21. HISTIDINE METABOLISM IN TOXAEMIA OF PREGNANCY. ISOLATION OF HISTAMINE FROM THE URINE OF PATIENTS WITH TOXAEMIA OF PREGNANCY¹

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In the course of various investigations it has been demonstrated that histidine is a normal constituent of the urine of pregnant women, that it appears in the urine at a very early stage of pregnancy and continues to be excreted during the whole of gestation [Kapeller-Adler, 1933; 1934; 1936]. The rate of histidine excretion seems to vary with the individual and to be dependent on the kind of food taken [Kapeller-Adler & Schiller, 1935]. No histidine is to be detected in the urine of pregnant animals [Kapeller-Adler & Herrmann, 1934]. Further studies revealed the existence of a relationship between the excretion of histidine and that of gonadotropic hormones in human pregnancy [Kapeller-Adler *et al.* 1935; 1936; 1937]. The increase in the gonadotropic hormones during human gestation was assumed to be the cause of the non-destruction of histidine so that this amino-acid passes the liver unaffected and, not being deaminated by the kidneys, is excreted in the urine. Whereas histidinuria is characteristic of normal human pregnancy a more or less distinct alteration in histidine metabolism occurs in toxic pregnancy. The clinical importance of this alteration is considered in a paper at present in the Press [Kapeller-Adler, 1940]. Briefly the results obtained in the study of 58 cases of toxæmia of pregnancy of various types indicate that histidinuria is not appreciably affected in cases of mild toxic pregnancy, but is considerably diminished in patients with serious symptoms of pre-eclamptic toxæmia; in these little or no histidine is to be found in the urine. So constant is this that a marked diminution in histidine excretion can be used as a diagnostic sign of severe toxæmia of pregnancy.

It seemed possible that in severe toxæmia the histidine instead of being excreted as such was being transformed into some other substance, probably one of pharmacological activity. In this connexion decarboxylation of histidine to histamine had obviously to be considered, and several attempts have been made to detect histamine in the urine from toxæmic patients as well as from cases of normal pregnancy. In this paper these attempts are described.

EXPERIMENTAL

The method used depended upon precipitation of the histamine with $\text{Zn}(\text{OH})_2$ [compare Ackermann, 1939], decomposition of the precipitate with H_2S , extraction of the resulting alkaline solution with amyl alcohol, re-extraction of the histamine from the amyl alcohol with $N/10 \text{ H}_2\text{SO}_4$ or $N/10 \text{ HCl}$, evapora-

¹ The substance of this paper was reported at a Meeting of the Biochemical Society in Edinburgh on 8 June 1940.

tion of this solution *in vacuo* and preparation of the histamine picrate or flavianate.

Exp. 1. 23 l. of urine of four patients with signs of severe toxæmia and no histidinuria were worked up. The urines were collected over chloroform and kept in the ice chest. Every 1000 ml. of the mixed urine were treated with 30 ml. of a saturated solution of freshly precipitated $\text{Zn}(\text{OH})_2$ in ammonia (sp. gr. 0.880). After standing overnight the precipitate was filtered off, thoroughly washed with water and dried in air. The dry white powder was treated with dilute H_2SO_4 and freed from zinc by H_2S . The ZnS was filtered off and carefully washed with water. The filtrate was freed from H_2S by air-suction, made strongly alkaline with NaOH and the NH_3 was removed by distillation *in vacuo* at 40° . This solution, completely free from NH_3 and having pH 9.5, was concentrated *in vacuo* to about 100 ml. and shaken 6 times with pure amyl alcohol, 50 ml. being used for each extraction. The combined amyl alcohol extracts were shaken 5 times with $N/10$ H_2SO_4 (in later experiments HCl) using 20 ml. each time. To remove traces of amyl alcohol the sulphuric acid solution was vigorously shaken with 25 ml. of chloroform. The resulting liquid was concentrated to a small volume under reduced pressure and then tested for the presence of histamine as follows:

(a) With a small amount of this solution Pauly's diazo reaction [1904] was carried out and a strong positive result was obtained. This very sensitive test, although not specific for histamine, being given also by other iminazole derivatives, seemed however in this case to indicate the presence of histamine because other iminazole derivatives are insoluble in amyl alcohol.

(b) Another part of the solution obtained was subjected to the reaction of Zimmermann [1929]. This test, which is specific for histamine, also gave a strong positive result.

(c) To the main part of the liquid above described an excess of a concentrated solution of flavianic acid was added. After some time a pale yellow precipitate was formed. The m.p. was 256° . This precipitate was purified by repeated crystallizations from hot water. Ultimately, a fraction of a milligram of a yellow crystalline substance, insoluble in water, was obtained and this had m.p. 260° , not depressed on admixture with an authentic specimen of histamine diflavianate. Under the microscope the material appeared as bright yellow characteristic prisms showing great similarity with crystals of histamine diflavianate. On account of the strong positive result of the Zimmermann reaction in the fluid investigated and of the fact that the isolated flavianate showed many physical properties characteristic of histamine diflavianate it was assumed that the base present might indeed be histamine. It was of course necessary to obtain further evidence for that assumption.

Exp. 2. 34 l. of urine of several patients with signs of severe toxæmia of pregnancy and no histidinuria were worked up in the manner above described. The flavianate isolated had after repeated recrystallization m.p. 260° , not depressed on admixture with histamine diflavianate. Unfortunately, the yield of the material obtained was again very small, amounting only to a fraction of a milligram. Since the identification of the compound, so far carried out, was not at all satisfactory a further attempt was undertaken to procure a greater yield.

Exp. 3. This time it was decided to start from the urine of only two patients with signs of very severe toxæmia of pregnancy and no histidinuria at all in the hope that under these conditions histaminuria might be so considerable as to allow of histamine being isolated in larger amounts. 52 l. of urine from the two patients were treated in the way described, the only difference being that the amyl alcohol extracts were shaken with $N/10$ HCl instead of $N/10$ H_2SO_4 . The

liquid thus obtained was evaporated to dryness *in vacuo* after the traces of amyl alcohol had been removed by chloroform. A hydrochloride was obtained which showed strong positive Pauly and Zimmermann reactions. After recrystallization from hot alcohol prisms were obtained which, under the microscope, seemed very similar to crystals of histamine dihydrochloride. This hydrochloride was converted into a picrate which after several recrystallizations from hot water melted at 238–242° with decomposition and brown-black colouring. It showed no depression of M.P. when mixed with histamine dipicrate. This experiment yielded 2.7 mg. of the picrate which, like histamine dipicrate, was soluble in hot water and insoluble in ether. Under the microscope the crystals of the isolated picrate, consisting of yellow plates, closely resembled those of histamine dipicrate. It may be pointed out that histamine picrate as well as histamine flavianate give directly a strong positive diazo reaction without having been previously freed from picric or flavianic acid. Unfortunately, the amount of the picrate obtained was not large enough for a micro-analysis but X-ray powder photographs of it were kindly taken by Dr C. A. Beevers, Chemistry Department, Kings Buildings, Edinburgh:

Fig. 1. Photographs of substance I (histamine dipicrate) and substance II (picrate isolated from toxic pregnancy urine).

Dr Beevers makes the following observation on these photographs:

(1) General features identical but II has some extra lines (continuous parts due to glass).

(2) I and II have at least nearly the same crystal structure. What differences there are may be due to (a) impurities in II crystallizing in quite a different way; (b) imperfect crystallization of I resulting in certain lines becoming blurred. This is supported by the fact that photograph I is generally more blurred than II.

(3) Lines are very numerous, showing a large unit cell (probably large molecules).

(4) Photographs were taken with Cu K X-rays (Ni filter to remove $K\beta$), $2\frac{3}{4}$ hr. exposure, 25 mA., 60 kW.).

Exp. 4. Whereas in the previous experiments urines of women suffering from pre-eclamptic toxæmia were used for the isolation of histamine this time the examination of the urine of a woman with hyperemesis gravidarum for the presence of histamine was undertaken.

The urinary output of 6 successive days (3000 ml.) of a woman in the 10th-12th weeks of pregnancy with distinct signs of hyperemesis gravidarum and with almost no histidinuria was the starting material. The urine was daily precipitated with $\text{Zn}(\text{OH})_2$ and the precipitate worked up in the manner described above, with only one slight modification. After the decomposition of the zinc salt by H_2S a few ml. of ammonia solution were added to the liquid containing dilute H_2SO_4 in order to ensure complete precipitation of ZnS . The precipitate was allowed to settle overnight and was then worked up according to exp. 3. The hydrochloride obtained showed strong positive diazo and Zimmermann reactions. In presence of a little dilute H_2SO_4 it was converted into the flavianate by means of a concentrated solution of flavianic acid. The bright yellow flavianate showed a strong positive diazo reaction and had m.p. 258° . On several recrystallizations a pure substance was obtained with constant m.p. 260° , not depressed on admixture with an authentic specimen of histamine diflavianate. The yield of the purified material was 10.9 mg. (Found (Weiler): C, 39.70; H, 3.6; N, 13.4%; required for $\text{C}_8\text{H}_9\text{N}_3(\text{C}_{10}\text{H}_6\text{N}_2\text{SO}_8)_2$: C, 40.58; H, 2.86; N, 13.26 %.)

Exp. 5. 39 l. of urine from four normal pregnant women with histidinuria were worked up as in exp. 4. The hydrochloric acid solution finally obtained, although showing a positive diazo reaction, did not yield any histamine diflavianate with flavianic acid. Obviously only traces of histamine were present.

It may be mentioned that Ackermann [1939] has isolated 0.9 mg. histamine dipicrate from 1000 l. of normal urine. Because of the very small amount of the compound he could not identify it accurately.

DISCUSSION

The experiments recorded above indicate that whereas normal pregnancy is characterized by histidinuria, in severe toxæmia of pregnancy the excretion of histamine in the urine seems to replace that of histidine. It should be stressed that the amount of histamine isolated certainly represents only a small part of the amount which may really be present in toxæmic pregnancy urine, since losses in the preparation cannot be avoided and, apart from that, not all the histamine can be recovered.

There is no doubt that the histamine appearing in toxæmia of pregnancy can only be derived from histidine which is normally present in large amounts throughout gestation. Histamine is presumably formed from histidine by decarboxylation. Werle [1936; 1937; 1938] has found that animal tissues, especially the kidneys, contain an enzyme which can alter histidine to histamine by decarboxylation. This enzyme which he called histidine decarboxylase appears to be more effective in absence than in presence of oxygen. But there is still another enzyme in the kidneys with an antagonistic action tending to the destruction of histamine. This enzyme, histaminase, first described and isolated by Best [1929; 1930; 1932; 1935] is effective only in the presence of oxygen.

Although no evidence has been advanced as yet as to the occurrence of histidine decarboxylase in the human body, the above-reported isolation of histamine from toxæmic pregnancy urine seems to suggest the presence of such an enzyme in man. For the conversion of histidine which is excreted in rather large amounts throughout normal pregnancy into histamine appearing in the urine of women with different manifestations of severe toxæmia of pregnancy can only be explained if the presence of a histidine decarboxylase in the human body is assumed.

Accordingly two factors seem to be responsible for the velocity of formation of histamine in histidine metabolism, namely, the activity of the histidine decarboxylase and that of histaminase. In a recent paper Werle [1940] reports that the activities of both enzymes are not only dependent on the presence or absence of oxygen but far more on the presence of various inactivators which regulate their action.

Thus the occurrence of histamine in the metabolism of pregnant women will finally be determined by the balance between histidine decarboxylase and histaminase.

The significance of the isolation of histamine from toxæmic pregnancy urine for the explanation of the pathogenesis and of the course of toxæmia of pregnancy will be discussed in another place.

SUMMARY

1. A chemical method has been described for isolation of histamine from the urine.

2. Four attempts have been made to isolate histamine from the urine of women with various manifestations of toxæmia of pregnancy. Whereas in the first two experiments only fractions of a milligram of histamine diflavinate have been isolated, exp. 3 yielded 2.7 mg. of histamine dipicrate from 32 l. of urine of two women with very severe toxæmia, identified by melting point, mixed melting point, the Zimmermann reaction and an X-ray photograph; in exp. 4, 10.9 mg. histamine diflavinate have been isolated from 3000 ml. of urine of a woman with severe hyperemesis gravidarum and identified by a micro-analysis.

3. From 39 l. of normal pregnancy urine with normal histidine excretion the same method yielded no detectable quantity of histamine.

4. The origin of histamine and the factors responsible for its occurrence in toxic pregnancy are discussed.

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Note added 20 February 1941. Further identification of histamine isolated from the urine of patients with toxæmia of pregnancy.

Since the above paper was submitted more histamine has been isolated from the urine of two toxæmic patients. 7700 ml. of urine of a patient with hyperemesis gravidarum yielded 34.6 mg. histamine diflavianate and 62.5 mg. of the same salt were prepared from 8500 ml. of urine of a woman suffering from serious pre-eclamptic toxæmia. In both cases the histamine diflavianate first obtained was purified and was then freed from flavianic acid by dilute hydrochloric acid and butyl alcohol, thus giving a pure histamine hydrochloride. This salt was converted again into the diflavianate which had m.p. 262–263°, not depressed on admixture with an authentic specimen of histamine diflavianate. (Found (Weiler): C, 39.71; H, 3.22; N, 12.96%; required for $C_5H_9N_3(C_{10}H_6N_2SO_8)_2$: C, 40.58; H, 2.86; N, 13.26%.)

Prof. A. J. Clark, Department of Pharmacology of the University, Edinburgh, kindly carried out biological tests with the preparations from both of these patients. In the case of the patient with hyperemesis the pure hydrochloride was used, whilst in the second case the hydrochloride contained traces of the flavianate. In tests on the guinea-pig's ileum, the guinea-pig's uterus and the rat's uterus the preparations from urine were identical in pharmacological action with similar amounts of authentic histamine.