

be made between diabetes mellitus and renal glycosuria, and diabetes mellitus may be diagnosed earlier than by the usual glucose tolerance test.

A number of cases, whose blood sugar rose above 180 mg. per 100 cc. blood and returned to normal in less than three hours, were shown by respiratory quotient curves, to be definitely diabetic.

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### THE EARLY DIAGNOSIS OF PREGNANCY BY METHODS OF PRECISION: FURTHER OBSERVATIONS ON SUGAR TOLERANCE TESTS: FINAL REPORT.

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In our preliminary report,<sup>1</sup> the literature was reviewed, different techniques were cited and finally, a series of 39 cases was presented using our own modification of the technique of Frank and Nothmann with a 95 per cent accuracy in the diagnosis of early pregnancy. Since that time the use of laboratory procedures for the early diagnosis of pregnancy has increased so enormously, that now we feel a survey of these methods, their possibilities and shortcomings, to be warranted. It must be remembered throughout this discussion that no laboratory method has yet been devised which is absolutely and infallibly diagnostic of the presence or absence of pregnancy (except Roentgen ray in the late months), and therefore the indication for the use of these tests is to strengthen the already present suspicion of pregnancy, or the probability of its absence. The

experimental work in this field has been dominated by two ideas. The first is based on the supposition that pregnancy causes a specific protein, possibly in the nature of a ferment, to appear in the maternal blood; the second, on the observation that pregnant women in the early months are prone to glycosuria, which is usually transient, harmless, and can easily be induced by the feeding of carbohydrate. Our attention will focus mainly about the latter group, for it is now fairly well proven that there is no protein specific to the pregnant female; at least so far as these tests can show.

The first group embraces the following:

I. *The Abderhalden Reaction.* This is based on the supposition that reaction will occur between the ferments in maternal blood serum when brought in contact with an extract of fresh placenta, using ninhydrin as the indicator. Its present status is best described in the conclusions of Smith and Shipley, "While the natural ferments present in serum are somewhat increased, there is no evidence that a specific ferment exists in pregnancy" and "the large number of positive results on the serums of men and nonpregnant women proves the test of no value for the diagnosis of pregnancy." Added to this, the actual technique of the test is so complex as to require an unusual degree of serologic skill in its performance.

II. *Erede's Anaphylactic Reaction.* This author proposes the theory that the "fetal proteins" in maternal blood should cause an anaphylactic reaction in smooth muscle, when perfused through the uterus of a sensitized virgin guinea-pig. No experimental work could be found to substantiate this thought.

III. *Costa's Novocain-Formalin Reaction.* To 1.5 cc. of 2 per cent novocain in citrated saline solution, add 3 drops of the blood to be tested, centrifuge, and add 1 drop of pure formalin. A gray precipitate appearing within fifteen minutes is considered diagnostic of pregnancy. The reports on several series of cases show that normal blood will react positively within three to fifteen minutes, and the test is also positive in infections and toxic conditions. It must therefore be discarded as allowing too large a factor of error.

IV. *Dienst's Reaction.* To 10 cc. of distilled water add 2 drops of the serum to be tested. Place 2.5 cc. of this mixture in a test tube, and add 1.25 cc. of a 33 per cent sodium hydroxid solution. The tube is shaken and 20 drops of copper sulphate solution added. A blue supernatant ring is supposed to be diagnostic of pregnancy. The most striking results come from Milan, Italy, where 130 women in the early months of pregnancy did not even show a convincing majority of positive reactions. No favorable report of this test has been found by us in the literature.

V. *Red Blood Corpuscle Sedimentation Test.* Into a syringe containing 0.2 cc. of 5 per cent sodium citrate solution, 0.8 cc. of the patient's blood is drawn. The mixture is then discharged into a specially calibrated upright tube. The length of time necessary

for the cells to pack below the 18 mm. mark is the observed standard. In the normal this is supposed to be one hundred and twenty minutes, and in the early months of pregnancy a much shorter time should be noted. This phenomenon has found its greatest use as a prognostic aid in gynecologic infections, and in pulmonary tuberculosis, but has proved entirely worthless in the diagnosis of early pregnancy, for which it was originally conceived by Fahraeus.

The reactions in the second group are:

I. *The Alimentary Glycosuria Test of Frank and Nothmann.* In pregnant women, after feeding 100 gm. of glucose on a fasting stomach, glycosuria without hyperglycemia will appear within the course of two hours. This fact is only noted with any constancy during the first three months of pregnancy, and immediately disappears following the separation of the placenta from the uterine wall, or upon the death of the fetus. This is the laboratory method to which we wish to call attention, and in a later paragraph will devote further discussion to it.

II. *The Roubitschek Adrenalin Test.* Feeling that the above procedure was too involved, this investigator fed his subjects only 10 gm. of glucose on a fasting stomach, and at the same time injected 0.5 mg. of adrenalin hypodermatically. The appearance of glycosuria within two hours was considered by him diagnostic of pregnancy. It soon became apparent in a larger series of cases, and from the reports of other workers, that no trust could be placed in this test, for the results were bizarre and inconstant. It has therefore been practically abandoned.

III. *The Phlorizin Test.* Phlorizin is a glucosid derived from the bark of the domestic apple tree, and is a favorite drug for inducing a lowered kidney sugar-threshold in laboratory animals. Since glycosuria without hyperglycemia had been noted constantly in pregnant women, Kamnitzer and Joseph reasoned that a phlorizin glycosuria should be relatively more easily induced in these patients than in the normal nonpregnant woman. That is to say, a threshold already low could be more easily lowered to the spilling point than could a normal one, and should necessitate a smaller dose of phlorizin to accomplish this purpose. They found this optimal dosage to be 2 mg. of the drug. After ascertaining that a preliminary urine specimen is free from sugar, they inject 2 mg. of phlorizin hypodermatically, the patient having fasted for twelve hours. If, within the next two hours, sugar appears in the urine, the presumption of pregnancy is strong. This test also loses any significance after the third month.

The simplicity of this technique, and its ease of administration, have appealed to the majority of workers in this field during the last two years, especially since the appearance of a proprietary preparation, "Maturin," which is a solution of 2 mg. of phlorizin and beta-eucain, in ampoules. In our preliminary report we tabulated

the results of work on this test, and showed that too many non-pregnant control cases reacted positively. The following table will add more convincing proof of this fact and will bear out the conclusion we then arrived at: "The phlorizin technique has yielded no satisfactory results." We had intended to continue our phlorizin series, but abandoned the idea in view of the number of reported failures with this test.

TABLE I.—RESULTS OF INVESTIGATORS USING THE PHLORIZIN TECHNIQUE.

Observer.	No. cases.	Pregnant.		Nonpregnant.		Men. Positive. (per cent.).
		Positive. (per cent.).	Negative. (per cent.).	Negative. (per cent.).	Positive. (per cent.).	
Kamnitzer and Joseph . . .	300	98	2	90	10	*
Craincianu and Goldenberg . . .	50	100	0	46	54	*
Klaften . . .	300	80	20	*	*	*
Perez and Brea . . .	116	85	15	95	5	*
Bronicoff . . .	300	74	26	96	4	72
	(1000 tests)					
Bodo . . .	175	86	14	84	16	*
Eastman . . .	136	90	10	92	8	*
Ewald . . .	150	78	22	97	3	*
Krauter . . .	149	74	26	80	20	*
Tomay . . .	?	91	9	73	27	*
Marbatto . . .	?	100	0	75	25	*
Rychlinsky and Gottlieb . . .	?	39	61	64	36	*

\* No tests performed.

**Discussion of Alimentary Glycosuria Test.** Convinced by our preliminary work that we were dealing with a fairly accurate procedure for aiding the early diagnosis of pregnancy, it became our aim to so simplify the actual technique of the test that it might be used in the routine of any general practitioner. The original technique of Frank and Nothmann was so cumbersome as to prohibit its use anywhere except in a hospital. Our present practice is as follows:

1. Patient shall take an average supper the night previous.
2. Collect first morning specimen of urine. This must be negative for sugar by Fehling's qualitative test, before the glycosuria test is begun.
3. Omit breakfast.
4. The calculated dose of table sugar is given, dissolved in two tumblers of water, flavored with half a lemon each. The dose is computed by using 7.5 gm. of table sugar for every 10 pounds of body weight, except that the maximum total must not exceed 150 gm. It has been our experience that Franklin granulated table sugar is best for this purpose.
5. Voluntarily voided specimens of urine are collected one hour and two hours after the dose is taken, and in cases going to operation

the same day, or under any nervous strain, a third hour specimen is also collected. These are tested for sugar by the Fehling's qualitative method.

6. We have largely dispensed with the collection of blood for sugar determination, but when necessary, have found that it should be done one and a half hours after the test is started. Our records show that the blood sugars of 33 pregnant women averaged 0.138; and of 19 nonpregnant averaged 0.125 during the test. If either of the hourly specimens of urine shows a definite reduction of the Fehling's solution such as would be termed "positive for sugar" in a routine urinalysis, the test is considered positive, and in conjunction with the clinical findings, strengthens the probability of pregnancy. The opposite is true for a negative test.

Certain pitfalls have been encountered in the course of these experiments which are worthy of mention. Several patients have not considered the "lemonade" appetizing enough and have relegated the second glassful to a convenient sink, or out the window. Others have become truly nauseated by the dose, and have vomited, making the test untrustworthy. In this connection it is well to note that false positives will almost inevitably result in patients who are intolerant of sugar in any form, as evidenced by a history of nausea on eating any sweet foods or candy, a fact noted by the patient before there is any suspicion of pregnancy. Patients with endocrine obesity, exophthalmic goiter, diabetes, or any severe hepatic disturbance, are not amenable to this test, because their carbohydrate metabolism is already abnormal.

It will be noted that a third hour specimen is counselled in certain cases. This is because we have seen patients vomit a large amount of the sugar solution as late as three hours after the test was completed, while being anesthetized for operation. This occurrence is probably due to pylorospasm, and an extra specimen will reduce the factor of error in such cases. Routine blood sugar determinations were not made in this series, because the early work had shown that this type of glycosuria is accompanied by normal glycaemic values. In only one case so far has this proved to be untrue. The patient was diagnosed pregnant with the aid of the test, and this was later confirmed, but during the observations her blood sugar rose to 228 mg. per 100 cc. of blood.

If the suspicion of another pregnancy arises during the lactation period, this reaction may be used as a diagnostic test, providing that all urines showing reducing substances shall be subjected to a yeast fermentation test for twenty-four hours to determine that glucose and not lactose is being excreted. Failure to take this precaution may lead to a false positive result, as in the following case: A lactating woman complained of gastric disturbances leading her to suspect another pregnancy, and her uterus was found slightly enlarged. After the sugar test, her urine was reported "positive

for sugar." At a subsequent examination the uterus was no larger than previously and at operation she was found to have multiple fibromyomata of the uterus. A recheck showed that no fermentation test had been done on the urine, which vitiated the value of the result.

It is important that credence be given only to those reactions in which there is either a frank reduction, or no reduction, of the Fehling's solution. The middle ground, reported by the laboratory as "trace of sugar" or "slight reduction of Fehling's," is very treacherous, and caused annoying error until the above criteria were established. Seven of our patients undergoing the test were reported to have a "trace" of sugar, most frequently in the specimen of the second hour. Of these cases, 3 were not pregnant, 2 were four and five weeks pregnant respectively, and 2 were one and three days postpartum respectively. Under these circumstances the test must either be repeated or discounted entirely.

Exclusive of the cases already cited, and a group of "dead ovum" cases to be detailed below, we wish to report a carefully followed-up series of 150 cases, of whom 88 were pregnant and 62 were not pregnant. Of the pregnant group, 83 women (94 per cent) reacted positively, and 5 (6 per cent) reacted negatively. In the non-pregnant group, 57 women (92 per cent) reacted negatively, and 5 (8 per cent) reacted positively. A truly specific test for pregnancy should not yield any positive results in nonpregnant women, and the failures in this series occurred in the following cases:

Two cases of pelvic inflammatory disease.

One patient whose period was overdue, but who menstruated normally ten days after a positive test.

One patient with the symptoms and gynecologic findings of an early ectopic pregnancy, and leukocytosis of 14,400. Abdominal section revealed absolutely normal pelvic genitalia.

One patient, thought to be pregnant, who proved to have a fibromyoma of the uterus.

Two women were successfully diagnosed pregnant at the third week after conception by means of this reaction. Our former conclusion that the test is of no value after the third month was also confirmed in this series.

Aside from its use in the early diagnosis of pregnancy, another phase of this reaction has proved of great value to us. It was stated by the original investigators, and corroborated in our work, that the death of the fetus, or the separation of the placenta from its implantation site will cause the reaction to become negative. The importance of this fact when applied to threatened abortions, or incomplete miscarriages with retention of a large residuum, is immediately obvious, especially to those who practise prompt evacuation of a dead ovum. In a series of 9 such cases ranging from the ninth week to the third month of pregnancy, a negative reaction

confirmed the suspicion of a dead ovum and enabled us to immediately evacuate the contents of the uterus. Thus, when a negative test supports the clinical history, we feel it is an invaluable indication for operative interference in cases of dead ovum, and suspected inevitable miscarriage. It has never caused us to make a mistake, and the economy it effects in days of hospital waiting is evident.

A recount of all the interesting patients met in this series is impossible in the allotted space, but certain outstanding ones may be appropriately cited. On August 1, 1925, a young unmarried woman presented herself with the history of coitus three weeks previously and amenorrhea for three days beyond her menstrual time. A test done at this time showed a "trace of sugar," and she was told to return in a week for further trial, when a definite positive was obtained. She was seen again on August 21st, and in the interval had suffered three days bleeding due to ergot, and had indulged in coitus once. The test was still positive. She reported again on September 8th, and showed the breast and gynecologic signs of early pregnancy, and the test was still positive. The patient induced an abortion a few days later. This is a most unfortunate aspect of the reaction in that it affords unscrupulous characters too much certainty at an early date in pregnancy. Here too it may be noted that unless there is a missed period between the suspected coitus and the date of the test, a negative result will be obtained, even in patients who later prove to be pregnant. This observation may help to substantiate the theory that the ovum of the first missed period matures into the fetus, as is already surmised from the fact that women who menstruate irregularly always pass beyond the calculated gestation time.

An interesting instance of this reaction after the third month was seen in Mrs. D., who in the fifth month after her last seen period fell over a chair, and was brought to the hospital. From the clinical picture it was evident that a miscarriage was inevitable, and as a routine we ran the glycosuria test, which was definitely positive. The explanation for this seeming paradox appeared with the fetus, for its heart was still beating after birth.

A patient aged forty years, came to the hospital complaining of three months' amenorrhea. She had the history and physical evidences of a hemiplegia four years before, and in addition, examination revealed an arteriosclerotic hypertension, and a fairly firm uterus enlarged to the size of a three months' pregnancy. In view of a positive sugar test, the diagnosis of early pregnancy, rather than early menopause with myoma, was made and an operation advised. A three months old fetus was removed by abdominal hysterotomy.

**Summary.** 1. The methods of precision for the early diagnosis of pregnancy are reviewed, and the alimentary glycosuria test shown to be the one in which most trust can be put.

2. An extremely simple technique for the performance of this test is offered, using table sugar, a lemon, and Fehling's solution as apparatus. The technical pitfalls of the test are outlined.

3. A new series of 150 cases is reported with 6 per cent error in the early diagnosis of pregnancy, and 8 per cent error in nonpregnant women. Although this percentage of error is larger than we experienced in our preliminary work, it still is smaller than that found with any other laboratory method for the early diagnosis of pregnancy, and compares favorably with such procedures as the Wassermann test in its factor of error. We therefore believe this test is useful, and if practised routinely, will aid appreciably in the diagnosis of pregnancy before the gynecologic signs appear.

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