and saturation of nicotinamide. Nicotinic acid, nikethamide and nicotinic acid-mono-ethyl-amide have a similar but quantitatively different action.

3. The hourly fluctuations of the nicotinamide methochloride elimination were studied and found to be identical in different persons and in the same person under different conditions.

4. The effect of a number of factors (food, alcohol, work) influencing the elimination of nicotinamide methochloride was studied.

5. The height of the nicotinamide methochloride elimination is determined by the intake of nicotinamide and related compounds and their use by the body, the presence of methyl-donators and the efficiency of the methylating mechanism.

6. The relation of nicotinamide methochloride eliminated in the urine to the nicotinamide ingested with the food indicates the presence of an extra dietary source of nicotinamide.

This work forms part of an investigation on nicotinamide deficiency carried out on behalf of the Air Ministry. We wish to thank Air Marshal Sir H. E. Whittingham, K.B.E., K.H.P., Director-General of the Medical Services of the Royal Air Force, for facilities provided, and Flight-Lieutenant G. A. Smart for collecting samples from airmen. Our thanks are also due to L.A.C.W. A. E. Wigglesworth for technical assistance and to members of the Scientific and Technical Staff of the Lister Institute who volunteered as experimental subjects.

We wish to acknowledge the gift of trigonelline from Dr B. S. Platt, of nicotinic acid mono-ethyl-amide from British Drug Houses Ltd. through Dr B. S. Platt, of pyridoxine and riboflavin from Glaxo Laboratories Ltd., of aneurin and nicotinamide from Roche Products Ltd., and of nicotino-nitrile from Dr J. Walker. One of the authors (R.A.C.) is a member of the Civilian Technical Corps of the Air Ministry attached to the Lister Institute of Preventive Medicine for an investigation of nicotinamide deficiency.

REFERENCES

- Coulson, R. A. & Ellinger, P. (1943). *Biochem. J.* **37**, Proc. xvii.
 ——— Glock, G. E. & Platt, B. S. (1942). *Biochem. J.* **36**, Proc. xix.
 ——— & Holden, M. (1944). *Biochem. J.* **38**, 150.
 Ellinger, P. & Coulson, R. A. (1943). *Nature, Lond.*, **152**, 583.
 ——— & Dojmi, L. (1935). *Liječn. Vjesn.* **2**, 6.
 ——— Hassan, A. & Taha, M. M. (1937a). *Lancet*, **2**, 755.
 ——— ——— (1937b). *Lancet*, **2**, 1188.
 Göbell, O. (1941). *Z. ges. exp. Med.* **109**, 96.
 Huff, J. W. & Perlzweig, W. A. (1943). *Science*, **97**, 538.
 Najjar, V. E. & Holt, L. E., Jr. (1943). *J. Amer. med. Ass.* **123**, 683.
 Neuwahl, F. J. (1943). *Lancet*, **2**, 348.
 Perlzweig, W. A., Bernheim, M. L. C. & Bernheim, F. (1943). *J. biol. Chem.* **150**, 401.
 Sarett, H. P. (1943). *J. biol. Chem.* **150**, 159.
 Single, S. A. & Sydenstricker, V. P. (1941). *Science*, **94**, 546.
 Wang, Y. I. & Kodicek, E. (1943). *Biochem. J.* **37**, 530.

Investigations on the Activity of the Histaminase in Normal and Toxaemic Pregnancy

By R. KAPELLER-ADLER, *Biochemical Laboratory of the Royal Infirmary, Edinburgh*

(Received 22 March 1944)

In some recent papers I have suggested that histaminase may play an important part in the metabolic changes occurring in normal and toxaemic pregnancy (Kapeller-Adler, 1941 a, b, c; Kapeller-Adler & Adler, 1943). From the experimental data obtained it has been assumed that histamine might be formed, in the metabolism of pregnant women, from the histidine present in large amounts throughout gestation, by the activity of histidine decarboxylase. In normal pregnancy most of the histamine formed is presumably destroyed by histaminase so that only traces escape destruction, to be excreted in the urine. It has further been suggested that in mild cases of pre-eclamptic toxaemia the activity of the histaminase may be impaired, and that more histamine may thus escape and be available to cause various kinds of damage.

Much of it, however, seems to be eliminated in the urine. In severe cases of pre-eclamptic toxaemia and in eclampsia a condition may arise where the activity of the histidine decarboxylase may be increased, whereas that of the histaminase may be completely inhibited. Thus much histidine would be converted into histamine which, not being destroyed by histaminase, would cause considerable damage, especially to the liver and kidneys. The latter would then lose the ability to excrete histidine and histamine, which would be completely retained in the tissues. The object of the present paper was to obtain evidence of the role played by the histaminase in normal and toxaemic pregnancy.

Marcou (1937) reported that the blood in pregnancy has an extraordinarily high activity in destroying histamine. Other investigators (Werle &

Effkemann, 1940; Zeller, Stern & Wenk, 1941a), working independently, came to the same conclusion and emphasized that this activity is characteristic only of pregnancy and does not occur in any other condition. According to Werle & Effkemann (1940) and to Zeller (1938 *a, b*), the histaminase reaction in the blood becomes positive in the very early months of pregnancy, remains positive until delivery, and disappears within the first few days of the puerperium. From numerous experiments Zeller (1938 *a, b*), Zeller, Birkhäuser, Mislin & Wenk (1939 *a*), Zeller, Schär & Staehlin (1939 *b*) and Zeller, Stern & Wenk (1940) claim that the histaminase discovered by Best in 1929 is identical with the diaminoxidase which Zeller himself described in 1938 and which he found to be able to destroy not only histamine but also other naturally occurring diamines like cadaverine, putrescine, agmatine, spermin, etc. Zeller suggests that the diaminoxidase reaction is an oxidative one according to the equation

Evidence that H_2O_2 is probably formed by this system was given by Stevenson (1943), who investigated the mechanism of the histaminase reaction upon histamine, putrescine and cadaverine. The purpose of this work was not only to study the histaminase activity in the serum of patients with normal and toxæmic pregnancy but also to investigate the activity of this enzyme in the placenta and its possible significance in the onset and course of labour. Danforth (1937) and Zeller *et al.* (1939 *a, b*) report the occurrence of the histaminase in the human placenta. Although in Danforth's experiments the amount of histaminase in the placenta showed some correlation with the efficiency of uterine contractions, the author did not think that the evidence was sufficient to draw a definite conclusion.

EXPERIMENTAL

One of the chemical methods for the estimation of the activity of the diaminoxidase (histaminase) in the serum devised by Zeller, Stern & Wenk (1941 *b*) is based upon the decoloration of a solution of indigo disulphonate by the H_2O_2 formed in the reaction between the diaminoxidase and its substrate. For this purpose Zeller advocates the use of cadaverine as substrate, since he found that histamine inhibits its own oxidation if present in superoptimal amounts. I have adopted this colorimetric method for my investigations after having made minor alterations in the amount of reagents. The required reagents are: (1) A solution of *m*/15 phosphate buffer (Sørensen) with pH 7.2. (2) A *m*/20 solution of cadaverine hydrochloride in *m*/15 phosphate buffer. (3) A solution of 20 mg. indigo disulphonate in 30 ml. of *m*/15 phosphate buffer.

Examination of serum. The serum is dialyzed against *m*/15 phosphate buffer in a refrigerator overnight. Haemolyzed sera must not be used.

To 3 ml. of the dialyzed serum is added 0.5 ml. of the *m*/20 cadaverine hydrochloride solution, and to 3 ml. of the control serum 0.5 ml. of the phosphate buffer solution is added. To each test-tube 0.2 ml. of the indigo disulphonate solution and 0.1 ml. of toluene are then added and oxygen is allowed to bubble through the solution for 1–2 min. The test-tubes are closed with a rubber stopper and incubated for 24 hr. at 37°. After that time the decoloration of the indigo disulphonate is examined. The initial blue colour of the control does not as a rule change, whereas the colour of the solution under assay has (a) completely disappeared or (b) diminished or (c) not changed at all. The estimation of the activity of the diaminoxidase (histaminase) is based upon the extent to which the indigo solution has been decoloured; thus +++ for complete decoloration, ++ for distinct decoloration, + for slight decoloration, 0 for no decoloration.

Examination of placenta. 10 g. of the thoroughly ground placenta are extracted with 25 ml. of 2.5% (w/v) NaCl solution and the extract dialyzed against *m*/15 phosphate buffer solution in the refrigerator for 24 hr. The precipitate is then centrifuged off. To 2.5 ml. of the placental extract 0.5 ml. of the *m*/20 solution of the substrate is added and then to each test-tube 2 ml. of the indigo solution and 0.3 ml. of toluene are introduced. The rest of the procedure is the same as that described above for serum.

RESULTS

Sera from 101 pregnant and non-pregnant subjects have been examined. Eight sera of non-pregnant subjects gave a completely negative result. Twenty-three mixed sera of women at various stages of gestation (12–40 weeks) showed a vigorous enzymic activity (++ to +++). In five cases the addition of 5 mg. of aneurin to 3 ml. of serum brought about a complete inhibition of the enzymic activity: no decoloration of the indigo solution occurred. This confirms the observation of Zeller *et al.* (1939 *b*) that solutions of the diaminoxidase were completely inactivated by the presence of aneurin. The results obtained in the investigation of the serum of six patients suffering from pre-eclamptic toxæmia were very surprising since, contrary to my expectation based on the previous work quoted above, they showed no diminished activity of the serum except in one case. Until then, following Zeller's work, I had measured the activity of the diaminoxidase (histaminase) by using only cadaverine as substrate. It became clear to me that, if any conclusion was to be drawn from the experimental work regarding histaminase metabolism in pregnancy, histamine had to be used as substrate. Therefore, it was decided to use *m*/20 solutions of histamine hydrochloride, as well as of cadaverine hydrochloride, as substrates in parallel tests.

The sera of nine non-pregnant women were first examined by this modified technique; the results obtained were completely negative. The investigation of 32 mixed sera from women at various stages of normal pregnancy (12–40 weeks) was then carried

out. As can be seen from Table 1 most sera showed the same enzymic activity with either substrate. In a few instances, however, the activity appeared to be slightly less with histamine as substrate. Added to five sera, aneurin inhibited the enzyme activity both when histamine and cadaverine were used as substrates.

Table 1. *Diaminoxidase activity of sera from normal pregnant women, with cadaverine and histamine as substrates*

(Activity estimated by degree of decoloration of indigo-disulphonate: +++ complete, ++ distinct, + slight, 0 no decoloration.)

No. of patients	Weeks of gestation	Diaminoxidase activity substrate		Activity of diaminoxidase in presence of aneurin
		Cadaverine	Histamine	
16	12-20	++ to +++	++ to +++	0
16	20-40	++ to +++	++ to +++	—

The results obtained in ten cases of mild pre-eclamptic toxæmia are presented in Table 2. While the results with cadaverine as substrate have been found to be on the whole comparable with those in normal pregnancy serum, this was not the case when histamine was used as substrate. In seven out of ten cases a distinctly diminished activity of the enzyme was found when the results were compared with the normal. Table 2 also shows results given

Table 2. *Diaminoxidase activity of sera from women with mild and severe pre-eclamptic toxæmia*

No. of patients	Weeks of gestation	Diaminoxidase activity substrate	
		Cadaverine	Histamine
Mild pre-eclamptic toxæmia			
3	28-40	++	Trace
1	40	+	Slight trace
3	38-40	++ to +++	+
3	34-37	+++	++
Severe pre-eclamptic toxæmia			
4	31-35	++ to +++	0
3	28-31	++ to +++	Trace
1	32	+++	+
2	33	+++	++
			} Still-birth

by the sera of ten patients with severe pre-eclamptic toxæmia. In most of the sera there was again little difference in the enzymic activity with cadaverine as substrate as compared with that in normal pregnancy. When, however, histamine was used as substrate, completely different results were obtained. In four out of ten sera no activity was observed and in three others only a trace. In the sera of two patients, however, a normal activity of the diaminoxidase was found irrespective of whether

cadaverine or histamine had been used as substrate. Both patients had stillbirths; intra-uterine death occurred several days before delivery and before the enzyme estimation had been carried out. The very-serious condition of both patients cleared up also a few days before delivery.

Table 3. *Diaminoxidase activity of sera from women with eclampsia*

No. of patients	Weeks of gestation	Diaminoxidase activity substrate	
		Cadaverine	Histamine
2	36-40	++ to +++	0
1	37	++	Trace

Table 3 shows the results obtained with the sera of three eclamptic patients. Whereas enzymic activity comparable with that in normal pregnancy was found with cadaverine as substrate, no activity was seen in two sera and only a trace in one serum when histamine was used as substrate. In four out of five cases of *hyperemesis gravidarum* negative enzymic reactions were obtained in the serum irrespective of the substrate used. In one of the cases normal activity was obtained with cadaverine whereas only a trace of activity was seen with histamine as substrate. The sera of four pregnant patients, three of them suffering from hypertension and one from cardiac failure, gave normal results with both substrates.

Placentæ from 45 women with normal and instrumental delivery were examined for histaminase activity, with cadaverine and histamine as substrates in parallel tests. Table 4 shows the results

Table 4. *Diaminoxidase activity of placenta from women with normal labour*

No. of patients	Diaminoxidase activity substrate	
	Cadaverine	Histamine
8	++ to +++	0
3	++ to +++	Trace
1	+++	+

obtained in the examination of twelve placenta from women with normal labour (with efficient uterine contractions). While enzymic activity was high in the experiments with cadaverine as substrate, the analogous experiments with histamine as substrate gave, in most cases, completely negative results. The results differed when placenta of eight women who had been delivered by caesarean section before the onset of labour were examined (Table 5); vigorous activity was found with both cadaverine and histamine as substrates.

In Table 6 are the results obtained on seven placenta from women who underwent caesarean section after a trial of labour. Whereas normal results

Table 5. *Diaminoxidase activity of placentae from women with caesarean section before the onset of labour*

No. of patients	Diaminoxidase activity substrate	
	Cadaverine	Histamine
1	+++	++
7	+++	+++

Table 6. *Diaminoxidase activity of placentae from women with caesarean section following trial of labour*

No. of patients	Diaminoxidase activity substrate	
	Cadaverine	Histamine
1	+++	+++
1	+++	++
3	+++	+
1	++	Trace
1	++	0

were again obtained with cadaverine as substrate, the findings with histamine were variable. With two out of seven placentae there were normal positive results, with three others a weak positive; one placenta showed a trace of enzymic activity and with another the enzymic reaction was negative.

Five placentae from women with prolonged labour were examined (Table 7). Strong activity was as usual observed with cadaverine as substrate. With histamine as substrate three placentae showed a strong enzymic reaction and two a weak one. Ten

Table 7. *Diaminoxidase activity of placentae from women with prolonged labour*

No. of patients	Diaminoxidase activity substrate	
	Cadaverine	Histamine
3	+++	++ to +++
2	+++	+

placentae from patients with pre-eclamptic toxæmia, who delivered themselves spontaneously, were examined. With cadaverine as substrate, vigorous enzymic activity was seen in all except one placenta; with histamine, no activity, or only a trace, was observed in all the placentae. In three cases of eclampsia cadaverine gave the usual strongly positive reaction; histamine, however, gave negative results, in two cases, and in one case showed only a trace of enzymic activity.

DISCUSSION

The results obtained in the investigations on pregnancy serum and on placentae make it clear that Zeller's claim that histaminase and diaminoxidase are identical should be thoroughly re-examined. The findings in the experiments with histamine and with cadaverine as substrates are on the whole so completely different that the question arises whether

the histaminase really is identical with the diaminoxidase.

As to the results obtained with the sera of non-pregnant women and of those with normal pregnancy, the conclusions of previous authors are confirmed. The histaminase reaction was found to be negative in the sera of non-pregnant individuals, and positive without an exception in normal pregnancy serum. The results found in the sera of women suffering from toxæmia of pregnancy, when histamine was used as substrate, were significant. As was to be expected from previous work quoted above, diminished histaminase activity was seen in cases of mild pre-eclamptic toxæmia, and only a trace of activity or a negative enzymic reaction was obtained in cases of *hyperemesis gravidarum*, in severe cases of pre-eclamptic toxæmia and in eclampsia. The two cases of severe pre-eclamptic toxæmia, in both of which intra-uterine death had occurred and in the sera of which normal histaminase activity was found, are interesting so far as the results obtained may be suggestive of a possible restoration of a normal histaminase reaction in the serum caused by the death of the foetus. The completely normal findings in the cases of hypertension and cardiac failure may suggest that a diminished activity or absence of the histaminase in the serum may be characteristic only of toxæmic pregnancy. More investigations, however, must be carried out on this subject. The results obtained in the investigations on 45 placentae with respect to histaminase activity may suggest that the amount of histaminase in the placenta might be correlated with the character of the labour, for the activity of the histaminase in the placenta was found to be inversely proportional to the uterine efficiency. Little or no histaminase activity was found in the placentae of women who had had a normal labour with very efficient contractions, whereas high enzymic activity was seen in the placentae of patients who had had to undergo a caesarean section before the onset of labour. Accordingly, the placentae of women with prolonged labour and poor uterine contractions, and of patients with primary or secondary uterine inertia, contained varying amounts of histaminase. No spectacular conclusions can yet be drawn from the findings with the placentae of women with pre-eclamptic toxæmia and eclampsia. In view of previous work histaminase activity might be expected to be much diminished or even completely absent in these cases. As all patients, however, had a spontaneous delivery, the fact that no enzymic activity or only a trace of it has been found in the placentae has therefore no significance, since, as shown above, placentae of women with normal pregnancy and spontaneous delivery do not contain histaminase. More work, therefore, will have to be done on these lines.

Although these experiments are far from complete, it appears possible from the data obtained that histaminase might play an important part at the onset and during labour. It may be suggested that histaminase, which is very effective in the placenta during pregnancy, might become inhibited at the end of pregnancy by the action of some compounds, possibly the sex hormones, which are known to play an important role at the onset of labour. It may be recalled that in 1937 (Kapeller-Adler, 1937) it was shown that the gonadotropic hormones inhibit the activity of the histidase.

SUMMARY

1. The activity of the histaminase in the sera and placentae of pregnant women has been studied with two different substrates, cadaverine and histamine, in parallel tests.

2. The histaminase reaction is negative in the serum of non-pregnant women.

3. In normal pregnancy serum the histaminase test was found to be positive without exception, irrespective of the substrate used.

4. The activity of the histaminase is diminished in mild cases of pre-eclamptic toxæmia and only a trace of activity or a negative enzymic reaction was obtained in cases of *hyperemesis gravidarum*, in severe cases of pre-eclamptic toxæmia and in eclampsia.

5. The results obtained in investigations on 45 placentae suggest a possible correlation of the amount of the histaminase in the placenta with the character of the labour, the activity of the histaminase in the placenta having been found to be inversely proportional to the uterine efficiency.

6. The results obtained render doubtful Zeller's claim that the histaminase and diaminoxidase are identical.

I am very much indebted to the obstetricians of the Simpson Memorial Maternity Pavilion, Royal Infirmary, Edinburgh, for permission to use the clinical material and to the resident and nursing staff of this institution for valuable assistance. I am especially thankful to Prof. R. W. Johnstone for his helpful criticism. My thanks are due to the Medical Research Council for a whole-time grant. I am also grateful to the Clayton Co. Ltd. for a generous gift of indigo disulphonate.

REFERENCES

- Best, C. H. (1929). *J. Physiol.* **67**, 256.
 Danforth, D. N. (1937). *Amer. J. Physiol.* **119**, 294.
 Kapeller-Adler, R. (1937). *Biochem. Z.* **293**, 207.
 — (1941a). *Biochem. J.* **35**, 213.
 — (1941b). *J. Obstet. Gynaec.* **48**, 141.
 — (1941c). *J. Obstet. Gynaec.* **48**, 155.
 — & Adler, E. (1943). *J. Obstet. Gynaec.* **50**, 177.
 Marcou, J. (1937). *Bull. Acad. Med. Roum.* **3**, 1.
 Stevenson, N. R. (1943). *J. biol. Chem.* **149**, 169.
 Werle, E. & Effkemann, G. (1940). *Arch. Gynaec.* **170**, 82.
 Zeller, E. A. (1938a). *Helv. chim. Acta*, **21**, 880.
 — (1938b). *Helv. chim. Acta*, **21**, 1645.
 — Birkhäuser, H., Mislin, H. & Wenk, M. (1939a). *Helv. chim. Acta*, **22**, 1381.
 — Schär, B. & Staehlin, S. (1939b). *Helv. chim. Acta*, **22**, 837.
 — Stern, R. & Wenk, M. (1940). *Helv. chim. Acta*, **23**, 3.
 — — — (1941a). *Schweiz. med. Wschr.* **71**, 544.
 — — — (1941b). *Schweiz. med. Wschr.* **71**, 1349.

A Study of the Determination of Glucuronic Acid by the Naphthoresorcinol Reaction, with the Photoelectric Absorptiometer

By S. W. F. HANSON, G. T. MILLS AND R. T. WILLIAMS, *Department of Biochemistry, University of Liverpool*

(Received 24 April 1944)

Methods for the determination of glucuronic acid in biological materials have appeared intermittently in the literature for the last 50 years. That none of these methods is entirely satisfactory is not surprising, since the reactions upon which they are based are not specific. The problem of the accurate determination of glucuronic acid in biological materials is therefore a difficult one.

Glucuronic acid may be estimated by the following methods: (1) those based upon measurement

of its reducing properties, e.g. by Shaffer-Hartman and Bertrand's reagents (Goebel & Babers, 1933); by Benedict's reagent (Quick, 1924, 1925); by the ferricyanide reduction method of Miller & Van Slyke (Fishman, 1938); (2) those depending upon the formation of furfural by the action of hydrochloric acid on glucuronic acid (e.g. C. Tollens, 1909; Haendel, 1929; Tanabe, 1938); (3) those depending upon the carbon dioxide set free by the decarboxylation of glucuronic acid with mineral acids and other