depends on the creatine content of the tissue. The belief that the presence of phosphocreatine is almost exclusively confined to muscular and nervous organs (Eggleton & Eggleton, 1929; Gerard & Tupikova, 1939) has given rise to the concept (Lipmann, 1941) that phosphocreatine has a connexion with the speed of action required by these organs. The occurrence of phosphocreatine in such organs as spleen, liver and kidney, on which no sudden demands for energy are made comparable to that placed upon, for example, skeletal muscle, suggests the desirability for some modification of this concept.

It would seem probable that phosphocreatine has a function other than that of acting as a reservoir of readily available phosphate-bond energy for the adenylic acid system and that it may contribute energy to endergonic reactions directly and without the mediation of the latter system.

SUMMARY

1. A method is described for the determination of phosphocreatine in protein-free tissue extracts.
2. Phosphocreatine has been shown to occur in all tissues investigated and the proportion of the total creatine present which is phosphorylated is of the same order irrespective of the tissue concerned.
3. Measurement of the phosphocreatine content of tissue by determination of acid-molybdate-labile phosphorus has been shown to be subject to error.

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REFERENCES

- Barker, H., Ennor, A. H. & Harcourt, K. (1950). *Aust. J. Sci. Res. B*, **3**, 337.
- Barker, H. & Ennor, A. H. (1951). *Biochim. Biophys. Acta*, **7**, 272.
- Eggleton, G. P. & Eggleton, P. (1929). *J. Physiol.* **68**, 198.
- Eggleton, P., Elsdon, S. R. & Gough, N. (1943). *Biochem. J.* **37**, 526.
- Ennor, A. H. & Stocken, L. A. (1948a). *Biochem. J.* **42**, 549.
- Ennor, A. H. & Stocken, L. A. (1948b). *Biochem. J.* **42**, 557.
- Ennor, A. H. & Stocken, L. A. (1950). *Aust. J. exp. Biol. med. Sci.* **28**, 647.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Franks W. R. (1932). *J. Physiol.* **74**, 195.
- Gerard, R. W. & Tupikova, N. (1939). *J. cell. comp. Physiol.* **13**, 1.
- Kerr, S. E. (1935). *J. biol. Chem.* **110**, 625.
- LePage, G. A. & Umbreit, W. W. (1945). In *Manometric Techniques and Related Methods for the Study of Tissue Metabolism*, p. 190, edited by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.
- Lipmann, F. (1941). *Advanc. Enzymol.* **1**, 99.
- McIlwain, H., Buchel, L. & Cheshire, J. D. (1951). *Biochem. J.* **48**, 12.
- Weil-Malherbe, H. & Green, R. H. (1951). *Biochem. J.* **49**, 286.

Histamine Metabolism in the Human Placenta and in the Umbilical Cord Blood

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In a recent paper (Kapeller-Adler, 1951) a new simple microchemical method and a new unit have been proposed for the estimation of histaminase activity in biological media. In the present work, this new method, along with the biological estimation of histamine, has been applied to the study of histamine metabolism in human placenta and in umbilical cord blood. A significant decrease in histaminase activity has been observed in placenta

of cases of severe toxæmia of pregnancy and a significant increase in placenta of women with twin pregnancy. The placental histamine content of women with severe toxæmia of pregnancy has been found to be greatly increased as compared with that of normal placenta. The results are related to previous observations made upon this subject with different methods. In view of contradictory findings concerning the action of histaminase on cadaverine (Kapeller-Adler, 1951), it was thought desirable to include observations on the effect of placental histaminase on this diamine in this paper.

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METHODS

Preparations of histaminase from placenta. The placentae were obtained as soon as possible after the confinement and were worked up within 24 hr. Superficial blood was removed by rapid rinsing with cold water and drying between sheets of filter paper. After the removal of membranes and the umbilical cord the organ was weighed and finely minced in an electric mincing apparatus. Thoroughly mixed pulp (40 g.) was ground in a mortar with 200 ml. saline. The fine suspension was transferred to a conical flask, was allowed to stand for 1 hr. and then filtered through a fluted filter

RESULTS

Histaminase activity in placentae

Placentae of women who had been delivered spontaneously or by a caesarean section at or before term, after a normal or toxæmic pregnancy, were examined for histaminase activity. Table 1 shows the results obtained in these investigations.

If the average results shown in the first line of Table 1 for the histaminase activity in normal placentae, delivered at term, are expressed in terms of $\mu\text{g.}$ of histamine inactivated per hr., it follows that 1 g. of placenta inactivates 45.54 $\mu\text{g.}$ of hist-

Table 1. *Effect of placental histaminase on histamine and cadaverine in parallel assays*

(Histaminase activity is expressed in permanganate units (P.U.) per g. placenta and per total placenta. 1 P.U. represents the amount of enzyme which, after 24 hr. at 37° and pH 7.2 in an atmosphere of O_2 with 1 mg. histamine hydrochloride as substrate and with an aqueous solution of indigodisulphonate, uses 0.1 ml. 0.002 N- KMnO_4 . 1 P.U. corresponds to the destruction of 0.46 $\mu\text{g.}$ histamine/hr. In this and succeeding tables the values given are means $\pm$ S.D. of a single observation.)

Cases		Histamine	Histamine	Cadaverine
Number	Description	(P.U./placenta)	(P.U./g. of placenta)	(P.U./g. of placenta)
63	Normal, at term	49 800 $\pm$ 10 700	99.0 $\pm$ 14.8	155.0 $\pm$ 3.2
14	35th-39th week of pregnancy	40 960 $\pm$ 12 500	90.2 $\pm$ 25.7	148.8 $\pm$ 42.1
1	20th week of pregnancy	—	122.0	145.5
13	Caesarean section 36th-40th week	36 300 $\pm$ 16 000	65.1 $\pm$ 20.6	118.6 $\pm$ 39.0
5	Mild toxæmia	38 640 $\pm$ 6 100	75.6 $\pm$ 9.6	155.8 $\pm$ 35.0
19	Severe toxæmia	22 520 $\pm$ 7 840	52.6 $\pm$ 15.9	120.3 $\pm$ 36.7
4	Twin pregnancy	98 040 $\pm$ 12 042	143.0 $\pm$ 23.6	187.5 $\pm$ 58.4
5	Twin pregnancy + toxæmia	54 920 $\pm$ 30 300	84.4 $\pm$ 60.8	143.0 $\pm$ 80.3

(Whatman no. 5). 1 ml. of the clear filtrate, corresponding to 0.2 g. placental tissue, was used for each determination. The volumetric histaminase method (Kapeller-Adler, 1951) was used.

Preparations of placental extracts for the biological estimation of histamine. 20 g. finely minced placenta are ground in a mortar with 30 ml. of 10% (w/v) trichloroacetic acid (TCA) and some silver sand, and then transferred to a conical flask. After 1 hr., the precipitate is filtered through a Büchner funnel, sucked completely dry, and then washed with 5 ml. of 10% TCA. This procedure is repeated twice, using 5 ml. of 10% TCA each time, the residue being sucked dry after each washing. The histamine content of the combined filtrate and washings is determined by the biological test of Barsoum & Gaddum (1935), as modified by Code (1937a). In every case, histamine was identified by tests with the antihistamine drug 'Mepyramine' (2-amino- N^2 -dimethylaminoethyl- N^2 - p -methoxybenzylpyridine) by the quantitative method described by Reuse (1948).

Simultaneous estimations of histamine in plasma and blood cells. Citrated blood (10 ml.) was centrifuged at 3000 rev./min. for 15 min. Only specimens completely free from haemolysis were tested. The plasma was very carefully separated from the cells, and the histamine content of both plasma and cells was separately determined by the biological method mentioned above. Similar histamine estimations were carried out with the blood from the umbilical cord.

The histaminase activity in umbilical cord serum. This was determined by the volumetric method (Kapeller-Adler, 1951).

amine/hr. and the total placenta, 22.90 mg. of histamine/hr.

Placentae of women with normal pregnancy, delivered before term (35th-39th week of gestation), showed a slightly lower histaminase activity. A strikingly high histaminase content was observed in the placenta of a woman who had to be operated on in her 20th week of pregnancy. Placentae of women, delivered by a caesarean section in the 36th-40th week of pregnancy, showed a lower histaminase activity than placentae of women who had been delivered spontaneously at a similar stage of pregnancy. This observation appears to point to an increased histaminase activity at the onset of labour and during birth, and does not confirm previous findings (Danforth & Gorham, 1937; Kapeller-Adler, 1944) when a higher histaminase activity was observed in the placentae of women after a caesarean section. Somewhat reduced histaminase activity was found in the placentae of women who had suffered from mild toxæmia of pregnancy. More apparent, however, was the decrease in histaminase activity in the placentae of women with severe pregnancy toxæmia. It should be mentioned here that three out of nineteen women of this group had had stillbirths. The histaminase content of placentae of

women with normal twin pregnancy was found to be much higher than in placentae of women with normal single pregnancy. The placental histaminase activity in twin pregnancy, complicated by pre-eclamptic toxæmia, was greater than that of women with toxæmia of pregnancy and only one foetus. It was further found in this work that the histaminase activity of 1 g. of a normal placenta, delivered at term, was about ten times stronger than that of 1 ml. of maternal serum in the 40th week of gestation.

The histamine content of human placentae

It can be seen from Table 2 that, whereas no difference was found between the placental histamine values of women after a normal and after a mild toxæmic pregnancy, the histamine in

Table 2. *Histamine content of placentae*

No. of placentae	Cases	Histamine of placenta ($\mu\text{g./g.}$)
22	Normal	0.10 ± 0.0104
4	Mild toxæmia	0.06 ± 0.0054
11	Severe toxæmia	0.40 ± 0.1700

placentae of cases with severe toxæmia was about four times higher than in normal placentae. The results obtained for the histamine and histaminase activity in placentae of women with severe pre-eclamptic toxæmia, as compared with the corresponding figures in placentae of women with normal pregnancy, seem to indicate that the histamine content of the placenta is inversely related to histaminase activity.

Histaminase and histamine activity in the umbilical cord blood

The serum obtained from the umbilical cord blood, tested for histaminase activity by the volumetric method, did not show any activity in any of the thirty-five examined cases. There was, however, a considerable histaminolytic effect in the maternal serum during labour (Table 3). In thirteen cases, the

Table 3. *Histaminase and histamine content of maternal and foetal blood*

Number of cases	Determination	Mother	Foetus
35	Histaminase (r.u./ml.)	7.1 ± 0.92	0
13	Histamine ($\mu\text{g./l.}$)		
	Cells	42.3 ± 16.1	57.7 ± 36.0
	Plasma	34.6 ± 12.2	32.7 ± 19.4

histamine content of the plasma and the blood cells was simultaneously investigated in the maternal and foetal blood. Results given in Table 3 show that the histamine content of the foetal blood cells was higher than that of the maternal blood cells, and that the plasma histamine was of the same order in

both the mother and the foetus. The fact that the foetal blood, unlike the maternal blood, does not show any histaminase activity, and that the foetal plasma contains a similar amount of histamine to that in the maternal plasma, must give rise to some speculation about the role of histamine during parturition. It may be worth while mentioning here that Rogers (1950) found the musculature of the human umbilical arteries to be exceedingly sensitive to histamine. It may be that histamine plays a part in the permanent occlusion of the umbilical arteries during birth. In Table 1, results are also presented which were obtained when placental histaminase acted on cadaverine and histamine in parallel assays. It follows from this table that the placental histaminase is much more active on cadaverine than on histamine. In contrast to this, histaminase made from hog kidney was found to be 4 times more active on histamine than on cadaverine (Kapeller-Adler, 1951). This table also shows that the results with cadaverine did not vary much in different types of case, while those with histamine did vary.

DISCUSSION

Danforth & Gorham (1937) were the first to report histaminase activity in the human placenta. Since then, many papers have been published on this problem (Marcou, Chiriceanu, Cosma, Gingold & Parhon, 1938; Zeller, Schär & Staehelin, 1939; Zeller, Birkhäuser, Mislin & Wenk, 1939; Effkemann & Werle, 1940; Ahlmark, 1944; Kapeller-Adler, 1944; Anrep, Barsoum & Ibrahim, 1947; Swanberg, 1948, 1950; Wicksell, 1949), but varying values for the histaminase activity in the placenta, ranging from 20 to 220 $\mu\text{g.}$ of histamine inactivated per g. of placenta per hr., were obtained by different workers. The results for histaminase activity obtained in this work by a microchemical technique agree with the findings of Anrep and his co-workers (1947) who, using a biological histaminase test, observed an inactivation of 20–30 $\mu\text{g.}$ of histamine per g. of placental tissue per hour. These workers also found the histaminase activity of 1 g. placenta to be 8–15 times greater than that of 1 ml. plasma. A similar observation has been made in this paper. Swanberg (1950) quotes a ratio of 20:1 for the histaminolytic power of 1 g. of placenta compared with that of 1 ml. of plasma. As regards the high histaminase concentration in the placenta of a woman delivered in the 20th week of gestation (Table 1), Swanberg (1948) and Wicksell (1949) also found that the histaminase content of the placenta rapidly increases in the first half of pregnancy. Anrep *et al.* (1947), on the other hand, concluded that placentae of different age and weight do not differ greatly in the histaminase concentration. The low activity of placental histaminase, which I have

found in cases of severe pregnancy toxæmia, does not agree with the results of Ahlmark (1944), who found more histaminase in some placentae of toxæmic women than in normal women. There are no previous reports on the incidence of high histaminase activity in the placentae of women with twin pregnancy. Since the placental histaminase in twin pregnancy was much higher, not only for the whole placenta, but also per g. of placenta as compared with results in single pregnancy, it seems as if not only the increase in the mass of placental tissue but also some qualitative differences in the tissue were responsible for the rise in histaminase activity in multiple pregnancy. It should be recalled here that Guido (1942) and Anrep *et al.* (1947) concluded that the placenta is the site of formation of histaminase during human pregnancy. According to the extensive studies of Swanberg (1948, 1950), the histaminolytic enzyme is formed in the decidua.

Reports on the histamine content of the human placenta are very scanty. Wicksell (1949) investigated the histamine content of nine placentae and reported values ranging from 0.16 to 1.5 μ g. histamine/g. placenta. The histamine contents of placentae reported here are much lower. The observation made in this paper that blood from the umbilical cord does not show any histaminase activity (Table 3) seems to agree well with results obtained by Swanberg (1948) and by Wicksell (1949), who both, using a biological histaminase test, found that the histaminolytic power of foetal blood was of the same order, or slightly higher, than that of non-pregnant persons. Wicksell (1949) also observed that the foetal blood contained more histamine than the maternal blood and that the foetal plasma histamine was of the same range as was the maternal plasma histamine. Wicksell explains this observation on the known fact that the amount of cellular elements is greater in the foetal than in the maternal blood and that the blood histamine mainly occurs in cells such as leucocytes

and platelets (Schwartz, 1936; Code, 1937*a, b*; Zon, Ceder & Crigler, 1939; Minard, 1941). The results shown in Table 3 of this paper, presenting separately the histamine content of the maternal blood cells and plasma and the foetal blood cells and plasma, seem to confirm this conception.

The discrepancy in the behaviour of histaminase towards histamine and cadaverine, depending on the origin of the enzyme, observed in this work as well as in preceding papers (Kapeller-Adler, 1944, 1949, 1951) is at present under investigation.

SUMMARY

1. The histaminase activity in 124 placentae of women with normal or toxæmic pregnancy has been investigated by a microchemical method, and the results have been considered in relation to those obtained by other workers with biological methods. The histamine content of some of the placentae has also been estimated.

2. The histaminase and histamine activity in the maternal and foetal blood have been simultaneously investigated, and the role that histamine might play in the permanent occlusion of the umbilical arteries during birth has been discussed.

3. Placental histaminase has been found to be more active on cadaverine than on histamine. Kidney histaminase and histaminase from human placentae differ in the relation between their actions on histamine and cadaverine.

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REFERENCES

- Ahlmark, A. (1944). *Acta physiol. scand.* **9** (Suppl.), 28.
 Anrep, G. V., Barsoum, G. S. & Ibrahim, A. (1947). *J. Physiol.* **106**, 379.
 Barsoum, G. S. & Gaddum, J. H. (1935). *J. Physiol.* **85**, 1.
 Code, C. F. (1937*a*). *J. Physiol.* **89**, 257.
 Code, C. F. (1937*b*). *J. Physiol.* **90**, 349, 485.
 Danforth, D. N. & Gorham, F. (1937). *Amer. J. Physiol.* **119**, 294.
 Effkemann, G. & Werle, E. (1940). *Arch. Gynäk.* **170**, 173.
 Guido, M. (1942). *Boll. Soc. ital. Biol. sper.* **17**, 687.
 Kapeller-Adler, R. (1944). *Biochem. J.* **38**, 270.
 Kapeller-Adler, R. (1949). *Biochem. J.* **44**, 70.
 Kapeller-Adler, R. (1951). *Biochem. J.* **48**, 99.
 Marcou, J., Chiriceanu, D., Cosma, G., Gingold, N. & Parhon, C. C. (1938). *Pr. méd.* **46**, 371.
 Minard, D. (1941). *Amer. J. Physiol.* **132**, 327.
 Reuse, J. J. (1948). *Brit. J. Pharmacol.* **3**, 174.
 Rogers, F. A. (1950). M.D. thesis (University of Bristol).
 Schwartz, A. (1936). *C.R. Soc. Biol., Paris*, **123**, 1181.
 Swanberg, H. (1948). *Acta physiol. scand.* **16**, 83.
 Swanberg, H. (1950). *Acta physiol. scand.* **23** (Suppl.), 79.
 Wicksell, F. (1949). *Acta physiol. scand.* **17**, 395.
 Zeller, E. A., Birkhäuser, H., Mislin, H. & Wenk, M. (1939). *Helv. chim. Acta*, **22**, 1381.
 Zeller, E. A., Schär, B. & Staehelin, S. (1939). *Helv. chim. Acta*, **22**, 837.
 Zon, L., Ceder, E. T. & Crigler, C. W. (1939). *Publ. Hlth Rep., Wash.*, **54**, 1978.