

THE EXPERIMENTAL PRODUCTION OF METAPLASIA AND HYPERPLASIA
IN THE SEROSAL ENDOTHELIUM OF THE VISCERAL PLEURA AND IN
THE ALVEOLAR EPITHELIUM OF THE LUNG OF THE RABBIT.

APPENDIX A. LESIONS OF THE LIVER AND OTHER ORGANS PRODUCED
BY THE INTRAPLEURAL INJECTION OF SUDAN III.

APPENDIX B. THE INCIDENCE OF SPONTANEOUS TUMOURS AND CERTAIN
OTHER LESIONS IN A CONSECUTIVE SERIES OF 700 RABBITS.

A THESIS

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presented by

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TABLE OF CONTENTS.

Page.

<u>INTRODUCTION</u>	1
<u>EXPERIMENTS WITH SUDAN III. IN OLIVE OIL OR IN LANOLIN, WITH AND WITHOUT BILE SALTS</u>	4
Historical summary	4
Preliminary investigations	6
Theoretical principles pertaining to later ex- periments.....	7
Experimental technic and general observations.	9
Summary of post-mortem reports	10
Microscopical observations	11
Summary of observations and conclusions	18
<u>EXPERIMENTS WITH SODIUM CHOLATE EMULSIFIED IN OLIVE OIL OR LIQUID PARAFFIN OR DISSOLVED IN PHYSIOLOGICAL SALINE</u>	18
<u>EXPERIMENTS WITH SOLUTIONS OF ELECTROLYTES</u>	23
Theoretical principles	23
Experimental methods	25
(1) Experiments with electrolytes possessing mono- valent cations	26
(a) Lithium chloride	26
(b) Sodium chloride	28
(c) Potassium chloride	29
(2) Experiments with electrolytes possessing bi-valent cations	30
(a) Magnesium chloride	30
(b) Calcium chloride	33
(c) Strontium chloride	37
(d) Barium chloride	39
(e) Ferrous chloride	40

	page
(3) Experiments with electrolytes possessing tri-valent cations	43
(a) Aluminium chloride	43
(b) Lanthanum chloride	47
(c) Ferric chloride	51
(4) An experiment with an electrolyte possessing a tri-valent anion	
Potassium ferricyanide	53
(5) An experiment with a mixture of sodium chloride and calcium chloride	54
Summary of observations and conclusions	54
<u>AN EXPERIMENT WITH DISTILLED WATER</u>	56
Summary of observations and conclusions	57
<u>EXPERIMENTS WITH SOLUTIONS OF NON-ELECTROLYTES</u>	58
(a) Cane-sugar	58
(b) Urea	59
Summary of observations and conclusions	60
<u>EXPERIMENTS WITH REPEATED INJECTIONS OF STRONTIUM CHLORIDE (3/4 N) AT INTERVALS OF 48 HOURS</u>	60
Summary of observations and conclusions	66
<u>DISCUSSION</u>	67
<u>SUMMARY OF OBSERVATIONS AND CONCLUSIONS</u>	91
<u>ACKNOWLEDGMENTS</u>	93
<u>REFERENCES</u>	94

APPENDIX A.

<u>LESIONS OF THE LIVER AND OTHER ORGANS PRODUCED BY THE</u>	Page
<u>INTRAPLEURAL INJECTION OF SUDAN III</u>	97

APPENDIX B.

<u>THE INCIDENCE OF SPONTANEOUS TUMOURS AND CERTAIN OTHER</u>	
<u>LESIONS IN A CONSECUTIVE SERIES OF 700 RABBITS</u>	103

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LIST OF ILLUSTRATIONS.

	Page
<u>PLATE I</u>	12a.
Fig. 1..... Rabbit 39/1927.	
" 2..... " 41.	
" 3..... " 41	
" 4..... " 41	
<u>PLATE II</u>	13a.
Fig. 5..... " 41/1927	
" 6..... " 41	
" 7..... " 41	
" 8..... " 41	
<u>PLATE III</u>	14a.
Fig. 9..... " 41/1927	
" 10..... " 41	
" 11..... " 46	
" 12..... " 46	
<u>PLATE IV</u>	15a.
Fig.13..... " 48/1927	
" 14..... " 49	
" 15..... " 50	
" 16..... " 50	
<u>PLATE V</u>	16a.
Fig.17..... " 54/1927	
" 18..... " 54	
" 19..... " 116	
" 20..... " 89	

<u>PLATE VI</u>			19a.
Fig. 21	Rabbit	183/1927	
" 22	Guinea-pig	7/1927	
<u>PLATE VII</u>			21a.
Fig. 23	Rabbit	A.134/1928	
" 23a.....	do.	(high power).	
" 24	"	A. 128.	
<u>PLATE VIII</u>			22a.
Fig. 25	"	B. 97/1929.	
" 26	"	B. 89	
<u>PLATE IX</u>			29a.
Fig. 27	"	A.199/1928.	
" 28	"	A.155	
" 29	"	A.297	
<u>PLATE X</u>			31a.
Fig. 30	"	A.283/1928	
" 31	"	A.285	
<u>PLATE XI</u>			34a.
Fig. 32	"	A.204/1928	
" 33	"	A.205	
" 34	"	A.179	
<u>PLATE XII</u>			36a.
Fig. 35	"	A.174/1928	
" 36	"	A.169	
" 37	"	A.171	
<u>PLATE XIII</u>			37a.
Fig. 38	"	A.146/1928	
" 39	"	A.186	
" 40	"	A.148	
" 41	"	A.190	

<u>PLATE XIV</u>			38a.
	Fig. 41a	Rabbit A.190/1928	
	" 42	" A.185	
<u>PLATE XV</u>			41a.
	Fig. 43	" A.344/1928	
<u>PLATE XVI</u>			42a.
	Fig. 44	" A.364/1928	
	" 45	" A.368	
	" 46	" A.366	
<u>PLATE XVII</u>			44a.
	Fig. 47	" A.194/1928	
	" 47a.....	" Do.	
	" 48	" A.197	
<u>PLATE XVIII</u>			46a.
	Fig. 49	" A.211/1928	
	" 50	" A.213	
<u>PLATE XIX</u>			49a.
	Fig. 51	" A.329/1928	
	" 52	" A.327	
	" 53	" A.356	
	" 54	" A.356	
<u>PLATE XX</u>			51a.
	Fig. 55	" A.373/1928	
	" 56	" A.374	
	" 57	" A.375	

PLATE XXI.....

53a.

Fig. 58 Rabbit A.377/1928

" 59 " A.378

" 60 " A.361

PLATE XXII.....

58a.

Fig. 61 " A.264/1928

" 62..... " A. 336

PLATE XXIII.....

64a.

Fig. 63 " B.13/1929

" 64 " B. 8

" 65 " B.18

PLATE XXIV.....

71a.

Fig. 66 Squamous epithelioma
of pleura (P.M.A761).PLATE XXVI.....

98a.

Fig. 67 Rabbit 47/1927

" 68 " 41

" 69 " 56

" 70 " 2

PLATE XXV.....

97a.

Fig. 71 " A.7/1928

(Coloured drawing of liver).

PLATE XXVII.....

99a.

Fig. 72 Rabbit A,7/1928

PLATE XXVIII....

104a

Fig. 73 " B.13/1929

" 74 Adenocarcinoma of uterus.

Rabbit 117/1927

Adenocarcinoma of kidney.

With the three exceptions noted, all the sections were stained
by Haematoxylin and Eosin.

The experimental studies which form the subject of this contribution are based upon the injection of a large variety of reagents into the right pleural sac of the rabbit. They were prompted, in the first instance, by curiosity in respect of the histogenesis of primary malignant tumours of the serous membranes with special reference to the pleura. This curiosity had been recently aroused by an analysis of three examples of those growths at the Western Infirmary of Glasgow, under the direction of Professor Muir.

In reckoning the means whereby the first objective might be approached, it was clear that some reagent must be sought which was capable of exciting active proliferation in the serosal endothelium. Ultimately it was shown that an emulsion of two highly complex substances - sudan III and sodium cholate - in olive oil could fulfil this requirement. At the same time, certain forms of metaplasia of the serosal endothelium were observed which, it is hoped, may help to explain the unusual characters of one of the three tumours cited - viz., a squamous epithelioma of the pleura. It was duly recorded that hyperplasia of the epithelium lining the marginal alveoli of the lung accompanied the changes in the serosal cells with great regularity.

These developments tended to encourage the hope that it might be possible to adapt the pleural sac as a "culture-chamber", so to speak, for the study of the conditions favouring the development of endothelial or of epithelial hyperplasia. The small success which had already been attained instigated a wider range of experiments with the purpose of analysing, if it were possible, those intimate changes in the cells which are liable to culminate in their division

or proliferation. Throughout the investigation it has been assumed that the orderly sequence of events which is illustrated in the mitotic division of any cell is determined by a definitive alteration in the equilibrium or in the integrity of that cell. Experimental necessity has demanded a more precise interpretation of that essential change. Unfortunately, nothing or next to nothing is definitely known about the constitution of the living cell. Under these circumstances it has seemed expedient to adopt as a working principle some phase of vital activity which is not entirely hidden in the mists of conjecture although it is swayed inevitably by every momentary change in the structure of the cell. Thus it has been assumed as a provisional hypothesis that cell division is a function of the permeability or of the surface tension of the cell membrane. This arbitrary choice of two vital phenomena of cellular activity with the avowed object of studying their participation in the process of cell division is not likely to pass unchallenged. Therefore it is well to emphasise that the hypothesis has been exercised merely for the purpose of formulating experiments which might reasonably be expected to test its merits. Likewise it might be explained that it is not desired to confuse the main issue by seeking to interpret the results in terms of "permeability" or of "surface tension". These dynamic phenomena are, themselves, highly complex and there is still wide divergence of authoritative opinion with reference to their control. It has been deemed more prudent in the later experiments to abandon abstruse biophysical terms in favour of simple, or relatively simple, reactions which are less inadequately understood. Thus the experiments with solutions of neutral salts

have been elaborated with the object of assessing the relative importance of physical and of chemical factors in the genesis of that type of hyperplasia which affects the epithelium lining the marginal alveoli of the lung of the rabbit under the experimental conditions ~~are~~ set forth in the text.

The epithelial proliferation which follows the intrapleural injection of an effective solution of an electrolyte seems to proceed strictly in accordance with a systematic or conventional plan. Towards the end of 24 hours the cytoplasm and the nuclei of the epithelial cells become swollen; during the next 24 hours nuclear hyperchromatism is intensified; and mitotic figures are probably most numerous on the third day. As a rule the proliferative process waxes in this methodical fashion until the fourth day and thereafter it wanes steadily so that the normal structure of the lung is restored more or less perfectly by the eighth day. It will be shown that the exhibition of a certain volume of a solution of strontium chloride consistently stimulates some measure of proliferation in the epithelial cells lining the marginal alveoli of the lung. Within the space of 48 hours the proliferating cells enter a "refractory" state such that they are indifferent to subsequent injections of the same solution while the process of involution which normally overtakes the epithelial hyperplasia arising from a single injection pursues its customary course. These circumstances, together, suggest that the proliferative phenomena tend to conform to physiological principles rather than to express an inherent pathological defect. Also they offer a relatively simple example of the complexity of the processes of cell-growth inasmuch as they indicate that the

initiation of epithelial hyperplasia, and the maintenance thereof, are governed by factors which are, in some respects at least, discrete.

The two terms "Metaplasia" and "Hyperplasia" recur very frequently in these pages and a few words of explanation are desirable concerning their significance. The term "Metaplasia" is employed in this contribution to indicate a transformation of one differentiated tissue into another, wherein the change has been completed and the reorganised cells have returned to a resting state. On the other hand, when the change is still progressing actively and is associated with numerous mitotic figures "Hyperplasia" has been considered a more apposite descriptive term. Thus the two terms are not mutually exclusive; they represent different phases of the same process.

The experiments are presented in the order of their execution. A brief summary of the results which appear to be significant follows the descriptive data of each group. Finally it is sought to correlate the principal observations in a general discussion.

Experiments with Sudan III in olive oil or in lanolin, with and without bile salts.

Historical summary.

Fischer (1906) was the first to observe and to record the growth-promoting properties of the fat-soluble dyes. He found that the subcutaneous injection of olive oil saturated with scharlach R (Scharlachöl) into the ear of a rabbit was attended by a notable degree of epithelial proliferation affecting the stratum Malpighii and by a marked increase of keratinisation. The new formation progressed until the histological picture became strongly reminiscent of squamous epithelioma, but it lacked the cardinal characteristic of autonomous growth and ultimate regression was the invariable rule.

Similar effects followed the injection of sudan III and of indophenol. He formed the opinion that "Scharlachöl" exercised a chemiotactic influence upon the squamous epithelium - possibly, too, upon the pulmonary epithelium - of the rabbit, whereas these tissues in other animals were insusceptible to the dye. In one rabbit the injection of a saturated solution of scharlach R in ether into four mammae was followed by a transformation of numerous gland lobules into islets of squamous epithelial cells in each of the injected breasts; the remaining breasts exhibited no such change.

These observations were confirmed and elaborated by many other workers including Powell White, Wacker and Schmincke, Ishio Haga, Stoeber, Stahr and Jores. By the injection of a variety of substances such as scharlach R in paraffin, amidoazotoluol in olive oil, oleic acid etc., proliferative phenomena have been excited in the squamous epithelium of white mice, of dogs and of man. Haga has demonstrated by the same means cellular proliferation in the epithelium of mucous membranes (e.g. tongue, stomach), in endothelia (e.g. endothelium of lymph vessels), and in the epithelium of bile-ducts and of the lacteal ducts of the breast. More recently, Fischer (1922) has described a very extensive series of experiments based upon the intravenous injection of oily media. In one group of these experiments, following the injection of a mineral oil, described as "Granugenöl", large necrotic foci were observed in the lungs. As these necroses underwent absorption, large cysts (Flimmerepithelblasen) were formed, which were associated in some cases with remarkable degrees of irregular epithelial hyperplasia. It is noteworthy that the intravenous injection of "Scharlachöl" failed to excite similar changes.

Preliminary Investigations.

In the light of Fischer's observations upon the local effects of injections of small quantities of scharlach R and of sudan III in olive oil subcutaneously in the ears of rabbits, it seemed not unreasonable to hope that the introduction of massive doses of the same dyes into rabbits, parenterally, by a route of slow absorption such as the pleural sac, might provoke some changes somewhere even if the original objective should defy approach. As a matter of fact, the local changes in the serosal endothelium were uniformly disappointing during the first six months of experiment. Meanwhile, on the other hand, it had been ascertained that in the course of six or eight weeks, in a proportion of the sudan III series of animals which survived, the retroperitoneal fat and the body fat in general ~~had~~ assumed a rich scarlet hue. In addition, there had been collected a graded series of lesions in the liver, ranging from focal or diffuse acute necrosis of the parenchyma, through intermediate stages of cirrhosis of the organ with regeneration of the parenchyma, up to and including one example of multiple nodular hyperplasia. Twelve examples of this lesion have now been secured, accompanied in a few instances by extensive proliferation of the bile-duct epithelium. However, these observations are incidental to this study and they will be reviewed briefly in an appendix.

As the experiments progressed it was found necessary to revise the opinion that sudan III dissolved or suspended in olive oil was incapable of producing proliferation in the epithelium lining the marginal alveoli. Whereas an impression had been

formed that the local changes arising from an intrapleural injection of this dye were confined to fibrosis of the subserosa and the formation of numerous "foreign-body" giant cells, a later series of experiments revealed definite evidence of epithelial hyperplasia yet the results continued to be capricious and unreliable.

Theoretical principles pertaining to later experiments.

The failure to make any progress at all towards the original objective compelled a resort to purely hypothetical considerations and these must be reviewed in some detail in order to explain the adoption of the next experimental step. Many hypotheses have been formulated in an attempt to explain the growth-promoting capacity of "fat-soluble" substances or, more strictly, of a diverse group of substances possessing the common property of solubility in fat solvents. Originally, Fischer attributed this capacity to some specific chemiotactic influence exercised by the reagent upon the epithelial cells. His view was endorsed by Stoeber, Rutschinski and Benthin, whereas it was opposed by Powell White and others. Stoeber and Wacker postulated a change in the chemical processes of the epithelial cell itself, and A. W. Meyer regarded disturbances of circulation and inflammatory processes as the causes of the cellular proliferation. Later Wacker and Schmincke suggested that the epithelial hyperplasia might be the effect of some alteration in the physical state of the "lipoid envelope" of the cell. The last hypothesis has now won the support of Fischer who quotes, by way of analogy, the observations of J. Loeb upon artificial parthenogenesis in the eggs of the sea-urchin promoted by hypertonic solutions and by "fat-soluble" surface-active agents. In the light of a long

series of experimental observations, Martinotti came to the conclusion that "all substances which promote epithelial proliferation 'in vivo' possess a special affinity for fats 'in vitro'", and his view was correlated by Fischer with the conception of the cellular membrane as a "lipoid envelope". This comparatively simple interpretation of the constitution of the cell membrane has been generally abandoned as the result of more recent investigations and most authorities (Leathes, Bayliss, Lillie) are agreed that the cell membrane must be regarded as a lipoid-protein integral of the greatest complexity. Nevertheless, there seems to be equal agreement that the "lipoid" component plays an essential part in controlling the vital phenomena of the permeability and of the surface tension of the membrane of the living cell. On the other hand, it has been demonstrated by McClendon that in the process of fertilisation of the egg cell the membrane becomes considerably more permeable to electrolytes, evidenced by the increase of electrical conductivity of a mass of eggs of the sea-urchin on fertilisation. There is other evidence of increased permeability in the escape of pigment observed by Lillie, who regards the essential factor in the artificial segmentation of the egg under the influence of certain salts as an increase in the permeability of the cell membrane.

In the course of the present series of experiments, it seemed expedient to submit Wacker and Schmincke's hypothesis to a crude experimental test - with special reference, perhaps, to the permeability of the cell membrane or to the surface tension of the cell membrane - by adding to the sudan III a substance or substances which might be expected radically to alter those physical attributes of the living cell. On these grounds, it was elected to add bile salts

whereupon a series of changes became apparent in the serosal endothelium and in the pulmonary epithelium lining the marginal alveoli.

Experimental technic and general observations.

The experimental series of animals which are to be considered here comprises sixteen rabbits. The inoculum was prepared as follows for the whole group:-

Sudan III (B.D.H.)	16 grms.
Olive oil	160 c.c.
Sodium cholate ^x (B.D.H.) 5 per cent.aq.soln.	16 c.c.

The resulting mixture or emulsion was autoclaved. Each rabbit was anaesthetised in turn and 10 c.c. of the emulsion were injected into its right pleural sac with antiseptic and, as far as possible, aseptic precautions. In order to minimise the risk and the extent of injury to the surface of the lung, the injections were performed postero-laterally, near the inferior angle of the right scapula, with a trocar and cannula. The trocar was withdrawn as soon as the intercostal tissues were penetrated, and then the cannula was manipulated gently until its point moved freely in the pleural sac. The period of survival varied within comparatively wide limits, but two distinct crises were liable to supervene, either of which might be fatal - namely, acute necrosis of liver and hydrothorax with collapse of lung. Ten rabbits died or were killed between the 10th and 16th days; the remaining six between the 35th and the 47th days of experiment.

^x- The "bile salts" used have been the British Drug Houses' preparation, sold under the name of "Sodium cholate (synonym: Sodium tauroglycocholate)." In later experiments these have been purified by solution in alcohol and filtration: ether has then been added to the point of producing a turbidity, when the salts have been crystallised out at 0°C.

Rabbit 39 . . .	D. 11th day.	Rabbit 48 . . .	D. 13th day.
" 40 . . .	K. 16th day.	" 49 . . .	D. 11th day.
" 41 . . .	K. 35th day.	" 50 . . .	K. 42nd day.
" 42 . . .	D. 41st day.	" 51 . . .	D. 46th day.
" 43 . . .	D. 11th day.	" 52 . . .	D. 14th day.
" 44 . . .	K. 37th day.	" 53 . . .	D. 45th day.
" 46 . . .	D. 11th day.	" 54 . . .	D. 15th day.
" 47 . . .	K. 15th day.	" 55 . . .	D. 10th day.

Microscopically, cellular metaplasia or cellular hyperplasia or both became apparent in the serosal endothelium of the visceral pleura or in the pulmonary epithelium in every animal. In general, the greatest epithelial hyperplasia was disclosed in the peripheral alveoli, immediately subjacent to the serosa. These facts have encouraged the belief that the changes in the serosal endothelium may be the result of a direct action of the reagent upon the surface cells whereas the alveolar changes may be induced by the local diffusion of the reagent through the superficial tissues of the lung.

Summary of post-mortem reports.

Generally speaking, the most consistent feature was a remarkable loss of weight during the first few weeks after the injection. At the end of ten days or a fortnight the animals became emaciated, having lost as much as one-third of their original body weight in spite of the fact that their appetites had not seemed to be grossly impaired. For instance, the weight of Rabbit 47 fell from 2.25 kgm. to 1.5 kgm. in fifteen days. Invariably the retroperitoneal fat was reduced to a minimum. The liver was usually enlarged and pale, sometimes attaining a weight of 180 grams, but it lacked the common characteristics of a "fatty liver"; it was typically tough in consistence and dry, not soft and greasy, while it presented a chestnut rather than a yellowish colour; there was a negative iron reaction. In many cases the organ was the seat of necrosis, either focal or

diffuse; multiple nodular hyperplasia of the parenchyma was found in Rabbit 50 after 42 days. The bulk of the inoculum remained unabsorbed in the right pleural sac; it was associated in every case with a copious serous effusion which was frequently bilateral. The surface of the right lung was smeared more or less uniformly with the dye so that its naked-eye appearances could not be studied; the degree of collapse of the lung was governed by the extent of the effusion. For the purposes of microscopical investigation it was considered adequate to preserve only a portion, perhaps one half or one third, of the right lung of each animal, and this procedure was followed. Unfortunately, later experience proved the necessity of preserving the whole lung for a complete examination of the surface of the organ since the changes at the apex might be more striking than those at the base, and conversely. It is not improbable, therefore, that some interesting phenomena have escaped observation in this series of animals.

Microscopical observations.

Rabbit 39 - (11 days). The substance of the lung is collapsed. There is uniform hyperplasia of the epithelium lining the marginal alveoli, characterised by swelling, nuclear hyperchromatism and cubical formation of the epithelial cells (Fig.1). Discontinuously there is round-cell infiltration of the periphery of the organ. The subserosa is thickened by vascular granulation tissue and the serous surface is covered by a cellular exudate. For the most part, the serosal endothelium is desquamated but a small strip of it is still intact; a few cells retain their flattened form while others have assumed a columnar shape.

Rabbit 40 - (16 days). The substance of the lung is only partially collapsed. Around the periphery of the organ there is a striking degree of epithelial proliferation in alveolar formation, such that the alveoli are lined by several layers of cubical or columnar cells or their lumina may be entirely filled by those cells. The subserosa is thickened by granulation tissue embracing "foreign-body" giant cells. The serosal endothelium is almost completely destroyed but it survives, or has undergone regeneration, in one short strip as a stratified layer of flattened cells. The pleural surface is covered by an abundant cellular exudate.

Rabbit 41 - (35 days). The substance of the lung, in general, is collapsed and congested. The marginal alveoli are lined by cubical or columnar cells which present a notable contrast to the cells lining more deeply situated alveoli (fig.2). The subserosa is represented by a broad zone of granulation tissue which includes a few "foreign-body" giant cells and a certain number of lymphangiectatic spaces. Also multiple cystic formations are present in the thickened subserosa immediately external to the limiting elastic lamina of the lung; the integrity of this lamina has been clearly demonstrated by Weigert's elastic tissue stain. The "cysts" vary in size from 50 μ or more up to 1mm. in diameter; they are frequently closely apposed to one another. With very few exceptions, that margin of each space which abuts upon the pulmonary tissues is lined by flattened endothelial-like cells whereas the opposite border, directed towards the pleural sac, is lined by cubical or columnar cells (fig.3). The transition from one cell type to the other is a very gradual one. Here and there the lining cells assume a stratified formation of squamous type. Two cysts are lined entirely by a

Fig. 1. Rabbit 39 - sudan III/sodium cholate, 11 days - The pleural surface is covered by a cellular exudate. The subserosa is thickened by granulation tissue which embraces two irregular alveoli, lined by tall columnar epithelium and probably derived from "pockets" of serosal endothelium. The epithelium lining the marginal alveoli of the lung is swollen and cubical. (X 100).

Fig. 2. Rabbit 41 - sudan III/sodium cholate, 35 days - The serosal endothelium is desquamated. The subserosa is thickened by granulation tissue and surrounds fat spaces and small lymphangiectases. The subjacent pulmonary epithelium is cubical or columnar in shape whereas the cells lining the more deeply situated alveoli are not affected. (X 80).

Fig. 3. Rabbit 41 - the same - The serosal endothelium is again lacking and the subserosa is thickened. Three closely apposed cystic formations are present superficial to the limiting elastic membrane of the lung; they are lined by cubical or columnar cells on that margin which is directed towards the pleural sac, and by flattened cells along the pulmonary border; the lumen of one contains nondescript cellular debris. The lung is partially collapsed and, in this field, the pulmonary epithelium exhibits no hyperplasia. (X 75).

Fig. 4. Rabbit 41 - the same - There is metaplasia of serosal endothelium from a slight degree of swelling thereof to globular or columnar cells. (X 400).

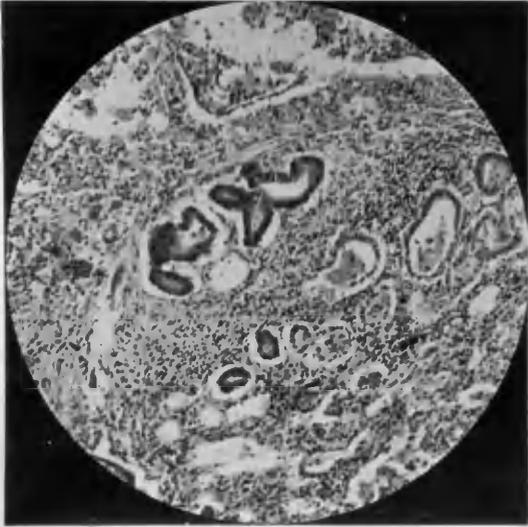


Fig. 1.

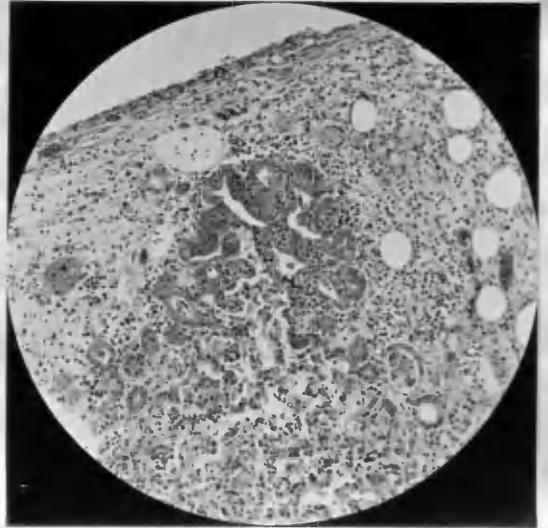


Fig. 2.

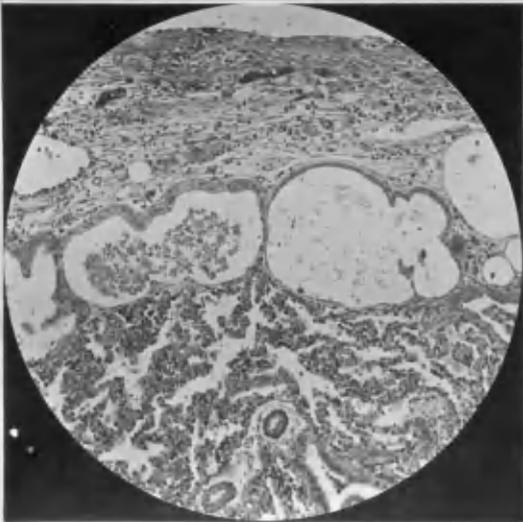


Fig. 3.

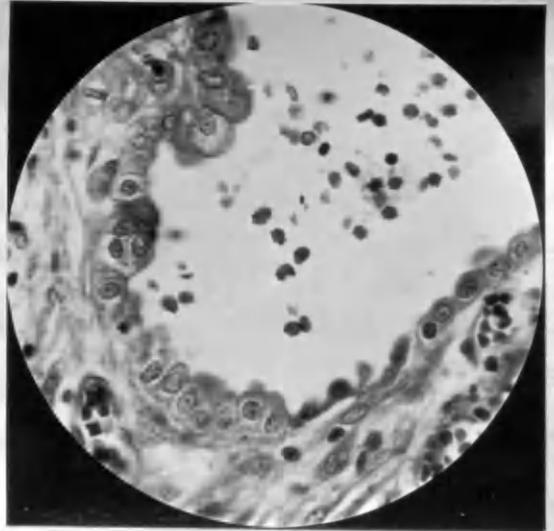


Fig. 4.

transitional epithelium. The lumina of several cysts are occupied by disintegrating desquamated cells: the majority of these cells form a nondescript mass filling the centre of the space, but a few cells at the periphery present a rounded form, with relatively abundant cytoplasm, and a small pyknotic nucleus, tending to recall the characters of desquamated serosal cells. The continuity of the serosal endothelium is interrupted and over certain areas of the pleural surface it is lacking altogether (figs. 2 & 3); elsewhere it presents a great variety of appearances which can be illustrated by reference to five closely adjacent fields. The first field (fig. 4) shows an abrupt transformation from slightly swollen serosal endothelium to epithelial-like cells of relatively high columnar type. The second field (fig.5) which is continuous with the first presents an unbroken sequence of columnar cells. Towards the boundary of the third field (fig.6) the epithelial layer is becoming stratified while it maintains continuity with an invagination into the thickened subserosa. In the fourth field (fig.7) at a small interval from the third, the stratification is plainly visible; it has assumed a transitional type which is more familiar in the lining of the urinary tract. The fifth field (fig.8) is interposed between the third and the fourth; in this case the stratification has acquired a squamous type. Under a lower magnification (fig.9) it is disclosed that the superficial layer is continuous with a comparatively extensive proliferation into the thickened subserosa. In general, it has not been possible to demonstrate any intercellular bridges, or "prickles", between those squamous-like cells, but one exception is illustrated in the next photograph (fig.10) - an oil-immersion field showing the left lip of the small crater upon the

PLATE II.

Fig. 5. Rabbit 41 - sudan III/sodium cholate, 35 days - In this field, continuous with the preceding (fig.4), the thickened subserosa is covered by an unbroken sequence of columnar cells. (X 400).

Fig. 6. Rabbit 41 - the same - Towards the left boundary of this field the epithelial layer is beginning to assume a stratified formation while it maintains continuity with an invagination of epithelial cells in the thickened subserosa. (X 400).

Fig. 7. Rabbit 41 - the same - In this field at a small interval from the last (fig.6), the visceral pleura is covered by a stratified epithelium of transitional type. (X 400).

Fig. 8. Rabbit 41 - the same - Intervening between the two preceding fields (figs. 6 and 7) a short strip of the pleural surface is covered by a stratified epithelium of squamous type. (X 400).

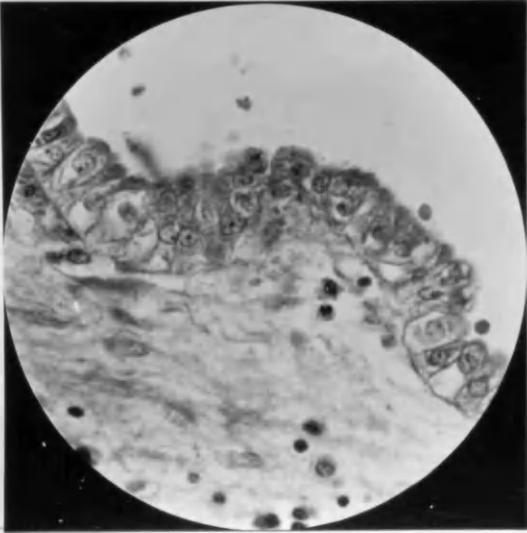


Fig. 5.

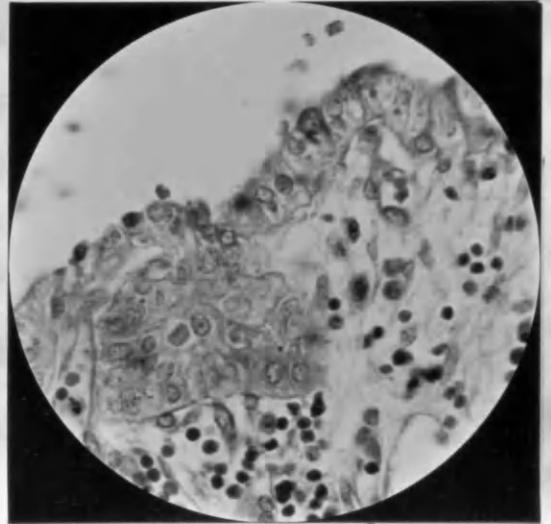


Fig. 6.

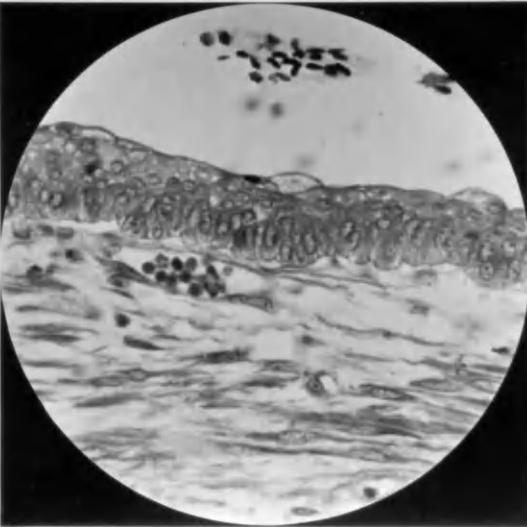


Fig. 7.

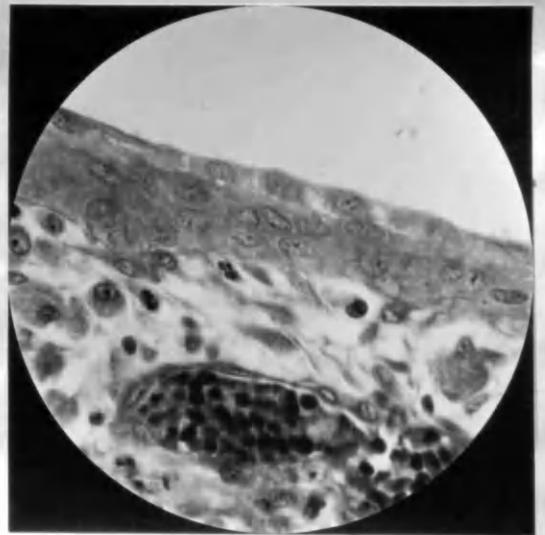


Fig. 8.

pleural surface in the preceding figure (fig.9). The prickles are faint and lack perfect symmetry but they are present.

Rabbit 42 - (41 days). The substance of the lung is partially collapsed. The epithelium lining the marginal alveoli is swollen and cubical but the changes are less conspicuous than in the preceding rabbit. The subserosa is thickened by vascular granulation tissue. The serosal endothelium is entirely desquamated.

Rabbit 43 - (11 days). The lung is partially collapsed. The epithelial cells lining the marginal alveoli are swollen and cubical and their nuclei are hyperchromatic. The subserosa is thickened by vascular granulation tissue. Discontinuously the serosal endothelium has assumed a columnar form.

Rabbit 44 - (37 days). The changes correspond closely with those described in Rabbit 42.

Rabbit 46 - (11 days). Over a wide area of the costal surface of the lung there is a more or less uniform dilatation of the peripheral alveoli, the lining cells of which have assumed a cubical or columnar form. The dilatation is so regular in its configuration that the pulmonary tissues present a scalloped border, as it were, beneath the thickened pleura. At two places, one of which is illustrated (fig.11), the epithelial proliferation becomes so extensive that it fills the whole of a low-power field. Within these foci, the peripheral elastic lamina is shown to be intact by Weigert's elastic tissue stain whereas the interalveolar elastic fibres are ruptured and fragmented. A third focus of cellular hyperplasia and irregular alveolar formation (fig.12) at some considerable distance from the two preceding is present within the substance of the lung; the appearances suggest that it might have developed around a bronchiole.

PLATE III.

- Fig. 9. Rabbit 41 - sudan III/sodium cholate, 35 days - The whole extent of the squamous-cell metaplasia of the serosal endothelium is shown under a low power, and the manner of its invagination in the thickened subserosa. Many plasma cells are present in the surrounding granulation tissue. (X 80).
- Fig.10. Rabbit 41 - the same - In this field from the left lip of the small crater upon the pleural surface in the preceding photograph (fig.9) intercellular bridges or "prickles" can be identified. Heidenhain's Iron Haemotoxylin. (X 750)
- Fig.11. Rabbit 46 - sudan III/sodium cholate, 11 days - The pleural surface is covered by cellular debris and the serosal endothelium is lacking. There is irregular hyperplasia of the epithelium lining the marginal alveoli, and affecting also, in this field, the epithelium of deeper alveoli. (X 70).
- Fig.12. Rabbit 46 - the same - Another focus of irregular fibro-epithelial proliferation is shown within the substance of the lung, at some considerable distance from the surface. (X 70).

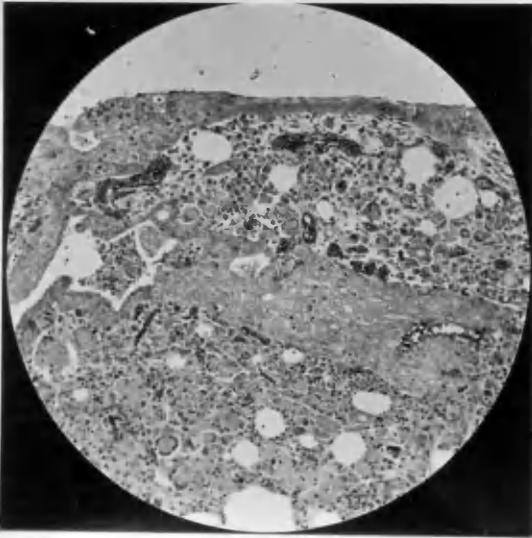


Fig. 9.

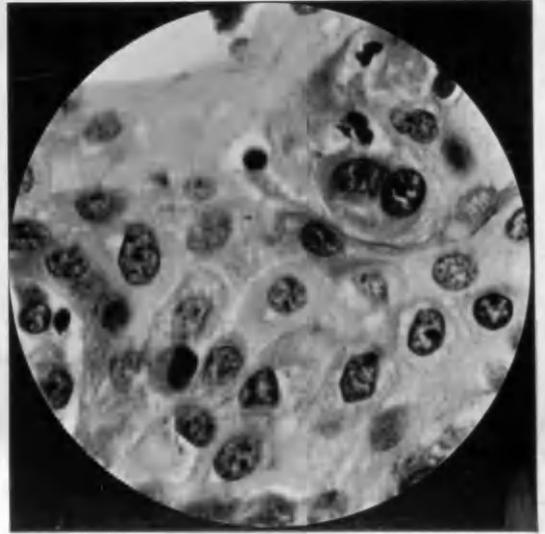


Fig. 10.

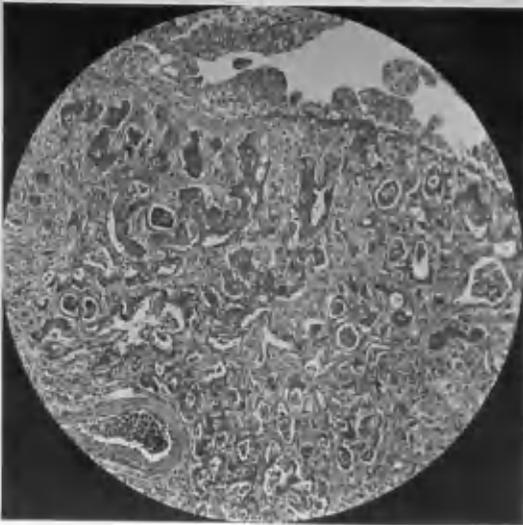


Fig. 11.

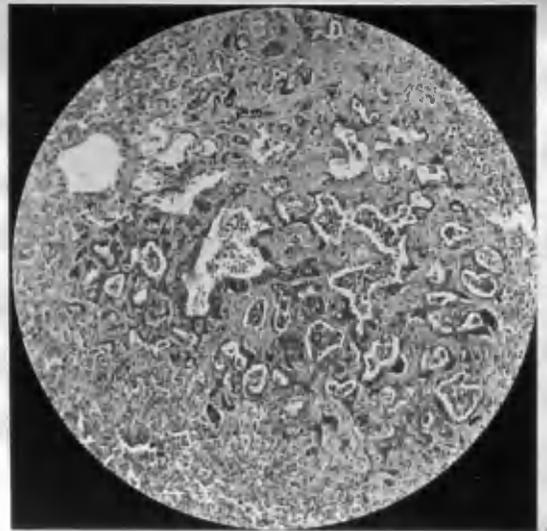


Fig. 12.

No manner of connection has been traced between these three foci of proliferation.

Rabbit 47 - (15 days). In general the changes correspond to those described in the preceding case. There is capillary bronchitis with early broncho-pneumonic consolidation of the lung.

Rabbit 48 - (13 days). The substance of the lung is collapsed. The marginal hyperplasia of the alveolar epithelium is clearly represented and the subserosa is thickened by fibrous tissue. A small strip of the pleural surface is covered by a stratified layer of flattened cells; thence, there extends a wide area of irregular fibro-epithelial proliferation far into the substance of the organ, comprising alveoli lined by one or more layers of cubical cells or solid columns of spheroidal cells (fig.13). Discontinuously around the periphery there is a rich infiltration of lymphocytes.

Rabbit 49 - (11 days). See rabbit 39 for description of changes. (fig.14).

Rabbit 50 - (42 days). The substance of the lung is collapsed. The subserosa and the interlobar septa are greatly thickened by fibrous tissue. Two types of metaplasia and of hyperplasia are observed. The one is a mild variant of the common type (fig.15), whereas the other is represented by a formation which has been unique in this series of experiments. There is an extensive development of tubular or gland-like structures, lined by a double layer of cells, a basal flattened layer and a superficial columnar layer, and surrounded by an abundant connective tissue stroma (fig.16). This formation tends to be reminiscent of the structure of a foetal lung.

Rabbit 51 - (46 days). The subserosa is thickened by fibrous tissue

PLATE IV.

Fig.13.

Rabbit 48 - sudan III/sodium cholate, 13 days - The pleural surface is covered by a stratified layer of flattened cells. Subjacent to it, there is wide-spread epithelial hyperplasia of an aberrant type, lining irregularly shaped alveoli. Such a lesion is believed to arise from the combined effects of trauma and of the fat-soluble dye. (X 70).

Fig.14.

Rabbit 49 - sudan III/sodium cholate, 11 days - The thin tapering extremity of the anterior border of a lobe is shown. The serosal endothelium is lacking and the pleural sac contains cellular debris and fat or oil. There is well-marked hyperplasia of the epithelium lining the marginal alveoli. (X 90).

~~Fig.15. Rabbit 49 - sudan III/sodium cholate, 11 days - There is a striking degree of irregular epithelial hyperplasia in alveolar formation; the lumina of certain alveoli are filled with spheroidal cells. (X 60).~~

Fig.16.

Rabbit 50 - the same - The serosal endothelium is lacking. Numerous tubular or gland-like structures are present surrounded by an abundant connective tissue stroma. The acini are lined by a double layer of cells, a basal flattened layer and a superficial columnar layer, and, by appropriate staining methods, it has been shown that many of them are circumscribed by an elastic lamina. (X 100).

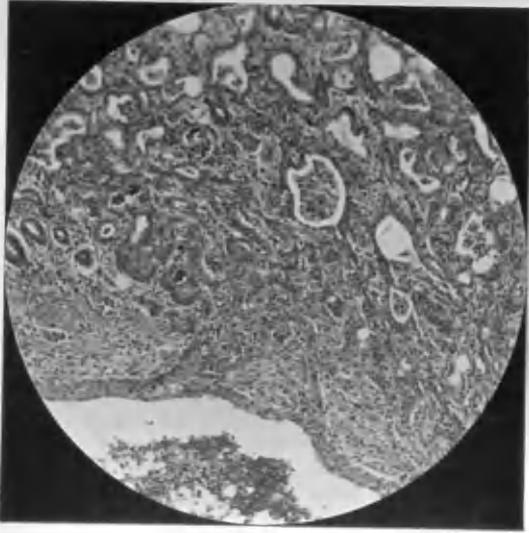


Fig. 13.



Fig. 14.

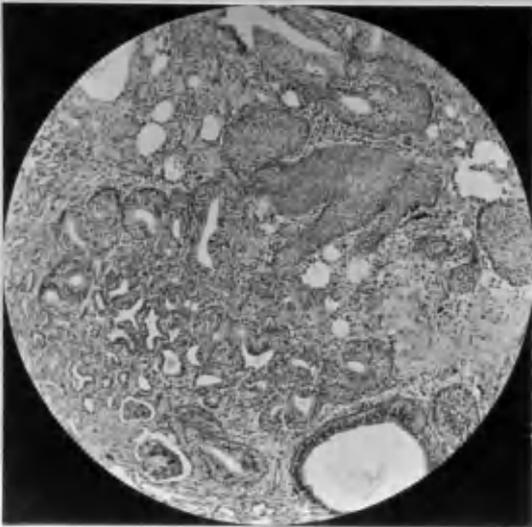


Fig. 15.

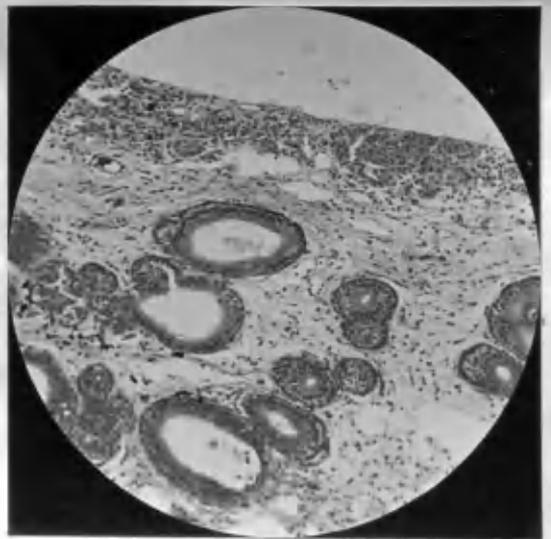


Fig. 16.

which envelops a number of cysts lined by stratified epithelium of transitional or squamous type, and filled with cellular debris. Metaplasia and hyperplasia of the alveolar epithelium are observed discontinuously around the periphery of the lung.

Rabbit 52 - (14 days). The changes correspond more or less closely to those described in Rabbit 39.

Rabbit 53 - (45 days). See Rabbit 51.

Rabbit 54 - (15 days). There is a striking degree of cellular proliferation, at one place forming solid columns of spheroidal cells, and at another place irregular alveoli lined by cubical or flattened cells. Two closely adjacent fields are shown. The first (fig.17) illustrates the superficies of the lung and includes the altered serosa which is covered by a mass of debris and cellular exudate lying within the lumen of the pleural sac. The serosal endothelium has assumed a stratified formation and from its deep surface cellular processes are continued into a broad belt of granulation tissue which represents the thickened subserosa. More deeply the cellular processes become luminated and form irregular alveoli (fig.18); it is clear that they have transgressed beyond the limiting elastic membrane of the lung since they impinge upon fragmented strands of elastic tissue.

Rabbit 55 - (10 days). See Rabbit 39.

The subsequent history of those changes has been followed over a period of sixteen months, in other series of rabbits. Complete regression has been the rule. The process of involution overtakes the swollen hyperplastic cells slowly, being presaged for a month, more or less, by eosinophil staining of their cytoplasm and nuclear pyknosis. Ultimately they seem to undergo disintegration in the

- Fig. 17. Rabbit 54 - sudan III/sodium cholate, 15 days - The pleural sac contains a cellular exudate. The surface of the visceral pleura is covered by a stratified layer of flattened cells; thence cellular processes pass into the thickened subserosa, presenting some resemblance to the papillae of the cutis. (X 110).
- Fig. 18. Rabbit 54 - the same - More deeply the epithelial proliferation assumes a more irregular type in alveolar formation. (X 70).
- Fig. 19. Rabbit 116 - sudan III/sodium taurocholate/lanolin, 16 months - A small clump of aberrant spheroidal cells attached to the wall of a cyst in the thickened subserosa is illustrated. No other evidence of epithelial hyperplasia has been found in this lung but the subserosa and the interlobar septa are thickened by hyaline connective tissue. (X 200).
- Fig. 20. Rabbit 89 - sudan III/lanolin followed eight weeks later by sudan III/sodium cholate/olive oil, 16 months - This field shows two large cystic formations, left and right respectively, lined by tall columnar mucus-secreting cells and filled with cellular debris. The middle third of the field is occupied by small irregular alveoli, lined by cubical cells, surrounded by connective tissue and associated with a very rich lymphocytic infiltration. (X 100).

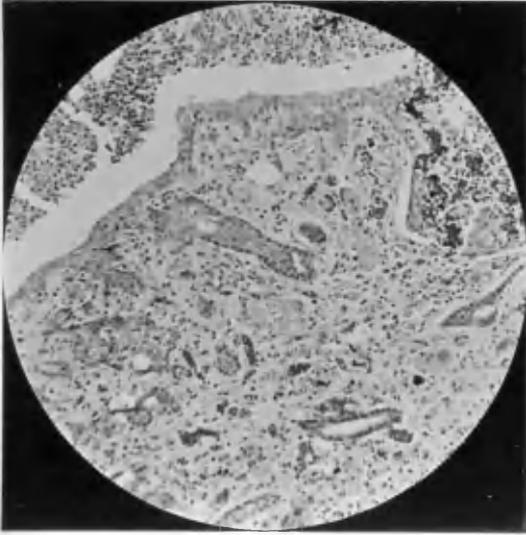


Fig. 17.

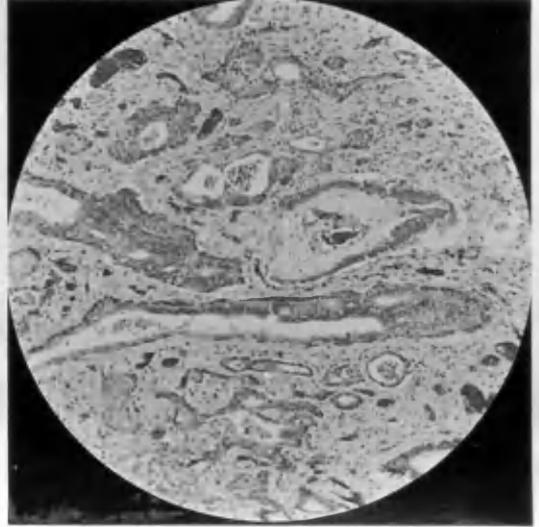


Fig. 18.

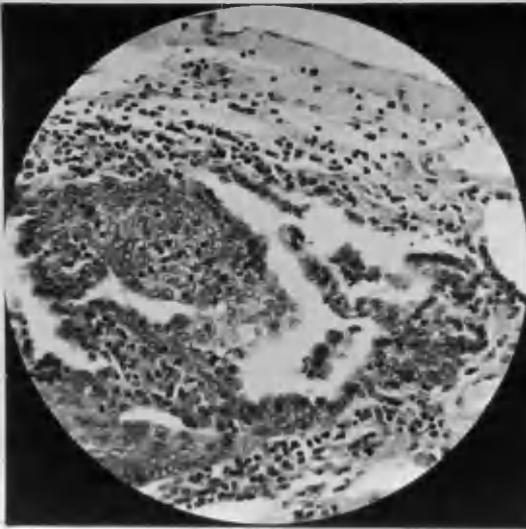


Fig. 19.



Fig. 20.

presence of a rich lymphocytic infiltration and, by and by, the normal structure of the lung is restored apart from the presence of a few fibrous cicatrices. The fibrous thickening of the subserosa and of the interlobar septa, accompanied in most cases by "foreign-body" giant cells, is a conspicuous feature throughout. Two examples of squamous-cell metaplasia of the serosal endothelium are worthy of special mention. They occurred in Rabbits A.23 and A.24 at the end of eleven weeks. In one case (A.23) the "prickles" are remarkably clear and symmetrical, while keratohyalin granules are present in the other case. Also two animals, out of twenty which survived the intrapleural injection of sudan III and bile salts and which were killed sixteen months later, provide exceptions to the rule affecting the regression of the metaplasia of the serosal endothelium and pulmonary epithelium. Just a few islets of aberrant spheroidal cells, possessing healthy vesicular nuclei, are ~~observed in the thickened subserosa of Rabbit 116 (fig.19).~~ An entirely different formation is present in Rabbit 89. In this case numerous cysts are found within the substance of the lung, lined by tall columnar mucus-secreting cells and encapsulated by fibrous tissue. These major cysts are surrounded by a fringe of atypical alveoli, lined by one or more layers of cubical cells (fig.20). In neither of the two cases, however, is there any evidence of active growth and no mitotic figures are recognised.

It might be added that the inoculum of sudan III tends to become enclosed by a thick fibrous capsule, usually about the base of the lung, after three or four months and subsequently the animals gain a kilogram or more in weight.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

- (1) Extensive and varied metaplasia of the serosal endothelium covering the visceral pleura, and of the epithelium lining the marginal alveoli, of the lung of the rabbit can be produced by injecting into the pleural sac a mixture of sudan III and sodium cholate in olive oil. In two exceptional cases, these changes have been witnessed sixteen months after the injection but, as a rule, they undergo regression within three or four or six months.
- (2) Sudan III in olive oil, without the sodium cholate, is relatively ineffective.
- (3) There seems to be little doubt that the sodium cholate plays an important part in the development of the metaplastic phenomena.

EXPERIMENTS WITH SODIUM CHOLATE EMULSIFIED IN OLIVE OIL OR LIQUID PARAFFIN OR DISSOLVED IN PHYSIOLOGICAL SALINE.

These experiments were undertaken in the routine analysis of the components of the mixture which had proved effective in the preceding investigations. The intrapleural injection of olive oil produces no notable reaction in the serosal endothelium or in the pulmonary epithelium. On the other hand, an unmistakable reaction is produced by the injection of olive oil emulsified with bile salts, and autoclaved. The inoculum was prepared as follows:-

- Olive oil 150 c.c.
- Sodium cholate (5% aqueous soln)..... 15 c.c.

A series of 12 rabbits was injected, each with 10 c.c. of the emulsion, into the right pleural sac. The appearances of the lung on the fifth day of the experiment (Rabbit 183) are illustrated

(fig.21); they are comparable with those described in the sudan III/bile salts series but the changes are short-lived, disappearing within ten days. It might not be irrelevant to mention in passing that changes of the same type can be produced in the guinea-pig by the injection of an emulsion containing a larger proportion of bile salts, thus:-

Olive oil..... 30 c.c.

Sodium cholate (10% aqueous soln.) 10 c.c.

Each guinea-pig received 2.5 c.c. of this emulsion into its right pleural sac. After 72 hours (Guinea-pig 7) the alveolar epithelium is altered uniformly around the periphery of the lung and numerous mitotic figures bear witness to a state of unusual excitement (fig.2

So far as the efficacy of the bile salts was concerned, however it was clear that no reliable deductions could be drawn from these observations, on account of the impossibility of ensuring adequate ~~controls. Olive oil, dispersed in an aqueous medium,~~ is hydrolysed on autoclaving to a greater extent than the unemulsified oil, and the increased content of free fatty acid introduced a serious fallacy. In the first instance, it was sought to overcome this difficulty by neutralising the inoculum with N/NaOH. The event proved that the proliferation of the epithelial cells lining the marginal alveoli of the lung was not abated, yet it remained impossible to assign the processes of cell-division to the direct action of the bile salts and to exclude the intervention of free oleic acid and its sodium salt, formed by hydrolysis of the oil within the pleural sac. On these grounds, it seemed expedient to employ a non-saponifiable substance as the vehicle for the introduction of the bile salts. For this purpose liquid paraffin was

PLATE VI.

Fig.21. Rabbit 183 - olive oil/sodium cholate, 5 days - The serosal endothelium has been almost completely regenerated. There is distinct hyperplasia of the pulmonary epithelium lining the marginal alveoli, characterised by swelling and nuclear hyperchromatism; the epithelium lining the more deeply situated alveoli is normal in appearance. The lung is partially collapsed. (X 70).

Fig.22. Guinea-pig 7 - olive oil/sodium cholate, 3 days - the serosal endothelium has been desquamated. The unstriped muscle of the subserosa is infiltrated with leucocytes or destroyed. There is active hyperplasia of the marginal epithelium of the lung evidenced by numerous mitotic figures. (X 300).

PLATE VI.

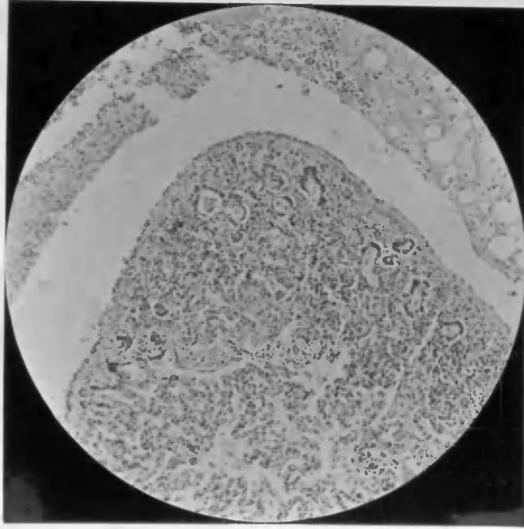


Fig. 21.

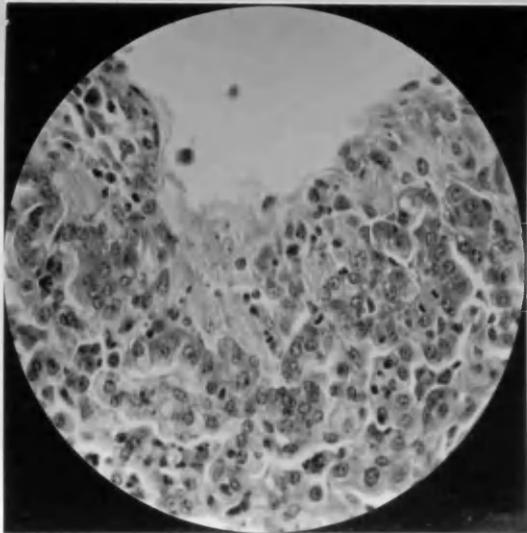


Fig. 22.

chosen. An emulsion of 100 c.c. liquid paraffin and 10 c.c. of a 5% aqueous solution of sodium cholate was autoclaved and 10 c.c. were injected into the right pleural sac of each of ten rabbits. The animals were killed at intervals of 24 hours, more or less, up to ten days. The descriptive data which follow refer to the microscopical appearances of the right lung; the duration of the experiment is indicated in each case.

Rabbit A.132 - (24 hours). The most conspicuous feature is an infiltration of the sub-serosa and of the periphery of the lung by polymorphonuclear leucocytes. There is an appreciable degree of swelling of the serosal endothelium, where it has not been desquamated; here and there, likewise, swelling of the epithelial cells lining the marginal alveoli is observed. In one section there is more distinct indication of increased epithelial activity, evidenced by a few mitotic figures and by more uniform swelling of the cells and of their nuclei. The substance of the lung in general is emphysematous.

Rabbit A.133 - (48 hours). There is well marked hyperplasia affecting the serosal endothelium and the epithelium lining the marginal alveoli over a large proportion of the surface of the lung. The epithelial changes are the more conspicuous. Mitotic figures are not very numerous but nuclear hyperchromatism is a consistent feature. There is a serous exudate upon the surface of the visceral pleura mingled with scanty polymorphonuclear leucocytes. Throughout the lung substance the capillaries are congested and an albuminous exudate is observed in the alveoli.

Rabbit A.134 - (72 hours). More active manifestations of increased cellular activity become apparent, characterised by mitotic figures

in a relatively large number of epithelial cells lining the marginal alveoli. These cells have assumed a cubical form; their nuclei are swollen and hyperchromatic. The same changes are observed in the serosal endothelium (fig.23). Here and there an irregular mass of chromatic substance is found extracellularly within the zone of active proliferation. The process of hyperplasia is discontinuous but it occurs in strips of considerable extent at intervals over the whole surface of the lung; also it is limited strictly in its distribution to the serosal endothelium and to the epithelium of the marginal alveoli.

Rabbit A.135 (96 hours). The cubical formation and the nuclear hyperchromatism of the peripheral epithelial cells are maintained and a few mitotic figures are still observed. The general impression is formed however that the cellular activity is beginning to wane.

From the fourth day onwards the epithelial proliferation undergoes a process of rapid involution so that the normal structure of the lung is restored by the seventh or eighth day.

The injection of 10 c.c. of liquid paraffin, without bile salts, has failed consistently to excite epithelial proliferation. Occasionally a perceptible degree of swelling has been noted in the serosal endothelium and in the peripheral epithelium during the first two or three days but the changes have been relatively insignificant. A characteristic field from the lung of rabbit A.128 (72 hours) is presented (fig.24) for contrast with fig.23.

At a very early stage in these experiments, a series of injections was made with 1% and with 0.5% solutions of sodium cholate in

PLATE VII.

Fig.23. Rabbit A.134 - liquid paraffin/sodium cholate, 72 hours - The serosal endothelium is swollen and there is slight thickening of the subserosa. There is well-marked hyperplasia of the epithelium lining the marginal alveoli of the lung characterised by swelling cubical formation and nuclear hyperchromatism, and not affecting the deeper epithelium. (X 130). Cf. Fig.24.

Fig.23a. A higher magnification of the same field shows numerous mitotic figures in the epithelial cells. (X 300).

Fig.24. Rabbit A.128 - liquid paraffin alone, 72 hours - there is no appreciable swelling of the marginal epithelium and the appearances of the lung are nearly normal. (X 130). Cf. Fig. 23.

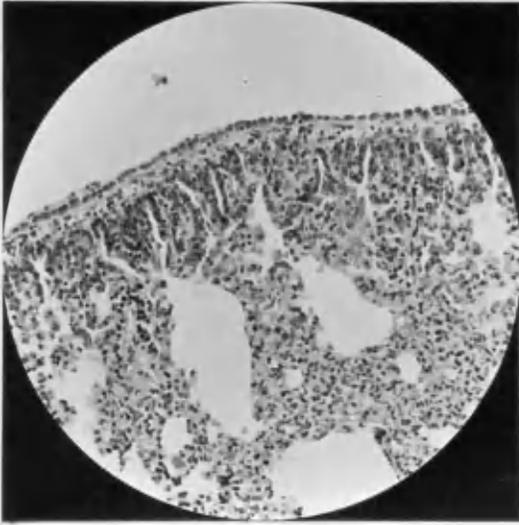


Fig. 23.

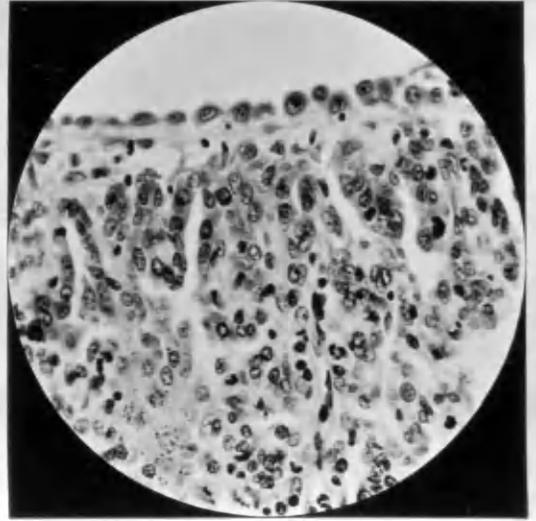


Fig. 23a.

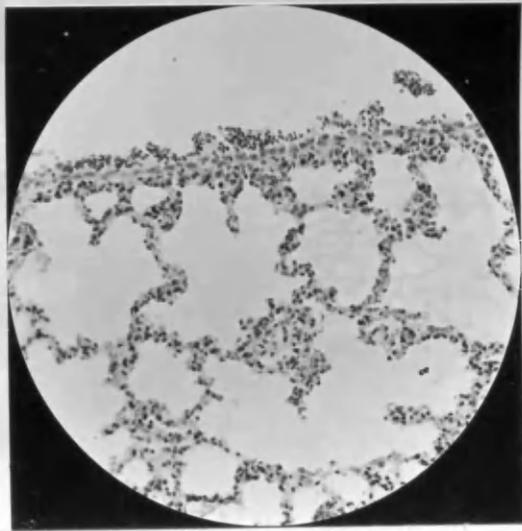


Fig. 24.

distilled water or in physiological saline. Again and again the animals died with acute pulmonary oedema. The emulsification of the bile salts was adopted in later experiments mainly in the hope that the reagent might prove less lethal if it were dispersed in an oily medium. When active proliferation was obtained under those circumstances, it was supposed for a time that the reagent might be effective only when it was exhibited in a comparatively weak solution over a period of some duration - e.g., 24 hours - since the emulsion seemed to "break" slowly. However, these impressions have been proved to be erroneous during the past few months and it has been shown that epithelial changes of the same order (figs. 25 and 26) can be produced by the intrapleural injection of 1% (5 c.c.) or of 0.5% (10 c.c.) solutions of sodium cholate in physiological saline, whereas the saline alone produces no reaction. The acute pulmonary oedema which had been such a stumbling-block at an earlier date was due to superficial puncture of the surface of the lung by the point of the trocar.

These results encouraged the view that the proliferative phenomena were dictated by the direct action of the bile salts upon the parent cells. The necessity then arose to try to determine whether the cellular hyperplasia was caused by the physical properties or by the chemical nature of the bile salts. The complexity of this reagent alike in its chemical structure and in its physical properties rendered it desirable, or preferable, to exploit alternative methods of experimentation with simpler reagents in order to achieve this end.

PLATE VIII.

Fig.25. Rabbit B.97 - 1% sodium cholate in physiological saline (5 c.c.), 95 hours - The regeneration of the serosal endothelium has been completed. The subserosa is thickened by oedematous granulation tissue. There is marked hyperplasia of the epithelium lining the marginal alveoli of the lung. (X 200).

Fig.26. Rabbit B.89 - 0.5% sodium cholate in physiological saline (10 c.c.), 73 hours - The serosal endothelium is discontinuous. The subserosa is oedematous and infiltrated with leucocytes. There is active hyperplasia of the peripheral epithelium of the lung. (X 200).

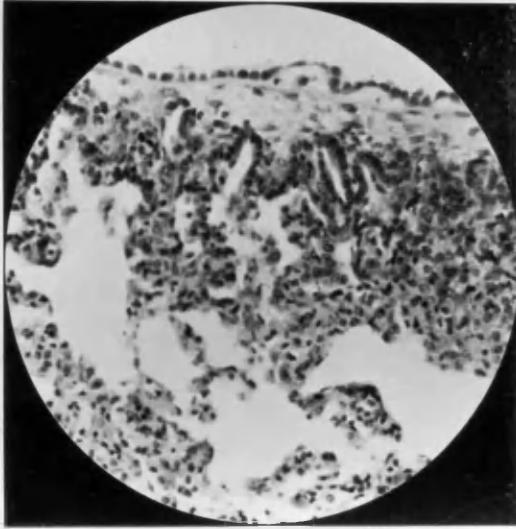


Fig. 25.

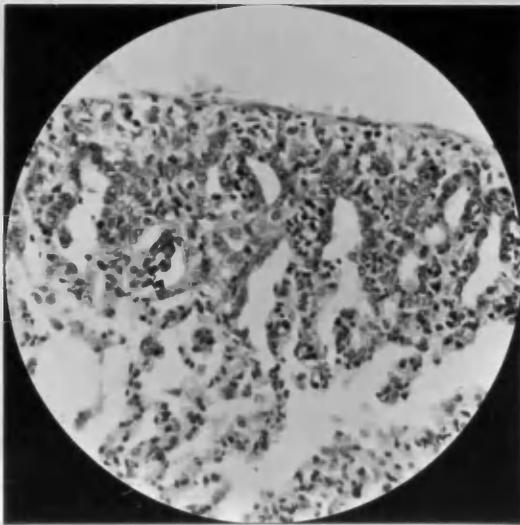


Fig. 26.

EXPERIMENTS WITH SOLUTIONS OF ELECTROLYTES.

Theoretical principles.

In general terms, the object of the experimental procedure had resolved itself into an attempt to analyse the effects of altering the environment of the serosal endothelium and of the epithelium lining the marginal alveoli of the lung 'in vivo' by the injection of simple substances, utilising the pleural sac as a natural "culture chamber". The injection of a medium, such as olive oil or liquid paraffin, however inert it might be in a biological sense, precluded a clean experiment since it remained as a "foreign body" in the pleural sac after the active agent had exhausted itself. This circumstance could be averted only by the employment of an agent or agents which were effective in aqueous solution.

The prevailing views in respect of the constitution of the cell membrane seem to favour a sieve-like structure, whereof the mesh is composed of a most intimate combination of protein cholesterol and phosphatide. Leathes has emphasised the importance of the part played by the lipoids in controlling the vital phenomena of the permeability and of the surface tension of the membrane of the living cell, yet he does not deny to the proteins a subsidiary role. Therefore it seemed possible that some visible changes might be excited in the serosal endothelium and in the epithelium lining the marginal alveoli of the lung by the intrapleural injection of reagents which might be expected to exercise a selective effect upon the proteins of their cell membranes. In this connection, the later work of Loeb has directed special attention to solutions of neutral salts. His observations are reviewed in one of his last monographs on the proteins (1924). He sought to apply Donnan's theory of membrane

equilibria to the interpretation of the colloidal behaviour of proteins with such success that he was enabled to derive the influence of electrolytes on certain important physical properties of proteins quantitatively and mathematically. For the present purpose it will suffice to quote one principle which he established "The addition of a neutral salt to a protein solution (not at the isoelectric point) depresses the osmotic pressure and viscosity of the protein solutions, and the degree of swelling of gels, and this effect increases with the valency of that ion of the salt which has the opposite sign of charge to that of the protein ion, but is independent of the chemical nature of the ion." It follows that the cation of the neutral salt will be effective when the protein is on the alkaline side of its isoelectric point.

As the hydrogen-ion concentration of the body fluids of the rabbit lies clearly on the alkaline side of the iso-electric point of most animal proteins (pH 4.5 to pH 6) it seemed justifiable to project a series of experiments based upon the intrapleural injection of neutral salts in chemically equivalent proportions. For the same reason it was decided to employ salts possessing mono-, bi-, and tri-valent cations and a common anion, so that the effect of increasing the valency of the cation might be studied as an isolated factor, as nearly as possible. These requirements were fulfilled by the selection of a 3/4 normal solution of sodium chloride, of strontium chloride, and of aluminium chloride respectively. At the same time, subsidiary experiments were performed with less concentrated solutions of each salt. Later, attention was directed to the effects produced by the chlorides of lithium and potassium, of magnesium and calcium, and of iron (ferrous and ferric) and lanthanum in order to test the consistency of the "valency rule".

experimental methods.

All the solutions were autoclaved (with the exception of the iron salts) prior to injection, which was made as carefully as possible into the right pleural sac by trocar and cannula according to the method previously described (page 9). Special care is demanded because even a minor injury of the lung is liable to be followed by an acute pulmonary oedema and sudden death of the rabbit. It has not been practicable to autoclave the solutions of ferrous chloride or of ferric chloride on account of their instability and, with the object of excluding as many living organisms as possible from the inoculum, concentrated solutions ($3/4$ N.) of each salt have been diluted in the required degree with sterile distilled water in sterile flasks. Also a routine practice has been adopted of aerating the inoculum by repeatedly filling and discharging a syringe from and into a beaker and of warming the solution to body temperature (or thereby) just before it has been injected. Perhaps the importance of these two steps has been exaggerated. Their effects as adjuvants of any given epithelial reaction have not been extensively studied, but it has been shown more recently that a non-aerated $1/20$ N. solution of ferrous chloride, at room temperature, produces a degree of proliferation which is not appreciably less than that produced by a warmer aerated solution.

The animals were killed by a blow on the back of the neck in series at intervals of 24 hours. The violence of this manner of death has been associated in exceptional cases with petechial haemorrhages in both lungs. When the pleural sac is opened the lung collapses spontaneously. Profiting by a suggestion, kindly

offered by Professor Muir, I have ligatured the trachea before cutting the chest wall so that this collapse has been reduced to a minimum. Next, the pericardial sac was opened and the heart excised. The left bronchus was ligatured before removing the left lung. The right lung was then fixed as a whole in Zenker's fluid for three or four hours before the organ was cut into four, five or six segments for complete microscopical examination. The effects of strontium chloride have been studied in the largest series of animals but two or more independent investigations have been made with each of the other salts upon each day of experiment. The results have been remarkably consistent from day to day and it is believed that the following descriptive data represent fair samples of the cellular reactions, which have been observed in the right lung. It might be emphasised that each of the animals comprised within this series of experiments received only a single intrapleural injection.

(I) Experiments with electrolytes possessing mono-valent cations - the chlorides of (a) lithium, (b) sodium, and (c) potassium.

(a) LITHIUM CHLORIDE. (LiCl, 2H₂O).

3/4 normal solution LiCl - 5.9% - 5 c.c. - 6 rabbits.

The animals were killed at intervals of 24 hours on the 1st, 2nd, 3rd, 4th, 5th, and 6th days respectively. The right pleural sac was dry in every case. One rabbit (A.308) bled freely from the nose when it was killed and its right lung was found to be the seat of multiple small haemorrhages; its left lung was normal in appearance. In other rabbits the right visceral pleura was mildly congested until the third day; no other gross change was apparent throughout the experiment.

Microscopical observations.

Rabbit A.308 - (48 hours). There is irregular congestion of the substance of the organ, with multiple small haemorrhages which have produced no reaction on the part of the adjacent tissues. Discontinuously around the periphery there is slight swelling of the epithelium lining the marginal alveoli with some oedema of the subserosa and polymorphonuclear infiltration. There is no evidence of active epithelial hyperplasia.

Rabbit A.309 - (70 hours). The substance of the organ is normal in appearance. Around the periphery there are oedema of the subserosa and swelling of the serosal endothelium, again discontinuous and associated with a minor degree of swelling of the alveolar epithelium. There is no evidence of active epithelial hyperplasia.

In the other rabbits of this series (A.307, A.310, A.311, A.312) the appearances of the lung are practically normal.

3/20 normal solution - LiCl - 1.18% - 25 c.c. - 6 rabbits.

The animals were killed at intervals of 24 hours on the 1st, 2nd, 3rd, 4th, 5th, and 6th days respectively. In every case the right pleural sac was dry and the appearances of the right lung were normal apart from some degree of irregular congestion during the first three days of experiment.

Microscopical observations.

Rabbit A.303 - (71 hours). There is evidence of capillary bronchitis and early broncho-pneumonic consolidation of the lung, associated with albuminous exudate into the marginal alveoli and swelling of the epithelium lining them. Also there is haemorrhage within the substance of the lung surrounded by a zone of epithelial

hyperplasia.

Similar changes are present on the 5th day (Rabbit A.305), whereas the lungs of the other members of the series, A.301, A.302, A.304 and A.306, are practically normal in appearance. At most, there is a slight degree of swelling of the marginal epithelium and there is no evidence of active hyperplasia.

(b) SODIUM CHLORIDE. (NaCl).

3/4 normal NaCl - 4.3 per cent - 5 c.c. - 9 rabbits.

The animals were killed on the 1st (1), 2nd (2), 3rd (2), 4th (1), 5th (1) and 6th (2) days respectively. In two cases a small quantity of blood-clot was found in the pleural sac as a result of trauma during injection. Until the fourth day there was fairly distinct hyperaemia of the visceral pleura. The pleural sac was dry throughout.

Microscopical observations.

Rabbit A.163 - (24 hours). There is slight swelling of the epithelium lining the marginal alveoli of the lung associated with mild hyperaemia and a moderate leucocytic infiltration of the periphery of the organ.

Rabbit A.198 - (48 hours). The substance of the lung in general is normal in appearance. There is clear evidence of hyperplasia of the epithelium lining the marginal alveoli, characterised by swelling and by nuclear hyperchromatism; mitotic figures are present in small numbers. The hyperplasia is found in small superficial plaques at wide intervals.

Rabbit A.199 - (72 hours). The lung is emphysematous. Otherwise its appearances are normal but hyperplasia of the marginal epithelium

is disclosed over one small strip of the surface of the organ (fig. 27).

From the fourth day inclusive the appearances of the lung are normal.

3/8 normal NaCl - 2.15% - 12.5 c.c. - 3 rabbits.

The animals were killed on the 1st, 3rd and 4th days respectively. In each case the pleural sac was dry and the visceral pleura exhibited a minor degree of irregular congestion. Microscopically slight degrees of swelling are noted in the serosal endothelium and in the marginal epithelial cells but they are almost negligible. There is no nuclear hyperchromatism and mitotic figures are not present.

0.145 normal NaCl (physiological saline) - 0.85% -
25 c.c. - 4 rabbits.

The animals were killed on the 1st, 3rd, 4th and 6th days. To the naked eye the right lung presented a normal appearance in each case and the pleural sac was dry and healthy. Microscopically no gross change is apparent in the serosal endothelium or in the pulmonary epithelium lining the marginal alveoli. A typical field showing the periphery of the lung is illustrated (fig.28) from rabbit A.155 killed on the third day; the organ is emphysematous; there is no evidence of epithelial hyperplasia.

(c) POTASSIUM CHLORIDE. (KCl).

3/4 normal solution - KCl - 5.6% - 5 c.c. - 6 rabbits.

One animal died a few minutes after the injection with acute pulmonary oedema; the others were killed at intervals of 24 hours from the second day onwards. Irregular congestion of the surface

PLATE IX.

Fig.27. Rabbit A.199 - $3/4$ N. NaCl, 72 hours - Over the greater part of the surface of the lung, the pulmonary epithelium exhibits only a minor degree of swelling. In this field, however, there is some oedema of the subserosa, accompanied by distinct hyperplasia of the marginal epithelium. (X 200).

Fig.28. Rabbit A.155 - 0.145 N. NaCl (physiological saline), 72 hours - The lung is emphysematous, otherwise normal in appearance. There is no epithelial hyperplasia. (X 100).

Fig.29. Rabbit A.297 - $3/4$ N. KCl, 76 hours - The serosal endothelium is swollen and the subserosa is slightly oedematous. There is only a minor degree of swelling of the marginal epithelium and there is no active hyperplasia. (X 200).

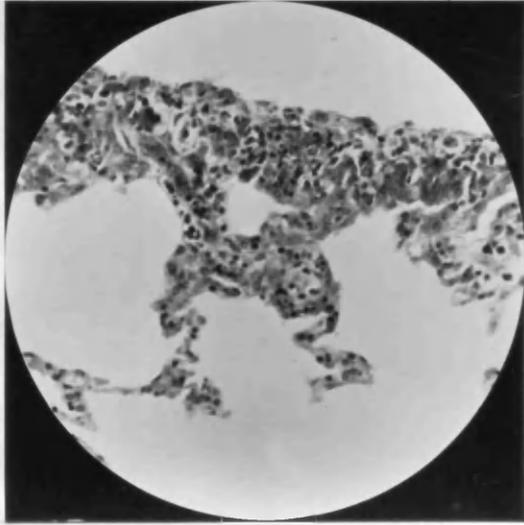


Fig. 27.

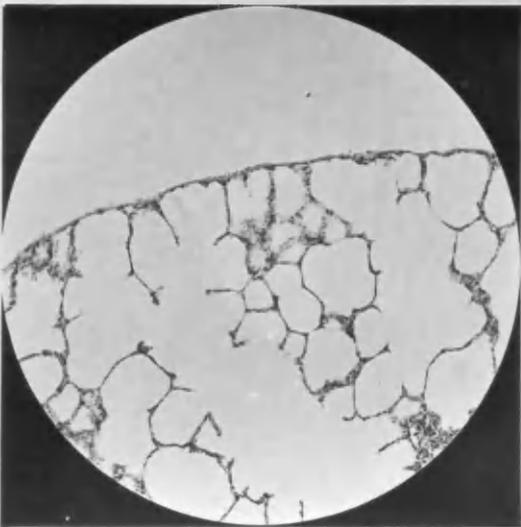


Fig. 28.

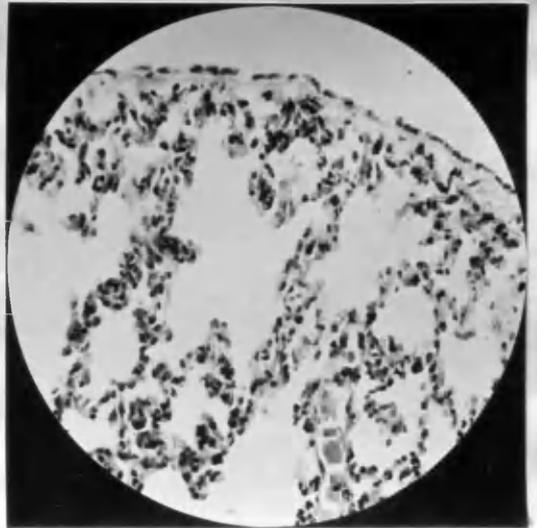


Fig. 29.

of the right lung was observed after 48 hours, but thenceforth the appearances of the organ were normal. The right pleural sac was invariably dry.

Microscopically, a minor degree of swelling of the epithelium lining the marginal alveoli is noted discontinuously around the periphery of the lung on the second day, but the changes do not proceed further as the experiment advances. Even on the third day (fig.29 - Rabbit A.297) there is no active hyperplasia of the epithelium.

3/20 normal solution - KCl - 1.12% - 25 c.c. - 6 rabbits.

Three animals died - two within a few minutes and the third within 24 hours - as a result of the injection; the survivors were killed after 48, 76 and 100 hours respectively. In the last three the pleural sac was dry; the lungs were moderately emphysematous, otherwise normal.

Microscopically, there is no epithelial hyperplasia. Here and there, slight swelling of the marginal epithelium is recognised, but in general the changes are almost entirely negligible.

(2) Experiments with electrolytes possessing bi-valent cations - the chlorides of (a) magnesium, (b) calcium, (c), strontium, (d) barium, and (e) iron (ferrous).

(a) MAGNESIUM CHLORIDE. (MgCl₂,6H₂O).

3/4 normal solution - MgCl₂ - 7.63% - 5 c.c. - 15 rabbits.

The animals were killed at intervals of 24 hours on the first (2), 2nd (3), 3rd (3), 4th (3), 5th (2), and 6th (2) days. A small quantity of free fluid was found in the right pleural sac of two

rabbits (1st and 3rd days respectively); in all the other cases both sacs were dry. There was irregular congestion of the surface of the right lung on the 1st and 2nd days of experiment; on the 3rd day the surface of the right lung was wrinkled in two animals; from the fourth day onwards the appearances of the right lung were normal.

Microscopical observations.

Rabbit A.284)
" B. 23) - (24 hours). The substance of the lung in general is normal in appearance. Discontinuously, around the periphery there is a minor degree of swelling of the epithelium lining the marginal alveoli associated with an emigration of polymorphonuclear leucocytes. The serosal endothelium is largely intact presenting no gross changes.

Rabbit A.283)
" B. 24) - (48 hours). The serosal endothelium is swollen and
" B. 25)
the subserosa is congested and oedematous and infiltrated with polymorphonuclear leucocytes; the epithelium lining the marginal alveoli has assumed a cubical shape and nuclear hyperchromatism is a conspicuous feature (fig.30 - Rabbit A.283).

Rabbit A.285)
" B. 26) - (72 hours). There is oedema of the subserosa with
" B. 27)
some swelling of the serosal endothelium. Around the periphery of the lung there is nearly uniform hyperplasia of the alveolar epithelium characterised by swelling, nuclear hyperchromatism and occasional mitotic figures. It is remarked, however, that the nuclei of many of the swollen epithelial cells are becoming pyknotic in the earliest phase of their involution. Also irregular masses of chromatin, or of a chromatin-like substance, are observed lying

PLATE X.

Fig.30. Rabbit A.283 - 3/4 N. MgCl₂, 48 hours - there are well-marked swelling and nuclear hyperchromatism of the epithelium lining the marginal alveoli. The periphery of the lung is infiltrated with leucocytes. (X 200).

Fig.31. Rabbit A.285 - 3/4 N. MgCl₂, 72 hours - the epithelial hyperplasia is maintained but the nuclei of the swollen cells are becoming pyknotic in an early phase of involution. A dumb-bell shaped mass of chromatin is shown, lying extracellularly, near the centre of the field. (X 200).

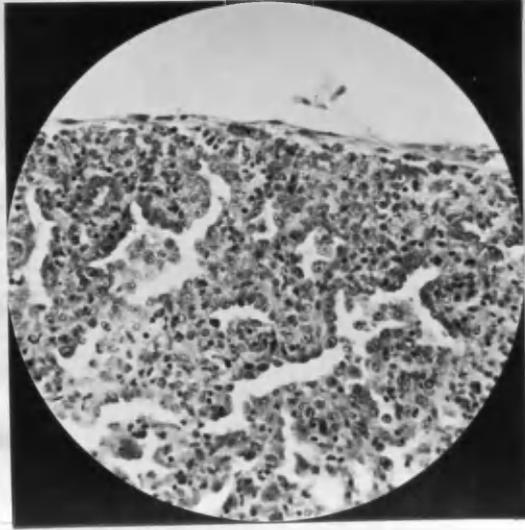


Fig. 30.

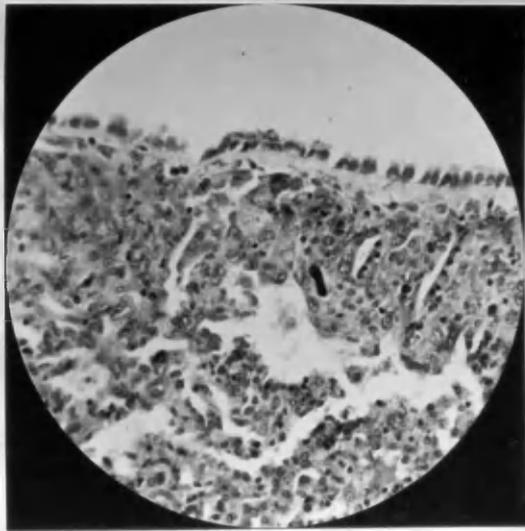


Fig. 31.

free between the cells. (fig.31 - Rabbit A.285). Apart from the altered appearances of the marginal zone of the lung, the structure of the organ in general is normal.

Rabbit A.286)

" B. 28) - (96 hours). Some swelling of the serosal endothelium
" B. 29)
is present with slight oedema of the subserosa. The marginal epithelium is still swollen but nuclear pyk^onosis is more general associated with a lymphocytic infiltration of the periphery of the lung. The epithelial hyperplasia is undergoing involution.

Rabbit A.287)

" B. 30) - (5 days). Over a large part of the surface of the lung there is no evidence of epithelial hyperplasia at all, and the appearances of the organ are normal. Elsewhere there is swelling of the marginal epithelium with nuclear pyknosis and these areas are infiltrated with lymphocytes. The subserosa is slightly thickened by fibrous tissue.

Rabbit A.288)

" B. 31) - (6 days). The same changes are present but the swelling of the epithelium is still more erratic than in the preceding case.

3/20 normal solution MgCl₂ - 1.52% - 25 c.c. - 6 rabbits.

One animal died from acute pulmonary oedema as the result of accidental lung puncture. The others were killed at intervals of 24 hours on the 2nd, 3rd, 4th, 5th and 6th days. In every case, the right pleural sac was dry and the right lung was normal in appearance apart from some degree of emphysema.

Microscopically, swelling of the alveolar epithelium is observed discontinuously around the periphery of the lung on the 2nd and 3rd days of experiment, but the changes are almost negli-

gible. On the 4th, 5th, and 6th days of experiment the appearances of the lung are practically normal.

(b) CALCIUM CHLORIDE. (CaCl₂)

3/4 normal solution CaCl₂ - 4.1% - 5 c.c. - 10 rabbits.

Two animals were found dead on the second day of experiment (about 36 hours); the remainder were killed on the 1st (2), 2nd (1), 3rd (1), 4th (2), 5th (1) and 6th (1) days respectively. A copious serous effusion was found in the right pleural sac in every case with minor degrees of effusion into the left sac and into the pericardium. During the first two days of experiment only congestion of the right visceral pleura could be detected with the naked eye, but thenceforth wrinkling puckering and opacity of the surface of the right lung became more and more conspicuous. In one or two cases the same wrinkling and opacity were observed on the surface of the left lung, but more commonly the latter presented a normal appearance.

Microscopical observations.

Rabbit A.209 - (22 hours). There is some slight swelling of the epithelium lining the marginal alveoli, but the most conspicuous features are oedema and hyperaemia of the subserosa with leucocytic infiltration and congestion of the lung. Very few surviving endothelial cells are present on the surface of the visceral pleura.

Rabbit A.204 - (48 hours). There is well-marked hyperplasia of the marginal epithelium characterised by swelling and nuclear hyperchromatism of the epithelial cells; mitotic figures are not uncommon. The same features are found in epithelial cells more remote from the surface. The subserosa and the substance of the lung are congested and oedematous (fig.32).

Rabbit A.205 - (71 hours). There is uninterrupted hyperplasia of the epithelium lining the marginal alveoli over the whole surface of the lung. The subserosa is oedematous and infiltrated, in mild degree, by polymorphonuclear leucocytes. The substance of the lung is congested and moderately oedematous (fig.33).

Rabbit A.179 - (4 days). The epithelial hyperplasia is remarkably uniform over the whole pulmonary surface. Nuclear hyperchromatism is a consistent feature whereas mitotic figures are not numerous although they are found with comparative ease. With considerable regularity the hyperplastic epithelium forms sheets of pyramidal cells which bear a superficial resemblance to the "prickle" cells of skin (fig.34). A minor degree of proliferation is observed in the serosal endothelium.

Rabbit A.207 - (5 days). There is fairly uniform hyperplasia of the peripheral epithelium characterised by swelling and cubical formation; mitotic figures are scanty and nuclear hyperchromatism is less conspicuous. There is no oedema of the lung which is nearly normal in appearance.

Rabbit A.208 - (6 days). There is distinct epithelial hyperplasia around the periphery of the lung characterised by swelling and by cubical formation; nuclear hyperchromatism is relatively inconspicuous and mitotic figures are very rare. Frequently the swollen epithelial cells present pyknotic nuclei and an eosinophilic cytoplasm and are undergoing desquamation and disintegration. In one section the margin of the lung is densely infiltrated with lymphocytes which surround degenerating epithelial cells.

3/8 normal solution CaCl₂ - 2 % - 12.5 c.c. - 4 rabbits.

One animal died as a result of accident during the 2nd day

PLATE XI.

Fig. 32. Rabbit A.204 - 3/4 N. CaCl₂, 48 hours - The subserosa is congested and oedematous. The epithelial cells lining the marginal alveoli form two or three layers in the activity of their proliferation and they exhibit a marked degree of nuclear hyperchromatism. (X 400).

Fig. 33. Rabbit A.205 - 3/4 N. CaCl₂, 71 hours - The hyperplasia affects the marginal epithelium almost exclusively and it is evidenced more or less uniformly over the whole surface of the lung. (X 110).

Fig. 34. Rabbit A.179 - 3/4 N. CaCl₂, 96 hours - This field, from the tapering anterior border of the lower lobe, shows a pleural surface above and below. The proliferating epithelial cells are pyramidal in shape and bear a superficial resemblance to "prickle" cells. (X 220).

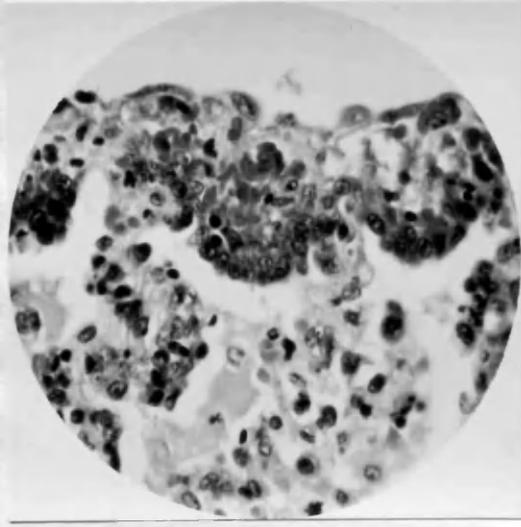


Fig. 32.



Fig. 33.

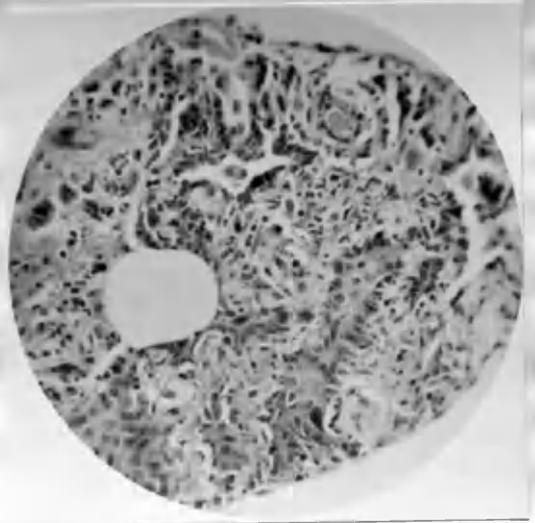


Fig. 34.

(about 36 hours); the others were killed on the 1st, 4th and 6th days respectively. From the first to the fourth day there was marked oedema of the tissues of the mediastinum associated with an effusion of serous fluid into the right pleural sac; during the same period there was congestion, with more or less distinct wrinkling and opacity, of the visceral pleura. On the sixth day there was some little puckering of the costal surface of the right lower lobe and the right pleural sac was dry.

Microscopical observations.

Rabbit A.172 - (24 hours). There are distinct swelling and cubical formation of the epithelial cells lining the marginal alveoli, with hyperaemia of the subserosa and emigration of a small number of polymorphonuclear leucocytes. The substance of the lung is congested and partially collapsed.

Rabbit A.175 - (? 36 hours). There are clear manifestations of increased activity in the peripheral epithelial cells; mitotic figures are present in small numbers. The hyperaemia of the subserosa and the more general congestion and oedema of the lung are maintained.

Rabbit A.174 - (4 days). The hyperplasia of the epithelial cells lining the marginal alveoli attains a striking degree; it is characterised by nuclear hyperchromatism and by cubical formation; mitotic figures are rare (fig.35).

Rabbit A.173 - (6 days). The substance of the lung, in general, presents a normal appearance. Around the periphery there is some evidence of epithelial hyperplasia but it falls far short of that described upon the 4th day. Oedema and slight proliferation of

fibroblasts are observed in the subserosa.

3/20 normal solution CaCl₂ - 0.8% - 25 c.c. - 4 rabbits.

One animal died on the third day; the others were killed on the 1st, 4th and 6th days respectively. On the third and fourth days of experiment there was a copious sero-fibrinous effusion into the right pleural sac accompanied by a gelatinous oedema of the tissues of the mediastinum; the visceral pleura of the right lung was wrinkled and slightly opaque; the pericardial sac contained an excess of fluid and its parietal layer was congested. On the first day congestion of the visceral pleura was the most conspicuous feature, whereas the appearances of the lung were more or less normal on the sixth day and the pleural sac was dry.

Microscopical observations.

Rabbit A.168 - (24 hours). The most outstanding features are oedema and congestion of the lung. There is a distinct degree of swelling of the epithelial cells lining the marginal alveoli but nuclear hyperchromatism has not yet developed.

Rabbit A.169 - (72 hours). There is very uniform hyperplasia of the epithelium lining the marginal alveoli around the periphery of the lung. It is characterised by swelling of the cells which have assumed a cubical or columnar form, and by a marked degree of nuclear hyperchromatism; mitotic figures are not uncommon (fig.36). There is a mild hyperaemia of the subserosa associated with emigration of polymorphonuclear leucocytes; the substance of the lung in general is oedematous.

Rabbit A.171 - (4 days). The epithelial hyperplasia is maintained; withal mitotic figures are few. The substance of the lung is con-

PLATE XII.

- Fig. 35. Rabbit A.174 - 3/8 N. CaCl₂, 4 days - There is oedema of the subserosa. The peripheral epithelial cells are cubical in shape and their nuclei are hyperchromatic. (X 400).
- Fig. 36. Rabbit A.169 - 3/20 N. CaCl₂, 72 hours - The peripheral alveoli are occupied by an albuminous exudate, polymorphonuclear leucocytes and a few desquamated epithelial cells. There is well-marked nuclear hyperchromatism of the marginal epithelium and mitotic figures are present in considerable numbers; four are included in this field. (X 400).
- Fig. 37. Rabbit A.171 - 3/20 N. CaCl₂, 4 days - The nuclear hyperchromatism is maintained and is readily recognised under a low power. Mitotic figures are scanty. There is some oedema of the substance of the lung and sometimes the red blood corpuscles in the smaller vessels seem to form homogeneous thrombi. (X 100).

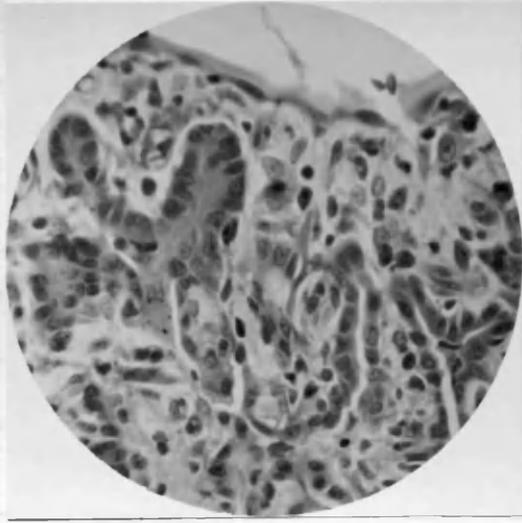


Fig. 35.

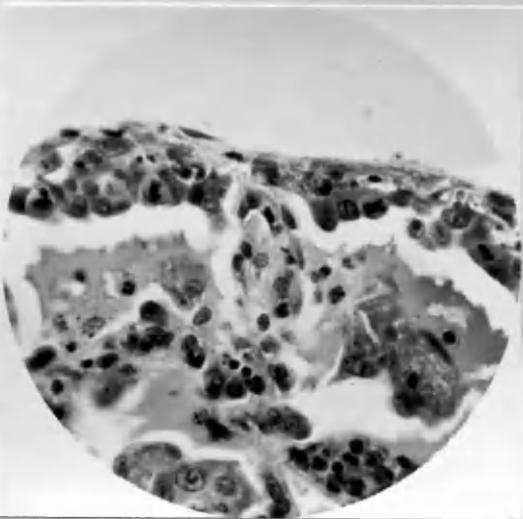


Fig. 36.

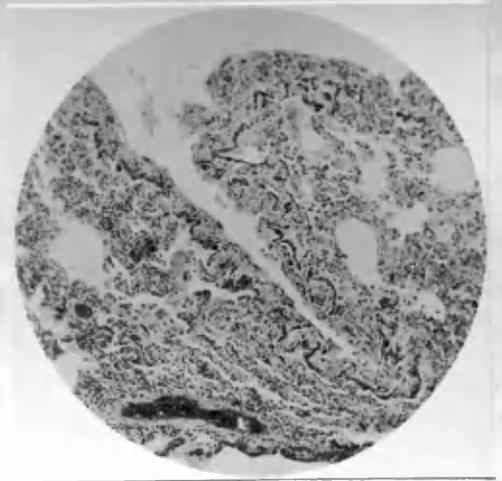


Fig. 37.

gested and the red blood corpuscles seem to form homogenous capillary thrombi (fig.37).

Rabbit A.170 - (6 days). In general the appearances of the lung are nearly normal and only the thin anterior borders exhibit epithelial hyperplasia. No mitotic figures are observed.

(c) STRONTIUM CHLORIDE. (SrCl₂,6H₂O).

3/4 normal solution SrCl₂ - 9.99 % - 5 c.c. - 14 rabbits.

The animals were killed on the 1st (1), 2nd (3), 3rd (3), 4th (3), 5th (1), 6th (1), 8th (1) and 10th (1) days respectively. At the end of 24 hours there was a marked degree of irregular congestion of the surface of the right lung; during the 2nd, 3rd, and 4th days wrinkling and opacity of the visceral pleura were consistent features. In the last four members of the series the naked-eye appearances of the lung were uniformly normal. Oedema of the mediastinal tissues was noted in one case on the fourth day. A small quantity of free fluid was found in the pleural sac on two or three occasions, but as a rule the cavity was dry or nearly dry. A small ecchymosis upon the surface of the lung, presumably due to the point of the trocar, was a comparatively rare occurrence.

Microscopical observations.

Rabbit A.146 - (20 hours). There is a slight degree of swelling of the epithelium lining the marginal alveoli. The nuclei of the serosal cells tend to stain faintly and the serosa is covered by a thin film of fibrinous exudate. The most conspicuous feature is a leucocytic infiltration of the periphery of the lung (fig.38).

Rabbit A.186 - (48 hours). There is more or less uniform hyperplasia of the epithelium lining the marginal alveoli, characterised by

PLATE XIII.

- Fig.38. Rabbit A.146 - 3/4 N. SrCl₂, 20 hours - There is oedema of the subserosa, associated with an emigration of leucocytes. There is distinct swelling of the alveolar epithelium around the periphery of the lung. (X 400).
- Fig. 39. Rabbit A.186 - 3/4 N. SrCl₂, 48 hours - The oedema of the subserosa is more clearly marked and the swelling of the alveolar epithelium is more pronounced. A few mitotic figures are present; one is illustrated. (X 400).
- Fig.40. Rabbit A.148 - 3/4 N. SrCl₂, 72 hours - Regeneration of the desquamated serosal endothelium is almost completed, and, in this field, the serosal cells are swollen and cubical and stratified. The subserosa is slightly thickened by granulation tissue. The subjacent alveolar epithelium is swollen and exhibits nuclear hyperchromatism. (X 300).
- Fig.41. Rabbit A.190 - 3/4 N. SrCl₂, 96 hours - The serosal endothelium has been restored and the swelling of the cells is beginning to subside. The subserosa is thickened. The nuclear hyperchromatism and the cubical formation of the epithelium lining the marginal alveoli are clearly represented. (X 220).

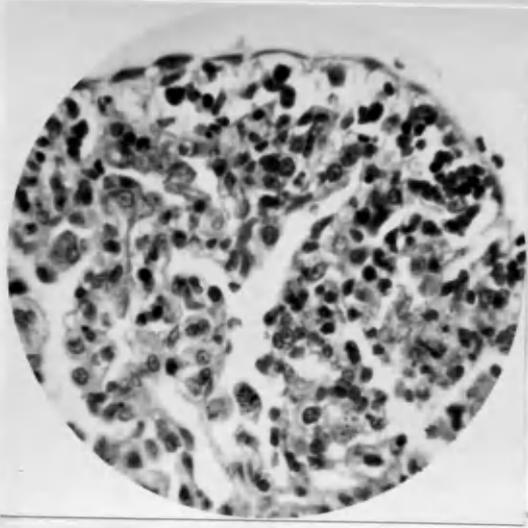


Fig. 38.

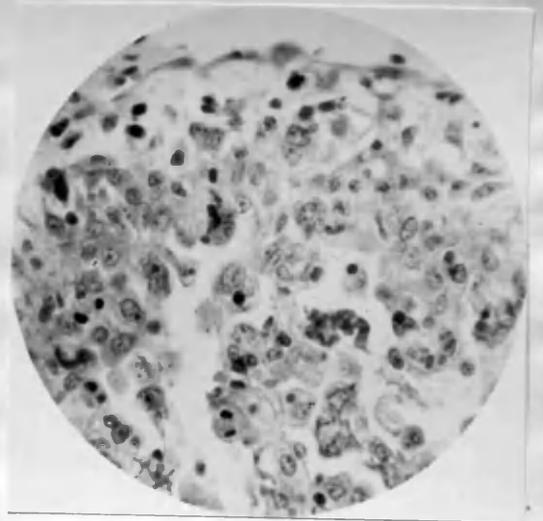


Fig. 39.

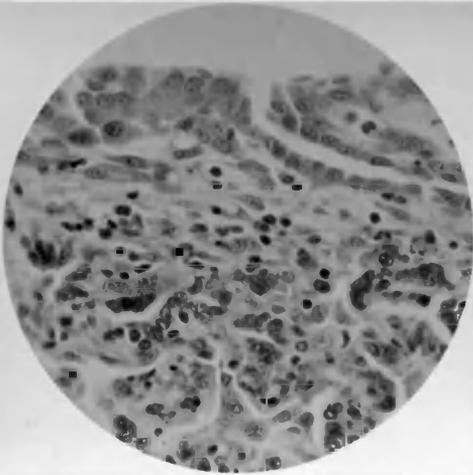


Fig. 40.

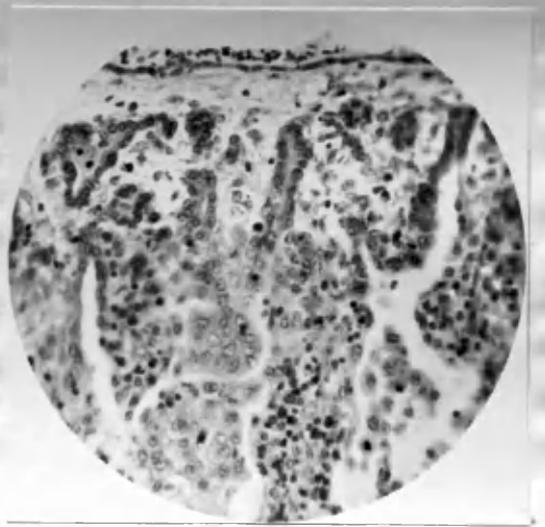


Fig. 41.

swelling of the epithelial cells, nuclear hyperchromatism, and by a fair number of mitotic figures. The serosal endothelium is also swollen. In many situations the subserosa is oedematous and is infiltrated by a small number of polymorphonuclear leucocytes (fig. 39).

Rabbit A.148 - (72 hours). There is well-marked nuclear hyperchromatism of the serosal endothelium and of the epithelium lining the marginal alveoli, associated with more advanced proliferation of the epithelial cells (fig.40). This proliferation, however, is discontinuous and is interrupted by strips of lung presenting a more or less normal appearance.

Rabbit A.190 - (96 hours). A most uniform degree of hyperplasia of the epithelium lining the marginal alveoli is found over the whole surface of the lung (fig.41). The epithelial cells are swollen and cubical or columnar in shape; their nuclei stain deeply; mitotic figures are not uncommon. Here and there, likewise, the serosal endothelium is the seat of active proliferation.

During the fifth and sixth days of experiment mitotic figures are rarely encountered; the nuclear hyperchromatism becomes less conspicuous until it ceases to be significant, and the normal appearances of the lung are restored by the seventh or eighth day. It might be said that the cellular hyperplasia undergoes a process of involution.

0.146 normal solution SrCl₂ - 1.94 % - 25 c.c. - 14 rabbits.

The animals were killed on the 1st (1), 2nd (3), 3rd (3), 4th (3), 5th (1), 6th (1), 8th (1) and 10th (1) days respectively. In five cases there was evidence of a minor degree of trauma upon the

PLATE XIV.

Fig. 41a. Rabbit A.190 - $3/4$ N. SrCl₂, 96 hours - The depression or fissure on the surface of the lung represents one of the "wrinkles" described under the naked-eye observations. The uniformity of the epithelial hyperplasia is shown. (X 130).

Fig. 42. Rabbit A.185 - 0.146 N. SrCl₂, 72 hours - There is only a slight degree of swelling of the marginal epithelium, and that discontinuously over the surface of the lung. (X 100).

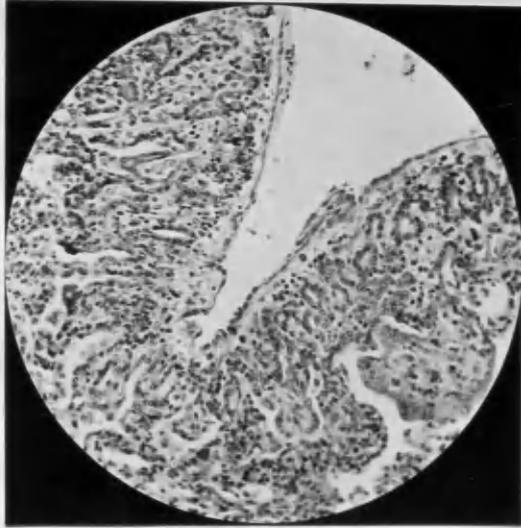


Fig. 41 a.

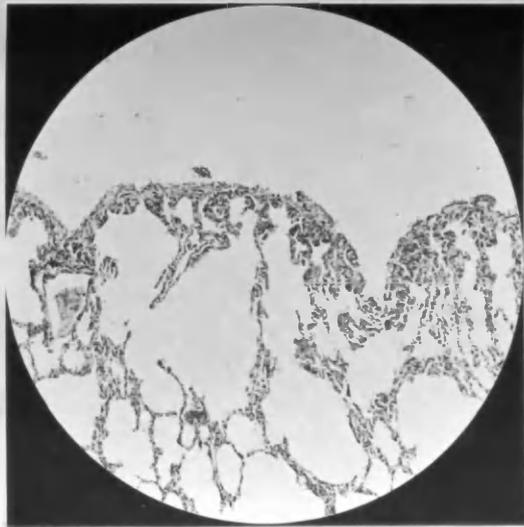


Fig. 42.

surface of the right lung; there was a small blood-clot in the pleural sac of one rabbit. The serous cavity was invariably dry. On the second day of experiment the visceral pleura was congested in one case and slightly wrinkled and opaque in another; the same wrinkling was noted in one rabbit on the third day. The surface of the lung presented a normal healthy appearance in the remaining eleven rabbits.

Microscopical observations.

In those three animals which exhibited congestion wrinkling and opacity of the visceral pleura, varying degrees of proliferative activity are disclosed in the epithelial cells lining the marginal alveoli of the lung. The cellular changes are almost negligible in the other eleven members of the series; there are no mitoses, and no nuclear hyperchromatism and the trace of swelling in the peripheral epithelial cells is liable to be overlooked. A typical field from the lung of a rabbit (A.185) which was killed on the third day is illustrated (fig.42).

(d) BARIUM CHLORIDE. (BaCl₂, 2H₂O).

3/20 normal solution BaCl₂ - 1.83% - 25 c.c. - 6 rabbits.

The rabbits of this series recovered from the anaesthetic but every one died within half-an-hour after a single convulsive movement. In each case the pleural sac contained a small quantity of free fluid and the right lung was congested. No gross trauma was observed.

The lung of one rabbit has been examined microscopically. The serosal endothelium is entirely desquamated and the substance of the organ is richly congested; no other distinctive changes are present.

(e) FERROUS CHLORIDE. (FeCl₂,4H₂O).

3/4 normal solution FeCl₂ - 7.46% - 5 c.c. - 6 rabbits.

All the animals recovered from the immediate effects of the injection but three were found dead next morning; a fourth died after 48 hours; a fifth was found dead on the third morning and the sixth on the fourth day. In the last three rabbits of the series there was a copious effusion of fluid into both pleural sacs; in each case the surface of the right lung was richly pigmented of a dark brown or chestnut colour, wrinkled and lack-lustre; similar changes were observed on the surface of the left lung.

Microscopical observations.

Rabbit A.313 - (48 hours). There is a zone of necrosis extending more or less uniformly around the periphery of the lung and involving a depth of one or two or three alveoli. These alveoli contain little or no albumen but an abundant exudate of leucocytes is present; their lining epithelium is indistinguishable; their intervening septa impregnated with golden-brown pigment. The subjacent zone is very cellular. The increased cellularity is due partly to emigrated leucocytes and partly to collapse of alveoli associated with swelling of their lining epithelium. The serosal endothelium is completely desquamated. The substance of the lung, in general, is extremely oedematous and presents extravasations of blood with or without necrosis. The bronchioles are filled with polymorphonuclear leucocytes.

Rabbit A.318 - (?60 hours). A marginal zone of lung tissue, to a depth of two or three alveoli, has undergone necrosis and the peripheral interalveolar septa are impregnated with golden-brown

pigment. Immediately below, there is another zone of intense congestion with haemorrhages; also in this situation the epithelial cells are swollen and their nuclei are hyperchromatic, but these features are not very conspicuous. The central part of the organ is nearly normal in appearance.

Rabbit A.317 - (?80 hours). In the right lung the^{same} changes are observed as in the preceding animal, with the addition that there is evidence of an early broncho-pneumonic consolidation. In the left lung, on the other hand, there is well-marked epithelial hyperplasia upon the costal surface whereas the mediastinal surface presents a marginal zone of necrosis and little or no epithelial proliferation.

3/40 normal solution FeCl₂ - 0.746% - 10 c.c. - 3 rabbits.

The animals were killed after 2, 3, and 4 days respectively. In each case a small quantity of free fluid was found in the right pleural sac, while the left sac was dry. The surface of the right lung was invariably pigmented of a chestnut hue, wrinkled and opaque, and covered in some degree by a gelatinous exudate.

Microscopical observations.

Rabbit A.343 - (48 hours). There is a narrow peripheral zone of necrosis involving, perhaps, a depth of one alveolus. A sinuous strand of golden-brown pigment is observed which seems to correspond to the marginal elastic lamina. There is distinct swelling of the epithelium lining the subjacent alveoli. The substance of the lung in general is extremely congested and oedematous and presents multiple haemorrhages.

Rabbit A.344 - (72 hours). The substance of the lung is oedematous

PLATE XV.

Fig. 43. Rabbit A.344 - 3/40 N. FeCl₂, 72 hours - The periphery of the lung is impregnated uniformly with free iron. Stained by potassium ferrocyanide and HCl, counterstained by carmalum. (X 4).



Fig. 43.

and partially collapsed. The peripheral zone is impregnated with golden-brown pigment which yields a brilliant Prussian blue reaction (fig.43). There is well-marked hyperplasia of the epithelium lining the marginal alveoli and it is worthy of note that the deposit of iron coincides almost exactly in its depth with the cellular proliferation. It should be emphasised, however, that the iron is distributed intercellularly, and none has been demonstrated within the proliferating cells even after oxidation of the sections for 48 hours with hydrogen peroxide. Multinucleated "giant-cells" or syncytia are observed within the lumina of a number of the peripheral alveoli.

Rabbit A.345 - (96 hours). The serosal endothelium has been **entirely** desquamated. There is uniform hyperplasia of the epithelium lining the marginal alveoli of the lung associated with a deposit of free iron between the proliferating cells. This deposit is less conspicuous than in the preceding cases. The substance of the lung is extremely oedematous. The pleural surface is covered by a fibrinous exudate.

1/20 normal solution FeCl₂ - 0.5% - 10 c.c. - 6 rabbits.

The animals were killed at intervals of 24 hours on the 2nd (2), 3rd (2) and 4th (2) days respectively. A certain quantity of free fluid was found in the right pleural sac in every case, but it was most abundant on the 4th day, when it was accompanied by a gelatinous exudate. Likewise, in every case the surface of the right lung was pigmented, of a chestnut colour, wrinkled and opaque. The left pleural sac was uniformly dry and the left lung was normal in appearance.

PLATE XVI.

Fig. 44. Rabbit A.364 - 1/20 N. FeCl₂, 47 hours - The serosal endothelium is destroyed. There is swelling of the marginal epithelium accompanied by leucocytic infiltration and impregnation of the periphery with iron pigment. (x 200).

Fig. 45. Rabbit A.368 - 1/20 N. FeCl₂, 71 hours - There is well-marked epithelial hyperplasia, uniformly around the periphery of the lung, associated with proliferation of fibroblasts in the subserosa. (X 200).

Fig. 46. Rabbit A.366 - 1/20 N. FeCl₂, 96 hours - The epithelial hyperplasia is maintained. The deep fissure on the surface represents part of a "wrinkle". (X 200).

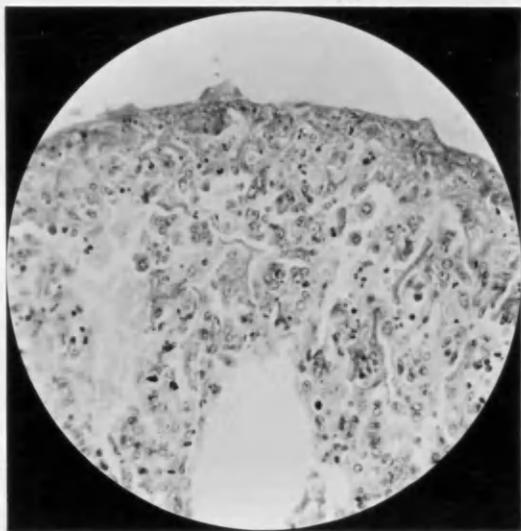


Fig. 44.

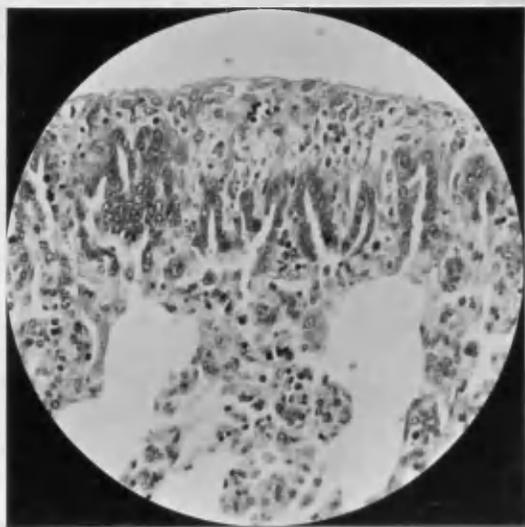


Fig. 45.

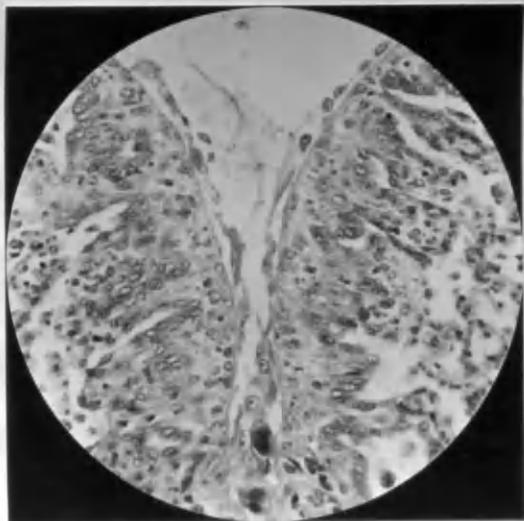


Fig. 46.

Microscopical observations.

Rabbit A.364)
" A.367) - (47 hours). The serosal endothelium is entirely desquamated. The periphery of the lung is impregnated with free iron. The epithelium lining the marginal alveoli is swollen and presents numerous mitotic figures. The outer third of the organ is congested and oedematous, whereas its central part is less abnormal (fig.44 - Rabbit A.364).

Rabbit A.365)
" A.368) - (71 hours). The substance of the lung is more or less normal in appearance. There is a striking degree of epithelial hyperplasia, characterised by swelling of the cells, nuclear hyperchromatism and numerous mitotic figures uniformly around the periphery of each lung (fig.45 - A.368); free iron is deposited between the proliferating cells. In one lung (A.368) "giant cells" or syncytia are observed within the marginal alveoli.

Rabbit A.366)
" A.369) - (96 hours). The central part of the lung is nearly normal in appearance; its outer third is congested and oedematous. The subserosa and the septa between the marginal alveoli are impregnated with free iron. There is well-marked epithelial hyperplasia around the periphery of the organ but mitotic figures are rare (fig. 46 - Rabbit A.366). In one lung (A.366) syncytia are a very conspicuous feature in the marginal alveoli.

(3) Experiments with electrolytes possessing tri-valent cations - the chlorides of (a) aluminium, (b) lanthanum and (c) iron (ferric).

(a) ALUMINIUM CHLORIDE. (AlCl₃).

3/4 normal solution AlCl₃ - 3.3% - 5 c.c. - 6 rabbits.

Three animals died during the first day (? 18 hours); another

died during the second day (? 44 hours); the two survivors were killed on the third and fourth days respectively. There was always some degree of pleural effusion. Invariably the surface of the right visceral pleura was lustreless, and pigmented to a deep brown colour. From the first day there was an extreme degree of wrinkling of the pulmonary surface. On the third and fourth days there was oedema of the tissues of the mediastinum. As a rule the left lung was the seat of irregular congestion and of oedema.

Microscopical observations.

Rabbit A.193)
" A.195) - (? 18 hours). There is a zone of necrosis involving
" A.196)
the serosa and the marginal alveoli of the lung. In two cases this zone is richly infiltrated with polymorphonuclear leucocytes; not so in the third. The substance of the lung in general is congested and oedematous.

Rabbit A.192 - (? 44 hours). There is a marginal zone of necrosis destroying the serosal endothelium and the epithelium lining the peripheral alveoli. The subjacent epithelial cells show some degree of swelling and large rounded masses of chromatic substance are observed here and there; mitotic figures are present in small numbers. The lung in general is oedematous.

Rabbit A.194 - (72 hours). Beneath a peripheral zone of necrosis there is very marked hyperplasia of the pulmonary epithelium, characterised by swelling and by cubical formation and by large numbers of mitotic figures. Occasionally the zone of necrosis is interrupted yet the cellular proliferation is maintained (fig.47). The lung is extremely oedematous.

Rabbit A.197 - (4 days). The same manifestations of epithelial pro-

PLATE XVII.

- Fig. 47. Rabbit A.194 - 3/4 N. AlCl₃, 72 hours - There is a zone of necrosis destroying the serosal endothelium and penetrating the surface of the lung to a depth of one or two alveoli. Subjacent to the necrotic zone the surviving alveolar epithelium exhibits a striking degree of hyperplasia, characterised by swelling and cubical formation, nuclear hyperchromatism, and numerous mitotic figures. (X 400).
- Fig. 47a. Another field of the same lung. The superficial zone of necrosis is reduced to a minimum, yet the same proliferative phenomena are apparent in the epithelium lining the peripheral alveoli. (X 275).
- Fig. 48. Rabbit A.197 - 3/4 N. AlCl₃, 4 days - The superficial zone of necrosis is undergoing organisation. There is well-marked hyperplasia of the subjacent alveolar epithelium whereas the deeper and more remote epithelium is unaffected. (X 110).

PLATE XVII.

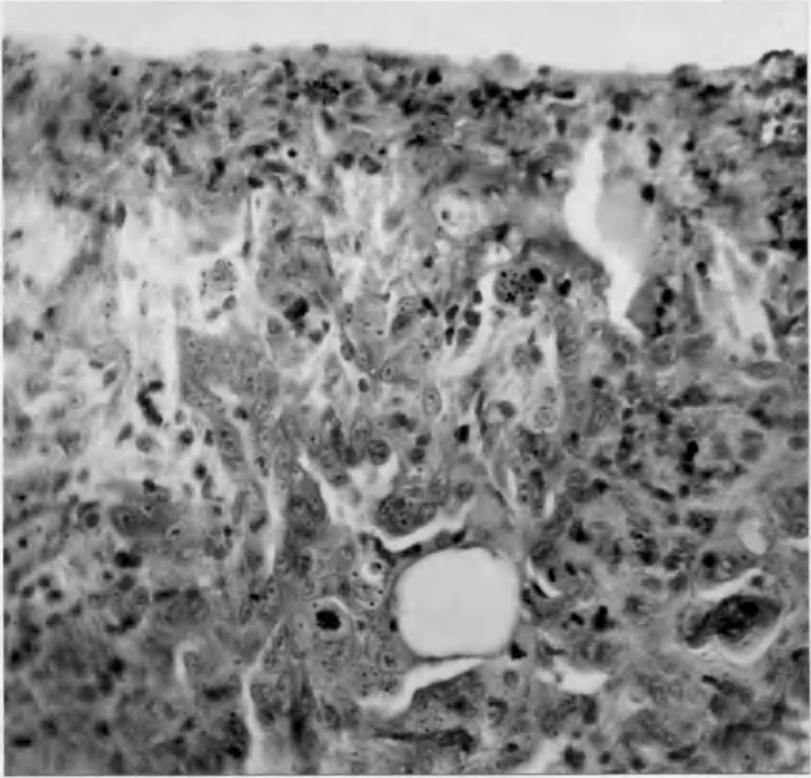


Fig. 47.

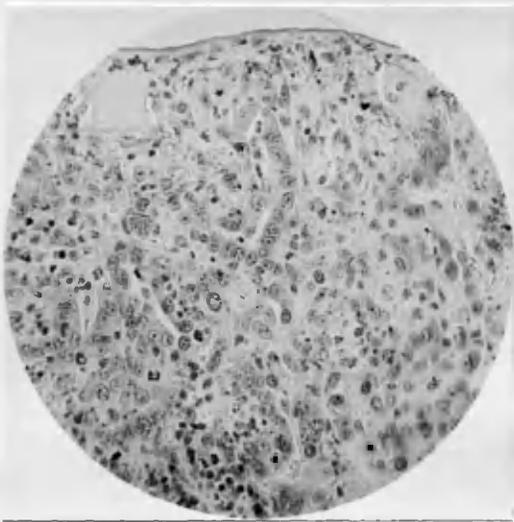


Fig. 47 a.

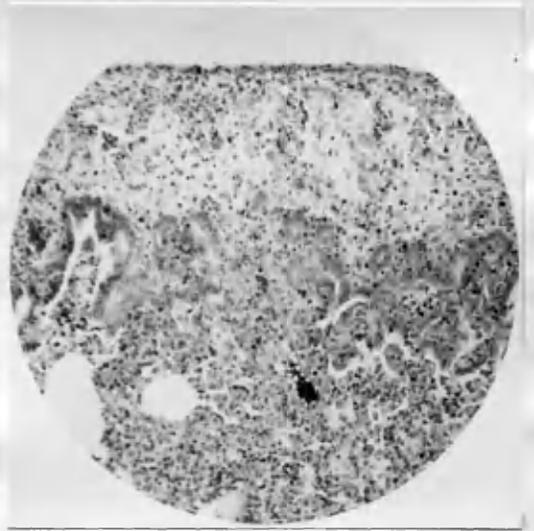


Fig. 48.

liferation are present. Organisation of the superficial zone of necrosis is proceeding (fig.48).

3/20 normal solution AlCl₃ - 0.66% - 5 c.c. - 6 rabbits.

The animals were killed at intervals of 24 hours from the beginning of the experiment with one exception which died during the second day. A serous effusion was found in the right pleural sac in three cases; in the other three the sac was dry. Irregular congestion of the visceral pleura was a constant finding whereas definite wrinkling and opacity were observed only on the fourth and fifth days.

Microscopical observations.

Rabbit A.221 - (19 hours). The lung is extremely oedematous. There is no perceptible swelling of the marginal epithelium and there is no necrosis of the superficies of the organ. The serosal endothelium seems to have been desquamated entirely.

Rabbit A.220 - (? 36 hours). There is distinct swelling of the epithelial cells lining the peripheral alveoli but it never attains a striking degree. The lung is extremely oedematous.

Rabbit A.216 - (67 hours). The lung is extremely oedematous. There is fairly uniform hyperplasia of marginal epithelium but in addition numerous foci of irregular epithelial proliferation are discovered within the substance of the organ associated with leucocytic infiltration.

Rabbit A.218 - (90 hours). There is little or no hyperplasia of the epithelium lining the marginal alveoli. On the other hand large areas of irregular fibro-epithelial hyperplasia are found within the substance of the lung surrounding foci of necrosis. No distinc-

tive vascular lesion has been identified. The organ in general is congested and oedematous.

Rabbit A.217 - (5 days). There is uniform hyperplasia of the peripheral epithelial cells; their nuclei are vesicular and hyperchromatism is lacking. Small foci of fibro-epithelial proliferation within the substance of the lung are observed in one section. The organ is congested.

Rabbit A.219 - (6 days). A narrow zone of granulation tissue is observed on the surface of the lung. Beneath it there is well-marked epithelial hyperplasia characterised by a cubical formation of the cells and by nuclear hyperchromatism; mitotic figures are scanty. The substance of the organ tends to be collapsed and congested; it shows no oedema and no necrosis.

3/40 normal solution AlCl₃ - 0.33% - 10 c.c. - 6 rabbits.

The animals were killed at intervals of 24 hours up to six days, with one exception which was found dead on the fifth day. A moderate serous effusion was discovered in the right pleural sac on the second and the sixth days whereas none was found on the other days; in two or three cases a small quantity of brown debris, apparently altered blood, was present on the surface of the lung. After 24 hours a marked degree of irregular congestion was observed in the visceral pleura; thenceforth wrinkling and opacity of the surface of the lung were invariably present.

Microscopical observations.

Rabbit A.210 - (20 hours). There is just a perceptible degree of swelling of the epithelial cells lining the marginal alveoli. The serosal endothelium appears to have been desquamated entirely. The

PLATE XVIII.

Fig. 49. Rabbit A.211 - 3/40 N. AlCl₃, 44 hours - The serosal endothelium has been entirely desquamated and the pleural surface is covered by an albuminous exudate. The subserosa is oedematous and infiltrated by leucocytes. There is well-marked hyperplasia of the epithelium lining the marginal alveoli. (X 400).

Fig. 50. Rabbit A.213 - 3/40 N. AlCl₃, 92 hours - The pleural surface is covered by a sero-fibrinous exudate. The subserosa is oedematous. There is a striking degree of epithelial hyperplasia very uniformly around the periphery of the lung and nuclear hyperchromatism is a conspicuous feature. (X 400).

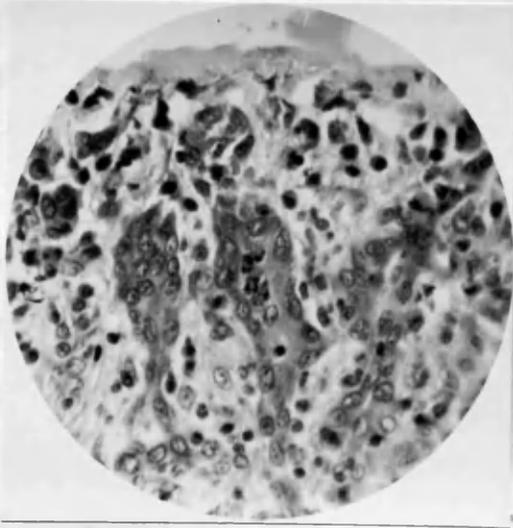


Fig. 49.

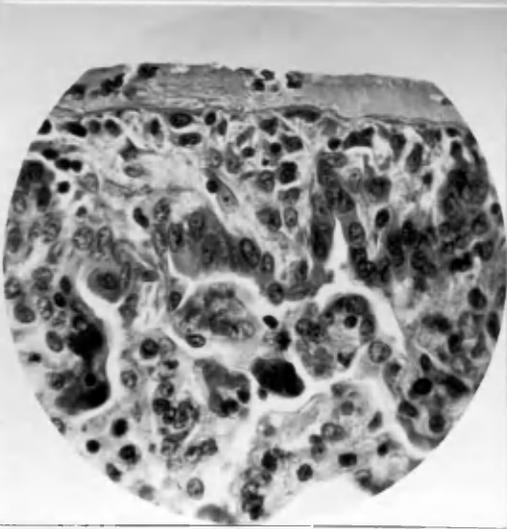


Fig. 50.

substance of the lung in general is extremely oedematous.

Rabbit A.211 - (44 hours). The serosa has undergone necrosis and forms a thin hyaline layer on the surface of the lung; it is covered by fibrinous exudate. There is distinct hyperplasia of the epithelium lining the marginal alveoli (fig.49). The substance of the lung is congested.

Rabbit A.212 - (68 hours). There is marked oedema of the lung accompanied by discontinuous hyperplasia of the epithelium lining the marginal alveoli. Throughout the substance of the lung foci of irregular fibro-epithelial proliferation are disclosed.

Rabbit A.213 - (92 hours). There is abundant hyperplasia of the epithelium lining the marginal alveoli although mitotic figures are rare. There is slight oedema of the subserosa. The tissues of the lung in general present a normal appearance (fig.50).

Rabbit A.214 - (5 days). There is uniform swelling of the marginal epithelium over the whole surface of the lung; the nuclei of the epithelial cells are swollen and vesicular but not hyperchromatic; mitotic figures are rare. The substance of the lung is congested and not oedematous.

Rabbit A.215 - (6 days). There is a narrow zone of granulation-tissue around the periphery of the lung with well-marked epithelial hyperplasia immediately subjacent to it. The nuclei of the hyperplastic cells are pyknotic and their cytoplasm richly eosinophilic. The substance of the lung is congested but exhibits no oedema.

(b) LANTHANUM CHLORIDE. (LaCl₃).

3/4 normal solution - LaCl₃ - 6.14% - 5 c.c. - 6 rabbits.

One animal died on the fifth day; the others were killed on

the 1st, 2nd, 3rd, 4th and 6th days respectively. At the end of 24 hours a small quantity of sero-fibrinous exudate was found in both pleural sacs; the exudate increased in amount during the next 48 hours and it was very abundant on the last three days. Even on the first day the surface of the right lung was wrinkled, grey and lack-lustre; the wrinkling became more marked as the experiment progressed. Similar changes were observed on the surface of the left lung following the diffusion of the salt into the left sac.

Microscopical observations.

Rabbit A.325 - (27 hours). The substance of the lung is oedematous and intensely congested. There is a zone of necrosis extending to a depth of two or three alveoli around the greater part of the surface of the organ; beneath it there is another zone of intense hyperaemia with or without extravasation of blood. The epithelial cells lining the subjacent alveoli, and also the marginal alveoli where the necrosis is interrupted, are distinctly swollen.

Rabbit A.326 - (48 hours). A similar zone of necrosis is present, but it is infiltrated with polymorphonuclear leucocytes. The nuclei of the subjacent epithelial cells are hyperchromatic. The substance of the lung is congested and oedematous.

Rabbit A.329 - (72 hours). In general the same changes are observed. At this stage, however, there is well-marked hyperplasia of the epithelium lining the marginal alveoli or subjacent to the necrotic zone. Nuclear hyperchromatism is a conspicuous feature and mitotic figures are common (fig.51).

Rabbit A.327 - (96 hours). The changes resemble those of the preceding day, with the addition that the hyperplastic epithelial

cells are more cylindrical, and often contain multiple nuclei (fig. 52).

Rabbit A.328 - (5 days). Organisation of the necrotic zone is proceeding. The epithelial hyperplasia is maintained over a large part of the surface, but a new feature is added in the respect that many of the marginal alveoli contain "giant-cells" or masses of syncytium. These syncytia are derived from the epithelial lining of the alveoli and they appear to be formed by fusion of adjacent cells, since it is possible sometimes to detect, by careful focussing, a honey-comb structure, representing the bases of discrete cells which merge superficially into a homogeneous continuum of protoplasm.

Rabbit A.330 - (6 days). Syncytia are found in a large number of the peripheral alveoli; elsewhere the epithelial proliferation is maintained.

3/40 normal solution LaCl₃ - 0.61% - 10 c.c. - 12 rabbits.

The animals were killed in pairs on the 1st, 2nd, 3rd, 4th, 5th and 6th days respectively, with two exceptions - one was found dead on the first day and another on the third day. In every case there was some free fluid in the right pleural sac, increasing in quantity during the first three days; later, the effusion was bilateral in four cases. As a rule the surface of the right lung was grey in colour, lack-lustre, and wrinkled. When these changes were less marked, as they were in four rabbits on different days (including the rabbit which was found dead within 24 hours), the visceral pleura presented irregular congestion and the lung was oedematous. Only six lungs were examined microscopically in this series; the others were preserved as naked-eye specimens.

PLATE XIX.

- Fig. 51. Rabbit A.329 - $3/4$ N. LaCl_3 , 72 hours - There is a narrow zone of necrosis destroying the visceral pleura. The pulmonary epithelium lining the marginal alveoli exhibits well-marked hyperplasia, characterised by swelling, nuclear hyperchromatism and numerous mitotic figures. (X 200).
- Fig. 52. Rabbit A.327 - $3/4$ N. LaCl_3 , 96 hours - The hyperplasia is maintained. The epithelial cells are becoming enlarged, cylindrical and multinucleated. (X 200).
- Fig. 53. Rabbit A.356 - $3/40$ N. LaCl_3 , 5 days - The peripheral alveoli are occupied by "giant-cells" or syncytia. The substance of the lung is partially collapsed. (X 75).
- Fig. 54. Rabbit A.356 - the same - The surface of the lung is shown. There is a cellular exudate in the pleural sac. The marginal alveoli are filled with syncytia, one of which appears to be lying free. In such a case as the last, however, it is often possible, by careful focussing, to distinguish a honey-comb-like structure in the syncytium at its attachment to the wall of the alveolus. These appearances suggest that the "giant-cells" are formed by a process of fusion of adjacent epithelial cells. (X 260).

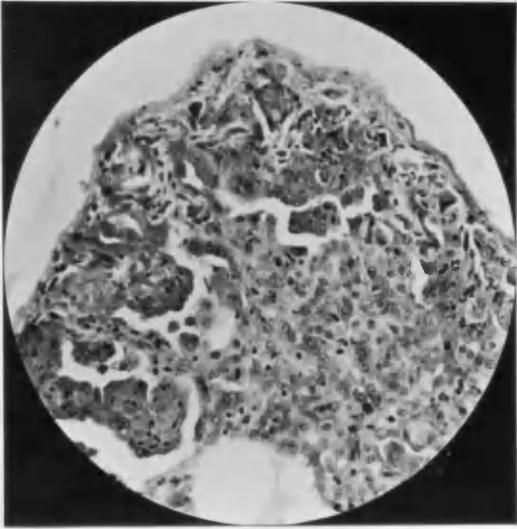


Fig. 51.

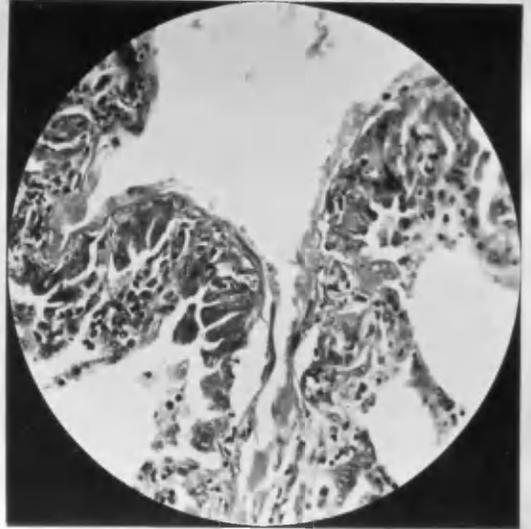


Fig. 52.

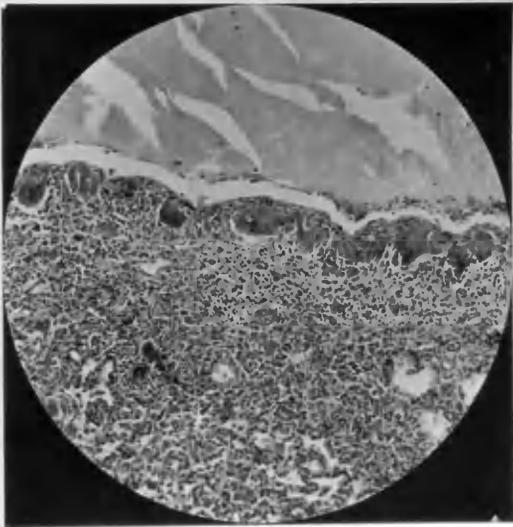


Fig. 53.

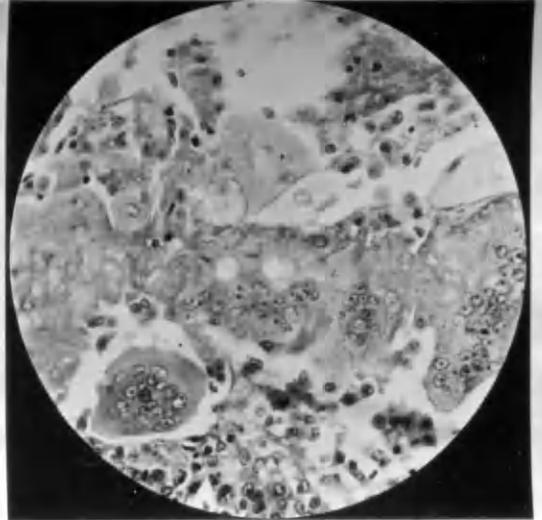


Fig. 54.

Microscopical observations.

Rabbit A.347 - (? few hours). The substance of the lung is extremely oedematous and congested. There is no necrosis of the surface of the organ. No distinct changes are observed in the marginal epithelium.

Rabbit A.346 - (47 hours). Similar changes are present, with the addition that the marginal epithelium is swollen.

Rabbit A.354 - (? 60 hours). The swelling of the marginal epithelium is more uniform and more conspicuous. The substance of the lung is oedematous and congested and shows areas of haemorrhage and foci of necrosis.

Rabbit A.349 - (96 hours). There is well-marked hyperplasia of the epithelium lining the marginal alveoli, characterised by swelling, nuclear hyperchromatism, and numerous mitotic figures. "Giant-cells" or syncytia are frequently found around the periphery within the alveoli. There is widespread fibro-epithelial proliferation within the substance of the lung such as might arise in relation to a central necrosis. The oedema and congestion of the organ are maintained.

Rabbit A.356 - (5 days). The formation of syncytia is the most striking feature and a very large number of the marginal alveoli are filled with these masses of multinucleated protoplasm (figs 53 & 54). Although the epithelial hyperplasia, with or without syncytial formation, occurs very uniformly around the periphery of every section, yet it appears to have reached a stationary or even a regressive phase. The nuclei of the swollen epithelial cells are pyknotic and the cytoplasm is richly eosinophilic; the same applies to the syncytia, in many fields.

Rabbit A.351 - (6 days). The foregoing changes are reproduced and a process of involution is overtaking the hyperplastic epithelium.

(c) FERRIC CHLORIDE. (FeCl₃).

3/4 normal solution FeCl₃ - 4.06% - 5 c.c. - 6 rabbits.

Four animals died within 30 minutes of the injection; the two others lived for an hour only. All had recovered from the anaesthetic and death was not due to accidental lung puncture. The right pleural sac contained a small quantity of free fluid in each case and the surface of the right lung was puckered and brown.

One lung (A.322) has been examined microscopically. The serosal endothelium is entirely desquamated. The surface of the organ is thrown into deep folds and is heavily impregnated with brown or black pigment; the pulmonary capillaries are engorged with blood and the alveoli are filled with albuminous exudate.

1/20 normal solution FeCl₃ - 0.27% - 10 c.c. - 9 rabbits.

The animals were killed on the 2nd (3), 3rd (3) and 4th (3) days respectively. In every case some free fluid was found in the right pleural sac, increasing in quantity on the 3rd and 4th days, when it was accompanied by fibrinous exudate. The surface of the right lung was invariably wrinkled and opaque. As a rule, the visceral pleura was lightly pigmented, of a chestnut colour.

Microscopical observations.

Rabbit A.340)

" A.370) - (48 hours). The appearances of the lung in general

" A.373)
vary from one rabbit to another; in one case there is some oedema with congestion, whereas the other two lungs are rather emphysematous. The serosal endothelium is entirely desquamated in all

PLATE XX.

Fig. 55. Rabbit A.373 - 1/20 N. FeCl₃, 48 hours - The fissure represents one of the "wrinkles". There are well-marked swelling and nuclear hyperchromatism of the epithelium lining the marginal alveoli. (X 200).

Fig. 56. Rabbit A.374 - 1/20 N. FeCl₃, 71 hours - The serosal endothelium is lacking. There is proliferation of fibroblasts in the subserosa associated with an emigration of leucocytes. The epithelial hyperplasia is more advanced. (X 200).

Fig. 57. Rabbit A. 375 - 1/20 N. FeCl₃, 95 hours - There is proliferation of fibroblasts in the subserosa. The epithelial hyperplasia is maintained, or increased, and there is no evidence of nuclear pyknosis. (X 200)

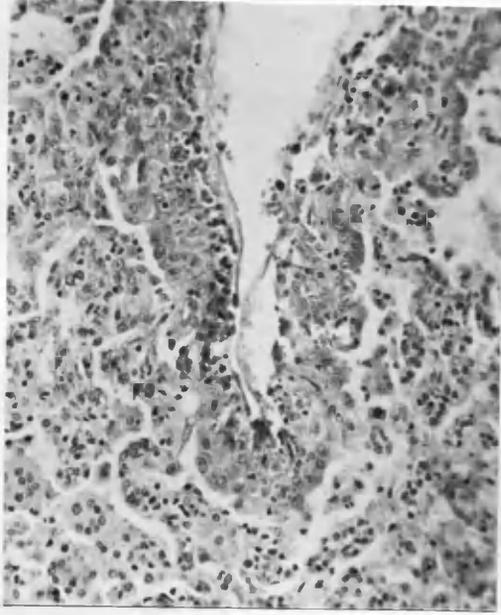


Fig. 55.

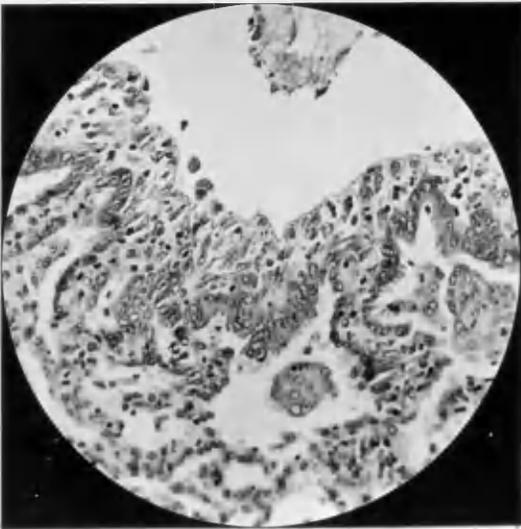


Fig. 56.

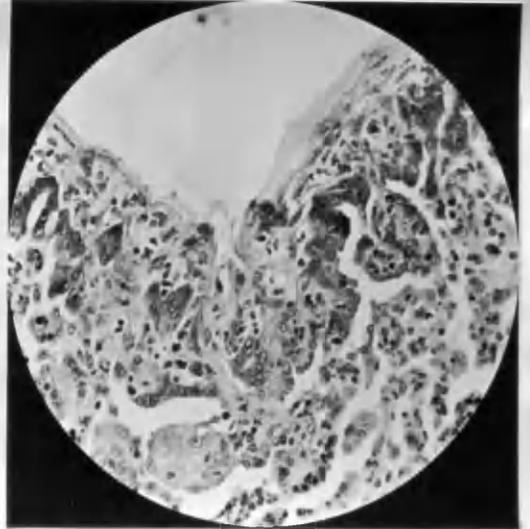


Fig. 57.

cases. Likewise, uniformly, there is hyperplasia of the epithelium lining the marginal alveoli characterised by swelling, nuclear hyperchromatism and a sufficient number of mitotic figures to be found readily (fig.55 - A.373). There is no evidence of a deposit of free iron at the periphery of the organ (Cf. A.364 and A.367 following the injection of ferrous chloride).

Rabbit A.341)

" A.371) - (71 hours). Again the appearances of the substance
" A.374)
of the organ are erratic, sometimes normal and sometimes oedematous.

The epithelial hyperplasia in general is more striking and mitotic figures are comparatively numerous. The subserosa is becoming thickened by the proliferation of fibroblasts (fig.56 - A.374). There is no deposit of free iron in the peripheral tissues, and only the scanty macrophages which are present yield a positive prussian blue reaction. It has not been possible to demonstrate any iron within the proliferating epithelial cells, even after oxidation of the sections by hydrogen peroxide for 48 hours.

Rabbit A.342)

" A.372) - (95 hours). The surface of the lung is covered by
" A.375)
a fibrinous exudate, but the substance of the organ is more or less normal in appearance. There is a very striking degree of hyperplasia affecting the epithelium lining the marginal alveoli, although mitotic figures are becoming rare. The nuclear hyperchromatism is well maintained, however, and there are no clear signs of impending involution. The subserosa is thickened by the proliferation of fibroblasts (fig.57 - A.375).

(4) An experiment with an electrolyte possessing a tri-valent anion

POTASSIUM FERRICYANIDE. (K₃Fe (CN)₆).

1/20 normal solution K₃Fe (CN)₆ - 0.55% - 10 c.c. - 6 rabbits.

The animals were killed in pairs on the 2nd, 3rd and 4th days respectively. In all cases both pleural sacs were dry and healthy in appearance; the lungs likewise were normal to the naked-eye apart from some emphysema.

Microscopical observations.

Rabbit A.376)
" A.379) - (46 hours). The serosal endothelium is swollen, but it is intact over the greater part of the visceral pleura. There is distinct swelling of the epithelial cells lining the marginal alveoli. In all other respects the appearances of the lungs are normal.

Rabbit A.377)
" A.381) - (70 hours). There is distinct swelling of the marginal epithelium discontinuously and at wide intervals over the surfaces of the lungs. Occasionally this evidence of excitement proceeds to nuclear hyperchromatism, but not a single mitotic figure is discovered by a careful examination (fig.58 - A.377). Otherwise the appearances of the organs are normal.

Rabbit A.378)
" A.380) - (96 hours). Similar changes are present characterised only by a minor degree of swelling of the marginal epithelium (fig.59 - A.378). For the most part the appearances of the lungs are normal.

PLATE XXI.

Fig. 58. Rabbit A.377 - 1/20 N. K3 Fe (CN)₆, 70 hours - to the naked eye the appearances of this lung were normal. Likewise, microscopically the substance and the greater part of the periphery of the organ is normal in appearance. The field illustrated here is exceptional and may be taken as an example of the maximal effects produced by this reagent. There is swelling of the marginal epithelium associated with nuclear hyperchromatism, but no mitotic figures have been disclosed by a careful examination. (X 200). Cf. fig. 56, page 51a.

Fig. 59. Rabbit A.378 - 1/20 N. K3 Fe (CN)₆, 95 hours - the serosal endothelium is nearly intact. The subserosa is slightly oedematous. There is only a minor degree of swelling of the epithelium lining the marginal alveoli and that in spite of the fact that a field has been chosen which illustrates the maximal reaction. (X 200). Cf. fig.57, page 51a.

Fig. 60. Rabbit A.361 - distilled water (25 c.c.), 96 hours - there is more pronounced thickening of the subserosa, due partly to oedema and partly to proliferation of fibroblasts. Also there are well-marked swelling and nuclear hyperchromatism of the epithelium discontinuously around the periphery of the lung. (X 200).

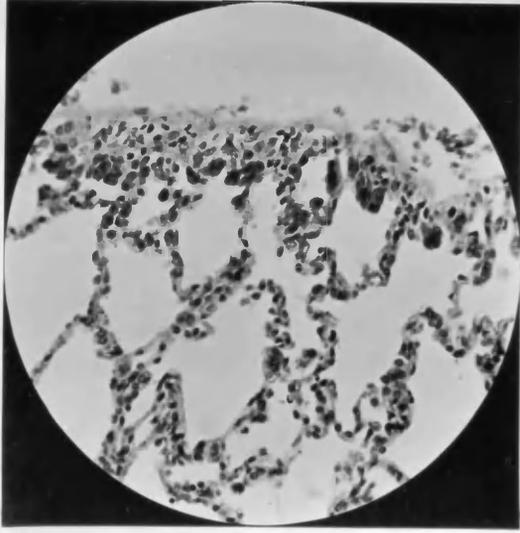


Fig. 58.

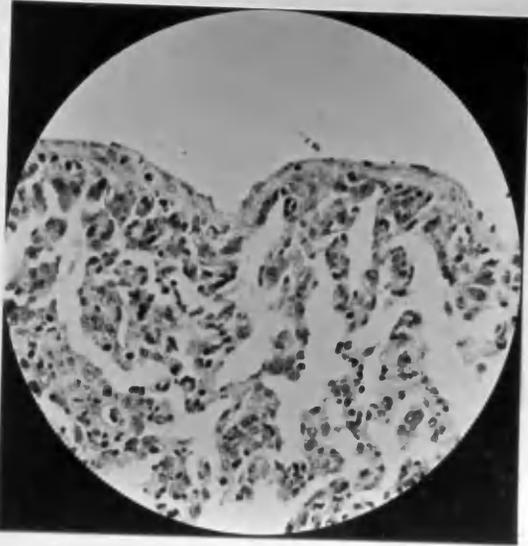


Fig. 59.

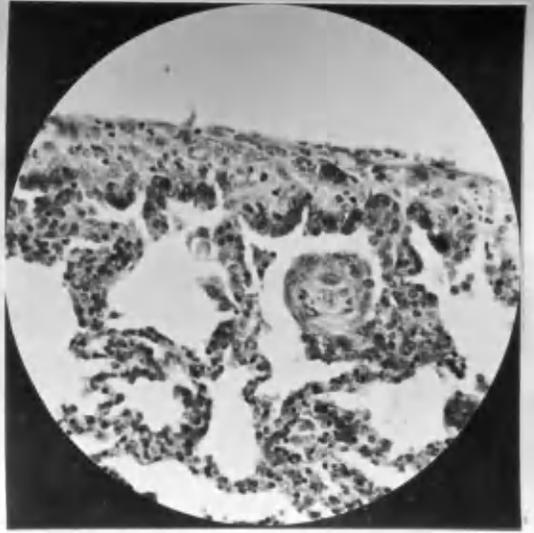


Fig. 60.

- (5) An experiment with a mixture of sodium chloride and calcium chloride.

3/4 N Sodium Chloride, 3/4 N Calcium Chloride, of each 5 c.c. -
6 rabbits.

One animal died an hour after the injection from the effects of acute pulmonary oedema. The remaining five were killed on the 2nd (1), 3rd (2) and 4th (2) days respectively. In every one of the series of five there was a considerable pleural effusion associated with partial collapse of the right lung, and the surface of this lung was wrinkled, puckered and opaque.

Microscopical observations.

On the second day there is well-marked hyperplasia of the epithelium lining the marginal alveoli, characterised by swelling and a cubical or columnar formation of the cells, by nuclear hyperchromatism and by fairly numerous mitotic figures. The same features are present in greater or less degree on the third day. On the fourth day mitotic figures are found with greater difficulty but the hyperplasia is still maintained. At all stages of the experiment the lung is congested and oedematous.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

- (1) The three electrolytes, possessing mono-valent cations - LiCl, NaCl and KCl - each in 3/4 normal solution (5 c.c.) produce only a minor degree of swelling of the epithelium lining the marginal alveoli of the lung. Mitotic figures are usually absent and always rare.
- (2) The three electrolytes, possessing bi-valent cations - MgCl₂,

CaCl_2 and SrCl_2 - each in $3/4$ normal solution (5 c.c.) produce well-marked epithelial proliferation ~~and~~ on the third or fourth day of experiment. On the other hand, when the same salts are exhibited, each in $3/20$ normal solution (25 c.c.) the epithelial reaction is relatively slight or altogether lacking. The effects of calcium chloride are more striking than those of strontium chloride, and likewise strontium chloride seems to be more potent than magnesium chloride. Nevertheless the reactions produced by these three salts are essentially of the same order, being characterised by abundant epithelial hyperplasia without any visible evidence of necrosis. The epithelial hyperplasia undergoes more or less complete involution within eight days.

(3) The three electrolytes, possessing tri-valent cations - AlCl_3 , LaCl_3 and FeCl_3 - each in $3/4$ normal solution (5 c.c.) produce necrosis of a narrow zone of tissue around the periphery of the lung, destroying the visceral pleura and penetrating the surface of the organ to a depth of one or two or three alveoli. In animals which survive the injection for three or four days very active signs of proliferation become apparent in the epithelium immediately subjacent to the necrotic zone. When the same salts are exhibited, each in $3/40$ or in $1/20$ normal solution (10 c.c.) there is well-marked hyperplasia of the epithelium lining the marginal alveoli of the lung without any visible evidence of necrosis.

(4) Contrasting with the preceding observation, an electrolyte possessing a tri-valent anion - $\text{K}_3\text{Fe}(\text{CN})_6$ - in $1/20$ normal solution produces only a minor degree of swelling of the epithelium lining the marginal alveoli not exceeding that elicited by an electrolyte

possessing a mono-valent anion - e.g. KCl.

(5) The epithelial hyperplasia increases as the valency of the cation of the salt increases whereas it is not enhanced by increasing the valency of the anion under the same experimental conditions.

(6) There is no evidence of any inhibition of the reaction typically produced by calcium chloride (3/8 N - 10 c.c.) when sodium chloride is added to the inoculum in chemically equivalent proportion. In other words, it would seem that there is no antagonism between sodium and calcium ions, under those conditions, in so far as the production of hyperplasia in the alveolar epithelium of the lung of the rabbit is concerned.

AN EXPERIMENT WITH DISTILLED WATER.

DISTILLED WATER - 25 c.c. - 6 rabbits.

The animals were killed on the 1st, 2nd, 3rd, 4th, 5th and 6th days respectively. Throughout the experiment, both pleural sacs were dry. The right visceral pleura was the seat of irregular congestion during the first 48 hours; thereafter it was normal in appearance. Also the spleen was swollen and congested during the first two days only.

Microscopical observations.

Rabbit A.358 - (24 hours). Around the periphery of the lung there is slight hyperaemia, becoming more marked locally. There is a minor degree of swelling of the epithelium lining the marginal alveoli here and there, but the changes are never striking.

The pulp of the spleen is engorged with blood; numerous giant-cells (? megakaryocytes) are present; there is active haemophago-

cytosis.

Rabbit A.359 - (48 hours). The subserosa is slightly oedematous. Discontinuously around the periphery of the lung the epithelial cells lining the marginal alveoli are moderately swollen. Otherwise the appearances of the lung are normal.

Rabbit A.360 - (72 hours). There is swelling, with nuclear hyperchromatism, of the marginal epithelial cells fairly uniformly over the surface of the lung; in other respects the organ is normal in appearance.

Rabbit A.361 - (96 hours). Swelling of the marginal epithelial cells, and nuclear hyperchromatism thereof, is a conspicuous feature over small areas of the surface (fig.60); elsewhere it is not present and the periphery of the lung is normal. The subserosa is thickened in some degree by oedematous granulation tissue.

Rabbit A.362 - (5 days). The appearances of the lung are practically normal. There is no epithelial hyperplasia and even the swelling of the cells is minimal.

Rabbit A.363 - (6 days). One small superficial focus of fibro-epithelial proliferation is observed - it is probably traumatic in origin. Here and there a minor degree of swelling is detected in the marginal epithelium subjacent to plaques of fibrous thickening of the subserosa; in other respects the appearances of the lung are normal.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

Following the injection of a large volume of sterile distilled water, there is distinct hyperplasia of the alveolar epithelium discontinuously around the periphery of the lung; it attains a

maximum on the fourth day. It is not improbable that these changes arise from endosmotic phenomena. They acquire greater interest since they suggest that any "hypotonic" solution might tend to produce minor changes of the same type. Thus the swelling and other manifestations of increased cellular activity described after the exhibition of 10 c.c. of a 1/20 normal solution of potassium ferricyanide (page 53) might be partially due to the "hypotonicity" of the inoculum.

EXPERIMENTS WITH SOLUTIONS OF NON-ELECTROLYTES:- (a) Cane-sugar and (b) Urea.

(a) CANE-SUGAR.

3/4 molar solution - Cl₂ H₂O 11 - 25.66% - 10 c.c. - 12 rabbits.

Two animals were found dead within 24 hours. The survivors were killed in pairs on the 2nd, 3rd, 4th, 5th and 6th days. A bilateral pleural effusion was found in each of the two rabbits which failed to survive the injection and it was accompanied by oedema of the lungs. From the second day onwards the pleural sacs were uniformly dry and the lungs were normal in appearance apart from a minor degree of congestion of the visceral pleura on the second day.

Microscopically there is no evidence of hyperplasia of the epithelium lining the marginal alveoli of the lung throughout the period of the experiment (fig.61 - Rabbit A.264 - 4 days). Congestion and oedema of the organ are observed on the first day and the congestion is present in less degree on the second and third days. Beyond that date the appearances of the lung are normal. The perivascular lymphatics surrounding the larger vessels are

PLATE XXII.

Fig. 61. Rabbit A.264 - 3/4 M. cane-sugar, 96 hours -
The appearances of the lung are normal.
(X 200).

Fig. 62. Rabbit A.336 - 3/4 M. urea, 96 hours - The
appearances of the lung are normal. There
is no evidence of epithelial hyperplasia.
(X 200).

PLATE XXII.

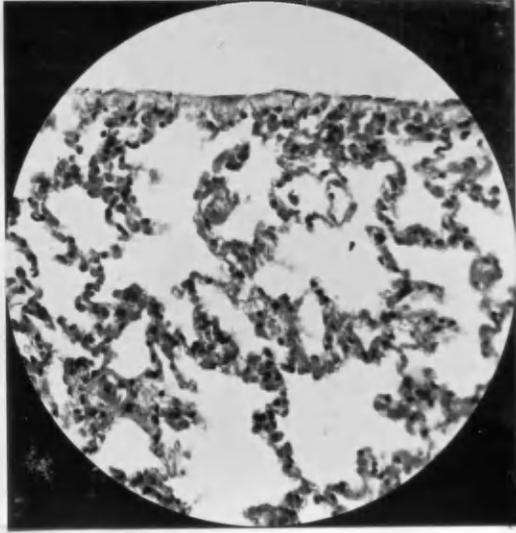


Fig. 61.

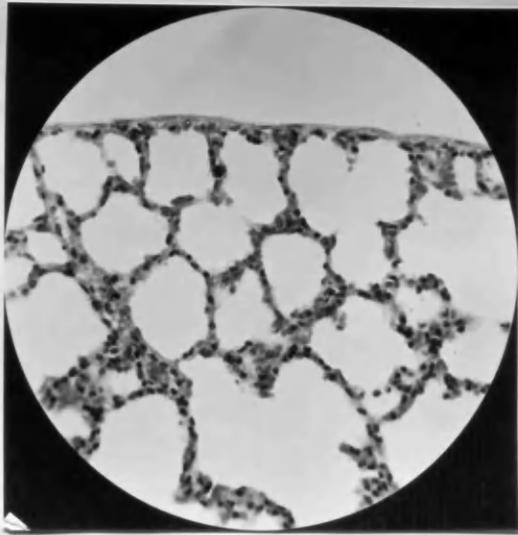


Plate. 62.

distended with an eosinophil matrix on the third day. The serosal endothelium is almost completely intact even on the first day.

(b) UREA.

3/4 molar solution CO(NH₂)₂ - 4.50% - 10 c.c. - 8 rabbits.

The animals were killed on the 1st (1), 2nd (1), 3rd (2), 4th (2), 5th (1) and 6th (1) days. A mild degree of hyperaemia of the visceral pleura was noted on the first day. The right pleural sac was dry in every case but one (A.332 - 48 hours). In this rabbit there was a copious effusion into both pleural sacs and into the pericardial sac while the surfaces of both lungs were congested and wrinkled; the other organs were normal in appearance with the exception that an ovoid "tumour" of the size of an almond, or thereby, was found in each uterine horn towards its free extremity.

Microscopical observations.

Rabbit A.332 - (48 hours). The tumour of the uterus presents the characters of an adeno-carcinoma. In the right lung numerous miliary metastases are observed in the perivascular lymphatics comprising small groups of mucus-secreting tumour cells; otherwise the organ exhibits only a minor degree of irregular congestion; there is no hyperplasia of the epithelium lining the marginal alveoli.

The other lungs of this series are entirely normal in appearance (fig.62 - Rabbit A.336 - 4 days) apart from a small superficial focus of proliferation arising from trauma in one or two of the rabbits.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

These two non-electrolytes - cane-sugar and urea - each in 3/4 molar solution (10 c.c.) produce no hyperplasia of the epithelium lining the marginal alveoli of the lung. This result suggests that plasmolytic or oxosmotic phenomena possibly play a relatively unimportant part in the production of epithelial proliferation.

EXPERIMENTS WITH REPEATED INJECTIONS OF STRONTIUM CHLORIDE (3/4 N. solution) AT INTERVALS OF 48 HOURS.

3/4 normal solution SrCl₂ - 10% - 5 c.c. - 12 rabbits.

The injection was repeated at intervals of 48 hours according to the following scheme:-

	Number of injections.	Duration of experiment from date of 1st injection.
Rabbit A.240	1	3 days.
" A.238	2	5 days.
" A.237	3	7 "
" A.229	4	9 "
" A.231	5	11 "
" A.234	6	11 "
" A.232	7	12 "
" A.230	7	14 "
" A.233	7	16 "
" A.236	7	18 "
" A.239	7	20 "
" A.235	7	22

All the animals survived the injection and they were killed

in rotation 3 days after the last injection up to the fifth; the sixth injection followed the fifth at an interval of 24 hours and the sixth rabbit (A.234) was killed 48 hours later. The remaining animals were killed 1, 3, 5, 7, 9 and 11 days respectively after the seventh injection. The right pleural sac was dry in all cases but one - A.238. In this case only a small quantity of free fluid was present. A scanty fibrinous exudate was observed in A.232 and A.236. In the latter there had evidently been some injury to the lung as some blood clot was also present. In the first rabbit of the series (A.240) the surface of the upper lobe of the right lung was wrinkled; minor changes of the same type were noted in the second rabbit (A.238); later small white plaques of thickening were observed in the visceral pleura with or without small sub-pleural ecchymoses which were believed to correspond with groups of injections. In some cases the appearances of the right lung were normal apart from some degree of emphysema.

Microscopical observations.

Rabbit A.240. There is hyperplasia of the epithelium lining the marginal alveoli, characterised by swelling of the cells and by nuclear hyperchromatism almost uniformly over the surface of the lung. Mitotic figures are scanty. In all other respects the appearances of the lung are normal.

Rabbit A.238. The epithelial hyperplasia is less uniform, but it is clearly marked.

Rabbit A.237. There is only a slight degree of swelling of the marginal epithelium, and that at considerable intervals over the surface.

Rabbit A.229)
" A.231) - Discontinuously there is swelling of the marginal
" A.234)
epithelium associated with plaques of proliferating fibroblasts
in the subserosa but, in general, these changes are negligible.

Rabbit A.232)
" A.230) - The subserosa is thickened by fibrous tissue.
The peripheral pulmonary epithelium is swollen and exhibits dis-
tinct nuclear hyperchromatism but these changes fall far short of
those described in A.240. Foci of fibro-epithelial proliferation
are discovered within the substance of the lung in relation to
haemorrhages of a few days' standing. There is a strand of baso-
phil substance which appears to follow the course of the elastic
lamina circumscribing the lung.

Rabbit A.233)
" A.236)
" A.239) - The appearances of these lungs are normal apart
" A.235)
~~from some fibrous thickening of the subserosa and, at the most, a~~
minor degree of swelling of the marginal epithelium subjacent to
the plaques of fibrosis.

This experiment was revised with the addition of two fresh
controls at each injection in order to ensure that each injection
was individually effective. Two rabbits were killed on the third
and fourth days respectively after the last injection along with
the controls which had received that injection alone. The injec-
tions were repeated at intervals of 48 hours and are indicated
serially in the following table by the letters "A" to "F".

		Number of injections.		Injections given.		Duration of experiment from date of 1st injection
Rabbit	B.1	1	A	3 days.
"	B.2	1	A	4 "
"	B.3	2	AB	5 "
"	B.4	2	AB	6 "
"	B.5	3	ABC	7 "
"	B.6	3	ABC	8 "
"	B.7	4	ABCD	9 "
"	B.8	4	ABCD	10 "
"	B.9	5	ABCDE	11 "
"	B.10	5	ABCDE	12 "
"	B.11	6	ABCDEF	13 "
"	B.12	6	ABCDEF	14 "

CONTROLS.

Rabbit	B.13	1	B	3 "
"	B.14	1	B	4 "
"	B.15	1	C	3 "
"	B.16	1	C	4 "
"	B.17	1	D	3 "
"	B.18	1	D	4 "
"	B.19	1	E	3 "
"	B.20	1	E	4 "
"	B.21	1	F	3 "
"	B.22	1	F	4 "

The results obtained in the control animals can be summarised briefly. As a rule the right pleural sac was dry; occasionally

it contained a small quantity of free fluid with or without some blood clot. In one rabbit (B.13) there was a bilateral blood-stained pleural effusion while both lungs were studded with metastases from a primary growth of the uterus.

Microscopical observations.

Rabbit B.13. The tumour of the uterus presents the characters of an adeno-carcinoma. The right lung and its visceral pleura are extensively infiltrated with miliary metastases of mucus-secreting tumour cells while there is slight, but distinct, hyaline thickening of the subserosa; there is no swelling, not to mention hyperplasia, of the epithelium lining the marginal alveoli (fig.63).

The remaining eleven rabbits, without exception, show distinct evidence of hyperplasia of the peripheral epithelium sometimes uniformly but more often discontinuously over the surface of the right lung, characterised by swelling of the epithelial cells, nuclear hyperchromatism, and numerous mitotic figures on the third day of experiment.

The right pleural sac was dry or contained only a trace of free fluid in the series of ten rabbits which received more than one injection (B.3 to B.12, both inclusive); a small quantity of blood clot was present in three accompanied by one or two small sub-pleural ecchymoses upon the costal surface of the lung. As a rule the surface of the right lung was bleached and more pale than that of the left; in other respects it was normal in appearance presenting neither wrinkling nor opacity.

Microscopical observations.

Rabbit B.3)
" B.4) - Discontinuously over the surface of each lung there

PLATE XXIII.

Fig. 63. Rabbit B.13/1929 - 3/4 N. SrCl₂, 72 hours - The visceral pleura is infiltrated with acini of mucus-secreting tumour cells - a metastasis from an adenocarcinoma of the uterus. There is no hyperplasia of the pulmonary epithelium at any point of the surface of the organ. (X 200).

Fig. 64. Rabbit B.8/1929 - killed 4 days after the fourth injection of 3/4 N. SrCl₂ - There is hyaline thickening of the subserosa. A small lymphangiectatic space is present filled with lymphocytes. There is just a little swelling of the epithelium lining the marginal alveoli and the nuclei of the epithelial cells are more or less pyknotic; there is no active hyperplasia. This field may be taken as an example of a reaction well above the average, following the repeated injection of SrCl₂. (X 200).

Fig. 65. Rabbit B.18/1929 - killed 4 days after a single injection of 3/4 N. SrCl₂ (D) (control) - there is active hyperplasia of the epithelium discontinuously around the periphery of the lung, characterised by swelling, nuclear hyperchromatism and cubical formation. (X 200).

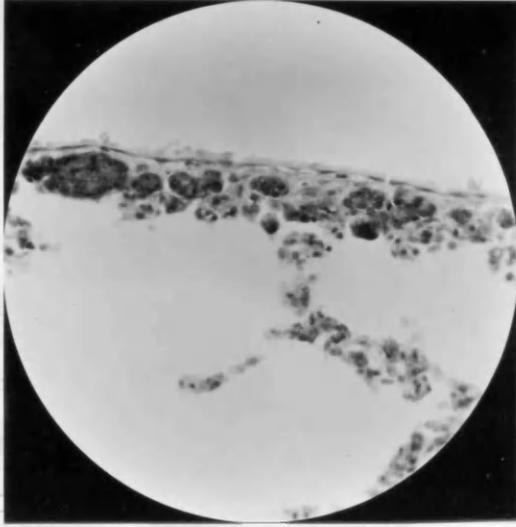


Fig. 63.

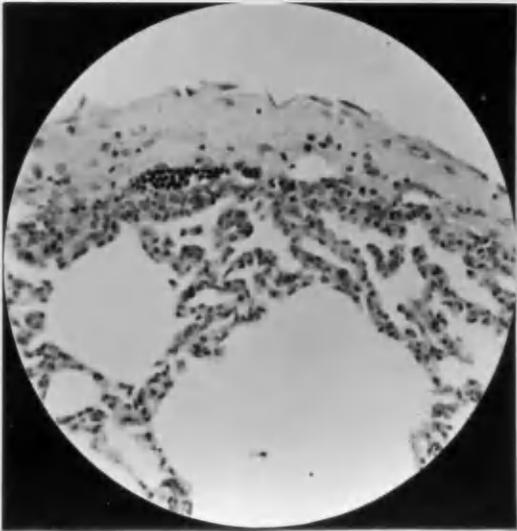


Fig. 64.

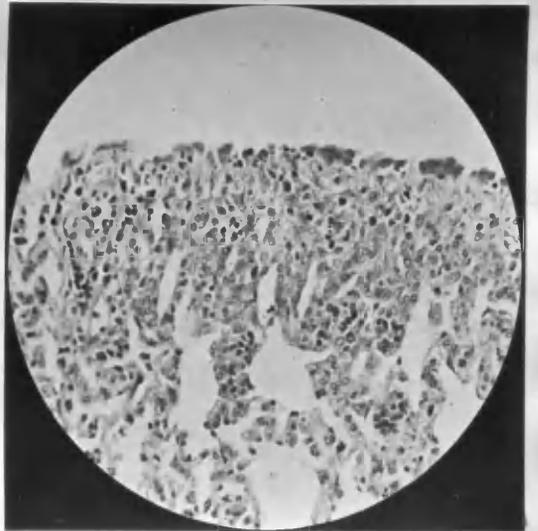


Fig. 65.

is distinct swelling of the marginal epithelium; the nuclei of the swollen cells are becoming pyknotic and their cytoplasm pale or eosinophilic; the periphery of the organ is infiltrated in some slight degree with lymphocytes; and the subserosa is becoming thickened by the proliferation of fibroblasts.

Rabbit B.5)
" B.6) - The swelling of the epithelial cells and the nuclear pyknosis are still observed with or without lymphocytic infiltration at comparatively wide intervals over the surface. These changes are confined almost exclusively to strips underlying thickened plaques of fibrous tissue in the subserosa. There is no evidence of active hyperplasia at all comparable with that noted in the controls (B.15 and B.16). Over extensive areas the appearances of the periphery of the lung are normal.

The same changes are found in the remaining six members of this series. In all cases a large proportion of the periphery of the lung is normal in appearance, but there is discontinuous thickening of the subserosa by fibrous tissue accompanied by swelling of the subjacent pulmonary epithelium with or without lymphocytic infiltration. Occasionally, small lymphangiectatic spaces filled with lymphocytes are present in the fibrous plaques. The swollen epithelial cells exhibit no evidence of active hyperplasia; their nuclei are pyknotic and their cytoplasm is pale or eosinophilic, presenting a striking contrast to the proliferating cells, with their vesicular nuclei, which are witnessed so consistently in the control animals, killed at the same interval after a single intrapleural injection of the same solution (figs. 64 & 65).

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

It has been shown in earlier experiments (page 37) that the intrapleural injection of 5 c.c. of a 3/4 N. solution of strontium chloride is followed regularly by hyperplasia of the epithelium lining the marginal alveoli of the lung. The hyperplasia pursues a very definite cycle, attaining a maximum on the third or fourth day and undergoing involution during the next four days. The present series of experiments was designed to demonstrate the effects of repeating the injection at intervals of 48 hours - i.e., at a period short of the maximum stimulation produced by a single injection. It has transpired that the process of involution which normally overtakes the hyperplasia arising from a single injection is not caused to deviate from its customary course by subsequent injections. Beyond a period of six days from the date of the first injection the appearances of the lung are practically normal and they have remained normal even when the injection has been seven times repeated. It would seem that the proliferating cells arising from a single injection of 5 c.c. of a 3/4 normal solution of strontium chloride become "refractory" or indifferent to subsequent injections of the same solution.

DISCUSSION.

Certain methods are set forth in the accompanying protocols, whereby hyperplasia and metaplasia in the serosal endothelium of the visceral pleura and in the alveolar epithelium of the lung of the rabbit can be produced with unfailing regularity. The reagents employed represent, for the most part, three groups of substances which are known to be endowed with special importance in biological phenomena, viz., (a) lipid alterants, of which sudan III is an example in virtue of its fat-solubility; (b) surface-active, or cytolytic, organic compounds of which sodium cholate is an example; and (c) colloid-alterants, represented by the neutral salts. These three groups of substances are capable of influencing protoplasmic action in profound degree apparently as the result of some physical or chemical alteration in the constitution of the protoplasm, yet their effects are not necessarily destructive, or even injurious, inasmuch as vital processes may resume their normal course after the reagent has been removed. The most familiar example of the reversible effects of certain members of the lipid-alterant group is presented by the phenomenon of general anaesthesia. The fundamental principles involved in these reactions are infinitely complex, affecting, as they do, the permeability and the surface tension of cell membranes, the application of Donnan's theory of membrane equilibria, the precipitation of colloids, the viability of cells and so forth. The difficulties are so great that it has not yet been possible to interpret with certainty even in the isolated egg cell of an invertebrate the essential nature of that vital process which expresses itself in the segmentation of the

egg. These difficulties are magnified many times in the mammal when it is sought to investigate the changes underlying or determining the proliferation of a community of somatic cells in their natural environment wherein a constant effort is maintained to restore their normal equilibrium. Consequently, it is not justifiable at this juncture to attempt to correlate the experimental results obtained in the rabbit with any particular biophysical process or processes. Nevertheless, it is believed that the general principle which has been exploited in these experiments - namely the utilisation of the pleural sac as a natural "culture chamber" for the study of the conditions promoting or favouring the proliferation of the serosal endothelium and of the pulmonary epithelium - promises to yield more precise information with reference to those conditions than that which is available now. The pleural sac is well adapted to an investigation of this kind. Being a potential space, it can accommodate an inoculum of any volume up to 25 c.c, and, provided that the reagent is dissolved in an aqueous medium, absorption can proceed so that the normal anatomical relationships are restored within an hour. Meantime the whole surface of the lung has been exposed to the action of the reagent and it is possible to trace the effects of the latter, as they unfold themselves, by the microscopical examination of the affected lung. While it is not permissible to ignore the counter-attack of the body fluids and the host of defensive mechanisms residing within the cells themselves, yet a comparative analysis of the gross effects of kindred substances - e.g., the neutral salts - on a reasonably extensive scale may elucidate, in some small part, the general nature of their action.

The first series of experiments was undertaken - sudan III in olive oil with and without bile salts - in the hope that some light might be shed upon the difficult problem of the histogenesis of primary malignant tumours of the serous membranes. Bearing in mind Fischer's experience that the epithelial proliferation which followed the subcutaneous injection of sudan III into the ears of rabbits underwent complete regression within three or four weeks, it was not anticipated that the intrapleural injection of the same reagent would produce a tumour formation. At the most, it was imagined that the inoculum might initiate certain ephemeral proliferative phenomena which might form a morphological link between simple swelling of serosal endothelium, such as occurs in the presence of a subacute inflammatory process, and the aberrant type, or types, of growth which are identified as primary tumours of the serous membranes. In other words, a vague idea was entertained that this reagent might be capable of unmasking some of the properties, or potencies, lying latent in healthy serosal cells. The results obtained by the injection of sudan III and sodium cholate together, have surpassed those expectations. Assuredly, no tumour formation has been produced, yet a complete transformation of the characters of the serosal cells has been witnessed over periods ranging from thirteen to seventy-seven days. Thus the visceral pleura has been observed, covered in one field by tall columnar epithelial cells, in another field by a transitional epithelium, and by stratified squamous epithelium in another field, all in the same lung, which still presents upon its surface strips of flattened endothelium. Moreover the altered characters of these cells tend to merge into one another in transitional

zones. In interpreting these phenomena as they befell in rabbit 41, for example, two alternative explanations suggest themselves.

(1) the epithelial formation upon the surface of the visceral pleura has developed from the serosal endothelium directly by a process of metaplasia.

(2) the superficies of the lung has been breached by the point of the trocar in the act of performing the intrapleural injection, thereby permitting an outcrop of alveolar epithelium upon the abraded surface and a process of metaplasia has overtaken the dislocated epithelium.

The second view enjoys two advantages in the respects that it does not conflict with the embryological axiom concerning the "specificity of the germ layers," and that metaplasia from the columnar epithelium of a bronchus to stratified squamous epithelium is an event of common experience. There is no doubt that in a majority of cases, and that in spite of the exercise of great care, some degree of injury is inflicted upon the surface of the lung, but it is necessary to assume that the reagent injected can provide an adequate stimulus for the outgrowth and for the survival of a specialised epithelium upon a serous surface. The arguments in favour of this view lose something of their virtue when it is remarked that no islets of squamous epithelium have been found within the substance of the lung in any rabbit of the series, although hyperplasia affecting the pulmonary epithelium has been an invariable rule.

In my opinion, the most important clue to the source of the metaplastic epithelium covering the visceral pleura of rabbit 41

is provided by a study of the cystic formations in the thickened subserosa of the same rabbit. The anatomical distribution of these "cysts" immediately external to the limiting elastic membrane of the lung and the characters of the desquamated cells occupying their lumina admit little room for doubt that they represent small endothelial "pockets". These "pockets" are of common occurrence in any serous membrane which has been the seat of a subacute or chronic inflammatory process; their lining is derived from the proliferation of one or two surviving serosal cells. Attention has already been directed to the gradual transition from flattened cells lining the pulmonary margin of each space to high columnar cells lining the pleural margin - as a result, perhaps, of the diffusion of some of the elements of the inoculum through the superficial tissues. If the original identity of these morphologically different units were conceded - and the concession seems to be justifiable - it might be possible to sustain an argument in favour of a sequence of metaplastic phenomena emanating directly from serosal endothelium and proceeding from columnar cells to stratified squamous and to transitional epithelium. This view gains a considerable measure of support from the fact that two of the subserosal cysts are lined entirely by transitional epithelium, and that other adjacent cysts are partially lined by stratified plaques of squamous-like cells in contact, at their margins, with columnar cells.

Obviously the argument tends to subvert the preconception of the specificity of the germ layers but the circumstantial evidence supporting the argument is believed to be conclusive.

PLATE XXIV.

Fig. 66. P.M. A.761, Western Infirmary, Glasgow.
Macroscopically, the visceral pleura of the right lung was thickened more or less uniformly by white fibro-cellular tumour tissue, and no alternative primary growth could be discovered. Histologically, the tumour presents the characters of a squamous epithelioma. Stained by Heidenhain's Iron haematoxylin. (X 500).

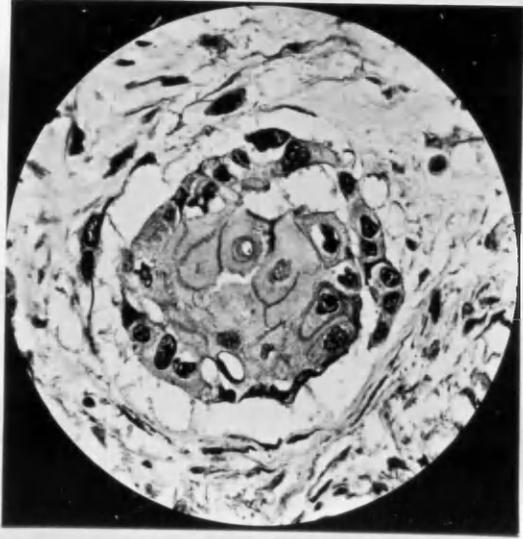


Fig. 66.

The incidence of stratified squamous epithelium upon the surface of the visceral pleura, usurping the place of serosal endothelium, has acquired a greater interest on account of the peculiar characters presented by one (P.M.A.761 West. Inf.) of the three primary growths of the serous membranes which prompted this series of investigations. An oil immersion field of a section of the growth is illustrated (fig.66); it exhibits the histological structure of a squamous epithelioma. The other two tumours - one affecting the pleura and the other the peritoneum - were diagnosed as endotheliomata of the serous membranes. The prevailing attitude towards a primary squamous epithelioma of the pleura might be summed up in the words of Robertson, who was inspired to write in more general terms that "all tumours of the pleura are secondary, representing extensions, implantations, or metastases from an unrecognised, or latent primary source, usually the lungs." On the contrary, if any conclusion can be drawn from these experimental studies at all, the possibility of the development of a primary squamous epithelioma of the pleura can not be eliminated as a rare natural event.

Hyperplasia and metaplasia of the epithelium lining the marginal alveoli of the lung are invariably found in association with the metaplasia of the serosal endothelium. They are characterised by swelling, nuclear hyperchromatism, and cubical or columnar formation of the epithelial cells. These changes are probably less permanent than the serosal changes but they have been observed after the experiment had been in progress for fifty

days and they make their appearance within four days. In many cases, their development is so uniform around the periphery of the lung that the latter seems to present a scalloped border, as it were, although it is circumscribed by an intact peripheral elastic lamina. The marginal distribution of these changes, their uniformity upon the mediastinal aspect of the lung as well as upon the costal surface, and the integrity of the elastic lamina seem to testify that the hyperplasia is a result of the diffusion of some of the elements of the inoculum through the tissues of the visceral pleura. On the other hand, the proliferative lesions which are more local in their distribution, tending to penetrate the substance of the lung more or less deeply and to disrupt the interalveolar elastic laminae, are probably due to the combined effects of the inoculum and of accidental trauma. Both types of epithelial reaction, alike, the superficial and the penetrating, are aberrant in the respect that they do not conform to simple reactive phenomena which are recognised and described as the results of mechanical injury or chronic inflammatory processes. In the writer's experience they have been reproduced with the closest precision, perhaps, in the rabbit by the intrapleural injection of another lipid-alterant, or "fat-soluble" substance - viz., an ethereal extract of tar. The tar employed was a very active "carcinogenic" agent (in mice), and it was exhibited as a 1% solution of the ethereal extract in olive oil (5 c.c.); its effects were enhanced by the addition of bile salts. Reverting to the changes produced by the injection of sudan III and bile salts, their essential

nature is unknown but it would appear that the morphological alterations in the serosal endothelium and in the pulmonary epithelium may occasionally express some permanent, or semi-permanent, disturbance in the biological state of the cells. Whereas it is customary for the metaplastic pulmonary epithelium to resume its normal characters some time after the encapsulation of the sudan III in the pleural sac, within three months or thereby, the lungs of two rabbits contain islets of aberrant epithelium after the experiment had been in progress for sixteen months although the natural environment of those cells had probably been restored a long time before.

These experiments had been preceded by a preliminary series, based upon the intrapleural injection of sudan III, in olive oil (or lanolin) without bile salts, but the results were disappointing. The subserosa became thickened in greater or less degree by the development of granulation tissue; "foreign-body" giant cells were common; the serosal cells tended to become swollen and heaped up, forming layers two or three deep, but they exhibited no aberrant proliferation; the lung tissues presented varying degrees of collapse according to the extent of the pleural effusion which was an invariable sequel of the injection. Later, in revising the experiments, considerable degrees of irregular epithelial hyperplasia have been found in the lung following the injection of the same dye in olive oil but the results have been capricious whereas consistent changes had been produced in a consecutive series of sixteen rabbits in which bile salts had been added to the inoculum. As the investigation progressed, a

strong impression was formed that the addition of the bile salts tended to promote and tended to maintain the cellular proliferation, but some time elapsed before an experimental proof of this view could be obtained. In the first instance, it was shown that minor degrees of the same type of metaplasia and hyperplasia can be produced in the alveolar epithelium of the lung by the intrapleural injection of an autoclaved emulsion of olive oil and bile salts not only in the rabbit but also in the guinea-pig, whereas olive oil, alone, is ineffective. However, these results might not be accepted as conclusive evidence of the efficacy of the bile salts since olive oil, dispersed in an aqueous medium, is hydrolysed on autoclaving to a greater extent than the unemulsified oil and the increased content of free fatty acid introduces a serious fallacy. The same objection is applicable to another series of experiments in which the emulsion was neutralised by $N/NaOH$ prior to its injection. Metaplasia and hyperplasia of the alveolar epithelium are again observed but it is impossible to exclude the intervention of free oleic acid and its sodium salt, formed by hydrolysis of the emulsified oil within the pleural sac. These difficulties were finally overcome by the substitution of a non-saponifiable substance, viz., liquid paraffin, as the vehicle for the introduction of the bile salts, in emulsion, into the pleural sac. Liquid paraffin, alone, produces only a trace of swelling of the epithelium lining the marginal alveoli, whereas the same volume of an emulsion of liquid paraffin and bile salts is followed by progressive hyperplasia which attains a maximum on the third or fourth day and, thereafter, undergoes involution. Although these results seemed to justify the view that the pro-

liferative phenomena were determined by the direct action of the bile salts upon the parent cells yet they were open to another criticism, namely, that the experiment was not a clean one since the paraffin remained in the pleural sac as a "foreign-body" after the bile salts had been absorbed. Therefore, it was decided to test again the effects of introducing the bile salts in solution in physiological saline. Little hope of a successful issue was entertained since earlier experience had proved that even a trivial injury of the surface of the lung in the presence of a solution of bile salts is very liable to be followed by an acute pulmonary oedema, and sudden death of the animal. However, it has been demonstrated now that active proliferation of the alveolar epithelium can be produced by the injection of solutions of sodium cholate in normal saline, 1% (5 c.c.) and 0.5% (10 c.c.) and that it attains a maximum on the third or fourth day. On the other hand, physiological saline (25 c.c.) produces no proliferation and even a 3/4 normal solution of sodium chloride (5 c.c.) occasions only a trace of hyperplasia. These observations admit no shadow of doubt concerning the efficacy of sodium cholate in the production of hyperplasia and metaplasia in the alveolar epithelium of the lung of the rabbit, whether the reagent is exhibited in weak solution over a more prolonged period, as in an emulsion, or in a relatively concentrated solution for a short period. It is noteworthy that the reaction pursues the same cycle under both conditions. Sodium cholate is an extremely "surface-active" agent. A few years ago, it was generally believed to exercise its biological effects upon fats and lipoids

but more recently Ponder has arrived at the conclusion that its haemolytic properties are due to the fact that "sodium taurocholate, and also sodium glycocholate in certain concentrations, form compounds with the proteins of the cell envelope". Be that as it may, for the present purpose it would seem to be of greater moment to try to assess the relative importance of the physical properties and of the chemical nature of the bile salts in the genesis of the epithelial proliferation which follows their exhibition. However, the complexity of this reagent alike in its chemical structure and in its physical properties promised to create insuperable difficulties in the interpretation of any results which might be forthcoming. Therefore it was decided to elaborate an alternative series of investigations with simpler reagents, whereby it might be possible, perhaps, to discriminate between chemical and physical agencies with a greater measure of confidence. The event has proved that metaplasia and hyperplasia of the epithelium lining the marginal alveoli of the lung, histologically of the same order as that determined by sodium cholate, can be produced with equal certainty by the intrapleural injection of a variety of neutral salts in aqueous solution. Yet there is one grave difficulty besetting their interpretation.

In the course of the examination of a series of rabbits' lungs, exceeding 600 in number, following the intrapleural injection of a variety of reagents, it has been noted that a small accidental trauma, whether it be situated superficially or more deeply, is attended regularly on or before the third day by active proliferation of the surrounding epithelial and connective

tissue cells. The same changes develop around any recent focus of necrosis within the lung substance. For instance, Pinkerton has described lesions of this kind after the intratracheal injection of fatty acids; Fischer, after the intravenous injection of "Granugenöl"; and in the present series of experiments deep-seated foci of proliferation have been recognised around central foci of necrosis following the intrapleural injection of certain solutions of aluminium chloride. It would appear to be a universal rule that the death of a few cells within any community of somatic cells is followed by some degree of proliferation regeneration or repair originating in the adjacent surviving cells, and numerous authors have postulated diverse "growth-promoting" substances derived from the dying cells. Caspari speaks of them as "nekro-hormones." In my opinion, this conception is not constructive, yet it is the crux of the present experimental investigation. Is it possible experimentally to assign the processes of cell-division to the direct influence of a simple agent upon the parent cells, and to exclude the intervention of disintegration products of dead or dying cells? Apart from the members of the aluminium chloride and of the lanthanum chloride series of animals - and, in their case, a logical explanation is forthcoming - no necrosis affecting either individual cells or groups of cells has been disclosed in the epithelial lining of the peripheral alveoli by a careful microscopic examination. Nevertheless, progressive changes which are characteristic of an increased growth capacity of these cells, viz., swelling, nuclear hyperchromatism, and a more or less large number of mitotic figures, have declared themselves during the

first four days of experiment after the intrapleural injection of certain solutions of neutral salts. Loeb and Lillie have shown that segmentation of the unfertilised egg of the sea-urchin can be induced by hypertonic solutions of neutral salts under conditions which exclude the operation of any other factor. The limitations of our present knowledge, however, will not admit any fruitful discussion of an experimental analogy which might appear to subsist between the female sex cell of an invertebrate and the somatic epithelial cell of the lung of a rabbit.

A considerable range of preliminary experiments with intrapleural injections of a $3/4$ N solution of strontium chloride (5cc.) had produced hyperplasia of the epithelium lining the marginal alveoli. This salt was chosen in the first instance because it had proved most effective (with barium chloride) in Loeb's experience in promoting artificial parthenogenesis in the unfertilised egg of the sea-urchin. It yielded results in the rabbit which were remarkably consistent and it seemed to create no constitutional disturbance. These observations encouraged the hope that the pleural sac might be adapted to form a culture chamber, so to speak, for the study of the behaviour of the peripheral cells of the lung under altered environmental conditions. It was accepted that the semi-permeable membrane or membranes of the visceral pleura would offer no real barrier to the diffusion of the electrolytes which it was proposed to inoculate. On the contrary, they would serve a useful purpose in protecting the epithelial cells of the lung from the sudden in-rush of a hypertonic salt solution, for it had been observed that the serosal endo-

thelium was desquamated in large part possibly as the result of this interference. The conditions of the experiment demanded that only those changes which were marginal or submarginal should be reckoned in interpreting the effects of the various reagents. For the present, the more remote changes which were clearly secondary to traumatic or necrotic lesions must be ignored.

When these impressions had been formed, a fresh series of 14 rabbits was injected each with 5 c.c. of a $3/4$ N solution (9.99%) of strontium chloride. At the same time a control series of 14 rabbits received each 25 c.c. of a 0.146 N solution (1.94%) of the same salt. It is clear that the total quantity of strontium chloride injected (0.49 gm.) was practically constant. The animals were killed in pairs or groups of three at intervals of 24 hours. A marked contrast is presented by the microscopical observations which have been recorded in the two series. With three exceptions, the cellular reaction to the larger volume of dilute solution is slight; frequently it is barely perceptible. On the other hand, the smaller volume of concentrated solution has never failed to produce active manifestations of epithelial hyperplasia, sometimes limited in its extent but, as a rule, distributed more or less uniformly over the whole surface of the lung. These manifestations - swelling of the epithelial cells, nuclear hyperchromatism, and mitotic figures - are progressive in their development up to the third or fourth day; thereafter they tend to disappear in the reverse order so that the normal appearances of the lung are re-established by the sixth or seventh or eighth day. These results directed attention to the importance of the concentration of the reagent and they seemed to indicate

that physical factors play a part not less significant, perhaps, than chemical factors in the genesis of cellular proliferation. This deduction could be submitted to an independent test. Loeb (1924) has shown that neutral salts exercise a specific effect upon certain important physical properties of proteins "in vitro" - their membrane potentials, their viscosity, their swelling and their osmotic pressure - and that the cation alone is effective when the hydrogen-ion concentration lies on the alkaline side of the isoelectric point of the protein concerned. The isoelectric point of most animal proteins is included within a range from pH 4.5 to pH 6. Therefore it was assumed as a working hypothesis that the proliferative phenomena following the intrapleural injection of a hypertonic solution of strontium chloride might be due to the activity of the bivalent cation of the salt. It has also been shown, however, by the same author, that the specific effect exercised by a neutral salt upon a protein increases rapidly with increasing valency of the cation of the salt whereas the chemical nature of the cation plays a negligible part. Hence it became desirable to test the effects of neutral salts possessing mono- and tri-valent cations, e.g. sodium chloride and aluminium chloride respectively.

An intrapleural injection of 25 c.c. of a 0.145 N solution (0.85%) of sodium chloride produces no evidence of epithelial proliferation in the lung. The changes which follow an injection of 12.5 c.c. of a $\frac{3}{8}$ N solution (2.15%) of the same salt are limited to minor degrees of swelling of the epithelial cells lining the marginal alveoli. On the other hand, clear manifes-

tations of increased activity in the epithelial cells lining the marginal alveoli of the lung are excited on the second or third day by an injection of 5 c.c. of a $3/4$ N solution (4.3%) but, in respect of their extent and uniformity, they fall far short of those observed and recorded after an injection of strontium chloride in chemically equivalent proportion. The importance of the concentration of the reagent is again emphasised.

Conversely, 5 c.c. of a $3/4$ N solution (3.3%) of aluminium chloride kill the serosal endothelium and a marginal zone of pulmonary epithelium "in situ". The activity of the cellular proliferation which ensues in the subjacent epithelial cells within three days and which is maintained over the fourth day surpasses anything which has been observed throughout the preceding experiments with neutral salts possessing monovalent and bivalent cations. Plainly there is here a zone of dead tissue which might yield an abundance of "hekro-hormones", and their participation in the reaction cannot be excluded. Nevertheless it is worthy of note that 10 c.c. of a 1 in 10 dilution of the $3/4$ N solution can and do consistently produce marked hyperplasia of the epithelial cells lining the marginal alveoli without any visible evidence of necrosis, in adjacent epithelial cells.

In the next place, two series of experiments were made with non-electrolytes, viz., cane-sugar and urea. They were designed to show whether any solution possessing a high osmotic pressure was capable of producing epithelial proliferation. Inasmuch as a $3/4$ molar solution of NaCl had excited little more than swelling of the cells whereas a $3/40$ molar solution of CaCl₂ provokes

well-marked hyperplasia, it might seem that plasmolytic phenomena play a subsidiary part in the reaction. However, the uncertainty which prevails in regard to the biological behaviour of sodium and of calcium ions discounts any conclusion affecting the influence of the osmotic pressure of solutions of their salts. Serious difficulties are met in trying to isolate osmotic phenomena as a factor in the production of the epithelial hyperplasia, under any circumstances, because the fate of any fluid inoculum in the pleural sac can only be guessed. The solution is absorbed rapidly but the rate of absorption is unknown; at the same time it is likely to be diluted by the effusion of tissue fluids but the rate of dilution is also unknown. In view of these qualifications, it must suffice for the present to record the fact that a "hypertonic" solution ($3/4$ M. - 10 c.c.) of cane-sugar or of urea produces no epithelial proliferation within the period of six days.

Proceeding to the study of the effects of a wider range of electrolytes, it has been demonstrated that the mono-valent cations potassium and lithium behave like sodium; the bi-valent cations, magnesium and calcium, like strontium; and the tri-valent cation, lanthanum, like aluminium. It has been a routine practice to inject 5 c.c. of a $3/4$ N. solution of the chloride of each metal in order to keep the concentration of the anion constant. Under those conditions, potassium chloride or lithium chloride produces only a minor degree of swelling of the epithelium lining the marginal alveoli. Magnesium chloride produces swelling and nuclear hyperchromatism of the epithelial cells within 48 hours; the hyperplasia is maintained over the third day but already the

nuclei of the swollen cells are becoming pyknotic in the earliest phase of their involution. Lanthanum chloride excites a much more striking degree of epithelial proliferation on the third day; the proliferation is maintained over the fourth day and tends to culminate in "giant-cell" or syncytial formation on the fifth day.

Since these results indicate that the efficiency of an ion in producing epithelial hyperplasia is related to its valency, it was proposed at this stage to test the effects of injecting the compounds of an element which gives rise to two series of salts and to observe whether the "valency rule" was maintained. For this purpose, ferric chloride and ferrous chloride were employed. At the same time it was hoped that it might be possible to trace the fate of the injected iron microchemically. The main object of these experiments has been frustrated by the instability of ferrous chloride owing to the readiness with which it becomes oxidised to the ferric state in the tissues while it is precipitated in part as ferric hydroxide. The whole surface of the lung becomes impregnated uniformly with this precipitate - intercellularly not intracellularly - and it is noteworthy that its distribution coincides more or less precisely in its depth with a narrow marginal zone of epithelial hyperplasia. Following the exhibition of both salts, each in 1/20 N. solution (10 c.c.), there is progressive proliferation of the marginal epithelium during the first four days of experiment but, in general, the changes produced by the ferric salt are more striking than those produced by the ferrous. Hitherto it has not been possible to demonstrate any iron within the proliferating cells even after

prolonged oxidation of the sections with hydrogen peroxide. Also it was elected to test the effects of a salt possessing a tri-valent anion. For this purpose potassium ferricyanide was chosen - 10 c.c. of a 1/20 N. solution. In these experiments the naked-eye appearances of the lungs were uniformly normal. Microscopically, a very large proportion of the surface of every lung is normal in appearance but discontinuously there is a minor degree of swelling of the marginal epithelium scarcely exceeding that produced by 25 c.c. of a 3/20 N. solution of potassium chloride. A 1/20 N. solution of potassium ferricyanide is "hypotonic" to the body fluids and might occasion some slight reaction by a certain degree of endosmosis. In this connection it is interesting to note that the intrapleural injection of 25 c.c. of distilled water is not without effect ~~although~~ although the epithelial reaction is again discontinuous over the pulmonary surface.

The special object of these experiments with solutions of neutral salts has already been set forth. They form a corollary to the experiments with emulsions and solutions of bile salts. It has been demonstrated that bile salts can provide an adequate stimulus for the proliferation of the epithelial cells lining the marginal alveoli of the lung under certain experimental conditions. It promised to be difficult, if not altogether impossible, to discriminate between the physical properties and the chemical structure of this most complex reagent in respect of their capacity to promote cell division. This difficulty could be evaded only by the employment of simple reagents which were more or less equally effective. Theoretical considerations prompted

the use of neutral salts. It has transpired that chemically equivalent proportions of three groups of neutral salts, possessing monovalent, bivalent, and trivalent, cations respectively, produce increasing manifestations of cellular proliferation in the epithelial cells lining the marginal alveoli of the lung of the rabbit as the valency of the cation of the salt increases. This gradation conforms to Loeb's "valency rule" with reference to the effects exercised by neutral salts upon certain physical properties of proteins when ^{the} hydrogen-ion concentration lies on the alkaline side of the isoelectric point of the proteins concerned. Also in conformity with this principle, the proliferation is not enhanced by increasing the valency of the anion ($K_3Fe(CN)_6$) under the same experimental conditions and non-electrolytes (cane-sugar and urea) produce no proliferation at all. These observations taken together offer clear evidence in favour of the view that physical factors play an important, if not an essential, part in promoting hyperplasia in the marginal epithelium of the rabbit's lung.

On the other hand, the valency of the cation of the salt is not the only determining factor. If the valency of the cation were alone involved, then it should follow that all bi-valent cations might be expected to produce equivalent effects. Magnesium, calcium, and strontium, alike, produce well-marked epithelial proliferation without necrosis; in that respect they form a homogeneous group but the reaction of each seems to be specific in some measure. Thus the proliferation produced by magnesium is believed to attain its maximum on the 2/3rd day

whereas the maximal effects of strontium and calcium are disclosed on the 3/4th and 4th days respectively. The differences of reaction detected by the naked-eye are not less convincing. The wrinkling and opacity of the visceral pleura following the injection of magnesium are usually trivial; strontium rarely fails to produce well-marked wrinkling of the surface of the upper lobe of the lung and often the wrinkling affects the whole surface of the lung uniformly; with both metals alike the right pleural sac is almost invariably dry and there is no apparent constitutional disturbance. Under the same conditions calcium is likely to kill a few of the more susceptible animals and it is attended always by a copious bilateral pleural effusion and by opacity and a striking degree of wrinkling or puckering of the surface of the right lung or even of both lungs. It is safe to say that calcium produces a far more intense reaction, locally and constitutionally, than strontium or magnesium. It is more difficult to differentiate the reactions of the two latter metals but it is believed that magnesium produces less sustained effects than strontium. This general estimate is based upon a careful microscopical examination of the right lung of 45 rabbits (strontium - 30; magnesium - 15). It is not possible to correlate these discrepancies with any common attribute of the metals, such as their valency. As the experiments have progressed a general impression has been formed that the biological behaviour of the elements, with special reference in this instance to the production of epithelial proliferation, tends to harmonise with their classification in the periodic table. In other words, it

might seem to be possible that the biological properties of a metal are governed in some degree by its atomic weight which has long been known to preside over its chemical reactions. However, the real merits of this impression can be assessed only by a much more extensive series of investigations.

Attention has also been directed towards that biological antagonism which is known to prevail between certain ions. For example, the freshly excised heart of a frog, when perfused with an isotonic solution (0.75%) of sodium chloride in distilled water, rapidly ceases to beat but its excitability is restored and its contractions renewed by the addition of a trace of calcium chloride to the perfusion fluid. Bullock and Cramer, in their studies upon the mechanism of bacterial infections, have shown that the "defence-rupturing" action of calcium chloride is abolished by sodium citrate. Also, Cramer has studied the effects of sodium ions and of calcium ions on the growth of a transplantable mouse carcinoma (strain No.63 of the Imperial Cancer Research Fund). Calcium ions, in his experience, exercise a very distinct inhibitory effect upon the growth of subsequent grafts, but they are antagonised in respect of this property by sodium ions. The essential nature of this antagonism, whether it be physical or chemical, is not clear. It must suffice to say that it is not demonstrable under the experimental conditions of the present investigation. Only five animals have been examined after the intrapleural injection of sodium chloride and calcium chloride together, in chemically equivalent proportions. In every case there is well-marked hyperplasia of the epithelium lining the marginal alveoli of the lung. The action of the calcium ions is

not abolished; indeed, there is reason to doubt whether it is modified in any significant degree. This result is consistent with those which have been recorded after the injection of $3/8$ N sodium chloride (10 c.c.) and of $3/8$ N calcium chloride (10 c.c.), separately, and it harmonises with the view that physical factors do play a large part in the production of that transient epithelial hyperplasia which forms the subject of this contribution. Likewise no antagonism is apparent between calcium ions and potassium ions when they are exhibited in chemically equivalent proportions ($3/4$ N solution - of each 5 c.c.).

In conclusion, one striking contrast in the effects produced by sudan III and sodium cholate on the onehand and by solutions of the electrolytes on the other hand must be emphasised. The fat-soluble dye, dissolved in olive oil and emulsified by bile salts, excites an epithelial reaction, the duration of which is measured in weeks or in months, whereas the characteristic reaction produced by a $3/4$ N solution of strontium chloride (5 cc.), for example, attains a maximum within four days and undergoes involution within eight days. Superficially, this discrepancy might appear to be quite logical since the solution of strontium chloride is completely absorbed after a short interval and the sudan III is absorbed so slowly that it remains in contact with the surface of the lung for an indefinitely long period until it becomes encapsulated with fibrous tissue. The results of more recent investigations tend to contradict that plausible explanation. The fugitive nature of the changes produced by a single injection of strontium chloride is not due to the rapid absorption and exhaustion of the reagent, for the process of involution

which normally overtakes the hyperplasia following a single injection of the salt pursues its customary course regardless of subsequent injections, repeated at intervals of 48 hours. Beyond a period of six or eight days from the date of the first injection, the appearances of the lungs have been practically normal and no active proliferation has been witnessed even when the injection had been repeated seven times. It would seem that the proliferating cells arising from the first injection of strontium chloride become "refractory" or indifferent to subsequent injections of the same solution. A large series of experiments is in progress with the object of determining the scope and, as far as possible, the nature of this "refractory state", as it affects the alveolar epithelium of the lung. An important principle is believed to be involved. Two reagents, a lipid-alterant and a colloid-alterant, which might be expected to exercise selective effects upon the cell-lipoids and upon the cell-proteins respectively, can initiate epithelial hyperplasia and metaplasia with equal facility: but the reaction produced by the lipid-alterant is sustained for a period of weeks or months, in the presence of an excess of the reagent, whereas one injection of the colloid-alterant inhibits the action of a second injection 48 hours later. It is hoped that these results may provide a basis for the study of the biological conditions which favour the maintenance of aberrant epithelial hyperplasia in the mammal for there can be little doubt that, in some respects at least, this problem is entirely distinct from that affecting the initiation of the hyperplasia.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS.

Certain methods are described, based upon the intrapleural injection in the rabbit of certain biological reagents, whereby hyperplasia and metaplasia of the serosal endothelium, covering the visceral pleura, and of the alveolar epithelium of the lung can be produced.

(1) a. Sudan III, dissolved in olive oil and emulsified by sodium cholate (bile salts) induces a transformation of the serosal endothelium into columnar, transitional, or stratified squamous epithelium, while the pulmonary epithelium lining the marginal alveoli of the lung undergoes metaplasia to a tall columnar type. These changes are generally maintained for two or three months but they have been witnessed in two exceptional animals after sixteen months. The dye is absorbed from the pleural sac very slowly and incompletely and the residue remains in contact with the surface of the lung for an indefinitely long period until it becomes encapsulated by fibrous tissue.

b. Sudan III, dissolved in olive oil but not emulsified by bile salts, is relatively ineffective.

(2) Sodium cholate, whether emulsified in olive oil or in liquid paraffin or dissolved in physiological saline, produces well-marked hyperplasia and metaplasia of the epithelium lining the marginal alveoli of the lung. These changes are transient, attaining a maximum within four days and undergoing involution within eight days.

(3) Certain solutions of various electrolytes produce reactions which conform in their histological characters and in their

evolution with the reaction determined by bile salts. The hyperplasia increases as the valency of the cation of the salt increases, but it is not enhanced by increasing the valency of the anion. It has not been possible to demonstrate any antagonism between sodium and calcium ions, or potassium and calcium ions, in respect of the production of epithelial proliferation when they are exhibited in chemically equivalent proportions (3/8 N solutions).

(4) Minor degrees of the same type of epithelial hyperplasia are produced by the intrapleural injection of large volumes of distilled water.

(5) "Hypertonic" solutions (3/4 M - 10 c.c.) of two non-electrolytes - cane-sugar and urea- produce no reaction.

(6) It is not possible to sustain the transient reaction produced by 5 c.c. of a 3/4 N solution of strontium chloride by repeating the injection at intervals of 48 hours. On the contrary, there is some reason to believe that the proliferating cells arising from the first injection become "refractory" to subsequent injections of the same solution.

(7) The significance of physical factors in the genesis of the epithelial proliferation is discussed and attention is directed towards certain observations which may affect the maintenance or the stability of the reaction.

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APPENDIX A.

LESIONS OF THE LIVER AND OTHER ORGANS PRODUCED BY

THE INTRAPLEURAL INJECTION OF SUDAN III.

PLATE XXV.

Fig. 71. Rabbit A.7/1928 - sudan III/olive oil, 233 days -
A portion of the left lobe of the liver is shown.
The organ was enlarged, weighing rather more than
100 grams (wt. of rabbit - 2.3 kgm.). It presented
a light chestnut colour and its surface was uni-
formly nodulated while its consistence was firm
and its substance was intersected with broad strands
of vascular connective tissue. There was no
significant enlargement of the spleen; the animal
was well-nourished; its retroperitoneal fat was
stained pink; there was no ascites. Drawn by
Miss E. M. Wright. (Natural size).



FIG 71.

In addition to the local changes produced in the serosal endothelium and in the alveolar epithelium of the lung of the rabbit by the intrapleural injection of sudan III in olive oil, with or without the addition of sodium cholate, certain remote effects in other organs are worthy of more detailed description.

At a very early stage in the investigation, it was disclosed that the liver of the rabbit is peculiarly susceptible to injury by this fat-soluble reagent and presents, within a few days, areas of necrosis either focally or diffusely throughout the parenchyma. These lesions are clearly visible to the naked eye in a majority of the experimental animals but sometimes they can not be recognised. Almost invariably, however, there is a notable increase in the consistency of the organ, associated in many cases with a remarkable increase in its size and weight. For example, in one rabbit (128/1927 - Wt. 2.05 kgm.) which died on the tenth day of experiment, the liver weighed 186 grams (average normal wt. - 70 to 80 grams). Also, in typical cases, the liver assumes a light chestnut colour. As a rule, there is little evidence of fatty infiltration microchemically. Histologically, the liver exhibits a considerable variety of pathological changes. Frequently, the cells are vacuolated as if they contained an excess of fluid, manifesting a form of hydropic degeneration (fig. 67 - Rabbit 47); or their cytoplasm may be opaque and eosinophilic, presenting crescentic masses of "intracellular hyaline" (Mallory); elsewhere small groups or large areas of cells have undergone necrosis, more particularly in the central and intermediate zones of the lobules (fig.67);

PLATE XXVI.

- Fig. 67. Rabbit 47/1927 - sudan III/bile salts/olive oil, 15 days - A portal tract is shown at the upper border of the field, infiltrated with round cells. A small focus of acute necrosis of the parenchyma can be recognised encroaching upon each of the two lateral borders of the field. There are numerous examples of hydropic degeneration of the liver cells, represented by large clear spaces arising from the fusion of adjacent cells. Below the centre of the field there is a clump of surviving liver cells of rather large size; above the centre, there is another group of smaller liver cells which are participating actively in the regeneration of the parenchyma. (X 100).
- Fig. 68. Rabbit 41/1927 - sudan III/bile salts/olive oil, 35 days - There is wide-spread necrosis of the parenchyma and only a few surviving liver cells are present in immediate relation to the portal tracts. In other fields there is slight proliferation of bile duct epithelium but there is little or no regeneration of liver cells. (X 50).
- Fig. 69. Rabbit 56/1927 - sudan III/olive oil, 22 days - In this liver, a process of focal necrosis is being overtaken by regeneration of the parenchyma. The proliferating liver cells are relatively small in size; they surround and appear to be replacing, or encroaching upon, the central necrotic tissue, whereas the resting liver cells around the periphery of the field are larger in size. (X 70).
- Fig. 70. Rabbit 2/1927 - sudan III/lanolin followed a month later by sudan III/olive oil, 2 months - The regeneration of the parenchyma has been completed culminating in a nodule of hyperplasia. Mitotic figures are very rare. A striking contrast is presented between the small deeply-staining liver cells, which have participated recently in the regenerative process, and the larger resting cells around the periphery of the field. (X 70).

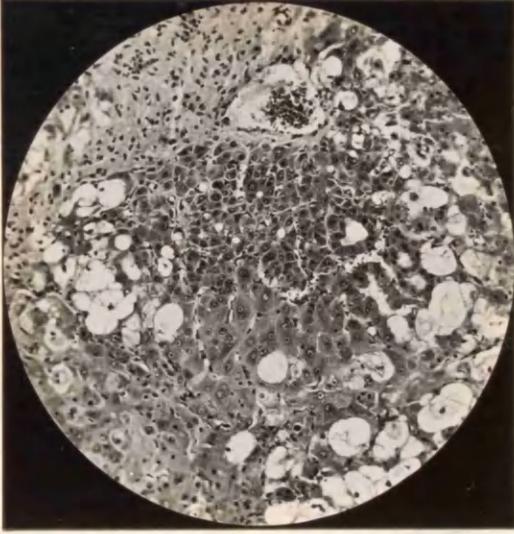


Fig. 67.

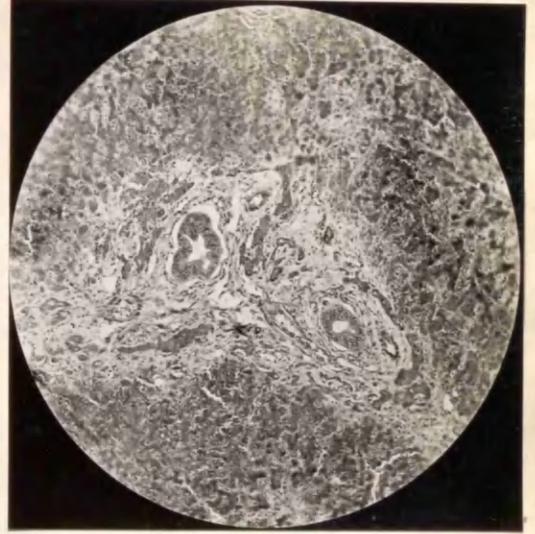


Fig. 68.

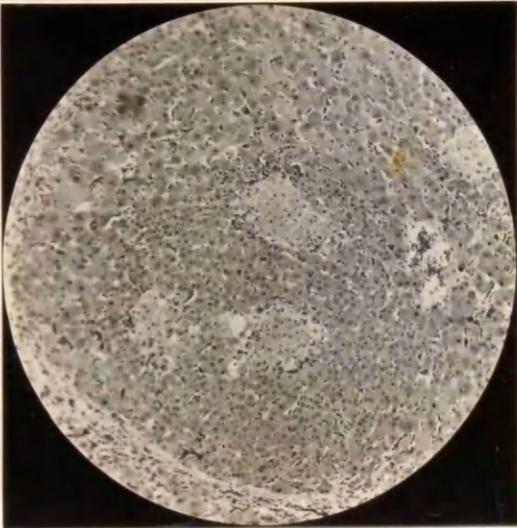


Fig. 69.

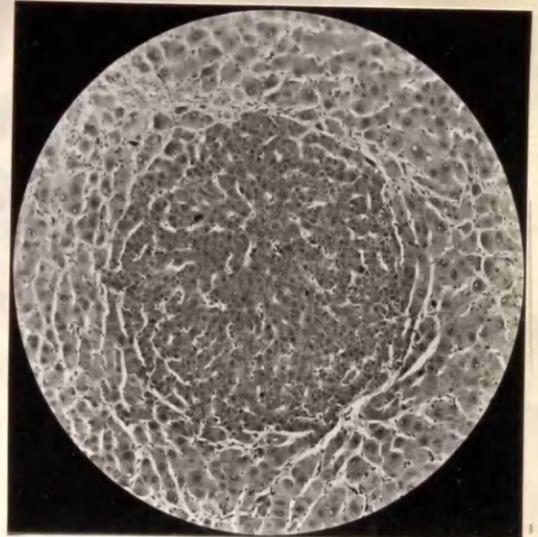


Fig. 70.

generally a few surviving cells are found in immediate relation to the portal tracts (fig.68 - Rabbit 41). Regeneration of the parenchyma proceeds from these surviving liver cells, often with great rapidity. Thus active proliferation, characterised by numerous mitotic figures, has been witnessed on the fifteenth day of the experiment (fig.67) and occasionally it has proceeded so far within twenty-one days as to merit the designation of multiple nodular hyperplasia (fig.69 - Rabbit 56). More commonly, however, this condition does not become clearly established until six or eight weeks have elapsed (fig.70 - Rabbit 2/1927). As the necrotic foci undergo absorption the surface of the organ becomes more and more irregular and nodulated (fig.71 - Rabbit A.7/1928). This animal was killed after the experiment had been in progress for eight months. Microscopically, the parenchyma is intersected by broad strands of vascular connective tissue, and there is abundant proliferation of bile duct epithelium, which may assume a very aberrant and cellular character (fig.72 - Rabbit A.7); alternatively, it may simulate an adenomatous formation. During active regeneration the proliferating liver cells are small in size, possessing hyperchromatic nuclei and slightly basophilic cytoplasm, whereas the resting cells which have survived the injection are considerably enlarged and their cytoplasm stains faintly. In later stages, after a year or more, the cirrhosis and the nodulation of the organ are still present, but it is less easy to discriminate between the new-formed cells and the old as no discrepancy is apparent in their size and staining reactions, and the prolongation of the experiment to a period of sixteen months in twenty animals has not revealed a single example of

PLATE XXVII.

Fig. 72. Rabbit A.7/1928 - sudan III/olive oil,
233 days - In this field, which shows
one of the portal tracts, the wide-spread
proliferation of the bile duct epithelium
is illustrated. The epithelium is flattened
and tends to assume a spindle shape. (X 200).
Cf. fig. 71, page 97a.

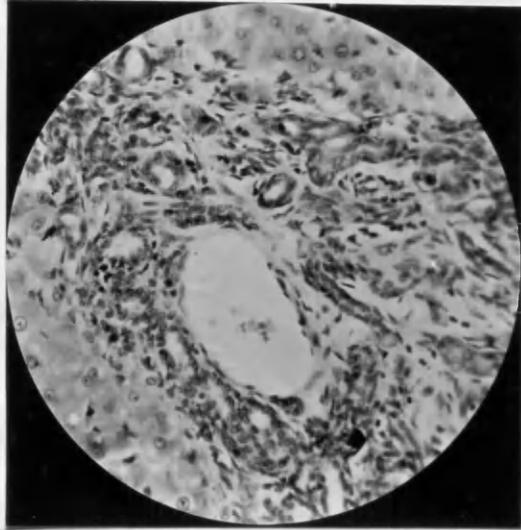


Fig. 72.

primary liver-cell carcinoma. There is no doubt that these lesions in the liver are determined by the sudan III. A large number of control experiments have been made with intrapleural injections of olive oil and lanolin, with and without bile salts. and the results have been uniformly negative. Also, in the course of subsequent experiments with electrolytes and so forth, a consecutive series of 500 rabbits has been carefully examined with reference to the natural incidence of cirrhosis of the liver. A slight degree of chronic hepatitis, characterised by a mild round-cell infiltration of the portal tracts, is not uncommon but not one example of multiple nodular hyperplasia or of multilobular cirrhosis has been encountered.

Certain sequelae of these experimental lesions might be mentioned. Thus ascites has been relatively common after three months in the presence of well-marked cirrhosis. Beyond six or nine months, however, an adequate collateral circulation seems to be established as the ascites tends to disappear. Exceptionally a considerable enlargement of the spleen has been observed but a minor degree of congestion and little or no enlargement are more characteristic. One rabbit bled so freely for several days from a large haemorrhoid, just above the anal sphincter, that it became moribund.

The wide range of individual susceptibility, exhibited by the rabbits to the dye, has precluded any straightforward conclusions with reference to the effects of varying the dose of the reagent. On the other hand, it has been established that a single massive dose of sudan III - e.g., 1gm dissolved or suspended in olive oil

or in lanolin - can produce in the same series of rabbits a great variety of lesions of the liver, conforming to the types of acute and subacute yellow atrophy as they occur in the human subject and proceeding to multilobular cirrhosis and multiple nodular hyperplasia. These observations coincide with those made by Davidson following the exhibition of coal tar, either by external application to the ear of the rabbit or by subcutaneous injection, and they tend to support his conclusion that the lesions are essentially of the same order and represent different degrees or stages of the same pathological process. A profound disturbance of fat metabolism seems to be induced under the present experimental conditions. It has already been pointed out that the retroperitoneal fat and the body fat in general may assume a rich scarlet hue. In other rabbits the injection is succeeded by a rapid mobilisation of the fat from its usual depots so that the latter are exhausted more or less completely and the animal becomes emaciated. It is not known how the staining of the distant abdominal fat is effected but that fat might well be the vehicle whereby the dye is transported to the liver cells.

Also it has been found that sudan III is very liable to disturb the course of pregnancy but it is uncertain whether it acts directly as an abortifacient or indirectly through the toxæmia arising from the lesions of the liver. In any case, a large proportion of pregnant animals die at an early stage of the experiment (4th to 8th day); the survivors either abort or the embryos are destroyed and retained as hæmorrhagic moles. In some of these moles the syncytium has survived and seems to have flourished in spite of the fate which has overtaken the

embryos. In the later stages of pregnancy, the death of the foetus and miscarriage appear to be the rule but again the dead foetus may be retained in the uterus. Thus three examples of "lithopœdion" have been observed in this experimental investigation.

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APPENDIX B.

THE INCIDENCE OF SPONTANEOUS TUMOURS, AND CERTAIN OTHER
LESIONS, IN A CONSECUTIVE SERIES OF 700 RABBITS.

These brief notes are appended for the information of other workers who are interested in the general pathology of the rabbit and to illustrate the chief characteristics of the experimental stock which has been employed in the preceding investigations. No definite data are available with reference to the age of any particular animal but, apart from those rabbits which were kept under observation for a year or more, perhaps one hundred in number, the average age might be estimated at nine to fifteen months. As a group, they were healthy and well-nourished, mostly weighing from 2 to 2.5 kilos. Coccidiosis has been relatively uncommon. Cysticercosis (? *Cysticercus tenuicollis*) has occurred very frequently in the peritoneal sac, and also in rare instances in the pleural sac, but even a comparatively heavy infestation does not appear to be associated with any notable constitutional disturbance; the lesions originating in the capsule of the liver are represented by calcified foci. Pseudo-tubercle has occurred in a small minority of the rabbits.

A minor degree of chronic hepatitis, characterised by slight round-cell infiltration of the portal tracts, has been frequently observed microscopically in the absence of any gross naked-eye changes. There has been no instance of multilobular cirrhosis or of multiple nodular hyperplasia outside that series of rabbits which received sudan III intrapleurally.

The kidney is not susceptible to injury by sudan III. Sporadic examples of cirrhosis of this organ, either focal or more diffuse, and unilateral or bilateral, have been encountered

PLATE XXVIII.

Fig. 73. Rabbit B.13/1929 - spontaneous tumour of uterus. The tumour presents the characters of an adenocarcinoma; it has infiltrated the whole thickness of the myometrium and impinges upon the peritoneal coat. (X 200). Miliary metastases were recognised in both lungs. Cf. fig.63, page 64a.

Fig. 74. Rabbit 117/1927 - spontaneous tumour of kidney. The tumour presents the histological characters of an epithelial growth of a fairly benign type, but a ~~small metastasis has been found~~ in the same kidney. It bears a striking resemblance to the "adeno-carcinoma of the renal blastema" (Ewing) as it occurs in the adult human subject. This rabbit was kept under observation for 16 months after the intrapleural injection of sudan III/lanolin but there is no reason to believe that the inoculum has been concerned in the production of the growth. (X 200).

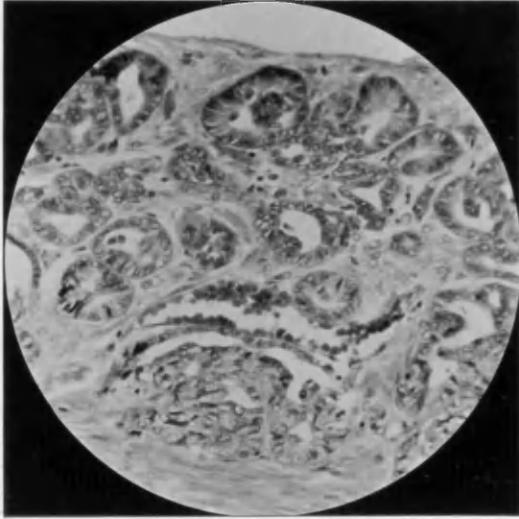


Fig. 73.

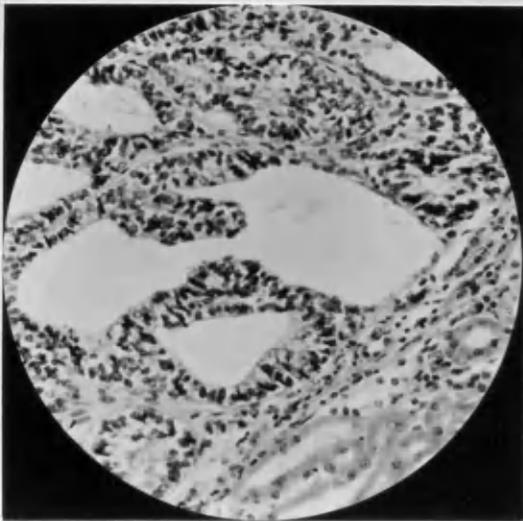


Fig. 74.

at long intervals. One beautiful specimen of congenital cystic kidney, affecting the cortices of both organs alike, was obtained; the liver was healthy in appearance.

With reference to the incidence of spontaneous tumours, eight growths have been recognised and recorded. They have been confined to two organs - viz., the uterus (5) and the kidney (3). The uterine tumours comprise one myosarcoma and four adenocarcinomata. The myosarcoma attained the size of a golf ball at its primary site and innumerable metastases were disseminated throughout the liver, the omentum, the spleen and the lungs; the myocardium was the seat of a secondary growth and another secondary tumour, which had undergone superficial ulceration, was found subcutaneously in the thigh of the left hind limb. Two of the adenocarcinomata were associated with metastases in the lungs and in the visceral pleurae (figs. 73 and 63 - Rabbit B.13/1929).

Two of the three tumours of the kidney present the histological characters of epithelial growths (fig.74 - Rabbit.117/1927) conforming to the type of the "embryonal adenocarcinoma of the renal blastema" (Ewing) as it occurs in the adult human subject. Unfortunately the third tumour was not examined histologically and it has been temporarily mislaid but its naked-eye characters were the same as in the other two cases.

Finally, attention might be directed to the frequent occurrence of emboli of bone marrow in the pulmonary arteries. This phenomenon is apparently due to the violent struggles of the animals in their death agony, following a blow on the back of the neck. The emboli are sometimes large enough to block

the larger vessels and they are interesting because they have been observed repeatedly in the absence of fracture of any of the long bones.

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