XXIV. BLOOD ENZYMES. IV. STUDIES ON THE MALTASE OF DOG'S SERUM: INFLUENCE ON ACTIVITY OF THE REACTION OF THE MEDIUM, AND OF THE STATE OF DIGESTION.

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IN previous communications, data relating to the distribution, the activity, and the influence of the action of heat on the enzyme maltase, as met with in mammalian blood, have been worked out. From the point of view of distribution, these investigations have shown that among mammals there exist two groups: a positive blood maltase group and a negative blood maltase group—man belonging to the latter [Compton, 1921, 2]. As concerns its activity, and its temperature constants (optimum temperature and destruction temperature), the data so far brought to light concern chiefly the enzyme as met with in dog's blood, the dog being a representative animal of the positive blood maltase group.

In the present communication some further questions of importance in connexion with this enzyme are investigated: notably, the influence of the reaction of the medium upon its activity, with a view to ascertaining what is the optimum reaction of the medium for the study of the maltase of dog's blood; what variations, if any, of activity are to be found when the blood under examination is collected respectively in the fasting condition and during digestion; and what respectively is the response of the enzyme to the action of heat in these two conditions.

INFLUENCE OF THE REACTION OF THE MEDIUM UPON THE ACTIVITY OF THE ENZYME.

This investigation was carried out at the optimum temperature of the enzyme, 55° in an action lasting for 16 hours [Compton, 1922].

The practical details of the experiment were as follows: 0.9 cc. of serum from the dog 2-*P*, in the fasting condition for 24–36 hours, were carefully measured with a pipette into a 10 cc. measuring flask and the volume completed to 10 cc. with pure (redistilled) water. This solution was added in 1 cc. portions to each of six clean sterile test-tubes, containing 90 mg. of pure maltose (hydrated) and either 4 cc. of pure water or of a solution containing

a known quantity of acid or alkali. Five drops of toluene were added and the tubes transferred to a water thermostat regulated at or about 55° ($54.8-55.0$). After a few minutes, to allow of expansion and escape of vapour while the tubes took up the temperature of the water-bath, they were closed with clean corks which had been washed and sterilised with boiling water. At the end of 16 hours, the enzyme action was stopped by plunging the tubes into boiling water for five minutes, the corks having been previously withdrawn. The tubes were then cooled, the contents diluted to 50 cc. and a Bertrand sugar estimation was made on 20 cc. of the dilution. Fig. 1 shows the results. The serum was seven days old when the experiment was undertaken, this being an interval which is without much appreciable deteriorative effect on the enzyme as met with in the serum.

The curve shown in Fig. 1 indicates that in the conditions of the experiment the merest trace of alkali is detrimental to the enzyme action, while on the contrary the addition of acid increases the efficiency of the enzyme, until a certain point is reached, corresponding to the presence of 0.15 cc. of $N/100$ H_2SO_4 per 0.09 cc. of serum, beyond which the addition of more acid is in turn detrimental to the action. The action, therefore, passes by a maximum situated in the acid region. This optimum works out at 1.66 cc. of added $N/100$ H_2SO_4 per cc. of dog's serum, in a reaction mixture of total volume 55.5 cc.

Hydrogen ion concentration measurements, by the colorimetric method, revealed that this addition of acid to the medium brought about a p_H of approximately 6.6: the natural reaction, without any addition of acid, being 7.4.

It is of interest that this optimum reaction of the medium for a maltase of animal origin is, as regards p_H , practically identical with that found by us for the maltase of vegetable origin met with in *Aspergillus oryzae* [Compton, 1915].

INFLUENCE OF THE STATE OF DIGESTION ON THE ENZYME'S ACTIVITY.

For this investigation, the following comparative experiment was carried out. The dog 2-*P* was allowed to fast during 24–36 hours, previous to the withdrawal of some blood by venous puncture from the jugular vein. It was then fed with a meat meal rich in fat, and 3–4 hours later another specimen of blood was taken. Both specimens of blood were centrifuged as soon as a beginning of clotting was visible. The supernatant serum was pipetted off and conserved in a sterile test-tube in presence of a few drops of toluene. The serum from the digestion specimen was opaque and chylous, owing to the fat partaken of in the meal; the fasting specimen gave a transparent, slightly pink tinted, serum. Parallel experiments were set up with these two freshly collected serums, as follows. Into two series of four test-tubes, were weighed 90 mg. of pure maltose, water was added to each series in quantities of 4.9, 4.8, 4.6 and 4.2 cc., after which the volumes were completed to 5 cc. by the addition respectively of 0.1, 0.2, 0.4 and 0.8 cc. of serum. Five drops of toluene were added to each tube, and they were incubated for 16 hours in a thermostat regulated at approximately 47° (47.5–46.9). The enzyme was then destroyed by heat as previously, and the proportion of maltose hydrolysed in each tube estimated. The experiment gave the result shown by the curves of Fig. 2.

As regards the activity of the two specimens, Fig. 2 brings to light a certain anomaly. It will be seen that, over the range of doses studied, for small doses—for instance, for enzyme concentrations up to 0.1 cc. serum per 5 cc. reaction mixture—the digestion specimen is but slightly more active than the fasting specimen, while for doses between 0.1 and 0.75 cc., the enzyme of the digestion specimen is less active. Beyond 0.75 cc. of serum, the digestion specimen again shows increased activity.

As concerns the activity here revealed by the middle portion of the fasting curve, this is in accord with the findings of Kumagai [1913], who states that the hydrolysis of maltose by dog's serum is a little quicker, and the minimum active dose a little feebler, in the fasting condition than after food. We ourselves in a former study [Compton, 1922] observed in the case of a fresh digestion specimen and a 48 hours old fasting specimen, from the same dog 3-*P*, that the digestion specimen was throughout its course more active in maltase than the fasting specimen; but in this case the latter being somewhat aged the experiment was not deemed conclusive; for it was not certain then if the time the serum had been kept before testing might not be a contributory factor.

INFLUENCE OF THE STATE OF DIGESTION ON THE OPTIMUM TEMPERATURE
OF BLOOD MALTASE.

Two parallel series of temperature studies were carried out 24 hours later with the two foregoing specimens. The quantity of serum employed was 0.09 cc. To measure out this dose conveniently a dilution was made: 1.35 cc. of each of the two specimens was made up with water to 15 cc. in measuring flasks, and 1 cc. of each was distributed respectively into two series of six test-tubes, containing 90 mg. of weighed maltose dissolved in 4 cc. of water and five drops of toluene. The tubes were incubated in six thermostats regulated at temperatures varying between 42° and 58°. After 16 hours the enzyme action was stopped by placing the tubes in boiling water. On estimating by Bertrand's method the proportion of maltose hydrolysed, the two series gave the results shown in Fig. 3.

This figure shows that the optimum temperature of the maltase of dog's blood is the same—55° for the time interval and the natural reaction of the medium—whether the specimen be taken when the dog is fasting or in digestion.

This finding is of special interest, in view of the fact that the optimum temperature of an enzyme is very sensitive to any p_H alteration of the medium used to determine it [Compton, 1915; 1921, 1]. It affirms the constancy of the p_H reaction of the blood—at least of the venous blood—of the individual; that this does not vary but remains constant even during digestion, when a variety of substances enter the blood stream from the breakdown by alimentary digestion of the complex molecules of food.

The result indicated by this study possesses the more interest for us in that we had already observed in previous work [Compton, 1922] under similar experimental conditions, at least of duration and of concentrations in enzyme and substrate, that the optimum temperature of the enzyme as met with in the blood of different dogs (2-*P*, 3-*P* and 4-*P*) was constant at 55°, in an action lasting for 16 hours whether the serum of the animals was tested fresh or a few days old; and also, in the case of one of the dogs (3-*P*), when the optimum temperature was determined on one occasion with a fresh digestion specimen, and on another with a fasting specimen two days old.

Now, enzyme preparations on ageing often show considerable loss of activity, especially when in aqueous solution; also, when preserved in the condition of a dry powder, they undergo transformations such as are revealed by changes in the optimum p_H reaction which they will support [Bertrand and Compton, 1921]. In the comparative experiment on optimum temperature described in this paper, by utilising specimens of blood withdrawn from the same dog and tested on the same day in exactly the same fashion, the experimental factors were rendered so nearly identical that any possible alterations of the enzyme which might result from ageing of the serum were reduced to a minimum and practically eliminated.

Our experiments of an earlier date and those of to-day, in the constancy of optimum temperature which they portray, cannot well be interpreted otherwise than in the fixity of the chemical, or p_H , reaction of the blood itself. They are but expressions of the same. The buffering of the blood is so perfect for the species that from individual to individual the optimum temperature of any contained ferments does not vary.

SUMMARY.

Optimum reaction of the medium for the maltase of dog's blood. In an action lasting for 16 hours at 55° (optimum temperature under these conditions for the natural reaction) in a substrate concentration of $M/20$ and an enzyme concentration of 0.09 cc. per 5 cc. reaction mixture, the optimum reaction is reached by the addition of 0.15 cc. of $N/100$ H_2SO_4 .

In respect of the enzyme requiring an acid medium in which to act to best advantage, no distinction is to be drawn between maltase of animal and maltase of vegetable origin.

State of digestion and activity of the enzyme. For all practical purposes the activity of the enzyme is the same whether the blood be collected during digestion or in the fasting state, only very slight differences being recorded, such as might be expected in oscillations about normal limits.

State of digestion and optimum temperature of the enzyme. The optimum temperature is unaffected by the state of digestion of the animal at the time the blood is withdrawn for investigation (whether fasting, or immediately after a meal), being 55° in an action lasting for 16 hours for an enzyme concentration of 0.09 cc. per 5 cc. reaction mixture.

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