INFLUENCE OF TEMPERATURE ON THE AMYLASES OF COLD- AND WARM-BLOODED ANIMALS

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(With Three Text-figures)

I. INTRODUCTION

A certain amount of work has been carried out on the interesting problem of the temperature relations of the enzymes of poikilothermous animals, but no general agreement has been reached as to whether they are possibly better adapted to act at lower temperatures than the corresponding enzymes of the birds and mammals. Many of the previous workers have confined themselves to determinations of the optimum temperatures of different enzymes and have concluded that a lower optimum temperature entails better adaptation to low temperatures. Riddle (1909) introduced Mett's tubes into the stomachs of a representative series of vertebrates and concluded that the rate of digestion by pepsin decreased at low temperatures in the higher forms. Kenyon (1925) made an extensive study of the enzymes occurring in poikilothermal vertebrates and compared the rates of peptic digestion at room temperature and 37° C. He found that in fishes, Amphibia and reptiles digestion was uniformly more rapid at 37° C., with the exception of the pickerel which apparently showed no change. Müller (1922) and Pjatnitzkij (1930), from studies of the optimum *p*H and optimum temperature of frog pepsin, concluded that it was identical with that of warm-blooded animals. Oya & Harada (1926) found that the amylase from the caecae of *Seriola* had a similar optimum temperature to mammalian amylase, and they also showed that the temperature coefficient is greater below 20° C. than at higher temperatures. Chesley (1934) discussed the significance of determinations of the optimum temperature of enzyme activity, and he tabulated the conclusions reached by various workers using this method. He showed that, unless the experimental conditions are rigidly controlled, the optimum temperature is a meaningless term, as it is rapidly susceptible to variations in *p*H, ionic concentration, digestion period and the enzyme-substrate ratios. Chesley showed the effect of the digestion period on the optimum temperature by determining the activity at different intervals. He found that menhaden amylase after 10 min. digestion at 45° C. had an optimum at 40° C., while after 25 min. the optimum had fallen to 35° C. The enzyme substrate ratio causes even greater changes in the optimal point, and Ernstrom (1922) showed that it is also affected by changes in the electrolytic content of the digest.

Some interesting findings have been recorded by workers on invertebrates with regard to the changes in the optimum temperature of enzyme activity over prolonged digestion periods. Berrill (1929), working on ascidians, found that after 1 hour the optimum temperature of the amylase was 45° C., but with an increased digestion period it gradually fell until after 57 hours it was 13° C. Berrill correlated this with the normal digestion period of the animals at different temperatures, and concluded that the food was normally retained in the gut until the enzymes were 75 per cent destroyed. Nicol (1930) sought for evidence of a similar change in the optimum temperature for the enzymes of *Sabella pavonina*, but with negative results. She found that the optimum temperature for amylase activity remained at 29° C. for digestion periods of from 10 to 26 hours. The significance of this work in relation to the life of the organism is discussed by Pantin (1932) and Yonge (1935). Chesley (1934) also investigated the temperature relations of the amylases of human saliva, terrapin pancreas and menhaden pancreas under carefully controlled experimental conditions and over a wide range of temperature. He found that the optimum temperature was somewhat lower for the cold-blooded animals and attributed this to the lower heat coagulation point of the proteins causing the enzyme to be dragged down, although itself little affected. His figures show that menhaden amylase is better adapted and that of the terrapin less well adapted to act at low temperatures than human saliva, thus disagreeing with Riddle's (1909) conclusions for pepsin.

In view of the contradictory nature of much of the work on this problem it was decided to undertake a comparison of human salivary amylase and that from the pancreas of the common frog (*Rana temporaria*). The experimental procedure of Chesley was adhered to in the main, although the determination of activity was carried out by a different method. In addition, a prolonged digestion experiment has been carried out on a sample of frog pancreatic amylase, to study its effect on the temperature of optimum activity.

II. COMPARISON OF HUMAN SALIVARY AND FROG PANCREATIC AMYLASES

(*a*) *Material and Methods*

Owing to the small size of the pancreas it was necessary to employ about sixty frogs to obtain a reasonable quantity of extract. The pancreas was rapidly removed from the animals and weighed, after absorbing the superficial moisture with blotting paper. The glands were then mixed with powdered glass and ground up in a mortar, 50 per cent alcohol in the proportion of 10 c.c. to each gram of tissue was added and left to extract for 24 hours. The tissue residue was removed by straining through fine muslin.

Human saliva was obtained by chewing on paraffin wax, and was diluted five times with 50 per cent alcohol. This was allowed to stand for 24 hours before use so that the conditions should be comparable in each case. Human saliva in this

dilution was found to have approximately the same activity as the frog pancreatic extract.

A 2·5 per cent starch solution of the following composition was used as a substrate. 25 g. of soluble starch were dissolved in 600 c.c. of boiling distilled water, 200 c.c. of phosphate buffer (*p*H 6·9) were added and 2·92 g. of sodium chloride (0·05 *M*), the total volume then being made up to 1 l. with distilled water.

A series of constant-temperature baths was arranged at the following temperatures: 1·0, 8·5, 14·5, 24·0, 36·0, 45·5 and 57·0° C. Duplicate determinations were made at each temperature with frog amylase and at the two lower temperatures with ptyalin. The experimental procedure was as follows. 50 c.c. samples of the substrate were pipetted into 100 c.c. flat-bottomed flasks, and these were suspended in the respective water baths for at least half an hour before the beginning of the experiment to attain a constant temperature. 1 c.c. of the amylase under investigation was then accurately measured into each flask, at 2 min. intervals, and digestion allowed to proceed for 30 min. The glucose formed was estimated by the method of Hagedorn & Jensen as described by Cole (1928). The enzyme action was stopped at the end of the digestion period in the following way. A number of 6 × 1 in. tubes each containing 10 c.c. of distilled water was placed in a vigorously boiling water bath and at the end of the 30 min. period two 0·1 c.c. samples were rapidly pipetted from each flask and added to these tubes containing hot distilled water. As an extra precaution each tube was boiled up over a flame immediately after addition of the sample to ensure the complete destruction of the enzyme. The normal procedure for the Hagedorn-Jensen method was then followed. Very good agreement was obtained between the samples from the duplicate flasks at each temperature, and it seems very probable that the increase in glucose content found represents the true activity of the enzyme. Control flasks to which no enzyme had been added were placed in several of the baths, and these were all found to have a constant glucose content, showing that the original starch used was not free from glucose. The glucose formed by enzyme activity was found by subtracting the glucose content of the control from that found in the digest.

(*b*) *Experimental results*

The data obtained from two experiments on frog amylase and ptyalin are shown graphically in Fig. 1. The temperature coefficients (Q 10) calculated from these two curves are shown in Table I, where values obtained from a second experiment with ptyalin are also included. These two determinations give very concordant results, despite the fact that the saliva used in the second experiment was rather more active than in the first. It is evident from Fig. 1 that not only have both amylases an optimum activity at approximately 48° C. but also that their actual activities at 0° C. are practically the same. Above 48° C. the activity falls rapidly showing the heat destruction of the enzyme. Between 10 and 40° C. it appears as if the activity of the frog amylase is slightly greater than that of ptyalin; thus at 20° C. 31·5 per cent more glucose is produced by the former in the same time. The temperature

coefficients (Table I) are also uniformly lower for the frog amylase over this part of the range.

Table I. *Temperature coefficients for the action of frog pancreatic amylase and ptyalin*

Temperature range °C. ...	0–10	10–20	20–30	30–40	40–50
Frog pancreas	2·19	1·79	1·48	1·32	1·12
Human saliva (1)	1·61	1·83	1·69	1·48	1·23
Human saliva (2)	1·63	1·84	1·77	1·47	1·18

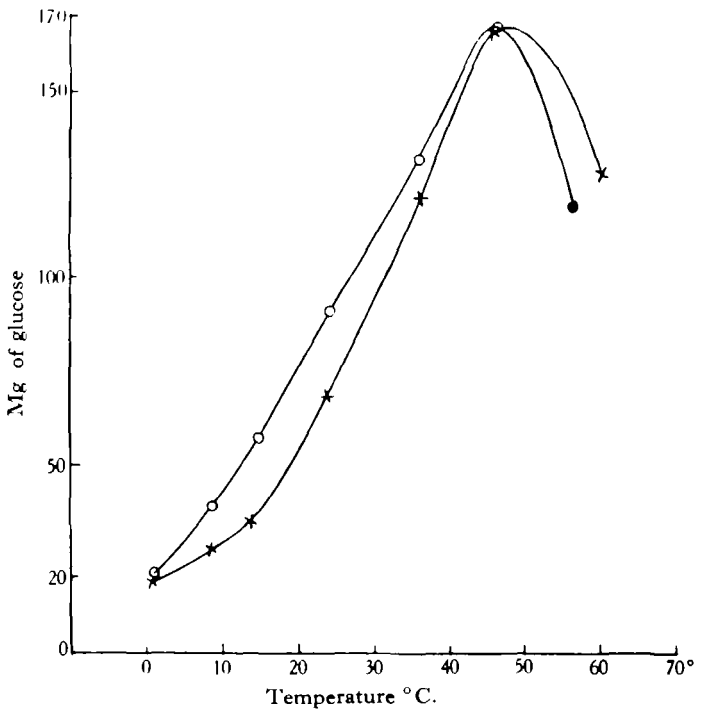

Fig. 1. Graph showing the effect of temperature on amylolytic digestion.
⊙ Frog pancreatic amylase. × Human salivary amylase.

It is of interest to note that both frog pancreatic amylase and ptyalin retain approximately 12 per cent of their maximal activity at 0° C. Ernstrom (1922) observed that the action of ptyalin was not completely inhibited at 0° C. Ernstrom also obtained the temperature coefficients (Q 10) of ptyalin over the range 0–45° C., and his results agree fairly closely with those found in the present investigation with the exception of the coefficients between 0 and 10° C. Ernstrom's values for two samples of saliva are 2·3 and 2·7, as against 1·61 and 1·63 found in the two independent determinations recorded here. Chesley (1934) also finds that the temperature coefficients between 5 and 15° C. for fish, reptilian and human amylases

lie between 2·5 and 3·5. It may be that this difference is due to slight differences in the experimental conditions.

Table II shows the temperature coefficients recorded by Ernstrom (1922) and Chesley (1934) for human saliva compared with the values found in this investigation.

Table II. *Temperature coefficients for human saliva recorded in the literature*

Range °C.	0–10	10–20	20–30	30–40	40–45	Ernstrom (1922)
Q 10	2·7	2·2	1·9	1 6	1 2	
Range °C.	5–15	15–25	25–35	35–45	45–63	Chesley (1934)
Q 10	2 4	2·1	1 6	1·5	1 1	
Range °C.	0–10	10–20	20–30	30–40	40–50	This paper
Q 10	1·62	1·83	1·73	1·47	1·20	

(*c*) *Conclusions*

It is apparent that under standard conditions the optimum temperature of the amylase is the same in frog and man. At temperatures below the optimum, however, it would seem that the frog amylase is relatively more efficient. During the summer months when the frog is most active, the temperature will usually vary between 10 and 20° C., and it has been shown that over this range the frog amylase is approximately 30 per cent more effective than ptyalin. The rate of digestion at 0° C. is approximately equal in both amylases, but this is probably not of biological significance as the frog is not normally feeding under such conditions, although it is apparent that the amylase at least can still function at such low temperatures.

III. INFLUENCE OF TIME ON THE OPTIMUM TEMPERATURE OF FROG AMYLASE

(*a*) *Methods and results*

The pancreatic extract was made in exactly the same way as in the previous experiments except that 50 per cent alcohol was added in the proportion of 30 c.c. to each gram of tissue. The substrate used was a 5 per cent starch solution buffered at *p*H 6·9 with phosphate buffers and containing 0·05 *M* sodium chloride. The experimental procedure was unchanged, duplicate digests being used at each temperature and the glucose formed was estimated by the Hagedorn-Jensen method at suitable intervals. In view of the extended period of digestion 25 c.c. of chloroform water was added to each digest, to prevent putrefaction. The activity was determined at 8, 19, 30, 40,and 49° C. after periods of 1, 7, 20, 28, 46 and 68 hours.

Table III shows the amount of starch digested at each temperature after different intervals, and in Fig. 2 the temperature curve of the enzyme activity after each period has been plotted.

It can be seen that there is but little change in the optimum temperature. After 1 hour it appears to be slightly above 50° C.; after 7 hours it is about 48° C.; after

Table III. *Record of experiment on the influence of the digestion period on amylase activity*

Temp °C.	Duration of experiment (hours)						
	1	7	20	28	46	68	
	mg.	mg.	mg	mg.	mg.	mg	
8	26	42	93	114	183	235	Glucose content of digest (mg.)
19	30	72	191	249	340	451	,,
30	34	106	290	390	532	673	,,
40	40	157	399	500	707	877	,,
49	76	179	419	545	697	836	,,

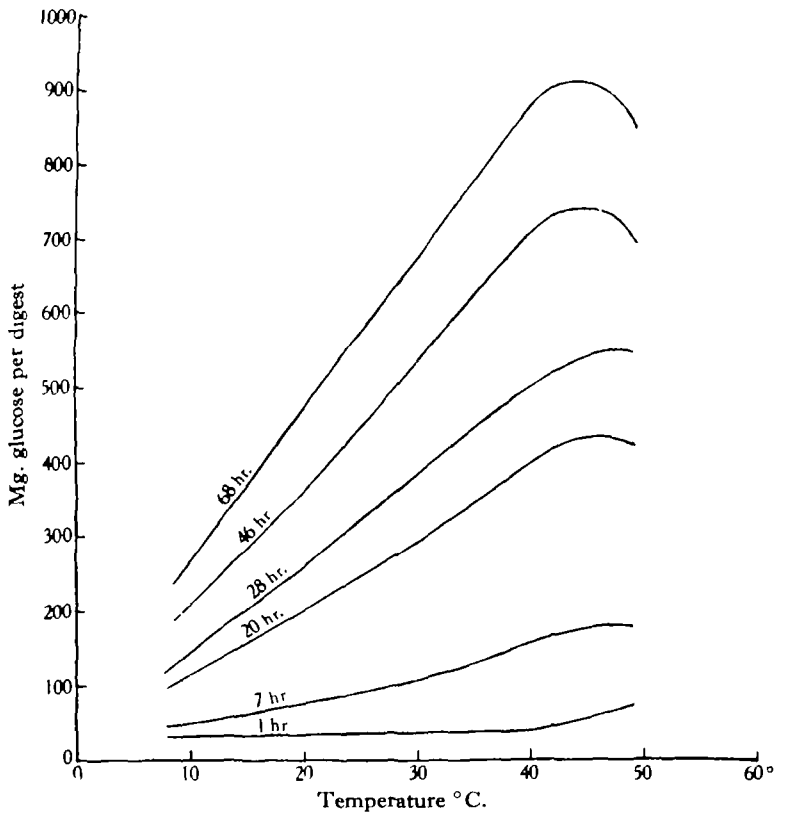

Fig 2. Graph showing the effect of temperature on the activity of the frog (*R. temporaria*) pancreatic amylase during different digestion periods.

20 hours 46° C.; after 28 hours 47° C.; after 46 hours 44·5° C.; and after 68 hours 44° C. In Fig. 3 the titration curve at each temperature has been plotted, and it is apparent that the enzyme shows little change in activity. The change is most marked in the 49° C. curve which cuts that for 40° C. after 40 hours, but even at 68 hours the enzyme was still active at this temperature, as shown by the continued upward trend of the curve. As regards the other curves there does not appear to be

any differential temperature effect, as they all follow an approximately similar course. This absence of a differential temperature effect will account for the practical constancy of the optimum temperature shown in Fig. 2. Thus it appears that the frog pancreatic amylase is relatively thermostable.

Frost (1932) conducted feeding experiments with American frogs (*Rana clamitans* and *R. sylvatica*). He found that the stomach was apparently emptied on

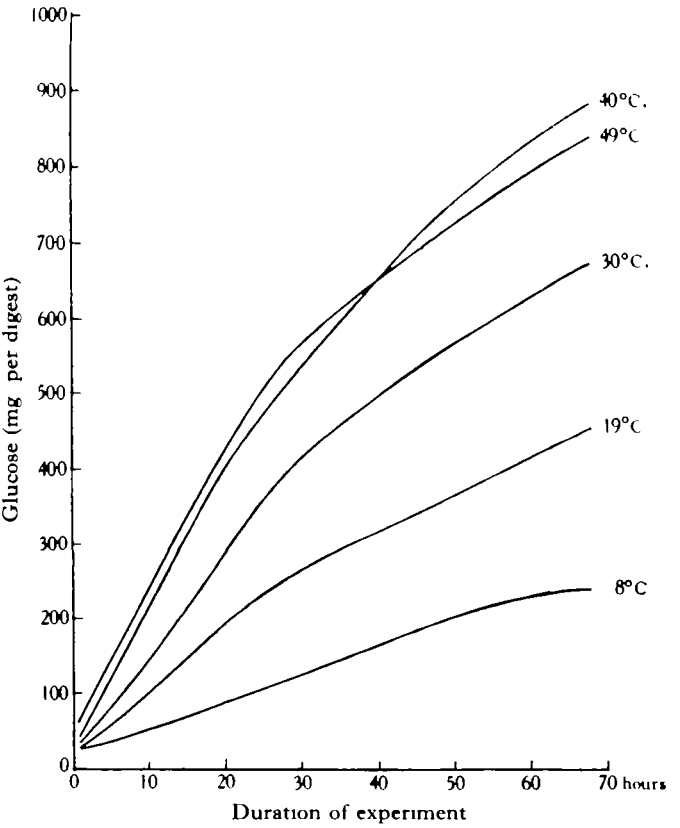

Fig. 3 Activity of pancreatic amylase of *R temporaria* at different constant temperatures and digestion periods.

the average once every 2 or 3 days. The rate of passage of food was more rapid if food was abundant and slower if the stomach was not full. In the former case whole insects were sometimes found in the faeces, while when food was limited insects were often digested beyond recognition. Such experiments do not give any information as to the length of time the food remains in a particular part of the alimentary canal, neither is the temperature at which the experiments were made recorded. It would, however, appear that the pancreatic amylase would not normally act for a longer period than 48 hours.

(*b*) *Conclusions*

There does not appear to be any evidence to indicate that the frog shows any such temperature adaptation as regards its digestive enzymes as that found by Berrill (1929) in ascidians. In the period during which the frog normally retains food in its gut, the amylase, at least, is thermostable and there is no relation between the period of digestion and the heat inactivation of the enzyme. In this respect the conditions in the frog appear to be more comparable with those described by Nicol (1930) in polychaets.

Berrill, however, makes an interesting observation with regard to enzyme thermostability. He suggests that: "It is possible that a permanent increase in the stability of the digestive enzymes would be turned to advantage through a more prolonged retention of the food within the gut." This is suggestive when considered in connection with Frost's (1932) observation that the passage of food through a frog's alimentary canal is much slower on a limited diet. In this case it might seem that the animal is making use of the stability of its enzymes to extract all the nourishment possible from the food available.

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