

A RE-EXAMINATION OF THE VALUE OF THE
KAPPELLER-ADLER PREGNANCY DIAGNOSIS TEST (HISTIDINURIA)
AS AN INSTRUMENT OF DIFFERENTIAL DIAGNOSIS WITH
SPECIAL REFERENCE TO MENTAL DISEASES.

by

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It is generally accepted that the presence of the aminoacid histidine is not to be recognised by any means now at our disposal in the urine of the healthy non-pregnant human subject. The work of Edlbacher (1) would seem to have shown conclusively that the reason for this is that the liver either contains or elaborates the ferment histidase which is specifically concerned with the destruction of histidine present in the body. Apparently this ferment is found only in the liver, not only of the human subject, but also in those of other animals. Edlbacher has further shown that histidine is not deaminised by the kidney, or if so to no appreciable extent.

Histidine, however, is present in considerable quantity in the urine of the pregnant human female. Kapeller-Adler (2) succeeded in demonstrating experimentally that liver tissue derived from the pregnant human female does not destroy histidine, whereas that obtained from the non-pregnant subject did. Further, she has successfully shown that the excretion of histidine in the urine of the pregnant woman is definitely correlated with the excretion of the gonadotropic hormones. For this reason she postulated a causal connection between the increase in the production and excretion of the gonadotropic hormones and the excretion of histidine. Later, Kapeller-Adler/

Adler (3), in a series of experiments, was able to demonstrate that the gonadotropic hormones restricted to a marked extent the effectiveness of histidine destruction by liver tissue. She therefore postulates that the increase in the gonadotropic hormones during pregnancy is the cause of the non-destruction of histidine, so that this aminoacid passes through the liver unaffected, and, not becoming deaminised by the kidneys, passes out with the urine.

As long ago as 1909, Pauly (4) had devised a test by means of which the presence of histidine in the urine could be demonstrated. This test takes advantage of the fact that when histidine is mixed with an alkaline solution of diazotised sulphanic acid a cherry-red colour results. This test has proved, in the hands of many people, to be exceedingly sensitive, but unfortunately this colour reaction is not specific for histidine; it is given by all iminazol bodies and also by such a variety of things as aromatic hydrozyl acids, polyphenols, tyrosine and the colouring matters of the urine (comp. Hunter) (5).

A more refined test was developed in 1908 by Knoop (6) which was based upon the observation that when histidine, to which bromine water is added, is warmed, a reddish or even a dark wine-red colour develops, and eventually a flocculent, black precipitate is produced. Though this test is not nearly so sensitive as that of Pauly's it has been shown to be absolutely/

absolutely specific for histidine. Its great disadvantage lies in the fact that the personal factor enters very largely into the appreciation of the result since the depth of the colour varies from time to time and case to case.

In 1933 Kapeller-Adler (7) devised a colorimetric method for demonstrating the presence of histidine. The solution in which the presence of histidine is suspected is treated with a solution of bromine in acetic acid and to it thereafter is added a mixture of ammonia and ammonium carbonate in solution. When heat is applied a coloration ranging from red to purple slowly develops, if histidine be present, and the depth of the colour varies according to the quantity of histidine. It has been shown that this test, like that of Knoop, is quite specific for histidine. Its sensitivity is such that by means of it histidine present in a concentration of 1:50,000 is recognisable.

In the course of further investigations Kapeller-Adler was able to show that histidine, recognised by means of this test, is a normal constituent of the urine of the pregnant female. She was therefore able to make use of this test as a chemical test for the diagnosis of pregnancy (8). A brief account of the technique of the Kapeller-Adler chemical test for pregnancy is given.

Technique of the Kapeller-Adler Chemical Test for Pregnancy./

Technique of the Kapeller-Adler Chemical Test for Pregnancy.

Reagents required -

- (a) Bromine reagent:- Add 5 ccs. bromine to 500 ccs. acetic acid (glacial) and add 1000 ccs. distilled water to this solution. Store this mixture in a brown glass bottle.
- (b) Mixture of Ammonia and Ammonium Carbonate:- 400 ccs. ammonia solution (specific gravity .880) are mixed with 200 ccs. of a 10% ammonium carbonate solution.
- (c) Potassium iodide starch paper.
- (d) n/10 Potassium permanganate solution.
- (e) 10% sulphuric acid.

Since it is desirable to have the urine to be tested as concentrated as possible, the first morning specimen is preferable.

Technique.

Add sufficient bromine reagent to 5 ccs. urine to change the original colour to orange-red, then to orange-yellow and finally to a pale yellow. The latter colour indicates the necessary slight excess of bromine. This excess is tested for by potassium iodide starch paper; the fluid is then left for 5 minutes during which the excess bromine disappears. (Test with potassium iodide starch paper - no blue stain). Now add .5 ccs. of the mixture of ammonia and ammonium carbonate solution to the new bromine free urine, shake the fluid and place the test/

test-tube containing it into a beaker of boiling water for half a minute. In the urines containing histidine a deep reddish-violet or blue-violet colour appears. The histidine-free urines do not change their colour.

Treatment of alkaline urines.

To 5 ccs. urine add 2 ccs. 10% sulphuric acid and sufficient n/10 potassium permanganate to change the urine to a pink colour which will persist for $\frac{1}{2}$ - 1 minute. The urine is allowed to stand for a few minutes until it becomes clear and almost colourless. Now add a few drops of the bromine reagent until the fluid becomes pale yellow. Test with potassium iodide starch paper for the slight excess of bromine. Leave for 5 minutes during which time the excess bromine disappears. Add 1 cc. of the mixture of ammonia and ammonium carbonate, shake the mixture and place it in a beaker of boiling water for 1 minute. The urines containing histidine become a red colour while the histidine-free urines remain yellow.

Histidinuria has been observed in conditions other than pregnancy. Kapeller-Adler herself had found it to be present in a case of Cushing Syndrome in which there was also an increase in the excretion of gonadotropic hormones. She also found it in cases of hydatidiform mole, again, of course, associated with an abnormally high excretion of gonadotropic hormone.

Valle/

Valle (9), when investigating cases of liver cirrhosis, terminal stages of precoma hepaticum, catarrhal jaundice, cholecystitis, and carcinoma of the stomach with metastases in the liver, succeeded in obtaining a positive histidine reaction in one case only, a case of precoma hepaticum in which there was a serum bilirubin content of 5.2 mg. per cent and severe jaundice.

Chomet (10), examining a series of 53 urines of non-pregnant patients, obtained two positive reactions. One of these was in the case of a woman with slight frostbite, and the other a woman with a lutein cyst of the ovary.

Stern (11), examining 54 varied gynaecological cases found histidine in the urine of three; one a case of cervicitis, the second a climacteric metrorrhagia, and the third an ovarian tumour.

Schimmelpfeng (12) applied this test to cases of mental disease and got a high proportion of positives in the case of melancholics.

Norpoth (13), as would be expected if the hypothesis of Kapeller-Adler is valid, found histidinuria in several cases in which the anterior pituitary was functioning abnormally and in which there was an over-production of the gonadotropic hormones. Incidentally, Norpoth agrees with Kapeller-Adler in regarding the presence of gonadotropic hormones in excess as the cause of such histidinuria.

Finally/

Finally, Tschopp and Tschopp (14) in a series of 300 tests obtained a positive histidine reaction in 85. The specimens used in these tests were derived from a great variety of sources; some from pregnant women, some from apparently healthy individuals, and the rest from such as were suffering from different derangements.

A review of this scanty literature makes it abundantly clear that it is not possible to state that histidinuria is characteristic of any particular condition other than that of normal pregnancy (or a hydatidiform mole). Nevertheless, the suggestion that perhaps if the "improved" Kapeller-Adler test were used it might be possible invariably to demonstrate histidinuria in certain pathological states, seemed to be sufficient reason for making another series of observations. It will be agreed that if histidinuria can be shown to be an invariable concomitant of a certain pathological condition, a test such as this might constitute a very welcome diagnostic reinforcement.

Material and Methods.

326 urines derived from 193 males and 133 females were examined by means of the Kapeller-Adler test. Each specimen was subjected to the test on at least two separate occasions in an attempt to obviate any possible mistake. If the specimen itself was highly coloured or contained much solid material, it was/—

was always cleared by means of the treatment described for use in the case of alkaline urines. As a preservative, chloroform was used. The specimens are classified below according to the absence or presence of pathological conditions found in the donor.

Healthy Persons.

As a basis for comparison and as a test of the test to be used, 12 urines (4 from males and 8 from females) were examined. According to the test no histidine was present.

Normal Pregnancy.

Specimens derived from 15 women, pregnant for different periods (2-9 months) were tested. In every case a positive reaction was obtained. In two strongly alkaline specimens from cases of late pregnancy the reaction was definitely weak, but after preliminary treatment with n/10 solution of potassium permanganate in acid solution, a typical positive reaction was obtained.

Schizophrenia.

36 specimens: 31 male and 5 female.

In all save two definite standard negative results were obtained. In the case of one male urine a weak positive reaction resulted. This urine was tested several times subsequently and on every occasion, save one when a weak positive was obtained, the reaction was negative. In the case of one female urine the first test showed traces of histidine, but the test/

test being repeated thereafter gave only negatives.

Melancholia.

13 specimens: 3 male, 10 female.

In no case and at no time was histidinuria observed.

General paralysis of the insane.

9 specimens: 5 male, 4 female.

7 gave definite negatives. One male and one female urine gave weak positives which were obtained again on repetition of the test. It is not reasonable to suggest that anti-syphilitic treatment to which the patients had been subjected should so affect two of these specimens as to yield a weak histidinuria, whilst the other seven similarly treated should remain unaffected.

Paranoid Reaction.

5 specimens: 2 male, 3 female.

In no case and at no time was histidine found in the urine.

Epilepsy.

6 specimens: all men.

In no case and at no time was the histidine demonstrable.

Manic depressive psychosis.

25 specimens: 10 male, 15 female.

In the case of 24 of these the results were always negative. In the case of one woman in a state of chronic mania, histidine was found in the urine on the first examination but not/

not thereafter.

Korsakow psychosis.

2 specimens: 1 male, 1 female.

No histidinuria was ever found.

Cardio-vascular diseases.

28 specimens: 18 male, 10 female.

6 congestive cardiac failure: 4 male, 2 female.

3 arteriosclerosis: 2 male, 1 female.

1 angina pectoris: 1 female.

1 coronary infarction: 1 male.

2 aortic aneurysm: 2 male.

1 coronary thrombosis: 1 male.

3 rheumatic carditis: 1 male, 2 female.

10 myocarditis: 6 male, 4 female.

1 Adam Stokes' syndrome: 1 male.

In none of these 28 specimens, repeatedly examined,
was a positive reaction obtained.

Respiratory diseases.

39 specimens: 24 male, 15 female.

5 lobar pneumonia: 3 male, 2 female.

1 pulmonary infarction: 1 male.

20 pulmonary tuberculosis: 11 male, 9 female.

1 silicosis: 1 female.

3 bronchitis: 2 male, 1 female.

3/—

- 3 asthma: 3 male.
- 1 bronchiectasis: 1 male.
- 1 lung abscess: 1 male.
- 4 pleurisy: 2 male, 2 female.

In 37 of these cases the results obtained were always definitely negative, but in the case of one woman suffering from severe broncho-pneumonia, histidine was demonstrable in the urine on one occasion, whilst in the case of a male with dry pleurisy slight traces of histidine were once recognised.

Hepatic diseases.

- 13 specimens: 11 male, 2 female.
- 8 cirrhosis of the liver: 7 male, 1 female.
- 1 biliary colic: 1 male.
- 2 catarrhal jaundice: 2 female.
- 2 carcinoma: 1 male, 1 female.

In all cases save one no histidinuria was ever demonstrated. The exception proved to be of exceptional interest. This was the case of a male with marked jaundice and pronounced hypertrophy of the liver. When his urine was first examined it gave a very strong positive histidine reaction. Examination of his blood sugar showed a typical liver lag curve, and he also had a marked glycosuria, but no acetone bodies were present in his urine. His Van den Berg gave a biaphasic response and his icteric index was 65. As the condition improved under treatment and as jaundice faded,

a/

a series of Kapeller-Adler tests showed that his histidinuria became progressively weaker. Finally a standard negative was obtained.

Alimentary diseases.

28 specimens: 18 male, 10 female.

8 duodenal ulcer: 7 male, 1 female.

1 carcinoma of the pancreas: 1 male.

2 colitis: 1 male, 1 female.

1 carcinoma of the oesophagus: 1 female.

1 carcinoma of the pelvic colon: 1 male.

2 carcinoma of the stomach: 2 male.

1 carcinoma of the rectum: 1 female.

5 carcinoma of the palate: 3 male, 2 female.

1 carcinoma of the tongue: 1 female.

5 carcinoma of the fauces: 3 male, 2 female.

1 appendicitis: 1 female.

Of these 28 specimens all but one gave standard negative reactions. In the case of a male with a carcinoma of the fauces, traces of histidine were found on two occasions, whilst on a further three the result was negative.

Diabetes mellitus.

14 specimens: 8 male, 6 female.

In no case was histidine found in the urine.

Diseases/

Diseases of the Endocrine System.

10 specimens: 6 male, 4 female.

5 thyreotoxicosis: 3 male, 2 female.

4 pituitary dysfunction: 2 male, 2 female.

1 carcinoma of the thyroid: 1 male.

In three cases out of the 10 a positive histidine reaction was obtained. One was that of a woman suffering from acromegaly. This specimen gave a very strong positive reaction repeated in a series of five daily tests. A specimen derived from a man with a dysfunction of the pituitary gave a positive reaction on three occasions, whilst on others the result was negative. In the urine of a woman from whom a tumour of the pituitary had been removed, no histidine was found, but unfortunately it had not been possible to apply this test before operation.

Diseases of the Blood.

7 specimens: 4 male, 3 female.

1 hypochromic microcytic anaemia: 1 female.

3 hyperchromic macrocytic anaemia: 1 male, 2 female.

2 secondary anaemia: 2 male.

1 aleucaemic leucemia: 1 male.

The histidine reaction was negative in all cases.

Diseases of the Genito-urinary system.

30 specimens: 17 male, 13 female.

1 cystitis: 1 female.

3 renal colic: 2 male, 1 female.
8 nephritis: 5 male, 3 female.
7 pyelitis: 4 male, 3 female.
3 carcinoma of the bladder: 2 male, 1 female.
2 carcinoma of the prostate: 2 male.
2 carcinoma of the penis: 2 male.
2 carcinoma of the cervix: 2 female.
2 carcinoma of the ovary: 2 female.

In 25 of these a definite negative result was observed. In one case of gonococcal cystitis a positive histidine reaction was obtained, and in a case of acute nephritis a weak positive reaction occurred. In the case of a patient with a carcinoma of the penis the reaction was a definite positive. The same result was obtained by repeated tests on different days.

Rheumatic diseases.

9 specimens: 6 male, 3 female.
4 rheumatoid arthritis: 3 male, 1 female.
1 rheumatic fever: 1 female.
3 acute rheumatism: 2 male, 1 female.
1 chronic rheumatism: 1 male.

The histidine reaction was negative in all 9 cases.

Allergic diseases.

3 hay fever: 2 male, 1 female.
The result was negative in all 3 cases.

Diseases/

Diseases of the central nervous system.

5 specimens: 3 male, 2 female.

1 cerebral abscess: 1 male.

1 cerebral tumour: 1 male.

2 cerebral haemorrhage: 1 male, 1 female.

1 spinal tumour: 1 female.

No histidine was found in the urine of these 5 cases.

Miscellaneous Diseases.

15 specimens: 7 male, 8 female.

2 tonsillitis: 2 female.

1 epithelioma of the lip: 1 female.

1 epithelioma glands of the neck: 1 male.

1 anxiety state: 1 female.

1 hysterical vomiting: 1 female.

1 hysteria: 1 female.

2 migraine: 1 male, 1 female.

2 alcoholism: 2 male.

1 Pick's disease: 1 female.

1 sunstroke: 1 male.

1 recurrent attacks of syncope: 1 male.

1 multiple myelomatosis: 1 male.

In all 15 cases the histidine reaction was negative.

Results/

Results.

The specimens derived from normal healthy persons, some male, some female, invariably gave a standard and definite negative Kapeller-Adler reaction. The specimens from cases of normal pregnancy equally invariably gave a standard positive reaction. The remaining 299 were obtained from cases showing one or other of a great variety of derangements. A positive reaction was obtained in 14 cases only. These were as follows:

2 Schizophrenia: 1 male, 1 female.

2 General paralysis of the insane: 1 male, 1 female.

1 Chronic mania: 1 female.

1 Cirrhosis of the liver: 1 male.

2 Carcinoma: 2 male.

1 Pleurisy: 1 male.

1 Broncho - pneumonia: 1 female.

1 Gonococcal cystitis: 1 male.

1 Nephritis: 1 male.

1 Acromegaly: 1 female.

1 Pituitary dysfunction: 1 male.

Of these 14 positives the reaction approached the standard only in 4. These were in the cases of specimens obtained from patients suffering from acromegaly, cirrhosis of the liver, carcinoma of the penis, and gonococcal cystitis. A weak positive reaction was found in one case of schizophrenia, in/

in two cases of general paralysis of the insane, in one of severe broncho pneumonia, in one carcinoma of the fauces, in one with disturbed pituitary function, and nephritis. In one case of schizophrenia, one of mania, and one of dry pleurisy only traces of histidine are demonstrable. It is interesting to note that in only one case the positive reaction obtained remained uniformly strongly positive on subsequent tests during an appreciable period of time. This was a case of acromegaly. Norpoth (13) also records that histidine is constantly present in the urine of acromegalic patients. From this point of view, therefore, acromegaly would seem to be the only condition which in respect of histidine excretion is similar to normal pregnancy.

Discussion.

Though it is recognised that the series of examinations on which these discussions must be based is necessarily numerically small and that no single class within the series is in itself satisfactorily large, the author is forced by circumstances to bring this investigation to a premature end. Consequent upon the outbreak of war, and the associated dislocation of the existing medical services, it is impossible for the present to extend this investigation to statistically desirable proportions. However, it would seem warrantable to draw certain tentative conclusions from the results obtained.

It/

It is reasonable to assume that in certain cases some disorder of the pituitary is the cause of the histidinuria that was recognised. In two cases in which there was discernible pituitary disease present, discernible hyperfunctioning of the pituitary can be regarded as being responsible for the positive reaction obtained. In such cases, therefore, the test for histidine excretion might possibly be used in corroboration of the usual clinical diagnoses. The positive reaction obtained in the case of severe cirrhosis of the liver is probably to be explained by the reasonable postulate that the destruction of the liver tissue had impaired the effectiveness of the enzyme histidase. Were this so, then histidinuria would necessarily follow.

It may be that in the two cases of carcinoma associated with the positive Kapeller-Adler reaction, the cachexia itself was responsible for a metabolic disturbance of such magnitude that the elaboration and functioning of histidase became disorganised. In the remaining cases it would seem to be that the positive result obtained is to be regarded as an indication of temporary disturbance of the histidase function of the liver.

The contribution that this paper has to make would seem to be that the results obtained, whilst agreeing with those of Valle, Chomet, Stern and Morpoth, are completely in conflict with those of Schimmelpfeng and Tschopp. The former claims to have/

have found invariably histidine in the urine of patients suffering from melancholia, and according to his conclusions this test might have been regarded as possessing a value in differential diagnosis. In no case of melancholia in the present series, carefully examined by the refined Kapeller-Adler technique, was histidine found in the urine.

Tschopp and Tschopp obtained a positive histidine reaction in no less than three out of eight cases of healthy non-pregnant individuals. In the 12 cases of this category included in this present series, every one gave a very definite and unequivocal negative. The Tschopp's got 5 positive histidine reactions in a series of 28 cases of cardiovascular disease. The 28 cases of the same pathological group included in the present series invariably gave a definite negative.

Among the 63 patients suffering from diseases of the respiratory system examined by the Tschopps, 18 gave a positive histidine reaction. Among the 39 cases of the same category included in the present series, 1 gave a weak positive, another an extremely weak positive, and all the rest gave definite negatives. The Tschopps obtained 28 positives in a series of 67 cases of diseases of the alimentary system. Of the 28 specimens from the same clinical group included in the present series only one showed slight traces of histidine in the urine. Of 18 cases of liver diseases, the Tschopps found 8 positives. In the present series histidine could be demonstrated in only one out of 13 similar cases. 8 out of 18 cases of allergic diseases/

diseases proved to be positive when examined by the Tschopps. Of the three similar cases included in the present series, not one showed a trace of histidine.

There is thus very grave discrepancy between the results now reported and those published by the Tschopps. It seems most reasonable to ascribe this disagreement to differences in the actual procedures employed in the tests. Since both Tschopp and the present author were using the test devised by Kapeller-Adler, and since the latter has had the privilege of working directly under the guidance of Dr. Kapeller-Adler herself, it is surely possible for him to maintain that the results presented in this paper probably possess the greater degree of reliability.

The results obtained from this enquiry in themselves are purely negative. It is hoped that they may prove of value to others who find themselves interested in the same general problem.

Summary.

326 urines (193 males, 133 females) were tested by means of the newest Kapeller-Adler test for the presence of histidine. In the case of normal pregnancy the reaction was invariably positive; in those derived from non-pregnant healthy individuals it was equally invariably and unequivocally negative.

In/

In 14 out of 299 cases of diseases of various kinds, histidinuria was demonstrated.

It would seem that histidinuria is indicative of dysfunction of the pituitary or of the liver for tests for histidinuria cannot claim to possess any real value in differential diagnosis of diseases.

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