LXXXIV. BLOOD ENZYMES. I. ON THE OCCURRENCE OF MALTASE IN MAMMALIAN BLOOD.

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WHILE studying the enzyme content of the blood of certain higher animals, I have frequently had occasion to observe that the ferment maltase in the blood of mammals is not a constant feature, but appears to be rather a function of the animal species. Present in the blood of one mammal, the ferment may be absent from that of another; for instance, while dog's blood serum, when tested *in vitro* under suitable experimental conditions, always shows hydrolytic activity on maltose, the serum of man, under similar conditions, I have never found to be active. As this is an observation which does not appear hitherto to have been recorded or to have attracted attention¹, I propose in the present paper to give a brief account of the experimental evidence on which it is based.

The experiments related are taken from certain general studies on blood enzymes, which have occupied me over a period of several years [Compton 1915, 1]. This explains why in what follows the experimental conditions are not uniform throughout, the activity in maltase of the different blood sera studied being determined under conditions of temperature and concentration in maltose (see accompanying Table) fixed by the main investigations in hand. From the qualitative point of view, however, in which sense the present communication is mainly put forward, these varying conditions do not in the least detract from the general results arrived at.

EXPERIMENTAL.

Blood was withdrawn as aseptically as possible by venous puncture (man, cat, dog, goat, horse, rabbit), by heart puncture (guinea-pig, rat), by bleeding at the slaughterhouse (ox, pig, sheep), and rapidly centrifuged so as to obtain a clear serum. The latter was pipetted off into a clean sterile test-tube, toluene added—one drop per cc. of serum—to ensure sterility, the tube closed with a clean sterile cork to prevent evaporation, and the mixture vigorously shaken for a few seconds. Conserved under these circumstances the serum remained as a rule sterile during subsequent weeks and months, and that whether in

¹ The idea indeed exists in the literature that in a general way maltase is to be met with in the blood, side by side with amylase and glycogenase [compare Gley 1920].

the ice-box or at room temperature: a matter of great convenience in protracted studies. The serum is referred to in the Table as "fresh" when used immediately, or at most within 2 hours of its separation from the clot; while, when used after standing a few days the age is stated. After shaking, the serum was allowed to stand a short time at the temperature of the laboratory before use, then suitable quantities were withdrawn by pipette and measured off into already prepared test-tubes containing 90 mg. of pure maltose (hydrated) dissolved in sufficient pure water to make, with the serum added, 5 cc. total solution. The tubes thus prepared had 5 drops of toluene added to each, after which they were plunged into a water thermostat regulated at a constant temperature¹. Closed at once with clean sterile corks they were incubated for 16 hours; they were then withdrawn, uncorked, and the enzyme action stopped by heating for 6 minutes in boiling water. On cooling, the contents of each tube were diluted to 50 cc. and the proportion of maltose hydrolysed determined by Bertrand's method on 20 cc. of the diluted mixture.

The following Table gives a *résumé* of the experimental findings:

Animal	No.	Vol. of serum employed cc.	Maltose concentration of reaction mixture	Temperature of experiment ° C.	% of maltose hydrolysed in 16 hrs.	Information relating to the age of the serum when used, how it was conserved, etc.
Pig	1	{ 0.1 0.4 0.8	M/20	46.0	83.8 92.5 94.3	3 days, in presence of toluene, at Lab. temp.
	1	{ 0.02 0.05 0.1 0.2	M/20	46.5	28.2 76.8 75.1 85.5	8 days, Lab. temp.
	1	{ 0.05 0.1 0.2 0.4 0.8	M/20	47.0	25.4 39.1 50.9 63.5 78.5	Fresh, after centrifugation. Male, in fasting condition 48 hours
	2	{ 0.1 0.2 0.4 0.8	M/20	47.0	47.9 68.4 78.5 80.3	1 day, in presence of toluene, at Lab. temp. Male, under 2 years, fasting 24 hours
	3	{ 0.05 0.1 0.2 0.4	M/20	50.0	22.5 25.4 39.1 52.5	6 hours, in ice-chest. Male, state of digestion unknown
Dog	4	{ 0.05 0.1 0.2 0.4	M/20	50.0	37.5 57.1 65.2 71.7	6 hours, in ice-chest. Pregnant female, state of digestion unknown
	5	{ 0.1 0.2 0.3 0.6	M/20	50.0	70.0 82.0 89.0 96.1	1½ days, in ice-chest. Non-pregnant female, state of digestion unknown

¹ In the absence of information, at this stage, regarding the optimum temperature of maltase of animal origin, the temperature employed in these experiments has been mostly one in or around 47°, that being the optimum temperature of the vegetable maltase met with in *Aspergillus oryzae* in an action of 16 hours' duration and P_H 7.2 [Compton 1914; 1915, 2].

Table (continued)

Animal	No.	Vol. of serum employed cc.	Maltose concentration of reaction mixture	Temperature of experiment °C.	% of maltose hydrolysed in 16 hrs.	Information relating to the age of the serum when used, how it was conserved, etc.
Goat	1	0.06	M/50	42.5	11.4	1½ days, in ice-chest
Sheep	1	0.1	M/50	47.0	16.9	Fresh
Horse	1	0.2	M/20	46.0	15.5	4 days, at Lab. temp.
		0.4			24.0	
		0.8			45.0	
	2	0.1	M/20	47.0	11.4	6 days, at Lab. temp.
		0.2			19.7	
		0.4			35.9	
Ox	1	0.8	M/20	45.5	26.8	4 days, at Lab. temp.
Rat	1	0.12	M/20	50.0	4.5	11 hours, ice-chest
		0.24			16.9	
Cat	1	0.05	M/50	45.0	0	Fresh
	2	0.2	M/50	46.0	0	1 day, at Lab. temp.
Guinea-pig	1	0.25	M/20	40.0	0	20 hours in ice-chest in presence of chloroform
		0.5			0	
		1.0			0	
		2.0			0	
	2	0.06	M/50	47.0	0	6 hours, in ice-chest
Rabbit	1 and 2	0.1	M/20	32.0	0	Fresh
		0.4			0	
		0.8			0	
		1.5			0	
	3	0.5	M/20	32.0	0	Fresh
		1.0			0	
		1.5			0	
	4 and 5	0.8	M/20	46.5	0	1 day, at Lab. temp. Male and female ("pooled" specimen)
	6	0.06	M/50	47.0	0	Fresh, slightly haemolysed
	7	0.5	M/50	46.0	0	2 days, in ice-chest
Man	1 and 2	0.08	M/50	32.5	0	Fresh
	3	0.08	M/50	42.0	0	Fresh
	4	0.36	M/20	42.0	0	3 days, at Lab. temp. (positive Wassermann serum)
	5, 6, 7, 8 and 9	0.8	M/36	54.5	0	9 days, at Lab. temp. ("pooled" positive Wassermann sera)

It will be seen by an analysis of the foregoing Table that the enzyme maltase is present in the blood of the following animals:

Dog, Pig,
Goat, Rat,
Horse, Sheep;

while it is found to be lacking in the blood of the

Cat, Rabbit,
Guinea-pig, Man.

The great variation in enzymic activity which may be met with from dog to dog, under the influence of various modifying factors, is noteworthy. As to the relative richness in maltase of the specimens of serum from the 5 dogs, it is seen that in the case of the dogs (3), (4) and (5)—comparable together as to the temperature employed in testing—to produce in 16 hours at 50° a hydrolysis of 50 %, for instance, approximately 0.06 cc. of serum is required for dogs (4) and (5), and 0.35 cc. is required for dog (3); that is, in a general way the serum (3) may be said to be 5 times less active than either serum (4) or (5). Judged in a like manner, the serum of dog (2) is twice as rich in maltase as that of dog (1), 50 % hydrolysis being produced by 0.1 cc. of the former serum in 16 hours at 47°, while under the same experimental conditions 0.2 cc. of the latter serum is required to produce the same effect.

It is not our intention to enter here into the factors responsible for the variations observed from dog to dog, but merely to signalise the fact in passing. The question constitutes in itself a subject of sufficient interest and importance to be treated separately.

It is further to be noted that the serum of the pig is extraordinarily rich in the ferment maltase. To produce a 50 % hydrolysis of maltose under the experimental conditions here involved, only 0.03 cc. of serum is required, a dose considerably less than that for any of the other animals examined. This outstanding comparative richness of pig's serum in maltase was first pointed out by Doxiades [1911], although the presence of the enzyme in the blood serum of the pig has been known since the work of Tebb [1894].

Consider now the animals in the preceding Table in whose blood the enzyme has failed to be revealed: cat, guinea-pig, rabbit and man. It appeared to us necessary, before definitely concluding the ferment to be absent from the blood of these mammals, to investigate:

(1) Whether the enzyme might not be retained in the clot and cellular elements during the process of coagulation and ultimate separation of the serum; in which case by provoking a plasmolysis of the cells the enzyme might reasonably be expected to be liberated, if it were present, and thereby become evident.

(2) Whether the enzyme might not be present in some masked or latent state, such as the alkalinity of the serum might possibly produce; in which case one would expect the enzyme to be liberated, or activated, by acid neutralisation.

To test (1), the following experiments were undertaken with the blood of the guinea-pig and the rabbit:

(a) *Guinea-pig.* A guinea-pig was etherised, and after having the jugular cut, was bled into a dry clean centrifuge tube. The blood, on centrifugation, yielded about 6 cc. of serum, which was pipetted off as completely as possible from the clot. To the latter were added 6 cc. of pure water, and about 20 drops of chloroform, and, after mixing, it was set aside in the ice-chest for 24 hours; then centrifuged and the resulting supernatant dark red solution examined for

activity on maltose. Quantities of the haemolysed solution ranging from 0.25 cc. to 1.5 cc. were added to tubes containing 90 mg. of maltose and water to 5 cc. and the tubes incubated at 39° for 16 hours. No activity in maltase was exhibited under these conditions.

(b) *Rabbit*. Blood was withdrawn from the ear-vein, and centrifuged immediately to separate serum from clot. To the latter, after decanting off the serum, was added a volume of chloroform water equal to that of the serum removed, and after thorough admixture it was allowed to macerate overnight in the ice-chest. The haemolysed liquid of maceration was tested in quantities ranging from 0.5 to 1.0 cc. for activity in maltase, and was found to be quite inactive.

These two experiments show that maltase failed to be extracted from the blood cells of the clot, and, one is justified in assuming its absence as such.

To test (2), a pooled specimen of fresh human serum from three healthy persons was added in volumes of 0.15 cc. to test-tubes containing 90 mg. of maltose dissolved in 5 cc. of pure water which had previously been acidified by the addition of progressive volumes of N/H_2SO_4 : 0.1, 0.3, 0.6 cc.; and after 16 hours' incubation at 36°, a determination of the maltose hydrolysed was made by Bertrand's method. No hydrolysis was found to have occurred in any of the tubes¹.

This experiment seems to show that if a serum in the natural state does not indicate the presence of maltase, the enzyme may be taken as absent, since alteration of hydrogen ion concentration fails to bring it into evidence.

CONCLUSION.

Mammals can, therefore, be divided into two groups: according to whether they have, or have not, maltase in their blood. To the former group belongs the dog, to the latter the rabbit; the two laboratory animals which have hitherto been mostly used in connection with the experimental study of sugar transformations in the blood. The fact that man belongs to the non-maltase or "rabbit group" makes the observation of rather more than passing interest: and that whether viewed from the physiological, the pathological, or the medico-legal point of view.

The conception is illuminating in the explanation it affords of various contradictory statements met with in the literature. Thus, when it is stated—as the result of injecting maltose into the blood stream and studying its elimination by the urine—that the sugar of the blood cannot be maltose [see footnote p. 681], it is obvious the animal worked with must have belonged to the maltase or "dog group." In effect, this is so; for Dastre and Bourquelot [1884],

¹ In this connection it may be stated that according to certain of our experiments, to form the object of a subsequent communication, by alteration of the hydrogen ion concentration of dog's serum—which as we have seen is normally rich in maltase—by the addition of 0.15 cc. $N/100 H_2SO_4$ to a reaction mixture similar to the above, containing 0.1 cc. of serum, the activity of the maltase present is increased by 15% in an action of 16 hours' duration at 50°.

on whose work this statement is probably founded, working with the dog, found that most of the maltose injected was not voided by the kidneys but was utilised, with much the same facility as glucose. On the other hand, Philips [1881] is reported by Green [1899] to have found that when maltose is injected into the blood of an animal—species not stated, and I have been unable to trace the original publication—it is mostly excreted unchanged in the urine, much as the cane-sugar in a certain famous experiment on carbohydrate assimilation of Claude Bernard [1849]. There appears to me little doubt that in this case the animal worked with belonged to the non-maltase group.

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