoured unsuccessfully to transplant. Von Hippel (Verh.der deutsch.path.Ges.,1906) claimed to have transplanted a teratoma from the head of a twelve-day old rabbit embryo into the anterior chamber of the eye of rabbits. Petit (Travaux de la deuxieme conference Internationale pour l'etude du cancer, 1910, p.207) recorded two cases of rabbit tumours (1) a primary carcinoma of the lung, (2) a carcinoma of an accessory pancreas in the omentum.

Marie and Aubertin (Bull.de l'assoc.franc. pour l'etude de Cancer, 1911, IV, p 253) recorded a case of multiple tumours of both uterine cornua in a rabbit nine years old which had died of progressive emaciation. Though there were no matastases they regarded the tumours as "Epitheliomes cylindriques metatypiques", and they laid stress on the age of the animal and the fact that its fecundity was abnormal as it gave birth to a litter at the age of eight years. The Ninth Annual Report of the Imperial Cancer Research Fund (July 1911) briefly mentions the finding of a carcinoma of the mamma and a sarcoma of the subcutaneous tissues in rabbits. I understand that the latter was transplantable but the behaviour of the grafts was so disappointing, being absorbed after varying lengths of time, that no important observations could be made on the case. Stilling and Beitzke (Virch.Archiv.,1913,Bd.214, p 358) contributed an important paper on uterine tumours of rabbits. Stilling had an exceptionally large number of cases, 13 in all, and this was mainly due to the fact that he dealt exclusively with old animals. The youngest was 4 yours, the oldest 7 years, and most of the cases occurred between 5 and 6 years. He was of the opinion that if rabbits were allowed to live to an old

age we should probably find more tumours. He clung to the familial predisposition to cancer: all his animals were of the same breeding: he, in fact, endeavoured to create a tumour race. As most of his rabbits had undergone a previous laparotomy for some other purpose he was able to set a limit to the duration of the growth. In Lambert Lack's case the duration was certainly under a year as also in the case of Marie and Aubertin. In two of Stilling's cases it was within a year and in a third it was only a few weeks. The tumours found were myoma, adenomyoma, adenoma, and adenocarcinoma. The adenomata and adenocarcinomata occurred as single tumours in seven cases and as multiple tumours in six. The tumours almost always arose in the antimesometrial side of the uterus, but usually had an anterior or posterior part, rarely a mesometrial portion. There was no sharp distinction between the adenomata and the adenocarcinomata, for all gradations were found, as in my own case, between the simple tumour and the infiltrating distinctive cancer, not only in comparing tumours from different animals but also in comparing the tumours in a single animal. This is a point on which I should like to lay stress. Though in most cases there are quite distinct differences between typical benign and typical malignant tumours yet there is a very appreciable shadowy borderland in which the one class merges into the other. It seems to me a mistake to regard Cancer research as an endeavour confined to the study of malignant tumours or to attempt to find the cause of aberrant growth without realising that the divisions connoted by "Simple" and "Malignant" are purely empirical. Stilling in two instances unsuccessfully transplanted the tumours into other rabbits: but in four cases where he implanted

portions into the animal that bore the tumours he had two successes but did not carry on the transplantation to other animals.

Paine (personal communication) found a carcinoma of the testicle in a rabbit which he transplanted and carried on to a second generation in 1918.

These are all the references I can find to epithelial tumours of rabbits, and it will be seen that no one yet has succeeded in obtaining a carcinoma transplantable indefinitely. Several cases of transplantable sarcoma have been reported. Von Dungern and Coca described an endemic tumour of wild hares, on the real nature of which there was no general agreement amongst pathologists. Schultze (Verh.der.deut.path.Ges.,1913 p 358) described what he called a large cell sarcoma growing from the periosteum of the right mandible of a one-year old rabbit which he transplanted and carried on to the twelfth generation with a grafting success of 80 to 100 per cent. Sarcomata in animals, even in man, are not such definitely unmistakeable entities as carcinomata. There is always the thought at the back of our minds that the condition may be in any case a granuloma.

EXPERIMENTAL INVESTIGATION OF IMMUNITY TO TRANSPLANTATION OF MOUSE CANCER.

ON THE INDUCTION OF IMMUNITY TO CANCER INOCULATION.

After the earlier work on the experimental transference of spontaneous tumours had opened up new lines by which hitherto obscure phenomena might be investigated, and by means of which new facts might be brought to light, it was too readily assumed that the transplantability of a tumour was an inherent property of its malignant nature and that the power of indefinite propagation by repeated grafting from generation to generation transcended the laws governing the reproduction and growth of all other living tissues. All living things have their allotted span; after a certain time they die and perish; but in cancerous growth there are thought to be the potentialities of immortality - potentialities that never become actualities solely owing to the lack of appropriate environment.

The neoplasm studied as an entity, apart from the parent tissue that gave it birth and the unknown circumstances preceding its inception, or apart from the hosts that carried its descendent grafts, showed enormous variations from time to time in respect of rate of growth and grafting capacity, In one series of hosts it might gain a foeting in 100 per cent; in another, comparable in every respect, ~~the~~ the percentage success might be very small, falling even to zero and dying out; at one time the rate of growth might be extremely rapid, at other times tardy; or again, the grafts might go on increasing in size, or after attaining a maximum diminish to nothingness with more or less rapidity. Often the irregular fluctuations of rate of

growth and percentage of grafting success could be co-related. But whatever the general tendency of one particular strain may be, it is curious that other strains of tumour histologically identical may have a behaviour utterly dissimilar. This latter fact is an argument against the proposition that has often been erroneously assumed by some, namely, that the clinical course of tumours of certain histological types is more or less constant for those types.

While it must be admitted that certain definite characteristics may be attributed to strains of tumour cells yet it would be a mistake to neglect the fact that in considering the evolution of experimental neoplasms the economy of the host plays a paramount part. There are always two factors at work, namely, the inherent powers of the cancer cells, especially the capacity for apparently unceasing and purposeless multiplications and, on the other hand, the powers of the harbouring host, and the resultant will be the product of the play of the one upon the other.

Leaving to one side those features that have hitherto excited most interest, let us direct attention to the failures and the uncertainties of transplantation so as to view in proper perspective the success that has attended the various measures that have been adopted to bring about artificially diminished susceptibility to tumour inoculation.

Some spontaneous tumours inoculated in small fragments give a high degree of positive results even in the first generation, but this rather an uncommon experience. Generally the transplantability is established with difficulty. Many hundreds of animals may be inoculated and only one or two may

show progressive growth. Some, too, may show growth for a time with subsequent disappearance of the tumour by absorption. But it generally happens, though there are exceptions, that, where one has succeeded in obtaining growing grafts in the first generation the next generation gives a higher percentage of inoculation success which is progressively increased until a maximum for that particular strain is established. There is thus a gradually established adaptability of the tumour cells. They are all direct descendents of the original spontaneous tumour parenchyma, on each generation of which the environment of the successive series of hosts so far impressed itself as to destroy in a great measure the original individuality, without appreciably affecting the generic quality, of the tumour cells. That the individuality is not wholly destroyed is shown by the fact that recurring falls of percentage inoculation success occur. Further, the fact that histologically we can sometimes, as in the case of tumour "32", trace the predominance of specialised epithelium which in many series may become submerged or eliminated by less differentiated epithelium, points to the conclusion that individuality is not wholly lost by prolonged culture successfully in other hosts.

Grafting from the original tumour often entirely fails, even though a very large number of animals be inoculated with fragments. Hitherto, this absolute failure to transplant successfully has been attributed to inappropriate technique, such as damage to the tissue, inoculation of fragments too small or too large, or to the use of animal strains not similar to the provider,

and it has been considered that given suitable means of inoculation and a sufficient number of inoculations, often too limited by the mass of tissue at our disposal, we should succeed in transplanting every tumour. It is, indeed, well known that when a strain of tumour has been definitely established and the optimum grafting dose has been roughly determined, a diminution of the quantity of tissue inoculated in many cases fails to produce growth; on the other hand, an increase of that quantity may likewise be ineffectual in giving rise to a tumour. Thus we see that there is an optimum grafting dose for an inoculable tumour, and experience has shown that there is a different optimum for many. In cases where too many small a dose has been given, it is found that when a subsequent inoculation of optimum quantity is made the growth fails in the greater number of the cases. This shows that the graft originally introduced was not deprived of power of growth because our observations have demonstrated that tissue, which on inoculation produces a protection against subsequent inoculation of cancer cells, must be living. The conclusion is generally accepted that the absorption of a subminimal dose immunises the animal against an effective dose, just as in bacteriological immunisation a quantity of the virus so small as to be ineffective so stimulates the resistance of the body that it can cope with what otherwise would be a pathogenic quantity. The fact that a supermaximal dose likewise fails to produce growth, and arms the body against a subsequent optimum dose, has been accounted for by the supposition that a surplus has been absorbed and has protected the animal against the remainder. This theory of concomitant

immunisation has been ably supported by the workers of the Imperial Cancer Research Fund. Taking these two observations together, it has been concluded that the optimum grafting quantity for each tumour can only be arrived at after considerable experience of transplantation of the particular tumour and is in the first successes only a matter of chance, and hence failures to transplant a spontaneous tumour are to be attributed to ignorance of the optimum dosage. This explanation of failure is only a very partial one.

In great contrast to the difficulty of establishing grafts in other animals of the same strain is the success that practically invariably attends regrafting of a portion of the spontaneous tumour on to the animal in which it arose. Here, certainly, the size of the fragment transplanted is not of much moment. The environment is unaltered except in position, and it is the characteristic of cancer that position of origin, though giving the histological characters to the tumour, does not affect the ultimate situation in which portions of it may successfully lodge and grow. But the individuality of the animal in which the tumour arose, as compared with animals of the same species and of the same strain, reproducing itself in the tumour, originating from one or a group of its constituent cells, explains why the grafts should grow well in the provider and fail in other animals. It may be supposed that there are degrees in the depth to which this individuality of the whole animal is imprinted on its tumour derivative, and that even some tumours will

be more or less indifferent, in which case we should have an explanation of the success of primary transplantations. From the other view, we know from experiments in other directions that there is sometimes an indifference, at other times an active resentment of the animal body, against cells furnished by animals of the same species. This has been well brought out by the skin-grafting experiments of Schöne, by the experiment of Ehrlich in the production of isolysins to goats' blood, and by the work of various observers on the subject of isohaemolysis.. This is only another way of expressing the individuality of an animal's own tissues. The degree of transplantability we take, therefore, not to be a measure of the malignancy of a tumour, but a measure of the cell indifference, without reading into this indifference a want of histological or physiological differentiation.

Though in mice, as in human beings, and for that matter in other animals as well, malignant tumours most frequently arise towards middle life, and less frequently in youth or in old age, yet tumour grafts succeed best in young animals, and in the routine of experiment only young mice are employed. A transplantable tumour giving a high inoculation percentage success (according to our view, with its cellular individuality dormant,) ought to give a high percentage success with old animals. Such as a matter of fact we have found to be the case; at one period when tumour "No.63" was taking in every case the inoculation in old animals gave 100 per cent. success. In general, however, the older the animal the lower is the percentage of successful transplants. Thus, when the

transplantability of a tumour has been established, when the tissue has lost to a great extent its individuality by having the imprint of various hosts in succession upon it, the complaisance of the animals in which it may be transplanted diminished with the age of the animal, or in other words, the more differentiated and fixed the economy of the animal has become, the less must be the individuality of the tumour cells in order that successful growth may result.

The strain of mice has a great effect on the success of the graft. A tumour giving a high percentage of success in one particular strain may succeed badly or even fail with another strain. For example, Jensen's famous tumour, which gave a high take in Danish mice, gave at first a very low percentage in German and English mice, but it could be gradually accustomed in the environment of the latter till it succeeded well; afterwards it was found to be difficult of reinoculation into its original strain. The Flexner-Jobling rat tumour has never given the same high success in British rats that it has in American. In experiments with hybrid strains it has been found that transplants succeed better in first generations that in second generations.

Finally in ordinary routine inoculations with mice comparable in every respect and with an inoculable material practically homogeneous there are some mice which do not show growth. In others, again, temporary growth may be followed by absorption. We must admit a natural resistance sufficiently great to overcome the inherent proliferative potency of the cancer cells, a resistance which may not be always sufficiently strong in prevent the inception of the growth.

According to Schöne, who has done considerable work in the transplantation of normal tissues, in which cases, be it noted, cyclical variations in growth-capacity are absent as well as the factor of adaptability, heteroplastic transplantation does not succeed nearly so well as homoplastic grafting, and, furthermore, even in homoplastic grafting the transplant bears still some of its individual characteristics. Woglom found, as Bridré had previously noted, that preliminary inoculation of the splenic tissue of mice protected to a high degree against the subsequent inoculation of tumour tissue, but in further work he discovered the important fact that inoculation of the animal's own spleen had no protective effect whatever. From these observations it is evident that the conclusions formed regarding the state of immunity claimed to have been produced (and the resistance developed is beyond all question) by inoculation of various Living tissues, embryonic, normal adult, or malignant, are in need of considerable revision if essays in experimental therapy are to have any practical outcome.

Immunity, or more properly refractoriness, natural or exalted, is in the main merely an expression of biological resentment against tissues not proper to the animal's own economy.

THE ROLE OF THE SERUM IN CANCER BEARING ANIMALS.

It was natural to suppose if immunity could be induced in animals towards cancer inoculation that the serum of such subjects would contain a substance inimical to the life or proliferative powers of the cancer cell. This has been tested by several observers indirectly by the methods of investigating for antibodies, such as

complement deviation, and directly by subjecting the cancer cells before transplantation to the action of the serum of "immune" mice. By the direct method no evidence could be obtained of antibodies of known classification though the possibility of the existence of reaction bodies of other orders cannot be excluded. The direct method did not demonstrate any nocive action of the "immune" serum on the cells of the transplantable growths. Bashford and his co-workers have found that, whereas in the case of successful grafts in previously untreated animals there is a specific stroma reaction on the part of the host,- a stroma that acts as a nutritive basis for the parenchyma- yet in the case of immune animals no such stroma reaction is called forth, and they attribute the failure of the graft in the latter case, and the consequent explanation of immunity, to the failure of induced stroma reaction. It is somewhat difficult to comprehend an active reaction, admitting such for the moment, as being expressed by a passive inaction. Certain experiments have shown that when the inoculation of tumour cells is made into a parenchymatous organ of rich nutritive supply a considerable time elapses before the formation of adventitious stroma; all the while the inoculated cells go on multiplying; but if the inoculations are similarly made in animals rendered immune to tumour transplantation the proliferation of the introduced cells does not take place. This tends to show that the body fluids of "immune" animals contain some subtle substance inimical to the cancer cells. It is as reasonable to argue that the failure of stroma reaction is due to the checked proliferative powers or to the necrosis of the introduced cells as it is to attribute the death of the cells to the failure of stroma reaction.

Previous experiments having failed to demonstrate active antibodies in the serum of "immune" animals, it was thought that the serum of susceptible animals, on the other hand, might contain some substance advantageous to the growth of the cells. Indirectly, we have obtained evidence that in cancer subjects or cancer-bearers, reaction bodies do exist, though these substances may not be injurious to the tumour cells. The question could be investigated directly by acting on the cells of transplantable tumours with the serum of susceptible animals, and testing the grafting potency of such cells in comparison with similar material with normal serum. A second control is necessary, for normal serum itself might have a nocive action on the tumour cells. For this, untreated cells, or those mixed with physiological salt solution were employed. The grafting potency was estimated not only by the percentage inoculation success, but also by mensuration of the resulting tumours. Numerous experiments had to be performed before a true idea of the result could be obtained, because the serum of mice cannot be obtained aseptically with certainty, and bacterial contamination of the tumour emulsion vitiated the reading of many tests.

Experiment 1.

Under anaesthesia the tumour was removed from a mouse and the animal was bled. Both tumour and blood were stored in an ice-chest for 24 hours. The tumour after being broken up was divided into two portions, one of which was intimately mixed with the unheated serum. The two portions of tumour material were incubated at 37°C. for half-an-hour. The portion mixed with serum was injected into 7 mice, the untreated portion into 25 mice. The result of incubation was that 6 out of the 7 mice developed tumours, whilst in the controls, of 20 surviving 17 developed tumours, i.e., in both cases the result was approximately 85 per cent. The average size of the tumours was similar in each case. Before concluding that the action of a cancer bearer's serum on its own tumour cells is negative, we

shall have to see if the time of sensitisation of the cells has been sufficiently prolonged, and we must consider the possibility of insufficiency of complement in the serum. The numbers also may be too small from which to draw conclusions.

Experiment 2.

Two tumours were emulsified and intimately mixed, and to one portion of this emulsion the serum of the two animals was added soon after the clotting of the blood. The mixture was kept on ice for 18 hours, at room temperature for 4 hours, and, after the addition of the fresh serum of two normal mice, at 37°C. for 1 hour before inoculation. 32 mice were inoculated with this material. The other portion of tumour emulsion was kept for the same length of time under identical conditions of temperature and was inoculated into 22 mice. After the lapse of 21 days, 10 mice survived of those inoculated with sensitised cells, and 2 had small tumours, whilst in the controls 9 survived and 1 had developed a tumour. At the end of a month, in the former set 8 were alive and one was positive, in the latter also 8 survived, one having a tumour. There is practically no difference between them, but it is evident that the heavy mortality, 38 dying in 54, must have interfered with the ultimate reckoning to an unknown extent.

Experiment 3.

On this occasion the cancer-bearers' serum was heated to 57° ~~per cent.~~ to destroy complement and ~~and~~ also to minimise possible sepsis, and very minute precautions were taken to obtain fresh normal serum, as complement, in as aseptic a manner as possible. The cancer cells were left in contact with the reactivated serum for 24 hours, after which the mixture was centrifuged and the supernatant fluid removed. 34 mice were inoculated with these treated cells.

Another series of 44 mice were similarly treated with fresh serum only, but the tumour material in this case was from a growth of the same generation, of the same size, and considered to be practically identical.

Portion of the latter tumour was mixed with physiological salt solution and 50 mice as controls were inoculated.

The result was that in 10 days, in the series treated with cancer serum, 33 lived and 31 of these had tumour growth (94 per cent.), in the series treated with normal serum 40 lived and 22 had tumour growth (55 per cent.), and in the series treated with salt solution 43 lived and 17 had tumour growth (40 per cent.) At the end of 17 days, of the sensitised series, 30 out of 33 had tumours (90 per cent.); of the normal serum series, 20 out of 35 had growths (57 per cent.); and, of the control set, 15 out of 37 (43 per cent.) had growths. Further, the average size of the tumours in the sensitised series was considerably greater than in the others.

Though the results so fully bore out what we had expected - and perhaps wished - yet there was a source of fallacy that had escaped notice at the time. The tumour material used in the sensitised series was not indentical with that used in the two sets of controls, though, indeed, it was similar. The use of two separate tumours was quite inadvertent. They should have been intimately mixed, and it was only when one came to seek for possible sources of error before ~~accepting~~ accepting a wished-for result that, on reconstructing the experiment from memory, it was recollected that the mixture of tumours had not been made.

The difference in success between the series treated with normal serum and that treated with salt solution is perhaps an accidental one, for in a few of the latter, there was a sloughing of the integument over the graft, and its consequent expulsion before growth was established. These experiments were repeated several times, sepsis interfering with and spoiling the results, before two final satisfactory tests were obtained. The latter of these is given.

Experiment 4.

In this experiment one large tumour was used for all the inoculations. Three series of inoculations were made with (1) tumour cells plus reactivated cancer-bearers' serum, (2) cells plus normal serum, (3) untreated cells. In the first series there were 30 mice, in the second 30, and in the third 13.

After the lapse of 20 days, in the sensitised series 90 per cent showed tumours, in the series treated with normal serum 85 per cent showed tumours, and in the series with untreated cells 87 per cent were positive. The differences in percentage success are negligible, especially in view of the fact that the average tumour area of each series was in the three cases practically identical.

The conclusions can surely be drawn from these experiments that the serum of cancer-bearers does not contain any substances that encourage the proliferation of tumour cells, and that normal serum has no restraining influence on growth.

The experiments are of some importance in view of the claims of Freund and Kaminer to have isolated (1) from normal serum and normal tissues a fatty acid compound which has the property of destroying cancer cells and which they regard as the "Sehutzsubstanz"

of normal cells, and (2) from carcinoma serum and carcinoma cells an unsaturated fatty acid which replaces the normal fatty acid and which has the property of lysing normal cells. This they call the "Schutzsubstanz" of cancer cells. (vide Wien.Klin.Woch. 13 Nov.1919). As in their previous work before the war these authors in their numerous papers on the subject relied on colour reactions for the detection of the minute amounts of these substances - their observations did not seem to carry conviction. At any rate their work has never been corroborated.

INCREASE OF SUSCEPTIBILITY TO TUMOUR INOCULATION.

Simultaneously with Haaland, I had shown that a suspension of mouse-tumour cells which had been mechanically disintegrated and broken up, presumably without chemical alteration of their constitution, on injection into mice did not immunise against subsequent inoculation with homologous tumour tissue but, on the contrary, considerably increased the susceptibility.* The results we afterwards confirmed by Shattock. The susceptibility produced is probably not specific, but the mechanism deserves further examination.

The material injected is a suspension in normal saline solution of portions of tumour cells, and two factors may produce the result. It may be due to substances in solution - in other words, to an extract - or it may be due to an insoluble constituent of the cell. We performed two sets of experiments accordingly.

* Lancet, 9th April, 1910.

A. Experiments with EXTRACTS.

Experiment 1.

A tumour was rubbed up in a mortar with sand, and the mass suspended in 25cc. of normal salt solution. 1cc. of the fluid after centrifuging was injected intraperitoneally into each of 8 mice. 26 days afterwards the 6 mice surviving were tested by subcutaneous inoculation of fresh tumour material. The result was that five developed tumours. The controls gave a similar result.
A subsequent experiment on the same lines but in which the extract was allowed to stand for 24 hours appeared to show that the extract was toxic, for 17 animals out of 20 died within 24 hours, and the remaining 3 within 48 hours. This may have been due to other reasons because when repeated on a subsequent occasion no such result was obtained.

Experiment 2.

An emulsion of several tumours was mixed with 80cc. of normal salt solution, and the flasks containing it were kept on ice for 52 days - a somewhat longer period than in the case of the original experiment mentioned above. The turbid fluid was passed through a close-pored Doulton filter. As in the original experiments, three intraperitoneal injections were given, a period of 7 days elapsing between each; and 10 days after the last, a test subcutaneous inoculation with tumour cells was given. 24 mice, so treated with 3 injections of 0.75cc. of the filtrate, and 24 controls, were inoculated. Of the treated set, 16 out of 20 showed tumours (80 per cent.), and of the controls, 18 out of 23 were positive (78 per cent.). The sizes of the tumours in both cases were similar. The filtered extract therefore does not sensitise.

Experiment 3.

A tumour after being finely minced was divided into two equal portions. To one portion normal salt solution was added, the other portion was left untreated and both were stored in the ice-chest for 24 hours. At the end of that time the first was centrifuged and the supernatant fluid pipetted off and the cells were washed four times. 20 mice were injected with this portion of washed cells, and 20 were inoculated with the other portion. At the end of 18 days, of those treated with washed cells 15 were alive and all had tumours (100 per cent.), and of the controls 15 survived, 12 of which had tumours (80 per cent.)

We see, therefore, that the salt solution does not extract any substance from the cells which favours their growth because such extracted cells were even more potent than the untouched cells, so that it could not have been the soluble extract that

produced the supersensitiveness of our original experiments.

B. Experiments with INSOLUBLE CONSTITUENTS.

Experiment 1.

Tumour material having been finely minced was spread on watch-glasses in a thin layer and desiccated. A minute portion of the pulverised desiccated material in the dry condition was subcutaneously inoculated into 36 mice. Within a week 16 had died. The remaining 20 were then tested with an inoculation of fresh tumour emulsion. Only 9 survived for a fortnight, and of the tumours which arose in 5 of these, 4 were small and 1 was moderate in size. In 20 controls 19 showed good results growth, and in the remaining one it was late in appearing.

The desiccated tumour material would thus seem to be toxic, and it must be remembered that what is a minute dose of the powder represents a comparatively enormous quantity of fresh cancer material. The experiment shows that the desiccated material certainly does not increase but rather diminishes the susceptibility to tumour inoculation.

Experiment 2.

This is a repetition of the last. 21 mice were injected with the pulverised desiccated material each getting approximately 0.05gr. 13 survived 4 days and were inoculated with fresh tumour material. After 3 weeks 10 were alive and only one was positive with a small tumour (10%). Of the controls 10 were alive and 9 had good tumours (90%).

This experiment brings out even more conspicuously the fact that so far from increasing the susceptibility the desiccated material diminishes it.

These two sets of experiments do not provide an explanation of the sensitising effect of the suspension of disintegrated tumour material. The subject, has, however, been approached by a different method, but hitherto without success.

The previous experiments in increasing susceptibility, already referred to, were as follows:-

" Transplanted tumours of one particular strain were removed from several mice, and after being roughly disintegrated with small scissors were thoroughly triturated in a mortar with fine silver sand. The resulting pasty mass was transferred to bottles containing sterile saline solution and the whole shaken up. Strict aseptic precautions were, of course, observed throughout. The bottles were kept in a cold chamber for two days, and thereafter the subsequent fluid was decanted into test-tubes, which were kept at freezing point until required. This fluid remained turbid for at least a month, though there was a gradual accumulation at the bottom of the tubes; it was bacteriologically sterile and did not contain living cells as far as could be observed by microscopic examination or the results of injection. The chemical nature of the protoplasm was presumably not altered. (It may be noted that even advanced autolysis does not prevent growth on inoculation.)

The fluid was injected into the peritoneum of a number of mice on three occasions with ten days' intervals, employing for the first injection 0.5 cubic centimetre and 1 cubic centimetre for the last two. As the material was not standardised, it can only be said that in comparison with the ordinary quantity of tumour emulsion used for transplantations (in this case 0.05 cubic centimetre) it represented an excessively minute dose of epithelium. 24 days after the last intraperitoneal injection the 29 mice that survived were inoculated subcutaneously in the usual way with 0.05 cubic centimetre of fresh tumour emulsion. A similar number of controls were instituted.

The treatment, instead of increasing the resistance to test inoculation, had a marked effect to the contrary. In ten days' time, of the 27 treated mice that remained alive, 18 showed tumours; a week later, 21 out of the 27 (78 per cent.) had growing cancers. The controls gave 9 tumours in 27 mice (33 per cent.). There is a striking disparity between the two sets. But this disparity is all the more marked when we take into account the size of the tumours produced in both cases. By carefully mapping out the contours of the tumours on graph paper it was found that the average area covered by the tumours in the treated set was approximately 110 square millimetres, as compared with an area of 39 square millimetres in the case of the controls. The average mass is much larger.

The tumour used throughout these experiments was an adenocarcinoma -"No 63" of the Imperial Cancer Research Fund list. For a few months this tumour grew well, succeeding in a very high percentage of cases. Thereafter the successful "takes" rapidly diminished until it failed completely. This has proved a fortunate accident as far as these experiments are concerned. For my test inoculations I was confined to a single tumour 3½ months old which had been growing very slowly and which did not show the central necrosis so common in this strain. The sudden resuscitation in the treated series is all the more remarkable.

From these ~~preliminary~~ experiments it is evident that the injection of cancer cells which are physically disintegrated without probably being chemically altered has markedly affected the resistance towards living tumours cells. So far from conferring

anything in the nature of immunity it has undoubtedly exalted the susceptibility?

In Haaland's experiments, which were published in the preceding number of the Lancet, an increase of susceptibility was produced by similar means but as his tumour was "taking" in a high percentage of untreated controls the increase of susceptibility was not so evident as in my experiments where the testing tumour was in a dormant phase.

REPEATED INOCULATION OF REFRACTORY SUBJECTS.

Of the various means that have been adopted in the immunisation of mice against cancer material, the most potent, though perhaps the most haphazard, is by previous inoculation with cancerous material of the same kind. This material, to have any protective action against test inoculation, must be living, and as such, even though the usual taking dose be greatly reduced, it is liable to give rise to a tumour of itself. Deprived of life the tissue has no protective action. But in the analysis of the refractory state produced by the absorption of living cancer tissue we have material ready to hand in those cases that have failed to "take" in the routine inoculation of fixed optimum doses. It has long been known that in those animals that have failed to give tumours on inoculation, and in which the inoculated material has been absorbed, subsequent inoculation fails also. Numerous observers have agreed upon the point and it is practically undisputed. The protection is said to have an element of specificity in it. According to Bridré, Haaland, Michaelis, and Bashford, treatment with one tumour will not protect against a tumour of a different type. My own observations lead me to doubt that contention, and I am inclined to think that previous treatment with a tumour, which does not "take," in certain cases is as efficacious in protecting against another tumour subsequently inoculated into these as is treatment by normal or embryonic tissues. But however that may be, all are agreed that the highest protection against any

particular tumour is that produced by previous absorption of the same tumour.

It must be admitted that in the case of some tumours, perhaps in the case of the Jensen tumour, animals that prove refractory to one inoculation may prove refractory to all subsequent inoculations. In the case of others, it has been noted that there may be a relatively few of these refractories which fall to a second, and still less to a third. According to Russell the immunity deepens by repeated inoculation. If this were so it could not be proved, for we have no means of testing an accentuated resistance in animals which in the beginning were resistant. It seems to me that there is an obvious fallacy in the reckoning of increase of immunity, because we are not comparing two analogous sets of experiments. In passing, we must bear in mind the fact that refractoriness to inoculation increases with age, but even supposing our control animals were of the same age as the refractories (a precaution which has been observed by Russell and his colleagues) the conditions of inoculation in the two series are not comparable. If, having inoculated 100 mice with a certain tumour emulsion, we obtain a result of 50 positive and 50 negative, all we can say is that in 50 per cent. both the material and the soil happened simultaneously to be suitable for growth. But in the 50 negatives three conditions are possible - the particular portion of tissue injected might not have the necessary vitality, the natural resistance of the animal might

be high, or, by absorption of part of the material injected, the animals might be rendered refractory to the remainder. A uniform percentage success having established itself, we may conclude that the negatives result from natural or induced refractoriness. If now we reinoculate the 50 negatives with tumour emulsion of the same strain on a subsequent occasion, we shall be testing them with a substance slightly different from that with which they were tested on the first occasion, seeing that the material has been subjected to the environment of a different host. This may be a small point, but it has its possible bearings on the results obtained. Supposing the second inoculation proves negative in all the 50 negative to the first, we can only say that the resistance remains unaltered, being neither higher nor lower, but if a certain number of them prove positive on this occasion, say 10, the resistance is obviously being lowered from its original standard. If further, of the 40 negative to the second inoculation, 2 prove positive to a third inoculation, the resistance is being still further diminished. The general reading of such an occurrence is a surprising one. In this case it would be said that the susceptibility to inoculation is diminishing as shown by the diminishing percentage success, viz., 50 per cent. for one inoculation, 20 per cent. for two inoculations, and 5 per cent. for three inoculations. The results are here compared with previously untreated controls. If we consider that we are eliminating each time a certain number that ought to be counted

in the estimation, we shall see that the relative states of susceptibility are 50, 60, and 62 per cent., or in other words, the resistance is actually being decreased by subsequent inoculation. This assumes a point that has not been questioned, namely, that an animal susceptible to inoculation on one occasion would again be susceptible to a subsequent. That this is not actually so in all cases some of our experiments demonstrate. In most cases, if we remove a tumour from a "positive" animal, we shall have to wait perhaps a month or two to see that there is no recurrence before reinoculating on the opposite side, and the second will probably be positive. Removal of tumours and reinjection may be practised several times. If a time comes when inoculation proves negative we may have to attribute this refractoriness to the natural refractoriness that comes with age. All the time the death rate from operation and from natural causes has been so diminishing the original stock of positives that we have little ground on which to base conclusions. But if we find amongst the few that survive for a sufficient length of time, that a mouse which originally was positive, had its tumour removed, was then refractory to one or two inoculations, and finally became positive to a later injection, we are in a position to conclude that the susceptibility of a particular animal to cancer transplantation varies from time to time. I have actually shown this to be the case. This is further brought out by the detailed experiments which show how mice, originally negative, subsequently became positive.

Another point that seemed to deserve investigation was the duration of the supposed immunity. No observations on this point were available. Here again we are faced by the factor of refractoriness to tumour inoculation deepening with advancing age. The observations we have made on the point show that the induced refractoriness is of variable duration.

The broad conclusions from the experiments are that the individual is subject to fluctuations in susceptibility to tumour inoculation, and that nothing comparable to immunity, as we understand it in other departments of pathology, exists. There is a practical value in the experiments in the light they shed on the question of spontaneous diminution of malignant tumours or their secondary deposits, and the recrudescence of dormant relicts.

In the following experiments, animals were used which had been refractory to a previous inoculation, or in which a tumour had regressed and been absorbed. The inoculating dose, except where otherwise stated, was the optimum quantity particular to the tumour, and the inoculations were made on the right and left sides alternately. The cases are not selected in any way. As each reinoculation was made, a series of young mice were inoculated with the same material. These are not comparable controls, but merely serve to show on each occasion the maximum taking capacity of the tumour under the most favourable circumstances.

EXPERIMENTS WITH TUMOUR "No. 63."

This tumour is an adenocarcinoma, and gives usually a high percentage of success on inoculation. It does not show much tendency towards spontaneous absorption. Good growths, as a rule, are obtained by the 14th day; delayed growth is infrequent. The tumour emulsion is injected in quantities of 0.05 cc.

Experiment 1.

10 mice which proved negative to a first inoculation succeeding in 33 per cent., were inoculated on a second occasion, 66 days afterwards, with tumour material, giving a success of 95 per cent. in the controls. 9 lived sufficiently long to show the result; 7 were negative; 2 slowly developed tumours which slowly progressed.

The third inoculation was given 14 days later to 5 mice. In the controls 90 per cent. were positive. The result was, that 4 remained negative and 1 developed a large tumour.

Remarks. - In those falling to a second inoculation, the lateness of the growth in making its appearance and its slowness in developing, were notable. The original refractoriness still existed, though the result shows how it weakened. The one falling to the third inoculation, probably had its great refractoriness broken down by the third assault coming so soon after it had coped with the second.

Experiment 2.

A mouse, refractory to a first inoculation succeeding in 90 per cent., was reinoculated after a period of 115 days with tumour material, giving a success of 55 per cent. in the controls. It remained negative for 14 days and then developed a large tumour.

Remark. - The period that elapsed between the inoculations, during which the increasing refractoriness due to age might be expected to tell, did not accentuate the supposed induced refractoriness. Be it noted that the grafting capacity of the material employed was fairly low - 55 per cent., as against an original inoculation of 90 per cent.

Experiment 3.

Of two mice refractory to a first inoculation succeeding in 90 per cent., one was inoculated for a second time 60 days later, with tumour material giving a 55 per cent. success, and the other, 92 days later with material giving a success in the controls of 82-94 per cent.

In the first, a tumour appeared after 14 days and rapidly became large.

In the second, a very large tumour developed.

Remark. - In the first, the appearance of tumour growth was delayed, but the resistance quickly disappeared. In the second, the resistance seems to

have been lost in the greater lapse of time. But the grafting powers of the material used in each case make the comparison difficult.

Experiment 4.

A mouse, refractory to a first inoculation succeeding in 86 per cent., was inoculated on a second occasion, 38 days later, with tumour material giving a success of 55 per cent. in the controls. It proved still refractory.

A third inoculation was given 25 days later. In the controls 80 per cent. were positive. The result was that a small tumour arose and then disappeared.

Remark. - The second inoculation may have taken place during the time that an induced refractoriness existed. The material was less potent than on the first occasion; and on the third occasion, though the time elapsing is shorter still, yet the material is more potent. The resistance, however, though weakened is not lost.

Experiment 5.

A mouse which was positive to an inoculation giving a success of 90 per cent. in the series, had its tumour removed 55 days after inoculation. After a lapse of 3 months, during which no recurrence manifested itself, a second inoculation, succeeding in 98 per cent. of controls was given. The result was negative.

A third inoculation, with a control success of 84-100 per cent., was given 1 month later. Result, negative.

A fourth inoculation, with a control success of 84-92 per cent., was given one month later. The result was that a pea-sized tumour developed but disappeared.

A fifth inoculation, with a control success of 80 per cent., was given 15 days later. Result, negative.

Remark. - This is the only case, out of a series in which tumours were removed, that survived sufficiently long for us to see the effects of subsequent inoculation. Be it noted that though the animal was originally susceptible to tumour inoculation, yet it lost its susceptibility. The age factor must be borne in mind. The second inoculation was with material with an even greater grafting power than the first; in fact, all the inoculations were with potent material. A temporary loss of resistance occurred at the time of the fourth inoculation, and the fifth was therefore hurried, on the chance that this loss of resistance might be increased.

Experiment 6.

7 Mice which were refractory to a first inoculation succeeding in 55 per cent., were inoculated on a second occasion, 32 days later, with tumour material giving a success of 82-94 per cent. in the controls. The result was that 2 remained negative, and 5 developed tumours.

A third inoculation, with a control success of 84-100 per cent., was given to the 2 negatives 31 days later. 1 mouse died before sufficient time had elapsed to note the result; the other proved negative.

A fourth inoculation, with a control success of 84-92 per cent., was given 34 days later. The result was negative for a time, but later a tumour developed.

A fifth inoculation, with a control success of 80 per cent., was given 15 days later. Result, positive.

Remarks. - It is surprising that, in the case of the second inoculation, the majority (5 as against 2) should have proved positive. In this case the grafting material was much more potent than on the first occasion. Regarding the mouse that was negative to these inoculations, it was thought that the fourth inoculation was also infertile, because no growth was evident at the time the fifth was made. Ten days after the fifth inoculation, small tumours were found on both sides. Though the tumour of the fourth inoculation was thus extremely slow in appearing, yet when once started, it grew rapidly and outstripped the growth resulting from the fifth inoculation. It had histologically the same characters and, as far as one could judge, was not a spontaneous growth. It may be considered that the resistance was just sufficient to keep the growth of the fourth in abeyance until the resistance was broken down by the added assault of the fifth.

Experiment 7.

12 Mice which were refractory to a first inoculation succeeding in 82-94 per cent. were inoculated on a second occasion, 41 days later, with tumour material, giving a success of 50 per cent. in the controls. Result, all negative.

A third inoculation, with a control success of 84-92 per cent., was given 23 days later to 11 mice. The result was that 4 remained negative and 7 developed tumours.

A fourth inoculation, with a control success of 80 per cent., was given to the 4 negatives. 3 died before the result could be determined. The other remained negative.

Remarks. - In comparing this experiment with the preceding, we note that all were negative to a second as well as to a first inoculation. That the possible factor of potency of dose affects the result, as it might have done in the preceding case, can be discounted here, for the third inoculation to which the resistance of the majority succumbed (7 as against 4), is approximately of the same power as the first. Again, the proposition that a tumour of low virulence will protect against one of high virulence is not borne out in comparing the results of the second and third inoculations.

Experiment 8.

14 Mice, which were refractory to inoculation of tumour material that was septic and did not produce growths, were inoculated on a second occasion, 41 days later, with tumour material giving a success of 80 per cent. in the controls. 12 mice survived, and of these, 5 remained negative and 7 developed tumours.

A third inoculation, with a control success of 84-100 per cent. was given 38 days later to three of these negatives. 2 survived, 1 remaining negative, while the other developed a tumour.

A fourth inoculation, with a control success of 84-92 per cent., was given 15 days later. The result was that a tumour slowly developed.

Remarks. - It is interesting to observe that the septic condition of the emulsion injected on the first occasion prevented growth taking place. The mass was got rid of by sphacelation, but apparently some degree of protection was conferred because in inoculating on the second occasion with non-septic emulsion a take of 58 per cent. was obtained as against 80 per cent. in the controls. Again we see that repeated inoculation tends to weaken the resistance.

Taking all these experiments together merely from the point of view of the susceptibility of refractory mice towards subsequent inoculation, apart from the questions of length of duration of induced refractoriness, the effect of increasing age, and the changes in potency of inoculated material, we find that, regarding mice refractory to a first inoculation,

Of 44 at second inoculation 17 were positive and 27 negative.
Of 20 at third inoculation 9 were positive and 10 negative.
Of 3 at fourth inoculation 1 was positive and 2 negative.
Of 1 at fifth inoculation 1 was positive.

EXPERIMENTS WITH TUMOUR "No. 58."

This tumour is a transplantable adenocarcinoma which has not a high grafting capacity like "No. 63." Spontaneous absorption frequently occurs. It is of fairly slow growth. Transplantations are made from pin-head sized fragments, i.e., the optimum transplanting quantity is small.

Experiment 1.

The effect of reinoculation in the case of absorbed tumour was investigated at three stages. The first mouse had a cherry-sized tumour which took 1 month to disappear. It was reinoculated 1 month after complete absorption, but failed to take. The second mouse had a pea-sized tumour which persisted for 26 days before being completely absorbed. It proved negative to a second inoculation given 2

days after complete disappearance. The third had a tumour somewhat larger than a cherry, which, after reaching a maximum, started slowly to diminish. It was reinoculated soon after the commencement of the diminution, but the graft did not take, nor had it any influence in arresting or accelerating the absorption of the former tumour.

Remarks. - Mice which have had large or long-standing tumours that have been absorbed, are exceedingly refractory to subsequent inoculations, and the refractoriness is at its height during the time of absorption of the tumour.

Experiment 2.

14 Mice which were refractory to an inoculation that succeeded in 6 per cent. of the series, were inoculated on the second occasion, 30 days afterwards, with tumour material giving a success of 6 per cent. originally in the controls, the control tumours all being absorbed finally. All the animals proved still refractory.

A third inoculation, with a control success of 29 per cent. (11/38), was given 44 days later to 8 mice. 7 survived and all proved negative.

A fourth inoculation, the controls of which were carried off by epidemic was given to 5 mice, 46 days later. 3 survived. Result, 2 were negative; 1 showed a small temporary tumour.

A fifth inoculation, the controls of which gave 6 tumours in 13, only 1 of which was progressive, was given to 2 mice, 24 days later. The result was that 1 remained negative, whilst the other developed a cherry-sized tumour which was rapidly absorbed.

A sixth inoculation, the controls of which were all negative, was given 39 days later to 2 mice. The result was that the one which had been quite refractory throughout, still remained so, whilst the other, which showed a temporary tumour following the fifth inoculation, developed one again, which showed a maximum of 10 x 6mm., and after 20 days was absorbed.

Remarks. - Only temporary weakening of the original refractoriness was elicited by the last three inoculations. It is to be noted that in the case of the last injection, the potency of the tumour emulsion, while not sufficiently great to produce growth in fresh controls, was sufficient to give a temporary growth in one of the treated animals. This goes to show that its susceptibility had sunk below the normal.

Experiment 3.

14 Mice which were refractory to an inoculation that succeeded in 10 per cent. of the series (all tumours progressive), were inoculated on the second occasion 51 days later, with tumour material giving a success

of 29 per cent. in the controls. The result was negative.

A third inoculation, the controls of which showed the material to be septic, was given 46 days later, to 6 mice. 5 lived, and all were negative.

A fourth inoculation, giving 6 positives (only one progressive) in 13 controls, was given, 24 days later, to 3 mice. They all proved negative.

A fifth inoculation was given to one mouse but it died before result could be estimated. The controls were all negative.

Remark. - In this experiment repeated inoculation failed to give evidence of weakening of refractoriness.

Experiment 4.

4 Mice, which were refractory to a first inoculation that produced 6 per cent. of diminishing tumours in the series were inoculated on a second occasion, 44 days later, with tumour material giving a success of 29 per cent. in the controls. 3 lived. 2 remained negative and 1 developed a tumour which grew progressively.

A third inoculation, with tumour material which was septic as the controls showed, was given, 46 days later, without definite result.

Remark. - The second inoculation shows weakening of refractoriness.

Experiment 5.

23 Mice, which were refractory to a first inoculation that succeeded in 29 per cent. of the series, were inoculated on a second occasion, 46 days later, with tumour material which was septic. 19 lived but all were negative.

A third inoculation, with a control success of 6 (one progressive only) in 13, was given, 24 days later, to 7 mice. 5 lived. The result was that 3 remained negative, whilst 2 showed temporary pea-sized growths.

A fourth inoculation, the controls of which were negative, were given, 39 days later, to 3 mice. The result was that 1 was negative, while the other two developed small tumours which were absorbed.

Remark. - The refractoriness has definitely been lowered below the normal.

Experiment 6.

6 Mice out of a series which was refractory to the inoculation of a septic tumour emulsion were reinoculated 24 days later with tumour material giving a success of 6-1 out of 13 controls. 3 lived. All remained negative.

A third inoculation, the controls of which were all negative, was given 39 days later to 3 mice. The result was that 2 were negative and 1 developed a large transplantable tumour.

Remark. - This experiment shows clearly that the refractoriness was abolished by the third inoculation in one case. In fact, susceptibility had so far succeeded refractoriness that the tumour emulsion which produced no growth whatever in fresh controls gave a good growth in one of the treated series.

Experiment 7.

2 Mice refractory to a first inoculation giving a success of 6-1 in the series of 13 were inoculated a second time, 39 days later, with tumour emulsion that did not produce growth in the controls. No growth resulted.

The experiment could not be carried further as the strain died out, but the conclusion cannot be maintained that the refractoriness has deepened.

Experiment 8.

A mouse which had proved positive to first inoculation had its tumour removed by operation. After sufficient lapse of time, to see that no recurrence followed it was reinoculated on the opposite side. It proved refractory to this second inoculation. It was inoculated for a third time. The result was that a progressive tumour slowly developed.

Remarks. - This should be compared with the fifth experiment with tumour "No. 63." It demonstrates

that a susceptibility to one inoculation does not imply susceptibility to a second. The susceptibility was followed by refractoriness, which again gave place to susceptibility. This is the converse of the previous experiments, and supports the view that susceptibility and refractoriness in a particular animal are not constant but alternating characters.

In this tumour, the low potency of the cells is not so well suited as in the previous tumour for bringing out the diminution of refractoriness by repeated inoculation, but it does unmistakeably show that such a thing occurs. It has been on tumours of low grafting potency that the erroneous conclusions regarding the increase of immunity have mainly been built.

If we group the number of experiments together as before, according to the number of inoculations, we obtain the following result:-

Of 52 Mice refractory to 1 inoculation, 1 was positive 51 were negative to the second.

Of 20 refractory to 2 inoculations, 1 was positive, 1 was temporarily positive, and 18 were negative to the third inoculation.

Of 9 refractory to 3 inoculations, 3 were temporarily positive, and 6 were negative to the fourth inoculation.

Of 2 refractory to 4 inoculations, 1 was temporarily positive, and 1 was negative to the fifth inoculation.

Of 2 refractory to 5 inoculations, 1 was temporarily positive, and 1 was negative to the sixth inoculation.

EXPERIMENTS WITH "TUMOUR 32."

This tumour is a transplantable carcinoma of a mixed type, often showing a preponderance of squamous epithelium, at other times a preponderance of gland epithelium, sometimes both types being mixed. In our short experience of it, the adenocarcinomatous type has preponderated, and was mostly exclusive. The tumour was inoculated at a dose of 0.05cc. The transplantations are characterised by a very high initial percentage of successful results; the growth is generally very rapid, fairly large tumours being evident in 10 days; spontaneous absorption, which takes place rapidly, is very common. The experiments were not sufficiently protracted, owing to the dying out of the strain, to enable us to investigate fully the question of duration of refractoriness.

Experiment 1.

In the first inoculations, 100 per cent. of the series developed tumours. 2 mice were inoculated on a second occasion, 37 days later, with tumour material giving in the controls an initial success of 7 out of 13, only one of these being progressive. At the time of inoculation, 1 of these had a tumour

which diminished to the size of a pea; the other had a tumour which just disappeared. In both cases the results were negative.

A third inoculation was performed on the 2 mice, 101 days later. The controls gave an initial success of 2 out of 5, both tumours disappearing. The results were again negative.

Experiment 2.

In the first inoculation there was an initial success of 7 tumours in 13 mice. After the lapse of 101 days from the time of the first inoculation, a second was given to 2 mice; one of these had been negative throughout, the other had the remains of a diminishing tumour. The controls gave initially 2 in 5 but these two tumours were absorbed. The second inoculation was without effect.

Experiment 3.

In the first inoculation there was an initial success of 18 tumours in 42 mice, 12 of which progressed. 94 days after the first inoculation 6 mice were submitted to a second inoculation with material which gave an initial success of 2 in 5 with subsequent absorption of these two tumours. 5 of these mice had been negative to the first inoculation and proved refractory to the second; the sixth bore a tumour which was increasing at the time of second inoculation. In the latter case the second injection was negative, though the original tumour went on increasing.

Remarks. - It is somewhat strange that the original susceptibility of this animal, which evidently was not diminishing, because the tumour was increasing, should not have encouraged the growth of the material subsequently inoculated even though the grafting capacity was low. Such anomalies are not infrequent, and Ehrlich has laid undue emphasis on this phenomenon in his atreptic theory of immunity.

Experiment 4.

In the first inoculation the material was septic and no growths resulted. 44 days afterwards a second inoculation was given to 2 mice. The controls gave originally 2 in 5 with subsequent absorption. The results were negative in both cases.

Experiment 5.

The first inoculation showed an initial success of 100 per cent. in the series, but only one third of them progressed. 17 days after the first inoculation, a second was given to 5 mice with material which in the controls gave an initial success of 6 in 11, all finally being absorbed. Some of these 5 mice had tumours at the time of second inoculation, in others they had disappeared. The result in each case was as follows:-

Mouse A. - At time of second inoculation the tumour had diminished to half of its maximum. It went on

diminishing and disappeared. The second inoculation was negative.

Mouse B. - The tumour, originally the size of two peas, had diminished to the size of a pin's head. The second inoculation was negative.

Mouse C. - The original tumour had just disappeared. The second inoculation produced a temporary growth, small in size.

Mouse D. - The tumour had been steadily increasing, but suddenly it began to decrease and rapidly disappeared. The second inoculation produced a small growth that also rapidly disappeared.

Mouse E. - The original tumour had been of considerable size when it started rapidly to decrease. The second inoculation was performed before the disappearance. A small nodule resulted that also quickly disappeared.

A third inoculation, with material giving an initial success of 7 in 13, 1 of which was progressive, was given 44 days afterwards to 2 mice. The result was that in 1 a tumour of the size of two peas somewhat slowly appeared and lasted for 10 days before diminishing, and in the other a larger growth resulted which lasted for a month before absorption.

The experiments with this tumour are not sufficiently prolonged to bring more than slight evidence forward in support of the view that subsequent inoculations tend to weaken refractoriness, but in thelast of the series the observations go to support this theory. In these we see that the strongest "immunity" which we have found to result from the absorption of tumours that have reached an appreciable size, as contrasted with the refractoriness called forth by the absorption of material that has not exhibited marked proliferation, is not absolute nor lasting but is merely a passing condition.

EXPERIMENTS WITH TUMOUR "No. 37."

This tumour is a spindle cell sarcoma which arose during the transplantation of a carcinoma. A complete account of it is given by Haaland in the Third Scientific Report of the Imperial Cancer Research Fund. It gives a variable success on transplantation, sometimes reaching 100 per cent. Spontaneous absorption, even of fairly large nodules, is a common characteristic, and when this absorption takes place it is generally very rapid. The inoculating dose is 0.025cc.

With regard to some of the earlier inoculations the charts of controls were unfortunately destroyed. The strain had died out but was again revived by the kindness of Dr. Bashford.

To save repetition thelater controls are put together and will be referred to throughout by the corresponding letter.

Control A. - At the end of 16 days, 27 were positive (100 per cent.), but of these only one progressed, the rest slowly absorbed.

Control B. - In 14 days, 15 were positive out of 20. 2 progressed.

Control C. - In 18 days, 11 were positive out of 17. 3 progressed.

Control D. - In 14 days, 6 were positive out of 8. All slowly absorbed.

Control E. - In 17 days, 6 were positive out of 13. 2 progressed.

Experiment 1.

With a first inoculation giving a success of 83 per cent. in the series, 1 mouse developed a fairly large tumour which disappeared by the nineteenth day.

A second inoculation, Control A, was given 87 days later. Result, negative.

A third inoculation, Control B, was given 17 days later. Result, negative.

A fourth inoculation, Control C, was given 28 days later. The result was that a tumour developed which took fully a month to reach its maximum, and lasted for another month before being absorbed.

A fifth inoculation, Control D, was given 78 days later. The result was negative.

A sixth inoculation, control E, was given 54 days later. The result was negative.

Remarks. - The original state of the mouse was one of temporary susceptibility replaced by refractoriness, a refractoriness that lasted through two succeeding inoculations, but again reverted to susceptibility at the time of the fourth inoculation. On this occasion the susceptible period was of longer duration than the first test showed, but it gave place again to refractoriness.

Experiment 2.

The first inoculation had produced a medium-sized tumour by the tenth day. Within a week this had completely disappeared.

A second inoculation, control A, was given 38 days after the first. The result was a very large growth by the tenth day, taking over a month to absorb.

A third inoculation, control B, was given 17 days after the second and during the time that the former tumour was diminishing. The result was negative.

A fourth inoculation, control C, was given 28 days later. Result, negative.

A fifth inoculation, control D, was given 78 days later. Result, negative.

Remarks. - The condition produced by the absorption of the original tumour did not prevent the develop-

ment of a large tumour in consequence of the second inoculation, and in the latter case the time of diminution, an evidence of the potency of the refractory state, was greater than in the first instance. The result of the third inoculation would indicate here that the refractoriness is at its maximum during the time of diminution of a tumour, Susceptibility did not supervene afterwards.

Experiment 3.

10 Mice, refractory to a first inoculation which succeeded in 83 per cent. of the series, were inoculated on a second occasion, 19 days later, with the same material (kept on ice) as in the first instance. The result was that of the 9 that survived all were negative.

A third inoculation was given to 7 mice, 30 days later. The result was that 6 lived; 4 remained negative and 2 developed large transplantable tumours.

A fourth inoculation of double the usual dose was given to 4 mice, 14 days later. Result, negative.

A fifth inoculation was given to 2 survivors, 24 days later. Control A. The result was negative in one and a temporary tumour in the other.

A sixth inoculation, control B, was given to both 17 days later. Result, negative.

A seventh inoculation, control C, was given 28 days later. Result, negative.

An eighth inoculation, control D, was given 78 days later. The result was that one remained negative; the other gave a small temporary tumour. That which was negative died.

A ninth inoculation, control E, was given to the remaining mouse 34 days later. Result, negative.

Remarks. - The original refractoriness survived a second assault by the same material, which was still living and capable of producing growth, as was administered on the first occasion. On the third inoculation we find that the refractoriness, so far from being accentuated, has actually been abolished, so that in one third of the cases progressive growth resulted. The rest exhibit fluctuations in susceptibility throughout the succeeding inoculations.

Experiment 4.

6 Mice, which had had tumours as a result of a first inoculation, and in which the tumours were absorbed, were inoculated on a second occasion, 49 days later. 5 survived and all proved negative.

A third inoculation of double the usual dose was given, 14 days later. Only one survived and it proved negative.

Remark. - This short experiment shows no change from the refractoriness that supervened on the original temporary susceptibility.

Experiment 5.

10 Mice which developed tumours after a first inoculation, and in which the tumours were absorbed, were inoculated on a second occasion, 49 days later. 3 survived, and all were negative.

A third inoculation, of double the usual dose, was given 14 days later. Result, negative.

A fourth inoculation, control A, was given 24 days later. Result, negative.

A fifth inoculation, Control B, was given 17 days later. The result was that 2 remained negative, and 1 developed a large progressive tumour.

Remarks. - Notice that doubling the inoculation dose, though ineffective in producing growth, did not immunise the animals against subsequent inoculation of the usual dose.

Experiment 6.

4 Mice which developed tumours as a result of a first inoculation, and in which the tumours were absorbed, were inoculated on a second occasion, 44 days later, with double the usual dose. The result was that 1 developed a small tumour, and the other three were negative.

A third inoculation was given, control A, to 3 mice, 24 days later. All showed temporary nodules.

A fourth inoculation, control B, was given 17 days later. 1 died; 2 remained negative.

A fifth inoculation, control C, was given 28 days later. Result, negative.

A sixth inoculation, control D, was given 78 days later. Result, negative.

A seventh inoculation, control E, was given 34 days later. Result, negative.

Remarks. - Doubling the dose in one of the cases produced a small tumour. In the others the fluctuations towards suceptibility did not approach the original condition.

Experiment 7.

20 Mice which had been refractory to, or only gave small temporary tumours after a first inoculation, which altogether induced 13 tumours (only 1 progressive) in 32, were inoculated for a second time, Control B, 29 days later. 17 lived; 2 (1 of which had been temporarily positive to first inoculation) showed small temporary tumours; the remainder were negative.

A third inoculation, control C, was given 28 days later to 12 mice. 10 lived. Result, negative.

A fourth inoculation, control D, was given 78 days later to 2 mice. Result, negative.

Remark. - The refractoriness has been little changed throughout.

Experiment 8.

10 Mice, all of which were positive to a first inoculation succeeding at first in 75 per cent., but in which the tumours were absorbed, were inoculated on a second occasion, 28 days later. Control B. At the time of the latter inoculation, 2 still had diminishing tumours; in 3 these had just been absorbed. 7 survived. Result, negative.

A third inoculation, control E, was given 78 days later. Result, negative.

Remark. - Refractoriness persists.

Experiment 9.

10 Mice from a series in which 75 per cent. of positive results (mostly afterwards absorbing) were obtained, were inoculated on a second occasion, 28 days later. Control C. At the time of this inoculation, 3 were, and had been, negative; 3 had diminishing tumours; 1 had an increasing tumour; the remaining 3 did not survive long enough to be examined. The result was that all were negative; in the case of the increasing tumour, it also diminished.

A third inoculation, control D, was given 78 days to 3 mice. Result, negative.

A fourth inoculation, control E, was given 34 days later to 3 mice. Result, negative.

Remarks. - The refractoriness remained unaltered. The fact that a mouse showing an increasing tumour, so long after the absorption or disappearance had taken place in the others, had this tumour later absorbed is probably not to be attributed to the influence of the second inoculation which did not take, as it might have occurred otherwise.

Experiment 10.

5 Mice from a series of 17, in which a first inoculation produced tumours in 11 (3 of which progressed) were inoculated on a second occasion, 78 days later. Control D. Of these mice 2 had been negative to first inoculation, and 3, though negative at time of second inoculation had previously been positive. The result was that 4 remained negative, and 1 developed an increasing transplantable tumour.

A third inoculation, control E, was given 34 days later to 3 mice. Result, negative.

Remarks. - Definite change of refractoriness to susceptibility is evident, especially when it is remembered that the tumour material used in the second inoculation was not potent enough to produce increasing growths in the controls and yet succeeded in producing a large growing tumour in one of the treated mice.

Experiment 11.

8 Mice had had a first inoculation, which gave 7 tumours (all of which absorbed) in a series of 20, were inoculated a second time as follows: (a) 3 mice, originally negative, were reinoculated 35 days later. Control D. 2 lived andboth remained negative. (b) 5 mice, all of which originally had tumours slowly absorbed, were reinoculated 69 days after first. Control E. Result, negative.

Third inoculation, control E, was given to a survivor of the former, 34 days later.

Remarks. - Refractoriness unchanged.

Experiment 12.

4 Mice, from which the tumours resulting from a first inoculation had been absorbed, were reinoculated 34 days later. Control E. 3 lived. Result, negative.

Taking together the various experiments in series corresponding to the number of inoculations we obtain the result:

Of 57 refractory to 1 inoculation, 53 remained negative, 3 showed temporary tumours, and 1 was definitely positive after the second inoculation.

Of 29 refractory to 2 inoculations, 26 remained negative, and 3 showed temporary tumours after the third inoculation.

Of 17 refractory to 3 inoculations, 16 remained negative, and 1 showed a temporary tumour after the fourth inoculation.

Of 10 refractory to 4 inoculations, 8 remained negative, 1 showed a temporary tumour, and 1 was definitely positive after the fifth inoculation.

Of 4 refractory to 5 inoculations, 4 remained negative to a sixth inoculation.

Of 4 refractory to 6 inoculations, 4 remained negative to a seventh inoculation.

Of 2 refractory to 7 inoculations, 1 remained negative and 1 showed a temporary tumour after the eighth inoculation.

Of 1 refractory to 8 inoculations, 1 remained negative to a ninth inoculation.

The four sets of experiments with different tumours lend no support to the view that repeated inoculations deepen the refractoriness: on the contrary, they show conclusively that such a procedure increases the susceptibility. The failure of first inoculations is in most cases due to a natural insusceptibility to, or even active antipathy against, tissues foreign to the animal's economy. This state may last for an indefinite period or may give place to a condition of susceptibility. The fact that tumours develop and become absorbed may be due to a resistance somehow stimulated from dormancy tardily exerting itself, or it may be due to an inherent tendency of the tumour cells to necrobiosis, or it may be a new reaction induced by absorption of portion of the growth. A primary susceptibility, evidenced by successful growth, is not a per-

manent characteristic of the animal in all cases, but is sometimes a temporary phase that may be followed by insusceptibility Due weight must be paid to the observations that previous treatment by living embryonic or other tissues of animals of the same species confers a certain amount of protection against subsequent inoculation of tumour cells, as seen when the results of this inoculation ~~xfxtxxxxxxxxxxx~~ are contrasted with the results of inoculation of previously untreated controls. But all that such experiments prove is that the natural biological resentment to foreign tissues has been accentuated. Any refractoriness that may follow the absorption of an initial inoculation of tumour material is of the same order, so that it is not on these lines that we may seek for the successful inoculation of immunity to cancer.

ON THE SERUM DIAGNOSIS OF CANCER AND OF PREGNANCY.

THE PRACTICAL WORTH OF ABDERHALDEN'S METHOD WITH A CRITICAL EXPERIMENTAL STUDY OF THE THEORY OF PROTECTIVE FERMENTS.

Incorporating papers contributed to the British Medical Journal on 25th July and 15th August, 1914.

-:-:-:-:-:-:-:-

An amazingly fruitful period in the progress of our knowledge of pathological processes was ushered in with the discovery that the blood serum in certain germ-caused diseases possessed the property of destroying these organisms or of neutralizing their toxic powers. There resulted from this, inspired, helped, or hindered by the theories of conflicting schools, countless experiments that taught us to recognize the fact that the blood serum gives evidence of reaction bodies (immune bodies) to very diverse substances (antigens) that have gained access to the system. Diverse though these substances be at first sight, they can all be classified as complicated proteins. They may be bacteria, toxins, products of vegetable origin, tissues or tissue constituents of other animal species, but they are all foreign to the economy of the particular animal into which they have been introduced by the breaking down of some natural barrier of defence. It must not be hastily concluded, as is too often assumed, that the interplay of antigen and antibody in test-tube experiments is a replica of the reactions <u>in vivo</u>, or indeed is more than a side phenomenon, but by means of these test-tube experiments we are enabled to form some idea of the defensive mechanisms.

When we come to deal with substances less foreign to the body, such as the cells or cellular proteins of other animals belonging to the same species, the biological methods hitherto employed are generally useless to detect the presence of any reaction substances in the serum. That the system elaborates means of defence against

them is evident but we almost always fail to find evidences of that defence in the serum. It is true that occasionally such evidence may be forthcoming, but it is haphazard at the best. For example, Ehrlich, on one occasion, by injecting into a goat overwhelming amounts of the lysed corpuscles of other goats, produced in the serum a reaction body capable of setting free the haemoglobin of certain goats' corpuscles, but the experiment has never been successfully repeated, and had it not been that the name of this great authority was attached to the observation the possibility of its occurrence would have been disputed. At the most it is a rare experimental phenomenon, and no general conclusion can be drawn from it - a point that must be borne in mind when considering the details of Abderhalden's theory. Sometimes human serum exhibits a certain amount of haemolytic power towards the corpuscles of other individuals, and attempts have been made to connect this phenomenon with certain diseased states, but it is quite a normal occurrence, in no way connected with pathological conditions. Indeed normal human sera may be divided into four definite groups by this reaction. Crile of Cleveland claimed to be able to diagnose cancer by the haemolytic action of the serum of cancerous patients on normal human erythrocytes, but cancerous sera have the same group proportions as normal sera. Experiments in tissue transplantation, and especially the numerous experiments in mouse cancer inoculation have shown us that the normal resistance on the part of the host is very feeble, and though an artificial resistance may be induced by certain measures, it too is feeble and transitory, and no evidence of it whatever is traceable in the blood serum by any of our present methods.

Few and fortuitous as these results are with substances so closely allied as the tissues of other animals of the same species, they are absolutely negative when we attempt to trace reactions to injections to an animal's own tissues. A resistance of a kind to mouse cancer inoculation may be induced by the previous injection of mouse spleen, but the injection of the animal's own splenic tissue is followed by no resistance whatever. This experiment, which we owe to Woglom, is one of the most important in recent cancer research, and quite crushed the expectations entertained by enthusiasts of being able to induce a specific resistance to cancer along the lines of previous research. Again if a spontaneous tumour be removed from a mouse and kept in cultivation by successive transplantations through other mice, and during this time the original animal be "immunised" by mouse blood or mouse spleen, a transplantation back again into it will be successful (Murray). It shows no resistance even to its own tissues though they have been modified to some extent by the imprint of other hosts. In 1910 I endeavoured by injections of their own corpuscles, washed and unwashed, into two sets of three rabbits, subcutaneously or intramuscularly, every other day for a period of six weeks, to produce a haemolytic reaction body in the serum, but the experiments were quite negative. We have, in short, no experimental evidence along the lines on which we have gone that the blood serum shows any power of dealing with the cells of the body by producing reaction substances to them. We cannot, however, deny the possibility of the

metabolic products of injected cells being dealt with by some bodies that may be present in the serum. Let me take an example that will focus the problems. In cancer we have a progressive growth, beyond their natural confines, of cells that have sprung from the normal body cells and still retain many of the parent properties. Apart from the extremely rare instances of spontaneous cure, the body shows no resistance, as a whole, against these cells; no state of immunity is induced. They go on growing, and they finally produce death. Omitting those cases in which cancer prevents the discharge of some vital function, why should the disease be fatal? They presumably abstract from the circulation the same constituents as their normal progenitors, and probably not in seriously greater amounts. By microchemical methods we can demonstrate that they return to the economy, in some cases and to a much lesser extent, physiological substances similar to those of their progenitors, but as this functioning is very primitive we must conclude that they set free other metabolic products which either by their nature or by their amount, upset the regulating mechanisms of the body. It may quite well be that though there is no lethal reaction-body elaborated against them yet an attempt is made to cope with their products. And the question is, how is this attempt made? Are these products dealt with by the normal body cells, or does the blood serum acquire the power of breaking them up into less harmful constituents? If the latter could be shown to be the case we might hope to trace the changes. Here the genius of Abderhalden seemed to illuminate the beginnings of a road into the fruitful unknown.

Abderhalden imagines that just as the cells of the intestinal tract have at their disposal ferments which split up the complicated food constituents into simple products suitable for absorption, so the cells of the body, having taken what they require from the blood, build it up and break it down into suitable combinations by the help of ferments. Thus in normal circumstances. But if anything foreign gains access to the blood stream, without having undergone preliminary degradation in the alimentary canal, then new and specific ferments are elaborated capable of breaking it down into simple constituents. These new ferments, originating without doubt in the body cells, will be set free in the blood plasma, and as we can only detect a ferment by means of its specific activity, so their presence will be demonstrated if we trace what occurs when the ferment-containing serum is brought into contact with the foreign substance on which it acts, or to use the appropriate nomenclature, when the specific substrate is provided. If we add gastric juice to such a protein as egg-albumen we find that the latter is digested into peptones in virtue of the presence of the ferment pepsin. Similarly we test for the presence of proteolytic, or inverting, ferments in the blood by adding the plasma or serum to proteins, peptones, or sugar, and observing the changes produced in these substrates. We cannot recognize a ferment apart from its action. This being so, it is obvious that we are faced with the question, Given the changes we should expect from the action of a ferment, are these changes due to the ferment or are they due to other causes?

We shall only be entitled to assume ferment action when we can rule out of account the operation of all other factors producing the same result. This is the crux of Abderhalden's theory, and, as I shall show later on, he has not taken into account the extent of the other factors that contribute to the results on which he depends.

Abderhalden depends on two chief experiments for the basis of his theory. In the first place, he claimed to have observed in the blood plasma or serum of animals which had been injected with foreign peptone (that is, a peptone not derived from their own tissues) a ferment action on these peptones. This experiment was made by taking a mixture of the serum of the treated animals and a solution of the peptone, observing the polarimetric reading at once and noting the change produced after the lapse of varying periods of time. With the fresh serum of untreated animals or with the heated serum of treated animals (the heating would of course destroy the fermentative power) the initial rotation did not alter, whereas with the unheated serum of treated animals it increased. This observation he verified by repeated experiments. The second set of experiments dealt with the changes produced in mixtures of sugar solutions and the serum of animals which had previously had parenteral injections of sugar. In both cases the polarimetric readings were taken to demonstrate the presence of peptolytic or inverting ferments respectively. In neither case, if one may take as typical the graphic representations which he gives, was the rotation considerable. The extreme rotation is to be reckoned not in degrees, not even as a rule in tenths of a degree,

but in hundredths of a degree; and even supposing we were to admit that such small rotations were conclusive evidence of cleavage of the substrate by a ferment, yet even with the most delicate polarimeters and with the most experienced eyes it is gravely to be doubted if any one working with such a complicated substance as serum can be certain of reading with such meticulous exactitude. We know serum to be very unstable, and very slight departures from the strict isotonicity of added fluids are capable of producing evident changes in it. Generally speaking, it is questionable if polarimetric readings are more delicate than titration methods, and in such an important fundamental experiment it is unfortunate that Abderhalden neglected to bring forward the confirmatory evidence that could be supplied by such methods. He states, however, that the polarimetric readings in the experiments with protein are confirmed by the dialysation method, which will be considered in detail afterwards, and he admits that in the sugar injection experiments the desired results could only sometimes be obtained.

McVey (quoted by Browning, Applied Bacteriology) found that normal rabbit serum mixed with egg-white gave rise to diffusible substances reacting to ninhydrin, a reaction which was abolished by previously heating the serum to 60°C for an hour, and that the serum of a rabbit previously injected intravenously with egg-white acquired no enhanced power of producing dialysable substances.

I repeated in rabbits Abderhalden's original sugar experiments giving intravenous injections of saccharose on three occasions and bleeding the animals ten days after the last. Polarimetric readings were taken of mixtures of the serum and isotonic sugar

solution (6%) immediately and after incubation but in all cases I failed to find any increase of the original rotation. Further glucose titration experiments were made in comparing the results of sugar plus unheated "immune" serum and sugar plus heated "immune" serum as follows:- to 5 c.cm. of unheated and of heated serum were added 16.6c.cm. of isotonic saccharose solution: both sets of tubes were covered with toluol and incubated at 37°C for 2 days: the albumen was precipitated with absolute alcohol and iron oxide: the solutions were filtered and the filtrate tested for glucose by Bertrand's method. The amount of copper reduction was the same in both the heated and unheated serum series. Complement deviation tests were also applied. By preliminary trials it was found that 0.1 c.cm. of 6 per cent saccharose solution was a suitable dose for the antigen and tubes were set up using the antiserum in various doses from 0.005 c.cm. to 0.1 c.cm. The minimum haemolytic dose of the first complement used was 0.0075. After 2 hours incubation at 37°C the haemolytic system (sensitised ox corpuscles) was added. In no case was there the slightest deviation of complement. With another complement, the minimum haemolytic dose of which was 0.01, no deviation was detectable either. Thus by polarimetric, titration, and complement deviation tests, the serum of rabbits treated with sugar injections failed to show any evidence of inverting ferments. In view of the experiments of Weinland previous to those of Abderhalden, and of those of Röhman and Kumagai subsequently, there is some independent support for this part of his claim, but there is considerable divergence as to how the result

may be produced and as to what actually occurs.
Recently, however, even these corroborative experiments have been shown to be unreliable.

But however favourably we view these fundamental experiments, we cannot fail to notice that the reactions claimed are against substances quite foreign to the animal, which have gained access to the circulation in an abnormal way. And just as we have seen in discussing the reaction of immunity that there were reaction-bodies elaborated against proteins quite foreign to the animal body, scarcely any at all when the proteins were foreign to the animal but proper to the species, and none whatever when the proteins were derived from the animal itself, so it behoves us to be cautious before accepting a sweeping theory in which corresponding experimental links are wanting, all the more so because Abderhalden recently has brought his protective ferments into line with the immune bodies. That this is no captious criticism is evident from some experimental work published by Pincussohn and Petow. These observers found that the normal serum of an animal has the power of splitting up the muscle peptone of its own species or that of allied species, but manifests no such action against the muscle peptone of foreign species. Indeed, the average polarimetric rotation in these experiments was greater than the small rotation depended on by Abderhalden for the diagnosis of pregnancy. If this observation be correct, then the polarimetric readings which have been relied on in the diagnosis of pregnancy and diseased conditions must be received with great suspicion. Human serum will break down placental peptone, not because the serum is that of a pregnant woman

and the peptone is derived from a placenta but just because the serum and the peptone are from human beings.

In pregnancy there is a new organ interposed in the economy, existing only for a time.. It will take up from the blood certain constituents for its requirements and it will return to the circulation certain products of metabolism, and it is assumed that these are dealt with, not so much by endocellular ferments as by special specific ferments in the plasma elaborated ad hoc. It seems rational but it may or may not be true. Whether the placenta sets free into the blood characteristic proteins which are broken up by the supposed proteolytic ferments is a question vaguely discussed, but it seems to be assumed that the ultimate cleavage products of a ~~xxxxxx~~ protein are capable of calling forth a whole order of ferments acting specifically on all the higher combinations of these constituents. Thus, for example, the introduction of a peptone into the blood may call forth in response a ferment for the protein from which the original peptone was derived as well as a ferment for the peptone itself.

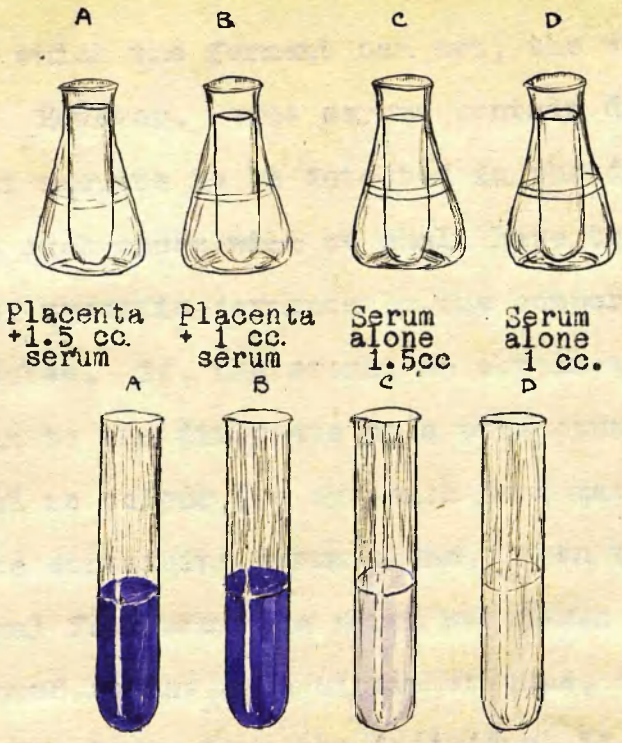
Two separate methods are employed for the detection of the presence of ferments, the polarimetric method by which the degradation of peptones may be observed, and the dialysation method by which the degradation products of proteins may be separated. The latter is the method on which most of the work has been done. According to Abderhalden, the results of the two methods always agree. I have used the dialysation method solely in the following experiments. If we take the procedure adopted in the diagnosis of pregnancy as an example, the technique as I have carried it out, is as follows :

Fresh healthy placental tissue from which the blood clot has been removed is cut up into pieces and washed until it is colourless. It is boiled several times in five times its volume of distilled water, until the water when filtered fails to give a colour when boiled with 1 c.cm. of a 10 per cent. solution of ninhydrin. The pieces of placental tissue may then be stored in sterile distilled water under a thick covering of toluol. These pieces contain the coagulated placental protein, in addition, of course, to other substances which are not of immediate importance, and they serve as substrate for the demonstration of the ferment action. The pieces of placenta ought to be boiled just before use because, though the water may be free of ninhydrin reacting substances when the tissue is stored, a certain amount of these substances is apt to form after a time. The serum to be examined for the presence of absence of specific ferments is obtained by removing a quantity of blood from the patient by venipuncture, and allowing this to clot spontaneously. As it is advisable to obtain serum quite free of bacterial contamination, special precautions have to be taken: my technique for this will be described later. Special parchment dialysation tubes are used, which are supposed to retain proteins and to allow peptones and amino-acids to diffuse through, though collodion tubes may be used instead and are probably better. These tubes are placed in 20 c.cm. of sterile distilled water in small Erlenmeyer flasks. Into one tube we put a teased-out portion of the placental tissue (roughly about ½ to 1 gram) and add to this a certain fixed quantity of the serum (either 1 c.cm. or 1.5 c.cm.), taking care to avoid any of the serum getting into the water

outside the tube. Into another tube, which is to serve as a control to this test, we put 1.5 c.cm. of the serum only.

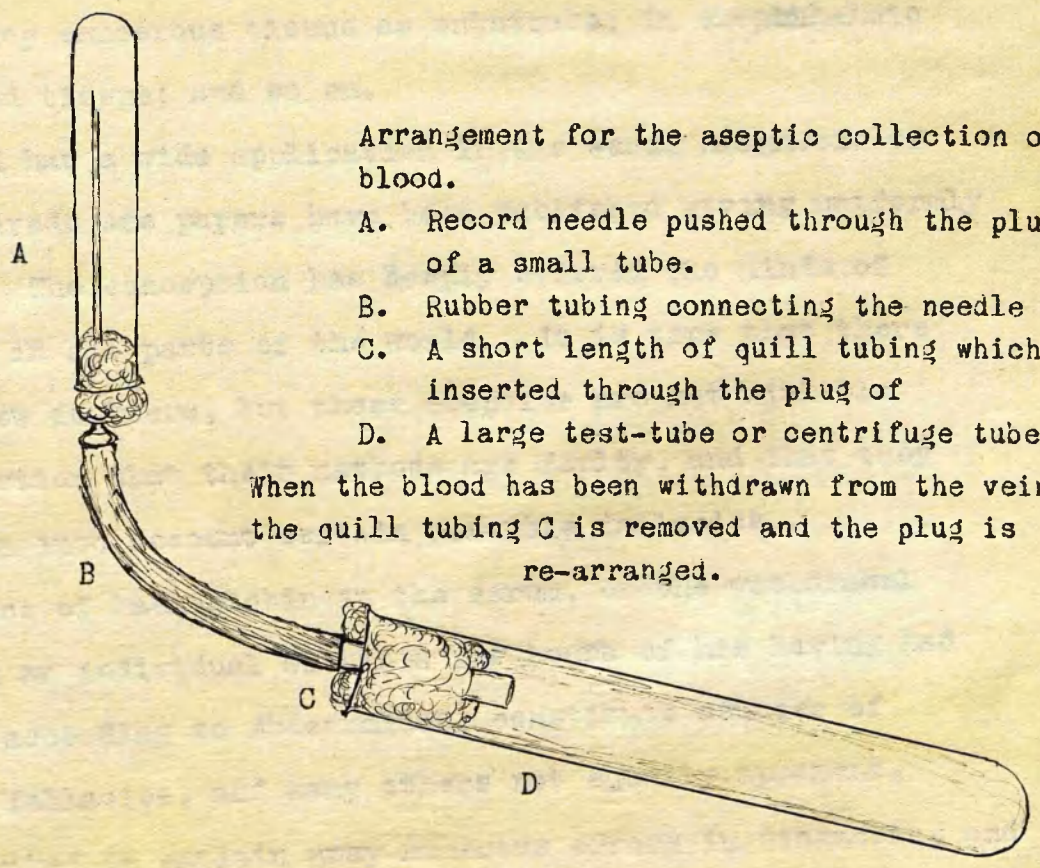
The tests and controls should be multiplied as far as the amount of serum will permit because equivocal results are sometimes met with that are probably due to variations of, or faults in the, dialysing tubes. The dialysing tubes should always be sterilised by boiling and kept in sterile water. In both sets we cover the contents of the tube and the surrounding water in the container with a thick layer of toluol in order to prevent evaporation and bacterial contamination. It is advisable not to trust to the toluol preventing bacterial growth. I have several times succeeded in getting cultures by inoculating broth with bacteria and covering the surface with a layer of toluol, but it probably prevents contamination of a sterile fluid medium. The tubes are incubated at 37°C. for sixteen hours. At the end of that time 10 c.cm. of each dialysate are removed and tested with 0.2 c.cm. of ninhydrin solution, the mixture being boiled with a few porcelain chips to prevent "bumping", for exactly one minute, timing it with a sandglass. A violet coloration, which may not be evident till the liquid cools, denotes the presence of peptones or amino-acids.

Supposing a specific proteolytic ferment is present in the serum, then the dialysate of the first tube will contain peptones and amino-acids, and these will be demonstrated by the violet colour with ninhydrin. If the ferment is absent, then there will be no production of peptones and no reacting substance will be found in the dialysate. On the other hand, the dialysate of the second tube, containing serum alone, will show no ninhydrin-reacting substance, because there is no



Dialysation tubes surrounded by distilled water in Erlenmeyer flasks.

A positive test.
The dialysates from the above have been boiled with ninhydrin solution.



Arrangement for the aseptic collection of blood.

A. Record needle pushed through the plug of a small tube.
B. Rubber tubing connecting the needle with
C. A short length of quill tubing which is inserted through the plug of
D. A large test-tube or centrifuge tube.

When the blood has been withdrawn from the vein the quill tubing C is removed and the plug is re-arranged.

substrate on which the ferment can act; the water will remain colourless. However, some serums contain diffusible substances in sufficient amounts to be detected in the dialysates by ninhydrin, and in such cases ~~will~~ we shall have to judge of the presence or absence of specific ferments by the comparative depths of colour in the two tubes. If, therefore, we obtain a violet colour when we add ninhydrin to the dialysate of a tube containing serum plus placenta, and no colour (or one much less marked) with the dialysate from the tube containing serum alone, then the conclusion is that the individual from whom the serum was taken is pregnant. If no colour is found in the case of either tube, or no comparative ~~of~~ difference of colour, then the individual is not pregnant. Similarly for the diagnosis of certain diseased conditions: in cancer we employ cancerous tissue as substrate; in exophthalmic goitre, thyroid tissue; and so on.

The method has a wide application in the serum diagnosis of disease. Hundreds are papers have been published giving uniformly good results. The conception has deeply stirred the minds of investigators in all parts of the world. It is true that there have been a few doubters, but these sceptics are met with the constant assertion that their methods are faulty, and that they have not taken into account certain possible fallacies..

The presence of haemoglobin in the serum, or the withdrawal of blood from an individual within a few hours of his having had a meal, may, according to Abderhalden, constitute sources of error. Such fallacies, and many others not equally apparent, have been invoked to explain away numerous errors in diagnosis, and

such being the case we must seriously ask if the method can be relied on for information in clinically doubtful cases. According to Abderhalden, the proper reading of the result is never wrong - a claim that reminds one of the Delphic oracle.

The method of performing the reaction, which has been sketched roughly above, is given in very voluminous detail in Abderhalden's Abwehrfermente des tierischen Organismus and in numerous publications in various journals. The method is by no means difficult, and the technique is easy to follow. A method that cannot be carried out by the average laboratory worker who exhibits ordinary care is not of much utility. Though I have worked with this reaction for more than eighteen months, I have rejected the results obtained during the first half of that period, so as to avoid the criticism of being unfamiliar with the technique. During that first period, however, the results were much more favourable to Abderhalden's claims than afterwards. The reason is not far to seek. Captivated by the theory which opened up for us great possibilities in cancer investigation, carried along by the host of communications that hailed the method as well-nigh infallible, perhaps intimidated by the scorn poured on the few that doubted, I accepted uncritically the good results, shut my eyes to those that were doubtful, and found excuses to reject those that were adverse - for a time. In the later period, when I was untrammelled by authority, it was found that the results were far from supporting the claims put forward. The technique given by Abderhalden was slavishly followed. His retort to that is: "I treat such assertions with the scepticism born of a rich experience." Such a reply might be overwhelming if

his own published technique were above reproach. I can only say that I have done my best to follow his directions without actually having made the customary pilgrimage to Halle to receive the direct apostolic revelations from the master.

In the following examples of the practical application of the method, before testing it on the much more important question of cancer diagnosis, I first investigated the reaction in pregnancy because the true diagnosis of the condition cannot long remain in doubt and we are soon enabled to verify our results. It is seldom that the condition is doubtful, and the serum diagnosis of pregnancy would only have a limited value. In cancer, on the other hand, a method of serum diagnosis would be of the utmost practical importance. I have tried most of the methods hitherto advocated in serum diagnosis and found them all worthless in practice. But if the procedure of Abderhalden was capable of giving good results in pregnancy it might reasonably be expected to give good results in cancer, though the relation of the placenta to the maternal organism is not strictly comparable to the relations existing between malignant tumour cells and the rest of the body. It is feasible to imagine that cancer cells give up to the circulation abnormal metabolic products.

@ @ @ @ @ @ @

In every case recorded in the following tables the true diagnosis of the case is known, either by subsequent events, surgical and histological verification, or by post mortem examination. To prevent

the knowledge of the condition of the patient furnishing the serum having any effect on our reading of the reactions - a vital point - the tubes were numbered, the numbers written down for later identification, and the tests conducted and the final readings made without being aware of the clinical diagnosis of the conditions. It is impossible to avoid bias otherwise. Again, the tests were manifolded. It sometimes happened that very strange results were recorded. For example, the controls might have deeper colours than the actual tests themselves, or the readings might be equivocal throughout any set.

Such cases were eliminated from our survey and the dialysation tubes corresponding were scrapped because it was imagined at first that the equivocal readings were due to flaws in them. That this is not so we found by later experience. The comparatively favourable figures shown in the first table are to some extent due to this rejection of adverse readings. Further, I have rejected the unfavourable readings, in those cases recorded, if I obtained a favourable set. The colours obtained with ninhydrin varied from light lilac to deep violet. In making a comparison between the tubes I have neglected weak tints, representing these as negative, except where there was a difference between the test and its control, and in this way also I have been generous towards the method. The deepest colours are represented throughout by +++.

TESTS WITH THE SERUM of PREGNANT WOMEN.

Sufficiently clear cut results were obtained in 36 cases of pregnancy from the second month onwards to a few hours after delivery. The exact condition of all these cases was known. It will be seen

from Table I that in two cases, Nos. 1 and 6, the reaction was quite negative. In the first case the serum was free of haemoglobin, the blood had been withdrawn more than four hours after a meal, and no disturbing factor could be discovered: yet the subsequent history of the case proved that the woman was two months pregnant at the time. The second negative (No.6) was interesting because at the time the test was performed there was doubt about the clinical diagnosis: the serum was fresh and free from haemoglobin: the further history of the case showed that the patient was then three months pregnant. Faint results were obtained in four cases viz. Nos. 5, 20, 30 and 28 (the serum in the last being haemoglobin-tinted), and perhaps a severe critic might reject them as positive results. In the other two cases Nos. 14 and 21, the positive result recorded with the higher doses of serum would have been missed, and the reaction returned as negative, if we had employed only the usual smaller doses. As so much stree has been laid on the presence or absence of haemoglobin in the serum, it may be stated that 9 of these sera (Nos. 4, 10, 12, 15, 17, 27, 28, 32 and 34) showed haemoglobin-tinting whilst the rest were quite normal. If as Abderhalden states, the presence of haemoglobin is apt to give a positive finding in a negative serum we ought to reject these results as being fallacious but as will be seen later, I do not think the presence or absence of haemoglobin comes into the matter at all. In Nos. 1, 34, 35, 36, more than four hours elapsed between the taking of a meal and the withdrawal of the blood; in the other cases I am unaware of the times elapsing.

Positive reactions would seem to be the rule in cases of pregnancy as

this table shows, even with the reservations above made, and I have found such positive reactions much more frequently in pregnancy than in any other condition I have examined. So far, therefore, the tests appear to afford promising support to Abderhalden's idea of guardian ferments.

TABLE I.

Age of pregnancy, etc.	Serum alone 1 c.cm.	Serum alone 1.5c.cm.	Placenta + 1 c.cm. Serum.	Placenta + 1.5c.cm Serum.
1. 2nd month	0	0	0	0
2. " "	0	0	+ + +	+ + +
3. " "(pelvic abscess)	0	0	+ +	+ + +
4. " "(pernicious vomiting)	-	0	-	+ + +
5. 3rd "	0	0	+	+
6. " "	0	0	0	0
7. " " (aborting)	0	0	+ + +	+ + +
8. " " "	0	0	+ +	+ + +
9. 5th "	0	0	+ +	+ + +
10. " " (albuminuria)	-	0	-	+ + +
11. 6th "	0	+	+ +	+ + +
12. 6th "(pernicious vomiting)	0	0	+ + +	+ + +
13. 7th "	0	0	+ + +	+ + +
14. " "	0	0	0	+ +
15. " "	0	0	+ +	+ + +
16. 8th "	0	0	+ +	+ + +
17. " "	0	0	+ +	+ + +
18. " "	0	0	+ + +	+ + +
19. 9th "	0	0	+ + +	+ + +
20. 9th "(threatened eclampsia)	0	0	+	+
21. Full term	0	0	0	+ +
22. " "	-	0	-	+ + +
23. " "	0	+	+ +	+ + +
24. " "	0	0	+ +	+ +
25. " "	0	0	+ + +	+ + +
26. " "	0	0	+	+ +
27. " "	0	+	+ + +	+ + +
28. " " (syphilitic)	-	++	-	+ + +
29. " " (eclampsia)	-	0	-	+ +
30. " " "	-	0	-	+
31. 3 hours after delivery	0	0	+ +	+ +
32. 12 " " "	0	0	+ + +	+ + +
33. 24 " " "	-	0	-	+ + +
34. Ectopic gestation (5th week)	0	0	+ + +	+ + +
35. Ectopic gestation (2nd month)	0	0	+ + +	+ + +
36. Ectopic gestation (10th week)	0	0	+ + +	+ + +

TESTS WITH SERUM FROM OTHER GYNAECOLOGICAL CONDITIONS (pregnancy reaction

We were fortunate enough in having two cases of chorionepithelioma to investigate. Those who support the specificity of Abderhalden's reaction agree that the serum in such cases may be expected to give a positive result because the cells of the tumour retain to a great extent the metabolic properties of their normal placental progenitors. In the first case the blood was withdrawn from the patient at the time of operation, and the result of the reaction was obtained before we had any suspicion of the real nature of the case. Hysterectomy was performed and on cutting into the uterus after it had been hardened we were surprised to see a small but typical chorionepithelioma. After an interval of twenty days the patient's serum was again examined and a positive result again obtained. The same result was found a month later still. At no time were there signs of metastases. The second case of chorionepithelioma, diagnosed as such, gave a positive reaction. In this case there was a vaginal recurrence a fortnight after the hysterectomy and when the blood was examined then the positive reaction still persisted. The patient died with pulmonary metastases. The serum from three cases of fibromyoma uteri, a uterine polypus, and vaginismus gave frankly negative results, but quite clear positive reactions were obtained in cases of endometritis, dysmenorrhoea, pyosalpinx, and fibromyoma. The case of endometritis (No.4) might possibly have been one of retained products of conception, as the curettings were not microscopically examined. The other three cases that gave clear positive results were not pregnant (the serum of two of them being re-examined with the same result), and are practical demonstrations of the unreliability

of the reaction. No. 6 was considered clinically to be a pregnancy of the third month; the reaction was positive; the case was followed up; she was not pregnant, and had not aborted. The last case ~~was~~ of uterine fibroid (No.11) is of interest because there was a conflict of opinion as to its nature, and the Abderhalden reaction was twice invoked to assist in the diagnosis. Twice it pointed to pregnancy; the Surgeon removed the uterus; it was a soft fibro-myoma, and there was no pregnancy. The times that elapsed between meals and the withdrawal of the serum in this case are unknown to me.

In all cases the serum was fresh: only in No.5 (which was frankly negative) was there haemoglobin staining. With the exception of No.11, the blood was withdrawn at the time of operation, and thus at least four hours must have elapsed since the last meal. Leaving out of account the two cases of chorionepithelioma and the case of endometritis (the nature of which is not beyond reasonable doubt) we find five negative and three positive results where pregnancy can certainly be excluded.

TABLE II.

Disease	Serum alone 1 c.cm.	Serum alone 1.5c.cm.	Placenta +1 c.cm. Serum.	Placenta +1.5 c.cm. Serum.
1. Chorion-epithelioma	0	+	+ +	+ +
2. Uterine polypus	0	0	0	
3. Vaginismus	0	0	0	0
4. Endometritis	0	0	++	+ +
5. Uterine fibroid	–	0,	–	0
6. Dysmenorrhoea	0	0	++	+ +
7. Pyosalpinx	0	0	++	+ +
8. Uterine fibroid	0	0	0	0
9. Uterine fibroid	0	0	0	0
10. Chorion-epithelioma	0	0	+++	+++
11. Uterine fibroid	0	0	+++	+++
	@	@	@	@ @ @

TESTS WITH SERUM FROM CANCER CASES (pregnancy reaction).

Several authors though giving general agreement to the value of Abderhalden's tests, have stated that cancer serum may break down placental protein, whilst others deny this. If both in pregnancy and in cancer there exists ferments in the serum capable of degrading placental and cancer-cell protein respectively we should expect a priori that these ferments would operate on allied substrates, though perhaps to a lesser extent, because none of the immune reaction bodies that we know is strictly specific. Such findings would not seriously affect the theory, but Abderhalden will have none of this. He says that one should never use a placenta in the pregnancy reaction that gives positive results with cancer serums, thereby admitting that such results may occur. Out of all the placentas which I have tested I have never yet encountered one that does not sometimes give a positive reaction with cancer serum and I doubt if it exists. The serum of 22 cancer cases was tested (table III), the first 10 being females and the other 12 males. In 7 cases the reaction was frankly negative, in 4 it was very faintly positive, and in 11 (i.e. in half the cases) it was definitely positive. Two of the sera were haemoglobin-stained, in one (No.7) there was a faint positive reaction, and in the other (No.17) the reaction was absolutely negative.

Duplicates of all the tests shown in the table gave identical readings. All the cases were operable and the blood was withdrawn at the time of operation when they were in a fasting condition. In No.6, where ninhydrin reacting substances were present in the dialysates of the tests and controls, dialysation of the serum against running water for a few hours failed to remove from the

serum all its dialysable ninhydrin-reacting constituents. I have frequently noticed this, and it is probably due to the progressive spontaneous cleavage of the serum proteins. I cannot vouch for the sterility of a serum after it has undergone dialysis against running water, but I do not think that bacterial contamination has much to do with the result.

TABLE III

Disease	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Placenta +1 c.cm Serum.	Placenta +1.5 c.cm. Serum
1. Carcinoma of Breast	0	0	0	0
2. " "	0	0	0	+
3. " "	0	0	+	++
4. " "	0	0	+	+
5. " "	0	0	0	+
6. Carcinoma of Breast (recurrent)	+	+	+	+
7. Epithelioma of Tongue	0	0	0	+
8. Carcinoma of splenic flexure	0	0	+	+
9. Carcinoma of Rectum	0	0	+	++
10. " "	0	0	0	0
11. Epithelioma of Lip (early)	0	0	++	++
12. Epithelioma of Tongue	0	0	+	+++
13. " "	0	0	0	++
14. Epithelioma of Tonsil	0	0	0	+
15. Epithelioma of fauces	0	0	0	0
16. Epithelioma of larynx	0	0	+	++
17. Epithelioma of Penis	0	0	0	0
18. Epithelioma of Bladder	0	0	0	0
19. Carcinoma of Rectum	0	0	0	0
20. " "	0	0	0	+
21. " "	0	0	++	++
22. " "	0	0	+	++

@ @ @ @ @

TESTS WITH SERUM FROM NORMAL and OTHER NON-PREGNANT CONDITIONS (pregnancy reaction).

A series of 31 cases, some normal, others suffering from different diseases, was examined. The accompanying table shows the conditions and the result of the test. The first 20 are females, the remaining 11 males.

There were frankly negative reactions in 19 of these, and at least 5 which were frankly positive, whilst the rest were weakly positive or gave different readings according as one considered the smaller or the larger doses of serum. Amongst the frankly negative cases, 5 sera were stained with haemoglobin (Nos.13,19,23,24, and 25 - the last being very deeply stained): there was no haemoglobin staining of the sera in any of the positive or doubtful cases. In Nos.1, 2, 9, 18, 21, 24, 25, and 26, the time that elapsed between the last meal and the withdrawal of blood is uncertain: two of these, a normal female and a normal male, gave positive reactions: the rest were quite negative. Doubtless, supporters of Abderhalden's claim would excuse these lapses by saying that the interval since the last meal must have been too short, and they would further neglect the adverse readings where an alternative favourable reading was available. But the results quite clearly demonstrate that when the diagnosis of a case is unknown the reaction can certainly not be relied upon to give indubitable information.

TABLE IV

Condition	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Placenta +1 c.cm. Serum	Placenta +1.5 c.cm. Serum.
1. Normal female	0	0	+	+
2. " "	0	0	0	0
3. Chronic appendicitis	0	0	+ +	+ +
4. " "	0	0	0	+
5. Cholecystitis	0	0	0	0
6. "	0	+	0	+
7. Cholecystitis (impacted stone)	0	+	+	+
8. Tuberculosis adenitis	0	0	0	0
9. Lupus	0	0	0	0
10. Lipoma of arm	0	0	0	+
11. Lipoma of Shoulder	0	0	0	+
12. Adenoma of Breast	0	+	0	++
13. Pyloric Stenosis	0	0	0	0
14. " "	0	0	0	0
15. Intestinal adhesions	+	+	+	+
16. Inguinal hernia	0	0	0	0
17. Hydrocele of hernia sac	0	+	+	+
18. Tertiary syphilis	0	0	0	0
19. Proptosis of sigmoid colon	0	0	0	0
20. Abnormality of caecum	0	+	0	+
21. Normal male	0	0	+	+
22. Wound of lip	0	0	0	0
23. Tuberculosis of lip.	0	0	0	0
24. Syphilitic glossitis	0	0	0	0
25. Locomotor ataxy	0	0	0	0
26. Tertiary syphilis	0	0	0	0
27. Pyloric stenosis	0	0	0	0
28. " "	0	+	0	+
29. Tuberculosis Peritonitis	0	+	+	+
30. Haemorrhoids	0	0	+	+ +
31. Chronic appendicitis	0	0	+	+ +

To sum up the results of the above 100 cases tested to prove the diagnostic worth of the dialysation reaction in pregnancy, we find that by the most generous reading possible, judging by two sets of experiments and not by the usual one, making allowances for all the hypothetical fallacies, we have still 17 most glaring mistakes in diagnosis, which of itself is a large percentage, sufficient to destroy the diagnostic worth of the reaction. But if we apply to the good results the same code of ethics by which we reject the undesired, then the whole theory becomes questionable.

@ @ @ @ @

PART II

APPLICATION OF ABDERHALDEN'S METHOD TO THE DIAGNOSIS OF CANCER

A considerable amount of work has been done on the serum diagnosis of cancer. The practical importance of this was early obvious to the originator of the reaction, and until the time that my paper appeared in 1914 practically all who had tested the method agreed as to its extreme diagnostic value. None of the observers reported on any large series of cases, and all seemed to accept uncritically the theoretical bases of the reaction. When the following series of experiments were undertaken, if I had any mental bias at all with regard to the worth of the test it was rather on the side of being favourably impressed by Abderhalden's claims, for the

preliminary examinations I had conducted with the sera of pregnant cases seemed to bear out the theory.

The cancer tissue was prepared in the same manner as placenta. Though I had an unusual amount of operative material at my disposal, yet comparatively little seemed to me suitable for the preparation of "substrates", because, owing to the irregularity of vascular supply of tumours and the frequent areas of degeneration, it seemed impossible to get tissues free from blood. However, we were fortunate enough in obtaining a certain number of highly cellular tumours without these defects. In all cases fresh operation material was used: postmortem tissues were never employed. In the following tables the details are given of 100 cases examined to test the method in the serum diagnosis of cancer.

In the first set of experiments (Table V) three different "substrates" were employed: (1) A large protuberant, non-ulcerated squamous-cell carcinoma of the scalp, extremely cellular, with fine supporting fibrous tissue trabeculae, the tumour being easily washed free of blood and being almost dead white; (2) a very cellular spheroidal-cell carcinoma of the breast with a minimal amount of stroma; (3) placenta.

This set of experiments is the most favourable I have found to the claims of Abderhalden. The 9 cancer serums all gave positive results with the epitheliomatous tissue - if anything, more markedly in the case of squamous-cell than in the other carcinomata - whereas the 12 non-cancerous cases were negative with the exception of the case of lipoma, and one might quite easily, if dominated by the claims made on behalf of the method,

have made excuses for this solitary lapse by blaming the technique. Unfortunately for this, the experiment was repeated in duplicate again with the same results. The mammary cancer was not such a favourable "substrate". It did not give as good results with the mammary cancer cases as did the epithelioma. With it 5 cancer serums were tested; 3 gave positive reactions, 2 were absolutely negative; of 5 non-cancer cases, 4 were negative, 1 was positive. With placenta, 8 cancer serums were tested, 7 gave positive reactions, 1 was negative; of the 7 non-pregnant, non-malignant cases 1 was positive. In all cases the serums were used fresh, and in no case was there any haemoglobin staining. In Nos. 2 and 13, in which no result adverse to the ~~others~~ reaction was obtained, the blood was withdrawn less than four hours after the last meal: in all the others, with the exception of the last three, in which I have no notes on the point, the blood was withdrawn at the time of the operation when the patient was in a fasting condition. It would seem that the epithelioma "substrate" gave more marked reactions with serum from cases of squamous-cell carcinoma than with serum from cases of glandular carcinoma, but the substrate prepared from mammary carcinoma did not act so strikingly with serum from cases of glandular carcinoma. Except in one of the pregnancy cases, the only one which gave a positive reaction with placenta, negative results were obtained with both cancer substrates.

TABLE V.

Disease.	Serum alone.		Epithelioma + Serum.		Carcinoma + Serum.		Placenta + Serum.	
	1cc	1·5cc	1cc	1·5cc	1cc	1·5cc	1cc	1·5cc
1. Epithelioma of oesophagus	0	+	+	++	–	–	+	+
2. Epithelioma of penis	0	+	+	+++	–	–	–	–
3. Epithelioma of tongue	0	0	+++	+++	–	–	+	+++
4. Epithelioma of lip	0	+	+	+	–	+	++	++
5. Epithelioma of tonsil	0	0	++	++	–	0	–	++
6. Carcinoma of breast	0	0	+	+	+	+	+	+
7. Carcinoma of breast	0	0	+	+	–	–	0	0
8. Carcinoma of breast	0	0	+	+	0	+	+	+
9. Carcinoma of rectum	0	0	+	+	–	0	–	+
10. Lipoma of Shoulder	0	0	+	+	–	–	0	0
11. Cholecystitis	0	0	0	0	0	0	0	0
12. Chronic appendicitis	0	0	0	0	–	–	++	+++
13. Hysteria	0	0	0	0	0	0	0	0
14. Uterine polypus	0	0	0	0	0	0	0	0
15. Former epithelioma of lip	0	0	0	0	–	–	0	0
16. Parametritis	0	0	0	0	–	0	–	0
17. Endometritis	0	0	0	0	–	–	–	–
18. Two months pregnancy	0	0	0	0	–	+	+++	+++
19. Two months pregnancy	0	0	0	0	–	–	–	–
20. Seven months pregnancy	0	0	0	0	–	–	–	–
21. Nine months pregnancy	0	0	0	0	–	–	–	–

In four cases an epitheliomatous tumour of the tongue was used as "substrate", (See Table VI).

In the first three tests the results obtained were quite satisfactory: the reaction picked out the malignant from the non-malignant cases. But the last case was the crux of the whole examination: there was what was supposed to be lupus of the face, and there was an ulcerated patch that was not healing under x-rays; and the practical question arose whether or not epithelioma was developing. The reaction pointed to this being the case. As a matter of fact, it was not so. The patient gave a positive Wassermann reaction, and the ulceration healed under salvarsan.

TABLE VI

Disease	Serum alone 1 c.cm	Serum alone 1.5 c.cm.	Epithelioma + 1 c.cm. Serum.	Epithelioma +1.5 c.cm Serum.
22. Epithelioma of Tonsil	0	+	+	+ +
23. Syphilitic Glossitis	0	0	0	0
24. Normal male	0	0	0	0
25. ? Lupus of Face	0	0	+	+

None of the serums was haemoglobin-tinted. All were used fresh. In Nos. 23 and 24 the blood was withdrawn shortly after a meal (fifteen minutes after, in the case of No.24); in the first case it was withdrawn at time of operation; in the last case over four hours after the last meal.

@ @ @ @ @

In the twenty-six cases in Table VII a large, quickly-growing cellular carcinoma of the breast, with axillary masses was used as substrate. It was very thoroughly washed to free it of blood. It required twelve boilings before the water was free to ninhydrin.

TABLE VII

Disease	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Carcinoma +1 c.cm. Serum	Carcinoma +1.5 c.cm. Serum.
26. Carcinoma of breast	0	0	0	0
27. " "	0	0	+	+
28. Epithelioma of larynx	0	0	0	0
29. Carcinoma of Colon	0	0	+	++
30. Epithelioma of tongue	0	0	+	+
31. "	0	0	0	+
32. Epithelioma of penis	0	0	0	0
33. Carcinoma of rectum	0	0	0	0
34. Carcinoma of rectum (post-operation)	0	0	0	0
35. Intracystic adenoma of breast	0	+	+	++
36. Fibromyoma of uterus	0	0	++	++
37. " "	0	0	0	0
38. Tuberculosis of intestine	0	0	+	+
39. Tuberculosis of lip	0	0	0	0
40. Intestinal adhesions	0	0	+	+
41. Gumma of tongue	0	0	0	0
42. Pyloric stenosis	0	0	0	0
43. Cholecystitis	0	0	0	++
44. Cholecystitis (impacted stone)	0	0	0	0
45. Hydrocele	0	0	+	+
46. Pyosalpinx	0	0	0	0
47. Tertiary syphilis	0	0	0	0
48. " "	0	0	0	0
49. Normal male	0	0	0	0
50. " "	0	0	+	+
51. Normal female	0	0	0	0

Only in Nos. 32 and 44 was the serum haemoglobin-stained. In cases 34, 47, 48, 49, 50, 51, the blood was withdrawn less than four hours after a meal; in No. 34 it was withdrawn during a meal, and the serum was the most turbid I have ever seen, but it gave quite negative reactions.. In all the other cases the blood was withdrawn at the time of operation.

In this group, out of 9 cancer cases, positive reactions were obtained in 4, one of these being a mammary cancer: the other mammary cancer was negative. In 17 non-malignant cases which may serve as controls we have 7 positives and 10 negatives, which is approximately the same proportion as in the malignant cases. The only two sera which contained haemoglobin were both negative: only one of the six cases in which the blood was withdrawn less than 4 hours after a meal gave a false reaction. Case 38 was clinically considered to be carcinoma of the intestine, and a portion of gut was removed under that belief; the reaction was positive; histological examination showed it to be tuberculosis. On the other hand, No.39 - a small ulcer on the lip - was thought to be epithelioma; the reaction was negative and the histological examination showed it to be tuberculous.

(It may be of some interest to add that another pathologist who was testing Besredka's complement deviation method in tuberculosis was supplied by me with these two sera and he was given the clinical diagnosis corresponding: his results, which he communicated to me, agreed with the clinical diagnoses: it will be noticed that microscopic examination of the tissues removed proved them to be wrong.)

No. 40 - an abdominal mass - was diagnosed as cancer; the reaction was positive; operation showed it to be a case of old peritonitic adhesions. No. 35 was an interesting case; it was clinically considered to be malignant; the reaction was positive; histological examination showed it to be quite benign in nature.

It is as well to have the fullest possible information before deciding whether the reation is right or wrong.

@ @ @ @

A large epithelioma of the tongue with large glandular masses in the neck was used as substrate in the following 14 cases:

TABLE VIII.

Disease.	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Epithelioma + 1 c.cm. Serum	Epithelioma + 1.5 c.cm. Serum
52. Epithelioma of tongue	0	0	0	0
53. " "	0	0	0	0
54. Epithelioma of lip	0	0	+	+
55. Epithelioma of palate	0	0	0	0
56. Epithelioma of Oesophagus	0	0	0	0
57. Advanced carcinoma of rectum.	0	0	+	+
58. Advanced carcinoma of rectum	0	0	0	0
59. Syphilitic glossitis	0	0	0	0
60. Normal male	0	0	0	0
61. Tertiary syphilis	0	0	0	0
62. " "	0	0	0	0
63. Intestinal adhesions	0	0	0	0
64. Chronic constipation	0	0	0	0
65. Tuberculosis	0	0	+	+

In none was the serum haemoglobin-stained. In Cases 57, 58, 60, 61, 62, 64 the blood was withdrawn less than four hours after a meal; in the other cases it was removed at time of operation.

The reaction was positive in only 2 of the 7 cancer cases and negative in 6 non-malignant cases, but it was positive in a case of tuberculosis (histologically verified) which was thought clinically to have been lymphadenoma.

@ @ @ @ @

A carcinoma of the breast which contained much blood, no attempt being made to wash it free, was used as substrate in 8 cases:

TABLE IX

Disease	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Carcinoma +1 c.cm. Serum	Carcinoma +1.5 c.cm Serum.
66. Cancer of Breast	0	+	0	+
67. " "	0	+	+	+
68. Cancer of Uterus	0	0	0	0
69. " "	0	+	0	+
70. " "	0	+	0	+
71. " "	0	0	0	+
72. " "	+	+	+	+
73. Epithelioma of Palate	0	+	+	+

In none of these cases was the serum haemoglobin-stained. All the cases were advanced far beyond operability. Two of the very grievous hypothetical fallacies were purposely committed - the substrate was not washed free of blood and the blood in all cases was withdrawn soon after a meal. Two controls of non-cancerous serum were employed, but as they gave reverse results - that is, reactions with the serum tubes and none with the tubes of serum plus cancer - they were rejected. Nos. 65, 67, 68, 69, 71 (that is, 5 out of the 8) would be considered negative according to any method of reading the results, whilst the remaining 3 could only be considered positive by rejecting the readings of the tubes with the larger quantities of serum. As far as these tests go, there is no support lent to the idea that the two purposely-made mistakes of technique had anything to do with the false reactions; instead of these reactions being negative, they ought to have been doubly positive.

A rodent ulcer was used as substrate in 4 cases. one of these being the case from which the tumour material was removed: —

TABLE X

Disease	Serum alone 1 c.cm.	Serum alone 1.5 c.cm.	Rodent ulcer + 1 c.cm Serum	Rodent Ulcer + 1.5 c.cm. Serum
74. Rodent ulcer	+	+	+	+
75. Carcinoma of Rectum	0	0	0	+
76. Furunculosis	0	0	+	+
77. Syphilitic glossitis	0	+	0	+

In none of these was the serum haemoglobin-stained.

In the first two cases the blood was removed at time of operation; in the last two cases probably less than four hours elapsed between a meal and the time of veni-puncture. The reaction gave three false indications out of four. The blood serum of the rodent ulcer did not show evidence of any ferment activity against the protein of its own tumour.

@ @ @ @ @

A cellular carcinoma of the breast was used as substrate in the following 8 cases:

TABLE XI

Disease	Serum alone 1.c.cm.	Serum alone 1.5 c.cm.	Carcinoma + 1 c.cm. Serum.	Carcinoma + 1.5 c.cm. Serum.
78. Epithelioma of tongue	0	0	0	0
79. Carcinoma of Rectum	0	0	0	0
80. Epithelioma of bladder	0	+	0	+ +
81. Locomotor ataxy	0	0	0	0
82. Sacro-iliac disease	0	0	0	+
83. Renal calculus	0	0	0	+
84. Papilloma of rectum	0	0	0	+
85. Cholecystitis	0	0	+	+ +

In none was the serum haemoglobin-stained. In No. 81 the blood was removed less than four hours after a meal; ~~the~~ in the others, all operation cases, it was withdrawn at the time of operation.

In the 3 cancer cases, the reaction was positive only in 1, but it was positive in 4 out of the 5 non-malignant cases.

@ @ @ @ @

In another series (Table XII) the serums were tested simultaneously against four different cancers: (I) a carcinoma of the breast; (2) an epithelioma of the cervix which it was impossible to free from blood; (3) a protuberant epithelioma of the vulva; (4) an epithelioma of the tongue.

TABLE XII

Disease.	Serum alone		Mammary cancer + serum		Uterine Cancer + serum.		Vulvar Epithelioma + serum		Lingual Cancer + serum	
	1 c.cm	1·5 c.cm	1 c.cm	1·5 c.cm	1 c.cm	1·5 c.cm	1 c.cm	1·5 c.cm.	1 c.cm.	1·5 c.cm.
86. Carcinoma of Breast.	0	0	0	0	–	++	–	0	–	0
87. "	0	0	0	0	–	+	–	0	–	0
88. "	0	0	0	0	–	+	–	0	0	–
89. "	0	0	0		–	+++	–	0	–	0
90. "	0	0	0	0	–	+	–	0	–	0
91. "	0	0	0	0	–	0	–	0	–	0
92. Carcinoma of Uterus.	0	0	–	0	+	+	–	0	–	0
93. "	0	0	–		0	++	–	0	–	+
94. "	0	0	–	0	0	0	–	+	–	0
95. Carcinoma of Rectum	0	0	+	+	–	++	–	+	–	+
96. "	0	+	0	+	–	+	–	++	–	0
97. Carcinoma of Vulva	0	0	+	–	–	0	0	+	–	0
98. Dental cyst	0	0	0	+	–	0	–	0	–	0
99. Fistula in ano	0	0	0	+	–	0	–	+	–	0
100. Fibroid of Uterus	0	0	+	+	–	+++	–	0	–	+

None of the serums was blood-stained.

If we consider all the cancer tissue "substrates" together, and imagine that one will supply the deficiencies of another, we should find that in the fifteen cases cancer was picked out in all but one of the cases but by the same method we would be forced to agree that the reaction was positive in all three non-malignant cases.

With the mammary carcinoma substrate only 4 cancer serums out of 12 gave positive results, and all 3 non-malignant cases were positive; with the uterine cancer substrate 9 cancer serums out of 12

gave positive results, and 1 of the 3 non-malignant cases was positive; with the vulvar cancer substrate 4 cancer serums out of 12 were psoitive, and of the non-malignant serums 1 was positive; with the lingual cancer substrate 2 of the 12 cancer serums gave positive results, and of the non-malignant cases 1 was positive. The mammary cancer substrate only succeeded in picking out 1 of the 6 cases of mammary carcinoma, the uterine cancer substrate picked out 2 of 3 cases of uterine cancer, and the vulvar cancer substrate picked out the case of vulvar cancer.

@ @ @ @ @

To sum up, the results of the clinical application of Abderhalden's method to the diagnosis of cancer in 100 cases, 51 of which are known to be cancer and 49 non-malignant, show us that the reaction was positive with 28 cancer sera i.e. 55 per cent, and also positive in 18 of the non-cancer controls i.e. 37 per cent. The method, therefore, is without diagnostic value, either taken by itself or as an adjunct to other uncertain indications.

@ @ @ @ @

GENERAL CONSIDERATIONS of the REACTION.

It is necessary, in view of the adverse results here obtained, that contrast so strikingly with those of the brilliant and deservedly honoured originator of the theory and with those of his numerous disciples in all parts of Eupope and America, to search out the factors that led to the amazingly mistaken claims, as I consider the m

to be, and to show as well as I can, that my own results are not due to errors of technique or to biassed misunderstanding. It seems to me that the theory originated from the readings of some preliminary experiments. It may be that those readings unconsciously exaggerated the facts; the fundamental observations were not made sufficiently indubitable for a great structure to be reared upon them; and the observer, having satisfied himself that a certain result was to be expected, and knowing exactly what he had to deal with when identical experiments were repeated, could not avoid obtaining readings similar to the original. If the repetition of the experiments was entrusted to a disciple, as it probably was in a busy laboratory, there would be the greater chance of the original views being substantiated. Whether or not that be an ungenerous supposition, the fact remains that the fundamental experiments do not cover a sufficiently wide field, and it is obvious that a great jump was made from serum reactions to substances quite foreign to the animal body, to serum reactions to substances natural to the body. The experiments with the serum of pregnant women, viewed through the eyes of the impelling theory, would at first seem to justify this jump. The same phenomena were found, and these were rendered all the more plausible since the serum of many non-pregnant individuals failed to give them. False results would crop up, however, as the number of the latter cases increased. Surprised at these, an enthusiast would seek causes for rejecting them. If all biological laws were as dependable as the movements of the stars, then there would be little scope for the making of errors, and research would be unromantically monotonous. The unexpected false results would be associated with some accompanying phenomena, and a causal relationship between them would

be assumed, so that on the recurrence of false results and their accidental association with such conditions one would feel in a position to reject them. In the course of time other phenomena would be found in association with untoward results and again means would be found to reject them. This seems to have been the history of the Abderhalden reaction. Now it bristles with hypothetical fallacies that have well-nigh obscured the reaction itself.

@ @ @ @ @

During the currency of the clinical experiments and before any adverse criticism of Abderhalden's theory had appeared it seemed interesting to me to ascertain if by some other simple method of estimating the effects of proteolytic enzymes we could obtain confirmatory evidence. An application of the Sörensen-Schiff formaldehyde titration method suggested itself. This depends on the power of an aldehyde to alter the reaction of a neutral or amphoteric substance containing both an amino and a carboxyl group. Such a substance on titration with an alkali will be found to be neutral or very slightly acid, but on the addition of formaldehyde the amino group will be replaced by methyleneimino acid, and the definitely acid reaction of the latter can be estimated by alkali titration. If we take a simple amino acid like glycocol the reaction would be:

$$CH_2(NH_2).COOH + HCHO \rightleftharpoons H_2O + CH_2(N:CH).COOH$$

A peptone is composed of a mixture of amino acids and complicated substances with the characters of polypeptides, the latter being formed by the aggregation of two or several amino acids. Thus

glycylglycine, one of the simplest of polypeptides, is formed by the coupling of two molecules of glycocol:

$$CH_2(NH_2).COOH + CH_2(NH_2).COOH = H_2O + CH_2(NH_2).CO.NH.CH_2.COOH$$

and this polypeptide on hydrolysis will give us two molecules of glycocol. Now glycylglycine treated with formaldehyde will give us a methyleneimino derivative as follows:

$$CH_2(NH_2).CO.NH.CH_2COOH + HCHO = CH_2(N:CH).CO.NH.CH.COOH + H_2O$$

in which the amino group (NH_2) is replaced by the methyleneimino group (N:CH), and every molecule of the substance will require one molecular equivalent of sodium hydroxide to neutralise it to phenolphthalein. But as two molecules of glycocol result from the hydrolysis of glycylglycine these will react with formaldehyde to produce two molecules of methyleneimino acid, and these two latter will require twice as much sodium hydroxide for neutralisation as the methyleneimino derivative of glycylglycine, from which they have been obtained by hydrolysis, seeing that the derivative contains twice as much of the acid carboxyl group (COOH) as the original substance

$$CH_2(N:CH).CO.NH.CH_2COOH + 1\ NaOH = CH_2(N:CH).CO.NH.CH_2.COONa + H_2O$$

$$\downarrow H_2O$$

$$2CH_2(N:CH).COOH + 2NaOH = 2\ CH_2(N:CH).COONa + 2H_2O$$

If we treat a solution of a digestion product containing polypeptides and amino acids with formaldehyde we shall require a certain amount of sodium hydroxide for neutralisation, but if such a solution be previously hydrolysed by ferments we shall require a larger quantity of the alkali because amino acid and carboxyl groups will be set free. Hence if a certain peptone is digested with a substance in which we suspect a proteolytic enzyme, and the acidity after the addition of formaldehyde is estimated, the increase of acidity will enable to

detect the presence of the ferment.

In my experiments I endeavoured to compare the results of fresh and of heated serum. The latter was subjected to a temperature of 58°C for an hour in the belief that the specific proteolytic ferments of Abderhalden would be destroyed. At a later date Abderhalden himself confirmed the supposition that his ferments were rendered inactive at such a temperature. Placenta or cancer tissue was prepared in the ordinary way and then desiccated in vacuo over sulphuric acid and finally pulverised. Tubes were put up in parallel containing 1gr. of the powered "substrate" and 1 c.c. or 1.5 c.c. of the fresh serum in one case or the same amount of inactivated serum in the controls, together with 10 c.c. of distilled water. Toluol was floated on the surface of the tubes to prevent putrefaction and the tubes were incubated at 37°C for about 24 hours. 10c.c. of formaline previously neutralised to phenolphthalein by sodium hydroxide were added, and the mixture titrated. If a proteolytic ferment were present we should expect a difference of 2.5 to 4cc or more: but under any circumstances we should expect a slight rise in acidity either owing to the acidity originally present or to a slight degree of hydrolysis on exposure of a solution of peptone to 37°C for several hours. The following experiment out of several shows what happened with powdered placenta. The serum was from a pregnant woman and it gave a positive Abderhalden reaction by the dialysation method. A mixture of 1 gr. of placenta and 1.5cc of serum gave at once a good pink colour with 2c.c. of decinormal soda, the formalin added being neutralised till it gave just a very faint pink ~~colour~~ tint. With 1.5cc of fresh serum and 1 gr. of placenta and 20cc of distilled

water, the mixture being incubated for 27 hours, we got a titration of 10.2, and a titration of 10.7 where only 1 cc of serum was used. The controls with the heated serum gave titrations of 9.3 in both cases. In another series of experiments with heated and unheated sera and powdered gastric carcinoma tissue we got readings of 11.6 and 12.8 respectively (serum from a rectal carcinoma), 11 and 11 (serum from a mammary carcinoma), and 10.2 and 12.4 (serum from a case of cancer of the uterus). The use of such powdered material did not therefore give any indications of proteolytic activity on the part of the sera. We therefore obtained from the Hoechst Laboratory a specimen of placental peptone which was prepared under the directions of Abderhalden. This was employed in the quantities of 0.1 gr. with the results shown in the subjoined table:

TABLE XIII

Sörensen titration after 24 hours.

Serum from	1.5 cc fresh serum	1.5 cc heated serum	Difference
3 months pregnancy	4.2	3.6	0.6
Chorionepithelioma	3.7	3.5	0.2
Uterine carcinoma	3.95	3.5	0.45
Cancer of breast	4.05	3.5	0.55
Cancer of rectum	4.2	3.7	0.5
Normal case	3.8	3.4	0.4

The differences given in the table are not specific and are much too small in comparison with what one could expect if ferment activity had been in operation.

Michaelis and von Lagermarck (Deut.med.Woch.,1914,XL,317)

incubated the serum-substrate mixture and, after precipitating the proteins with colloidal ferric hydrate, estimated the amount of nitrogen in the filtrate. They were unable by this method to demonstrate any constant ferment action in the case of pregnant sera or any constant difference from other sera. Van Slyke and his collaborators (Journ. of Biol.Chem., 1915,XXIII, 377) precipitated the protein from the incubated serum-substrate mixtures with colloidal ferric hydrate and estimated by their "micro-amino method" the amount of free nitrogen in the evaporated filtrate. They found that practically every serum, whether from a pregnant or a non-pregnant individual, showed protein digestion when incubated with placental tissue prepared according to the rules laid down by Abderhalden, and that the range of individual variation in proteolytic activity was wide. The range covered by most normal sera was identical with that covered by most pregnant sera, though the average in the former was somewhat lower than the average in the latter, but the difference was so small that the reaction was quite indecisive for either positive or negative diagnosis of pregnancy. They also found that carcinoma tissue was digested to about the same extent as was placenta. A reading of their protocols would seem to indicate that human serum frequently if not always, shows a certain general proteolytic power. My figures just given support this, although, owing to the smallness of the differences, I am not disposed to attach much importance to the results. Since my paper was published numerous investigations have been made by biochemists employing various technical methods and an almost uniform agreement has been reached that there is no evidence in the serum of pregnant or diseased individuals or in

experimental animals of any specific proteolytic enzyme.

THE SUBSTRATE.

From the point of view of the theory of the reaction the proper preparation of the organ or tissue that is to serve as substrate for the "specific proteolytic ferment" is of the utmost importance. In the case of placenta, and similarly with other tissues, the fresh organ is washed free of blood until a "snow-white tissue is obtained". Let it be said at once that such a result is impossible. The method I have adopted is to remove the adherent blood clot, connect the umbilical vein with the water tap, and let the water flow through it for about two hours. At the end of that time the placenta should be practically colourless. The membranes are now removed, the placental tissue is cut up into walnut-sized portions, and these are kneaded in frequent changes of salt solution for another hour. Thereafter, the pieces, having been cut up into smaller fragments, are put into running water for a few hours. As these pieces lie in the bath they appear to be quite white, but if they are taken in bulk and the water expressed from them it will be found that they still preserve, no matter how long or how thorough the washing, a faint colour, which, in the case of the placenta, is a low-toned white or pale ivory. Every tissue that I have tried preserves a faint tint proper to the particular organ, and a "snow-white" condition cannot be produced. It is specially difficult to get cancers free from blood. I have used only fresh operation material and though there has been a large stock at my disposal it is seldom that we can get tumour material exactly suitable. It is very doubtful if the presence of small traces of blood - or even very large amounts, for that matter -

has anything to do with the false reactions. Even supposing we admitted the possibility of some obscure ferment in the serum capable of acting on the very minute traces of blood still left in the washed tissue, so as to give sufficient amounts of diffusible substances detectable with ninhydrin (a rather large supposition) - that is to say, giving a positive reaction where a negative reaction is to be expected - the hypothesis would not account for negative reactions where positives are to be expected according to the theory. I have obtained such negative reactions even in pregnancy. The tissue that is to serve as substrate is boiled in distilled water, to which a few drops of acetic acid are added, then for several times in distilled water alone, being well rinsed between each boiling, until the boiled water, which ought to be about five times the volume of the tissue, ceases to give a reaction when 10 c.cm. of it are boiled with 1 c.cm. of 10 per cent. ninhydrin solution. Any one may easily satisfy himself that even then the boiled water may show a violet colour when stronger concentrations of ninhydrin are used, and I have always gone on with the boiling until the water was colourless to as much as 2 c.cm. of the ninhydrin solution. Placental and other tissues vary very much in the number of boilings they require before they are suitable. I have often noticed the curious fact that the boiled water may give a colour with weak concentrations and none at all with stronger concentrations of ninhydrin. The explanation of that fact is a question for chemists, but it is a point to bear in mind that ninhydrin may fail to detect the presence of protein products simply because it is too strong, just as it may also fail to detect them because it is too weak. I have further

observed that the water of the tenth boiling of a tissue, for example, may be colour-free to ninhydrin, whilst that of the twelth may give the reaction. Once having got the water colour-free, if the tissue be allowed to stand, colour reactions may be ~~allowed to stand~~ obtained after varying periods of time, sometimes within a few hours. Some tissues seem to be very unstable in this respect. The ninhydrin reacting substances found in the boilings of tissues are diffusible through the dialysers, and even though we test the tissue immediately before use, as we always do, we may be sure that there is always a certain amount of diffusible substances from our substrate - apart from any other factor - capable of contributing to the result.

One of the hypothetical fallacies that Abderhalden has recently detected is that the connective tissue in the organ acting as substrate may itself be attacked and broken down by some intruding ferment; there may be some lesion of the connective tissues in the individual who furnishes the serum. This reduces the reaction to a farce, for there is no organ devoid of connective tissues and no organ disease incapable of implicating them. Besides, the latest method he adopts in the preparation of his substrates - the pounding of the tissues in a mortar - is calculated to get rid of as much parenchyma and to leave as much connective tissue as possible. The great difficulty in the preparation of cancerous material is to get rid of the blood and yet to leave the cancer cells in their connective tissue stroma. The more cellular the cancer the more easily are these cells removed by washing, and the fact that the connective tissue stroma renders the blood vessels of a malignant tumour so exceedingly tortuous militates against the freeing of the tissue from

blood. In this case there are only two ways of getting suitable "substrates" - either to cut very thin slices of the tumour, running the risk of losing the epithelial cells, or to reject tumour after tumour until we light upon something suitable at last.

It has been imagined that in the case of a blood effusion a ferment will be elaborated against the corpuscles of that effusion, and consequently, if any tissue employed as substrate should contain some blood, then the serum of that case would in these circumstances be capable of giving a false reaction. I have already pointed out that I could find no evidence of this in experiments on rabbits. I have, however, used as substrate pure blood clot, boiled free of ninhydrin-reacting substances, and tested this with fifteen serums, one of these being from a case of cerebral apoplexy, and most of the others from cancer cases in which local haemorrhages were evident, with the result that in no single case was there the slightest suspicion of a positive reaction. Again, a piece of cancerous tissue - a mammary carcinoma - containing much blood was forthwith boiled without any preliminary washing, and tested with various serums; it neither picked out all the cancer serums nor gave reactions with all the non-cancerous; it merely behaved, or misbehaved, as other substrates scrupulously prepared according to the regulations. (See Table VI) Lange (Biochem. Zeitschr. 1914, vol. LXI, p.193) amongst others also found that the presence of blood in the substrate had no influence on the result of the reaction. Bullock (Lancet, 30th Jan. 1915) subsequently found that by preparing two "substrates" from the same placenta, one being free of blood and the other boiled without the blood being removed, the same reactions were obtained in both cases.

But whatever theoretical objections be brought against the reliability of the substrates that I have used, whether it be that they were not absolutely snow-white, that they still still held in spite of our care minute traces of blood, that they were too unstable, or that they contained connective tissue, the criticism quite loses its points if we can get false reactions with "substrates" that are beyond suspicion. I tried, therefore, as "substrates", pieces of sterilized sponge, kaolin, and glass wool. In this way 39 serums were tested; I obtained 7 well marked positive results. Subsequently PLAUT (Muench.Med.Woch.1914 p.238) obtained positive results where he used as "substrates" kaolin, barium sulphate, talc, and Kieselguhr. Like him I have also noticed reverse effects (that is, reactions of the controls without substrate, and no reactions with the serum and substrate) in some cases, but the same thing has occurred several times in the ordinary procedures. It will hardly be argued by the most ardent believer in the Abwehrfermente theory that protected ferments are elaborated within the animal body capable of splitting up into peptones or amino-acids such substances as sponge, glass wool, and kaolin. Such an experiment is sufficiently striking to prove the worthlessness of the dialysation reaction and I am convinced that the important point in the reaction is that the substrate, owing to its physical nature, splits up the serum, and not so much the serum that splits up the substrate.

@ @ @ @ @

In all cases I have withdrawn at least 20 c.cm. of blood from the median basilic or cephalic vein direct into sterile centrifuge tubes. In the middle of the wool plug of the tube there is a length of glass tubing (2 inches of tubing of 0.5 cm. bore) projecting on both sides of the plug: the external end is connected with three inches of rubber tubing, at the other end of which is a wide bore Record needle: the latter is inserted through the wool plug of a Wassermann test-tube: and the whole apparatus is sterilized in the autoclave. The skin of the arm is well rubbed with ether and the vein rendered more prominent by the application of a tourniquet.

The blood is allowed to clot spontaneously, and the serum removed after a few hours or immediately by centrifugalization; it does not matter which. Some serums, even from normal cases, are haemoglobin-tinted on each occasion on which we examine the blood; it seems to be a healthy abnormality. We do not know the reason of this. Abderhalden has insisted from the beginning that haemoglobin-stained serum should not be used, as the presence of haemoglobin indicates that the corpuscles have broken up and shed into the surrounding fluid their endocellular ferments, some of which are bound to be proteolytic. Either this is a purely hypothetical objection, capable of substantiation or otherwise, or it has been invoked to discount false positive reactions, and, as haemoglobin-tinted serum is not uncommon, a relationship between the two has been drawn. The mere presence of haemoglobin does not give rise to reactions in the dialysate. I have carefully noted the behaviour of haemoglobin-tinted serum in over 20 cases, but false positive

reactions did not seem to be as frequent in these experiments as in the case of normally coloured serums.

Lange (loc.cit.) has arrived at the same conclusion.

Another point on which Abderhalden lays stress is that if the blood be withdrawn from a patient within four hours after a meal, the serum may produce false positive reactions. This, again, is a hypothetical objection, and by means of it one is enabled to explain away most of the adverse results. It may be said that the blood after a meal contains fugitive proteolytic ferments, or that there is a temporary accretion of free amino-acids. It is probable that the latter is true. Indeed, Costantino (Biochem. Zeitschr. 28th Sept., 1913) had shown that the blood of fed animals contains more amino-acid (formol-titratable nitrogen) than the blood of starved animals, but he found that the excess was anchored to the corpuscles, and was not free in the plasma.

Later Bullock (loc.cit.) dialysed the serum of fasting rabbits and of those which had been fed three hours beforehand, and found that the increase in amino acid in the latter series was too small to be detected in 2 c.c. of the serum.

In our own experiments, even supposing the presence of amino-acids in the serum had something to do with the production of colour reactions in the dialysate, yet a positive reaction is not reckoned by a mere presence of colour in a single dialysate, but by the comparison between the colours given by a test and by its controls; and the factor would be equal in both. I have always noted the relationship to time of last meal, and a consideration of the tables will show that the hypothesis does not hold good in practice. Still,

whatever objection there be to the usage of serum taken froma patient who is not in a fasting condition, it must be admitted that it is well-nigh impossible to amass any large number of observations under such ideal circumstances. Our habits are such - and this applied to Germany quite as much as to this Country - that seldom more than four hours elapse between meals of some sort, except when we are asleep, and any investigator would have little else to do who attempted to obviate the hypothetical fallacy. I have been fortunate in complying with the requirements in the great majority of my cases, for the blood was removed at the time of operation, when a minimum of six hours had intervened since the last meal. I have, on the other hand, examined blood taken actually during, or shortly after meals. It does not seem to make the slightest difference.

If a number of serums be tested, using dialysers of apparently equal permeability to peptone, it will be found not infrequently that the dialysates react to 0.2 c.cm. of ninhydrin solution - that is to say, they allow some constituent of serum to pass through in sufficient amount to be detected in the ordinary way. Other serums, again, do not give sufficient quantities to be detected with 0.2 c.cm. but if one tests the dialysates in these cases with slightly larger amounts of ninhydrin, it will be found that in practically every case some reacting substance has diffused through. This shows that serums though varying in their content of diffusible ninhydrin-reacting substances always furnish a certain amount to the dialysate. I have ~~dialysated~~ dialysed against running water for several hours serums which previously gave large amounts of reacting substances to the

distilled water, and finally dialysed this again against distilled water and I have found that this dialysis against running water did not succeed in wholly removing, sometimes not in appreciably diminishing, the diffusible substances. This shows that the serum is not a stable material, but is splitting up progressively; it is partly an inherent characteristic of serum, and is partly due to the action of the distilled water.

@ @ @ @ @

THE DIALYSATION TUBES.

Specially prepared dialysation tubes for the reaction were supplied by Schleicher and Schull No. 579a. These are supposed to be impermeable to protein, but permeable to peptones and other degradation products of proteins, and, though they are sold as having passed the tests, it is wise to satisfy oneself on the point. In the first consignments it was found that a fairly large percentage were permeable to protein, but in the more recent comparatively few failed to pass the tests that Abderhalden demands. They must be subjected frequently to these tests during the course of the experiments, and if the dialysers be numbered and at any time one is found faulty the cases in which it has been used since it last passed the examination will have to be rejected. In testing for the impermeability to protein, 2.5 c.cm. of a 5 per cent. suspension of fresh egg albumen are added to each tube, and this is dialysed

against 20 c.cm. of distilled water for sixteen or more hours, and the dialysates are tested for the biuret reaction. If the reaction is positive, the corresponding tube is rejected. It is very difficult, if not impossible, to be certain of detecting weak biuret reactions, and in every case I have repeated the test of the dialysate with ninhydrin. It is true that the two methods do not give strictly comparable results. It is considered by Leonor Michaelis and v. Lagermarck (Deut.med.Woch., 12th Feb. 1914, p.316) that the biuret reaction is not to be depended on, at least when performed in the way that Abderhalden recommends. They assert that they can in the same way obtain biuret reactions with distilled water, and they recommend the test of the dialysate with sulpho-salicylic acid. As this is a fairly delicate reagent for albumen I used it in the latter part of my work.

The second requisite is that they be permeable to peptones and all equally permeable. If we test a stock of dialysers with silk peptone, which is supposed to be a pure peptone, boiling the dialysates with 0.2 c.cm. of ninhydrin solution, we shall find that differences in depth of colour may be made out, showing that they are not uniformly permeable, and we must reject the extremes. The differences in permeability can be shown much better by using Witte's peptone, which is an impure peptone. The individual differences are very marked, and it is practically impossible to get a large series of absolutely comparable dialysers. At first sight it might be thought that by taking a large stock of dialysers, and by estimating the depth of colour in the corresponding dialysates by means of a colorimeter, we might arrange our dialysers in order of permeability.

Unfortunately this is not satisfactory for the reason that the actual colours, as well as the tints, differ, and no strict comparison can be made. What is more important is that the permeability of any single dialyser is not constant; it decreases or increases by use; and what are to-day strictly uniform tubes exhibit in the course of time various differences. We may gain an idea of their variation by numbering them and observing the results they give with serum from time to time. The differences between them can be brought out, too, by testing a small batch with one serum and noting the depths of the colour reactions of their dialysates with increased quantities of ninhydrin. We have, therefore, to take into account two factors affecting the reaction, namely, that with uniform dailysers different serums vary in the amount of diffusible substances and that with a single serum dialysers vary in the amounts of ninhydrin-reacting substances they allow to pass. The only way, therefore, to be sure of obtaining reliable readings in practice is to manifold the tests and controls as far as the quantity of serum permits, and to strike a just average. I have never depended on single tests; they are worthless. It will sometimes be found that the dialyser containing substrate plus serum gives to the dialysate a less amount of ninhydrin-reacting substances than the dialyser containing serum alone. Such results and dialysers must in kindness be rejected before giving an opinion on the diagnositc value of the reaction, but must be borne in mind when considering the worth of a theory that relies for its substantiation on such fickle means.

The following is a typical experiment out of several that will

show how positive reactions may come about:

The serum used throughout was from a non-pregnant female. The Placental tissue was boiled repeatedly until the water was colourless to 2.5 c.cm. of ninhydrin solution. The dialysation tubes were chosen as being as nearly uniform in their permeability to silk peptone as it was possible to judge from the depth of colour which their dialysates gave with ninhydrin. All the tubes were incubated for sixteen hours in the usual way, and thereafter each dialysate was boiled with 0.2 c.cm. of ninhydrin, the resulting colour was noted, then a further 0.2 c.cm. of ninhydrin was added, the test tubes again being boiled, and once again this procedure was repeated.

Tubes 1, 2, 3, 4, 5, contained equal amounts of placenta with 1.5 c.cm. of saline solution only. The dialysates remained quite colourless to as much as 3 c.cm of ninhydrin solution, so that here apparently we may rule out of account any contributory factor from the placenta alone.

Tubes 6 and 7 contained 1.5 c.cm. of the unheated serum only. With 0.2 c.cm. of ninhydrin the dialysates were quite colourless; with 0.4 c.cm. both gave an equal faint golden colour; with 0.6 c.cm. both gave a light violet colour, more marked in No.7 than in No.6. Taking the weaker as an arbitrary standard for the colorimeter, and giving it the value of 1, the depth of colour of No.7 would be represented as $1\frac{1}{2}$.

Tube 8 contained 1.5 c.cm. of unheated serum along with placenta. With 0.2 c.cm. of ninhydrin the dialysate gave just the faintest grey tint; with 0.4 c.cm. a deep violet colour was produced; with 0.6 c.cm.

the colour was very deep. The colorimeter value was represented as 9

Tubes 9 and 10 contained 1.5 c.cm. of heated serum only. With 0.2 c.cm. of ninhydrin the dialysates were colourless; with 0.4 c.cm. a light violet colour was produced in No.9, and a golden colour in No.10; with 0.6 c.cm. No.9 gave a marked violet colour - represented by the value 6 - and No.10 gave a light violet - represented by the value $1\frac{1}{2}$ on our empirical scale.

Tube 11 contained 1.5 c.cm. of heated serum ~~only~~ along with placenta. With 0.2 c.cm. of ninhydrin the dialysate was colourless; with 0.4 c.cm. it was a faint lilac; with 0.6 c.cm. the colour was violet and the colorimeter value was represented by $2\frac{1}{2}$.

TABLE XLV

Tubes	Contents	Ninhydrin reaction if dialysates 0.2	0.4	0.6	Arbitrary colour value of deepest tint.
1.	Plac.1 gr.+1.5 cc Saline	-	-	-	-
2.	"	-	-	-	-
3.	"	-	-	-	-
4.	"	-	-	-	-
5.	"	-	-	-	-
6.	Plac.1 gr.+1.5 cc fresh serum.	-	v.f.	+	1
7.	"	-	v.f.	+	$1\frac{1}{2}$
8.	"	v.f.	++	+++	9
9.	1.5 cc heated serum	-	+	+++	6
10.	"	-	v.f.	+	$1\frac{1}{2}$
11.	Plac.1 gr+1.5 cc heated serum	-	f.	++	$2\frac{1}{2}$

This experiment clearly shows (1) that dialysers, apparently uniform in their permeability to silk peptone, vary very much in permeability when actually tested with serum; (2) that all allow some ninhydrin-reacting constituent of serum to pass through; (3) that the heating of the serum does not abolish, though it may diminish, the liability to spontaneous cleavage, and (4) that the reaction is not a quantitative one. That the dialysers are liable to allow a certain amount of diffusible substances from serum to pass is practically, though not actually, admitted by Abderhalden himself, seeing that he lays such stress on the equal boiling of the dialysates. I tried the reaction in a few cases with collodion sacs instead of Schleicher and Schüll's dialysation tubes. These were prepared by smearing the inside of a test tube with a trace of glycerine on the point of the finger, pouring in and draining off celloidin dissolved in acetone (2.5, 5, or 10 per cent), detaching the collodion tube and testing it by water pressure. The results were quite as unfavourable to Abderhalden's theory as with the parchment thimbles.

We may gain an illuminating insight into the fallacies of the method by performing the following experiment suggested by certain observations of Frank, Rosenthal, and Biberstein (Muench.med. Woch., 22nd July, 1913). Into one dialyser let us put the substrate alone, and into another the specific serum alone, and dialyse them in the usual way, and we shall probably find that the dialysates in both cases do not give reactions with ninhydrin in the usual concentration. But let us, at the same time, put two dialysers, one containing substrate only and the other the serum only, into the same container

and we may find that in this case the dialysate gives a good violet colour with the usual amount of ninhydrin. This excludes the possibility of the operation of a ferment, and shows that the result is due, in this case, to the summation of two effects which by themselves were not capable of detection.

@ @ @ @ @

CONCLUSIONS.

The conclusions to which I have come after a careful investigation of Abderhalde's dialysation method are that its real fallacies are beyond control, and that the hypothetical fallacies which Abderhalden invokes to account for false reactions have no bases. Positive results are quite well accounted for by the facts that the "Substrates" alone, and the serums alone, give diffusible substances reacting with ninhydrin; that the dialysers vary enormously in the quantities of such substances which they allow to pass in a given time; that serums, influenced by the distilled water and by the presence of a tissue acting in virtue of its physical state, are progressively cleaved; and that it is not the substrate which is split by the serum so much as the serum which is split by the substance used as substrate. These very real fallacies quite destroy the value of the method, and they cast grave doubts on the whole theory.

Other investigators at a later date, using the polarimetric method confirmed the conclusions to which I had come.

But it may pertinently be asked why we obtained such a large number of positive results in the serum of pregnancy in comparison to

diseased conditions. As I said before, I may have been unconsciously generous to an attractive theory in my reading of the reactions, but I do not attach much weight to that explanation. It may be that pregnant serum more commonly possesses the property of being split up when some suitable physical accessory is added, such as placental tissue. But what seems more probable, and in saying this I do not detract from the general criticism nor give support to the theory of Abderhalden, is that there exists in serum, more frequently, though not exclusively, in pregnancy, a general proteolytic and peptolytic power which we can demonstrate by adding a suitable, though not necessarily a specific, protein or peptone. The serum of the normal guinea-pig is generally credited with a fairly strong proteolytic power. I tested the action of several guinea-pig serums on egg-albumen, coagulated cancer tissue, human blood clot, and human placenta, and it happened that only with the latter were strong reactions evident. That seems to show that placental protein is a suitable substrate for the demonstration of a general proteolytic power. Pincussohn and Petow, in the experiments previously cited, found that the normal serum of each species of animal had a peptolytic power against the muscle peptone of that species only, whereas the serum of the guinea-pig had a peptolytic power against the muscle peptones of all other species tested. We may thus even admit that, more in pregnancy than in other states, the human serum may have a proteolytic and peptolytic power against the proteins of human tissue more than against other proteins; even that certain proteins, owing to their physical configuration, are more suitable than others for a demonstration of these enhanced powers; and yet we may condemn Abderhalden's whole theory of specific guardian ferments.

THE KNOWLEDGE OF TUMOURS DURING CLASSICAL TIMES.

Doubtless there were great physicians in Greece before Hippocrates practised in the art and skilled in the science of medicine, but like the last Laird of Ravenswood their "names are lost for evermoe" and with them their teachings. The only remains of thought that come down to us, preserved by the rhapsodists, are the Homeric works and other poetical fragments. Medical discourses at no time have been of sufficient general appeal to be kept alive by oral tradition. In the nature of things a great contribution to art is a thing of beauty, apparent and appreciable for all time, whilst a great contribution to science, though it may be ephemerally distinct, and while it never passes into nothingness, is in the long run merged unrecognisably and anonymously into the growing mass of truth. We may delve with pleasure and great profit into the polite letters of long ago and feel refreshed by so doing, but the reading of scientific writings even a couple of generations old is as exhilarating a pastime as a visit to the tombs of our forefathers. However, it behoves us on occasion to make the dutiful pilgrimage, though we feel refreshed only when we return to the present. The scientific process is essentially a forming of the comparatively shapeless, and it is not without interest to watch the rough-hewing of the old pioneers, since in many departments of medical knowledge our ideas are, we must confess, still shamefully crude.

* * * *

In the early Greek writings tumour-growths as a whole were not sharply defined from other conditions; they were spoken of broadly as swellings. The word in common use was φῦμα, and it is a mistake to interpret this in any particular instance as cancer or even tumour in our sense, as some writers have done. For example, Wolff (Lehre von der Krebskrankheit) has cited the case of Atossa as being one of mammary cancer, and recently Ewing in his book on Neoplastic Diseases copies him in his error. The passage occurs in Herodotus, Hist., book III, chap.,138:-

> Ἀτόσσῃ, τῇ Κύρου μὲν θυγατρὶ καὶ Δαρείου δὲ γυναικὶ, ἐπὶ τοῦ μαστοῦ ἔφυ φῦμα, μετὰ δὲ ἐκραγὲν ἐνέμετο πρόσω. ὅσον μὲν δὴ χρόνον ἦν ἔλασσον, ἡ δὲ κρύπτουσα καὶ αἰσχυνομένη ἔφραζε οὐδενί, ἐπείτε δὲ ἐν κακῷ ἦν, μετεπέμψατο τὸν Δημοκήδεα καὶ οἱ ἐπέδεξε.
> (Atossa the wife of Darius and daughter of Cyrus had a swelling in the breast which subsequently ulcerated and spread. While

it was fairly small her modesty prevented her from telling anybody but when it grew worse she sent for Democedes and showed it to him.) Skilful beyond the physicians of his time as Democedes no doubt was, it is unlikely that the resources of his craft would have been able even temporarily to arrest a malignant disease. At that time - 520 B.C. - Queen Atossa would be somewhat under thirty years of age; she afterwards bore four children, and she was alive when Darius died thirty-five years later. (She comes on the stage in the Persae of Aeschylus as a rather forlorn motherly old body.) It is impossible that she had cancer of the breast.

* * * *

Hippocrates lived and taught at that most wonderful time in all the world's history when the gods showered on Greece their hoarded gems of marvellous glory, literary, philosophic, and artistic, gems that sparkle still undimmed on the bosom of civilisation. The knowledge of writing was then generally diffused and we own today the words of the great master. It is true that several of the writings attributed to him were the work of followers at different dates, and a good edition of the genuine writings is much needed. In those which are generally accepted as authentic there is strangely enough only one reference to tumours. This is not to be accounted for by the paucity of cancer in those days, for it was probably as prevalent then as it is now. It is more likely that some of the works of Hippocrates have been lost. The single reference is abrupt and brief, but even as it is it is sufficient to let us know that tumours were recognised though the difference between a simple and a malignant growth may not have been appreciated.

> Ὁκόσοισι κρυπτοὶ καρκίνοι γίνονται, μὴ θεραπεύειν βέλτιον· θεραπευόμενοι γὰρ ἀπόλλυνται ταχέως, μὴ θεραπευόμενοι δὲ, πουλὺν χρόνον διατελέουσιν. (Aphorisms, sect,. VI, no. 38)
>
> It is best to leave occult cancers alone, for if they are treated the result is fatal, whereas if they are left alone the patients may live for quite a long time.

The word καρκίνος like its Latin equivalent "cancer" meant in ordinary speech a crab. We shall see later when we come to Archigenes the reason for the medical adoption of the term as descriptive of certain tumours. It is here used in

in literature for the first time in its present medical sense. The expression "occult or concealed cancer" may mean either that the skin over it is intact or that it is situated in some internal organ, and subsequent writers take one or other of these meanings. The probability is, as far as we can now judge, that Hippocrates meant unulcerated growths. It is possible that he considered that ulcerated and unulcerated tumours were different pathological entities. Most of his successors would certainly seem to regard them as distinct. There is, however, no question as to the soundness of his advice in view of the surgical and therapeutic means that were then at the disposal of the physician.

In the fifth book of the Epidemics, which is of doubtful authenticity, there is the following reference to cancer:-

> Γυναικὶ ἐν Ἀβδήροισι καρκίνωμα ἐγένετο περὶ τὸ στῆθος, καὶ διὰ τῆς θηλῆς ἔῤῥεεν ἰχὼρ ὑφαιμος· ἐπιληφθείσης δὲ τῆς ῥυσιος, ἔθανεν.
>
> (A woman in Abdera had a cancer of the breast with a blood-stained discharge from the nipple. When the discharge ceased the patient died.)

It may be noted here that the word carcinoma had not the restricted meaning that we now employ but was equivalent to our word cancer - which is indeed but the Latin translation of the same word. In the passage quoted, which is repeated almost word for word in the seventh book of the Epidemics, we recognise a case of duct papilloma.

Reading through the other remains of Hippocrates we may, as we fancy, detect references to tumours but they are so confused with what are obviously non-malignant lesions that we cannot separate them out. For example, in the second book of the Diseases of Women there is a long account of uterine conditions amongst which cancer of the uterus is probably included though by no means clearly differentiated. However there is a passage where he undoubtedly connects the formation of mammary tumours with the onset of the menopause:-

> καὶ ἐν τοῖσι τιτθοῖσι φυμάτια ἐγγίνεται σκληρὰ, τὰ μὲν μέζω, τὰ δὲ ἐλάσσω· καὶ οὐκ ἐκπυοῦνται, σκληρότερα δε αἰεί· ἔιτα ἐξ αὐτέων φύονται καρκίνοι κρυπτοί. ..... καὶ ἐκ τῶν τιτθῶν ἐς τὰς σφαγὰς ὀδύναι διαίσσουσι καὶ ὑπὸ τὰς ὠμοπλάτας, καὶ δίψα ἴσχει, καὶ αἱ θηλαὶ καρφαλέαι, καὶ ἄυται πᾶν τὸ σῶμα

λελυπτυσμέναι εἰσὶ.

(Hard tumours are formed in the breast of various sizes. They do not suppurate but become progressively harder. These constitute the occult cancers. ..... From the breast pains shoot up to the neck and under the scapulae. The patients complain of thirst, the nipples become dry, and the whole body is emaciated.)

The above extracts are the only clear references to tumours that I have been able to find in Littre's text of the Hippocratean writings.

* * * *

Almost four centuries elapsed between the time of Hippocrates and the appearance of any important medical work. It is very remarkable that the next great writer should have been a Roman, for during the intervening period and for long afterwards the pursuit of medicine was confined to the Greeks. In Rome,,apart from the Greek physicians, there were certainly native practitioners of a kind, even specialists, but no Roman, if he had any pretensions to respectability, would have condescended to work that was performed by slaves. How comes it then that we find the immortal Celsus in that gallery? Almost certainly he was not a practising physician, at any rate in his native land. A patrician of wide culture and familiar with the teaching of Greek medicine, he was probably an author of rather versatile outlook, and might have written quite as brilliantly on any other subject. Possibly in his travels he visited the schools of Alexandria and gained there a practical experience of medicine, or he may have learned all his medical lore from the Greeks in Rome. We have no means of tracing the writings from which he borrowed his information; his predecessors with the exception of Hippocrates are merely names to us. Here we have an insight into the knowledge of tumours as it stood at the dawn of the Christian era. (De Medicina, book V, chap. 28: Targa's text).

Carcinoma vitium fit maxime in superioribus partibus, circa faciem, nares, aures, labia, mammas feminarum; et in jecore autem, aut splene hoc nascitur. Circa locum aliqua quasi puncta sentiuntur; isque immobilis, inaequalis tumet; interdum etiam torpet. Circa eum inflatae venae quasi recurvantur, haeque pallent, aut livent; nonnunquam etiam in quibusdam delitescunt; tactusque is locus,

aliis dolorem affert, in aliis eum non habet; et nonnunquam sine ulcere duior aut mollior est, quam esse naturaliter debet; nonnunquam iisdem omnibus ulcus accedit; interdumque simile iis est quae vocant Graeci κονδυλώματα aspredine quadam et magnitudine sua; colorque ejus ruber est aut lenticulae similis; neque tuto feritur, nam protinus aut resolutio nervorum aut distentio inseguitur. Saepe homo ictus obmutescit atque ejus anima deficit. Quibusdam etiam si id pressum est, quae circa sunt intenduntur et intumescunt. Ob quae pessimum id genus est . Fereque primum id fit quod κακόηθες a Graecis nominatur; deinde ex eo id carcinoma quod sine ulcere est; deinde ulcus; ex eo thymium. Tolli nihil nisi cacoethes potest; reliqua curationibus irritantur,et quo major vis adhibita est, eo magis. Quidam usi sunt medicamentis adurentibus, quidam ferro adusserunt, quidam scalpello exciderunt; neque ulla unquam medicina profecit; sed adusta protinus concitata sunt et increverunt donec occiderent; excisa etiam post inductam cicatricem tamen reverterunt et causam mortem attulerunt; cum interim plerique nullam vim qua tollere id malum tentent, sed imponendo tantum lenia medicamenta, quae quasi blandiuntur, quominus ad ultimam senectutem perveniant, non prohibeantur. Discernere autem cacoethes quod curationem recipit, a carcinomate quod non recipit, nemo scire potest nisi tempore et experimento. Ergo ubi primum id vitium notatum est, imponi debent medicamenta adurentia; si levatur malum, minuunturque ejus indicia, procedere curatio potest et ad scalpellum et ad ustionem; si protinus irritatum est, scire licet jam carcinoma esse, removendaque sunt omnia acria, omnia vehementia.

(The most frequent sites for cancer are the upper parts, especially about the face, nose, ears, lips, and the breasts of women; but it may occur also in the liver or the spleen. Occasional stabbing pains are felt around the part. The tumour is fixed and irregular and the part is practically functionless. The surrounding veins are swollen and tortuous, pale or livid, and in some cases they cannot be made out. Some are painful and others painless to touch, and usually unulcerated tumours are harder or softer than the normal tissues. Often an ulcer is superadded. Occasionally it resembles

what the Greeks call "knuckle tumours" from their configuration and consistence. In colour it is red or dun. Surgical interference is dangerous for shock or nervous excitement is the direct result. An injury to the part may produce speechlessness or faintness, and in some cases pressure causes tension of the surrounding tissues. This is the worst form of the disease. The first stage of the disease generally is what the Greeks call "cacoethes"; this is followed by the unulcerated form of carcinoma; then succeeds the stage of ulceration; and finally we have the thymium or fungating stage. None of the forms except the cacoethes can be successfully removed; the others are aggravated by treatment, and the more violent the means adopted the worse it becomes. Some surgeons use caustic applications, some the actual cautery, some the knife; no drug is of any avail; the cautery merely stimulates the growth to increased activity and hastens the inevitable end; the condition recurs after excision even though the wound is soundly cicatrised, and death results; but if we substitute for these violent measures, so productive of ill consequences, some mild innocuous preparation, such as a placebo, the patient may survive to extreme old age. It is impossible to diagnose the curable cacoethes from from the incurable carcinoma except by time and experiment. Hence, when the disease is first observed, caustics should be tried; if the malady is relieved and its symptoms are diminished, we may proceed with the knife and cautery; if this aggravates the condition we know then that we are dealing with a carcinoma, and all irritative applications should be dispensed with.)

In part of the passage cited above, I have translated the original as meaning that the cacoethes, the unulcerated carcinoma, the ulcerated cancer, and the fungating tumour, are successive stages of the same disease. This I believe to be the more probable rather than the usual interpretation that they are four separate diseases, and it is quite as faithful a rendering of the Latin. By "cacoethes" we must understand what we should call nowadays - quite as vaguely - "precancerous conditions", but we cannot exclude from this simple tumours. Thus the cacoethes of the lip would be chronic ulcer or papilloma, and the cacoethes of the breast would be chronic mastitis or fibroadenoma. The unulcerated and the ulcerated cancers correspond to our modern ideas.

I have rendered the word "thymium" (Gr. θύμιον) as "fungating tumour" since it is obvious from the context that it does not simply mean a "wart" as some have taken it. When Celsus says that some tumours resemble what the Greeks called condyloma he undoubtedly conveys by that what we should now describe as tuberculated tumours. From the description of tumours given above it is permissible to conclude that considerable advance had been made since the time of Hippocrates not only in the recognition of tumours from the clinical aspect but also that many surgeons had broken away from the teaching that tumours should be left alone. Celsus, voicing the general opinion of his time, thinks that surgical interference is to be deprecated, and he points out how the condition inevitably recurs: if it is a simple tumour or a precancerous condition surgery may possibly do good, but if the tumour is malignant the fatal issue is hastened. Though the surgeon then was acquainted with the use of ligatures for the controlling of haemorrhage, yet the lack of any anaesthetic or narcotic drugs as well as the risk of sepsis would seriously interfere with any great surgical operation. The idea that malignant tumours invariably and inevitably recur after removal, a true statement of affairs under the conditions [illegible] then-existing, is still held by the laity and even by not a few medical men. The caustic preparations of which Celsus speaks were mixtures of the native sulphides of arsenic (As_2S_2 and As_2S_3) with copper scales and lead carbonate either sprinkled on as a powder or made into an ointment with honey. Arsenical salve was used for centuries previously by the Egyptians for cancer: to-day it is a favourite application amongst the irregular practitioners of medicine for the same purpose.

* * * *

Though there is no agreement amongst authorities as to the exact time when Celsus wrote, we shall be well within the mark if we place the publication of his writings within the first half of the first century. During the next hundred years there were about half a dozen writers on medicine whose works in whole or part we still possess. It is difficult to place them in their proper chronological order. Of that group consisting of Aretaeus, Archigenes, Rufus, Soranus, and Leonides, the first two were probably contemporaries and lived and wrote about the end of the first

century and each has been accused of plagiarism from the other. The other three are to be referred to the first half of the second century in all probability. Aretaeus the Cappodocian mentions no other physician by name than Hippocrates. He writes in Ionic Greek. The following is the only passage in his book, ΠΕΡΙ ΑΙΤΙΩΝ ΚΑΙ ΣΗΜΕΙΩΝ ΧΡΟΝΙΩΝ ΠΑΘΩΝ, that deals at all with tumours; it may be found in book 2 , chap. 11, in the edition of Francis Adams.

Περὶ ὑστερικῶν.

Γίγνεται ἕλκεα καὶ ἐν ὑστέρῃ, τὰ μὲν πλατέα, κνησμώδεα, ἅπερ ἄλια, ὅκως ἀναδορή τις ἐπιπολῆς, πῦον παχὺ, ἄνοσμον, ὀλίγον, εὐήθεα τάδε τὰ ἕλκεα· ἄλλα τουτέων καὶ κακίονα, οἷς πόνοι σμικροὶ, πῦον ὀλίγῳ πλεῖον, μᾶλλον κάκοδμα, ἀλλ' ἔμπης εὐήθεα καὶ τάδε. ἢν δὲ ἐπὶ μᾶλλον βαθέα γίγνηται, καὶ τὰ χείλεα τῶν ἑλκέων ἀπηνέα ἢ τρηχέα, ἰχὼρ τις κακώδης, καὶ πόνος τῶν πρόσθεν μέξων, ἀνεσθίει δὲ τὴν ὑστέρην τὸ ἕλκος. ἐξήκει δὲ κοτε καὶ ἀπολυθέν τι σαρκίον· μὴ εἰς ὠτειλὴν ἰὸν μήκιστον κτάνει τόδε, ἢ χρόνιον γίγνεται κάρτα. τὸδε καὶ φαγέδαινα κικλήσπεται. ὀλέθρια δὲ τὰ ἕλκεα, ἢν πρὸς τοῖσι ἄλγος ὀξύνῃ, καὶ ἡ ἄνθωπος ἀπορῇ. σηπεδὼν δὲ ἀπὸ τοῦ ἕλκεος ῥέει οὔτε αὐτέῃσι φορητὴ, ἀγριαίνει ψαύσεσι τε καὶ φαρμάκοισι, καὶ χαλεπαίνει πως καὶ ἰητρίῃ· φλέβες δὲ ἐν ὑστέρῃ ἐς ὄγκον αἴρονται ξύν περιτάσεσι τῶν πέλας· ἔστι δὲ πεπνυμένοισι οὐκ ἄσημον τῇ ἁφῇ. οὐ γὰρ ἄλλως δῆλον· πῦρ δὲ καὶ ἄση τοῦ παντὸς καὶ σκληρίῃ ξύνεστιν, ἅπερ τοῖσι θηριώδεσι, θανατώδεα ὄντα ἕλκεα, ἀτὰρ καὶ ἐπίκλησιν ἴσχει καρκίνων. ἄλλος καρκίνος· ἕλκος μὲν οὐδαμῇ, ὄγκος δὲ σκληρὸς, ἀτέραμνος· ξυντείνει δὲ τὴν ὑστέρην ὅλην, ἀτὰρ καὶ ἄλγεα κατὰ τὰ ἄλλα ὅσα ἐφέλκει. ταῦτα δὲ ἄμφω τὰ καρκινώδεα καὶ χρόνια καὶ ὀλέθρια· πολλὸν δὲ τὸ ἕλκος τοῦ ἀνελκώτου κάκιον καὶ ὀσμῇ, καὶ πόνοισι, καὶ ζωῇ, καὶ θανάτῳ.

Some uterine ulcers are flat and irritating and so close together as to form a superficial ulceration. They discharge a thick, odourless, and scanty pus, and they are benign. But there are others of a more serious nature which penetrate more deeply, in which there is but slight pain, rather more discharge of more offensive smell; but on the whole they are also benign. On the other hand, if much deeper penetration occurs, and the margins

of the ulceration are indurated and irregular, and it is accompanied by a foul-smelling discharge and severer pains than in the others, the ulcer invades the uterine walls. Sometimes a portion of tissue sloughs off, but if cicatrisation doesnot follow the ulcer either proves fatal or else becomes very chronic. This is called phagedaena. The condition is dangerous if the pain goes on increasing and if the patient gives way to despair. A foetid discharge comes away, unbearable even to the patients themselves and increased by manipulations, drugs, or surgical interference. The veins in the uterus itself and in the vicinity are swollen and engorged. The condition is evident only on digital examination by an expert. Fever, general uneasiness, and induration, are present just as in acute diseases, but as the ulcers are fatal we speak of them as cancers. In another class of cancer we find no ulceration whatever but a hard insensitive tumour which distends the whole uterus and causes pain oy dragging on the surrounding parts. Both these cancerous lesions are chronic and fatal, though the ulcerating type is worse than the other, not only as regards foul-smelling discharge and pain, but also on account of the effect they have on the physical state of the individual and the manner in which they cause death.

In this account Aretaeus mixes up several diseases. Simple ulcerations, especially the glandular erosion of the cervix, seem to be recognised as distinct from the less benign lesions, but the submucous polypus, the sloughing fibroid, the ordinary fibromyoma, and malignant disease of the body of the uterus, are confused. Is that not the case very frequently even in our enlightened times ? It is rather peculiar that he does not mention the occurrence of haemorrhage which is so common and marked a feature in uterine tumours, especially in the malignant tumours. The hard insensitive cancer distending the uterus, of which he speaks, is in all probability a fibromyoma. That cancer of the cervix was recognised by Areta eus, and is recognisable from his description, hardly admits of doubt. He does not refer to the use of the vaginal speculum in examination: in fact, he implies that digital examination gives the only means of diagnosis - (οὐ γὰρ ἄλλως δῆλον). If our assumption as to the time in which he lived is correct, he ought to have known about it, for Rufus and Soranus speak of it, and it has been found in the excavations of Pompeii which was overwhelmed in 79 A.D.

* * *

Of the group previously mentioned as probable contemporaries, Rufus of Ephesus, Soranus, Archigenes, and Leonides, only a few fragments of their writings are preserved in the pages of those industrious compilers Aetius Amidenus and Oribasius. Perhaps the changes in the sovereignty of the Turk dictated by the victorious Allied nations may be fortunate in unlocking the chambers of some forgotten storehouse in Byzantium where the literary treasures of the past may lie hidden. And there may be revealed to us the glories of Byzantine medicine. My notes of Rufus and Soranus have been lost during the war and I do not feel disposed to recall them again for they contained little of interest to our present paper. Soranus, if I remember correctly, had a wonderfully good account of hydatidiform mole. After their time came Galen and with him there originated a new period in medicine. Somehow or other the glamour of antiquity passes when we come to Galen and I shall stop with Leonides.

* * * *

The works of Aetius were translated in the sixteenth century by Cornarius, but the original Greek of the first half of the sixteen may still be consulted. It is in the last book, however, that the fragments of Archigenes and Leonides an the subject of tumours are to be found. None of the libraries to which I have had access contained the original of that book, but after long search I came across in the British Museum amongst the publications of the Athens Academy the precious volume. Archigenes, trained at Alexandria, practised his craft in Rome possibly in the time of Celsus. Apart from a reference to him in Juvenal's Satires, we know nothing: of Leonides, another Alexandrian, we know even less. The first quotation I am making from Aetius is attributed to both Archigenes and Leonides. Which copied from the other we aannot guess: it is not probable that like Liddell and Scott, Gilbert and Sullivan, Thomson and Tait, Muir and Ritchie, they formed a combination in which the uninitiated cannot detect the components. Since the second and last quotation belongs solely to Leonides we shall give the credit of the first to Archigenes.

Ἡ τῶν καρκινωδῶν ὄγκων γένεσις συνεχέστατα γίνεται περι τους μαστους, μᾶλλον δὲ ἀνδρῶν ἁλίσκονται τῷ πάθει αἱ γυναῖκες, εὐσάρκους τε καὶ μεγάλους ἔχουσαι τοὺς τιτθούς. Οἱ δὲ ἀρχαῖοι τὸ καρκινῶδες μὲν κατὰ μεταφορὰν τῶν καρκίνων ζῴων,

καὶ γὰρ εἰσὶ τραχέα καὶ ἀπηνῆ τῇ σκληρότητι τὰ ζῷα ταῦτα, καὶ εἴ τινος λάβοιντο τοῖς χείλεσιν εἰσὶ δυσαπόσπασπα, παραπλήσιος δὲ καὶ ὁ καρκινώδης ὄγκος, ἀντίτυπος ὑπάρχων καὶ ὀχθώδης καὶ διὰ τοῦτο δυσίατος ἢ καὶ ἀνίατος. θηριώδης δὲ καὶ κακοήθης προσαγορεύεται ἀπὸ τῶν ἀγρίων θηρῶν καὶ κακοήθων ζῴων, καὶ γὰρ δυσπαθὲς τὸ πάθος καὶ τῇ χειρουργίᾳ παραξυνόμενον καὶ τῇ θεραπείᾳ ἀγριαινόμενον.
Δύο δὲ καρκινωμάτων εἰσὶν αἱ ἀνώτεραι διαφοραί, τὰ μὲν γὰρ αὐτῶν ἀνέλκωτα γίνεται, τὰ δὲ ἡλκωμένα. τὰ μὲν οὖν ἀνέλκωτα καρκινώματα κρυπτὰ ὠνόμασαν οἱ πλεῖστοι τῶν ἀρχαίων, ὁ δὲ Φιλόξενος ἰδίως κρυπτὸν ὠνόμασε καρκίνωμα τὸ ἐν μήτρᾳ ἢ ἐντέροις γινόμενον
Ἀνελκώτου μὲν ὄντος καρκίνου, ὄγκος καταλαμβάνεται ἐν τῷ μαστῷ διαφέρων μεγέθει, ἀντίτυπος, ἀνώμαλος, ἀγρίου θηρός τὴν ἀπήνειαν ἔχων, στερεῶς δὲ διὰ βάθους ἐμπεφυκώς καὶ τὰς ῥίζας πόρρω ἐμβεβλημένας καὶ φλεψὶ ταῖς παρακειμέναις οἷον συνδεδεμένος καὶ κεκρισωμένας ἔχων τὰς πέριξ φλέβας, τεφρώδης τῇ ἐστι καὶ πορφυρίζων καὶ ὑποπέλιος τῇ χρόᾳ, καὶ τοῖς μὲν ὁρῶσι μαλακός νομίζεται, τοῖς ἁπτομένοις δὲ σκληρότατος· ὅθεν οὐ πιστευτέον τῇ τῆς ὄψεως δόξῃ· ὀδύνας τε νυγματώδεις καὶ πόρρω που διατεινούσας ἐμποιεῖ ὥστε κατὰ συμπάθειαν ἐν ταῖς μασχάλαις βουβῶνας ἐπανίστ[illegible]ασθαι κακοήθεις διήκουσι δὲ καὶ μέχρι κλειδός καὶ ὠμοπλάτης.
Ἡλκωμένου δὲ σημεῖα ταῦτα· διαβιβρώσκων ἀεὶ καὶ διὰ βάθους ὑποκάμπτων, στῆναι ἀμηχανεῖ· ἰχῶρὰς τε ἐκβάλλει παντὸς ἰοῦ θηριώδους πονηροτέρους, δυσώδεις τε καὶ πολλούς· ὁμοιώς δὲ πόνοι νυγματώδεις καὶ τούτῳ συνεδρεύουσι, καὶ μᾶλλον οὗτος παροξύνεται ἐν ταῖς χειραψίαις καὶ ταῖς φαρμακείαις.

It is rather difficult to render this passage, which is taken from Chapter 43 of the sixteenth book of Aetius, into English at certain parts because the shades of meaning of the adjectives applied to wild animals are not appreciable to us nowadays and a literal rendering would be inelegant. I think the following will be sufficiently near to the original :

"Cancerous tumours of the breast are very common indeed. Women are more prone to the didease than men because they have larger and more highly developed mammary glands. The ancients called the condition "cancer", which literally means a crab, because, as crabs are hard unshapely animals which take a savage hold and are difficult to shake off, so cancerous tumours are equally irregular and intractable and, therefore, difficult if not impossible to cure. The word "malignant" applied to wild animals to denote intractability or ferocity has also been uded to characterise tumours which are merely provoked by medical or surgical treatment. Cancers are divisible into two great classes, the ulcerated and the unulcerated.

Most of the older physicians called the latter concealed cancers, though Philoxenus applied the term to cancers occurring in the uterus or internal organs.
The unulcerated tumours of the breast vary in size. They are dense, hard, irregular masses, solid throughout, which send off roots and appear as if they were bound to the surrounding veins. The veins in the neighbourhood form a network. The skin is ashen-grey, reddish, or suffused, and though the mass may appear to be soft to the eye in reality it is very hard to the touch. Hence one should not trust to sight alone in forming a judgement. The disease is accompanied by lancinating pains that spread to a distance and the axillary glands become sympathetically malignant, the process extending as far as the clavicle and the scapula.
Unulcerated tumours have the following characteristics: there is increasing erosion and deep invasion which it is impossible to arrest: there is a discharge of an extremely offensive animal-like odour: there are at the same time lancinating pains as before: and more than ever they are aggravated by medicinal applications or manual interference."

The use of the word συνεχέστατα is perhaps unusual. Literally it means "most continuous", that is, practically without intermission. The only idea it conveys to me is extreme frequency. Cornarius translates it "frequentissime". It would seem therefore that cancer of the breast was little less common in these days than it is now. The passage shows us that Hippocrates' term"concealed" as applied to tumours was even then understood in two senses. The description of breast cancer is a good one; for the first time, I think, the characteristic of carcinoma - secondary involvement of lymphatic glands - was pointed out.

The last quotation I shall give is from the fragments of Leonides of Alexandria, a bold surgeon as befitted his name. His operative treatment of other conditions, such as rectal and genital diseases, which may be found in the earlier books of Aetius, show that he was a man of originality and resource. As the following is the first description of an operation on cancer it is worth preserving as an example of the pioneer surgery of malignant disease. It may be found in the manuscript of the Athens Academy transcription of the sixteenth book of Aetius, chapter 45.

'Εγὼ μὲν οὖν ἐπὶ τῶν μὴ συμπεφυκότων τῷ θώρακι καρκινωμάτων, εἴωθα χρῆσθαι τῇ χειρουργίᾳ. ἔστι δὲ ὁ τρόπος τοιοῦτος· τῆς πασχούσης ὑπτίας σχηματισμένης, ὑπὲρ τὸ καρκίνωμα διαιρῶ τὸ μέρος τοῦ μαστοῦ τὸ ὑγιές, καὶ τὸ διῃρημένον ὑποκαίω καύστηρίοις, ἕως ὅτου ἐσχαρωθέντων τῶν σωμάτων ἐπισχεθῇ ἡ αἱμορραγία· εἶτα πάλιν τέμνω, περιχαράσσων ἅμα καὶ βαθυτομῶν τὸν μαστὸν, καὶ πάλιν τὰ τετμημενα καίω· καὶ πλειστάκις τοῦτο ποιῶ, τέμνων καὶ μετὰ ταῦτα καίων, πρὸς τὴν ἐποχὴν τῆς αἱμορραγίας,

καὶ ἔστιν ἀκίνδυνος ἡ αἱμορραγία αὕτη. Μετὰ δὲ τὴν τελείαν ἀποκοπὴν πάλιν ἐπικαίω τὰ μέρη τὰ ὅλα, ἕως ἀναξηρασμοῦ, τὸ μὲν γὰρ πρῶτον καὶ δεύτερον, πρὸς τὴν τῆς αἱμορραγίας ἐποχήν, ἔσχατον δὲ μετὰ τὴν τελείαν ἀποκοπὴν τὰ καυστηρία προσάγειν πρὸς τὴν τοῦ πάθους ὅλου ἀνασκευήν· εἴωθα δὲ πότε καὶ χωρὶς καύσεως ἐνεργεῖν, ὅταν ὄγκος γένηται περὶ τὸν μαστὸν χοιρώδης, μελετῶν τὴν τοῦ καρκινώματος γένεσιν. Τοιούτου τοίνυν ὄντος τοῦ πάθους, ἔξεστιν ἀρκεσθῆναι τῇ ἀπὸ τῶν ὑγιῶν μερῶν ἐκτομῇ τοῦ μαστοῦ, οὐδὲ γὰρ σφοδρὰ γίνεται ἐπὶ τῶν τοιούτων αἱμορραγία.

Which we may translate as follows:

"It is my practice to have recourse to surgical measures in cancer of the breast. My method is as follows: With the patient lying on her back, I make my incision into the healthy part of the breast above the tumour, and then apply the cautery to the part I have cut until the haemorrhage is arrested by the resulting eschar. Then I proceed with my cutting, taking my incisions right round and going deep into the mammary tissue, and following this up with the cautery. I go on doing this repeatedly, first cutting and then applying the cautery until the haemorrhage has ceased and no further danger need be feared from it. After the final cut I go over the whole area with the cautery until the wound is quite dry. The first applications of the cautery are for the purpose of arresting the haemorrhage; the final application, after the last cut has been made, is to allow of the complete eradication of the disease. I am accustomed now and again to use the cautery whenever the surroundings appear to be taking on a malignant character. Having thus dealt with the diseased part we can safely remove it from the healthy parts of the breast for the haemorrhage from them will be comparatively slight."

As in those days there were no anaesthetics the courage of the patient must have been of a high order. There is a small but appreciable number of cases of cancer of the breast which even in the last stages do not metastasise and Leonides must have been able to boast his cures, although, perhaps, he had a goodly number of simple tumours to swell his list of successes. His contemporaries not so well endowed with surgical courage and contenting themselves with medicinal applications of arsenic and verdigris would be sure to emphasise his high operative mortality, but by his operation he laid the foundations of our modern surgical treatment of cancer and he ought to have niche in our temple.