SYMPATHOMIMETIC ACTIVITY IN THE ISOLATED FROG'S HEART (RANA TEMPORARIA)

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(Received 28 August 1951)

(With Seven Text-figures)

In a previous paper (Smith, 1951) the occurrence of various types of relation between temperature and the pulse rate of the isolated frog's heart was reported. Five main types of curve were observed and their chief features may be briefly summarized as follows. Type A showed an exponential relation between frequency and temperature, and corresponded to the 'winter' curve originally described by Barcroft & Izquierdo (1931). Type B was rather similar to A, but instead of the rates lying along an even curve they lay on two straight lines which intersected at about 10° C. Type C showed a linear relation between frequency and temperature over the range investigated and the value of the observed temperature coefficient ($Q_{10}$) was about 2-5. This form corresponded to the 'summer' curve of Barcroft & Izquierdo (1931). Type D was of infrequent occurrence, and in this case the pulse rates for the upper and lower temperatures lay on straight lines of very similar slope, but which had different origins at 7° C. Type E again showed a linear relation between pulse rate and temperature, but in this case the observed temperature coefficient was significantly lower than that found for type C (2-1). It was further shown that pulse rate curves of type B or type D could be produced by treatment of type E hearts with an extract of anterior pituitary gland in the presence of added adrenaline. Type C curves were obtained from type E hearts when they were perfused with Ringer containing anterior pituitary extract, adrenaline and thyroxine. To explain these results it was suggested that there was a synergistic action between an anterior pituitary principle and adrenaline which was inhibited at temperatures below 10° C. Further, as types A, B and C pulse rate curves could be obtained from normal hearts in the absence of external adrenaline, the hypothesis was advanced that the isolated heart was under the influence of an active sympathomimetic substance even when it was being perfused with unmodified Ringer.

In the course of further work on this problem experimental evidence has been obtained which throws more light on the genesis of these various pulse rate curves. In the main this work supports the basic hypothesis put forward in the earlier paper, but it is necessary to correct some of the tentative conclusions previously drawn. It is now apparent that the transformation of type E to other types of curve is not a specific action of pituitary extract, but can also be obtained by treatment with ascorbic acid or liver extract. In addition, evidence has now been
obtained which makes it seem very probable that not only is the isolated heart subject to persistent sympathomimetic activity, but that adrenergic material is synthesized by the heart tissues and the observed form of the temperature-pulse rate curve depends on the level at which equilibrium is established between production and inactivation of this material. In the present paper experiments on the action of certain adrenergic blocking agents on types A and B hearts are also described. Finally, various forms of temperature-amplitude curves which correspond to the different types of temperature-pulse rate curves are given and their significance discussed.

MATERIAL AND METHODS

The isolation and perfusion of the hearts used in this work was carried out in the manner previously described (Smith, 1951). The hearts were perfused, at a temperature of about 12°C, for at least 1½ hr. before observations were made. Except where otherwise stated, each experiment was started at approximately 7°C, and when a stable rate had been attained, the temperature was progressively raised to the upper limit of the range used (usually 19 or 21°C). Observations of pulse rate were then made at approximately 2°C intervals over the range, the temperature being kept constant at the desired level for 5 min. before a recording was made. A control experiment in which the heart was perfused with either unmodified or adrenaline-containing Ringer was always made initially to determine the form of the natural temperature-pulse rate curve. In the majority of cases the Ringer was then withdrawn from the apparatus and the extract or drug under investigation added. The pH of the medium was checked and readjusted to 7-6, if necessary, by adding phosphate buffer. The modified perfusate was then reintroduced into the apparatus, and the heart perfused for at least 20 min. before recordings of the pulse rate were started. The only case where this procedure was departed from was in those experiments involving the use of ergotoxine. Here 2 l. of Ringer were made up, of which 1 l. was used for the control experiment, while the required amount of ergotoxine ethanesulphonate was added to the other litre. This solution was well shaken at intervals before it was required for perfusion, and as far as could be ascertained the material had completely dissolved.

Liver extract. The extract of frog's liver used in some of the experiments was prepared in the following way. After the heart had been isolated the required amount of liver was weighed out (usually 100 mg.) and ground up with sand. About 100 ml. of alkaline Ringer (pH 8-5) were then added and the material left to extract at room temperature. After the completion of the control experiment this crude extract was added to the perfusate without any further treatment.

Adrenaline. When external adrenaline was required in the perfusate it was added by appropriate dilution of a solution of commercial adrenaline hydrochloride (Liquor Adrenalinæ Hydrochloridi, B.P.). This solution contains adrenaline stabilizers in the form of chlorbutol (0·5%) and sodium metabisulphite (0·05%).

Mechanical response of the heart. In the majority of experiments kymograph records were taken at each observational temperature, in addition to timing the
rate of beat with a stop-watch. The records were obtained by means of an isotonic lever connected to a fine pin in the tip of the ventricle. The movements of the lever were recorded on the drum by a small, glass, frontal writing point. When quantitative data on the mechanical response of the heart were needed for the construction of temperature-amplitude curves the total excursion of the lever was measured. The greater part of this movement was, of course, due to ventricular systole. The same recording system was not used in all experiments, so that no comparison can be made between the absolute heights of the mechanical response of different hearts. In comparing the temperature-amplitude curves for different hearts, attention must therefore be directed to the general form of the curve rather than to the numerical value assigned to the amplitude. The recording conditions remained unchanged throughout a series of experiments on any one heart, and in this case any variations in the absolute heights may be regarded as significant.

**EXPERIMENTAL RESULTS**

(i) *Modification of type E curves*

In the previous paper (Smith, 1951) the action of extracts of anterior pituitary gland on the linear, low $Q_{10}$ type of temperature-pulse rate curve was described. It was found that such treatment caused an increased frequency response at higher temperatures so that the temperature coefficient was increased and curves of types $D$ or $B$ were produced. Numerous experiments have subsequently been made to test the action of ascorbic acid and liver extract on the type $E$ heart, and all the results conform very closely with those presented below. In addition, several control experiments have been made to investigate the possibility of spontaneous changes occurring in the form of various pulse rate curves during prolonged perfusion with unmodified Ringer. These have shown that there is no significant change in the relation between frequency and temperature up to 28 hr. after isolation (see also Carter, 1933; Smith, 1951).

Fig. 1 illustrates the typical response of the type $E$ heart to liver extract in the presence of external adrenaline. The preliminary control experiment, when the heart was beating in Ringer plus adrenaline (1 in $10^7$), showed a linear relation between frequency and temperature over the range 7–17°C. A Ringer-extract of 100 mg. of frog’s liver was then added to the medium and the temperature-pulse rate curve determined about 4 hr. later. In this case the frequency acceleration between 7 and 9°C. was very similar to that found initially, but from about 10°C. upwards a marked increase was apparent, so that the final curve consists of two straight lines which intersect at 9.5°C. The value of $Q_{10}$ between 7 and 17°C. increased from 2.21 to 2.60.

Fig. 2 shows the production of a very similar type of curve from an original type $E$ by the addition of ascorbic acid (5 mg./l.) to the perfusate. Adrenaline at a concentration of 1 in $2 \times 10^7$ was added prior to both experiments. In the case of both anterior pituitary and liver extract the increase in frequency seemed to be
confined to the higher temperatures entirely, but there was a definite tendency when ascorbic acid was used for the acceleration of pulse rate between 7 and 9°C. to be slightly higher than in the control experiment. This is the case in the example shown in Fig. 2, where the \(Q_{10}\) of the initial type E line was 1.85, but the projection of the 7 and 9°C. rates to 17°C. in the second experiment would give a line with a \(Q_{10}\) of 2.30.
A not uncommon feature shown by hearts in January and February, when the incidence of type E curves was high, was the initial rise of frequency on an apparently normal type E line, but at about 12° C. the acceleration gradually started to decline and in some cases even became negative, so that the pulse rate at 20° was actually lower than that at 12° C. This effect has been observed with hearts beating in unmodified Ringer as well as in the presence of added adrenaline.

Another feature commonly seen in these hearts was the reversibility of this decrease in rate when the temperature was lowered again. A typical curve of this type is shown in Fig. 3A. On raising the temperature from 7° C, the frequency increased linearly up to 12° C., and if it had continued on this course it would have given a $Q_{10}$ of 2.04. There was, however, a marked falling away below this line at the higher temperatures. When the heart was rapidly cooled again the rates at first lay on a lower straight line, but with further temperature reduction there was a relative increase in rate so that the points were coincident with the original line obtained when the temperature was rising. The point on the temperature scale at which this relative increase in frequency appeared was not fixed, but depended on the rate of cooling. For instance, when the heart was quickly cooled to 7° C. the observed frequency was still significantly lower than that observed initially, but if further...
records were made with the temperature steady at this level, then a progressive increase could be observed until the initial rate was regained.

The action of ascorbic acid on hearts of this type was very revealing, as can be seen from Fig. 3, B. After addition of ascorbic acid to the medium, the pulse rate acceleration from 7 to 12°C again gave a straight line ($Q_{10} = 2.10$), but instead of the frequency falling off at the higher temperatures there was now a marked extra acceleration apparent, so that the curve became type $A$. Furthermore, on quickly cooling the heart there was again a lag in the pulse rate change, but it was in the opposite direction to that observed in the first experiment. When the temperature had fallen to 9°C, the rate was still appreciably higher than in the upward run, but on keeping the temperature steady at this level for about 10 min. the original rate was regained, and when the temperature was now reduced to 7°C the original path was followed. In both these experiments the heart was beating in unmodified Ringer and this was the only occasion on which addition of ascorbic acid, in the absence of added adrenaline, led to the immediate appearance of a type $A$ curve. A very similar reversal of the direction of the delayed frequency change when the temperature was reduced again has several times been observed when the typical action of ascorbic acid or liver extract was produced in the presence of external adrenaline. Barcroft & Izquierdo (1931), in their original experiments on the temperature-pulse rate curve, also found a difference in rate at a particular temperature when the direction of temperature change was reversed. Carter (1933) found that this difficulty could be obviated if the temperature were kept constant for at least 5 min. before recording the rate. This would appear to be due to the same phenomenon as that shown in Fig. 3, for if the temperature were reduced more slowly and by small intervals, there would probably be no difference between the rising and falling temperature curves.

Several experiments have also been made to investigate the action of ascorbic acid on type $E$ hearts after more prolonged exposure, both with and without external adrenaline. The record of such a group of experiments is shown in Fig. 4. The control experiment, made 2 hr. after isolation in unmodified Ringer, gave a type $E$ curve with some falling off in the rate at higher temperatures which was reversible on cooling again. Another experiment was made after the heart had been beating in the same medium plus ascorbic acid (5 mg./l.) for 2½ hr. The resulting curve (Fig. 4, B) is now a quite typical type $E$ with no indication of either decreasing acceleration at the higher temperatures or a change-over to type $A$ or type $B$. The heart was left under perfusion overnight in the same medium, and another determination made 24 hr. after isolation. It is apparent that there was now a further definite change in the temperature-pulse rate curve (Fig. 4, C). The projection of the line joining the 7 and 9°C rates would give the same coefficient as before (2.16), but there was a marked increase in the rate of beat at the higher temperatures so that the observed coefficient became 2.72. Thus the same result was obtained by longer exposure to ascorbic acid in the absence of external adrenaline as was shown almost immediately when adrenaline was added to the medium (Fig. 2).
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Even when a modification of the type $E$ curve was produced almost at once by ascorbic acid or liver extract in the presence of adrenaline it was possible to observe a continuing action over a longer period. In the experiments shown in Fig. 5 the control curve ($A$) with unmodified Ringer was a normal type $E$ which was unchanged after exposure to an extract of frog’s liver for $1\frac{1}{2}$ hr. (curve $B$). The addition of adrenaline ($1$ in $10^7$) to the medium, however, led to the appearance of a type $D$ curve (Fig. 5, $C$), this being the same picture as that previously described after treatment with anterior pituitary extract (Smith, 1951). This heart was perfused overnight and a further experiment made 25 hr. after isolation, but without adding any fresh adrenaline to the medium. The curve obtained is of the same type as that which was obtainable only in the presence of adrenaline on the previous day (Fig. 5, $D$). Adrenaline ($1$ in $10^7$) was now added to the medium and another determination made. It is apparent that the presence of external adrenaline now carried the development of the pulse rate curve a stage further, as the increase in frequency over the whole temperature range was now linear and the $Q_{10}$ value high ($2\cdot38$), that is, a type $C$ curve was produced. A very similar response to adrenaline after prolonged exposure to ascorbic acid is also shown in Fig. 3. As already mentioned, this heart was exceptional in that a type $A$ curve was produced after treatment with ascorbic acid for $2\frac{1}{2}$ hr. in the absence of added adrenaline. The temperature-pulse rate curve given by this heart 24 hr. after isolation and again in the absence of adrenaline is shown at Fig. 3, $C$. This may be regarded as rather more advanced than that obtained the previous day (Fig. 3, $B$), as the increased

![Figure 4](image_url)
acceleration appears a little earlier and the general form is type B rather than type A. On addition of adrenaline (1 in 2 \times 10^7) to the medium, however, the response was very similar to that shown in Fig. 5, as all the observed rates now lie close to a straight line with a \( Q_{10} \) of 3.04.

![Graph](image)

Fig. 5. Action of extract of frog's liver on type E heart, 3 frog, 12 February 1951. A, × — ×, 1\( \frac{1}{2} \) hr. after isolation, heart perfused with unmodified Ringer. B, ○—○, 4 hr. after isolation and 1\( \frac{1}{2} \) hr. after adding extract of 100 mg. frog's liver to the perfusate. C, □——□, 5\( \frac{1}{2} \) hr. after isolation, heart perfused with same medium plus adrenaline 1 in 10\(^7\). D, Δ——Δ, 25 hr. after isolation, perfused with same medium but no fresh adrenaline added. E, ▽——▽, 28 hr. after isolation, same perfusate containing adrenaline 1 in 10\(^7\).

(ii) *Action of adrenergic blocking agents*

All the preceding experiments have been directed towards the modification of type E curves by treatment with various agents, but it is obviously equally important to ascertain whether the process can be reversed by suitable treatment of types A or C hearts. In a previous paper (Smith, 1951) preliminary experiments made to investigate the action of an adrenergic blocking agent, ergotamine tartrate, on the temperature-pulse rate curve were mentioned. The results obtained were equivocal, but there was in some cases an indication that the curve was changed in the direction of the type E form. Nickerson & Nomaguchi (1950) have subsequently published an account of a detailed investigation into the action of adrenergic blocking agents on the frog's heart. They found that only in the winter could the adrenaline-induced cardio-acceleration be blocked by ergot alkaloids or \( \beta \)-haloalkylamines. In the summer, however, blockage could be produced if the heart
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were subjected to the combined action of one of the above agents and sodium fluoro- or iodo-acetate. In view of this the action of ergotoxine, combined with iodoacetic acid, on the temperature-pulse rate curve has been investigated. The record of such an experiment is shown in Fig. 6. This heart was isolated from a frog which had received five injections of sodium thyroxine (0.2 mg. each) during the preceding 10 days. The control curve obtained with the heart beating in Ringer containing ascorbic acid (5 mg./l.) and adrenaline (1 in 10⁷) was of the winter or A type (Q₁₀ 2.6). The frog was given thyroxine and ascorbic acid added to the perfusate to ensure optimal conditions for the appearance of the increased cardio-acceleration at higher temperatures. A perfusate containing ascorbic acid and adrenaline at the same concentration together with ergotoxine ethanesulphonate (1.5 mg./l.) and iodoacetic acid (2.0 mg./l.) was then substituted. The pH of the medium was adjusted to 7.6 immediately before the start of the experiment. It is apparent from Fig. 6, B, that the extra acceleration found at the higher temperatures in the first experiment has been largely inhibited, so that the curve is now linear up to 15°C, with a slight rise appearing at 17 and 19°C (observed Q₁₀ 2.09). At the end of the second experiment 0.02 ml. adrenaline (1:1000) was added directly to the cannula, and the inotropic and chronotropic response of the heart recorded. The maximum frequency attained is shown in Fig. 6, B, and it is evident that there was a definite, though small, response (+2.5 beats per min.). This slight increase in rate after added adrenaline does show, however, that the
chronotropic reaction to adrenaline was not completely blocked, and hence the slight departure from linearity of the second curve in the upper temperature range could have been due to a sympathomimetic action breaking through the blockade. Nickerson (1949) and Nickerson & Nomaguchi (1950) have shown that the ergot alkaloids block the chronotropic response to adrenaline competitively. If, as suggested below, there is increased synthesis of adrenergic material in the heart with rising temperature it is not unnatural to find some adrenergic stimulation breaking through in this way. When adrenaline was added to the cannula at the end of the second experiment (Fig. 6, B) there was a very pronounced increase in amplitude. This is in agreement with the work of Nickerson & Nomaguchi (1950) who found that, when the chronotropic response was completely blocked, the inotropic response to adrenaline was quite unimpaired.

Stefan (1928) investigated the action of ergotamine on the isolated frog’s heart. He found that while a commercial solution of ergotamine tartrate (1 in 10⁴) caused a significant reduction in the rate of beat, a solution of pure ergotamine tartrate of the same strength led to the appearance of a considerably higher pulse rate on warming than was found in any of the control hearts. Stefan stated that difficulty was experienced in dissolving the ergotamine in the second case. In one experiment I also observed a stimulatory action of ergotamine. This heart when perfused with Ringer containing ergotamine tartrate (1 in 10⁶) showed little change in rate at the lower temperatures, but above 11° C. there was very marked progressive increase in both pulse rate and amplitude compared with the initial control experiment. It is suggested that these results may be due to exposure of the heart to a stimulatory rather than an inhibitory concentration of ergotamine (Jang, 1941).

(iii) Temperature-amplitude curves

The fact that the increased frequency response at higher temperatures shown by experimental and normal hearts was very often accompanied by a noticeable increase in the amplitude of beat (Smith, 1951), led to an examination of a large number of kymograph records to see if there were any consistent differences in the amplitude changes shown by the various types of heart. It was found that, while there was no absolutely fixed form of the temperature-amplitude curve corresponding to each type of temperature-pulse rate curve, there was nevertheless a reasonably good conformation to a typical pattern in each case. Temperature-amplitude curves, which may be regarded as fairly typical, for different pulse rate curves are shown in Fig. 7. Curve A is representative of the amplitude changes shown by ‘summer’ hearts (type C), where there is usually a continuous increase over the whole range. There is, however, a distinct indication of an intermediate plateau at about 12° C. which is probably of some significance as it occurred regularly. Curve B is based on the record of a ‘winter’ heart (type A), and there is a typical initial decrease in amplitude from 7 to 9° C. Above this point there is a sharp increase in amplitude which parallels the extra acceleration in rate found in this part of the range. There is a distinct flattening of the amplitude curve at
17 and 19° C., and in some cases the amplitude recorded at 19° C. was less than that at 17° C. Curve C corresponds to a type E pulse rate curve which showed no falling off in rate at the higher temperatures. Here again there is a decrease in amplitude over the lower part of the range, and while there is some increase in the mid-region, it is very small compared with that shown by curves A and B. Curve D was obtained from measurements of a record from a heart which showed marked falling off in rate above 12° C. Here the amplitude of the beat falls progressively and fairly steadily over the whole range. It is apparent that this series of temperature-amplitude curves shows a continuous trend towards the reduction of the temperature coefficient of the mechanical response. These curves show the

Fig. 7. Temperature-amplitude curves obtained from experiments on isolated frogs' hearts which gave different types of temperature-pulse rate curves. A, ×—×, from an experiment which gave a linear, high Q_{10}, pulse rate curve (type C); ♀ frog, 18 December 1947. B, ⊙—⊙, from an experiment which gave an exponential pulse rate curve (type A); ♂ frog, 26 May 1950. C, □—□, from an experiment which gave a linear, low Q_{10}, pulse rate curve (type E); ♀ frog, 11 May 1951. D, △—△, from an experiment in which there was marked falling off in pulse rate acceleration at higher temperatures (cf. Fig. 3); ♀ frog, 17 February 1950.
following values for $Q_{10}$ (amplitude) over the range 7–17°C.; curve $A$ 1.90, curve $B$ 1.73, curve $C$ –1.03, and curve $D$ –1.40. It is generally considered that the amplitude of the mechanical response of the heart would be expected to decrease with increasing temperature owing to the added frictional losses at faster rates of contraction (Clark, Eggleton, Eggleton, Gaddie and Stewart, 1938). It would seem, therefore, that curve $D$ may be the simplest expression of the relation between amplitude and temperature, although even here there is probably a disproportionate decrease in the mechanical response at higher temperatures. As the reversal of the expected amplitude changes in the other types is also accompanied by an increased frequency response it seems logical to attribute both to the internal release or synthesis of sympathomimetic material.

**DISCUSSION**

In the previous paper (Smith, 1951) it was suggested that the isolated frog’s heart, beating in unmodified Ringer, might be under the influence of sympathomimetic substances of endogenous origin. It is considered that the experimental work described on the preceding pages provides confirmation for this hypothesis. The depression of the extra frequency response in the upper temperature range by a combination of adrenergic blocking agents and the correlation found between the temperature-pulse rate and temperature-amplitude curves strongly suggest that sympathomimetic activity is involved. Perhaps the clearest evidence for the synthesis rather than the release of such material is provided by the anomalous type $E$ hearts such as that shown in Fig. 3. In these hearts the frequency at 7°C. appears to be stable and, indeed, on raising the temperature the acceleration at first follows the expected course, but then a progressive and reversible falling away supervenes. Although this falling off is reversible by lowering the temperature again, there is a marked time lag before the original rate is regained. This is consistent with the conception that the original rate at 7°C. is partly due to stimulation of an adrenergic nature, but as the temperature is raised the active substance is progressively inactivated and so the initial acceleration is not maintained. On cooling the heart the rate of inactivation will decrease again, but the adrenergic stimulation cannot attain its former level until resynthesis of active compound has had time to take place. On the basis that in these hearts inactivation or destruction of adrenergic material is the dominant process at higher temperatures, it would be expected that addition of a substance such as ascorbic acid would at least partially protect the active substance. Numerous workers have shown that ascorbic acid has a marked stabilizing action on adrenaline, both *in vitro* and *in vivo* (Bacq, 1936; Welch, 1934; Clark & Raventlos 1939). In the experiment shown in Fig. 3, it might seem that the only process involved was this protective action. Thus, when the temperature is raised in the presence of ascorbic acid, not only is the active material present at the lower temperature stabilized as the temperature rises, but also the higher temperature promotes synthesis of more material, so that the heart shows an increased chronotropic response and an exponential type of curve is produced.
Further evidence that synthesis of active material is promoted at the higher temperatures, in addition to protection from inactivation being afforded, is provided by the behaviour of the heart on rapidly cooling in the presence of ascorbic acid. At about 9°C the rate was still appreciably higher than that found with a rising temperature. When the temperature was kept constant at this level for about 10 min. the rate gradually fell to the initial value and was then stable. This type of behaviour suggests that sympathomimetic material was present in greater amount at the higher temperatures, and that the excess slowly disappeared after rapid cooling, until the normal balance between production and inactivation for that particular temperature was attained.

The heart shown in Fig. 3 was, however, the only case where an immediate change-over to an exponential curve was produced by ascorbic acid in the absence of external adrenaline. All other hearts examined show more clearly that the action of ascorbic acid is twofold. For instance, it can be seen from Fig. 4 that a type E curve which showed slight falling off above 12°C. was first of all converted into a typical type E curve by ascorbic acid (Fig. 4, B). Not only did this curve not show any decrease in acceleration over the range examined, but also on rapidly cooling again the rate of beat agreed with that observed when the temperature was rising. This is the effect which would be expected if ascorbic acid were simply preventing adrenergic material from being inactivated. After the heart had been beating in the same medium for about 20 hr., however, a further change in the temperature-pulse rate curve was apparent. The acceleration between 7 and 9°C. was unchanged, but with continued rise in temperature the rate began to increase more rapidly, and the curve became exponential or type A. When the temperature was again rapidly reduced there was a delayed frequency change similar to that shown in Fig. 3. In cases such as this the delayed production of the exponential curve suggests an action of ascorbic acid or liver extract on the synthesis of endogenous adrenergic compounds. Beyer (1942) found that ascorbic acid promoted the synthesis of sympathomimetic amines in vitro, and suggested that it might bring about a step in the synthesis of adrenaline from its precursors. Bourne (1936), after considering the cytological distribution of ascorbic acid, also suggested that it might play a part in adrenaline synthesis.

Previously (Smith, 1951), it was suggested that the action of an extract of anterior pituitary gland on the type E temperature-pulse rate curve was due to the occurrence of synergism between an anterior pituitary principle and the exogenous adrenaline which appeared to be necessary for the production of the effect. It is obvious that this hypothesis requires modification in view of the later work. In the first place it is not a specific action of pituitary extract, as very similar effects are obtainable with either ascorbic acid or liver extract. It is not, however, suggested that the action is necessarily due to the same substance in each case. Ascorbic acid is widely distributed in animal tissues, the pituitary gland being one of the richest sources (King, 1938), and it was at first thought that the action of pituitary and liver extracts on the heart might be due to the presence of ascorbic acid. Samples of Antoxylin (Oxo Ltd.), which were proved to be active on type E hearts,
have been tested for ascorbic acid content by both the dichlorophenol-indophenol and dinitrophenylhydrazine methods (King, 1951). Both methods showed that there was no detectable ascorbic acid in the extract, so that its activity must depend on the presence of some other factor. It has already been pointed out that no change in the temperature coefficient of the basic line joining the rates at 7 and 9° C. was caused by extracts of anterior pituitary gland or liver, even when this coefficient was particularly low. When the control curve showed low values for this coefficient (1·80–2·00) addition of ascorbic acid generally led to its increase to about 2·20. This may perhaps be due to the fact that the protective action is a minor function in the case of the tissue extracts, the main action being on the synthesis of adrenergic material, whereas in the case of ascorbic acid both actions are prominent. In the earlier work (Smith, 1951), when type E hearts were treated with Young's pituitary extract in the presence of external adrenaline, the normal response was different from that obtained with ascorbic acid, in that, although an extra frequency response occurred at about 10° C., the pulse acceleration stabilized again on a higher line of low Q_{10} at about 15° C. (cf. Fig. 5 after liver extract). This could also be attributable to the fact that synthesis of adrenergic material was stimulated by the pituitary extract, but as inactivation was not inhibited a new equilibrium was established at a higher frequency level.

The dependence of the immediate appearance of an increased pulse rate at higher temperatures, after treatment with various agents, on the presence of external adrenaline in the medium still requires explanation. It is obvious that the time factor is involved, as external adrenaline is not required when the heart has been treated for longer periods. Usually the required concentration of adrenaline was obtained by adding 0·1 ml. of a commercial adrenaline hydrochloride solution to a litre of Ringer. This operation would also result in the addition of 0·05 mg. of sodium metabisulphite to the medium. The possibility therefore cannot be ignored that it was the stabilizer, rather than the adrenaline itself, which facilitated the more rapid appearance of an increased pulse rate in these experiments. Even if the commercial stabilizer should prove to be ineffective as regards promotion of the increased frequency response it is possible that the added adrenaline would afford some measure of protection to the endogenous material by competing for the inactivating enzymes. The behaviour of the hearts shown in Figs. 3 and 5 after the addition of external adrenaline is not inconsistent with the conception that extra protection of endogenous adrenergic material occurred. The heart shown in Fig. 5 gave a type D curve after 3 hr. in a medium containing liver extract and adrenaline. A curve of very similar form was obtained 20 hr. later without adding any fresh adrenaline, but when adrenaline was added at this stage the chronotropic response at higher temperatures was enhanced and a type C curve produced. The sequence of events shown in Fig. 3 after treatment with ascorbic acid is rather similar. Here again addition of external adrenaline 22 hr. after adding ascorbic acid caused the transformation of a type B into a type C curve. It has been shown previously that commercial adrenaline hydrochloride solution alone at this concentration did not change the form of the type E
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curve. It is therefore possible that the above changes, and the more rapid appearance of an increased pulse rate in a type E heart, may be due to increased stabilization of the endogenous material synthesized after treatment with ascorbic acid or liver extract. In experiments previously described (Smith, 1951), it was found that such adrenaline solutions could either lead to no change or an increase in the observed temperature coefficient of the isolated heart, and moreover an increase was usually only observed in those hearts where the natural coefficient was relatively low. Carter (1933), using solutions of pure adrenaline, did not find any alteration of the pulse rate curve. Again it seems possible that this discrepancy may be due to increased stability of endogenous material in my experiments. If the presence of a stabilizer in the adrenaline solution used in my original experiments was indeed a factor in the appearance of an increased pulse rate shortly after treatment with pituitary extract, then the use of such solutions was a fortunate chance, as otherwise this action of pituitary extract could easily have remained undetected.

When an analysis of the seasonal distribution of the various types of temperature-pulse rate curves in terms of thyroid activity was made (Smith, 1951), it was assumed that the occurrence of a type E curve indicated inactivity of the gland. This conclusion was based on the fact that at that time it had not been possible to obtain type C curves by treatment of such hearts with anterior pituitary extracts. It now appears, however, that type C curves can be produced, in the presence of external adrenaline, after longer exposure to ascorbic acid or liver extract. In order to clear up this point histological preparations have been made of thyroid glands from a considerable number of frogs which gave type E curves. Examination of such sections revealed marked signs of activity in some of the glands. In an inactive thyroid the epithelium is extremely flattened and the follicles large and distended with colloid, whereas in several of these 'type E' glands the epithelium was of a columnar type and the follicles were smaller and not turgid with colloid. It is apparent, therefore, that the type E curve cannot be regarded as a direct indication of the thyroid state, as the operative conditions for the appearance of this form seem to involve a defect in the synthesis of adrenergic material by the heart tissues, which is apparently not influenced by the thyroid hormone.

An interesting correlation has been found between the thyroid condition and the occurrence of a decrease in pulse rate acceleration at higher temperatures shown by some type E hearts. As stated previously (Smith, 1951), type E hearts were regularly obtained in January and February. It is now apparent that there is also a fairly high incidence of this form from September to November, but at this time it is only shown by hearts isolated from female frogs. These autumn, female, pulse rate curves are of the linear, low $Q_{10}$ type with no indication of decreasing acceleration in the upper temperature range, and the thyroid glands from these frogs showed marked histological signs of activity. In January and February, however, a considerable proportion of the type E curves did show falling off in frequency (Fig. 3, A), and all thyroids from such frogs had an obviously more inactive appearance. In those cases where there was no significant departure from linearity in the
January and February type E hearts the thyroid had a very similar appearance to that found in the females in the autumn. Further evidence that lack of thyroid hormone is not the primary factor leading to the appearance of a type E curve is provided by the failure of addition of thyroxine alone to the medium to change the form of these curves (Smith, 1951). Type C curves can be produced from type E, in the presence of external adrenaline, either by long exposure to ascorbic acid or by adding thyroxine to the medium in addition to anterior pituitary extract. This suggests that the action of thyroxine is also one which potentiates sympathomimetic activity, so that the type C relation is produced earlier in the presence of thyroxine than when the other agents alone are employed. Bacq (1936) has shown that, in vitro, thyroxine inhibits the destruction of adrenaline in solution. It seems very probable, therefore, that the action of thyroxine on the pulse rate curve is due to its protective action on sympathomimetic material in the heart. Obviously, if the increased synthesis of such material at higher temperatures is lacking, as it appears to be in the type E heart, then addition of thyroxine alone should not alter the form of the curve, except that, in those cases where the rate falls away with increasing temperature, there might be some amelioration of this condition. If the principal role of the thyroid hormone in this connexion is that of a stabilizer of sympathomimetic material in the heart, then the correlation between inability of the type E heart to maintain a constant acceleration over the whole temperature range and thyroid inactivity is not unexpected.

ANALYSIS OF THE OBSERVED TYPES OF TEMPERATURE-PULSE RATE CURVE

From the preceding discussion it is apparent that the normally observed relation between pulse rate and temperature is the result of the interaction of several complementary and antagonistic processes. It has been shown that rising temperature affects three more or less independent systems, all of which contribute to the observed frequency at a particular temperature. These three systems may be briefly defined as follows:

(1) Changing temperature has a direct action on the pacemaker mechanism of the heart such that the rate of beat increases with temperature. At the present time there is little direct information available as to the characteristics of this basic relation. It may, however, be tentatively assumed that the temperature-pulse rate curve obtained from hearts isolated from frogs after long-term hypophysectomy is an expression of this basic relation alone (Smith, 1951; and unpublished). These hearts show a linear relation between frequency and temperature over the range 7–21°C, with a mean value for the temperature coefficient \(Q_{10}\) of about 2.00. The rate of beat at 7°C is in general fairly constant at about 13 per min.

(2) It has been suggested that much of the experimental work on the heart leads to the conclusion that substances having sympathomimetic properties are synthesized by the heart tissues. Increase in temperature also accelerates this synthesis, so that the resulting chronotropic action is more marked at the upper end of the range. This adrenergic stimulation summates with the natural rate of
the heart so that not only is the mean rate at 7°C. higher (about 17 per min.), but the observed temperature coefficient is also increased. There is some evidence to suggest that changing temperature either acts at two points in the synthesis of active material, or that two independent mechanisms are involved. The temperature-amplitude curves for type C or 'summer' hearts usually show a definite plateau at about 12°C. (Fig. 7, A), which would seem to indicate that there is an initial potentiation of adrenergic stimulation which is checked at this point, to be followed by a further increase in activity as the temperature rises beyond 13°C. A similar resurgence of adrenergic activity is also shown by many of the temperature-amplitude curves for type A and normal type E hearts (Fig. 7, B and C).

(3) The third process, which is antagonistic in its action on the pulse rate to the two preceding, is the inactivation of sympathomimetic material. These reactions are also accelerated by a temperature increase, and in view of the relative constancy of the pulse rate at 7°C. in hearts of all types (Smith, 1951) it would seem that inactivation is depressed to a very low level at such a temperature. The extent to which the observed temperature-pulse rate curve is modified by these inactivation processes is dependent on several factors, which may act either by protecting the active material or by stimulating its synthesis. Anterior pituitary and liver extracts appear to owe most of their activity to their ability to promote synthesis, while thyroxine probably acts by virtue of its 'antioxygène' properties (Bacq, 1936). Ascorbic acid falls into a different category as it shows a dual action which can be attributed to adrenaline stabilization and promotion of synthesis.

Nickerson & Nomaguchi (1950) put forward a hypothesis to account for the difference in form of the summer and winter temperature-pulse rate curves. This was based on their suggestion that in the winter frog's heart there is only one pathway for the production of the energy substrate which is necessary for the appearance of the chronotropic response to adrenaline. In the summer heart a second labile pathway is also operative. In view of this they suggest that in the winter form the production of utilizable acetate may be the limiting factor for heat-induced cardioacceleration. The relation between temperature and pulse rate is therefore indirect instead of direct as it is in summer when the supply of energy substrate is not the limiting factor. The experimental results described here and in the earlier paper (Smith, 1951) do not, however, support this theory. There is no indication that energy substrate is the limiting factor at any temperature in the winter heart, as a chronotropic response to added adrenaline was always obtainable and the form of the temperature-pulse rate curve was not necessarily changed. It would also be difficult to account for the occurrence of the type E curve on the basis of substrate lack and for its conversion to the other types by the agents employed. Nickerson & Nomaguchi found that this utilizable acetate was a specific energy source for the chronotropic response, and a deficiency did not affect the inotropic response to adrenaline. In this paper, however, it has been shown that the various forms of temperature-pulse rate curve are accompanied by characteristic differences in the temperature-amplitude curve. This would be an unexpected correlation if the form of the temperature-pulse rate curve were dependent on the availability of utilizable
acetate. The conception that synthesis or release of sympathomimetic material leads to a chronotropic response which is superimposed on a basic relation between temperature and frequency is in better accord with the experimental results. The existence of the necessary enzyme systems for such synthesis in certain organs is indicated by the work of Beyer, Blaschko, Burn & Langemann (1950), Schmiterlöw (1951) and Blaschko (1942).

A general analysis of the temperature-pulse rate curve based on the available experimental data can be made in the following terms. In all normal isolated hearts the observed rate of beat at 7°C. is partly due to adrenergic stimulation. In the summer heart (type C) the active material is protected from inactivation by the presence of thyroid hormone and, in addition, rising temperature has a marked stimulatory action on its synthesis. In consequence, the typical linear relation with a high temperature coefficient is obtained. In the winter heart (type A) the level of circulating thyroid hormone is lower, and hence inactivation of the sympathomimetic substance is more rapid. Between 7 and about 12°C. increased destruction under the influence of rising temperature is the dominant process, so that the temperature coefficient over this part of the range is low. At higher temperatures, however, synthesis is increased to such an extent that a positive inotropic and chronotropic response occurs again, and the observed curve is either exponential or in the form of two intersecting straight lines. The curve tends to reach a maximum at about 19°C., which may possibly be due to inactivation again becoming the dominant feature at temperatures higher than this. The fact that this tendency towards a decreasing pulse rate above 19°C. can be obviated by adding thyroxine to the medium (Carter, 1933), also suggests that inactivation of adrenergic material is the underlying cause.

The seasonal type E curve, which is linear in form with a low temperature coefficient, would seem to be primarily due to defective synthesis of adrenergic material at higher temperatures. In addition, the effect of inactivation of active material which was present at the lower temperatures can be clearly seen in type E hearts which showed a marked falling off in rate above about 12°C. These type E hearts may be regarded as providing further indirect evidence for the existence of two independent routes for the synthesis of adrenergic material. It is apparent that synthesis can take place at low temperatures (Fig. 3, A), but even when this material is protected by ascorbic acid the increasing chronotropic action at higher temperatures does not necessarily appear at once (Fig. 4, B). Although increased synthesis at higher temperatures may not be entirely lacking as judged by the inotropic response (Fig. 7, C), it seems that the threshold for the chronotropic response is not attained. As several agents have been found to alter the form of the type E curve it is not possible to define the factors leading to the seasonal appearance of this type. Further work on the problem is required to ascertain whether it is due to an endocrine change or a nutritional deficiency.
SUMMARY

1. It was found that addition of ascorbic acid or an extract of frog's liver to the medium perfusing hearts showing a linear, low $Q_{10}$, temperature-pulse rate curve (type $E$) led to an increased frequency response at the higher temperatures. By such treatment curves of types $A$, $B$ or $D$ were obtained (Smith, 1951).

2. In nearly all cases it was necessary to add adrenaline (1 in $10^7$) to the perfusate to obtain an early response of this nature. In the absence of external adrenaline a similar change was observed after longer treatment with ascorbic acid or liver extract (up to 20 hr.). The possible action of adrenaline in this respect is discussed, and it is suggested that it may afford protection to sympathomimetic substances in the heart tissues.

3. The occurrence of a decreasing acceleration of pulse rate at higher temperatures in certain types of hearts was observed. This phenomenon was reversible on lowering the temperature again, but there was a marked time lag before equilibrium was re-established. When such hearts were treated with ascorbic acid or liver extract, and type $A$ or $B$ curves produced, the direction of this delayed pulse rate change was reversed. The significance of this behaviour in relation to the hypothesis that sympathomimetic substances are synthesized by the isolated heart is discussed.

4. It is suggested that the observed modifications of the type $E$ curve produced by treatment with anterior pituitary extract, liver extract, or ascorbic acid were due to their action in promoting synthesis of adrenergic material by the heart. In the case of ascorbic acid there was evidence for an additional protective action.

5. It was found that treatment of types $A$ or $B$ hearts with ergotoxine and iodoacetic acid caused a definite change of the temperature-pulse rate curve towards the type $E$ form.

6. Temperature-amplitude curves were constructed for numerous hearts of various types, and it was found that distinct forms occurred in correlation with the different types of temperature-pulse rate curves. It has been shown that the frequency and amplitude changes are related in such a way that they can both be attributed to production or inactivation of sympathomimetic substances.

7. The action of thyroxine in modifying the form of the temperature-pulse rate curve is attributed to its protective action on adrenergically active compounds.

8. An analysis of the various forms of temperature-pulse rate curve has been made on the basis of the action of temperature on three independent systems: (i) the pacemaker mechanism of the heart, (ii) the synthesis of sympathomimetic material, and (iii) the rate of inactivation of such material. In the summer heart (type $C$) synthesis is active and the material formed is protected by the relatively high level of circulating thyroid hormone. In the winter (type $A$) form, owing to the lower activity of the thyroid, the effects of inactivation lead to an exponential relation between pulse rate and frequency. Prior to the breeding season and in female frogs in the autumn there is apparently defective synthesis of adrenergic
material and the type E relation appears. In January and February this type is often further modified, owing to thyroid inactivity, so that a constant acceleration is not maintained over the whole temperature range.

REFERENCES


