A critical study of the basis of Abderhalden's serum reaction / by W.E. Bullock.

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IMPERIAL CANCER RESEARCH FUND.

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THE great mass of literature dealing with the subject of this paper has been reviewed so recently by Abderhalden,[1] by Lange,[2] and others that it is unnecessary here to do more than to refer to special points and to emphasise the division of opinion as to the value of the reaction in the diagnosis of pregnancy and of disease. On the one hand, numerous writers[3] support Abderhalden's results and conclusions completely; one observer declared it to be possible to distinguish between a gastric and a duodenal ulcer.[4] On the other hand, a few writers[5] on the subject totally deny the existence of specific protective ferments. Between these extremes there are some who partially confirm Abderhalden's work, and others who corroborate the experimental results but offer another explanation of them.[6]

The chief object of the work to be described was to test the reliability of the reaction as applied to the diagnosis of cancer. But before carrying out the routine empirical tests devised by Abderhalden the experimental results on which the whole method is based were examined. The experimental results claimed are briefly: 1. By injecting animals with solutions of sucrose, maltose, or other sugars strange to the economy of the animal employed protective ferments are developed in the body which decompose the strange sugars and render them useful or at least innocuous to the animal. The evidence adduced that such ferments are produced is as follows. The blood serum of the normal animal is mixed with a solution of the sugar to be injected and the mixture examined with the polariscope; no alteration in the rotatory power of the solution takes place. When now the blood serum is obtained after the animal has received an

injection of the sugar and is mixed with the sugar solution as before, the rotatory power of the solution gradually changes, indicating a splitting up of the sugar. 2. When an animal is injected with a foreign protein a similar phenomenon is said to be observed. Ferments are produced which are able to break up the protein into peptones and amino-acids, acting in this way in a protective capacity. The optical method was again employed, in this case allowing the blood serum to act upon solutions of peptones prepared from the protein used to inoculate the animal.

These are the essential experimental results, and they are extended by speculation and reasoning by analogy to practical ends. In the case of pregnancy, for example, it is said that since cells of the placental villi may break off and pass into the maternal blood stream, and as these cells are made up of proteins strange to the mother, protective ferments may be produced. But pregnancy may be diagnosed very early (in the first month) by the method, and it is obvious that the total weight of placental cells which may in this time get into the maternal blood stream is excessively small; it is, therefore, unreasonable to assume that this process can be solely responsible for the production of the protective ferments. Further, in some animals—e.g., the mare—the relationship of the placenta to the mother precludes the possibility of cells of the villi escaping into the blood stream. Hence a long theory, based on endless assumptions, of the relationship between the maternal and fœtal metabolism is elaborated to show how protective ferments may be produced in the mother by simple products of the fœtal metabolism.[7]

By a similar process of reasoning the experimental results already mentioned have been made a basis for researches on practically every subject within the scope of medical science. It is all the more important, therefore, to repeat and consider carefully the experiments by which the existence of specific protective ferments is considered to have been established.

The optical method of demonstrating the ferments does not, on the surface, appear to be very satisfactory. In the first place, the maximum

rotations observed by Abderhalden are very small; in fact, only just outside the limits of error of the polariscope employed. In the second place, blood serum is chemically such a complex liquid that small changes in its optical activity are not necessarily significant. In this connexion it should be observed that Abderhalden states that blood serum may be kept indefinitely at 37° C. without any alteration in its optical activity, and it will be shown later that this statement comes into direct conflict with evidence obtained by the dialysis method. For these and other reasons the fundamental experiments with sugar as the substrate have not been repeated. In the last number but one of the *Journal of Biological Chemistry*[8] Hogan has published a paper on the parenteral utilisation of disaccharides, in which he comes to the conclusion that his data "yield no evidence whatever that any enzyme or any useful protective factor hitherto described is regularly developed in the animal body by the parenteral introduction of lactose or sucrose."

Turning now to the part of the experimental work in which albumins are used as the substrate, it is essential first to refer to the dialysis method which was employed to demonstrate the ferments. The technique adopted has been described in a previous paper.[9] In order, however, to prevent any misunderstanding it is necessary to emphasise several parts of the process. In the first place asepticity was observed throughout. The dialysis sacs, elongated Petri dishes, and pipettes were sterilised in the autoclave and used immediately they were cool. The substrate was sterilised before use by boiling for several minutes in distilled water; the blood was drawn off aseptically into sterile centrifuge tubes, and after centrifuging the blood serum was pipetted off into fresh sterile tubes. The contents of the dialysis sacs and the distilled water in which they were placed were both covered with a layer of pure toluene. In testing the dialysate with ninhydrin 10 c.c. of the liquid, to which 0·2 c.c. of 1 per cent. ninhydrin solution had been added, was boiled over the Bunsen flame for one minute.

It is essential, according to Abderhalden, to use

antigen and blood serum free of every trace of hæmoglobin. In experimental work with animals it is easy to obtain hæmoglobin-free serum. In order to do this the blood is collected quickly and centrifuged at 6000 revolutions per minute before coagulation occurs. The serum obtained is almost colourless and quite free of hæmoglobin. If the blood is allowed to coagulate before being centrifuged it is necessary to break up the clot, otherwise the serum will be mixed with fibrin in which red blood corpuscles are entangled. With human blood it is more difficult to obtain serum which is quite free of hæmoglobin owing to the fact that, as a rule, at least several hours elapse before the blood is centrifuged. The process by which the substrate may be obtained free of blood is fully described by Abderhalden in his book "Die Abwehrfermente."

Lange[2] states that in his experience the presence of hæmoglobin in the blood serum or in the substrate has no influence on the result of the reaction, and this opinion has been confirmed in the course of this work by numerous experiments. For example, a human placenta was taken and divided

TABLE I.

Sac.	Serum.	Substrate.	Ninhydrin test.	Sulphosalicylic acid test.
80	0·5 c.c.	HbO- free placenta.	–	–
81	1·0 ,,	,,	–	–
71	1 5 ,,	,,	+	–
74	0·5 ,,	Nil.	–	–
72	1·0 ,,	,,	–	–
82	1·5 ,,	,,	(+)	–
70	0·5 ,,	Bloody placenta.	–	–
83	1·0 ,,	,,	–	–
75	1·5 ,,	,,	+	–

into two parts, one of which was washed free of blood and prepared according to Abderhalden's directions, the other being boiled at once. Two

specimens of placental substrate were thus obtained, the one white and free of hæmoglobin, the other dark brown owing to the presence of altered blood. Blood was now obtained from a woman in labour and the experiment described in Table I. carried out.

The reverse experiment, in which hæmoglobin-free blood serum is obtained and divided into two parts, hæmoglobin being added to one part and both now incubated with and without placenta, was also performed. In this case also the presence of hæmoglobin exerted no influence on the reaction. Similar results were obtained with the blood and various substrates of the dog and rabbit. These experiments, though of the nature of preliminary investigations, were not carried out until a considerable proportion of the work described in this paper had been completed. Consequently in all the experiments to be described it is to be assumed that, unless stated to the contrary, the blood serum and substrate were hæmoglobin-free.

Aberhalden's experiments, on which the whole theory of protective ferments is based, were repeated in the following manner.

Half a dozen large healthy rabbits were taken, and the blood serum of each animal was allowed to act on coagulated egg albumin. The result of each experiment was negative, as the accompanying example shows. (Table II.)

TABLE II.

Sac.	Serum.	Saline.	Substrate.	Ninhydrin test.	Sulphosalicylic acid test.
81	1·0 c.c.	1·0 c.c.	Egg white.	–	–
82	1·5 ,,	0·5 ,,	,,	+	–
80	2·0 ,,	0	,,	++	(?)
76	1·0 ,,	1·0 c.c.	Nil.	–	–
73	1·5 ,,	0·5 ,,	,,	+	–
77	2·0 ,,	0	,,	++	(?

Thus the serum is without action on coagulated egg white. Following Abderhalden's method of procedure the rabbits were now injected intravenously (the second injection two

days after the first, and the third one day after the second) three times with egg albumin diluted with normal saline. The dose administered on each occasion was 2 c.c. of a 50 per cent. solution of albumin. The rabbits were bled as follows: 2 on the fourth day, 2 on the fifth, and 2 on the sixth day after the last injection. The sera obtained were tested against coagulated egg white, and in each case the result was negative. The accompanying table (No. III.) is typical:—

TABLE III.

Sac.	Serum.	Substrate.	Ninhydrin test.	Sulphosalicylic acid test.
76	0·5 c.c. rabbit No. 1	Egg white.	–	–
73	0·75 ,, ,, ,,	,,	(+)	–
72	1·0 ,, ,, ,,	,,	+	–
79	0·5 ,, ,, ,,	Nil.	–	–
83	0·75 ,, ,, ,,	,,	(+)	–
78	1·0 ,, ,, ,,	,,	+	–
71	0·5 c.c. rabbit No. 2	Egg white.	–	–
75	0·75 ,, ,, ,,	,,	(+)	–
82	1·0 ,, ,, ,,	,,	+	–
77	0·5 ,, ,, ,,	Nil.	–	–
80	0·75 ,, ,, ,,	,,	(+)	–
81	1·0 ,, ,, ,,	,,	+	–

A similar series of experiments was carried out, inoculating other sets of rabbits subcutaneously and intraperitoneally, and with the same results. In no case was any evidence obtained of the production of a protective ferment. The blood serum of the inoculated rabbits gave the precipitin reaction against solutions of egg white, and in the three cases tested anaphylactic shock was induced.

It will be noticed that in the tests with serum alone a positive ninhydrin reaction, progressively stronger with increasing doses of serum, is obtained. This result may be due either to the presence of amino-acids in normal rabbit blood or to a production of these substances by an autolytic enzyme. Abderhalden has demonstrated that amino-acids in minute amounts are normally

present in blood serum.[10] Working with very large quantities of blood—e.g., 50–100 litres—he was able to isolate glycocoll, alamin, prolin, leucin, &c., but owing to the energetic chemical methods employed there is a possibility that the amino-acids obtained were produced by the reagents used in the process. The question was therefore investigated by a new and simple method.

20 c.c. of blood were drawn from a rabbit's ear vein and rapidly centrifuged in order to obtain clear serum. The serum was pipetted off into a strong large sterile test-tube, which was immediately immersed in boiling water and kept there for 20 minutes in order to inactivate any enzymes which may be present, and to kill any organisms which may have contaminated the serum. The serum clotted, and a clear watery fluid separated. This fluid was pipetted off into sterile test-tubes, and again heated and then placed in two sacs and dialysed for 18 hours against 20 c.c. of distilled water in the usual way. The dialysates reacted faintly with ninhydrin and were negative to sulphosalicylic acid ; it was therefore concluded that blood serum does normally contain amino-acids. The experiment was repeated twice with rabbit's serum and with dog's serum with the same results.

In order to determine whether the blood content of amino-acids is increased during digestion an experiment was carried out in which rabbits which had fasted for 24 hours, and rabbits which had received a meal of oats and green stuff within three hours of the experiment, were bled and equal volumes of the serum obtained were treated in the manner already indicated. The dialysate from the fasting animal's blood serum gave a slightly fainter tint than the other dialysate, and from this result it is probable, considering the quantities of serum used, that any increase in the amino-acid content of the blood after a meal is too small to be detected in 2 c.c. of serum. It is possible, of course, that in an animal like the rabbit, with a great length of intestine and a large cæcum almost invariably loaded with partially digested food, the difference between a fasting animal and one in a state of digestion is very small. It was very noticeable that the dialysate from the serum of the animal in a state of digestion gave a temporary red colour when boiled with ninhydrin, this being due, probably, to glucose.

A further experiment was made to determine the influence of a previous bleeding.

An animal was bled, and the serum obtained was sterilised as before and the clear watery fluid pipetted off, and after being heated placed in the ice chest. Four hours later the animal was again bled, and the same volume of serum obtained and heated in the water bath. The clear watery fluid was pipetted off, heated, cooled in the ice chest, and 5 c.c. placed in a dialysis sac and dialysed against 20 c.c. of distilled water. In the same way 5 c.c. of the watery fluid from the first bleeding was dialysed against 20 c.c. of distilled water. After 18 hours the dialysates were tested with ninhydrin, and the reaction obtained with the dialysate from the serum of the second bleeding was much deeper than the other.

It may be concluded that amino-acids are washed from the tissues by the fluids which flow into the vascular system after a bleeding. These results do not preclude the possibility of a ferment action taking place in normal unheated blood serum. Indeed, it was observed that the depth of the ninhydrin reactions with fluid from 10 c.c. of serum was not more than equal to that usually obtained in an ordinary experiment with 1 c.c. of serum. In order to test for an enzyme action the following experiments were made.

An animal was bled aseptically, at least 30 c.c. of blood being taken, the blood immediately centrifuged, and the clear, watery, hæmoglobin-free serum was pipetted off into a sterile test tube. Into each of half-a-dozen or more sterile dialysis sacs 2 c.c. of blood serum was placed, and the sacs dialysed at different temperatures for 18 hours against distilled water. The temperatures chosen were as nearly as possible as follows : 5° (in chest), 20°, 30°, 40°, 50°, and 60° C. It was not always possible to set the baths at exactly these temperatures, but practically the temperatures employed were round about those stated. The baths used maintain the temperatures at which they are set to within half a degree during the 18 hours of the experiment.

The dialysates were tested in the usual way with ninhydrin, and the tints obtained gradually increased up to 40° C. and then diminished at the higher temperatures. The maximum was, in every experiment but one, round about 40° C. The results were compared by the method of dilution already described,[9] and the dilution numbers plotted

against the temperatures as shown in the curves below. The following are examples of the experiments :—

1. A dog was bled and the serum obtained by centrifugalisation. In order to be quite certain of the sterility of the serum it was filtered through a Berkefeld filter. The accompanying experiment was carried out (Table IV.). The

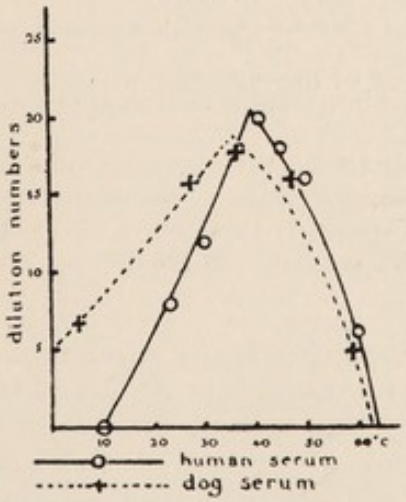
TABLE IV.

Sac.	Serum.	Temperature of incubation.	Duration of incubation.	Ninhydrin tests.	Dilution numbers.	S.S.A. test.
2	2 c.c.	4·5°	18 hours.	+	0·7 c.c.	–
32	,,	27·1°	,,	++	1·6 ,,	–
1	,,	36°	,,	+++	1·8 ,,	–
13	,,	47°	,,	++	1·65 ,,	–
5	,,	52°	,,	+(+)	1·4 ,,	–
12	,,	58°	,,	+	0·5 ,,	–

S.S.A. test = sulphosalicylic acid test.

curve for the dilution numbers is given in Fig. 1. From this experiment, in which in addition to using toluene every precaution was taken to exclude organisms, it is clear that an autolytic enzyme has been active.

FIG. 1.



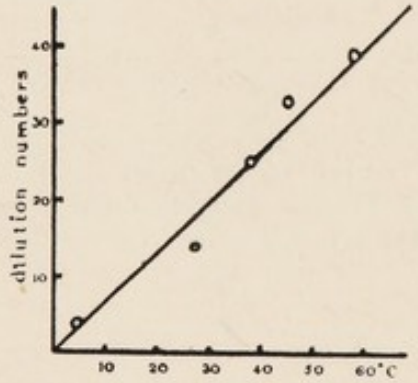
The dotted line shows the gradation in the depth of the ninhydrin reaction obtained with dog serum incubated at different temperatures. The dilution numbers give the volume of water added to 1 c.c. to bring the tint of the liquid to the degree of faintness of the weakest reaction.

2. The curve (Fig. 2) for the dialysis of a solution of peptone was obtained by dissolving 0·4 g. of Witte's peptone in 20c. c. of distilled water, and dialysing at different temperatures 2 c.c. of the solution against 20 c.c. of water thus (Table V.) :—

TABLE V.

Sac.	Temperature.	Duration of dialysis.	Ninhydrin test.	Dilution numbers.
13	4·5°	18 hours.	+	0·3 c.c.
5	27°	,,	++	1·4 ,,
2	38°	,,	+++	2·5 ,,
32	45°	,,	++++	3·3 ,,
12	55°	,,	+++++	3·5 ,,
1	58°	,,	++++++	3·9 ,,

FIG. 2.



Shows the effect of increasing temperatures on the dialysis of a dilute solution of Witte's peptone.

3. A bitch, three weeks pregnant, and a dog were bled and sera obtained. Aseptic precautions were taken, but the serum was not passed through a Berkefeld filter. The following tests were made (Table VI.). The curve is shown in Fig. 3.

It will be noticed from the curves (Fig. 3) that: (1) There is no evidence of digestion of the substrate either by the serum from the pregnant bitch or by that from the dog; (2) the serum from the bitch yielded a greater amount of amino-acids than that from the dog; and (3) the curve for dog serum alone is distinctly more irregular and rises to a smaller height than the curve for dog serum plus

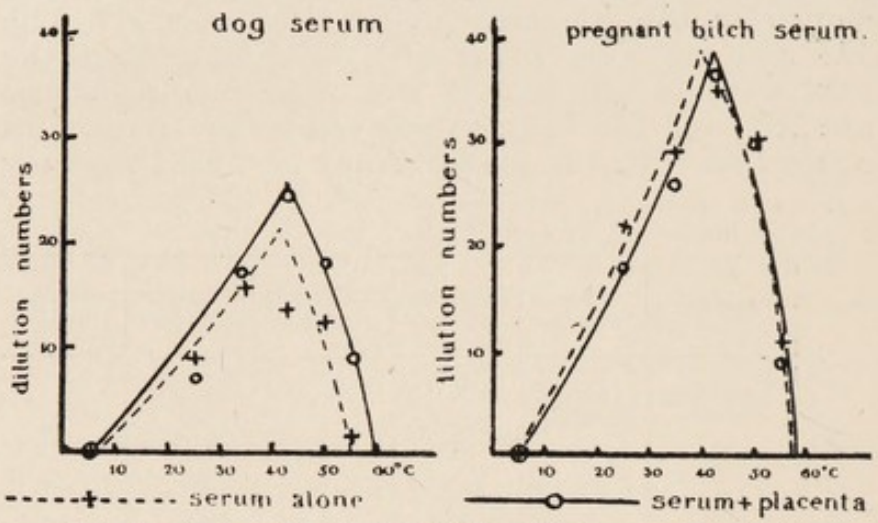
placenta. This is due to the fact, as has been shown in the previous paper,[9] that sacs 117, 116, 113, and 115 were made at a later date than the sacs used in the rest of the experiments (except sac 112), and had been autoclaved fewer times. In connexion with (2) above, it may be stated that my

TABLE VI.

Sac.	Serum.	Sub-strate.	Tempera-ture.	Ninhydrin test.	Dilution numbers	S.S.A. test.
16	2 c.c. dog serum.	Dog placenta.	5° C.	(+)	0 c.c.	–
112	,,	,,	25°	+	0·7 ,,	–
42	,,	,,	34·5°	++	1·7 ,,	–
20	,,	,,	42·5°	+++	2·4 ,,	(+)
27	,,	,,	50°	++	1·8 ,,	–
4	,,	,,	55°	+	0·2 ,,	–
7	,,	Nil.	5°	(+)	0 ,,	(+)
117	,,	,,	25°	+	0 8 ,,	–
43	,,	,,	34·5°	++	1·6 ,,	–
116	,,	,,	42·5°	++	1·4 ,,	–
113	,,	,,	50°	++	1·3 ,,	–
115	,,	,,	55°	+	0·2 ,,	–
24	2 c.c. bitch serum.	Dog placenta.	5°	(+)	0 ,,	–
25	,,	,,	25°	++	1·8 ,,	–
28	,,	,,	34·5°	+++	2 6 ,,	–
9	,,	,,	42·5°	++++	3·7 ,,	–
1	,,	,,	50°	+++	3·0 ,,	–
40	,,	,,	55°	+	0·9 ,,	–
26	,,	Nil.	5°	(+)	0 ,,	–
36	,,	,,	25°	++	2·2 ,,	–
12	,,	,,	34 5°	+++	2·9 ,,	–
13	,,	,,	42·5°	+++	3·5 ,,	(+)
5	,,	,,	50°	+++	3·0 ,,	–
2	,,	,,	55°	+(+)	1·1 ,,	–

colleague, Dr. Compton, estimated the content in the ferment maltase of both these sera and found a difference corresponding to that noted above. In view of the findings of Bertrand and Compton[11] the agreement between the maltase and proteolytic ferments in the two lots of serum is of considerable interest.

FIG. 3.



Showing that no degradation of placental tissue occurs either with dog or with pregnant bitch serum.

4. Blood serum of rabbits was examined in the same way with similar results. A normal Belgian hare was starved for 26 hours and then bled; the blood was centrifuged and the clear, hæmoglobin-free serum pipetted off. The serum was divided into two parts, to one of which 0·5 c.c. of hæmolysed red blood corpuscles was added. The sera were now filtered through separate Berkefeld candles and the following experiment carried out (Table VII.).

TABLE VII.

Sac.	Serum.	Temperature.	Ninhydrin test.	S S.A. test.
78	1 c.c. of HbO-free.	13·5°	–	–
80	,,	28·5°	++	–
83	,,	38·5°	+	–
79	,,	41·0°	–	–
73	,,	50·5°	–	–
82	,,	61·0°	–	–
76	1 c.c. containing HbO.	13·5°	–	–
81	,,	28·5°	++	–
77	,,	38·5°	+	–
74	,,	41·0°	–	–
72	,,	50·5°	–	–
75	,,	61·0°	–	–

It was not possible to obtain dilution numbers. The optimum temperature is here about 30° C.

5. About 40 c.c. of blood was drawn from the median cephalic vein of a healthy man (B.R G.R.) and hæmoglobin-free, yellowish serum obtained. This was dialysed in lots of 2 c.c. against water at different temperatures for 18 hours (Table VIII.). Fig. 1 shows the curve. The double dose of ninhydrin is required owing to the fact that human serum yields, dose for dose, a smaller amount of dialysable ninhydrin-reacting substances than the serum of the dog, the rabbit, the guinea-pig, or rat.

TABLE VIII.

Sac.	Serum.	Temperature.	Ninhydrin test (Dose 0·4 c.c.)	Dilution numbers.	S.S A. test.
98	2 c.c.	10°	+	0 c.c.	–
84	,,	23°	++	0·8 ,,	–
85	,,	30°	+++	1·2 ,,	–
90	,,	41°	++++	2·0 ,,	–
88	,,	45°	+++ (+)	1·8 ,,	–
86	,,	50°	+++	1·6 ,,	–
97	,,	60°	++	0·6 ,,	–

These results enable one to conclude that an autolytic ferment exists in the blood serum of rabbits, dogs, and human beings, and that it is responsible for some of the amino-acids indicated by the ninhydrin test. It does not follow, however, that it is impossible to diagnose pregnancy by Abderhalden's method. This requires to be tested directly, and for this purpose it was determined to use animal material owing to the ease with which blood serum can be obtained and male controls set up. Rabbits and dogs were tested. Blood was obtained from female animals both during the early days of pregnancy and immediately before or during delivery. In every case blood serum from a male was used as control. Altogether over 20 tests were made, and the results

obtained were concordant and invariably negative, as the following examples illustrate:

A pregnant rabbit and a buck were starved 18 hours and then bled. The serum was filtered through unglazed porcelain and the following tests made (Table IX.):—

TABLE IX.

Sac.	Serum.	Sub trate.	Ninhydrin test.	S.S.A. test.
21	½ c.c. doe.	Nil.	–	–
2	1 ,, ,,	,,	–	–
32	1½ ,, ,,	,,	+	–
12	2 ,, ,,	,,	++	–
5	½ ,, ,,	Rabbit placenta.	–	–
4	1 ,, ,,	,,	–	–
20	1½ ,, ,,	,,	+	–
27	2 ,, ,,	,,	++	–
13	½ c.c. buck.	Nil.	–	–
16	1 ,, ,,	,,	–	–
14	1½ ,, ,,	,,	+	–
43	2 ,, ,,	,,	++	–
7	½ ,, ,,	Rabbit placenta.	–	–
22	1 ,, ,,	,,	–	–
42	1½ ,, ,,	,,	+	–
25	2 ,, ,,	,,	++	–

Similar experiments, in which, however, the blood serum was not filtered, gave the same result. An example of a test with dog serum and dog placenta has already been given.

The difficulty of obtaining blood serum from human patients for the purpose of experimental work is very great, and renders it practically impossible to test the method as thoroughly as has been done for animals. It was, however, essential to make some experiments, and this was rendered possible by the kindness of Dr. Kettle, of St. Mary's Hospital, who procured four samples of blood, obtained during delivery. As a control to these tests in one experiment the blood serum of a

healthy adult male was used. In each test (including that with serum from male) it was found that the ninhydrin reaction of the dialysates of serum plus placenta was deeper than in the case of the dialysates from serum alone. The results here are therefore quite different from those already detailed above. The serum (from male and female) does break down placenta protein or in some way liberate amino-acids. But since this result had already been obtained in connexion with tests in cases of cancer in animals to be described later, it was decided, in spite of the small number of cases examined, not to continue the work.

The following example is typical. Blood obtained during labour was centrifuged and the serum, yellow in colour but free of hæmoglobin, was pipetted off (Table X.).

TABLE X.

Sac.	Serum.	Substrate.	Ninhydrin test.	S.S.A. test.
85	1 c.c. from woman on delivery.	Human placenta.	–	–
86	2 ,, ,, ,,	,,	++	–
84	1 ,, ,, ,,	Nil.	-	–
100	2 ,, ,, ,,	,,	(+)	–
98	1 c.c. male serum.	Human placenta.	–	–
90	2 ,, ,,	,,	++	–
97	1 ,, ,,	Nil.	–	–
84	2 ,, ,,	,,	(+)	–

In the published work dealing with the diagnosis of cancer by this method human material exclusively has been used. Here, again, the difficulty of providing sufficient controls has not been met. Where animal material is employed the difficulty does not arise. The following tables illustrate the results obtained in numerous experiments with rats, guinea-pigs, and rabbits.

1. Nine rats bearing tumours—rat tumour 9—which were growing progressively were starved for 20 hours and then bled and the blood centrifuged. The serum obtained was free of hæmoglobin, pale yellow in colour and clear. (Table XI.)

TABLE XI.

Sac.	Serum.	Substrate.	Ninhydrin test.	S.S.A. test.
116	0·65 c.c.	Rat tumour 9.	(+)	–
117	1·15 ,,	,,	+++	–
113	1·55 ,,	,,	++++	–
114	0·65 ,,	Nil.	?	–
112	1 15 ,,	,,	+	–
115	1·55 ,	,,	++	–

Incubated 20 hours.

2. Five normal rats were starved for 20 hours, bled, and serum obtained (Table XII.). Incubated 20¼ hours. The

TABLE XII.

Sac.	Serum.	Substrate.	Ninhydrin test.	S.S.A. test.
116	1·0 c.c.	Rat tumour 9	++	–
117	1·5 ,,	,,	++++	–
113	2·0 ,,	,,	++++++	–
114	1·0 ,,	Nil.	+	–
112	1·5 ,,	,,	++	–
115	2·0 ,,	,,	+++	–

same dialysing sacs were used as in the preceding experiment and in the same order.

3. Two rabbits, one a normal buck and the second a doe with an absorbing tumour, decreasing in 49 days from a size of $2\frac{3}{4} \times 2\frac{1}{2}$ inches to a size of $1 \times 1\frac{1}{2}$ inches at the time of the experiment, were starved for 18 hours and then bled. The sera were both free of hæmoglobin ; the normal serum was clear, that obtained from the tumour-bearing animal was opalescent. (Table XIII.) It will be observed

TABLE XIII.

Sac.	Serum.	Saline.	Substrate.	Ninhydrin test.	S.S.A. test.
80	0·5 c.c. tumour animal.	1·0 c.c.	Rabbit tumour.	(+)	–
76	0·5 ,, ,, ,,	1·0 ,,	,,	(+)	–
70	1·0 ,, ,, ,,	0·5 ,,	,,	++(+)	–
82	1·5 ,, ,, ,,	0 ,,	,,	++++(+)	–
83	0·5 ,, ,, ,,	1·0 ,,	Nil.	–	–
74	1·0 ,, ,, ,,	0·5 ,,	,,	++	–
73	1·5 ,, ,, ,,	0 ,,	,,	++++	–
81	0·5 c.c. normal animal.	1·0 c.c.	Rabbit tumour.	–	–
75	1·0 ,, ,, ,,	0·5 ,,	,,	+	–
79	1·5 ,, ,, ,,	0 ,,	,,	++	–
71	0·5 ,, ,, ,,	1·0 ,,	Nil.	–	–
72	1·0 ,, ,, ,,	0·5 ,,	,,	(+)	–
77	1·5 ,, ,, ,,	0 ,,	,,	+	–

that though the dialysates from tumour serum reacted to ninhydrin more strongly than the dialysates of normal serum, yet the differences between serum alone and serum plus substrate corresponded in the two sets of the experiment.

4. A guinea-pig tumour was washed free of hæmoglobin until the tissue was white, and was then extracted with chloroform to remove the large amount of fat usually found in this tumour. The product was then boiled in distilled water acidified with acetic acid, and again in distilled water until the water failed to react with ninhydrin. A guinea-pig bearing a tumour and a normal guinea-pig were bled from the heart and sera obtained. The animals were not

previously starved. (Table XIV.) In this experiment the reverse of what was shown in the last experiment is obtained; the serum of the normal animal

TABLE XIV.

Sac.	Serum.	Substrate.	Saline.	Ninhydrin test.	S.S.A. test.
72	0·5 c.c. normal animal.	Guinea-pig tumour.	0·5 c.c.	–	–
74	0·75 ,, ,, ,,	,,	0·25 ,,	++	–
71	1·0 ,, ,, ,,	,,	0	++++	–
83	0·5 ,, ,, ,,	Nil.	0·5 c.c.	–	–
73	0·75 ,, ,, ,,	,,	0·25 ,,	–	–
80	1·0 ,, ,, ,,	,,	0	+	–
76	0·5 c.c. tumour animal.	Guinea-pig tumour.	0·5 c.c.	–	–
82	0·75 ,, ,, ,,	,,	0·25 ,,	+	–
70	1·0 ,, ,, ,,	,,	0	++	–
77	0·5 ,, ,, ,,	Nil.	0·5 c.c.	–	–
79	0·75 ,, ,, ,,	,,	0·25 ,,	–	–
75	1·0 ,, ,, ,,	,,	0	+	–

Incubated 21 hours.

TABLE XV.

Sac.	Serum.	Substrate.	Ninhydrin test.	S.S.A. test.
4	0·5 c.c. immune serum.	Tumour 63.	–	–
5	1·0 ,, ,,	,,	+++	–
24	0·5 ,, ,,	Nil.	–	–
40	1·0 ,, ,,	,,	++	–
20	0·5 c.c. normal serum.	Tumour 63.	(+)	–
1	1·0 ,, ,,	,,	+++	–
21	0·5 ,, ,,	Nil.	–	–
9	1·0 ,, ,,	,,	+(+)	–

Incubated 19 hours.

produced a greater ninhydrin reaction than the tumour serum, but again in both sets of tests there is an indication of a degradation of tumour protein or of some physical action liberating amino-acids.

5 Two guinea-pigs were inoculated in the right axilla with an emulsion of mouse tumour 63 ; the dose in each case was 0·5 c.c. Sixteen days later these animals and two normal guinea-pigs were bled, and the sera allowed to act on mouse tumour 63 as substrate as indicated. (Table XV.)

Up to the present it has not been possible to obtain materials necessary to carry out similar experiments in cases of cancer in the human subject.

Results and Conclusions.

1. It was found impossible to cause the production in rabbits of a ferment which digests coagulated egg white by the injection subcutaneously, intraperitoneally, or intravenously of raw egg white.

2. A minute quantity of amino-acids is present in 10 c.c. of blood serum of rabbits and dogs, and it was found (in rabbits) that after a bleeding these substances are increased in amount.

3. An autolytic ferment is found in the blood serum of rabbits, dogs, and in human serum.

4. The serum of the pregnant rabbit is unable to break down rabbit placenta, and the serum of the pregnant bitch is unable to break down dog placenta. Human blood serum from the pregnant female and from the healthy male, incubated with human placenta, produced by chemical or physical action amino-acids.

5. Rat blood serum ("normal" and "tumour") breaks down rat tumour (J. R. S. & Rat 9). Guinea-pig serum ("normal" and "tumour") breaks down guinea-pig tumour and mouse tumour 63. The blood serum of the guinea-pig was not rendered more active in this property by a previous inoculation of the animal with mouse tumour 63.

The method devised by Abderhalden, even with the technical improvements described, is thus seen to be inadequate to distinguish normal from pregnant or cancerous sera. The fundamental

experiments adduced by Abderhalden do not, on repetition, give the results stated by him, and a proteolytic ferment normally present in the serum of mammals acting on tissue substrates of varying lability, prepared in accordance with his directions, in all probability accounts for his results and their confirmation by others.

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