THE SERUM DIAGNOSIS OF PREGNANCY AND OF CANCER:

A CRITICAL STUDY OF ABDERHALDEN'S METHOD.

BY

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II. THE PROTECTIVE FERMENTS IN CANCER: CRITICISM OF THE THEORY.

In the practical experiments on cancer, though I have had an unusual amount of operative material at my disposal, yet comparatively little seemed to me suitable, for reasons that will be explained later, and in the tests here recorded I have restricted myself to those in which good cellular tumours were available as "substrates." I have not used *post-mortem* material. The details of 100 cases examined to test the method in the serum diagnosis of cancer are as follows:

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

TABLE A.—*Epithelioma, Carcinoma, and Placenta.*

Disease.	Serum alone.		Epithelioma + Serum.		Carcinoma + Serum.		Placenta + Serum.	
	1 c.c.	1.5 c.c.	1 c.c.	1.5 c.c.	1 c.c.	1.5 c.c.	1 c.c.	1.5 c.c.
1 Epithelioma of oesophagus	0	+	+	++	—	—	+	+
2 Epithelioma of penis	0	+	+	+++	—	—	—	—
3 Epithelioma of tongue	0	0	+++	+++	—	—	+	+++
4 Epithelioma of lip	0	+	+	+	—	+	++	++
5 Epithelioma of tonsil	0	0	++	++	—	0	—	++
6 Carcinoma of breast	0	0	+	+	+	+	+	+
7 Carcinoma of breast	0	0	+	+	—	—	0	0
8 Carcinoma of breast	0	0	+	+	0	+	+	+
9 Carcinoma of rectum	0	0	+	+	—	0	—	+
10 Lipoma of shoulder	0	0	+	+	—	—	0	0
11 Cholecystitis	0	0	0	0	0	0	0	0
12 Chronic appendicitis	0	0	0	0	—	—	++	+++
13 Hysteria	0	0	0	0	0	0	0	0
14 Uterine polypus	0	0	0	0	0	0	0	0
15 Former epithelioma of lip	0	0	0	0	—	—	0	0
16 Parametritis	0	0	0	0	—	0	—	0
17 Endometritis	0	0	0	0	—	—	—	—
18 Two months pregnancy	0	0	0	0	—	+	+++	+++
19 Two months pregnancy	0	0	0	0	—	—	—	—
20 Seven months pregnancy	0	0	0	0	—	—	—	—
21 Nine months pregnancy	0	0	0	0	—	—	—	—

In Nos. 2 and 13 the blood was withdrawn less than four hours after the last meal. In all the rest, with the exception of the last three in which I have no notes on the point, the blood was withdrawn at the time of the operation when the patient was in a fasting condition. In no cases was the serum haemoglobin-stained.

This set of experiments is the most favourable I have found to the claims of Abderhalden. The 9 cancer serums all gave positive results with the epitheliomatous tissue—if anything, more markedly in the case of squamous-cell than in the other carcinomata—whereas the 12 non-cancerous cases were negative with the exception of the case of lipoma, and one might quite easily, if dominated by the claims made on behalf of the method, have made excuses for this solitary lapse by blaming the technique. Unfortunately for this, the experiment was repeated in duplicate again with the same results. The mammary cancer was not such a good *substrate*. It did not give as good results with the mammary cancer cases as did the epithelioma. With it 5 cancer serums were tested; 3 gave positive reactions,

2 were absolutely negative; of 5 non-cancer cases, 4 were negative, 1 was positive. With placenta, 8 cancer serums were tested, 7 gave positive reactions, 1 was negative; of the 7 non-pregnant, non-malignant cases 1 was positive. In all cases the serums were used fresh.

In four cases an epitheliomatous tumour of the tongue was used as a *substrate*.

TABLE B.—*Epithelioma of Tongue.*

Disease.	Serum alone, 1 c.cm.	Serum alone, 1.5 c.cm.	Epithelioma + 1 c.cm. Serum.	Epithelioma + 1.5 c.cm. Serum.
22 Epithelioma of tonsil	0	+	+	++
23 Syphilitic glossitis	0	0	0	0
24 Normal male	0	0	0	0
25 ? Lupus of face	0	0	+	+

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

The *substrate* behaved in the first three cases just as we hoped. The last case was the crux of the whole examination. There was what was supposed to be lupus of the face, and there was an ulcerated patch that was not healing under x rays; and the practical question arose whether or not epithelioma was developing. The reaction pointed to this being the case. As a matter of fact, it was not so. The patient gave a positive Wassermann reaction, and the ulceration healed under salvarsan.

In the following twenty-six cases a large, quickly-growing cellular carcinoma of the breast, with axillary

TABLE C.—*Cellular Carcinoma.*

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

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

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

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

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

	Disease.	Serum alone, 1 c.cm.	Serum alone, 1.5 c.cm.	Carci- noma, + 1 c.cm. Serum.	Carci- noma, + 1.5 c.cm. Serum.
66	Cancer of breast	0	+	0	+
67	" " " " " " " "	0	+	+	+
68	Cancer of uterus	0	0	0	0
69	" " " " " " " "	0	+	0	+
70	" " " " " " " "	0	+	0	+
71	" " " " " " " "	0	0	0	+
72	" " " " " " " "	+	+	+	+
73	Epithelioma of palate ...	0	+	+	+

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

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

Of these 100 cases tested to prove the worth of the method in the serum diagnosis of cancer, 51 are cancer and 49 are non-malignant. In the 51 cancer cases positive results were obtained in 28, that is, 55 per cent.; and in the 49 non-cancerous controls positive results were

TABLE H.—Four Different Cancers.

	Disease.	Serum Alone.		Mammary Cancer + Serum.		Uterine Cancer + Serum.		Vulvar Epithelioma + Serum.		Lingual Cancer + Serum.	
		1 c.cm.	1.5 c.cm.	1 c.cm.	1.5 c.cm.	1 c.cm.	1.5 c.cm.	1 c.cm.	1.5 c.cm.	1 c.cm.	1.5 c.cm.
86	Carcinoma of breast	0	0	0	0	—	++	—	0	—	0
87	" "	0	0	0	0	—	+	—	0	—	0
83	" "	0	0	0	0	—	+	—	0	0	—
89	" "	0	0	0	+	—	+++	—	0	—	0
90	" "	0	0	0	0	—	+	—	0	—	0
91	" "	0	0	0	0	—	0	—	0	—	0
92	Carcinoma of uterus	0	0	—	0	+	+	—	0	—	0
93	" "	0	0	—	+	0	++	—	0	—	+
94	" "	0	0	—	0	0	0	—	+	—	0
95	Carcinoma of rectum	0	0	+	+	—	++	—	+	—	+
96	" "	0	+	0	+	—	+	—	++	—	0
97	Carcinoma of vulva	0	0	+	—	—	0	0	+	—	0
98	Dental cyst	0	0	0	+	—	0	—	0	—	0
99	Fistula in ano	0	0	0	+	—	0	—	+	—	0
100	Fibroid of uterus	0	0	+	+	—	+++	—	0	—	+

None of the serums was blood-stained.

obtained in 18, that is, 37 per cent. The method, therefore, is without diagnostic value.

COMMENT.

It is necessary, in view of the adverse results here obtained, that contrast so strikingly with those of the brilliant and deservedly honoured originator of the theory and with those of his numerous disciples in all parts of Europe and America, to search out the factors that led to the amazingly mistaken claims, as I consider them to be, and to show as well as I can, that my own results are not due to errors of technique or to biased misunderstanding. It seems to me that the theory originated from the readings of some preliminary experiments. It may be that those readings unconsciously exaggerated the facts; the fundamental observations were not made sufficiently indubitable for a great structure to be reared upon them; and the observer, having satisfied himself that a certain result was to be expected, and knowing exactly what he had to deal with when identical experiments were repeated, could not avoid obtaining readings similar to the original. If the repetition of the experiments was entrusted to a disciple, as it probably was in a busy laboratory, there would be the greater chance of the original views being substantiated. Whether or not that be an ungenerous supposition, the fact remains that the fundamental experiments do not cover a sufficiently wide field, and it is obvious that a great jump was made from serum reactions to substances quite foreign to the animal body, to serum reactions to substances natural to the body. The experiments with the serum of pregnant women, viewed through the eyes of the impelling theory, would at first seem to justify this jump. The same phenomena were found, and these were rendered all the more plausible since the serum of many non-pregnant individuals failed to give them. False results would crop up, however, as the number of the latter cases increased. Surprised at these, an enthusiast would seek causes for rejecting them. If all biological laws were as dependable as the movements of the stars, then there would be little scope for the making of errors, and research would be unromantically monotonous. The unexpected false results would be associated with some accompanying phenomena, and a causal relationship between them would be assumed, so that on the recurrence of false results and their accidental association with such conditions one would feel in a position to reject them. In the course of time other phenomena would be found in association with untoward results and again means would be found to reject them. This seems to have been the history of the Abderhalden reaction. Now it bristles with hypothetical fallacies that have well-nigh obscured the reaction itself. Let us therefore consider the real as well as the fictitious fallacies.

The Substrate.

From the point of view of the theory of the reaction the proper preparation of the organ or tissue that is to serve as substrate for the "specific proteolytic ferment" is of the utmost importance. In the case of placenta, and similarly with other tissues, the fresh organ is washed free of blood until a "snow-white tissue is obtained." Let it be said at once that such a result is impossible. The method I have adopted has been to remove the adherent blood clot, connect the umbilical vein with the water tap, and let the water flow through it for about two hours. At the end of that time the placenta should be practically colourless. The membranes are now removed, the placental tissue is cut up into walnut sized portions, and these are kneaded in frequent changes of salt solution for another hour. Thereafter, the pieces having been cut up into smaller fragments are put in running water for a few hours. As these pieces lie in the bath they appear to be quite white, but if they are taken in bulk and the water expressed from them it will be found that they still preserve, no matter how long or how thorough the washing, a faint colour, which, in the case of the placenta, is a low-toned white or pale ivory. Every tissue that I have tried preserves a faint tint proper to the particular organ, and a "snow-white" condition cannot be produced. It is specially difficult to get cancers free from blood. I have used only fresh operation material and though there has been a large stock at my disposal it is seldom that we can get tumour material exactly suitable. It is very doubtful if the presence of small traces of blood—or even very large amounts, for that matter—has anything to do with the false reactions. Even supposing we admitted the possibility of some obscure ferment in the serum capable of acting on the very minute traces of blood still left in the washed tissue, so as to give sufficient amounts of diffusible substances detectable with ninhydrin (a rather large supposition)—that is to say, giving a positive reaction where a negative reaction is to be expected—the hypothesis would not account for negative reactions where positives are to be expected according to the theory. I have obtained such negative reactions even in pregnancy. The tissue that is to serve as substrate is boiled in distilled water, to which a few drops of acetic acid are added, then for several times in distilled water alone, being well rinsed between each boiling, until the boiled water, which ought to be about five times the volume of the tissue, ceases to give a reaction when 10 c.cm. of it are boiled with 1 c.cm. of 10 per cent. ninhydrin solution. Any one may easily satisfy himself that even then the boiled water may show a violet colour when stronger concentrations of ninhydrin are used, and I have always gone on with the boiling until the water was colourless to as much as 5 c.cm. of the ninhydrin solution. Placental and other tissues vary very much in the number of boilings they require before they are suitable. I have

often noticed the curious fact that the boiled water may give a colour with weak concentrations and none at all with stronger concentrations of ninhydrin. The explanation of that fact is a question for chemists, but it is a point to bear in mind that ninhydrin may fail to detect the presence of protein products simply because it is too strong, just as it may also fail to detect them because it is too weak. I have further observed that the water of the tenth boiling of a tissue, for example, may be colour-free to ninhydrin, whilst that of the twelfth may give the reaction. Once having got the water colour-free, if the tissue be allowed to stand, colour reactions may be obtained after varying periods of time, sometimes within a few hours. Some tissues seem to be very unstable in this respect. The ninhydrin reacting substances found in the boilings of tissues are diffusible through the dialysers, and even though we test the tissue immediately before use, as we always do, we may be sure that there is always a certain amount of diffusible substances from our substrate—apart from any other factor—capable of contributing to the result.

One of the hypothetical fallacies that Abderhalden has recently detected is that the connective tissue in the organ acting as substrate may itself be attacked and broken down by some intruding ferment; there may be some lesion of the connective tissues in the individual who furnishes the serum. This reduces the reaction to a farce, for there is no organ devoid of connective tissues and no organic disease incapable of implicating them. Besides, the latest method he adopts in the preparation of his substrates—the pounding of the tissues in a mortar—is calculated to get rid of as much parenchyma and to leave as much connective tissue as possible. The great difficulty in the preparation of cancerous material is to get rid of the blood and yet to leave the cancer cells in their connective tissue stroma. The more cellular the cancer the more easily are these cells removed by washing, and the fact that the connective tissue stroma renders the blood vessels of a malignant tumour so exceedingly tortuous militates against the freeing of the tissue from blood. In this case there are only two ways of getting suitable substrates—either to cut very thin slices of the tumour, running the risk of losing the epithelial cells, or to reject tumour after tumour until we light upon something suitable at last.

It has been imagined that in the case of a blood effusion a ferment will be elaborated against the corpuscles of that effusion, and consequently, if any tissue employed as substrate should contain some blood, then the serum of that case would in these circumstances be capable of giving a false reaction. I have already pointed out that I could find no evidence of this in experiments on rabbits. I have, however, used as substrate pure blood clot, boiled free of ninhydrin-reacting substances, and tested this with fifteen serums, one of these being from a case of cerebral apoplexy, and most of the others from cancer cases in which local haemorrhages were evident, with the result that in no single case was there the slightest suspicion of a positive reaction. Again, a piece of cancerous tissue—a mammary carcinoma—containing much blood was forthwith boiled without any preliminary washing, and tested with various serums; it neither picked out all the cancer serums nor gave reactions with all the non-cancerous; it merely behaved, or misbehaved, as other substrates scrupulously prepared according to the regulations.

But whatever theoretical objections be brought against the reliability of the substrates that I have used, whether it be that they were not absolutely snow-white, that they still held in spite of our care minute traces of blood, that they were too unstable, or that they contained connective tissue, the criticism quite loses its points if we can get false reactions with "substrates" that are beyond suspicion. I tried, therefore, as "substrates" pieces of sterilized sponge, kaolin, and glass wool. In this way 39 serums were tested; I obtained 7 well marked positive results. Recently Plaut¹ has also obtained positive results where he used as "substrates" kaolin, barium sulphate, talc, and *Kieselguhr*. Like him I have also noted reverse effects (that is, reactions of the controls without substrate, and no reactions with the serum and substrate) in some cases, but the same thing has occurred several times in the ordinary procedures. It will hardly be argued by the most ardent believer in the *Abwehrfermente*

theory that protective ferments are elaborated within the animal body capable of splitting up into peptones or amino-acids such substances as sponge, glass wool, and kaolin. Without for the moment denying the occurrence of specific protective ferments in the serum, I am convinced that the important point in the reaction is that the substrate, owing to its physical nature, splits up the serum, and not so much the serum that splits up the substrate.

The Serum.

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

Another point on which Abderhalden lays stress is that if the blood be withdrawn from a patient within four hours after a meal, the serum may produce false positive reactions. This, again, is a hypothetical objection, and by means of it one is enabled to explain away most of the adverse results. It may be said that the blood after a meal contains fugitive proteolytic ferments, or that there is a temporary accretion of free amino-acids. It is probable that the latter is true. Indeed, Costantino² has shown that the blood of fed animals contain more amino-acid (formol-titratable nitrogen) than the blood of starved animals, but he found that the excess was anchored to the corpuscles, and was not free in the plasma. Even supposing the presence of amino-acids in the serum had something to do with the production of colour reactions in the dialysate, yet a positive reaction is not reckoned by a mere presence of colour in a single dialysate, but by the comparison between the colours given by a test and by its controls; and the factor would be equal in both. I have always noted the relationship to time of last meal, and a consideration of the tables will show that the hypothesis does not hold good in practice. Still, whatever objection there be to the usage of serum taken from a patient who is not in a fasting condition, it must be admitted that it is well-nigh impossible to amass any large number of observations under such ideal circumstances. Our habits are such—and this applies to Germany at least quite as much as to this country—that seldom more than four hours elapse between meals of some sort, except when we are asleep, and any investigator would have little else to do who attempted to obviate the hypothetical fallacy. I have been fortunate in complying with the requirements in the great majority of my cases, for the blood was removed at the time of operation, when a minimum of six hours had intervened since the last meal. I have, on the other hand, examined blood taken actually during, or shortly after meals. It does not seem to make the slightest difference.

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

amount to the dialysate. If we dialyse against running water for several hours the serums which previously gave large amounts of reacting substances to the distilled water, and finally dialyse this again against distilled water, we shall find that this dialysis against running water has not succeeded in wholly removing, sometimes not in appreciably diminishing, the diffusible substances. This shows that the serum is not a stable material, but is splitting up progressively; it is partly an inherent characteristic of serum, and is partly due to the action of the distilled water.

The Dialysation Tubes.

Specially prepared dialysation tubes for the reaction are supplied by Schleicher and Schüll, No. 579A. These are supposed to be impermeable to protein, but permeable to peptones and other degradation products of proteins, and, though they are sold as having passed the tests, it is wise to satisfy oneself on the point. In the first consignments it was found that a fairly large percentage were permeable to protein, but in the more recent comparatively few failed to pass the tests that Abderhalden demands. They must be subjected frequently to these tests during the course of the experiments, and if the dialysers be numbered and at any time one is found faulty the cases in which it has been used since it last passed the examination will have to be rejected. In testing for the impermeability to protein, 2.5 c.cm. of a 5 per cent. suspension of fresh egg albumen are added to each tube, and this is dialysed against 20 c.cm. of distilled water for sixteen or more hours, and the dialysates are tested for the biuret reaction. If the reaction is positive the corresponding tube is rejected. It is very difficult, if not impossible, to be certain of detecting weak biuret reactions, and in every case I have repeated the test of the dialysate with ninhydrin. It is true that the two methods do not give strictly comparable results. It is considered by Leonor Michaelis and v. Lagermark that the biuret reaction is not to be depended on, at least when performed in the way that Abderhalden recommends. They assert that they can in the same way obtain biuret reactions with distilled water, and they recommend the test of the dialysate with sulpho-salicylic acid.³

The second requisite is that they be permeable to peptones and all equally permeable. If we test a stock of dialysers with silk peptone, which is supposed to be a pure peptone, boiling the dialysates with 0.2 c.cm. of ninhydrin solution, we shall find that differences in depth of colour may be made out, showing that they are not uniformly permeable, and we must reject the extremes. The differences in permeability can be shown much better by using Witte's peptone, which is an impure peptone. The individual differences are very marked, and it is practically impossible to get a large series of absolutely comparable dialysers. At first sight it might be thought that by taking a large stock of dialysers, and by estimating the depth of colour in the corresponding dialysates by means of a colorimeter, we might arrange our dialysers in order of permeability. Unfortunately this is not satisfactory for the reason that the actual colours, as well as the tints, differ, and no strict comparison can be made. What is more important is that the permeability of any single dialyser is not constant; it decreases or increases by use; and what are to-day strictly uniform tubes exhibit in the course of time various differences. We may gain an idea of their variation by numbering them and observing the results they give with serum from time to time. The differences between them can be brought out, too, by testing a small batch with one serum and noting the depths of the colour reactions of their dialysates with increased quantities of ninhydrin. We have, therefore, to take into account two factors affecting the reaction, namely, that with uniform dialysers different serums vary in the amount of diffusible substances and that with a single serum dialysers vary in the amounts of ninhydrin-reacting substances they allow to pass. The only way, therefore, to be sure of obtaining reliable readings in practice is to manifold the tests and controls as far as the quantity of serum permits, and to strike a just average. I have never depended on single tests; they are worthless. It will sometimes be found that the dialyser containing substrate plus serum gives to the dialysate a less amount of ninhydrin-reacting

substances than the dialyser containing serum alone. Such results and dialysers must in kindness be rejected before giving an opinion on the diagnostic value of the reaction, but must be borne in mind when considering the worth of a theory that relies for its substantiation on such fickle means.

The following is a typical experiment out of several that will show how positive reactions may come about:

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

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

Tubes 6 and 7 contained 1.5 c.cm. of the unheated serum only. With 0.2 c.cm. of ninhydrin the dialysates were quite colourless; with 0.4 c.cm. both gave an equal faint golden colour; with 0.6 c.cm. both gave a light violet colour, more marked in No. 7 than in No. 6. Taking the weaker as an arbitrary standard for the colorimeter, and giving it the value of 1, the depth of colour of No. 7 would be represented as 1½.

Tube 8 contained 1.5 c.cm. of unheated serum along with placenta. With 0.2 c.cm. of ninhydrin the dialysate gave just the faintest grey tint; with 0.4 c.cm. a deep violet colour was produced; with 0.6 c.cm. the colour was very deep. The colorimetric value was represented as 9.

Tubes 9 and 10 contained 1.5 c.cm. of heated serum only. With 0.2 c.cm. of ninhydrin the dialysates were colourless; with 0.4 c.cm. a light violet colour was produced in No. 9, and a golden colour in No. 10; with 0.6 c.cm. No. 9 gave a marked violet colour—represented by the value 6—and No. 10 gave a light violet—represented by the value 1½ on our empirical scale.

Tube 11 contained 1.5 c.cm. of heated serum along with placenta. With 0.2 c.cm. of ninhydrin the dialysate was colourless; with 0.4 c.cm. it was a faint lilac; with 0.6 c.cm. the colour was violet and the colorimetric value was represented by 2½.

This experiment serves to illustrate the facts that apparently uniform dialysers vary very much in permeability when actually tested with serum, that all allow some ninhydrin-reacting constituent of serum to pass through; that the heating of the serum does not abolish, though it may diminish, the liability to spontaneous cleavage, and that the reaction is not a quantitative one. That dialysers are liable to allow a certain amount of diffusible substances from serum to pass is practically, though not actually, admitted by Abderhalden himself, seeing that he lays such stress on the equal boiling of the dialysates. We may gain an illuminating insight into the fallacies of the method by performing the following experiment suggested by certain observations of Frank, Rosenthal, and Biberstein.⁴ Into one dialyser let us put the substrate alone, and into another the specific serum alone, and dialyse them in the usual way, and we shall probably find that the dialysates in both cases do not give reactions with ninhydrin in the usual concentration. But let us, at the same time, put *two* dialysers, one containing substrate only and the other the serum only, into the *same* container, and we may find that in this case the dialysate gives a good violet colour with the usual amount of ninhydrin. This excludes the possibility of the operation of a ferment, and shows that the result is due, in this case, to the summation of two effects which by themselves were not capable of detection.

The conclusions to which I have come after a careful investigation of Abderhalden's dialysation method are that the real fallacies of the technique are beyond control, and that the hypothetical fallacies which Abderhalden invokes to account for false reactions have no bases. Positive results are quite well accounted for by the facts that the "substrates" alone, and the serums alone, give diffusible substances reacting with ninhydrin; that the dialysers vary enormously in the quantities of such substances which they allow to pass in a given time; that serums, influenced by the distilled water and by the presence of a tissue acting in virtue of its physical state, are pro-

gressively cleaved; and that it is not the substrate which is split by the serum so much as the serum which is split by the substrate. These very real fallacies quite destroy the value of the method, and they cast grave doubts on the whole theory.

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

REFERENCES.

¹ *Muench. med. Woch.*, 1914, p. 238. ² *Biochem. Zeitschr.*, September 28th, 1913. ³ *Deut. med. Woch.*, February 12th, 1914, p. 316. ⁴ Frank, Rosenthal, and Biberstein, *Muench. med. Woch.*, July 22nd, 1913.

British Medical Association.

CLINICAL AND SCIENTIFIC PROCEEDINGS.

OXFORD AND READING BRANCH.

THE annual meeting of the Branch was held at Reading on July 23rd under the chairmanship of Sir WILLIAM OSLER, Bart., President.

Cases.

The following cases were shown in the out-patient department:—Dr. W. T. FREEMAN: Parasyphilis with nervous symptoms; fibroid phthisis, with skiagraph; a heart case with abdominal tumours. Dr. G. F. MURRELL: Two cases (brothers) of congenital absence of fingers and toes with marked family history. Drs. G. O. LAMBERT and G. S. ABRAM: Aneurysm of the aortic arch. Dr. E. P. CARMODY: A case suggesting pituitary disease. Dr. E. W. SQUIRE: Webbing of the second, third and fourth fingers of each hand. Dr. E. P. CARMODY: Enlargement of forearms and hands.

Specimens in the Pathological Laboratory.

Dr. R. DONALDSON showed chorion-epithelioma of testis, primary carcinoma of liver (child aged 3 years), epitheliomata of tongue and hand, sarcoma of calf's kidney, and various other interesting specimens. Dr. J. L. JOYCE showed a gall stone which caused intestinal obstruction in the small intestine. There were also a number of photographs of skin cases and skiagraphs of surgical cases.

Discussion on the Cases and Specimens Shown.

The cases aroused great interest. The case of abdominal tumour with a peculiar cardiac thrill and bruit was thought

by some to be a primary abdominal tumour with deposits in the myocardium. The heart condition had developed quite recently. Dr. Collier had examined the blood there and then, and had excluded spleno-medullary leukaemia. The cases of absence of fingers and toes showed a marked family history. All the four children of the father were similarly affected; the father was affected and earned his living by mowing golf greens, and his mother was also affected. The case—a male, aged 40—showing bilateral enlargement of the hands and forearms, with wasting, especially of the deltoids, spinati and pectorales muscles, was thought to be probably of pituitary origin. It was agreed by several members that the cases were of unusual interest and well worth coming from Oxford to see. Dr. R. Donaldson, the pathologist, was heartily thanked for the series of specimens.

Organization of the Clinical Laboratory.

Sir WILLIAM OSLER addressed the meeting on the organization of the clinical laboratory. He pointed out the impossibility in many cases of arriving at a diagnosis without a laboratory. The great defect in the present state of medical practice was the lack of facilities for carrying out the clinical methods learnt before qualifying. He regretted that the curricula in the medical schools were so crowded, and thought that in the third, fourth, and fifth years each student of medicine should have his own desk in a laboratory, and be completely equipped with apparatus which he could use to investigate his own cases. This method was being followed in the United States. Again, although great advances had been made in the furnishing of large hospitals in populous centres during the last fifty years, the growth of facilities for laboratory work had not been commensurate. There were still many hospitals in Great Britain of two or three hundred beds in which a surgeon was unable to get a section of a tumour cut for examination. A large building was not necessary for this purpose, but a budget was quite essential. The staff should consist of a whole-time man specially trained in microscopy and bacteriology, and a whole-time chemist would be a great advantage. Then again a special man was required for the very important work of electro-cardiac investigation. Hospital committees must be taught that these things were costly, but worth paying for. Sir William Osler said that he realized how difficult it was to conduct a general practice and still keep in touch with advances in medicine. The general practitioner might do his work very well, but he had not the time nor the special training necessary for research work; a research worker was urgently required in every centre of moderate size. He thought no general practitioner should send a specimen of urine away for another to examine. The Government was seriously considering the establishment in each centre of a laboratory for use by the general practitioners. He thought it would be best placed in connexion with a hospital, and that its use and the right to obtain a second opinion should be free of charge. He thought the salaries of the research men should rise with experience, and that "blind alley" posts should be carefully avoided.

A discussion followed, in which Drs. J. B. HURRY, W. COLLIER, G. C. TAYLOR, G. F. MURRELL, H. A. WHITELOCKE, and W. L. WAINWRIGHT took part; Sir WILLIAM OSLER replied.

Carcinoma and Radium.

Dr. HOLDEN read some notes on a case of carcinoma of the oesophagus treated by radium under Mr. Pinch. The growth had diminished in size, so that more food of thicker consistency was being taken and the general condition of the patient had improved, whereas the patient would, apart from gastrostomy, have otherwise died. The cost was roughly £30.

Annual Dinner.

The meeting then closed, and members repaired to the Caversham Bridge Hotel for the annual dinner.

THE Dean of the Royal Dental Hospital of London of the School of Dental Surgery, Leicester Square, W.C., announces that the next award of the Storer-Bennett Scholarship will be made early in 1915, and theses should be in the hands of the Dean by December 31st, 1914.