

The Serum Reaction in Pregnancy and Cancer by the Coagulation Method.

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IN the short time which has elapsed since the publication of Abderhalden's biological diagnosis of pregnancy, a vast number of observations have been carried out on the serum of pregnant patients, but from the difficulties which surround the test, the chief result of this work has been to demonstrate and correct errors in technique rather than to prove or disprove Abderhalden's claims. It is too early, therefore, to make any precise statement as to the clinical value of the test, but I nevertheless submit my results in the hope that they may throw some light on the difficult questions raised by this reaction.

The test is based on two principles: first, that the body reacts to the intravascular injection of albumen by the production of a ferment to digest the foreign substance; and second, that during pregnancy chorionic epithelium is carried into the circulation. Knowing these two facts, Abderhalden assumed that the presence of chorionic epithelium in the blood must call out proteolytic enzymes to split up the placental albumen into peptones and amino-acids. He has proved beyond doubt that this cleavage does occur; and, since it can also be shown that the ferment appears within the first few weeks of pregnancy and disappears within fourteen days after delivery, it is clear that it is specially produced for pregnancy and does not exist preformed in the blood. Further, the serum of healthy non-pregnant individuals does not contain a ferment capable of causing proteolysis.

A ferment can only be recognized by the products of its digestive action, and there are three methods by which we can demonstrate the disintegration of the chorionic albumen after it has been acted upon by a ferment-containing serum, namely:—

- (1) An optical method.
- (2) A dialyzation method.
- (3) A coagulation method.

I have no experience in the *optical method*, but it depends upon the change in rotation of the plane of polarized light before and after incubation of pregnant serum and placental peptone.

The dialyzation method. This is based upon the fact that an animal membrane is impermeable to albumen, yet the products of proteolytic digestion are readily diffusible. When, therefore, the

serum of a pregnant patient is incubated with placental tissue in a dialyzer the peptones and amino-acids will diffuse out and can be tested for in the surrounding water.

I have carried out the test by this technique a considerable number of times with satisfactory results, but since it is not the one which I have used throughout this series of cases it is unnecessary to describe it further.

The coagulation method. This method was used by Opie¹ in his work on the ferments in leucocytes, and has been applied to this reaction by Williams and Pearce.² The products of digestion are obtained by coagulating the residual albumen, the watery extract of which will contain the peptones, which are not, of course, coagulable by heat.

Preparation of the Placenta.

Fresh placenta without the membranes is cut into small pieces and washed free from all blood in salt solution. This process is much assisted by wrapping the tissue in a cloth and kneading it in a large basin of salt solution. The pieces are then thrown into ten times their volume of boiling water with a few drops of glacial acetic acid. After boiling for five minutes the water is strained off and the tissue well washed in distilled water. The whole process is then repeated until the filtered water extract gives no reaction for peptones. "Ninhydrin," the chemical name of which is triketohydindendydat, is the reagent used to test for these bodies. This salt gives a deep blue colour on boiling with albumen, peptones and amino-acids. The placental tissue may be regarded as peptone-free when 10 cc. of the filtered watery extract gives no colouration on boiling for five minutes with 1.0 cc. of a 1 per cent. solution of ninhydrin. The prepared placenta is stored in chloroform water in wide-mouthed sterile glass jars covered with a layer of toluol. The placenta will keep for two or three months, but since the albumen sometimes undergoes disintegration the tissue must be re-tested before each experiment is carried out.

The Serum.

About 10 cc. of blood is withdrawn from a vein by means of a sterile glass syringe, and gently transferred to a sterile centrifuge tube in such a manner as to avoid any frothing which will tend to cause hæmolysis. Clotting is hastened by incubation for half an hour at 37°C. The clear serum is obtained by slow centrifugalization. There is some danger of causing hæmolysis by centrifugalizing, so that it is better to avoid it if possible. Hæmolyzed serum is quite useless.

The Technique of the Test.

About 1 gram of the prepared placenta is pounded up in a sterile mortar and then transferred with sterile dissecting forceps to a sterile test tube to which is added 1.5 cc. of serum. A similar tube containing serum alone is put up as a control in order to make sure that it

does not contain disintegration products of albumen. Toluol is added to each, and the tubes, plugged with wool, are incubated at 37°C. for 20 hours. This test requires longer incubation than the dialyzation method. After incubation the toluol is pipetted off and the contents of the tubes are coagulated in a boiling water-bath after the addition of a few drops of 5 per cent. acetic acid; 10 cc. of distilled water is added to each tube and the coagulum well broken up and stirred with a glass rod. The filtered watery extract is then tested by boiling with 0.3 cc. of a 1 per cent. solution of ninhydrin for one minute. It is well to add a couple of glass beads to prevent bumping. If a blue or violet colour appears within half an hour the test is positive. The control, tested in the same way, should remain colourless, but, if some colour is present, provided that the "test" is a much deeper colour than the control, it may still be regarded as a positive reaction. As a matter of fact it very rarely happens that the controls do show any colour when the technique is used, unlike the dialyzation method in which it fairly often happens that one has to estimate between two shades of violet in comparing the "test" and the controls.

Fallacies.

Ninhydrin does not react to all dilutions of peptones and amino-acids, for, as Jellingham and Losee³ have pointed out, 0.00001 gram of these bodies must be present in 10 cc. of water in order to obtain a positive reaction with the reagent. It may happen, therefore, that traces of reacting substances are present in the serum and placental tissue but in such small quantities, that both, when tested alone, fail to give a reaction with ninhydrin, yet the sum of the two amounts may react when the sero-placental mixture is tested, although no digestion has taken place. This source of fallacy can be avoided to a very large extent by very careful preparatory testing of the placenta with an excess of the reagent (1.0 cc. of a 1 per cent. solution). The blood may contain cleavage products of albumen just after a large protein meal, in cachexia, when large hæmorrhages or effusions are being absorbed, and, possibly, in some cases of disturbances of metabolism or excretion. Blood must not therefore be taken after a meal, and the positive results obtained in the other conditions must be looked upon with some suspicion as being possibly due to this cause. Another common source of error lies in the use of hæmolysed serum which always gives a positive reaction. But as all sera give the blood spectrum it is sometimes difficult to know whether sufficient hæmolysis has taken place to render the serum valueless of the test, and I know of no way of determining it. From my experience with both the dialyzation and coagulation methods I am inclined to think that the latter method is much less susceptible to the lesser degrees of hæmolysis than the former. Finally, the most

careful attention to strict asepsis and cleanliness is essential throughout every step of the technique.

The following table amply confirms Abderhalden's positive results in pregnancy, and it will be seen that the reaction is obtained at all periods of gestation. It is especially interesting from a clinical point of view that ectopic gestation can be diagnosed by this means.

TABLE I.
REACTIONS AT VARIOUS PERIODS OF PREGNANCY.

Number.	Period of Pregnancy.	Result.
0 1 month —
1 2 „ +
3 3 „ +
1 4 „ +
1 5 „ +
3 6 „ +
0 7 „ +
5 8 „ +
6 9 „ +
3 Ectopic gestation +
1 Vesicular mole +
1 Missed abortion +

Total 25

Two cases of recent septic abortion gave negative results. Both patients presented the symptoms of severe septic infection with high temperature and rapid pulse, and the uterus contained decomposing placental tissue. Apart from faulty technique, which tends to produce positive rather than negative results, the only explanation which I can offer for these two anomalous reactions is one based upon the experiments of Opie.⁴ This observer demonstrated that the serum of *pus* contains bodies which have strongly anti-proteolytic powers. It is possible therefore that in acute septic infections similar substances are present in the *blood* which inhibit the action of the proteolytic enzymes of pregnancy.

Five sera were examined and gave positive reactions, but they are of no value for the purposes of this paper since the clinical diagnosis remains uncertain. They include three cases of pelvic hæmatocele in which sections of the tube fail to demonstrate evidence of gestation and which might have been cases of hæmorrhagic salpingitis. The two other patients gave histories suggesting recent abortion, but no products of conception were found upon microscopical examination of the curettings. A myoma undergoing red degeneration was found in the uterus of one of the patients and this fact alone may explain the positive reaction, for the histological

picture of necrobiosis is consistent with the results of ferment action, and the frequency of these changes in myomata during pregnancy lends some support to the suggestion that necrobiosis is caused by ferments.

The blood of 14 non-pregnant cases was examined to serve as controls, and one only, a man with chronic parenchymatous nephritis, gave a positive reaction. This was one of my early cases and may have been due to faulty technique.

TABLE II.
REACTIONS IN NON-PREGNANT PATIENTS.

Number.	Disease.	Result.
1	Salpingitis	Neg.
2	Salpingitis	Neg.
3	Uræmia	Neg.
4	Uræmia	Neg.
5	Cirrhosis of liver	Neg.
6	Cirrhosis of liver	Neg.
7	Cirrhosis of liver	Neg.
8	Optic atrophy	Neg.
9	Acute appendicitis	Neg.
10	Phthisis	Neg.
11	Aneurysm	Neg.
12	Tabes	Neg.
13	Chronic nephritis	+
14	Guinea-pig (male)	Neg.

The reaction can be applied not only for the detection of pregnancy, but also in the diagnosis of malignant growths, using cancer tissue in place of placental albumen. It has been claimed that carcinoma can be differentiated in this way from sarcoma, and I have one case of melanotic sarcoma which bears this out. This patient's serum digested one of her own glands which contained secondary deposits but did not react with carcinomatous tissue (*vide* Table IV.).

TABLE III.
THE REACTION IN MALIGNANT DISEASE.

Number.	Disease.	Result.
1	Carcinoma of cervix (early)	+
2	Carcinoma of cervix (late)	+
3	Carcinoma of tongue	+
4	Carcinoma of stomach	Neg.
5	Melanotic sarcoma (late)	+
6	Sarcoma of knee	Neg.

All of these carcinoma cases reacted except one of, presumably, advanced carcinoma of the stomach, but in which the abdomen was

not opened to verify the diagnosis. The sarcoma of knee did not react with a piece of gland from the case of melanotic sarcoma. This failure may have been due to the difference in the type of growth, but it is much more probable that the very small piece of tissue available for the test did not contain any, or enough, sarcomatous cells. The microscopic examination showed a very patchy arrangement of the new growth in the glands, and it would be easily possible, therefore, to have missed a secondary deposit.

But before we can diagnose either pregnancy or cancer with any degree of certainty it is necessary to determine whether the ferments of the serum of these patients are specific or not. In other words, Will the serum of carcinoma digest placental albumen, or the serum of pregnancy split up cancer tissue?

Abderhalden states that the ferments are absolutely specific and limited in their action to one particular albumen. Such a statement requires confirmation, and up to the present time contradictory reports have been published by the various workers who have experimented with this reaction. My results indicate that the reaction is not specific, although there is some evidence that the ferments react more strongly to their own type of albumen.

In the following table it will be seen that the serum of pregnancy and malignant disease was tested against various tissues. The albumen was obtained from the urine of a patient suffering from albuminuria of pregnancy, and it is interesting to note that her serum was the only one out of four pregnant patients which did not digest this substance. The observation may be of some importance in the ætiology of albuminuria of pregnancy, and I am carrying out some further observations on the subject. The carcinoma tissue was obtained from a case of advanced carcinoma of the cervix, and both this and the albumen were prepared exactly in the same way as the placental albumen.

TABLE IV.

SHOWING THAT THE FERMENTS ARE NOT SPECIFIC.

Period.	PREGNANCY.			
	Placenta.	Albumen.	Carcinoma.	Sarcoma.
Ectopic	+	—	+	—
Two months	+	—	Neg.	—
Missed abortion	+	—	Neg.	—
Vesicular mole	+	—	+	—
Six months	+	—	+	—
Six months	+	+	—	—
Eight months	+	+	—	—
Nine months	+	+	—	—
Nine months	+	Neg.	—	—

MALIGNANT DISEASE.

Disease.	Placenta.	Albumen.	Carcinoma.	Sarcoma.
Cancer of cervix	Neg.	—	—	—
Cancer of cervix (late)... ..	+	—	+	—
Cancer of cervix (early) ...	Neg.	—	+	—
General malignant disease ...	Neg.	—	—	—
Cancer of tongue (late) ...	+	++	+++	—
Cancer of stomach (late) ...	Neg.	—	Neg.	—
Melanotic sarcoma (late) ...	Neg.	—	Neg.	+
Sarcoma of knee (late)	+	Neg.	Neg.	Neg.

Out of the nine pregnant sera, six digested other albumens besides placenta, thus demonstrating that the ferments of pregnancy are *not* limited in their power of digesting albumen.

Of the eight cases of malignant disease, three digested placental tissue. These three cases included the one of sarcoma of knee, which I have already referred to as not reacting to the secondary gland of melanotic sarcoma. The digestion of the placenta shows that a ferment was present, and rather suggests that a positive result might have been obtained if suitable tissue had been available. Urinary albumen was not, however, digested by this serum, though a case of epithelioma of the tongue reacted strongly to it. Three sarcomata were negative to carcinoma tissue.

Thus out of 17 cases of pregnancy and malignant disease, nine sera digested albumens of a different type from that against which, *ex hypothesi*, they were produced. Hence if the observations are accurate the ferments are not specific.

Abderhalden believes that such results as these are due to faulty technique, and only further observation can settle the question.

As to the actual clinical value of the test, it must be remembered that we are still in the experimental stage, but I believe that it will prove a useful adjunct to the diagnosis of doubtful pregnancies, ectopic gestations, missed abortions, etc. It may also be useful in the early recognition of chorion-epithelioma following vesicular mole, as recently suggested by Williamson.⁵ Carcinoma and sarcoma may be diagnosed, though, so far as I can see, we shall not be able to differentiate them from pregnancy. This limitation, however, would not seriously interfere with the clinical usefulness of the test. Further research in this reaction may throw light on eclampsia and toxic vomiting of pregnancy. If it can be shown, for instance, in the latter condition that the ferments are absent or diminished in amount we shall have a rational basis for the treatment of this condition by the injection of the serum of healthy pregnant patients.

Conclusions.

- (1) The test is positive all through pregnancy.
- (2) It may be negative in pregnancy in the presence of severe septic infection.
- (3) With certain limitations it is possible to diagnose carcinoma and sarcoma, but not to differentiate them from pregnancy because the ferments are not absolutely specific.
- (4) The coagulation method is useful because it does not require special apparatus; it avoids the errors associated with faulty dialyzers, and it is not so susceptible to slight hæmolysis of the serum. This method, however, requires at least 20 hours' incubation and the use, in my experience, of 0.3 cc. of a 1 per cent. solution of ninhydrin in order to obtain positive results in pregnancy.

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