

available) one may estimate that in 100 grams of meat there are 27.16 grams of these six non-sugar-producing acids which on normal oxidation yield a respiratory quotient of 0.765 instead of 0.716 which was calculated. The lack of exact correspondence shows the comparative crudeness of the methods involved. It must also be remembered that the quantities present in meat of cystine and especially of serine, both of which are sugar formers, are unknown.

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On a simple method of diagnosing pregnancy, based upon the presence of specific enzymes in the urine.

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It has been clearly demonstrated by Abderhalden and the host of workers following in his footsteps, that protective enzymes are developed in the body as a result of the presence of a foreign protein in the blood stream, whether that protein be derived from placental tissue, or carcinoma in the body, or be introduced from without for experimental purposes.

These enzymes, proteolytic in nature, have been found in the serum: their function is to digest the foreign protein and split it into amino-acids, in which form it may properly circulate in the blood stream, and be absorbed by the cells.

While working with Dr. A. A. Bruère at the Royal Victoria Hospital, Montreal, on a method of performing the Abderhalden Cancer test without the use of dialyzing thimbles, it was observed that a serum which gave a strongly positive test after incubation for 20 hours, was negative on the following day. The suggestion was made that certain enzymes had dialyzed out, causing the further splitting of peptone and amino-acids into simpler bodies which would not give the Ninhydrin reaction. Theoretically one might now expect that an enzyme which is dialyzable through a parchment thimble would also pass through the kidney and be found in the urine.

It was at this stage that Professor Adami called my attention

to Kiutsi's work on urine diagnosis¹ in which this passage of specific enzymes through the kidney is made the basis of a widespread method for the diagnosis of disease.

Kiutsi's method is as follows:

"By filtering urin of a pregnant through animal charcoal, the urin is clarified and protein and peptone taken off, *i. e.*, let it be filtered through animal charcoal several times until Biuret reaction is no longer positive. Then 5 c.c. of so treated urin is put into a test tube. Into it 0.1 gr. of Kiutsi's placenta is added and the mixture is left for six hours or fourteen hours. After this the entire liquid is filtered through a filter paper into another test tube, and 2 c.c. of sodium hydroxid is added. After shaking the contents a little, the test tube is held by the left hand in a slanting position. With the right hand the copper sulphate solution is taken in 1 c.c. pipette. On letting the copper sulphate solution run down slowly by the inside of the tube, where two liquids meet, a brilliant purple color may be formed. Then the reaction is positive. But if no such coloration takes place, the reaction is negative."

Kiutsi claims to have "repeated this method hundred times, and never missed," and says "By this method the early pregnancy, the early abortion and the extra-uterus pregnancy could positively be detected, and its reliability put even myself to astonishment." He also states that he has been able to diagnose cancer, nephritis, tuberculosis of the lungs, renal glycosuria, and other diseases by this method, using the proper substrate in each case.

Kiutsi, while indicating the broad outlines of this method, is careful not to give any details regarding the preparation of his dried substrates, and has avoided pointing out the precautions which have to be taken to obtain uniform results. His paper is remarkable, and that not alone from the importance of the fundamental observation and the naïveté and quaintness of its diction. Undoubtedly the combination of the claim to have discovered a practically universal diagnostic method with what appears to be a purposeful silence regarding the finer but essential details of

¹ M. Kiutsi, "Kiutsi's Urindiagnosis by Means of 'Filtration Process.'" June 10, 1914. Printed by Bunyeido, Sapporo (Japan).

that method must create at first an unfavorable impression. Nevertheless the observations tallied so remarkably with our own, as to demand confirmation. The work, if correct, opens up such a large field for research that the writer sought to verify it by a similar series of experiments.

In the absence of any details as to the method of preparing the dry substrate, the following plan was adopted.

Two placentas were obtained, freed from blood by washing in saline solution, and then in running water, and treated according to Abderhalden's directions until the water in which the substrate had been boiled for five minutes gave no ninhydrin reaction.

One half of this substrate was placed in a sterile jar containing 50 per cent. glycerine in water, covered with toluol and kept in the ice-box; the other half was treated as follows:

It was minced finely, dried in the oven at 80° C. for 4 hours, and then over-night at 55° C. Next day it was ground to a fine powder in a sterile mortar and kept in a desiccator over calcium chloride for 3 days. The appearance of the substrate is that of a fine, dry, brown powder which can be kept in a closely stoppered bottle without fear of autolysis or bacterial action.

The use of animal charcoal as recorded by Kiutsi for the purpose of removing bodies giving a positive biuret reaction was found unsatisfactory. The method was slow and tedious, fresh filters of charcoal had to be employed for every sample of urine, and often the filtrate showed a purplish tint due to coloring matters absorbed from the charcoal. As a substitute method, shaking with kaolin was tried. Experiments were then conducted to determine

1. The amount of kaolin necessary to remove from the urine all bodies giving a positive biuret reaction.
2. The optimum amount of dry substrate to be employed.
3. The optimum incubation period.
4. The effect of the acidity or alkalinity of the urine on the reaction.
5. The effect of bacterial growth during the incubation of the urine to be tested.

As a result of these experiments the following method was devised, and has been found to be satisfactory.

A freshly passed specimen of urine from a pregnant woman is tested for albumin by the biuret test. If the test be positive 15 c.c. of urine are shaken with 0.3 gram of kaolin for 10 minutes on a mechanical shaker, filtered and tested again; the biuret test should now be negative. If it be still positive the process must be repeated. 10 c.c. of the biuret-negative urine are then neutralized with 1 per cent. acetic acid, or with 2 per cent. sodium carbonate solution, 0.2 gram of dried placenta added, and the whole well shaken. The shaking I find essential. 0.5 c.c. toluol is added to restrict bacterial growth. The mixture is incubated for 12 hours, filtered, and 5 c.c. tested by the biuret test. If negative the remaining 5 c.c. are left in contact with the substrate, incubated for 4 hours longer, and tested again.

Controls of the urine of the pregnant woman without substrate, of urine from a male with and without substrate, from a non-pregnant female with and without substrate, and of the substrate in 10 c.c. of distilled water, are set up and tested in the same way.

The urine of the pregnant female in contact with the substrate should give a positive biuret reaction: all the controls should give negative reactions. The color of a positive test varies from deep purple to lilac or rose in different cases. All blues and greens are negative.

Up to the present time 59 cases have been examined. Of these, 12 urines tested *before* the above technique had been fully developed, gave the following results: one male gave a negative reaction both with the blood serum and the urine; of the 11 pregnant females, only 2 gave a positive reaction with the urine although in every case the blood serum was positive. Since using the kaolin method, and adopting the precautions mentioned in the preceding paragraphs, the results have been very satisfactory.

URINES FROM 47 CASES EXAMINED.

Pregnant females.	29—all positive.
Males.	6—all negative.
Non-pregnant females.	9—8 negative, 1 positive (fibroids of uterus).
Ectopic gestation.	3—all positive.

These positive results in pregnant females were gained in the main from urines of cases estimated as from the 28th to 36th

weeks of gestation. Two cases were at about the third month.

It is perhaps scarcely necessary to point out that, as compared with Abderhalden's delicate and complicated serum test for pregnancy, this method is both simple and expeditious.

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The influence of chenopodium on the circulation and respiration.

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The intravenous injection of one to two per cent. emulsions indicates that the oil of chenopodium is a circulatory, as well as a respiratory depressant. Blood pressure fell after a dose of 0.02 c.c. per kilo was introduced into rabbits, cats and dogs, but the absolute, as well as the relative effect, varied in different individuals. While large doses produced a greater fall of blood pressure this was not always in proportion to the size of the dose. The volume of the kidney followed closely the blood pressure, thus indicating that the effect on the latter is of cardiac origin. That chenopodium is a cardiac depressant was also shown in experiments on the isolated frog heart. A solution of 1 : 2,000, of chenopodium, or its active principle, ascaridole, perfused for one to two minutes, caused a marked decrease of force, as well as frequency of cardiac action which was not always observed, however, when the perfusion time was reduced to half a minute only. It was frequently absent after the initial perfusion and sometimes when this was repeated once or twice after suitable intervals, but depression of the heart was usually produced when a sufficient number of perfusions were made in each case. Cardiac irritability after vagus stimulation which was tested in dogs was found to be decreased after chenopodium.

The action of chenopodium on respiration varied in different animals, being less effective in rabbits than in cats or dogs. Small doses, about 0.02 c.c. per kilo may produce respiratory depression in all animals. In some experiments the effect was observed after