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OBSERVATIONS ON SPERMINE OXIDASE OF MAMMALIAN PLASMA

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The enzyme spermine oxidase was first found in the blood plasma of the sheep (Hirsch, 1953) and of the ox (Tabor, Tabor & Rosenthal, 1954) but no systematic study of its occurrence has so far been made. Tabor *et al.* (1954) showed that the ox plasma enzyme can be highly purified and that the purified enzyme still acts not only on spermine and spermidine but also on a large number of amines which are substrates of the enzyme amine oxidase, e.g. tyramine, tryptamine and benzylamine. It has recently been shown (Bergeret, Blaschko & Hawes, 1957; Blaschko, 1958) that there exists in the blood plasma of some other mammals an oxidase which has a pattern of substrate specificities very similar to that of spermine oxidase, except that it is without significant action on spermine or spermidine (see also Kolb, 1957).

In order to understand why in the plasma of certain species there has arisen an ability to oxidize spermine, it was essential to obtain more information on the occurrence of spermine oxidase. Observations of this kind are described in this paper; some of them have already been briefly reported (Blaschko & Hawes, 1958; Blaschko, Ferro-Luzzi & Hawes, 1958).

METHODS

Samples of whole blood, plasma or serum were obtained from different sources. Whenever possible the samples were sent to Oxford in a Thermos flask on ice, but it appears that the enzyme is not readily destroyed at room temperature, and some of the samples from the Zoological Gardens, London, were sent by mail, and reached Oxford the following morning.

On arrival in the laboratory, the plasma or serum samples were usually dialysed for about 4 hr against 0·067 M sodium phosphate buffer, pH 7·4. This treatment did not affect enzymic activity, but it usually lowered the 'enzyme blank'.

The plan of the manometric experiment depended upon the amount of material available. In most experiments the total fluid volume in the main compartment of the flask was 1·6 ml.; this volume included the serum (or plasma) and the phosphate buffer. The side bulb contained 0·4 ml. of 0·05 M spermine tetrahydrochloride or water; the inner tube contained 0·3 ml. of N-KOH. In a few experiments, where little serum was available, smaller manometer flasks (of a volume of about 8 ml.) were used. In these experiments the total amount of fluid in the main compartment

was 0·63 ml., that in the side bulb 0·07 ml. and that in the inner tube 0·1 ml. In all experiments the gas phase was oxygen and the temperature was 37° C. For the calculation of the enzymic activity the initial rate of the reaction was used.

The classification of mammals used in this paper is that established by Gaylord Simpson (1945).

RESULTS

Distribution of plasma spermine oxidase

No significant amounts of spermine oxidase were found in the sera of man, dog, ferret, cat, lion, tiger, seal, kangaroo, Bennett's wallaby, Indian elephant or rabbit. It has been shown elsewhere (Blaschko, 1958) that some of these species contain an enzyme in plasma which will oxidize amines other than spermine or spermidine.

TABLE 1. Spermine oxidase in blood sera of ungulates

	Spermine oxidation
Order *Perissodactyla*	
Sub-order *Hippomorpha*	
Horse	–
Order *Artiodactyla*	
Sub-order *Suiformes*	
Pig	–
Sub-order *Tylopoda*	
Camel	+
Llama	+
Sub-order *Ruminantia*	
Family *Cervidae*	
Fallow deer	+
Family *Giraffidae*	
Giraffe	+
Family *Bovidae*	
Ox	+
Sheep	+
Goat	+

Our results with sera of the ungulates are shown in Table 1. Only serum or plasma of one species of perissodactyls has so far been examined; and this is the horse; no spermine oxidase activity has been found in this species. Of the artiodactyls, one non-ruminant species was tested, the pig; the serum of this species did not contain any spermine oxidase activity.

Spermine oxidase activity was found in the sera of members of two sub-orders of artiodactyls, Tylopoda and Ruminantia. Two tylopod species were examined, the camel and the llama. The camel serum was not sufficiently fresh to allow a quantitative statement on the amount of spermine oxidase present, but there was little difference in the rates of oxidation of spermine and benzylamine; spermidine was oxidized at a slightly higher rate. The results with the sample of llama serum, freshly obtained from the London Zoo, are shown in Fig. 1; it can be seen that here too there was little difference in the rates of oxidation of spermine and benzylamine. The rate of oxygen uptake with mescaline was not linear; it increased during the period of incubation. This is

similar to what happens when mescaline is oxidized by pig serum (Blaschko, 1958).

Of the sub-order Ruminantia altogether five species have been examined. (The samples of ox sera tested also included one of an Ankole bull obtained from the London Zoo.) In all these species spermine was oxidized at a rate higher than any other amine tested. Sera of all these species also oxidized spermidine but at a slightly lower rate. The results for two of these species, the

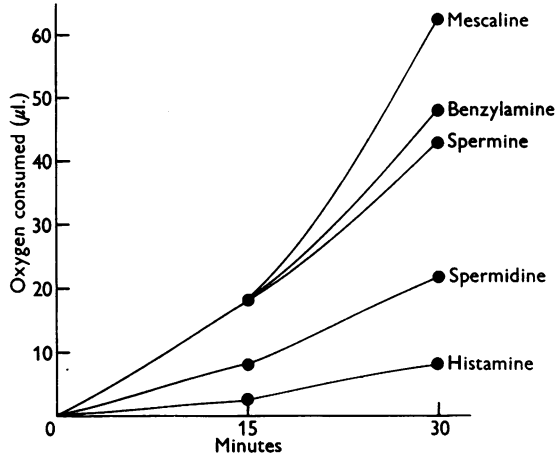

Fig. 1. Oxidation of amines by llama serum. Abscissa, time in min; ordinate, O_2 consumed (μl.). Initial substrate concentrations, 10^{-2}M; gas phase, O_2; temp. 37·5° C. Each flask contained 1·6 ml. of dialysed serum.

TABLE 2. Rates of oxidation of various amines by goat and giraffe sera. The figures give the μl. O_2 consumed by 1·6 ml. serum (dialysed) during the first 15 and 30 min respectively. Substrate concentrations 10^{-2}M

	Spermine		Spermidine		Benzylamine		Mescaline		Histamine	
Substrate	15 min	30 min	15 min	30 min	15 min	30 min	15 min	30 min	15 min	30 min
Goat serum	60	104	48	80	33	56	25	57	8	15
Giraffe serum	21	50	11	27	2	6	4	12	0	0

goat and the giraffe, are shown in Table 2. It can be seen that with the goat serum as source of enzyme the rates of oxidation decreased in the order: spermine, spermidine, benzylamine, mescaline, histamine. With giraffe serum benzylamine was rather poorly oxidized, and there was no measurable uptake of oxygen when histamine was tested as substrate.

Further observations on substrates of spermine oxidase will be separately reported. Cystamine and homocystamine are also among the substrates of the goat plasma enzyme (Bergeret & Blaschko, 1957). We have, in addition, found that the antimalarial compound Primaquin, a derivative of 8-aminoquinoline, is a substrate of spermine oxidase.

Spermine oxidase in young ruminants

The presence of spermine oxidase in the blood plasma of animals which possess a rumen (Tylopoda and Ruminantia) led us to investigate the spermine oxidase activity in the blood of young ruminants. It is known that the rumen only begins to function when animals go over from a milk diet to a mixed diet.

Samples of serum were obtained from nanny goats and from their kids, as soon after birth as was possible, and subsequently at weekly intervals. Our

TABLE 3. Spermine oxidase activity in sera of new-born kids and their mothers. Enzymic activity is in terms of μl. O_2 consumed by 1·0 ml. serum (dialysed) in 30 min, at a spermine concentration of 10^{-2} M

Trio no.	Hours after birth	Maternal	Kid no. 1	Kid no. 2
1	3	98	6	2
2	15	93	3	2
3	4	103	0	4
4	18	80	2	0

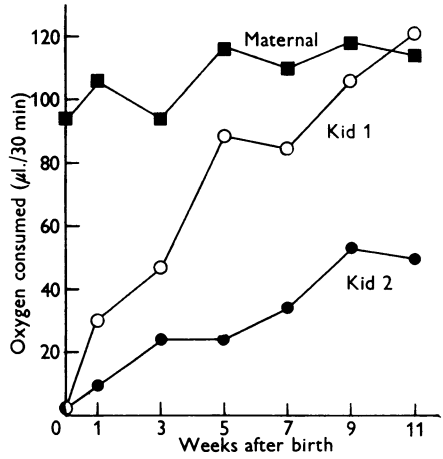

Fig. 2. Spermine oxidase in sera of nanny goat (■—■) and two female kids (○—○ and ●—●). Abscissa, time after birth in weeks; ordinate, O_2 consumed by 1·0 ml. of dialysed serum (μl./30 min).

results obtained with the new-born kids and their mothers are shown in Table 3. It can be seen that the activities with spermine as substrate in the maternal sera were uniformly high, varying from 80 to 103 μl./ml. serum in 30 min. Enzymic activity in the new-born animals was either absent or very low.

In each trio of animals determinations of enzymic activity were continued for several weeks after birth. In each series of observations, the enzymic activity in the sera increased; this is illustrated by the results given in

Figs. 2 and 3. Fig. 2 shows that there were individual differences in the rate of development of enzymic activity: one of the two kids had attained the maternal level after 11 weeks whereas the serum of the other animal had by then scarcely reached 50% of the activity of the maternal. There was also a slight upward tendency in the activities of the maternal serum. This observation was made in all the four maternal sera examined; the causes of this increase have not been further investigated.

Fig. 3 is taken from a trio where observations have been continued for a much longer period. It can be seen that even after 28 weeks the animals had not yet attained the level of activity of the maternal serum. In the experiments shown in Fig. 3, benzylamine was used as a second substrate in addition

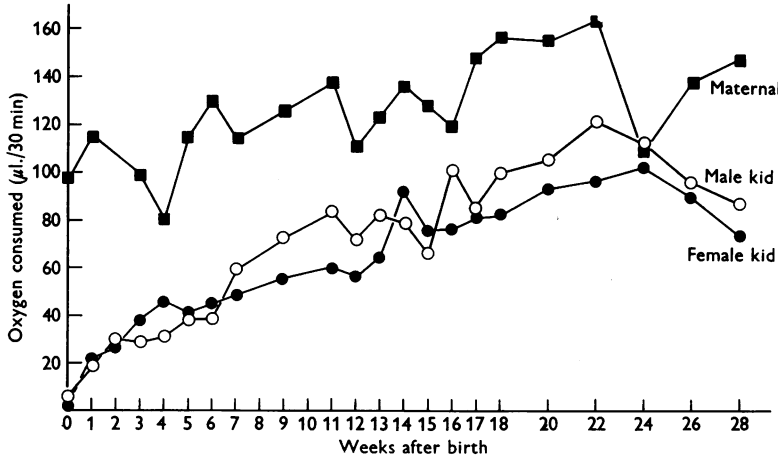

Fig. 3. Spermine oxidase in sera of nanny goat (■—■), female kid (●—●) and male kid (○—○). The male kid was castrated after the end of the second week. Units as in Fig. 2.

to spermine. Fig. 4 shows the results with both spermine and benzylamine in the maternal serum and in that of one of the two kids. The development of activity towards benzylamine paralleled that with spermine as a substrate—this is in conformity with the idea that the two amines are acted upon by one and the same enzyme. The results for the second kid (not shown) were very similar.

This study of the development of spermine oxidase has so far been carried out mainly in the goat, but a few preliminary experiments carried out on samples of calf serum indicate that the development of the enzyme in this species is somewhat analogous to that in the goat. The samples given to us were about $1\frac{1}{2}$ years old; they had been kept frozen, and only small amounts were available for the manometric experiments. Results are shown in Fig. 5 and suggest that the time scale of development of enzymic activity in the calf

may be different from that seen in the goat; however, this has not yet been investigated.

No spermine oxidase was found in a sample of goats' milk and in three samples of goats' colostrum.

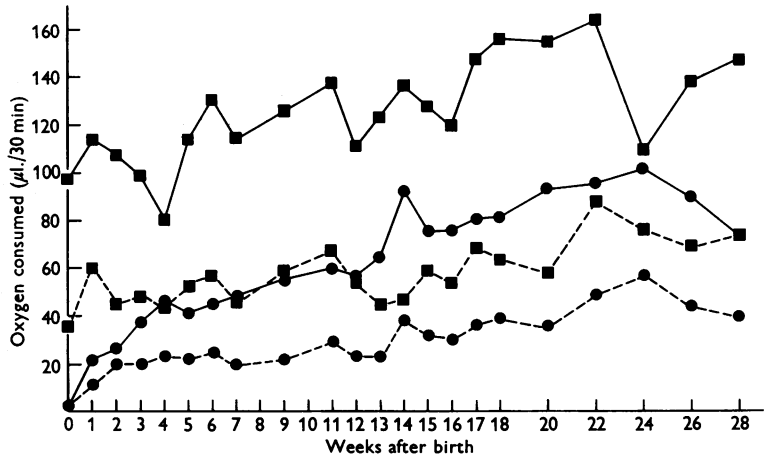

Fig. 4. Oxidation of spermine and benzylamine in maternal serum and kid serum (nanny goat and female kid of Fig. 3). ■—■ Nanny goat with spermine as substrate and ■- - -■ with benzylamine as substrate; ●—● kid with spermine as substrate and ●- - -● with benzylamine as substrate. Units as in Fig. 2.

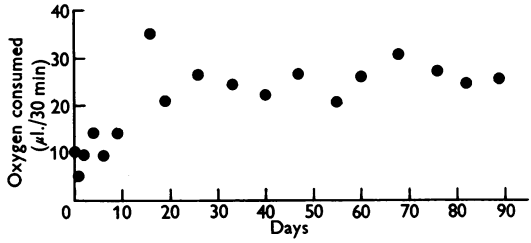

Fig. 5. Spermine oxidase activity of serum of new-born calf (colostrum-deprived); born 13. x. 56; spermine concentration, 10^{-2} M; gas phase, O_2; temp. 37·5° C; 0·5 ml. serum in each flask; abscissa, days after birth; ordinate, O_2 consumed (μl./30 min).

DISCUSSION

The experiments described above show that the ability to oxidize spermine is present in the plasma of all those ungulates which possess a rumen. In the classification of mammals introduced by Gaylord Simpson (1945) and followed by Young (1950), there are two suborders of the order Artiodactyla in which a rumen is present, the Tylopoda and the Ruminantia. In the blood plasma of all representatives of these two sub-orders spermine oxidase activity has been demonstrated. There seems to be a difference in the two sub-orders: in the

Tylopoda (camel and llama) the rate of oxidation of spermine was not higher than that of some of the other amines, whereas in all Ruminantia the rate of oxidation of spermine surpassed that of any of the other amines tested; spermidine was usually the second in the order of rates of oxidation. This preference for spermine and spermidine was particularly marked in the giraffe serum, where benzylamine was oxidized very slowly.

It is now known that spermine is a growth factor for a number of micro-organisms (Kihara & Snell, 1957). The rumen can be considered as a great container of micro-organisms, and the close association between the presence of a rumen and the ability of the blood plasma to oxidize spermine (and spermidine) could be understood if we assume that spermine (or a related compound) is also formed by some of the rumen organisms, and that the plasma enzyme acts on amine that reaches it from the lumen of this organ. It is known (Tabor & Rosenthal, 1956) that spermine is toxic, especially nephrotoxic, in mammals.

This interpretation is supported not only by our study of the distribution of spermine oxidase in mammals, but also by the observations made on the growing ruminants. As the rumen is not functioning in the new-born ruminant, our finding of the absence of spermine oxidase in new-born kids is therefore of interest. The ruminants belong to the great group of mammals which are born without a full supply of plasma proteins; this deficiency is partly made up by protein taken up in the first days of life from the colostrum. Spermine oxidase is not taken up in this way as it is absent from colostrum and milk. Further, the development of enzymic activity, at least in the goat, occurs within weeks or months rather than within a few days after birth.

The picture of the origin of spermine oxidase at which we arrive is what might be called 'adaptive evolution' of enzymic activity. In phylogenesis the rumen and spermine oxidase activity appear to have developed in parallel. The term 'adaptive' is based on the assumption, hitherto not proved, that spermine or a related polyamine is present or formed in the rumen.

We can, at present, not be certain whether the spermine oxidase of ruminant plasma is identical with the plasma enzyme that acts upon mescaline, benzylamine and other simple 'mono' amines, or whether it is closely related to this enzyme. However, a number of observations speak in favour of the existence of only one enzyme. There is the work of Tabor *et al.* (1954) who have shown that the spermine oxidase of bovine plasma retains the ability to act on benzylamine and other 'mono' amines even when purified 150–200-fold. To this we can add two further observations: first, amine oxidase activity was present in all species in which spermine and spermidine were oxidized, and, secondly, in the growing kid the ability of the blood plasma to oxidize benzylamine developed parallel to the spermine oxidase activity. It seems, therefore, that spermine oxidase arose through a modification of the substrate specificity

of an amine oxidase which is common to all ungulates and certain other mammals. The high activity with spermine and spermidine suggests that these amines are important substrates of the oxidase in the living ruminant.

SUMMARY

1. The distribution of the spermine oxidase activity in mammalian plasma has been studied.

2. Spermine and spermidine were oxidized in the sera of all ruminants examined, camel, llama, giraffe, fallow deer, ox, sheep and goat.

3. Spermine oxidase activity was not found in the sera of non-ruminants.

4. The ability to oxidize spermine and benzylamine is either absent or almost absent in the sera of new-born goats; enzymic activity is gradually acquired during the first months after birth.

5. It is suggested that spermine oxidase arises in phylogenesis, parallel with the evolution of the rumen, from an amine oxidase without significant action on spermine.

We are very grateful to Mr Oliver G. Jones, M.R.C.V.S., Curator of Mammals, Zoological Society of London, for his help. We owe the sample of camel serum, sent by air at 0° C, to Professor P. Hey of Baghdad; this sample was rather dark, indicating that haemolysis had occurred before centrifugation.

A first sample of goat and kid plasma was given to us by Dr J. L. Linzell, M.R.C.V.S., of Babraham, but the study of the enzymic activity in young ruminants owes much to the help given by Mr I. H. Pattison, M.R.C.V.S., of the A.R.C. Field Station, Compton, who has sent us samples of goat and kid serum at regular intervals during the past year. The samples of calf serum (deprived of colostrum) were given to us by Dr A. E. Pierce, F.R.C.V.S.

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