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CRITICAL REVIEW.

The Value of Abderhalden's Tests in the Diagnosis of Pregnancy.

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ALTHOUGH it cannot be said that the biological test for pregnancy devised by Abderhalden has so far attained its maximum development yet the time seems ripe briefly to review the present state of our knowledge upon this subject. The test has for its basis the principle that foreign substances when introduced into the blood stream excite the production of specific ferments in order to destroy them. The chemical changes which such specific ferments induce are capable of detection outside the body by the application of suitable methods. In order fully to comprehend such action we must briefly refer to the more minute processes of protein digestion, which can be followed in the gastro-intestinal tract, and also in experiments *in vitro*. The pepsin in the gastric juice attacks the proteins in the foodstuffs in the presence of hydrochloric acid, breaking them up into albumoses and peptones. These latter substances are further attacked by the trypsin in the pancreatic juice, and the erepsin in the intestinal secretion with the formation of peptides and amino-acids. The proteins in the food are therefore reduced by ferments in the alimentary tract, and the resulting products—the amino-acids—constitute the "Bausteine" of Abderhalden. These "Bausteine" or "building stones" are absorbed into the blood-stream, and each cell takes from the blood amino-acids in proportion to its individual requirements. Over this absorption and distribution of amino-acids the liver exerts a protective influence preventing any foreign elements from passing into the circulation by acting upon undigested protein. It also controls the quantity of these amino-acids entering the blood-stream. The lymphatic system likewise prevents the entrance of body cells into the circulation. The existence of such defences makes it possible to keep the blood fairly constant in composition, and also protects the blood from invasion. Any foreign material entering the blood-stream at once excites the

production of a specific ferment which breaks up the foreign substances into the molecules originally entering into their formation. Physiologically the introduction into the blood of foreign materials from the alimentary tract produces ferment changes which have a special character according to the chemical nature of the body introduced. An example is the change which cane sugar undergoes when introduced into the circulation through the intestinal tract. The cane sugar produces an increase in the ferment invertin which destroys it. Another similar phenomenon is the observed increase of fat splitting power of the serum after an excessive absorption of fat from the intestine. It will be seen therefore that not only does the foreign body give rise to ferment production, but the ferment so produced has a specific nature. Experimental work with these specific protective ferments indicates that they may be used not only for the diagnosis of pregnancy, but also for the diagnosis of various pathological conditions. The invasion of the blood-stream by chorionic villi, malignant tumour cells, broken-down gland tissue, bacillary proteins, and in fact any foreign cell is sufficient to excite the production of ferments. The action of such ferments is to digest the foreign cells and return them to the blood in their original state, *i.e.*, as the "Bausteine."

Foreign substances are known to produce anaphylaxis, and these are broken down and lose their individuality. The kidney tissue of a dog when introduced into the circulation of a second dog results in the production of a ferment capable of digesting kidney tissue. Therefore even those substances which actually form part of the normal tissue when thrown into the circulation excite the formation of ferments which bring about their own cleavage.

Schmorl, Veit and Weichardt have demonstrated that chorionic epithelium entered the circulation during pregnancy, but they did not state that this change took place during the first month of gestation. That chorionic villi are present in the fertilized ovum in the first month of pregnancy has, however, been proved by Peters, Stahl and Beneke, Brice and Teacher. With this knowledge Abderhalden evolved the two tests for the diagnosis of pregnancy. The presence of chorionic villi circulating in the blood of a pregnant woman as a foreign substance calls for the specific ferment. The serum of a pregnant woman should therefore be capable of digesting placental tissue with the production of amino-acids, and Abderhalden found this actually occurred. Two tests have been devised known respectively as the optical and the dialysation test, and have been subjected to a thorough examination by Abderhalden.

That placental tissue plays a prominent part in the production of a protective ferment has been proved conclusively by Abderhalden, by a number of animal experiments. The serum of a pregnant animal can be inactivated by heating to 60°C., thus demonstrating

that the ferment is destroyed by exposure to this temperature. On the other hand, the serum of the foetal blood, and foetal tissue, does not contain this ferment. An extract of human placental tissue in salt solution, and also human placental peptone was injected into dogs, rabbits, and guinea-pigs, either intravenously or intraperitoneally; the blood of normal animals mixed with placental peptone being also used. In the case of the dogs, two injections of 1 gm. of placental peptone were given on successive days, the blood collected eight days afterwards, and the serum tested against placental peptone by the optical method. In every case a breakdown of the placental peptone had occurred. The rabbits received four intravenous injections of 2 to 3.5 cc. of placental extract, and six days afterwards the serum when tested gave a similar result. The same changes occurred in guinea-pigs after injections of 0.6 cc. of placental extract into a shin vein. These results conclusively proved that a ferment is present in the blood-stream of pregnant animals, capable of detection by the optical method. The presence of this ferment in the blood-stream was further demonstrated by dialysis.

A large number of investigators have since clearly and sufficiently proved the value and reliability of these tests for pregnancy, and an extensive literature has already accumulated upon this subject. The application of the tests to pathological conditions, particularly cancer, tuberculosis, and nervous diseases, has also received attention.

The Optical Test.

The growing placenta is regarded as the agent providing the foreign protein substances which excite the production of a protective ferment in the maternal blood-stream. Owing to its proteolytic nature we are enabled to recognize the breakdown products which are the essential factors in both the tests described. The materials necessary are, first of all, the blood-serum of the patient to be examined, and a supply of fresh human placenta. The optical test requires the use of a good polarimeter capable of giving readings below 0.01° , and in addition special polarimeter tubes for maintaining a constant temperature. After some practice the readings are readily made, and differences of rotation determined. The material used upon which the ferment is to act is a 5 per cent. solution of placental peptone in physiological salt solution.

The optical test, depending as it does upon the disintegration of placental peptone by the specific ferment in the serum of pregnant women, is by far the most reliable of the two tests devised by Abderhalden. He has given full details of the procedure employed, and other workers who have used this test also refer in detail to the technique, particularly Abderhalden Freund and Pincussohn, Henkel, Macbrunni, Aeshner, Rubsamen, Freund and Brahm, Schafer, Bolaffio, Tschudnowsky, and the writer. With the

use of a good polarimeter, and careful preparation of the placental peptone, the results obtained are reliable, and with rare exceptions the results obtained by means of the optical test have provided a correct diagnosis. The degree of activity of the protective ferments can also be ascertained, and it is only by the use of this method that we are likely to obtain further information as regards the nature of such ferment changes. In the diagnosis of pregnancy greater stress should be laid upon a positive than upon a negative result of this test. In the series of cases investigated by the writer the optical test proved correct on every occasion, and in two cases where it was not performed owing to lack of material the result of the dialysation test was incorrect. Further, in a case where the dialysation test proved positive and the optical test negative, it was found that the patient had partaken of a meal one hour previously, and on repeating the tests later both proved negative. Abderhalden himself has investigated a large number of cases with perfectly concordant results, and, provided his procedure is followed in every detail, the same accuracy should be obtained. The large number of failures recorded are in all probability due to non-compliance with the details of the method. It will be seen from a perusal of the following account of the work of others upon the optical test that the results are on the whole quite satisfactory. Two sources of failure which I have observed in my own work should be mentioned here. In the first place, centrifugalization of the blood at high speed is very undesirable, since by this procedure the ferment is destroyed or carried down in the sediment. The best way of securing the serum is to place the blood in cold storage for about an hour, and then pipette off the clear serum which separates. The few remaining corpuscles in the serum can then be easily removed by gentle centrifugalization, preferably with a hand or water centrifuge. The variations in different samples of placental peptone may also give rise to erroneous results, and to avoid this every preparation should be tested from time to time against serum from a known case of pregnancy.

Abderhalden Freund and Pincussohn, working with the optical method, have reported numerous failures to obtain a positive reaction in pregnant women. They record 14 negative tests in 14 cases in the 9th and 10th months of pregnancy. These results are, however, not surprising as it is well known that the reaction becomes weaker in the later months of pregnancy. Abderhalden states that in his own work he has not experienced a single failure with the optical method, and attributes the errors recorded by Freund and Pincussohn to the placental peptone preparations which were used.

Henkel, in the women's clinic in Jena, investigated 40 cases and obtained reliable results in normal as well as pathological pregnancies. One case of extrauterine gestation with a positive reaction was of interest, as at operation a gestation sac was found on one side,

and an inflammatory tumour of the opposite appendages. Henkel gives a full account of the technique employed in both the optical and the dialysation methods. In the optical method he regards a rotation above 0.04° as a positive result. The work he carried out upon eclampsia is of interest as he found that the placenta of an eclamptic woman was not attacked by the serum from the same, and that only normal pregnant serum would split up eclamptic placenta. The cerebro-spinal fluid from a case of eclampsia, on the other hand, gave a very marked reaction with its own placenta, but only a weak reaction with normal placenta. The reverse was true of the bile from the same case.

Macbrunni's results include observations with both the optical and the dialysation method, and were applied to 100 cases. In 85 cases of pregnancy only one negative result was recorded, and two or three were doubtful. He also obtained positive results fourteen days after labour, and also after abortion. Only once did he find a positive reaction in the absence of pregnancy, and this was in a patient with a cystic ovary. Contrary to the results of Decio he found a positive dialysis test with foetal serum, and he suggests the possibility of the production of foetal protective ferments in the mother. Further, liquor amnii at times was found to give a positive reaction, but in the urine nothing definite could be determined. The cerebro-spinal fluid was negative in two cases of eclampsia. In albuminuria, severe vomiting, and eclampsia, the reaction of the protective ferments was neither very weak, nor very strong.

Aschner, working in Veit's clinic, has reported his results upon 61 cases of pregnancy with control observations on 59 other cases. He found 18 tests incorrect, *i.e.*, 30.5 per cent. In the first half of pregnancy 16 cases were investigated, 14 being positive and two negative, whilst in the later months of pregnancy, out of a total of 45 cases, all reacted positively. In one of the two failures recorded the test was controlled in Abderhalden's Institute, and gave a positive result. Of 12 non-pregnant cases a negative test was found in each instance, but in 14 cases of carcinoma of the uterus, 8 were negative, 3 positive to the dialysation method, but negative to the optical test, whereas the other three cases were slightly positive to dialysis, but no optical test was performed. This gives an error of 5 per cent. In 23 cases of disease of the adnexa 17 were negative, two positive, and the rest only faintly positive. He examined the sera of 4 cases of hæmorrhage in the climacteric due to metritis, and found 2 were positive, one faintly positive, and one negative. Five cases of amenorrhœa in the climacteric were examined, 4 being negative, but 2 were slightly positive with the dialysation method, one of these being negative with the optical test. Negative results were obtained in 4 cases of myoma, and 6 cases of chlorosis.

Both the optical and the dialysation methods were employed by

Rubsamen in his series of 94 cases. In the 100 tests applied, 38 were done by dialysis alone, 6 by the optical method alone, and 56 by both methods, and only one failure is reported. The 47 cases of normal pregnancy all gave a positive reaction, and this was most marked in the first half of pregnancy. The sera of 13 cases of eclampsia were tested, and 10 were found to digest placenta, but in the 3 severe cases no reaction was obtained. Rubsamen concludes that the test is entirely reliable, and advises the use of 1 cc. of serum only in emaciated subjects. In the non-pregnant series, 22 cases were investigated, including prolapse, myomata, adnexal tumours, parametritis, etc., and the reaction was always negative. In 14 of these cases both methods were applied.

Freund and Brahm employed both the dialysation and optical methods in their investigations. In 141 cases the optical test was applied in 134 instances, two tests each having been done in six of these cases. The dialysation test was used in 99 cases, and was negative on 29 occasions. In comparing the optical and dialysation methods agreement was found in 61 cases, 43 being positive and 18 negative, *i.e.*, out of a total of 92 cases, 31 did not confirm the results obtained by the optical method. The results were confirmed by the optical test in 72.4 per cent., and in the dialysation test in 66.7 per cent. They attributed their failures in the optical test to the changeable characters of the placental peptones with various sera. The cases included normal as well as pathological pregnant women, extrauterine gestation, and 17 cases of eclampsia in all stages of pregnancy, also several non-pregnant cases. Of three cases of ectopic gestation one was positive, and in the four cases of adnexal swellings one was positive to the dialysation test and negative to the optical test. It is of interest to note that they observed no excessive rotation in the eclamptic cases. They remark that the fresher the serum used the better the chance of accuracy in the tests employed.

Schafer made dialysation tests on 123 occasions, and controlled these by the optical method in 65 instances. The cases of pregnancy numbered 62, and of these 2 failed to react. In 11 of the non-pregnant series positive results were obtained, and many of these were suffering from malignant disease or myomata. The 65 optical tests which were carried out on these cases were generally correct, but one positive rotation was found in the serum from a case of uterine fibroid, and a negative result in a pregnant woman. The sera of two cases of pregnancy and two cases of carcinoma was found to split both carcinoma and placental tissue.

Bolaffio has utilized the optical method in his work, and reports correct results in 70—80 per cent. of pregnant women. In non-gravid conditions the optical test was always negative. From his results he concludes that a positive result is more valuable for affirming pregnancy, than a negative result for denying the existence of pregnancy.

Tschudnowsky's work includes results with both the optical and the dialysation method, both tests being applied in 26 cases, the dialysation test alone in 6, and the optical test alone in 8 cases. Of the 40 cases investigated, 17 were normal pregnant women, and all reacted positively to the optical test on 13 occasions, and the dialysis test on 10. The sera of 3 cases of tubal pregnancy were positive, twice to the optical test and three times to dialysis. A case of hydatidiform mole gave a positive optical test, but a negative dialysis test. As controls, three non-pregnant women and two males reacted negatively to both tests. A number of gynaecological conditions were investigated, including 5 cases of pyosalpinx, 5 endometritis, 1 ovarian cyst, 1 myoma, 1 carcinoma and 1 hæmatosalpinx. All of these reacted negatively to both tests with the exception of one case of pyosalpinx which gave a positive optical test.

The Dialysation Method.

Behne did not find the dialysation test absolutely specific for pregnancy in the 100 cases which he investigated. He deletes the first 40 cases because he thinks his procedure may have been defective. In this first series of 40 cases of pregnant women during the last weeks of pregnancy all except one reacted positively. Of the controls done upon 30 cases suffering from various diseases, 13 reacted positively, and 17 negatively to placental tissue, and those who gave a positive result nearly all had some suppurative affection of the genital tract. With the employment of his improved technique Behne used 60 sera, and in 12 pregnant women eight were positive, but two who were near to full term did not react. In 4 cases of ectopic gestation, confirmed by operation in each case, 3 were positive and one negative. The series of 15 cases, where infection followed from 7 days—3½ months after confinement, showed a positive reaction in 15, and two failed to react. As controls he took 26 women who were not at the time, and had not for a long time been pregnant, and found that half were positive and half negative. Three men suffering from advanced tuberculosis also reacted positively to the dialysation test. Although he found the reaction almost constantly positive in pregnant women, a large number of non-pregnant women suffering chiefly from inflammatory processes in the genital organs or elsewhere were found to give positive reactions. The same applied to males, but only in cases of pulmonary tuberculosis, or diseases of the liver.

Ekler obtained with the dialysis test constantly negative results in 25 non-pregnant individuals, and positive results in 37 pregnant women, making a total of 62 cases examined. Of his cases of pregnancy, 11 were in the first week, 5 in the second week, 6 in the third week, 3 in the fourth month, 2 in the sixth month; four were ectopic pregnancies, and six were incomplete abortions. He regards the test

as of especial importance in the diagnosis of early pregnancy, extra-uterine gestation, and amenorrhœa at the time of the climacteric.

Englehorn applied the dialysis test in 108 cases in all, and took the precaution always to withdraw the blood at a definite hour—4 p.m. His results with this method are, however, in striking contrast to those of other workers, and he concludes that the reaction is not specific, also that there is no justification for basing a diagnosis upon it. Of 60 pregnant women examined, 49 were positive and 11 were negative, the sera of the latter being taken in the last weeks of pregnancy. The sera of 48 non-pregnant cases showed positive results in 31 and negative results in 17. This author also gives his experience of the test as applied to carcinoma, having used both ovarian and liver carcinoma tissue as substrate. Twelve pregnant women were tested as to the latter tissue, and 10 reacted positively and 2 negatively. In 11 non-pregnant individuals 8 were positive and 3 negative, and amongst these latter there was actually one case of carcinoma. Using ovarian tissue, 3 non-pregnant women gave three positive results, whereas in 3 pregnant women under the same conditions the serum was positive in one instance, and negative in the other two cases. The results were also very diverse when foetal liver was used as substrate, both positive and negative tests being found.

Epstein has published an account of his work upon the sera of carcinoma and of pregnancy. In 37 cases of carcinoma 36 gave a positive reaction with carcinoma tissue, but all were negative to placental tissue. The sera of 17 pregnant women gave a positive reaction with placental tissue, but negative results with carcinoma tissue. These results are very striking as illustrating the specificity of the protective ferments.

Frank and Heimann, using the dialysation test with fish bladders and the biuret reaction, confirmed Abderhalden's results in 33 cases, where only one or two menstrual periods had been missed. Twenty-three of the patients returned later, and in 21 the positive reaction, which had been previously obtained, was confirmed. Those with a negative reaction, notwithstanding the amenorrhœa, were found subsequently to be non-pregnant in six instances. In the puerperium a positive reaction was obtained until the 7th day, but after the 13th day a negative test resulted, and it appeared immaterial whether at full term or after abortion. In a case of extrauterine gestation the test was positive, and operation showed an inflammatory condition in both adnexa, but no signs of an ectopic gestation sac. It was later found that this patient had had an abortion before admission to hospital. The same result was found in a second case which came under their notice. The authors conclude that the technique of the test is too difficult, and the results too uncertain, for it to be employed in general practice.

Gambaroff has investigated the dialysation method in its applica-

tion both to pregnancy and malignant disease. A positive result was found in 22 cases of pregnancy, and a negative in 10 normal sera. For the carcinoma test he used tissue from carcinoma of the ovary and uterus as the substrate, and in 54 cases of carcinoma found a negative result in 53, *i.e.*, 98.2 per cent.* In two cases of syphilis one gave a positive Abderhalden test, and one a negative.

Gutman and Druskin applied the dialysation test on 202 cases, performing in all 357 tests, but found some difficulties in technique in their earlier cases. With improved methods they applied 159 tests to 106 cases, and obtained correct results in 98 per cent. Of the two cases in which they failed one was that of a male with syphilis who gave a positive Wassermann reaction. The serum of this patient gave an indistinct biuret, and a doubtful ninhydrin reaction. The other was that of an ectopic gestation with a negative reaction. The total of 106 cases included 79 pregnant women and 27 non-pregnant individuals, including 11 males.

Heaney and Davis have reported their results both with and without the improvements in technique, particularly as regards the preparation of the placental tissue. Using, first of all, placental tissue, which had neither been re-boiled nor re-tested just prior to the test; they obtained positive results in four cases of pregnancy, but of 7 non-pregnant cases, 5 reacted positively and 2 failed to react. Of these 5, two were males with syphilis, one a woman who had been operated upon a week previously for tuberculous peritonitis, and had had the uterus and appendages removed a year before, and another a woman, clinically non-pregnant, with amenorrhœa for months, but with multiple rectal ulcers which were probably syphilitic in nature. After rendering their placental tissue free from diffusible substances they tested the sera of 17 individuals. Five of these were non-pregnant, but one of the five reacted positively. This patient had periods of amenorrhœa from causes not ascertained. Of the 7 women pregnant at all periods, two in the 6th and 14th week respectively, who subsequently developed all the signs of pregnancy, were negative. In 5 puerperal women two had been delivered 13 and 20 days prior to the test, and both were negative. The three other women were tested earlier in the puerperium and reacted positively. These writers question the value of the Abderhalden test on their findings.

Heilner and Petri do not regard the reaction as specific, as they found that whereas the serum of pregnant women gives a positive reaction other sera will also give the same result. This applies not only to placental tissue but to other tissues as well, since the sera of both pregnant and non-pregnant women give a positive reaction. They tested the blood of two individuals against various tissue proteins with negative results, but on injection of small quantities of

* Five cases of sarcoma were tested against sarcoma tissue with positive results but sarcoma serum was negative with carcinoma tissue and vice versa.

the serum into the same person the serum obtained a few hours afterwards now gave positive results with placenta, liver, muscle, and other tissues. It is quite conceivable that in their work they have neglected some important factors, particularly as the previous work of Petri has been shown to be erroneous. A reply to their criticism of the specificity of the protective ferments has been made by Abderhalden, and also by Plotkin.

Heimann confirms Abderhalden's work in every respect in the 100 cases which he examined, and regards the test as of value in the diagnosis of pregnancy.

Jamison and Cole have published the result of their work upon 50 cases. Of the pregnant women, 3 cases of 7—9 months gave a positive test, also 3 cases of normal pregnancy, two of ectopic gestation, and two abortions—one about six months and the other two months. One still-birth, on the other hand, gave a negative reaction. In a case of pernicious vomiting of pregnancy a positive result was obtained. In their controls one case—a non-pregnant woman in the menopause—was negative, and 10 females and 13 males with diverse diseases were also negative. There was one case of nephritis in their series with a positive reaction, and another patient reacted after a meal.

Jaworski and Szymanowski investigated 70 cases by the dialysis method, and as a result of their work they came to the following conclusions:—(1) That the reaction occurs in the early months of pregnancy and persists until 14 days after delivery; (2) the test is of value in the diagnosis of extrauterine gestation; (3) the serum of carcinoma patients does not split placental protein.

Jonas applied the test to 50 cases, and in only two did he obtain erroneous results, one negative reaction occurring in a woman after abortion, and one positive result in a case of fibroids. Two women with ulcerative tuberculosis were negative, 2 carcinomatous sera reacted negatively to placental tissue, and 3 pregnancy sera reacted negatively to carcinoma tissue.

Jellinghaus and Losee based their observations on the dialysation test on 563 individuals, including 370 pregnant women, and 193 non-pregnant. They divide their results into six series according to the actual nature of the technique adopted. In many cases their results were unsatisfactory owing to certain imperfections, but in the last series with many improvements in the method they obtained better results. The sera of 89 pregnant women gave 5·5 per cent. of negative reactions, and in 49 non-pregnant sera there were 8 per cent. of positive reactions. Their work represents an interesting and laborious attempt to master the technique, and as such deserves particular commendation.

Judd found that race conditions did not influence the biological test for pregnancy as the serum of coloured pregnant women reacted

equally well. He subjected a number of cases to the *Abderhalden* test, including one carcinoma of the cervix, several infectious diseases, typhoid, septicæmia, pericarditis, endocarditis, and gonococcal urethritis with negative results to placental tissue in every case.

Lichtenstein has utilized the dialysation test in 76 cases with very satisfactory results. In his series there were 42 pregnant women, including 6 tubal pregnancies and 4 cases of eclampsia; and 34 non-pregnant cases. The reaction was always negative in the non-pregnant individuals, and as three tests were improperly made, they were excluded. He found that the positive reaction of pregnancy disappeared during the third week of the puerperium. His results should be compared with those of *Henkel* with regard to eclampsia. The cerebro-spinal fluid and also umbilical blood failed to attack either normal or eclamptic placental tissue. The serum from eclampsia was found to split rapidly both normal and eclamptic placenta, whereas *Henkel* stated that only normal pregnant serum would reduce eclamptic placental tissue. *Lichtenstein* further observed that normal serum was not very different in its action from that of serum obtained from a case of nephritis in pregnancy, both upon normal and eclamptic placenta. A negative reaction was obtained with amniotic fluid from a non-eclamptic woman, and also with ascitic fluid from a non-pregnant individual. He concludes that the test is of value, but in spite of its exactness one should not be governed by it in making a diagnosis. *Lichtenstein* later performed 70 tests, and in only one instance did he find disagreement between the test and the clinical diagnosis. The reaction was found to be negative in a pregnant woman where the ovum had been dead for from 3 to 4 weeks.

Lindig's results with the dialysation method have been assailed by *Abderhalden* on account of his adoption of pulverized placental extract, which is known to give fallacious results owing to the presence of dialysable substances. *Lindig's* preference for this modification was based upon the ease with which such a dried preparation could be stored, and also upon its application as a more exact quantitative method. He also used the dried proteins of cancer, myoma, and dermoids. As a result of his work he came to the conclusion that there were proteolytic ferments in the sera of pregnant women which would digest not only placental albumen, but also the albumens, of the uterus, ovary and of tumours of the genitalia, and in a lesser degree of muscle. This proteolytic power of the serum was also found in cases of tumours of the genital tract, and perhaps also in inflammations.

King has also advocated the use of dried placental tissue in the dialysation test, and supports *Lindig* in this modification of the method.

Lewy only used the dialysation method, and in 20 cases found

this method correct. His series comprised a number of pathological conditions. In four cases of normal pregnancy the reaction was positive, also in 3 cases of ectopic gestation, pelvic hæmatocele, etc. In two cases of doubtful tubal pregnancy the reaction was positive, also in a patient with 7 months' amenorrhœa, and two cases of suspected abortion. In the following conditions he obtained negative results, viz., climacteric, salpingo-oöphoritis, right parametritis, retroflexed uterus (2 cases), and 14 days after abortion (3 cases). One woman 14 days after abortion gave a positive result.

Markus, using the earlier technique of Abderhalden's dialysation method, found a positive reaction in all of his 15 cases of pregnant and puerperal women, and also in 3 cases of ectopic gestation. His controls on 18 non-pregnant cases were all negative. The sera of 5 women suffering from carcinoma were tested against placental tissue with positive reactions in each case. With the use of the improved technique introduced by Abderhalden he obtained 20 positive reactions in 20 pregnant women, one of whom was only ten days past her expected period. Similarly he tested 11 carcinoma patients against placental tissue, and of these, 7 were negative, and 4 faintly positive. On using carcinoma of the uterus tissue and the sera from 8 cases of carcinoma, five were found to give a positive result, and three a negative, and these three had carcinoma of the intestinal tract. Of 7 pregnancy cases 5 of these did not react to carcinoma tissue, and only 2 were faintly positive.

A. Mayer relates a case, clinically one of ruptured ectopic gestation, in which the dialysation method gave a negative result before operation. He suggests the possibility that either there was a failure to furnish sufficient placental protein to excite a reaction, or death of the ovum supervened, since a positive reaction occurred in a clinically questionable case which at operation proved to have an unruptured gestation sac on one side and a ruptured sac on the other. He regards the contradictory results which have been published as due to bad work. In a case described of suspected recent abortion the Abderhalden test was positive, but was apparently unconfirmed by the examination of scrapings from the uterus. Renewed examination of the scrapings, however, revealed the presence of decidual tissue. The test was applied in eclampsia, and various complications of pregnancy, and there was no evidence to show that the protective ferments were not maintained in the blood in such conditions. The reaction is positive with extrauterine as well as normal gestation, but becomes negative in both after the tissues specially connected with pregnancy have lost their vitality due to death of the fœtus.

McCord reports results obtained upon 240 tests with practically no failure, the 5 per cent. of failures being attributed to errors in technique. He used dessicated placental powder, and celloidin

thimbles as dialyzers. Contrary to the results of Abderhalden this writer found that in animal work the controls were nearly always faintly positive even when all mechanical sources of error were removed. These results, however, do not apply to human serum as the controls are rarely positive when the blood is taken some hours after a meal. The author concludes that Abderhalden's dialysation test is both reliable and practical.

Parsamow, with the use of the earlier procedure of Abderhalden, made observations upon 100 cases, but adopted the later improvements in the method in 16 cases. He found not only a positive result in all cases of pregnancy, but also in a large number of other conditions, especially those with cysts, myomata, and some cases of carcinoma. On these findings he regards the test as not absolutely specific, but that a negative test is much more valuable in the differential diagnosis of some cases. His results would tend to show that a positive reaction is not absolutely specific for pregnancy, but in all probability his technique was defective in certain details.

Polano explains his contradictory results by stating that the serum of gestation contains more hæmolysins than does that of other blood. The boiled placental tissue and also the various foetal organs, especially the lung, show a greater hæmolytic activity towards the erythrocytes of pregnant than towards those of non-pregnant women. He found the sera of non-pregnant women positive to Abderhalden's test, and also obtained negative results in cases of pregnancy.

Piorkowski utilized both methods of Abderhalden and obtained correct results in 95 per cent. of his cases.

Petri regards Abderhalden's tests for pregnancy as very reliable. In experimental investigations with placental tissue he noted a positive reaction in the serum seven to eight hours after a subcutaneous injection. Injection of placental tissue intravenously, on the other hand, produced a positive result in 15 minutes. He concludes that a specific ferment arises even a few days after embedding of the ovum, and by this test we are thus able to establish the presence of gestation.

Schlimpert and Hendry have published results obtained upon 316 cases. In the first instance, they experienced difficulties in obtaining their placental tissue free from blood owing to the use of the soft Freiburg water, but this was overcome by the use of saline. The tests made upon 237 cases by the original Abderhalden method were doubtful, but with more careful technique the results were very striking. Of the 79 cases with the use of improved technique 40 were pregnant women, and 39 non-pregnant women, and in no case was a failure reported by the dialysation test; 28 of the pregnant women in this series had missed one period by about four days, and all reacted positively. Of 10 puerperal women, 9 were positive, the one negative case having been confined four weeks

previously; 8 were in the early puerperium, and one at the 13th day. Amongst the non-pregnant series, patients suffering from various diseases were included. The results obtained by these authors clearly prove the claims of Abderhalden when his improved technique is employed.

Schlimpert and Issel have recently shown that the serum of other animals is capable of splitting human placental tissue, particularly that of pregnant mares and pregnant sheep. These results may have an important bearing upon the utilization of the tests for the breeding of cattle.

Schmid has applied the dialysation method in 100 cases. In 14 cases of pregnancy he obtained positive results in every case, but in 19 non-pregnant cases, 5 were positive, 12 negative, and two doubtful. Using placental tissue, he obtained positive results in 4 cases of extrauterine pregnancy, and 2 positive reactions in 3 children. The sera of 16 cases of carcinoma of the uterus were tested against placental tissue, 5 being positive, 4 negative and 7 doubtful. Using ovarian, uterine, myomatous and glandular tissue, he almost invariably found negative reactions, one test being positive to ovarian tissue in the case of a pregnant woman.

Stange obtained positive results in all his 73 cases of pregnancy. In 5 cases of non-pregnancy the sera gave negative results on each occasion.

Steising claims to have separated the active ferment responsible for Abderhalden's reaction into an amboceptor and complement. He inactivates the sera by heating to 58°C. for an hour, and then re-activates it as desired by the addition of fresh male serum. He investigated in this way the sera of 10 pregnant women, and on this basis regards the technique as generally applicable to a large number of pathological conditions.

Schiff, in his work upon the Abderhalden test, brings forward strong evidence as to the value of the method. He employed the dialysation method in 49 cases in which the clinical diagnosis was not known. These cases included early and advanced pregnancies, climacterium, genital tumours, adnexal disease, eclampsia, abortion, puerperium, etc. In all cases of pregnancy he obtained a positive reaction, and in non-pregnant sera a negative reaction with the exception of two cases in which the sera were not in good condition owing to hæmolysis or prolonged exposure to the temperature of the room. Testing the sera of 12 pregnant women against carcinoma, he obtained negative results in every case. He contends that the method is of great diagnostic value, and emphasizes the importance of perfect technique in the application of the test.

Schwarz, following Abderhalden's method, found positive results in 21 pregnant women, and 4 puerperal cases. Of the 18 non-pregnant individuals some were suffering from tubal enlargements,

and 4 from uterine myomata, and these all gave a negative reaction. Similarly two males were negative. The test was applied in eight cases for differential diagnosis with correct results in every case.

Veit's work is of interest since he was the first to suggest the migration of placental elements into the maternal blood-stream, upon which the Abderhalden tests are based. He fully substantiates Abderhalden's observations even for early cases of pregnancy, and considers the reaction a valuable one for diagnosis. Since the reaction has a placental origin, it does not matter whether the ovum be alive or even present provided that living placental tissue is present. He cites the reaction with cobra toxins, and precipitins as analogous processes. According to Veit, the reaction is positive in animals in which the chorionic villi are not immersed in the maternal blood, but the chemical elements of the chorion-epithelium pass through the lymphatic system into the maternal circulation.

Williams and Pearce, employing the dialysation method, have never found the reaction negative in a known pregnant woman. They investigated 28 sera from pregnant women and 8 sera from post partum women, with positive results in every case. One of these latter cases was after an abortion. In addition they found that the serum of pregnant women reacts with tissues other than placenta, *e.g.*, kidney, heart, and uterus. The sera of two cases of nephritis—one of tabes and one of infection (carbuncle), and occasionally those of people in apparently perfect health, reacted positively to placental tissue and also to the other tissues above named. The inactivation of serum is said to produce a great diminution in the degree of reaction, but does not apparently cause it completely to disappear. They conclude that the test cannot be accepted as an accurate clinical method until it is more thoroughly investigated and all possible sources of error corrected. Their conclusions only apply to the dialysation method, and in this their technique is open to question, and Schwarz has recently stated that their results are valueless.

The Value of the Tests in the Diagnosis of Pregnancy.

Having proved that the blood of pregnant women or of recently delivered women contains a ferment specific for placental tissue, and also eliminated to a large extent the possible sources of error in the tests for demonstrating this ferment, the results should prove of value in clinical medicine.

In order to test the correctness of the methods used, the sera of 50 women who were known to be pregnant were investigated by the writer. Of these, 20 women were either in the last three months of pregnancy or had recently been delivered. In every case the optical test, and the dialysation test proved to be positive. The remaining 30 women were in the earlier months of pregnancy ranging from the eighth week to the fourth month, and all gave a positive

reaction with the dialysis test. The optical test was only applied to 10 of these cases, and was positive on every occasion.

In 30 non-pregnant women the reaction was always negative to both the dialysis and optical methods. These patients comprised women suffering from a variety of diseases, including 8 with malignant disease and 14 with syphilis. The sera of 30 males suffering from various diseases were also tested by both methods, and on no occasion was a positive reaction found. A large number of these men suffered from syphilis in its various manifestations.

The test has therefore been applied to 110 cases, in some instances on more than one occasion, and in every instance has proved to be correct.

Not only have these sera been used for the diagnosis of pregnancy, but a number have been tested for malignant disease and tuberculosis by the same test. In eight cases the sera of cancerous patients digested cancer tissue, but did not digest placental tissue. A larger number of cases have since been investigated by Brockman, working in my laboratory, with successful results. The sera of five cases of tuberculosis have also been investigated by means of the dialysation method, using an emulsion of human tubercle bacilli, with positive results in each case. One case of tuberculous salpingitis gave a positive reaction with the emulsion, and a negative result with placental tissue. The control sera in 10 cases gave negative results to tubercle, including one case of hydrosalpinx, which was proved at operation not to be tuberculous. Repeated attempts to apply the test for the diagnosis of syphilis have failed, there being no evidence of the presence of a specific ferment in the blood-stream capable of digesting syphilitic tissue.

The satisfactory results obtained by these tests in the demonstration of a specific ferment in the blood of pregnant women led to the application of the test in the diagnosis of pregnancy in special cases. In all, 18 cases were investigated for this purpose, and were as follows:—

| | |
|--|---|
| Suspected ectopic gestation | 3 |
| Pelvic and abdominal tumours where it was suspected that the whole or part of the tumour might be the pregnant uterus | 6 |
| Suspected chorion-epithelioma | 3 |
| Chorea in a woman of 21 | 1 |
| Heart disease with amenorrhœa | 1 |
| Nephritis with exacerbation of symptoms and amenorrhœa | 2 |
| Late puerperal sepsis | 2 |

The optical test was applied in almost every instance and proved correct. There were, however, two failures in the dialysation method, and these occurred in the two cases of inflammatory adnexal disease, where, unfortunately, owing to lack of material, the optical

test was not used. A full description of the clinical history of most of these cases has been given by Williamson in a previous publication.

General Summary.

It will be seen from the above summary that a large number of the workers engaged upon the serum diagnosis of pregnancy support the claims of Abderhalden that the serum of pregnant women contains a ferment capable of digesting placental protein. The work of Abderhalden has created a world-wide interest, and a steady stream of literature has issued from Germany, Russia, Italy, America and England. The work represents the greatest progress yet made in our knowledge of the metabolic processes which accompany pregnancy, and further opens up a wide field for future investigations. There seems no reason to doubt the existence of a specific ferment in the blood of pregnant women, and proof is forthcoming not only from experimental work, but also from the records of the cases investigated by the authors quoted above. The ferment is capable of detection in the blood of pregnant women from the sixth week until 15 days after delivery. In my own experience with both methods of diagnosis I have not obtained a single false result in a normal case of pregnancy from the eighth week until ten days after delivery. Further, in a large number of control sera from a variety of diseases negative results have been constantly found. The tests are therefore of value in the early diagnosis of pregnancy, and as such are of importance in obstetrical practice. The presence of living placental and syncytial tissue after the 15th day of delivery can be detected by means of these tests, and they are therefore of great value in late puerperal sepsis. This also applies in cases of chorion-epithelioma, which always gives a positive test as exemplified by cases quoted in the literature (Englehorn, Brucke). Paltauf described such a case in a woman aged 61 from whom a tumour (chorion-epithelioma) was removed which was acted upon by pregnant serum, but not by carcinoma serum. The case described by Williamson and the writer similarly gave a positive result with placental tissue, and a negative result with carcinoma tissue, and emphasized the importance of the combination of the clinician and chemical pathologist in the application of the test.

The value of the tests in cases of ectopic gestation has also been confirmed by several writers. In this connection it must be remembered that in a case of hæmatocele formation following tubal rupture the reaction may be negative, as in such a case the chorionic villi are incapable of being washed into the maternal blood-stream. The Abderhalden test is essentially a test for the presence of chorionic villi, and as long as these remain in the body, and come into contact with the maternal circulation the

reaction will be positive. The work of Abderhalden may prove of the greatest possible utility, however, in the advancement of our knowledge of the toxæmias of pregnancy. The writer's experience with these conditions fully confirms that of others as regards the deficiency of ferment met with in the serum. This is perhaps most strikingly illustrated by the optical test where only a slight rotation occurs. This deficiency may possibly explain the condition, but other factors are in all probability concerned. At the present time the writer is engaged upon an investigation of the liver and kidney ferments in these abnormal pregnancies. The present state of our knowledge would, however, lead us to suppose that the mechanism of protection has been exhausted, and an attempt should be made to supply the ferment. This could be possibly accomplished by the injection of normal human pregnant serum, or possibly the serum of pregnant animals could be utilized for the same purpose. The results of such injections could be easily followed by the optical test if necessary. In such a way we may be able to obtain further information as regards the causation and the treatment of the toxæmias of pregnancy. The above brief summary may be said to substantiate the chief claims of Abderhalden, and a perusal of the literature shows that the majority of observers support his views. The criticisms which have been levelled against the specificity of the protective ferments, and also the methods, have in the main received attention in papers by Abderhalden himself. Many of the earlier results have been shown to be erroneous, and later adverse comments have been sharply attacked by Abderhalden. The most recent contribution is that of Oeller and Stephan who have collected a number of instances from the literature of failure to confirm the value of the tests. They have divided up the subject into the chief clinical groups to which the methods have been applied, namely, obstetrics and gynæcology, psychiatry, bacteriology, and new growths. The authors quoted have all obtained diverse results, and on these grounds, as well as on their own work, they infer that the tests are not specific. Since Abderhalden has already satisfactorily dealt with their criticisms no further comment is necessary here. The chief source of fallacy in all these cases would appear to be not the methods themselves, but rather the actual worker. This applies not only to pregnancy, but to the diagnosis of malignant disease. The statement so frequently made that the serum of pregnant women will digest carcinoma tissue requires considerable investigation before any justification for such an opinion is claimed. In 10 cases in which this view has been put to the test in my laboratory no evidence of such a reaction has been obtained. It is now my practice to test every serum in this way, and also to utilize the tubercle test at the same time for control purposes.

No one who listened to the interesting address of Abderhalden at the International Congress of Medicine held in London could have

failed to be impressed by his admirable work, and the scrupulous care and attention to details which he has exhibited in perfecting the test. His readiness to impart information and the complete grasp of the whole subject which he displayed in conversation appealed to the writer. That his claims will receive due recognition appears inevitable, and his work has opened up a new field of research, the extent of which cannot be possibly gauged at the present time. Although a vast literature has already accumulated upon this subject much yet remains to be done; and it is only by co-operation and mutual help between the clinician and the chemical pathologist that progress can be made.

Conclusions.

1. The serum of pregnant women contains a specific ferment capable of digesting placental tissue, and this ferment can be detected from the eighth week of pregnancy until ten days after delivery, both by the optical and by the dialysation test.

2. That both tests should always be applied to the serum from the same case, and that the accuracy of the results depends entirely upon the most scrupulous care in details of technique.

3. That the tests appear to be of value in diagnosis, more especially in the following conditions:—

- (a) The early diagnosis of pregnancy.
- (b) The differential diagnosis between fibromyomata and pregnancy.
- (c) The diagnosis of ectopic gestation.
- (d) The diagnosis of chorion-epithelioma.
- (e) The presence of retained placenta.

4. That there is at present no justification for stating that the serum of pregnant women will digest other than placental tissue.

5. The claims of Abderhalden that the optical and dialysation tests are of value in the diagnosis of pregnancy are established.

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