Observations

on

The Sero-Diagnosis of Pregnancy by the Abderhalden Reaction

and on

The Relationship of this Reaction to other Immunity Reactions.

by

John P. McVey, M.C.,M.B.,Ch.B.

PREFACE.

These observations on the serum reaction of Abderhalden detailed in the following report were made in 1913-14. They were interrupted on the outbreak of war in August 1914. Although incomplete in certain points, the material has been presented in this form as an opportunity for further extension of the work will probably not arise again, and also, since the observations of others, published in the interval, confirm the definite conclusion which was arrived at, that the reaction although probably occurring more frequently in pregnancy than in any other condition is by no means peculiar to pregnancy or a specific indication of its presence.

April 1920.

The Sero-Diagnosis of Pregnancy.

The Defensive Reactions of the Blood Serum.

The invasion of the tissues of the living organism by bacteria or certain other foreign substances is provocative of a series of defensive reactions, varying greatly in character, yet closely related to one another. As a rule the tissues are called upon to deal only with bacteria and their toxins, but they are also capable, by alteration of their metabolic processes, of reacting to foreign protein substances which enter the tissues by other than the normal channels. According to the views of Abderhalden the reacting mechanism is not limited to this, the cellular elements of the animal's own tissues may become dissociated and, in a new locus prove foreign to the parent structure. The stimulus of such antagonistic substances is responded to by the appearance in the blood stream of anti-bodies which resolve the foreign matter into simpler elements.

The processes involved may be restricted merely to the neutralisation of the invading bodies as in the case of toxins. But when solid protein substances invade the tissues directly, more than neutralisation is required. Bacteria are inhibited and destroyed, the

union/

union of anti-body and antigen in this case not being reversible.

The various immunity reactions are therefore evidence of the reactive powers of the tissues towards antagonistic substances. The agglutinins and precipitins inhibit or immobilise bacteria. The Opsonins render the invading organism susceptible to the bacteriolytic action of the phagocyte. Not only toxins and bacteria are thus dealt with by the tissues, but foreign substances such as the snake venins and the vegetable poisons, ricin and abrin, can be neutralised by anti-bodies liberated into the blood serum by the tissues.

With protein matter derived from tissues, the action of the anti-body varies considerably. The reactions observed after red-blood corpuscles have been injected into an animal(Haemolysis, complement fixation) cannot be so easily demonstrated when tissue proteins are employed as antigen. The latter substances have proved in some cases to be incapable of producing anti-bodies to any marked degree, and when employed in a purified condition, are stated by Wells and other observers to possess practically no antigenic power at all. The mechanism possessed by the animal organism in/

in respect of such constituents has therefore to be investigated from another point of view.

Other Defensive Systems.

When the poisons mentioned above, viz., toxins, venoms and vegetable poisons, are passed into the gastro-intestinal tract, and not injected directly into the animal tissues, the animal may occasionally become immunised to them.

But this is not the usual course for proteid substances in the gastro-intestinal tract. By the action of the various ferments they are reduced to a less complex state in which they pass from the gut to the blood stream. The tissues therefore are not called upon under ordinary circumstances to deal with the grosser protein substances as the intestinal ferments, the lymph and the blood systems intervene. Harmonious substances only are supplied to them for the nutrition and reconstruction of the organism, the products of digestion reaching the tissues by the blood.

The parenteral introduction of proteins creates new conditions for the tissues, which must then deal directly with the unchanged substances. The results of the investigations so far indicate that, under such circumstances, the tissues do not respond so energetically by/

by the liberation of anti-bodies into the blood stream as when bacteria and their toxins are used.

The Work of Abderhalden.

Proteolytic and other Ferments of the Serum.

Abderhalden was led to investigate the behaviour of the tissues towards antagonistic proteins from considerations of the processes of digestion occurring in the intestinal tract. He conceived it possible that the tissue cells were possessed of ferments capable of splitting up protein and other substances into simpler and more harmonious elements.

For the investigation of the proteolytic properties of the blood serum, Abderhalden employed the optical and the dialysation method. For the former the protein substrate was hydrolised with strong sulphuric acid, and a solution of peptone obtained. The proteolytic serum and the peptone solution were mixed together and placed in the tube of the polariscope. After a period of incubation - 37·C. for 16-36 hours - a progressive deviation of the angle of rotation could be observed. In the dialysation method, the protein substance and the serum were mixed directly without previous hydrolysis of the substrate. The mixture was placed in the dialysation tube, and the products of the reaction diffused into/

into distilled water. The reaction was accelerated by incubating the mixture as in the polariscopic method. The presence of protein derivatives could be demonstrated in the dialysate, when the fermentation process had been in progress several hours.

The serum of normal animals was tested in the first instance. In the earlier experiments with dogs and rabbits, egg-white and horse serum were used as antigen. It was found that the sera of these animals had no action upon the antigen when the two substances were brought into contact with one another. There was no evidence that the egg-white or the horse serum was reduced in any way by the normal serum. Guinea pig serum proved to be exceptional, however, as in the normal state it is possessed of proteolytic powers. When, however, the dog or rabbit was injected with egg-white, either subcutaneously or intravenously, the serum of the animal was found later to develop a ferment capable of reducing the egg-albumin. Experiments with peptones, gelatine, and casein yielded similar results. The blood of the animal injected in each case was found to contain ferment-like properties when the serum and the corresponding antigen were allowed to act upon one another.

The substrate was broken down and the decomposition products/

products appeared in the dialysate, when the dialysation method was used. When fats and carbohydrates were employed as substrates no reaction took place. The selectiveness of the proteolytic properties of the abnormal serum was shown to be even more delicate still. The serum of an animal which had been injected with amino acids was found to have no action upon proteins. It was capable only of decomposing amino acids. But with the serum of the animal which had been injected with proteins, not only proteins but their derivatives could be broken down.

The Relationship of the Reaction to Fermentation.

The parallel tests made with yeast juice showed that the reaction corresponded very closely with the fermentation reactions. The nitrogenous contents of the dialysates from the tests of normal and abnormal sera were examined. An increase in the amount of ammonia nitrogen was observed in the dialysate of the abnormal serum.

The reaction is inhibited when the abnormal serum is exposed for a time to a temperature of 60.C. No alteration of the substrate takes place when it is exposed to the heated abnormal serum.

Controls.

A system of controls was adopted for all the tests.

In/

In both the dialysation and optical methods, serum and substrate were employed separately as control. The heated or inactivated serum was also used. With these controls no fermentative reaction could occur between serum and substrate. Any diffusible bodies, for instance, appearing in the dialysate during the dialysation test from these controls could not be considered to arise from fermentation. They were looked upon as an indication that the serum or the substrate had not been properly prepared. In the case of the former, a reaction in the dialysate of the fresh serum, suggested that the serum was either too old and that autolytic prosesses had set in, or that the formed elements of the blood had not been properly removed. With the inactivated serum, if it had been properly heated, the diffusible bodies were the result of autolytic decomposition of the serum proteins, and not of the substrate. The presence of diffusible bodies in the dialysing fluid when the substrate was employed alone indicated that the substrate had not been properly prepared. The crystalloid protein derivatives had not been completely removed in the preparation by washing and boiling. As a further control, parallel tests were always made with the optical and dialysation methods.

Reaction to Carbohydrate Antigens.

The response of the animal organism to injections of cane-sugar is more difficult to study as the sugar does not remain so long in the blood as the proteins. But it was observed by polariscopic methods that the serum of an animal which had been treated with cane sugar, was capable of reducing a cane sugar substrate.

Injections of soluble starch and of milk produced ferments in the blood which not only reduced these bodies, but also reduced cane-sugar. For raffinose, however, no ferment could be induced in the animal's serum, and with iodised albuminous substances a similar result was observed. The powers of response of the tissues of the animal organism to the invasion of antagonistic substances is therefore limited.

The work of Abderhalden with the carbohydrate ferments of the blood serum was stated to be independent of that of Weinland although of a later date. Weinland was also successful in producing defensive ferments in the serum of animals by the parenteral injection of cane-sugar.

Reaction to Fatty Antigens.

The methods employed in the study of the protein ferments were not applicable to the fat-splitting ferments of the serum. The alterations in the surface tension occurring/

occurring during the decomposition of fats afforded a means of investigating the activities of lipase in the blood. Where fats form a large proportion of an animal's food they appear freely in the blood soon after the meal. The fat ferment can then be shown to be present in the serum. During fasting it is very active, and after injections of foreign fat into the animal it is more active still.

As the absorption of fat at the site of injection is very slow this method was not very successful and the old method of gorging an animal to produce lipaemia was adopted in the subsequent investigations of the fat ferments of the serum. The actual increase of the fat-splitting powers of the serum was demonstrated, the reaction having the same specific characters as those of the protein or carbohydrate ferments. The serum from the fat-engorged animal had no action upon proteins or carbohydrates and reduced only the fatty antigens in feeding the animal.

The Application of the Reactions to the Diagnosis of Pregnancy and Disease.

As all these reactions present well marked specific characters, it was thought by Abderhalden that they might be employed as a diagnostic means in the detection of disease./

disease. In many pathological conditions, tissue elements and debris are set free in the blood and become antagonistic to the cells in other parts of the body. The cells respond by the discharge of defensive ferments into the blood stream and the antagonistic bodies are reduced to a condition permitting of their absorption by the tissues or of their discharge by the normal channels of execretion.

For such diseases as cancer, syphilis and phthisis, and the diseases affecting the glandular organs, the method appeared to be eminently suitable. In pregnancy the problem presented another aspect. The work of Schmorl and Veit showed that in pregnancy the chorionic villi were shed from the placenta and appeared free in the blood. As bodies antagonistic to the tissues of the mother their presence in the blood stream would stimulate the production of defensive ferments by the maternal cells. If Abderhalden were correct in this deduction, the serum from a pregnant patient should act on a placental substrate, and the diffusible bodies demonstrated in a dialysate could be considered as evidence of a state of pregnancy in the mother. In pregnancy the reaction could always be confirmed by the result of the clinical condition. Such an opportunity was not afforded so readily in diseased conditions, but the value of the method could be clearly estab/ lished by/

by the results of the reaction in pregnancy. The success of the considerable work of Abderhalden and his colleagues is advanced by him as evidence of the reliability of the reaction as a diagnostic means in pregnancy and disease.

Investigations of the Serum-Diagnostic Reaction of Abderhalden.

The investigations, detailed in the following report, were undertaken, to ascertain the value of the serum reaction of Abderhalden as a diagnostic means in pregnancy and in diseased conditions. An attempt was also made to determine the relationship of the reaction to the other immunity reactions. The dialysation method was employed. The substrates, sera, and dialysation tubes were all prepared and tested as described by Abderhalden. Any departure made from the methods practised by him had the object of effecting improvements in technique with a view to securing greater accuracy in results and greater facility in manipulation.

----------oOo----------

B

The Sero-Diagnosis of Pregnancy.

Preparation of Substrate.

The substrates used in the experiments were prepared from Placental tissue, Ox-blood corpuscles, and from the muscle and liver of the rabbit. Fresh tissue was invariably employed.

Placental Substrate:-

The placentas chosen were thick and firm in texture, and not unduly swollen nor oedomatous. The thin, small placentas, frequently met with, were usually very fibrotic. Some of them exhibited a gritty substance in their structure. Although they were easily washed free from blood, and the resulting substrate was apparently quite good they were not used, as it was considered that the content of fibrous tissue might be too high. It was found advantageous to select a fairly large specimen, as there was a considerable loss of material in the course of preparation. Placentas from syphilitic, eclamptic and haemorrhagic cases were rejected. Attention was also paid to the umbilical veins; large well developed veins facilitated the direct transfusion of the organ. The small tortuous veins burst very readily when the pressure was increased suddenly.

The placentas were obtained immediately on their expulsion/

expulsion from the uterus. No substrate was prepared from old or decomposing tissue. Many specimens, although obtained within a short time of parturition, showed signs of decomposition, and were discarded.

Transpusion of the Placenta:-

All blood-clot and superfluous membranes were removed. One of the umbilical vessels was then secured to the tap, and the placenta placed in a porcelain basin. As the wall of the vessel very readily gave way, on the slightest increase of pressure, the water was turned on gradually so as to slowly dislodge the clot from the vessels. In twenty to thirty minutes time the bulk of the blood was driven out of the organ which assumed a much lighter colour. The veins and the remaining membranes were then cut away and the placenta divided into small pieces.

Washing:-

The chopped placenta was put up in small sacks, made from linen or cotton, and firmly kneaded in copious quantities of normal saline solution, the water being changed from time to time as it became stained with the blood-debris. As the operation advanced, the tissue appeared whiter in colour, the water became less stained and greater force had to be exercised to express the blood. The washing was greatly hindered by the frequent bursting of the sack, and a good deal of material was lost thereby.

Linen/

Linen or cotton, as mentioned above, proved the most suitable fabric for this purpose. Later the placenta was more finely minced, and the washing continued in a greatly reduced volume of water. The whole process therefore required several hours before the water remained clear after a thorough kneading of the tissue.

Boiling:-

The substrate, retained in the sack, was boiled in an ample quantity of water, for about 30 minutes, and then transferred to cold water again, and the kneading resumed. With this the discoloration of the water reappeared. The double process of boiling and washing had to be continued for several hours before the water was obtained absolutely clear. To carry the process still further, the sack was dispensed with, during the boiling. The substrate had consequently to be filtered after boiling to permit of further washing. In addition the quantity of water was reduced to a minimum. By this means, it was possible to guage very accurately the disappearance of the staining from the residual water.

Placental tissue, when boiled in a glass beaker, gives rise to considerable "bumping" and much of the tissue is lost by the frequent spurting of the liquid and boiling over. It has therefore to be carefully watched, and boiled/

boiled rather slowly. The use of an enamelled pot obviates these mishaps, and allows the operation to be carried out more rapidly.

To expedite the washing process, and to render it less laborious, a rotary churn was obtained, which was driven by electric power. Large quantities of substrate could thus be easily manipulated , and the volume of wash-water accurately graduated. An appreciable decrease in the loss of tissue during the washing was now observable. This method was not very effective, however, when the placenta was too finely divided as the resulting mixture slowed down the churn considerably, and strained the motor. The best results were obtained when the tissue was merely chopped into small fragments.

By this method, the total time required in the preparation of an extract, was reduced to a few hours. The original manual method, occupying as it did in some instances several days, was not cleanly. Also with such an unstable substance as placental tissue, the reduction in the duration and the amount of manipulation was all important, as decomposition changes supervened on several occasions, and the substrate in preparation had to be dis- carded.

An inspection of the extract was made before the final tests/

tests with the Ninhydrin were applied. The tissue was spread out on a clean cloth and carefully scrutinised for any fragments of blood-clot or thrombosed vessels which may have escaped reduction during the washing. At this stage, it was generally found that very little clot had survived the washing. The substrate was now of a light grey colour. In no case was a "snow-white" substrate obtained from placenta. The ideal substrate as detailed by Abderhalden, was never realised. Eight specimens of placental substrate were prepared, of which two were mixed specimens.

The Ninhydrin Test.

A sample of the substrate was boiled in a minimum quantity of water for 5 minutes. The mixture was then filtered, and to 5ccs of the filtrate, Icc. of a 1% solution of Ninhydrin was added. After boiling for a minute, the test was allowed to stand for half-an-hour. With the successfully prepared substrate, the water remained clear, but in most cases, a violet coloration occurred in the initial tests, and the washing had to be resumed. Some specimens of placenta were encountered which persistently gave a positive reaction with Ninhydrin no matter how thoroughly they were washed. In other cases, where a negative reaction was successfully obtained, the positive reaction reappeared, after the substrate had been allowed to stand/

stand for a time. It is this latter characteristic which is responsible for much of the time expended in the preparation of placental extracts.

Whenever it was necessary to interrupt the washing-process for a time, the extract was placed in a beaker, and covered with boiled water and a thick layer of toluol. A piece of gauze was placed over the mouth of the beaker which was then placed in the ice chest.

Storage of Substrates.

The storage of the substrate in bulk under toluol, was considered unsatisfactory, as there always existed the risk of contamination when small quantities were frequently removed during the course of the experiment. Such a disadvantage was obviated by weighing the extract in equal amounts - 10grms.- into test-tubes, and sealing them in the blow-pipe. After sterilisation in the "Koch" the tubes were stored in the ice-chest.

Specimens so preserved were tested 9 months after, and proved to be satisfactory. Occasionally a batch of tubes were met which gave a faint coloration with Ninhydrin. After thorough boiling however the reaction was dissipated and the negative condition restored.

Muscle substrate.

The limb muscles of the rabbit were used, the sheaths and tendons being dissected off, before the tissue was minced. Washing and boiling occupied a very short time, the blood being soon eliminated. A negative Ninhydrin reaction was therefore easily obtained. Muscle extract was perhaps the easiest and most satisfactory to prepare, and when preserved in capsules remained negative throughout to the Ninhydrin test for a period of six months.

Liver extract.

At first some difficulty was experienced in preparing this extract. The fresh rabbit liver, when finely minced, was reduced practically to a juice in the churn. When cut into large pieces, it could not be washed satisfactorily free from blood. An intermediate stage of division had therefore to be arrived at. As the material was naturally small in amount, the washing had to be carried out carefully. The production of an extract from liver, reacting negatively to Ninhydrin proved as difficult a task as that of placantal extract. When once prepared, however, it remained very reliable.

Albumen.

The albumen used in the testing of the dialysers, and as a substrate in certain experiments, was prepared from fresh egg-white. All solid substances, consisting usually/

usually of fragments of pellicle and flaky material were removed. The fluid was then diluted to the percentage required.

Ox-blood Corpuscle Substrate.

This substrate was made as required and was not preserved. The fresh ox-blood was mixed with normal saline and centrifuged repeatedly. The operation was always a prolonged one before the negative reaction to Ninhydrin was obtained.

Stromata.

The ox-corpuscles were treated as above until a satisfactory negative reaction was obtained. They were then treated in a serum oven at 55-57°C, for intervals of 16 hours, with the result that the positive reaction reappeared. Centrifugal washing and the oven treatment were carried out alternately and eventually a substrate was produced which responded satisfactorily to all tests. When stored for some time in capsules in the ice-chest, this substrate would give a faint violet coloration with Ninhydrin. It was always washed therefore before being used in any test.

Preparation of Sera.

As pregnant sera were controlled with normal sera in the tests, it was often a difficult matter to obtain and prepare them within the time prescribed by Abderhalden. Non-pregnant cases were seldom to be found in the Maternity Hospital. The controls had therefore to be obtained in other hospitals. UsUally the sera were ready for the tests within ten hours after the blood had been withdrawn from the veins. The clinical condition of the patient was always noted, whether pregnant or otherwise. Generally it was arranged that the blood was drawn off before a meal and rarely after. About 16ccs. were taken as a rule. A sterilised all-glass syringe was used, the needle being relatively large in the bore. For the transport of the blood, thick walled test-tubes with well fitting rubber stoppers were employed. Before use they were washed out with saline or citrate solution. The skin surface over the cubital veins was prepared either with Iodine or Carbolic lotion.

The serum was allowed to separate from the corpuscles at room temperature, and was then drawn off into sterile tubes and centrifuged - at least twice - until no further sediment of corpuscles was obtained. When haemoglobin staining occurred, the serum was either rejected or the fact noted.

The/

The preservation of the sera for long periods was effected by refrigeration, the sera being sealed in small glass phials.

Controls. Inactivated serum.

In addition to the other controls employed in the experiments, inactivated serum was used. At first some difficulty was experienced in preparing this control, due chiefly to an insufficient exposure in the water-bath, but later the serum could be treated very effectively. A tubeful of the centrifuged serum was placed in a water bath and the temperature raised to 60C. A temperature lower than this was found to be ineffective. At 56C-58C, a positive result could be depended upon to occur. As regards time an exposure less than 20 minutes was useless, and in practice a period of 30 minutes was allowed. In no case was the serum exposed to a temperature exceeding 60C.

The onset of autolytic processes varied in many sera. Blood obtained from eclamptic cases and from the foetal circulation, appeared to undergo spontaneous decomposition very readily. In some sera haemoglobin staining proved a troublesome factor. Doubtless the vibration, and the variations in temperature occurring in transport hastened decomposition in many instances. It can be safely said however that the sera employed in the investigations were

of/

of the standard laid down by Abderhalden as regards age, and freedom from decomposition and haemoglobin products, since they were prepared within ten hours after their withdrawal from the patient.

----------oOo----------

The Sero-Diagnosis of Pregnancy.

The Ninhydrin.

Preparation of the Solution.

For the detection of the diffusible products of the placental and protein degradation occurring in the dialysate, a one per cent solution of Ninhydrin or Triketo-hydrindene-hydrate was used. It is soluble in water when boiled, and does not readily decompose. Distilled water was always used in its preparation. In order to avoid trouble from contamination 10ccs. only were prepared at one time. A sterile flask of coloured glass was employed for storing the solution.

When peptone solution (5ccs; ·5 per cent) is tested with ·2cc of a one per cent solution of Ninhydrin, the mixture being boiled in a test-tube over the open flame, the reaction usually requires one minute, but should the tube of solution be placed in a water bath and boiled, the violet coloration takes 4 to 5 minutes to appear. Throughout the investigations the Ninhydrin test was carried out in a water-bath, so that a series of dialysates could be tested with Ninhydrin at the one time, under perfectly uniform conditions as to duration and degree of heating. Before placing the tubes in the bath the water was brought

to/

to boiling point, and the duration of the boiling was accurately controlled. The bath was fitted with a perforated lid to accomodate the tubes properly. The even distribution of the heat was secured by a couple of Bunsen burners. On the completion of the reaction in the bath, the tubes were allowed to stand in a rack for 30 minutes, before the results were noted. By this method of boiling, no solution was lost at any time though boiling-over, an accident which readily occurs when the direct flame is used, as recommended by Abderhalden. A further point is that, in my experience, it is impossible to heat any tube uniformly over an open flame.

To arrive at an idea of the limits of the reaction, a series of tests were made, where the peptone and the Ninhydrin solutions were varied in strength; the effect of dialysation on the time of the reaction was also studied.

TEST I.

At the outset, in order to compare the effect of the various methods of conducting the test on the reaction-time, solutions of Witte's peptone in distilled water of different strengths were tested with one per cent ninhydrin solution/

solution -

(a) after dialysation,

(b) without dialysation.

In (a) and (b) the water-bath was used.

(c) without dialysation, the direct flame being used.

In (b) and (c) 10ccs. distilled water were added to compensate for the extra amount of fluid employed in (a) as dialysing fluid. The time for the development of the maximum tint of the solution by the three methods is shown in Table I; in (a) the dialysation was carried out at 37°C. for 16 hours. Practically the result is therefore to be expressed as 10.4.1. The degree of permeability to peptone of the dialysers, the volume of the dialysing fluid, and the duration of dialysation, are all factors of importance in the case of (a). The dialysers were chosen from standardised specimens whose rate of permeability was known. (4. Standardisation of Dialysers). The time of dialysation is that adopted by Abderhalden in the test for the sero-diagnosis of pregnancy, viz: 16 hours.

TEST 2/

TEST 2.

The influence of dialysation was tested with solutions of peptone of varying concentration. The reactions were tested in the bath.

Table 2. the peptone varying from 3 per cent - ·5 per cent, dialysed 16 hours.

Table 3. the peptone varying from ·1 per cent - ·6 per cent not dialysed.

When the solution of peptone is placed in the dialyser, nothing below ·5 per cent peptone is likely to give sufficient in the dialysate for a positive reaction. Without the dialyser the effective limit is about ·3 per cent. A 1 per cent solution, without the dialyser gives an "XXX" reaction; with the dialyser the colour is reduced to "XX", i.e. after 16 hours dialysation.

TEST 3. Table 4.

The effect of variations in the concentration of Ninhydrin was tested with a ·5 per cent peptone solution. From ·05cc to 1cc of the 1 per cent ninhydrin solution was employed; the water was decreased from ·95cc. to 0cc. inversely with the ninhydrin in order to yield a constant volume. The amount of peptone solution used was 10ccs. The mixtures were not dialysed, but boiled direct in the bath.

With/

With •2cc. ninhydrin, the "XXX" (very marked) reaction appeared in 10 minutes, and with 1cc. it appeared in 4 minutes. With •4cc. the result was practically the same as with •2cc. but reached the maximum tint in 7 minutes. With •05cc. the result was very faint after 15 minutes boiling. In an ordinary test this result would be considered nil, and with the dialyser such a concentration would have no effect.

TEST 4.

Water from the tap, and also a specimen of distilled water were tested with ninhydrin with negative result. (see table 5).

The Colour of the Reaction.

An attempt was made to construct a colorimetric scale for the reaction, but owing to the variations of the dialysing tubes this was found to be scarcely practicable. In the tests enumerated above, (Tables 1 to 5), Witte's peptone was used. Later a silk peptone was employed for the standardisation of the dialysers. ("Seiden-peptone, Höchst). Solutions of the latter peptone gave a more distinctly violet coloration than the Witte's peptone, with which the reaction varied somewhat. Generally it produced a reddish-violet tinge; at other times the reaction was distinctly blue.

During the investigation of sera from pregnant cases with ninhydrin, the usual result obtained at full time approached closely to that given by 5ccs. of •5 per cent peptone solution, and •2cc. of 1 per cent ninhydrin. The latter reaction was therefore used as a standard and is denoted as "XX" or "M", equal to "Marked" in the tables. The faint reaction of weaker dilutions, eg.•3 per cent peptone, is denoted by "X". Frequently during the tests of pregnant cases, the coloration was much more pronounced. (VM. or XXX.).

Occasionally variations in the colour of the reaction were noticed, but they were never very pronounced. They were usually a faint red and occurred for the most part/

part in the fresh serum controls, usually where the serum was more than ten hours old. They are recorded in the text by small letters, "x.,xx.,xxx," which indicate the degree of the reaction.

TABLE I.

Solution of Witte's Peptone, in distilled water, 5ccs.
Solution of Ninhydrin, 1 per cent., 2cc.
Water, 10ccs. - in series (a) used as dialysing fluid.
in series (b) and (c) added direct to peptone solution to compensate that used in (a).
Incubation for 16 hours at 37·C (series (a)).

Peptone Percentage.	(a) Water-bath. Dialysed.	(b) Water-bath. Not Dialysed.	(c) Direct Flame. Not Dialysed.
3 per cent.	10 minutes.	4 minutes.	1 minute.
1·5 per cent.	do.	4·5 do.	do.
·5 per cent.	do.	6 do.	do.

In the three series the colour reaction on the completion of the test was as follows:-

With 3 per cent peptone, "XXX" or "Well Marked".
With 1·5 per cent peptone, "XXX" or "Well Marked".
With ·5 per cent peptone, "XX" or "Marked".

TABLE 2. Amount of Dialysate tested. = 5 ccs.

Solution of Witte's Peptone, I·5ccs. Ninhydrin 1 per cent ·2cc. - " ,
Water, as Dialysing fluid, 10 ccs.
Dialysed 16 hours at 37·C. Toluol Used.

Tube No.	Peptone percentage.	Progress of Ninhydrin in Bath. X.	XX.	XXX.	Total. Time of Reaction.
I.	3 per cent.	3 minutes.	5. minutes.	10. minutes.	10.
2.	3 " "	3.	5.	10.	10.
3.	1·5" "	3.	7.	14.	14.
4.	1.5" "	3.	7.	14.	14.
5.	1 " "	3.	7.	10.	10.
6.	·5 " "	6.	10.		10.
7.	·5 " "	6.	11.		11.

TABLE 3. Test without Dialysers.

Peptone, 5ccs., percentage varied. Not dialysed.
Ninhydrin., 2cc., 1 per cent solution.
Boiled 10 minutes in bath.

Peptone.					
.1 percent.	·2 per cent.	·3 percent.	·4 per cent.	·5 per cent.	·6 per cent.
-	Very faint.	X.	XX.	XX.	XXX.

TABLE 4.

Ninhydrin, - 1 per cent solution.
Peptone, (Witte's) •5 per cent.
Solutions not dialysed.
Peptone percentage, constant, (Ninhydrin percentage Varying.
Total ~~Volume~~ constant.
(Water amount varying.

Peptone.	10ccs.	10ccs.	10ccs.	10ccs.	10ccs.	10ccs.
Water.	•95cc.	•9cc.	•8cc.	•6cc.	•3cc.	o•cc.
Ninhydrin.	•05cc.	•1cc.	•2cc.	•4cc.	•7cc.	1.cc.

Exposure in Water Bath.
Time and Development of Reaction.

Ninhydrin percentage.	X.	XX.	XXX.	Total Time.
.05 per cent.	15 minutes.	-	-	15 minutes.
•1 do.	2 do.	3 minutes.	15 minutes.	15 do.
•2 do.	3 do.	4 do.	10 do.	10 do.
•4 do.	- -	4 do.	7 do.	7 do.
•7 do.	- -	- -	4 do.	4 do.
1 do.	- -	- -	4 do.	4 do.

TABLE 5. Tested against water, (a) distilled water, (b) tap water.

Ninhydrin, varying from, ·05cc - ·5. @ 1 per cent solution.

Boiled in water-bath from 4.30 pm. to 4.45 pm.

Control - 5 per cent peptone, 5ccs.

H20.	5ccs.	5ccs.	5ccs.	5ccs.	5ccs.	5ccs.	Peptone 5ccs.
Distilled H20.	do.	do.	do.	do.	do.	do.	do.
Nin.	·05cc.	·1cc.	·2cc.	·3cc.	·4cc.	·5cc.	·5cc.
Result.	-	-	-	-	-	-	XXX.

D.

The Sero-Diagnosis of Pregnancy.

The Dialysing Tubes.

The tubes used in the first instance, the No.579A of Messrs. Schleicher and Schull of Düren, measured 50 X 16mm. They were not standardised when obtained from the manufacturer, and proved generally to be very hard, and by no means permeable to peptone. A large number had to be tested before a suitable series could be arranged. In the later experiments, the standardised tubes made by Rudolf Schöps, of Halle, were employed. They were certainly much softer and more permeable to peptone, but varied as much in their permeability for peptone, as those originally obtained from Schleicher and Schull. They were therefore standardised in the laboratory before use.

Standardisation of the Dialysers.

The tubes were standardised as regards:

(a) Impermeability to albumen:

(b) Permeability to peptone:

(c) Rate of diffusion of peptone.

A careful preliminary examination of all the tubes was made for minute perforations.

One/

One tube out of 48 tubes tested was rejected on this account.

Impermeability to Albumen.

Four sets of tubes, 48 in all, were tested, the first three with serum, the fourth with egg-white albumen.

Standardisation ~~contd.~~

The sera for tests A, B & C were obtained from normal females. The blood was approximately 10 hours old in each case. The serum was allowed to separate in the ice-chest, and was centrifuged twice in a sterile tube. B and C were haemoglobin free; A was haemoglobin stained.

The egg-white albumen was freshly prepared, and was diluted down to 20 per cent with water.

Procedure.

Instead of Erlenmeyer flasks as employed by Abderhalden, glass jars with well fitting stoppers were substituted. The watch-glass cover used with the Erlenmeyer was thus dispensed with and the risk of infection of the contents of the flask reduced. The jar and stopper were sterilised by boiling. The jars were numbered serially, and a mark was made corresponding to 20cc content in order to facilitate filling with the dialysing fluid.

The/

The dialysing tubes were sterilised for each test by boiling in water for one minute. When stored they were immersed completely in boiled water in a jar and a thick layer of toluol was superimposed.

For the albumin test the sterile dialysing tube was charged with 1cc. serum, or 1.5cc. albumin solution and then thoroughly washed externally with a stream of distilled water to remove any trace of serum which might have accidently contaminated the outside in the process of filling.

The wall of the tube was then compressed with a forceps about 1cm. from its orifice and the inner edges of the mouth were rinsed carefully with water. The tube was then lowered into the (stoppered) jar, containing 16ccs. of distilled sterile water. As an antiseptic seal, a thick layer of toluol was run on to the surface of the water, and also into the tube. About 2cms. of the tube projected above the surface of the water. The manipulation of the dialysers was facilitated by the use of forceps, the point of which was bent obtusely on the limbs to an angle of about 35°.

Incubation.

The series of twelve tubes, when thus prepared, were incubated @ 37°C, for two consecutive periods of 16 hours each./

each. At the end of each period, 5ccs. of the dialysate were drawn off with a pipette, and tested for the presence or absence of albumen by the biuret test.

To the biuret test all the units in sets "A" and "C" were negative.

In set "B" tube "9" gave a positive reaction to the biuret in both tests.

In set "D" tubes 8 and 11 were twice positive.

The tubes giving the positive reactions were rejected and replaced by others which were tested and found to be impermeable to albumen.

For the biuret test, 5ccs. of the dialysate were mixed with 2·5ccs. of caustic soda solution (33 per cent). To this 1cc. of copper sulphate solution (1·500) was added. Of the forty eight tubes tested the dialysates of three of them were observed to give the violet coloration of the reaction. They were rejected therefore, being permeable to albumen.

Permeability to Peptone.

For the permeability test, a ·5 per cent solution of Witte's peptone was used in series A and B, and for series C and D silk-peptone was employed. The latter series had already been standardised by the manufacturer before being issued.

Procedure.

The/

Procedure, contd.

The jars were sterilised by boiling, after which they were filled with 10ccs. of distilled water, as dialysing fluid. The dialysing tubes were in the first instance washed freely under the tap, and then boiled for one minute in a beaker, each tube being boiled separately. The tubes were then charged with 5ccs. of peptone solution, and, after their external surfaces had been washed with distilled water, they were placed in the jars. The dialysing fluid and the contents of the tubes were covered with a layer of toluol. The incubation of the tubes at 37°C lasted 16 hours.

The Ninhydrin Test.

For the ninhydrin test, 5ccs. of the dialysate were mixed with •2cc. of the 1 per cent ninhydrin in a test-tube and the mixture boiled in the water-bath. As soon as the colour of the solution attained a degree equal to that of the control (described later) ie. equal to XX, the test-tube was removed from the bath. The time and the progress of the reaction were noted. The test-tubes were boiled in pairs, so that they could be observed more easily. The control employed was made as follows - 5ccs. of the •5 per cent peptone solution were added to 10ccs. distilled water and thoroughly shaken. To 5ccs. of this

mixture/

mixture •2cc. of the ninhydrin solution were added. The solution was boiled in the water-bath for 10 minutes, when it assumed the violet colour already described. Before the final reading of the tests was made the tubes were allowed to stand in a rack for 30 minutes.

Analysis of the Tests.

Series A.

These tubes were not standardised and in the ninhydrin test occupied an average of 9•25 minutes per tube, before the reaction was completed.

3 tubes required 9 minutes each.

2 - - 10 - -

2 - - 11 - -

Average for 7 tubes - ~~9~~ 9·6 minutes each.

2 tubes required 6 minutes.

2 - - 8 -

Average for 4 tubes - 7 minutes.

A standard reaction-time of 10 minutes was aimed at, and dialysers which produced a dialysate requiring boiling for more than 11 minutes or less than 9 minutes were accordingly rejected. In series A tubes No. 3,4,7, 9,10, had to be replaced by others which approached the standard more closely.

Series B/

Series B. Not Standardised.

This test proved the most unsatisfactory; practically only three of the tubes were sufficiently permeable to peptone.

(a) Tube No.1 was almost impermeable to peptone.

(b) The remaining eleven tubes averaged 11.9 minutes. Of these Tubes No.8,10,12, averaging 11 minutes, alone were unsatisfactory. The others were not used.

Series C.

These were standardised tubes and were much softer than those in series A and B.

(a) Average for 12 tubes 9.6 minutes.

(b) 6 tubes averaged 10 minutes

or (c) 9 tubes averaged 9.8 minutes.

All these tubes were used except Nos.8,10 & 12, which were either too soft or too hard.

Series D.

These were also standardised and proved the most satisfactory of all the tubes tested.

(a) Average for 12 tubes 9.6 minutes.

All these tubes were used.

A retest of these tubes was made after they had been employed for 10 tests. For sterilisation purposes, they had not been boiled, at the utmost, for more than a total of/

of 15 minutes, during all the tests.

The average for the 12 tubes was raised thereby to 10•9 minutes. Four tubes (Nos.4,5,8,11) had increased their average to 12•75 minutes and had to be replaced.

----------oOo----------

The Sero-Diagnosis of Pregnancy.

To test the diagnostic value of the serum-reaction in pregnancy, a series of investigations were made in which 109 sera in all were examined. Of these, 76 sera were obtained from pregnant cases, before or after parturition. As controls, 13 sera from healthy normal individuals and a similar number from gynaecological cases were employed. The remaining seven sera were from the foetal circulation. All the sera were prepared as described in the previous section relating to sera. The blood was drawn under aseptic conditions and the corpuscles removed by coagulation and subsequent centrifuging. The occurrence of haemoglobin staining in several of the sera is noted in the appended tables. The bulk of the sera were less than 10 hours old when tested. In some instances, however, it was not found possible to prepare the sera for the tests in such a short time as often great difficulty was experienced in obtaining satisfactory sera from non-pregnant cases as controls. With a few of the sera more than twenty-four hours elapsed before they could be used. This however was of rare occurrence. Reference to the exceptional cases is made in the text.

Pregnant/

Pregnant Cases.

All the sera from cases at the ninth month of gestation (nineteen in number) were procured within twenty-four hours before the delivery of the patient occurred. Those from cases in the later months of pregnancy (twelve) were obtained from hospital in-patients awaiting operative or other treatment. Out-door and dispensary patients, who were from two to five months pregnant furnished the other sera (twenty-two). Care was taken to select only those cases which were free from complications such as nephritis and clinically obvious syphilis.

Eclampsia. (Six cases).

The blood was collected when the patient was undergoing venesection, prior to saline-transfusion. As it was found that the blood from these patients lysed spontaneously much more rapidly than that from cases of normal pregnancy, the serum was separated in as short a time as possible and often the test was proceeded with, in the absence of control-serum, from a non-pregnant patient.

Abortion. (Eight cases.)

Without exception, in all the cases the uterus had not/

not been explored or the products of conception evacuated before the blood was drawn off. Two cases had suffered severely from haemorrhage before their admission to hospital. Generally the pregnancy had lasted from seventy to eighty-four days before the interruption occurred.

Puerperium. (Nine cases).

The blood was obtained from:-

2 cases on the fourth day after delivery;

6 cases on the seventh day;

1 case on the tenth day.

Foetal Circulation. (Seven cases).

For this series the blood was collected from the placental segment of the cord immediately upon the severance of the umbilical vessels after the foetus had been expelled from the mother. All the sera were from full time pregnancies.

The bulk of the above mentioned cases were patients of the Maternity and Samaritan Hospitals, Glasgow.

Gynaecological Cases. (Thirteen).

Pregnancy was excluded in all these cases by careful clinical examination.

The blood from the eight cases of new-growth was drawn off several days before the patient was operated on.

The/

The clinical diagnosis was confirmed by the patholigical examination of the specimens after the operation. The four endometritic cases were examined in the wards prior to operative treatment or in the dispensary in the case of out-door patients. A bacteriological examination of the gonorrhoeal case was not made, the diagnosis being merely clinical.

Sera from normal females (eight) were obtained from dispensary patients who were free from septic processes or from tumours. Usually they attended for the adjustment of a pessary for a uterine displacement.

Four of the sera from adult males were procured from medical officers or attendants of the above mentioned institutions. One serum was drawn from a patient suffering from locomotor-ataxia.

Procedure.

Preliminary:-

The laboratory in which the investigations were conducted was reserved for the work alone and no bacteriological or other work was performed in it. An incubator was also set apart for the tests. Contamination therefore from bacteriological and other sources was to a great extent excluded, as Abderhalden stipulates should be the case. All apparatus employed, such as jars, test-tubes, pipettes, forceps, was sterilised in boiling water, while the dialysing tubes were boiled for one minute in distilled water immediately before use. Only proved tubes were used (cf. Standardisation of Dialysing tubes). They were restandardised after every ten tests for impermeability to albumen and permeability to peptone, and those showing much alteration in their permeability were rejected. As a dialysing fluid, sterile distilled water was used in all the tests.

The serum from pregnant cases was tested in conjunction with that from non-pregnant cases; generally three of the former were grouped with one of the latter. Occasionally a non-pregnant case was not available, and the other sera were then tested alone.

Method/

Method.

A routine method for the preparation of the substrate, serum and dialysers, and for the performance of the experiment, was adopted in order to reduce to a minimum the manipulation of the specimens, and the time required in their preparation.

(a) All the apparatus having been sterilised, the jars were filled with 20ccs. distilled water as dialysing fluid, and then numbered.

(b) The placental substrate was removed from the capsules and dried between a couple of sheets of filter paper. To 10 grms. of the substrate 20.ccs. of distilled water was added and the mixture boiled for 10 minutes in a beaker. After filtering it was dried and divided into •5grm. quantities. The filtrate was preserved and tested with ninhydrin for the presence of diffusible protein products. To 5ccs. of the filtrate •5cc. of the 1 per cent ninhydrin solution was added and the mixture boiled for 10 minutes in the water-bath. Where the violet coloration indicative of positive reaction occurred, the placental tissue was boiled again until a negative reaction was obtained. A positive reaction to ninhydrin was often observed at this stage of the procedure when the water from the substrate was tested. But it was rarely observed after

the/

the first boiling, and was at all times got rid of before the substrate was used in the tests. On a few occasions this proved impossible and the specimen was rejected.

In the earlier experiments •5grm. of substrate was used in the tubes, but later the quantity was increased to 1grm. Larger amounts were frequently used (3 to 5 grm.), where placental tissue was used as a control, without serum. The substrate was introduced into the dialysing tubes by means of a pair of forceps and was never touched directly with the hand.

(c) The serum prepared as already described, was divided into two equal portions, one half being heated in the water-bath at 60•C for 30 minutes in order to inactivate the ferments. The serum was pipetted into the dialysers with sterile pipettes. 1•5ccs. being placed in each tube.

(d) The exterior of every tube and also the inner edges of its orifice were carefully washed under a stream of distilled water after the tube had received the serum or placenta or both. This precaution was necessary to prevent contamination of the dialysing fluid from serum or placental tissue which might have soiled the exterior of the tube during the filling process. The tubes when filled were immersed in the dialysing fluid in the jars. Toluol was then poured over the surface of the water and into the tubes.

(e)/

(e) When a series had been completed as above described, the tubes were placed in the incubator and kept at 37•C for 16 hours.

Order of Test.

The tubes were arranged according to the scheme detailed below.

Unknown Serum for Diagnosis.

Tube No.(I) (Placental Tissue:- •5grm.
(Serum (fresh) 1•5ccs.

(2) (Placental Tissue:- •5grm.
(Serum, inactivated:- 1•5ccs.

(3) (Serum (fresh) 1•5ccs.

(4) (Serum, inactivated:- 1•5ccs.

(5) (Placental Tissue:- •5grm.

(Control)
Known Serum from Non-pregnant Case.

(6) (Placental Tissue:- •5grm.
(Serum (fresh):- 1•5ccs.

(7) (Placental Tissue:- •5grm.
(Serum, inactivated:- 1•5ccs.

(8) (Serum (fresh):- 1•5ccs.

(9) (Serum,inactivated:- 1•5ccs.

As autolytic processes were liable to occur in the blood within a very short time after it was withdrawn from the patient, usually within twenty hours, but seldom less than ten hours - the serum had to be separated from the corpuscules in as short a time as possible, and introduced into/

into the dialysers without delay. It was not possible to differentiate by the ninhydrin reaction the diffusible products resulting from the autolytic decomposition of the serum albumins from those derived from placental tissue by the interaction of the serum and placenta. To avoid this complication fresh serum was always used. Seldom more than ten hours elapsed after it was withdrawn from the patient before it was placed in the dialysers.

Seven (Six) sera were examined to determine the time of onset of spontaneous decomposition. The details of this investigation are given later.

Controls.

As the test depends upon the demonstration of ninhydrin reacting bodies in the dialysate, after the interaction of serum from the pregnant individual and of placental tissue, three types of control are possible;

(a) A serum from a non-pregnant individual which, being presumably devoid of the proteolytic ferments, will have no digestive action upon placental tissue. No diffusible protein products should occur in the dialysate when such a serum is brought into contact with placental substrate and incubated.

(b) The proteolytic ferments in the serum of a pregnant person are rendered inactive by exposure to a temperature of/

of 60°C. Such a serum can have no action upon placental tissue and (if incubated with it in the dialysing tube) the dialysate of the control ought not to contain ninhydrin reacting bodies.

(c) The serum, fresh or inactivated, and the placental substrate when placed in the dialyser separately ought not to produce ninhydrin reacting bodies in the dialysate. Should a positive reaction occur, it is due (1) in the case of the serum to diffusible protein products resulting from the autolytic decomposition of the serum proteins, (2) in the case of the substrate to decomposed protein, eg. blood, the substrate not having been properly prepared.

The Ninhydrin Test of the Dialysate.

On the completion of the incubation, 10ccs. of the dialysate were removed with a sterile pipette from each jar, care being taken to leave the toluol. The contents of the pipettes were placed in sterile test-tubes which had been carefully selected, so that they were all uniform as regards size, bore and the thickness of the glass. To each tube .2cc. of a one per cent solution of ninhydrin was added. The tubes were then placed in the water and boiled for ten minutes after which they were removed and allowed to stand in a rack for thirty minutes. During this period it was usually noticed that where the reaction occurred/

occurred the violet tint of the solution became more pronounced. No note of the result of the test was made until the half hour had elapsed. In cases where the result was doubtful a further 10ccs. of the dialysate were drawn off and boiled with ninhydrin.

Explanations of Symbols Occurring in the Following Tables.

Dialyser Contents.

S:- Serum.

S':- Serum, inactivated.

P:- Placenta.

SP:- Serum and Placenta.

S'P:- Serum, inactivated, and Placenta.

Ninhydrin Test of Dialysate.

X:- Faintly Marked. Reaction.

XX:- Marked Reaction.

XXX:- Very Marked Reaction.

0:- No Reaction.

Anomalous Reactions:- the colour reaction in such cases departed from the usual violet coloration and was either reddish or pinkish blue.

x:- Faintly Marked.

xx:- Marked.

XXX:- Well Marked.

Notes as to haemoglobin staining and the age of the sera are to be found in the column headed "Remarks".

F.

The Sero-Diagnosis of Pregnancy.

Synopsis of Cases Examined.

TABLE 6.

Source of Serum.	Total Cases Examined.	Result of Tests.	
		Negative.	Positive.
Pregnancy.	53.	14.	39.
Eclampsia.	6.	2.	4.
Foetal-Circulation.	7.	2.	5.
Puerperium.	9.	4.	5.
Abortion.	8.	2.	6.
Normal Females.	8.	5.	3.
Endometritis.	4.	3.	1.
Ovarian Cyst.	1.	1.	0.
Uterine Fibroid.	3.	2.	1.
Carcinoma Uteri.	4.	3.	1.
Gonorrhoeal Vaginitis.	1.	1.	0.
Males.	5.	3.	2.

TABLE 7.

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
1.	X ara. (Nine Months)	XX.	0.	0.	0.	0.	11 hours old.
2.	1 ara. (Nine Months)	XX.	X.	0.	X.	0.	do.
3.	1 ara. (Nine Months)	XX.	0.	0.	0.	0.	do.
4.	Male.	0.	0.	0.	0.	0.	do.
5.	X ara. (Nine Months)	XXX.	X.	0.	X.	0.	9 hours old.
6.	X ara. (Nine Months)	XX.	0.	0.	0.	0.	do.
7.	X ara. (Eight Months)	XX.	0.	0.	0.	0.	do.
8.	Endometritis.	0.	0.	0.	0.	0.	13 hours old.
9.	Abortion. (Three Months)	XX.	X.	X.	X.	X.	Placental substrate negative to ninhydrin before test was made.
10.	X ara. (Six Months)	X.	0.	0.	0.	X.	
11.	1 ara. (Five Months)	X.	0.	X.	0.	X.	
12.	Normal Female.	0.	0.	0.	0.	X.	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
13.	1 ara. (Nine Months)	XX.	0.	0.	0.	0.	Twelve hours old.
14.	X ara. (Three Months)	0.	0.	0.	0.	0.	do.
15.	Male.	XX.	0.	0.	0.	0.	do.
16.	1 ara. (Eight Months)	XX.	-	-	-	-	19 hours old.
17.	Abortion.	X.	-	-	-	X.	Haemoglobin Stained.
18.	Eclampsia.	XX.	X.	-	X.	X.	Placental substrate negative when tested with ninhydrin.
19.	Gonorrhoeal Vaginitis.	0.	-	-	-	X.	
20.	X ara. (Nine Months)	XX.	-	-	-	X.	
21.	Puerperium.	XX.	-	-	-	-	Fourth Day.
22.	Abortion.	XX.	X.	-	-	-	
23.	1 ara.	XX.	0.	0.	0.	0.	
24.	Male.	X.	0.	0.	0.	0.	

TABLE 7. (Contd).

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
25.	X ara. (Six Months)	X.	X.	0.	0.	0.	
26.	Normal Female	0.	0.	0.	0.	0.	Haemoglobin Stained.
27.	X ara. (Eight Months)	XX.	X.	0.	X.	0.	
28.	X ara. (Nine Months)	0.	0.	0.	0.	0.	
29.	Endometritis.	XX.	X.	0.	0.	0.	Sixteen hours old.
30.	Ovarian Cyst.	0.	0.	0.	0.	0.	Sixteen hours old.
31.	X ara. (Four Months)	0.	0.	0.	0.	0.	do.
32.	X ara. (Two Months)	0.	0.	0.	0.	0.	do.
33.	Abortion.	X.	0.	0.	0.	0.	Haemoglobin Stained.
34.	Normal Female.	X.	X.	0.	X.	0.	
35.	Endometriris.	0.	0.	0.	0.	0.	
36.	X ara. (Six Months)	XXX.	X.	X.	X.	0.	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
37.	1 ara. (Eight Months)	XX.	x.	0.	0.	0.	
38.	X ara. (Nine Months)	0.	X.	-	-	-	Haemoglobin Stained.
39.	1 ara. (Three Months)	X.	-	-	-	-	
40.	Abortion.	0.	-	-	-	-	
41.	X ara. (Nine Months)	XXX.	-	-	-	-	17 hours old.
42.	X ara. (Three Months)	0.	-	-	-	-	17 hours old.
43.	Abortion. (Three Months)	0.	-	-	-	-	17 hours old.
44.	Male (Loco-motor Ataxia)	0.	-	-	-	-	9 hours old.
45.	X ara. (Five Months)	X.	-	x.	-	-	12 hours old.
46.	Eclampsia.	0.	-	-	-	-	12 hours old.
47.	Uterine Fibroma.	0.	0.	0.	0.	0.	do.
48.	Normal Female	XX.	-	X.	-	-	do.

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'. P.	S.	S'.	P.	Remarks.
49.	1 ara. (Nine Months)	0.	-	-	-	-	
50.	Eclampsia.	X.	-	x.	-	-	
51.	Normal Female.	0.	0.	0.	0.	0.	
52.	Endometritis.	0.	-	-	-	-	
53.	1 ara. (Three Months)	X.	X.	X.	X.	-	
54.	Puerperium.	0.	-	-	-	-	Tenth Day.
55.	Eclampsia.	XX.	-	-	-	-	Haemoglobin Stained.
56.	1 ara. (Four Months)	X.	-	-	-	-	
57.	Eclampsia.	0.	-	-	-	X.	
58.	1 ara. (Three Months)	0.	X.	-	-	X.	
59.	Abortion.	X.	0.	0.	0.	0.	
60.	Abortion.	X.	0.	0.	0.	0.	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
61.	Puerperium.	0.	0.	0.	0.	0.	Seventh Day.
62.	Foetal Circulation.	XX.	0.	0.	0.	0.	
63.	Normal Female.	X.	0.	0.	0.	0.	Haemoglobin Stained.
64.	X ara. (Nine Months)	XXX.	x.	0.	0.	0.	
65.	X ara. (Eight Months)	0.	0.	0.	0.	0.	
66.	Uterine Fibroma.	X.	X.	X.	X.	-	25 hours old.
67.	Male.	0.	0.	0.	0.	0.	9 hours old.
68.	Eclampsia.	XX.	-	-	-	-	do.
69.	1 ara. (Three Months)	X.	-	-	-	-	Haemoglobin Stained.
70.	1 ara. (Nine Months)	0.	-	-	-	-	Haemoglobin Stained.

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
71.	X ara. (Five Months)	X.	-	-	-	-	
72.	Puerperium.	X.	-	-	-	-	Fourth Day.
73.	X ara. (Five Months)	0.	-	-	-	-	
74.	Normal Female.	0.	0.	0.	0.	0.	
75.	X ara. (Nine Months)	XX.	-	x.	-	-	
76.	Puerperium.	XX.	-	-	-	-	Seventh Day.
77.	1 ara. (Five Months)	0.	-	-	-	-	
78.	Foetal Circulation.	0.	-	x.	-	-	
79.	1 ara. (Nine Months)	X.	-	-	-	-	
80.	X ara. (Nine Months)	XXX.	-	-	-	-	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
81.	Foetal Circulation.	X.	-	-	-	-	Haemoglobin Stained.
82.	Foetal Circulation.	XX.	-	-	-	-	Haemoglobin Stained.
83.	Puerperium.	0.	0.	0.	0.	0.	Seventh Day.
84.	X ara. (Eight Months)	X.					
85.	Carcinoma Uteri.	0.	0.	0.	0.	0.	
86.	Puerperium.	XX.	-	-	-	-	Seventh Day.
87.	X ara. (Eight Months)	0.	0.	0.	0.	0.	Haemoglobin Stained.
88.	ara. (Three Months)	X.	X.	X.	X.	0.	Haemoglobin Stained.
89.	X ara. Three Months)	X.	-	-	-	-	
90.	Foetal Circulation.	0.	0.	0.	0.	0.	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
91.	1 ara. (Three Months)	X.	-	-	-	-	11 hours old.
92.	1 ara. (Five Months)	XX.	-	-	-	-	11 hours old.
93.	Foetal Circulation.	X.	-	X.	-	-	25 hours old.
94.	X ara. (Four Months)	X.	-	-	-	-	11 hours old.
95.	Puerperium.	XX.	-	-	-	X.	Seventh Day.
96.	Normal Female.	0.	-	-	-	X.	
97.	ara. (Five Months)	X.	-	-	-	X.	
98.	X ara. (Three Months)	XX.		X.	-	-	
99.	Uterine Fibroma.	0.		X.	-	-	
100.	Carcinoma Uteri.	0.	0.	0.	0.	0.	

TABLE 7. (Contd.)

Case No.	Source of Serum.	S.P.	S'.P.	S.	S'.	P.	Remarks.
101.	X ara. (Nine Months)	X.	- 0.	0.	0.	0.	
102.	X ara. (Six Months)	0.	0.	0.	0.	0.	
103.	Carcinoma Uteri.	XX.	0.	0.	0.	0.	
104.	Puerperium.	0.	0.	0.	0.	0.	Seventh Day.
105.	1 ara. (Nine Months)	XX.	0.	0.	0.	0.	
106.	Foetal Circulation.	XX.	0.	x.	0.	0.	Haemoglobin Stained.
107.	X ara. (Three Months)	XX.	X.	X.	X.	0.	
108.	Carcinoma Uteri.	0.	0.	0.	0.	0.	Haemoglobin Stained.
109.	X ara. (Six Months)	XX.	0.	0.	0.	0.	

The Sero-Diagnosis of Pregnancy.

Analysis of Results.

TABLE 8.

Pregnancy. Total fifty-three cases.

Fourteen sera reacted negatively ie. when the fresh serum and placental extract were allowed to interact no diffusible bodies which reacted with ninhydrin appeared in the dialysate. In the later months of pregnancy the reaction was more marked than in the earlier months. Twenty-four sera from cases from the sixth to the ninth month gave a positive reaction. In eighteen of these the reaction was "marked" or "well marked". Of the twenty-two cases between the second and fifth months, seven gave a negative reaction, twelve reacted faintly and in three only was the reaction "marked". The case from the second month of pregnancy was not secured at a later date for further examination. No cases were obtained at a period of gestation earlier than the second month pregnancy.

The dialysates of the serum-controls of six of the cases reacted positively with ninhydrin, although in one of these cases the serum produced no diffusible bodies in the dialysate when it was exposed to placental extract.

TABLE 8. (Contd).

Analysis of Results of Pregnant Cases Examined.

Number of Cases Examined - 53.

Duration of Pregnancy.	Grade of Reaction.				
	0.	X.	XX.	XXX.	Total.
Nine Months.	4.	3.	8.	4.	19.
Eight Months.	2.	1.	4.	0.	7.
Six Months.	1.	2.	1.	1.	5.
Five Months.	2.	4.	1.	0.	7.
Four Months.	1.	2.	0.	0.	3.
Three Months.	3.	6.	2.	0.	11.
Two Months.	1.	0.	0.	0.	1.

Eclampsia: total six cases.

The dialysates of three of the sera reacted markedly with ninhydrin and one faintly. Of the serum-controls, two produced a reaction. In one (18) the controls of inactivated serum reacted and in the other (50) the reaction was produced by the fresh serum control.

Abortion: total eight cases.

Six cases were positive but two only were "marked. In one case the serum controls produced a reaction with ninhydrin when all three controls as well as the placental control reacted.

Blood from Foetal Circulation: Seven cases.

Two cases were negative. Three sera were haemoglobin stained. The fresh serum control reacted in No.(106). In case No.93, where it also reacted the serum was twenty-five hours old.

Cases in Puerperium: Nine Cases.

Five cases gave a positive reaction. In none of the controls was a dialysate observed to react with ninhydrin.

The serum from the case at the tenth day of the puerperium, and three of those from the seventh day were negative in reaction.

Gynaecological/

Gynaecological Cases.

Endometritis: Four cases.

One case (29) was positive. The serum was sixteen hours old and in the "Inactivated serum and placenta" control the dialysate reacted positively with ninhydrin.

New Growths: Six Cases.

Two of the sera reacted positively. In two of the cases the serum controls reacted, viz: Nos. 66 and 99. In the former case the serum was twenty-five hours old, all the serum controls yielding diffusible bodies in the dialysates.

Normal Females: Eight Cases.

Five cases gave a negative reaction. Of the three positive cases, the "inactivated" serum-control in one (34) and the "fresh" serum-control in another (48) produced a dialysate which reacted with ninhydrin. The latter serum was twelve hours old.

Adult Males: Five Cases.

Two out of five cases were positive. No.15 had been tested on two occasions previously with similar result before it was included in the series. As it presented a marked degree of lipaemia, another serum

was/

was selected (No.44) which also showed some lipaemia. The latter serum however produced no diffusible bodies when exposed to placental substrate. None of the serum-controls in these cases reacted with ninhydrin.

Serum Controls.

The dialysates of the serum-controls in twenty-eight cases reacted with ninhydrin as follows:-

in all three controls -	6
in the "inactivated serum" controls -	12
in the fresh serum-controls -	10

A reaction with ninhydrin occurred in the dialysate of the placental-extract control on four occasions involving thirteen sera.

Age of Sera.

Two sera were twenty-five hours old when tested and gave a positive reaction in the serum-controls. Three sera seventeen hours and one nineteen hours old produced no reaction in the dialysates of the serum-controls.

Reactions occurred in the controls of sera which were nine, eleven, twelve, and sixteen hours old respectively.

Haemoglobin /

Haemoglobin Staining.

Fourteen sera were examined which were stained with haemoglobin. In nine cases the reaction was positive, and in three cases the serum controls reacted positively.

H.

The Sero-Diagnosis of Pregnancy.

Factors Regarding the Serum, Substrate and Dialysation influencing the Abderhalden Serum Reaction.

Age of the Serum and Temperature of Inactivation.

The age of the serum, or the period at which it is examined after it is withdrawn from the patient, and the temperature employed to render it inactive, influence to a great extent the reaction of the dialysates of the serum-controls towards ninhydrin.

The influence of the factors discussed above was investigated by a series of tests which were made at varying intervals after the blood was withdrawn from the patient. "Inactivation" was studied by varying the temperature of the water-bath from 50-60 degrees Centigrade, and the period of exposure from 15 to 60 minutes.

The behaviour of the sera already investigated suggested that these factors varied somewhat with sera from different sources. The sera from normal cases were apparently more stable or less liable to autolytic decomposition than those from pregnant and other patients.

In/

In eclampsia and in the case of blood from the foetal circulation the serum proved to be less stable than that from patients with uncomplicated pregnancy.

Abderhalden stipulates that the serum for the inactivated serum controls should be heated at 60.C, but he does not indicate how long it should be exposed to that temperature.

Freezing of the Sera.

The effect of freezing temperatures on the sera was investigated in order that sera might be properly stored for further examination. Eight sera were stored and examined at intervals of three, five and seven months. In addition to the above investigations, a quantitative examination of the relationship of serum and a placental extract was made, and also of the progress of the reaction during prolonged periods of dialysation

Age of the Serum. TABLE 9.

Seven serum were kept at room temperature for varying intervals of time, after which they were examined by the dialysation method. The blood was withdrawn from the patient under aseptic conditions, and stored in sterile test-tubes which were sealed with cotton wool. Three sera were obtained from pregnant patients, two from cases/

cases of eclampsia, one from the foetal circulation, and one from a normal female.

The sera from cases 1-5 were separated immediately from the clot by centrifuging after they were withdrawn from the patient. The blood from cases 6-7 was divided into two parts, one of which was centrifuged and the serum separated; with the other, the serum was allowed to remain in contact with the clot for a time viz: 10 to 25 hours. The sera were tested with placental extract at intervals of ten, fifteen and twenty-five hours, by the dialysation method. Controls of fresh and inactivated serum were employed.

In all the sera, little effect on the ninhydrin reaction was noticeable, when the test was made within fifteen hours after the blood was withdrawn from the patient. The serum from the eclamptic case (No.5) produced a reaction in the dialysates of the serum controls after it had stood fifteen hours before being tested. When however an interval of twenty-five hours elapsed before the serum was examined, a reaction to ninhydrin occurred within the dialysates of the serum controls in practically all the cases.

In cases (6) and (7) where the portions (b) of the blood were not immediately centrifuged and the serum

was/

was allowed to remain in contact with the clot for ten and twenty-five hours respectively, a reaction occurred with all the dialysates after a period of ten hours. These portions of serum were haemoglobin stained.

TABLE 9. The Age of Serum.

Case No.	Source of Serum	Degree of Ninhydrin Reaction. S.P.	S'.P.	S.	S'.	P.	Remarks.
1.	X ara. 10 hours old.	XX.	0.	0.	0.	0.	
	15 do.	XX.	0.	0.	0.	0.	
	25 do.	XXX.	0.	XX.	0.	0.	
2.	X ara. 10 hours old.	X.	0.	0.	0.	0.	
	15 do.	X.	0.	0.	0.	0.	
	25 do.	XX.	XX.	XX.	XX.	0.	
3.	X ara. 10 hours old.	XX.	0.	X.	0.	0.	
	15 do.	XX.	0.	X.	0.	0.	
	25 do.	XX.	X.	XX.	X.	0.	
4.	Eclampsia. 10 hours old.	XX.	0.	0.	0.	0.	
	15 do.	XX.	X.	0.	0.	0.	
	25 do.	XX.	XX.	XX.	0.	0.	

TABLE 9. (Contd.)

ase 0.	Source of Serum.	Degree of Ninhydrin Reaction. S.P.	S'.P.	S.	S'.	P.	Remarks.
	Eclampsia.						
	10 hours old.	XX.	XX.	0.	0.	0.	
	15 do.	XX.	XX.	X.	X.	0.	
	25 do.	XX.	XX.	XX.	X.	0.	
	Foetal Circulation.						
	(a) 10 hours old.	XX.	0.	X.	0.	0.	(6) (For 10-25 hours respectively).
	25 do.	XX.	XX.	XX.	XX.	0.	
	(b) 10. do.	XX.	XX.	XX.	XX.	0.	(1) (Retained in contact with clot, Haemoglobin stained).
	25 do.	XX.	XX.	XX.	XX.	0.	
	Normal Female.						
	(a) 10 hours old.	0.	0.	0.	0.	0.	
	25 do.	0.	0.	X.	0.	0.	
	(b) 10 hours old.	XX.	0.	XX.	0.	0.	(b)Retained in contact with clots for 10 & 25 hours respectively. Haemoglobin stained.
	25 do.	XX.	XX.	XX.	XX.	0.	

Inactivated Sera.

Four sera from pregnant cases (sera) were examined.

(a), (b) and (c) were fresh sera less than ten hours old: (d) was twenty-five hours old before it was used. All were divided into four portions (a), (b) and (d), were each heated in the water-bath at 50°, 55°, 57°, 60°C for thirty minutes. With (c), the water-bath was maintained at a temperature of 60°C and the portions of serum were exposed at this temperature for varying intervals of time, viz: 15, 20, 30, 60 minutes. 1·5ccs of the heated serum were placed in the dialysing tube with 1grm. of placental extract. As a control 1·5ccs. serum were dialysed in a tube alone without the placental extract. Twenty ccs. of distilled water were used as dialysing fluid. Toluol was used as in the previous experiments. The sera were incubated for 16 hours at 37C. With the first three sera (a,b,d,) only those portions of serum heated at 60°C for sixty minutes were completely inhibited, so that no reaction occurred between the dialysates and ninhydrin. With those heated at 57°C, diffusible bodies reacting with ninhydrin occurred in the dialysates, but to a less extent than when the sera had been heated at 50°C. In (d) however, the reaction was present in all the dialysates heated from 50-60C. Serum C gave reactions in the dialysates of the

portions/

portions heated at 60°C for 15 and 20 minutes respectively. No reaction occurred when the serum was heated for 30 and 60 minutes.

TABLE 10. The Inactivation of Sera.

Sera (a), (b), (d) exposed in water-bath for thirty minutes; temperature varied.

Degree of Reaction.

Temperature of Inactivation.	(a)		(b)		(d)	
	S'P.	S'.	S'P.	S'.	S'P.	S'.
50°C.	XX.	0.	XX.	0.	XX.	X.
55°C.	X.	0.	XX.	0.	XX.	X.
57°C.	0.	0.	X.	0.	X.	X.
60°C.	0.	0.	0.	0.	0.	0.

Serum (c) exposed in water-bath at 60°C.

Time of exposure varied.

	Duration of Exposure.	Degree of Reaction.
1·5ccs. serum at 60°C.	15 minutes.	XX.
	20 do.	XX.
	30 do.	0.
	60 do.	0.

Refrigeration of Sera.

Eight sera were selected and stored in glass phials in the refrigerator for periods of three, six and seven months. Two of the sera (7 & 8) were seventeen and twenty-five hours old respectively, before they were placed in the refrigerator. The others were fresh sera, that is, they were less than ten hours old. Serum No.8 was haemoglobin stained. Before the sera were placed in the refrigerator, portions of each were tested by the dialysation method, and the reaction of the resulting dialysates towards ninhydrin recorded. When removed from the refrigerator they appeared to have undergone little change as regards colour and transparency. The sera were carefully thawed, and afterwards their action upon placental extract was examined by incubating in dialysing tubes. When the ninhydrin reaction of the dialysates was compared with that of the unrefrigerated portions previously tested, little change was noted except that the reaction in Nos.7 & 8 was more pronounced than in the first test. Some discrepancy was also noted in the reaction of the serum controls.

The sera selected were from pregnant cases. In all of them, except in the case of the abortion, the pregnancy was uncomplicated.

Pregnancy,	5.
Abortion,	1.
Puerperium,	1.
Foetal Circulation,	1.

TABLE 12. Refrigerated Sera.

Case No.	Source of Serum.	S.P.	S'P.	S.	S'.	P.	Remarks.
1.	1 ara. (a)	X.	0.	0.	0.	0.	Fresh Serum.
	(b)	X.	0.	X.	0.	0.	In refrigerator six months.
2.	X ara. (a)	XX.	0.	0.	0.	0.	Fresh Serum.
	(b)	XX.	0.	0.	0.	0.	In refrigerator six months.
3.	X ara. (a)	XX.	0.	0.	0.	0.	Fresh Serum.
	(b)	XX.	X.	0.	X.	0.	In refrigerator seven months.
4.	Foetal Circulation.						
	(a)	X.	0.	0.	0.	0.	Fresh. Refrigerator six
	(b)	X.	0.	0.	0.	0.	months.
5.	Abortion.						
	(a)	X.	X.	0.	0.	0.	Fresh. Refrigerated
	(b)	X.	0.	0.	0.	0.	three months.

TABLE 12 (contd.)

ıse No.	Source of Serum.		S.P.	S'.P.	S.	S'.	P.	Remarks.
).	Puerperium.	(b)	X.	0.	0.	0.	0.	Refrigerated 3 months
.	X ara.	(a)	XX.	0.	0.	0.	0.	Fresh. Refrigerated 3 months.
		(b)	XXX.	X.	XX.	X.	0.	
.	1 ara.	(a)	X.	0.	0.	X.	0.	Fresh. Refrigerated 7 months.
		(b)	XX.	X.	0.	0.	0.	

The Relationship of Serum and Substrate in the Reaction.

The relationship of serum and substrate in the reaction was studied by a series of quantitative tests, serum and placenta being varied in amount.

(1) Fresh serum and placental extract were placed in separate dialysing tubes, 2·5ccs. serum, and 2·5grms. placental extract being used. The tubes were immersed in dialysing fluid in the same jar. Toluol was added as before and incubation was carried out at 37.C for 20 hours. Three control tests were conducted simultaneously.

(a) Fresh Serum 2·5ccs.)Dialysed in separate
Placental Extract 2·5grms.) tubes in same jar.

(b) Fresh Serum 2·5ccs.)Dialysed in the same
Placental extract 2·5grms.) tube.

(c) Fresh Serum 2·5ccs.

(d) Placental extract 2·5grms.

At the completion of the period of incubation, 5ccs. of the dialysate from each of the tests were removed and tested with 2cc. of a one per cent solution of ninhydrin, the four tubes being retained in the water-bath for ten minutes. With tubes (a), (c) and (d) no coloration appeared. In (b) the dialysate reacted positively.

(II)/

(II). In this series three tests were made in which the placental substrate and serum from a pregnant case, were employed in varying amounts. Placental extract in each series was kept constant in amount; viz: ·1, ·5, 2·5 grms. respectively. The serum was varied in decreasing doses from 2·5ccs. to ·1cc. As control tests 2·5ccs. of fresh serum and 2·5grm placental extract were dialysed in separate tubes. Incubation lasted sixteen hours at 37°C. Five ccs. of the dialysate were tested with ·2cc of the one per cent solution of ninhydrin.

In series (1) with ·5grm. placental extract a reaction occurred with the serum varying in dose from 2·5ccs. to .5cc. In test (2), the reaction was limited to between 2·5ccs. and 1cc. serum. In test (3) a reaction occurred with 2.5ccs. serum alone. In none of the tests did a reaction take place with ·25cc. or ·1cc of serum. The minimum limit therefore of serum and placenta with which a reaction can occur is ·5cc serum and ·5grm. placental extract.

TABLE 11.

Relation of Serum and Substrate in the Reaction.

Test 2.

Series 1.

Substrate. (Placental)	•5grm.	•5grm.	•5grm.	•5grm.	•5grm.	•5grm.
Serum.	2•5ccs.	1•5ccs.	1cc.	•5cc.	•25cc.	•1cc.
Reaction of Dialysate. (Ninhydrin)	XX.	XX.	X.	X.	0.	0.

Series 2.

Substrate (Placental)	2•5grm.	2•5grm.	2•5grm.	2•5grm.	2•5grm.	2•5grm.
Serum.	2•5ccs.	1•5ccs.	1cc.	•5cc.	•25cc.	•1cc.
Reaction of Dialysate.	XX.	XX.	X.	0.	0.	0.

Series 3.

Substrate. (Placental)	•1grm.	•1grm.	•1grm.	•1grm.	•1grm.	•1grm.
Serum.	2•5ccs.	•5ccs.	1cc.	•5cc.	•25cc.	•1cc.
Reaction of Dialysate.	X.	0.	0.	0.	0.	0.

Incubation at 37°C for 16 hours in each case.

Controls.

In the three series the following controls were employed, and dialysed separately.

Serum (fresh) 2•5ccs.
Placental Substrate 2•5grm.

The reaction was negative in each case.

No controls of inactivated serum were used.

Influence of the Duration of Dialysation on the Reaction.

The following tests were made to determine the influence of the duration of dialysation on the production of ninhydrin reacting bodies in the dialysate, when the serum of a pregnant patient is allowed to react on placental substrate. In each case the serum used was from a full time pregnant patient.

TEST 1.

Three grms. of placental extract and three ccs. of serum were placed in a dialysing tube and dialysed in 20ccs. of distilled water for five days. At the end of each period of twenty-four hours, five ccs. of the dialysate were removed and tested with one per cent ninhydrin solution. The reaction of the first two tests was "well marked". On the third day no reaction was obtained, but a further five ccs. of the dialysate tested on this occasion yielded a reaction with ninhydrin. The volume of dialysing fluid was restored by the addition of twenty ccs. of distilled water and the incubation continued. On the two succeeding days the dialysate produced no coloration when boiled with ninhydrin. The remaining ten ccs. were also tested on the fifth day with like result. The same experiment was repeated on two occasions later, but no difference was noted in the behaviour of the dialysate towards ninhydrin; the results agreed with those given above.

Factors Regarding Serum &c.

Summary of Experiments on:-

(1) Age of the Serum.

Serum tested fifteen hours after the blood is withdrawn from the patient, gave little evidence of reaction in the dialysates of the serum-controls, provided the serum had been separated from the clot shortly after withdrawal of the blood. When the serum was twenty-five hours old when the test was begun practically all the dialysates of the controls reacted to ninhydrin. If left in contact with the clot, the serum produced a reaction in the controls when ten hours old.

(2) Inactivation.

The action of the serum in virtue of which it produced ninhydrin reacting dialysates when in contact with placental substrate, was inhibited by heating in the water-bath at not less than 60 degrees centigrade and for not less than thirty minutes.

(3) Refrigeration.

Freezing of the sera did not affect the reaction when the sera were placed in the refrigerator in a fresh condition. No autolytic decomposition of the serum therefore appeared to take place at that temperature.

(4) /

(4) No reaction occurred when less than •5grm. placental extract and •5cc. of pregnant serum were dialysed together. A reaction occurred when •1grm. substrate and 2•5ccs. serum were used.

(5) Duration of Reaction.

The interaction of serum and placental extract ceases to liberate ninhydrin reacting bodies into the dialysing fluid after about three days dialysation.

The Relationship of the Abderhalden Reaction to other Immunity Reactions.

The suggestion has frequently been made that the phenomena associated with the Abderhalden reaction were related to the other anti-body reactions. The following investigations were undertaken to examine the relationship and although incomplete, for reasons already stated, were advanced sufficiently far to demonstrate the fact that no constant relationship can be said to exist between the Abderhalden reaction and other immunity reactions.

Substrates.

The substrates employed were employed as already described (cf.Part B: Preparation of Substrates). The egg-albumen was employed fresh. All solid material and pellicle were removed and the mixture diluted to t twenty per cent with sterile distilled water.

The red corpuscles from ox-blood were fresh and were washed with salt solution in order to get rid of the serum. They were also employed in the heated condition/

N.B.- A summary of my observations on this question has been already published by Prof.C.H.Browning in his book on "Applied Bacteriology".

condition, being alternately washed and treated in the serum oven at 55°C - 57°C for periods of sixteen hours until they no longer reacted with ninhydrin.

The substrates prepared from the muscle and liver of the rabbit were prepared previously and were stored in capsules in the ice chest. The last mentioned substrates were dialysed with the sera of animals immunised with other antigens to test the specificity of the reaction. Five ccs. of the substrate were boiled with five ccs. of distilled water for thirty minutes, after which the mixture was filtered. To the filtrate ·2cc. of a one per cent ninhydrin solution was added. The solution was boiled in the water-bath for ten minutes. Where a violet coloration indicated the presence of reacting bodies, the substrate was washed again until the fluid reacted negatively. In no case was the substrate used until it reacted negatively with ninhydrin.

The albumen substrate was tested by adding the ninhydrin direct to five ccs. of the albumen without previous boiling of the latter. The mixture was then treated in the water-bath as described above. Rarely was a positive reaction observed.

Rabbits./

Rabbits.

Six rabbits were employed. Two of them were females and were not pregnant. All were healthy, and free from apparent disease. The blood was withdrawn under aseptic precautions, and the serum was usually separated within three to five hours later. No specimen of serum was haemoglobin stained. In each case the serum of the normal animal was tested with a substrate by the dialysation method before any injection was performed.

Dialysation.

Dialysation was carried out at 37°C for three periods of twenty-four hours each. At the end of each period five ccs. of the dialysate were removed and tested with ninhydrin.

Controls.

The controls employed were similar to those used previously for the reaction in pregnancy.

Inactivated Serum.

Generally the serum was inactivated at 60°C for thirty minutes, but in two cases (Serum from rabbit C and E) the serum was inactivated by heating at 57°C for one hour. Fresh serum and substratr were also dialysed separately as controls.

Reaction/

Reaction before and after Immunisation. (Antigen, Egg White Albumen 20%)

Rabbits A, B, C, and F, were injected intravenously with immunising doses of egg white albumen, each dose consisting of 5ccs of a twenty per cent solution in water.

Rabbits A and B received each 2 doses.

Rabbit C received 1 dose.

Rabbit F received 3 doses.

An interval of twenty-four hours elapsed between each dose. No attempt was made to have the rabbits in a fasting condition, before the blood was drawn off.

Rabbit A. Normal Serum. (Tables 12 and 13.)

The Serum was tested before the animal was injected. It produced no ninhydrin reacting bodies when 2•5ccs. serum were dialysed with 1cc of 20 per cent albumen for twenty-four hours. At the end of forty-eight hours dialysation, the dialysate gave a faint reddish coloration with ninhydrin which was more pronounced after dialysation had been continued for seventy-two hours. The test with 1•5ccs serum yielded no reaction until after seventy-two hours dialysation.

Immunised/

Immunised Serum.

Two days after the above test was made the animal received an intravenous injection of five ccs of egg white (20%). A second injection was given twenty-four hours later. The animal was bled on the following day. After twenty-four dialysation reactions occurred where 2•5ccs and 1•5ccs of the serum respectively were allowed to interact with albumen. The reactions of the two subsequent days were more pronounced but faint. When 1cc of serum was used with albumen no reaction was observed until the end of the third day.

With rabbit muscle, the reaction observed with the normal serum was very faint after seventy-two hours dialysation and with the serum obtained after injection the reaction, although it appeared on the second day was also faint.

Rabbit B. (Tables 12-15.) received two injections of egg-white (20% solution) of 5ccs each at twenty-four hours interval. The normal serum when dialysed with 5cc of egg-white solution produced a faint reaction when 2.5ccs of serum were used, the reaction appearing after forty-eight hours dialysation. With 1•5cc of the serum no reaction occurred at the end of the third day of dialysation.

The/

The serum from the immunised animal obtained twenty-four hours after the second injection and also seven to ten days later, reacted with the albumen substrate after two days incubation, but only when 2•5ccs were used. No reaction occurred with 1cc of serum.

When muscle substrate was used the serum of the normal animal showed only a faint reaction after the third day. After the injection no reaction occurred Rabbit C. (Tables 13-14) received one injection intravenously of 5ccs of 20 per cent egg-albumen. The normal serum produced a reaction with egg-white substrate with 2•5cc and 1cc serum respectively when dialysation had been in progress for forty-eight hours. The reaction was faintly marked.

One hour after the animal was injected 5ccs of the blood were removed and tested with egg-white but no reaction occurred even after three days incubation.

The serum obtained twenty-four hours after the animal had been injected was examined. When 2•5ccs of serum and 5ccs of albumen were incubated together the dialysate reacted positively to ninhydrin in twenty-four hours. Forty-eight hours later the reaction was marked. With rabbit muscle a reaction was not observed until after two days incubation. The control test in which the

muscle/

muscle substrate was dialysed with serum inactivated at 57°C produced a dialysate after three days invubation which reacted with ninhydrin.

Rabbit F. (Tables 14-15.) received three injections of 20 per cent egg-white of 5ccs each at intervals of twenty-four hours. The serum of the immunised animal was tested with egg- white as substrate on the second, fifth and tenth days after the third injection. A reaction occurred between serum and substrate when 2·5ccs of serum were used but no reaction occurred when 1cc was used. With the former quantity the reaction was observed after twenty-four hours dialysation. On the following day th dialysate gave a similar result. With rabbit liver substrate the serum had no effect until the second day of incubation, when a faint reaction occurred.

The serum of the normal animal produced no ninhydrin reacting bodies in the dialysate when 1cc of serum was incubated with albumen.

Reaction with Red blood Corpuscles as Antigen.

Two rabbits, D and E were injected with washed ox-blood corpuscles. D received one dose of corpuscles of 5ccs/

5ccs. E received three doses of 5ccs each at intervals of five days.

Normal Serums. (Tables 16, 17, 18)

The serum from the normal animals was tested with the washed corpuscles as substrate.

The amounts of serum used were 2·5ccs, 1·5ccs, ·25cc. One cc of the substrate was used dialysed with the serum. With serum D a reaction with ninhydrin was noticed after the second days dialysation, when 2·5ccs. + 1·5ccs. serum were used. No reaction took place with ·25cc. of serum. The serum of rabbit E was more active as the dialysate (2·5ccs.serum) tested at the end of the first day reacted faintly and on the following day somewhat more pronounced. With 1·5cc. the result was only fainly marked on the second day. A negative result was observed in the dialysate when ·25cc. of serum was incubated with red-corpuscles. The rabbit liver substrate and 1cc of the normal serum from E yielded a dialysate in twenty-four hours which reacted positively with ninhydrin.

Serum/

Serum from Immunised Animals.

The result of the injections in both animals was to intensify the reaction so that when the blood was withdrawn twenty-four hours after an injection, the sera and red blood corpuscles produced ninhydrin reacting bodies in the dialysates after twenty-four hours interaction. This result was observed with sera from both animals when 2·5cc and 1·5ccs. were employed. Where rabbit liver substrate was used the reaction was the same as with the normal serum in the caes of rabbit E. The test was repeated five and ten days later but no difference was observed.

Reaction after Haemolysis.

Throughout all the tests an effort has to be made to avoid the occurrences of haemoglobin staining in the sera, as Abderhalden has repeatedly stated that diffusible bodies reacting with ninhydrin appear in the dialysate when such sera are used. In the haemolytic reactions it might be expected that the reaction of the dialysates might correspond with these reactions. The investigations detailed in Tables 19 and 20 were made to ascertain if, during the lysis of sensitised ox-blood corpuscles by fresh guinea-pig's complement, the products/

products of the reaction were diffusible and appearing in the dialysate would react with ninhydrin.

TABLE 18.

The guinea-pig's serum was fresh and was used four hours after the blood was obtained from the animal. It was haemoglobin free. It was employed in increasing doses, viz: 25cc. ·6cc., 1·5ccs., with ·5cc. ox red corpuscles, and ·5cc of a powerful immune body from the rabbit. In addition to the controls usually made in haemolytic tests, controls of inactivated serum were used, the guinea pig's complement being heated at 60°C for one hour. After the complement had been in contact with the red corpuscles - with or without immune body - for one hour, at 37°C the contents of the test tubes were transferred to the dialysing tubes and dialysed in distilled water for sixteen hours. It is to be noted that in all the tubes containing both immune body and fresh guinea pig's serum along with the red cells complete haemolysis had occurred: in the other tubes there was no haemolysis. The dialysates were tested by boiling with ninhydrin, ·2cc of one per cent ninhydrin being added to 5ccs of the dialysate. A positive reaction occurred only where 1·5ccs. of complement were dialysed

with/

with •5cc. red blood corpuscles, irrespective of whether lysis occurred or not. No reaction was observed where the serum employed was inactivated.

TABLE 19.

The test was repeated using only 1•5cc. of complement. The dialysates reacted as in the previous test, the ninhydrin reaction bearing no relation to the lysis of the red blood corpuscles.

Summary of Observations.

Reactions with Albumen as Antigen.

Four rabbits were treated with egg-white albumen, injected intravenenously. The sera of the normal animal had little action upon albumen substrate until dialysation had been in progress for forty- eight hours. Quantities of serum amounting to 2•5ccs and 1•5ccs. had to be employed. After immunisation with albumen had occurred, the sera (2•5ccs + 1•5ccs.) reacted with albumen after twenty-four hours dialysation, except in the case of the serum of animal B. No reaction was observed with the serum of the latter until the serum had been in contact with the substrate for two days.

Reactions with Red Corpuscles as Antigen.

Two rabbits were injected with 5ccs. of washed ox-

blood/

ox-blood corpuscles. The normal serum of one was less active upon the ox-blood substrate than that of the other, which produced a ninhydrin reacting dialysate after twenty-four hours incubation. In both cases the reaction was rendered more pronounced by the immunisation of the animal with ox-blood, and was observed on testing the dialysate after one days dialysation.

The action on rabbit muscle substrate and on ox stromata by normal and immune sera was very slight. With rabbit liver substrate a more marked reaction occurred. The reaction in all cases with rabbit sera was faint when compared with that occurring in the examination of human sera in pregnancy and other conditions. Dialysation was therefore continued for longer periods, two th three days incubation being required in most cases before a dialysate was obtained which reacted with ninhydrin.

Reaction after Haemolysis.

The lysis of washed red-corpuscles from ox-blood under the influence of specific immune body of the rabbit along with fresh guinea pig's serum (complement) was not succeeded by the production of a ninhydrin reacting dialysate until twelve doses of guinea pig's complement were employed. The quantity of serum used rather than the/

the occurrence of lysis determining the result, since similarly reacting dialysates were obtained when the immune body was omitted.

The Relationship of the Abderhalden Reaction to other Immunity Reactions.

TABLE 12. Normal Rabbits: A and B.
Serum tested four hours after withdrawal, haemoglobin free.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.		
		24.	48.	72.
	Rabbit A.			
1.	Albumen 1cc: Serum 2·5cc.	0.	X.	X.
2.	do. 1cc: Serum 1·5ccs.	0.	0.	X.
	Rabbit B.			
3.	Albumen 1cc: Serum 2·5ccs.	x.	X.	X.
4.	do. 1cc: do. 1·5ccs.	0.	0.	0.
5.	Serum Fresh (A) 1cc.	0.	0.	0.
6.	Serum (A) (60°C) 1cc.	0.	0.	0.
7.	Serum Fresh (B) 1cc.	0.	0.	0.
8.	Serum (B) (60°C) 1cc.	0.	0.	0.
9.	Albumen 1cc.	0.	0.	0.
11.	Rabbit Muscle 1grm. Serum (A) 2·5cc.	0.	0.	X.
12.	Rabbit Muscle 1grm. Serum (B) 2·5cc.	0.	0.	X.

TABLE 13.

Rabbit A. injected twice with 5ccs. egg-albumen (20% solution)
Serum obtained 24 hours after last injection.
Serum haemoglobin free, employed when 4 hours old.
Serum inactivated at 60°C for 60 minutes.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.		
		24.	48.	72.
1.	Albumen 1cc. Serum 2·5cc.	X.	X.	X.
2.	Albumen 1cc. Serum 1·5cc.	X.	X.	X.
3.	Albumen 1cc. Serum 1cc.	0.	0.	X.
5.	Albumen 1cc. Normal Rabbit Serum (C)2·5ccs.	0.	X.	X.
6.	Albumen 1cc. Normal Rabbit Serum(C)1cc.	0.	X.	0.
4.	Albumen 1cc. Serum (60°C) 2·5cc.	0.	0.	0.
7.	Albumen 1cc. Normal Rabbit Serum(C)(60°C)	0.	0.	0.
8.	Albumen 1cc.	0.	0.	0.

TABLE 14.

Rabbit C injected with egg-albumen (5ccs. of 20 % Solution)
Serum two hours old; haemoglobin free. (Obtained 24 hours after injection).
Serum inactivated at 57°C for 60 minutes.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.		
		24.	48.	72.
1.	Albumen 1cc: Serum 2·5ccs.	X.	X.	XX.
2.	do. 1cc: do. 1cc.	0.	X.	X.
3.	do. 1cc: do. ·25cc.	0.	0.	0.
4.	do. 1cc: do. (57°)2·5	0.	0.	0.
5.	Serum (Fresh) 1cc.	0.	0.	0.
6.	do. 57°C 1cc.	0.	0.	0.
7.	Albumen 2ccs.	0.	0.	0.
8.	Alb.1cc:Normal Rabbit Serum 1cc. (F)	0.	0.	0.
9.	Rabbit Muscle 1grm. Serum 2·5cc. (C)	0.	X.	X.
10.	Rabbit Muscle 1grm. Serum 1cc. (C) (57°C)	0.	X.	X.

TABLE 15.

Rabbit B received two injections of Egg Albumen(5ccs. 20%)
Rabbit F received three injections Egg Albumen(5ccs. 20%).
Interval between injections, 24 hours.
B. Blood withdrawn on 1st, 7th, and 10th days after last injestion
F. Blood withdrawn 2nd, 3rd, 10th days after last injection.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.	
		24.	48.
	Rabbit B.		
1.	Albumen 1cc. Serum 2·5ccs.	0.	X.
2.	Albumen 1cc. Serum 1cc.	0.	0.
3.	Albumen 1cc. Serum 2.5ccs. (60°C)	0.	0.
4.	Serum (60°C) 1cc.	0.	0.
5.	Serum (Fresh) 1cc.	0.	0.
6.	Albumen 1cc.	0.	0.
	Rabbit F.		
7.	Albumen 1cc. Serum 2·5ccs.	X.	X.
8.	Albumen 1cc. Serum 1cc.	0.	0.
9.	Albumen 1cc. Serum (60°C) 1cc.	0.	0.
10.	Rabbit Liver 1grm.	0.	X.
11.	do. do. 1grm. Serum (60°C) 1cc.	0.	0.

TABLE I6.

Normal Rabbits D and E.
Serum two hours old: haemoglobin free.
Serum inactivated at 60°C for 60 minutes.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.	
		24.	48.
	Rabbit D.		
1.	Red Blood Corpuscles 1cc. Serum (Fresh) 2·5ccs.	0.	X.
2.	Red Blood Corpuscles 1cc. Serum (Fresh) 1·5ccs.	0.	X.
3.	Red Blood Corpuscles 1cc. Serum (Fresh) ·25cc.	0.	0.
4.	Red Blood Corpuscles 1cc. Serum (60°C) 1·5cc.	0.	0.
5.	Serum (Fresh) 1·5ccs.	0.	0.
6.	do. (60°C) 1·5ccs.	0.	0.
7.	Red Blood Corpuscles 1cc.	0.	0.
	Rabbit E.		
8.	Red Blood Corpuscles 1cc. Serum Fresh 2·5ccs.	X.	X.
9.	Red Blood Corpuscles 1cc. Serum Fresh 1·5cc.	0.	X.
10.	Red Blood Corpuscles 1cc. Serum Fresh ·25cc.	0.	0.
11.	Rabbit Liver 1grm Serum Fresh 1cc.	X.	X.
12.	Red Blood Corpuscles 1cc. Serum (60°C) 1cc.	0.	0.

TABLE 17.

Rabbit D. injected with Red Blood Corpuscles (Washed).
Serum Five Hours old Haemoglobin Free.
Serum inactivated at 60°C for 30 minutes.
Animal bled on 1st, 5th & 10th days after last injection.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.-	
		24.	48.
1.	Red Blood Corpuscles 1cc. Serum Fresh 2·5ccs.	X.	X.
2.	Red Blood Corpuscles 1cc. Serum Fresh 1·5ccs.	X.	X.
3.	Red Blood Corpuscles 1cc. Serum (60°C) 1·5cc.	0.	0.
4.	Red Blood Corpuscles 1cc.	0.	0.
5.	Serum (Fresh) 1cc.	0.	0.
6.	Serum (60°C) 1cc.	0.	0.
7.	Rabbit Liver 1grm. Serum (Fresh) 1·5cc.	0.	X.
8.	Rabbit Liver 1grm. Serum (60°C) 1·5ccs.	0.	0.
9.	Heated Corpuscles 1cc. Serum Fresh 1cc.	0.	0.
10.	Heated Corpuscles 1cc. Serum (60°C).	0.	0.

TABLE 18.

Rabbit E. injected twice with Red Blood Corpuscles.
Serum separated three hours after withdrawal.
Interval between injections, 5 days.
Serum inactivated at 57°C for 6 minutes.
Animal bled on 1st, 5th and 10th days after injection.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.	
		24.	48.
1.	Red Blood Corpuscles 1cc. Serum Fresh 2·5cc.	X.	X.
2.	Red Blood Corpuscles 1cc. Serum Fresh 1·5ccs.	X.	X.
3.	Red Blood Corpuscles 1cc. Serum (57°C) 1·5ccs.	0.	0.
4.	Red Blood Corpuscles 1cc.	0.	0.
5.	Serum Fresh 1cc.	0.	0.
6.	Serum (57°C) 1cc.	0.	0.
7.	Rabbit Liver 1grm. Serum Fresh 1grm.	X.	X.
8.	Rabbit Liver 1grm. Serum (57°C) 1cc.	0.	0.
9.	Heated Corpuscles 1cc. Serum Fresh 1cc.	0.	X.
10.	Heated Corpuscles 1cc. Serum (57°C) 1cc.	0.	0.

TABLE 19.

Guinea Pig's Serum, 4 hours old (Haemoglobin Free). Treated with washed ox R.C.s and I.B. ·5cc. for 1 hour at 37°C before being placed in dialysing tubes.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction after Dialysation. Hours.	
		24.	48.
1.	R.C's, ·5cc: IB. ·15cc: G.P.C. ·25cc.	C.	0.
2.	RC's, ·5cc: IB ·15cc: GPC ·6cc.	C.	0.
3.	RC ·5cc: IB ·15cc: GPC 1·5ccs.	C.	X.
4.	RC's ·5cc: IB·15cc: GPC 1·5ccs. (60°C)	0.	0.
5.	RC's,·5cc: GPC,1·5ccs.	0.	X.
6.	RC's ·5cc: GPC,1·5ccs(60°C)	0.	0.
7.	RC. ·5cc.	0.	0.
8.	RC's,·5cc: IB,·15cc (unlaked)	0.	0.
9.	Na Cl.·5cc: IB,·15cc: GPC,1·5cc.	0.	0.

C = Complete Haemolysis

TABLE 20.

Guinea Pig's Serum 4 hours old, Haemoglobin free.
Guinea Pig Serum treated with RC's,·5cc and IB,·15cc, for 1 hour before dialysation.

Tube No.	Contents of Dialysation Tubes.	Ninhydrin Reaction.
1.	RC's,·5cc: IB,·15cc: GPC,1·5ccs.	X.
2.	RC's,·5cc: IB,·I5cc:	0.
3.	RC's,·5cc: IB,·I5cc: GPC,1·5ccs(60°C)	0.
4.	RC's,·5cc: GPC,1·5ccs.(60°C)	0.
5.	RC's,·5cc: GPC,1·5ccs.	X.

THE NATURE OF THE ABERHALDEN REACTION.

The following represents the attempts which have been made by Aberhalden to explain the pregnancy reaction. The work of Schmorl and Veit directed attention to the conditions obtaining in pregnancy where tissue elements gained access to the blood stream at the placental site, from the chorionic villi. Although these elements do occasionally occur in the free condition in the blood and must then be regarded as foreign elements in the plasma, it must be remembered that this probably takes place only under exceptional circumstances and cannot be considered therefore as a general explanation of the presence of defensive ferments in the serum of the pregnant animal. Aberhalden himself stated that the serum of the mare was capable of reducing placental extract although in the mare the chorion was not related to the placental site as in other mammals; thus the villi cannot escape into the maternal circulation. From the placental site therefore products of protein decomposition must be supposed to enter the circulation of the mother and were considered as the cause of the phenomena associated with the reaction.

It is difficult to understand however, from the above reasoning why defensive ferments against placenta should necessarily arise in pregnancy when a whole organism is being developed with the assistance of the placenta which must supply the actively /

actively growing foetus with building stones provided by the maternal tissues rather than pour into the maternal circulation antigenic products of the disintegration of embryonic tissue.

The direction of the metabelic tide is towards the placenta, and the escape of the formed elements of the chorionic villi or of the products of protein decomposition into the maternal circulation might be expected to play little if any part in the maternal organism in the sense of stimulating it to produce it antisubstances. The whole question of the synthesis of the manifold specific tissues of the foetus is practically untouched either by Abderhalden or other workers and even Miescher's observations do not greatly illuminate the problem.

According to F. Miescher immediately prior to the period of sexual activity in the salmon a decomposition of the muscle proteins occurs Histones developed from the muscle albumens are observed in the testes where later they are transformed into protamines. Is the developement from the tissues of the muscles therefore made possible by a mechanism which converts antagonistic proteins into bodies which are not antagonistic to the blood serum. The observations of Miescher are adduced by Abderhalden as evidence of the power of the tissues to control the admission of foreign substances to the blood stream. But should defensive ferments exist/

THE NATURE OF THE ABDERHALDEN REACTION.

exist in the serum of the salmon, where no foreign materials are allowed to enter it during the maturation of the testes?

The work of the biologists is further confirmed by the work of Van Slyke, Vinograd, Villchur, and Losee. By investigating the Amino-Nitrogen after the interaction of placenta and serum, they found that the amount of Amino-Nitrogen resulting from the reaction could not be accounted for merely by the digestion of the substrate. Further the variations of normal and pregnant sera overlap so considerably that the reaction has little diagnostic value whatever.

The serum reaction of Abderhalden is demonstrated more frequently in pregnancy than in other conditions. It has also been demonstrated with the serum from normal individuals. Two factors have an important bearing on the reaction, viz:- the amount of serum and of substrate used, and the time at which the serum is examined after it is withdrawn from the host.

When serum and substrate are employed below a certain minimum quantity no reaction takes place. In enzyme action, quantity has little effect upon the reaction, as the smallest amount of enzyme acts as well as the largest amount, and the reaction does not cease until the substrate has been completely reduced or a position of equilibrium established.

The physical charcters of the substrate rather than the chemical /

H Transfer to Page 113.

THE NATURE OF THE ABDERHALDEN REACTION.

chemical or morphological characters appear to determine the occurrence of the Abderhalden reaction. Placental substrate, albumen and liver substrate produce a reaction more readily than muscle substrate. None of these substrates are reduced by the serum of an immunised animal if too small a dose of serum is employed. Charcoal, kaolin, and glass wool have been employed as substrates, and when serum is incubated with them, the dialysate reacts with ninhydrin as when organised substrates are used. Leitch and Plaut observed that these substances reacted with some sera and not with others.

When the serum is dialysed alone, diffusible bodies occur in the dialysate if the se rum is incubated long enough. The presence of the substrate appears only to hasten the reaction. Between pregnant serum and normal serum the difference is apparently only one of time, the substrate inhibiting for a time the reaction with the normal serum, probably by the simple absorption of something from the serum. The end result therefore is the occurrence of some change in the serum and not in the substrate during the course of dialysation and incubation. As a result of his work on the Abderhalden reaction De Waele arrived at a similar conclusion. The existence of defensive proteolytic ferments in the blood serum in pregnancy and in other conditions is still a debateable /

debateable point and so far the evidence points to the contrary. #

CONCLUSIONS.

(1). The reaction occurs more frequently in pregnancy than in other conditions. It is more marked in the later months of pregnancy than in the earlier months.

(2). Serum from normal individuals and from those suffering from pathological conditions may react with placental substrate.

(3). The reaction occurs readily in eclampsia and with the blood from the foetal circulation.

(40. The reaction is dependent on the amount of serum and of substrate used.

(5). It is inhibited by heating the serum at 60.C for at least thirty minutes.

(6). The serum of normal rabbits reacts with such substrates as egg-white, rabbit muscle and liver, and with the washed corpuscles from ox-blood.

(7). The immunisation of rabbits with these substrates increases the reaction but little.

(8). Heated /

Transfer from page 111

THE NATURE OF THE ABDERHALDEN REACTION.

CONCLUSIONS.

(8). Heated serum from normal and immunised rabbits fails to give the reaction.

(9). The lysis of sensitised oxblood corpuscles with guinea pig's complement does not release diffusible bodies from the corpuscles to any extent, and so does not produce a ninhydrin reacting dialysate after dialysation.

(10). The reaction is apparently dependent on the physical characters of the substrate rather than on the morphological characters, the serum undergoing change and not the substrate.

(11). The claim of a specific character for the reaction has not been established. The reaction has no diagnostic value in pregnancy or in disease.

The Nature of the Abderhalden Reaction.

References.

E. Abderhalden, Abwehrfermente des tierischen Organismus (1913-14).

De Waele, Zeitschrift f. Immunitatsforsch. Bd.xxi. 1914. P.83.

Leitch, Brit.Med.Journ.vol.ii.1914.pp161,330.

Plaut, Munch.med.Woch.1914, p238.