LXVI. BLOOD ENZYMES. III. ON THE GLYCOGENOLYTIC ACTIVITY OF MAMMALIAN SERA— WITH REMARKS ON SERUM TOXICITY.

By ARTHUR COMPTON.

From the "Laboratoire de Chimie Biologique," Institut Pasteur, Paris; the Laboratories of the Imperial Cancer Research Fund, London; and the Surgical Unit, St Mary's Hospital.

(Received June 20th, 1923.)

THE present communication treats of the enzymes, or enzyme complex, of the blood responsible for the breaking down of glycogen by hydrolysis. The end-products of glycogen hydrolysis being either maltose or glucose, according to the hydrolytic agents employed, it is assumed that at least two distinct enzyme systems are involved in the complete breaking down of glycogen to glucose: one, *glycogenase*, whose function would be to transform the glycogen first into maltose; and the other, *maltase*, concerned with the transformation of the maltose into glucose.

Now, it has been shown by us [Compton, 1921] that mammals differ considerably, from the species point of view, in the content of their blood in the last-mentioned ferment. For instance, when tested *in vitro*, the sera of man, the cat, the rabbit and the guinea-pig are entirely devoid of maltose splitting power; whilst sera from the dog, the horse, the ox, the goat and the pig—particularly the last-mentioned mammal, the blood of which is very rich in the ferment—normally contain *maltase*. Thus it comes about that, in terms of the enzyme content of their blood, there are two groups of mammals: a *positive blood maltase* group, and a *negative blood maltase* group.

On the other hand, the sera of all mammals which we have thus far investigated are capable of breaking down glycogen, with the production of either maltose or glucose. Whether the end-product of the hydrolysis of glycogen by mammalian blood serum is glucose, or maltose, must evidently depend upon the blood maltase group to which the animal belongs.

The results which follow concern the hydrolysis of glycogen by the sera of various mammals in a 16 hours' action at approximately 46–47°, the mammalian species studied comprising representatives of the two groups previously defined by us: (1) the *maltase* group: the dog, the horse, the ox, the pig, and the sheep; and (2) the *non-maltase* group: the cat, the rabbit, the monkey, and man.

It will be seen from the results set forth that here again, as in the case of *maltase*, mammals differ among themselves in the content of their sera in *glycogenolytic power*—as measured by the amount of glycogen hydrolysed in a given time at a given temperature by a given quantity of serum—the activity, moreover, being fairly characteristic of the species.

EXPERIMENTAL.

Preparation of Glycogen. Twenty-two rabbits were fed during a week largely on carrots, and the animals were then killed. The livers were removed immediately, minced, and plunged into an equal weight of 60 % potassium hydroxide, and the mixture heated up to boiling for 5 hours. An equal volume of water was added, and the entire mixture allowed to cool. Contrary to the methods usually described, it was now precipitated directly without previous filtration, by the addition of one and a half volumes of alcohol. After standing overnight, the precipitate was collected by centrifugation, and washed well with 70 % alcohol. The resulting, fairly white, precipitate was now heated on the water-bath for $1\frac{1}{2}$ hours with about six times its own weight (moist) of water to dissolve it, and was then filtered by suction while still hot through two layers of starch-free filter paper on a Buchner funnel. The solution thus obtained was rapidly cooled under the water-tap, and neutralised with concentrated hydrochloric acid, and about 3–5 cc. excess of acid added to precipitate any extraneous protein matter. The solution was next filtered anew through a Buchner funnel, an olive-green residue and a whitish opalescent solution resulting. The filtrate, and washings, some $2\frac{1}{4}$ litres in all, were poured into 4 litres of 95 % alcohol and the mixture allowed to stand overnight, the resulting white flocculent precipitate being collected and washed with 60 % alcohol. The precipitate (moist) was redissolved in ten times its weight of distilled water, and precipitated a third time with $1\frac{1}{2}$ volumes of alcohol, the opalescent solution being poured into the alcohol. Next morning, the precipitate was collected by centrifugation, washed twice with 70 % alcohol, and then with absolute. It was now dried in a vacuum over sulphuric acid. 120 g. of the dried pure product were thus obtained.

Complete Hydrolysis of Glycogen—its Glucose Content. According to Pflüger [1904], hydrolysis by 2·2 % hydrochloric acid on the water-bath for 3 hours completely transforms glycogen into glucose, and he utilised this as a method for estimating glycogen in tissues. In Pflüger's method the weight of glucose found after hydrolysis is multiplied by 0·927 to find the corresponding quantity of glycogen.

30 mg. of the foregoing specimen, when completely hydrolysed by prolonged heating with 2–3 % hydrochloric acid on the water-bath gave as the result of four experiments the following glucose numbers in mg., the latter being estimated by Bertrand's method: 29·8, 28·6, 29·2, 28·4—mean 29·0. This signifies that 100 mg. of the specimen when completely hydrolysed would yield 96·7 mg. approx. of glucose. From this it follows that 96·7 mg. of glucose found, as the result of the enzymic hydrolysis of 100 mg. of the specimen, corresponds to 100 % hydrolysis when the end-product is glucose. In other words, under such conditions 1 mg. of glucose found represents $\frac{100}{96.7} = 1.034$ % hydrolysis.

Again, since 1 mg. of glucose is the equivalent of $\frac{342}{360} = 0.95$ mg. of maltose, it will be evident, when the end-product is maltose that 1 mg. of maltose found as the result of the enzymic hydrolysis of 100 mg. of the specimen will represent a percentage hydrolysis of $\frac{100}{97.6 \times 0.95} = 1.09$ approx.

Method of Procedure. The method of determining the glycogenolytic activity of the different sera investigated was as follows: 100 mg. of glycogen were carefully weighed out into each of four, or more, clean dry test-tubes, and after adding 2 cc. of redistilled water to each tube, the latter were heated for about 2 minutes in boiling water to dissolve the glycogen. The tubes were rapidly cooled and the contents of each completed with water and varying doses of serum to 5 cc. Three drops of toluene were added, the tubes plunged into a water-thermostat regulated about 46–47°, and the tubes having acquired the temperature of the thermostat were closed with clean sterile corks. After incubation for 16 hours, the corks were removed and the enzyme action stopped by heating for 7 minutes in boiling water. When cold, the contents of each tube were diluted to 50 cc., and 20 cc. were withdrawn for a sugar determination by Bertrand's method. The percentage of glycogen hydrolysed was then calculated in terms of glucose, or of maltose, by the use of one or other of the preceding factors, according as the serum under investigation appertained to a positive, or a negative blood maltase group of mammals.

BLOOD MALTASE POSITIVE GROUP.

Under this category, sera from the dog, the horse, the pig and the sheep were investigated.

Dog. Three dogs were investigated (2-P, 3-P and 4-P). The animals were, as a rule, in the fasting condition for 24 hours previous to withdrawal of the blood for testing. The animal being immobilised, the blood was collected from the jugular vein into a sterile syringe, and centrifuged immediately to obtain serum for the tests.

The animal 2-P was examined, as to the richness of its blood in glycogenolytic activity, on three separate occasions; 3-P twice; and 4-P twice. The results obtained are set forth in Table I:

Table I.
Glycogen hydrolysed %, by the blood serum of the following dogs:

Dose of serum employed	2-P			3-P		4-P	
	1	2	3	1	2	1	2
0.1	29.2	23.7	—	23.7	—	33.0	—
0.15	—	—	34.4	—	35.6	—	47.0
0.2	40.8	37.7	—	35.2	—	53.0	—
0.3	—	—	50.9	—	45.4	—	65.1
0.4	52.2	52.3	—	42.9	—	69.3	—
0.6	—	—	66.6	—	60.5	—	69.8
0.8	65.9	66.7	—	57.6	—	71.8	—
Age of serum	2 days	Fresh	Fresh	2 days	Fresh	1 day	Fresh
Temp. at beginning and end of exp. °C.	47.4–46.8	47.5–46.9	47.2–47.4	47.0–46.8	47.2–47.4	47.4	47.2–47.4

Fig. 1 gives the graphical representations of these numbers; from which it will be seen that the activity for small doses of serum, whether the same individual or different animals of the same species be studied, represents a more or less definite degree of activity—there being but little separation between the individual curves in this region. For larger doses, the curves tend to separate, suggestive perhaps that the enzyme content of the blood is not absolutely fixed, but that it varies at times within certain limits. For the individual, however, the enzyme content of the blood does appear to be remarkably fixed. This is well brought out in the case of the dog 4-*P* (Fig. 1), the activity curves of which relate to two specimens collected at an interval of 1 week, the two curves being practically identical. The same thing is also seen, though perhaps to a less marked degree, in connection with the dogs 2-*P* and 3-*P*. In regard to 2-*P*, an interval of 1 week separated the specimens 1 and 2, and an interval of 2 weeks the specimens 2 and 3; while for 3-*P* an interval of 44 days separated the specimens 1 and 2.

Horse. The figures with two different specimens of horse serum, both utilised 4 days after collection are set out in Table II.

Table II.

Dose of serum employed	Glycogen hydrolysed %	
	1	2
0.4	—	1.2
0.8	1.8	2.6
Temp. of exp.	47.4-47.2	46.0-45.9

On graphical representation these figures give the curve represented in Fig. 2, which proves the glycogenolytic power of horse's serum to be extremely feeble.

Ox. Only one specimen of ox serum was examined. This was used 2 days after collection, the temperature during the action being $45.5-45.7^{\circ}$. The results are shown in the curve in Fig. 2.

Pig. One specimen only of pig's serum was examined, the serum at the time of testing being 15 days old; temperature 46° . See the corresponding curve in Fig. 2.

Sheep. A specimen of sheep's serum, shed 26 days, and preserved sterile in presence of toluene, was tested for glycogenolytic power at a temperature of 46.5° . See the curve in Fig. 2.

BLOOD MALTASE NEGATIVE GROUP.

Under this heading, sera from the cat, the monkey, the rabbit, and man were investigated.

Cat. Only one specimen was examined, the serum used being 4 days old; temperature $46.4-44.5^{\circ}$. See Fig. 2.

Monkey. This examination relates to a specimen of blood obtained from a *Macacus cynomolgus*. The serum was fresh when used, that is to say, as soon as clotting began the serum was at once separated from the clot by centrifugation, and the activity experiment then put up. The specimen was without any action on maltose in doses of 0.2 and 0.4 cc. in an action of 16 hours' duration at 46° , thereby proving, as was to be anticipated, that the monkey belongs to the blood maltase negative group of mammals. See Fig. 2.

Fig. 2.

Rabbit. Four specimens of rabbit's sera were examined. Blood was collected from 2 rabbits at a time, the blood being "pooled," in connection with specimens 1, 2 and 3, so as to obtain sufficient serum for these and other experiments undertaken with it. The 4 specimens, therefore, correspond in all to 7 rabbits. Table III gives the numbers obtained, and the respective curves are indicated in Fig. 3.

Table III.

Dose of serum employed	Glycogen hydrolysed %			
	1	2	3	4
0.1	8.0	—	—	14.4
0.2	16.9	—	—	—
0.3	—	—	24.4	—
0.4	—	31.0	—	—
0.5	33.6	—	—	37.5
0.8	—	41.0	—	—
1.0	—	—	—	55.0
Age of serum	1 day	1 day	4 days	Fresh
Temp. of exp. °C.	47	46-47	47	46.0-47.4

Man. The activity of normal human serum in glycogenolytic power was tested on three occasions as follows: (1) with a single specimen of serum; (2) with a "pooled" specimen of seven negative Wasserman sera; and (3) with a "pooled" specimen of eight negative Wasserman sera. The figures obtained are given in Table IV.

Table IV.

Dose of serum employed	Glycogen hydrolysed %		
	1	2	3
0.4	4.4	8.0	15.5
0.8	20.8	20.8	25.8
Age of specimen	1 day	6 days	3 days
Temp. of exp. °C.	47.2	47.2	45.6-45.7

When these numbers are represented graphically, the two curves of the right-hand portion of Fig. 3 are obtained. There is the suggestion of a period of lag portrayed by the curve relating to specimens 1 and 2. Whether such findings are accidental, or real, will require further investigation to settle.

DISCUSSION OF RESULTS.

From a consideration of Fig. 1, and the first section of Fig. 2, it will be seen that positive blood maltase mammals, at least the limited number studied by us, when classified in terms of decreasing glycogenolytic activity of their blood give rise to a series, as follows: pig, dog, ox, sheep, horse.

This series presents a slightly different arrangement to that of the corresponding series representing decreasing activity in *maltase*, the latter, under the same experimental conditions being: pig, dog, goat, sheep, horse and ox [Compton, 1921]. A noteworthy point of contrast here, which merits attention, is the comparative richness of the serum of the horse in *maltase*, as compared with its extremely feeble *glycogenase* content. Perhaps, indeed, it would be more in accordance with the real facts, in this connection, to speak of glycogenolytic power; because, however feeble this may be, increased activity can always be brought out by suitable p_H alterations of the medium.

Do the foregoing series of sera, of diminishing enzyme content, correspond to any other known properties of sera? Among possible properties one thinks of toxicity. It is a well-known fact that the blood of one animal when transfused into an animal of a different species, is often very toxic: a toxicity which in

the first instance was attributed to the corpuscles. But it has been proved, to quote Richet [1921], that "the real toxic element is the serum itself." It may then be asked, if the serum of one animal contains substances which are toxic for an animal of another species, may not the differing enzyme content of sera play a rôle in this phenomenon? As a matter of fact the toxicity of various sera for man, on intravenous or subcutaneous injection, is given by Richet [1921], in terms of diminishing toxicity, as: dog, goat, ass, horse. Horse serum, then, is the least toxic of mammalian sera for man; and we have shown that, of all the sera studied by us, it possesses the feeblest glycogenolytic power.

Fig. 3.

But, this conception, of serum toxicity being a function of enzyme content, will be found to rest already on solid ground, if examined from quite a different aspect: the action of heat upon the toxicity of serum. In the case of the most toxic sera known, namely those of the eel—whose serum kills in the dose of $\frac{1}{20}$ th of a cc. per kilogram—the lamprey, certain vipers and cobras, whose serums are almost as toxic as their venoms, Richet states that when heated to 58° , or even lower, the toxic properties of such sera are mostly abolished; so much is this so, that the use of heated sera to-day tends to become general. The progressively destructive action of heat on any solution of enzymes hardly requires being insisted upon.

When the mammals of the negative blood maltase group are studied, it is seen from Fig. 2 (right-hand section) and Fig. 3 that they arrange themselves, in terms of decreasing glycogenolytic activity of their blood, as follows: cat, monkey, rabbit, man.

It is of some interest that man, in this group, should be lowest in the scale of blood glycogenolytic function. Of course that may simply be accidental, and further studies may reveal negative blood maltase mammals of still lower glycogenolytic power. But if not, then it points to the possible fact—allowing for exceptions, like the rabbit above—that loss of glycogen hydrolysing power of blood occurs as we ascend in the mammalian scale.

I would mention here, in passing, that Twort and Archer [1923] have found a practically identical arrangement for animals of our negative blood maltase group, so far as the content of their blood in *lipase* (monobutyrylase) is concerned. In terms of decreasing *monobutyrylase* content the negative blood maltase mammals studied by these workers arrange themselves in the following series: guinea-pig, cat, rabbit, man.

But it may be asked to what purpose are enzymes met with in blood? Some authorities, like Carlson and Luckhardt [1908] consider that they serve no essential end in the body processes, that they are mere "discards" of the tissues on the road to destruction or elimination, and that such differences as are noticeable, for instance in the amylase of blood, at different times appear to be of an accidental nature; or again, as stated by Moeckel and Rost [1910], that they play no rôle in the economy, that oscillations in the amylase of blood are merely expressive of variations in the digestive processes in some organ of the body.

I find it difficult to subscribe to the views thus expressed, that blood enzymes play no rôle in the economy, or that they are simply waste products on their way to elimination and destruction. That these enzymes in the blood are functionally active, and that they are active to different—but more or less definite—degrees for different mammalian species, is at least suggestive that they are present in the blood for their own specific purposes. As shown particularly for the individual dog, successive examinations, under as nearly as possible constant experimental conditions, reveal a remarkable constancy of enzymic activity. For the individual animal, the glycogenolytic activity of the serum gives one the impression of bearing the stamp, so to speak, of the individual; the activity curve is, in this intimate way, perhaps more characteristic of the individual than it is even of the species. In other words, the individual stands portrayed in the enzymic activities of his blood.

It is a pleasure here to express my special indebtedness to M. Albert Frouin who kindly supplied me with the specimens of dog's blood for the investigation, also to Dr C. Levaditi who furnished me with the blood of the monkey; while my thanks are due to Mlle L. Castlenau for help with some of the sugar estimations.

REFERENCES.

- Carlson and Luckhardt (1908). *Amer. J. Physiol.* **23**, 148.
Compton (1921). *Biochem. J.* **15**, 681.
Moeckel and Rost (1910). *Z. physiol. Chem.* **67**, 433.
Pflüger (1904). *Pflüger's Arch.* **103**, 169.
Richet and Richet (1921). *Traité de Physiologie Medico-Chirurgicale*, 783–786 (Paris).
Twort, C. C. and Archer (1923). *Lancet*, **104**, 1102.