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THE VASOCONSTRICTION CAUSED BY A PYROGEN*

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It is uncertain whether or not a circulating constrictor substance plays any part in the production of fever. The matter was discussed earlier by one of us (Grant, 1935). Since then little new evidence has been published, but that which has suggests that in both man (Perera, 1941) and the rabbit (Douglas, 1954) fever can occur without the release of a constrictor substance into the blood stream. However, during the last war we witnessed gross constriction develop in the vessels of the feet during a febrile transfusion reaction in a patient suffering from a week old crush fracture of the 4th to 6th thoracic vertebrae, with complete sensory and motor loss from the 4th segment downwards and incontinence of urine and faeces. More recently, we observed rabbits during the febrile reaction following 5 or 6 days after inoculation with neurotropic herpes virus by our colleagues Boyse, Morgan, Pearson & Wright (1956). The vessels of the previously denervated ear became grossly constricted. This observation prompted us to examine the problem afresh in the rabbit, using, among others, the methods recently devised by us for the detection and assay of constrictor substances in the blood (Armin & Grant, 1955, 1957). Briefly, we find that during the fever that follows the intravenous injection of an *Escherichia coli* vaccine, the circulating blood acquires constrictor activity which is due to the release of several substances into the blood stream.

METHODS

Rabbits with three-quarter-lop ears were used mainly. One ear was denervated at least a week beforehand by excising the superior cervical ganglion and portions of the great and posterior auricular nerves at the base of the ear.

In a few preliminary experiments unanaesthetized animals were observed in their cages. Their ears were inspected and the rectal temperature measured from time to time. Mainly, however, the rabbits were observed in a box (45 × 15 × 15 cm) open in front and on top. The sides and rear of the box were of wood and the base of sheet metal. The top of the box was covered with a movable rubber sheet. A loosely fitting rubber collar was placed on the rabbit's neck. The sides of this collar, prolonged for 15 cm and slotted near their free ends were attached by these slots to a stud

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on each side of the box. This method of restraint allowed sufficient freedom for the animal to change its posture. Usually the rabbit sat quietly with only occasional movements during the 2–3 hr required.

The box was placed on a table, the temperature of which could be regulated. During a control period of at least half an hour the table temperature was adjusted to maintain the rabbit sufficiently warm so that the vessels of the normal ear were relaxed and rectal temperature (measured by an indwelling thermal junction) remained steady or nearly so.

The ears, depilated by clipping with scissors, were joined by a strip of gauze and collodion across their anterior margins. They projected above, and were held upright by, the front edge of the rubber cover of the box. Ear temperature was measured by a copper-constantan thermal junction attached by surgical plaster to the skin (previously cleaned with ether) towards the tip of the ear and between the central artery and the anterior edge of the ear. The central artery and most of the blade of the ear were thus left open to examination by transmitted daylight. Liquid paraffin smeared on the skin rendered the vessels more clearly visible. A visible change in the calibre of the central artery is followed within a few seconds by a corresponding change in ear temperature. In most instances ear temperature was recorded every minute, but, to simplify, only 5 min temperatures are shown in the figures. Inspection of the ear is important, because differing changes in the calibre of the various classes of vessels provide an indication of the nature of the circulating constrictor substance affecting them (Armin & Grant, 1955).

Blood pressure and pulse rate were measured either by a capacitance manometer through a fine polythene catheter (internal diam. 0·28, external 0·61 mm, Clay Adams and Co., New York, no. P.E. 10) in the central artery of the normal ear or by a mercury manometer through a larger catheter (internal diam. 0·58, external 0·965 mm., Clay Adams and Co., no. P.E. 50) in a femoral artery. The fine catheter was inserted through a needle after a local injection of procaine. The femoral catheter was inserted while the animal was lightly anaesthetized with pentobarbital sodium (Nembutal, Abbott Laboratories); at least an hour was allowed to elapse for recovery from anaesthetic before proceeding with the experiment. The catheters were maintained patent by a slow infusion of sodium chloride solution 0·9% (w/v). For experiments under anaesthesia, we used the combination of phenobarbital sodium (Luminal, Bayer Products) and pentobarbital sodium previously described (Armin & Grant, 1955).

Repeated 0·1 ml. blood samples for blood-sugar estimations (micromethod of King & Wootton, 1956) were drawn from a three-way Perspex tap inserted into the length of the fine catheter in the ear artery of the unanaesthetized animal. Before the sample was taken the catheter was cleared of saline by drawing off a volume of about 0·1 ml. saline followed by blood. The total blood loss for each sample was under 0·3 ml. so that many samples could be taken at 5 min intervals without materially affecting blood volume. For larger blood samples and for transferring small blood samples to a test ear the catheter was inserted into the marginal or central vein of the ear (Armin & Grant, 1955). Plasma ultrafiltrates were prepared by the method of Armin & Grant (1957).

The pyrogen was a vaccine prepared from a strain of *Escherichia coli* (N.C.T.C. 6064). Stock cultures were subcultured into Hartley digest broth from which infusion agar slopes were inoculated. These were incubated during the night, and the organisms washed off with normal saline made with glass-distilled water, centrifuged, re-suspended in saline, centrifuged again and finally re-suspended in saline. This suspension was then heated at 60° C for 1 hr. By the use of Brown's opacity tubes (Burroughs Wellcome and Co., Beckenham, Kent) it was estimated that the suspension contained 3×10^9 organisms/ml. It was then distributed in 1 ml. ampoules; the ampoules were sealed and heated at 60° C for 1 hr. Sample ampoules were tested for sterility. The vaccine dose ranged from about 0·005 to 0·3 ml./kg, but was usually 0·1 ml./kg. The dose was diluted to 0·5 or 1 ml. with saline and injected usually into the marginal vein of the denervated ear.

RESULTS

Unanaesthetized rabbits

The preliminary observations showed that the intravenous injections of the vaccine regularly provoke a febrile reaction. We could detect no significant difference between the reaction displayed by animals unrestrained except by their cages and that displayed by those restrained by collar and box.

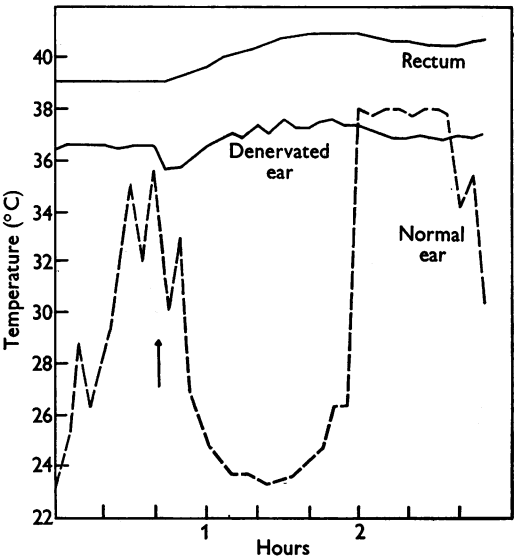

Fig. 1. Rabbit, 2·5 kg, unanaesthetized. Chart of rectal and ear temperature. Room temperature 20·2–20·8° C. At arrow, *E. coli* 0·01 ml./kg injected.

The general features of the reaction

These are as follows and are illustrated by Figs. 1–3. Usually the injection into the denervated ear does not disturb the rabbit; exceptionally it provokes transient restlessness. Quickly after the injection the vessels of the normal ear contract strongly and the ear temperature falls steeply; any oscillations in calibre present before injection cease. Usually the vessels of the denervated ear also quickly contract, but only slightly and transiently. Rectal temperature soon begins to rise and reaches its height, usually about 2° C above initial level, by about 1 hr after the injection. Thereafter it fluctuates slowly before falling to normal levels after several hours. The rise of temperature is associated with shivering, with a slowed or unchanged respiratory rate and with an increased pulse rate (Fig. 2). Blood pressure usually shows no definite change; it may rise slightly and temporarily or, exceptionally, fall slightly immediately after the injection. As rectal temperature approaches its maximum height, the vessels of the normal ear relax and thereafter usually

remain more or less dilated, but sometimes they show considerable fluctuations in calibre. At this time also the vessels of the denervated ear again contract slowly and progressively and ear temperature falls steadily over about half an hour. Blood pressure usually shows no change; it may fall slightly. The constriction of the denervated vessels persists until rectal temperature begins to fall.

Though individual rabbits display variations, the severity and duration of the febrile reaction is in general related to the dose injected. With doses of up to 0·1 ml./kg the rabbit is not obviously disturbed. During the second hour

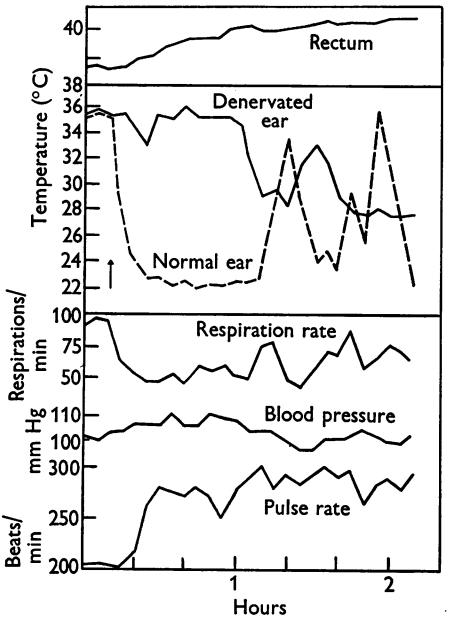

Fig. 2. Rabbit, 2·5 kg; unanaesthetized but given 1·0 ml. Nembutal 2 hr previously for insertion of catheter into femoral artery. Chart of rectal and ear temperatures, and of respiration rate, blood pressure and pulse rate. Room temperature 20·1–20·4° C. At arrow, *E. coli* 0·024 ml./kg injected.

an occasional rabbit may become a little restless or, on the other hand, unusually quiet and may droop the head on the forepaws for a time. The reaction is over by about 6 hr after the injection. With a dose of 0·2 ml./kg or more the animal becomes dyspnoeic and prostrated, lying on its side and passing urine and copious loose faeces. Blood pressure at this time may fall considerably. The animal may not be fully recovered the following morning. An occasional animal has died during the night following the injection. In most instances, however, we did not follow the reaction in detail beyond the first 2 hr after injection, since within this time the changes in the denervated ear vessels in which we were particularly interested had already taken place.

Denervated ear

From this general account it will be seen that the vessels of the denervated ear respond differently from those of the normal ear. They show two phases of constriction, which are not due to a fall of blood pressure, (i) an early constriction developing quickly after the injection and (ii) a delayed constriction developing about 50 min after the injection. Each phase requires separate description.

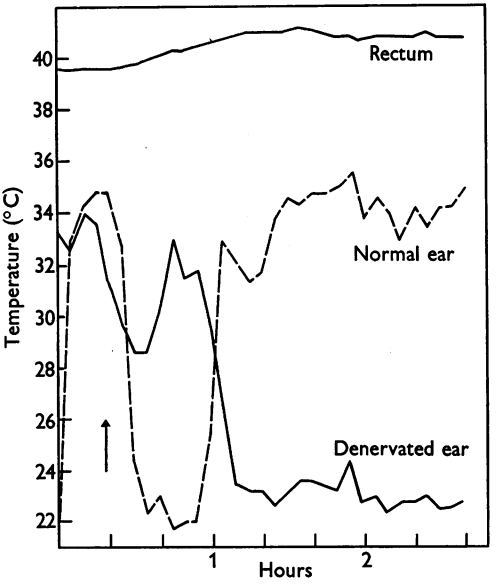

Fig. 3. Rabbit, 2·5 kg, unanaesthetized. Chart of rectal and ear temperatures. Room temperature 21–21·5° C. At arrow, *E. coli* 0·5 ml./kg injected.

The early constriction rarely fails. Though it may follow almost immediately after injection it usually begins after a delay of a minute or two; occasionally the delay is as long as 5 min. In general, the larger the dose the more promptly the constriction begins and the greater is its degree. Figs. 1–3 illustrate the temperature changes associated with this constriction and its subsequent relaxation in three animals with widely differing dosage. Fig. 3 shows that with a large dose the early constriction may not have entirely passed off before the delayed constriction supervenes. But even with the usual dose of 0·1 ml./kg the degree and duration of the early constriction vary considerably. Usually it is small and transient and accompanied by temperature fall like those of Figs. 1 and 2, and only exceptionally is great enough to cause a fall like that in Fig. 3.

The constriction affects mainly the central artery and its chief branches. There is little or no apparent change in the network of smaller visible vessels

and the ground tone of the ear does not pale as would be expected if the constriction were due to a circulating adrenaline-like substance. Rather, on several occasions when the arterial constriction has been greater than usual we have detected a slight flushing of the ground tone. Arterial constriction without change in the minute vessels suggests the action of a substance like 5-hydroxytryptamine; arterial constriction with minute vessel dilatation suggests a histamine-like substance.

The delayed constriction occurs regularly, except after small doses of the vaccine (see Fig. 1). It begins ½–1 hr after the injection, commonly at about 50 min, at the time when rectal temperature is nearing its height and the vessels of the normal ear are relaxing. The constriction is progressive and ear temperature falls steadily to remain a few degrees above that of the room. Exceptionally, as in Fig. 2, it may be interrupted by a temporary relaxation. Usually it persists until defervescence begins, several to many hours later, depending upon the dose injected. With the usual dose of 0·1 ml./kg the vessels are relaxing 4–6 hr after the injection.

In contrast to what is seen in the early phase, during the late phase the constriction of the central artery (to a third or a quarter of its initial diameter) and its main branches is associated with reduction in the calibre of the network of smaller visible vessels and with a definite paling of the ground tone of the ear. These vascular changes suggest the action of an adrenaline-like substance.

Hyperglycaemia

We can confirm the earlier observations that the vaccine injection causes hyperglycaemia. The rise of blood sugar, however, does not occur till after the beginning of the delayed constriction; no rise accompanies the early constriction even when this is greater than usual (cf. Fig. 8). Like the vascular changes in the denervated ear, this hyperglycaemia also suggests the release of an adrenaline-like substance during the delayed constriction.

Cooling

So far we have been dealing with rabbits warmed during an initial control period to maintain rectal temperature constant or nearly so. The injection of the vaccine is followed by a considerable rise of rectal temperature. This pyrexia, however, is not necessary for the development of constriction in the denervated ear. Thus, if heating is withdrawn during the control period, rectal temperature slowly falls. The vaccine injection checks the fall and then rectal temperature rises slowly. But in spite of the absence of the usual pyrexia the usual vascular reaction takes place in the denervated ear vessels. Fig. 4 illustrates this; in this example, when the delayed constriction begins at about 45 min, rectal temperature is only about 0·2° C above that when the injection is given.

Second dose

The above description applies to rabbits which have not previously received an injection of the vaccine. The injection of a second dose after an interval of at least a week provokes an unusually strong early constriction leading to a temperature fall of 5–10° C. The constriction is associated with a definite flushing of the ground tone and sometimes also by a conspicuous segmental oscillation in diameter of the central artery, suggesting the effect of a histamine-like substance. On the other hand, the delayed constriction is not exaggerated when a second dose is given. Rather its onset is longer delayed, it develops more slowly and is less in degree; paling of the ear is less distinct.

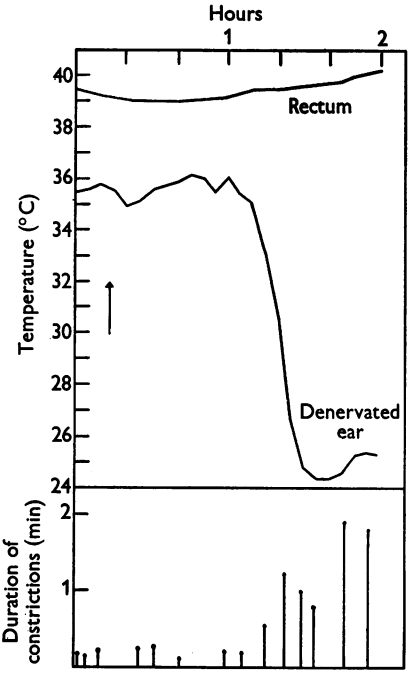

Fig. 4. Rabbit, 2·9 kg; unanaesthetized; heating withdrawn. Catheter passed to near heart from marginal ear vein and connected to the test ear of another rabbit. Chart of rectal and denervated ear temperatures and of durations of constrictions provoked by blood transfers from donor to test ear. Room temperature 22° C. At arrow, *E. coli* 0·03 ml./kg injected.

Condition of vaccine

The reaction of the denervated ear vessels is also modified by ageing of the vaccine. These observations have been made at intervals since the end of 1954. During this period we have used four batches of vaccine from the same stock culture of *E. coli*. This was because after periods of about 9 months the vaccine,

though still provoking the pyrexia and the early constriction, caused only a slight or no delayed constriction. On each occasion the injection of freshly prepared vaccine gave the expected delayed constriction.

Anaesthetized rabbits

Although the two phases of constriction occur in the denervated ear while the unanaesthetized animal remains quiet and, so far as we can tell by its appearance and behaviour, unalarmed, yet it is possible that emotional and muscular activity might be at least in part responsible for the constriction. We have therefore repeated these experiments on anaesthetized animals.

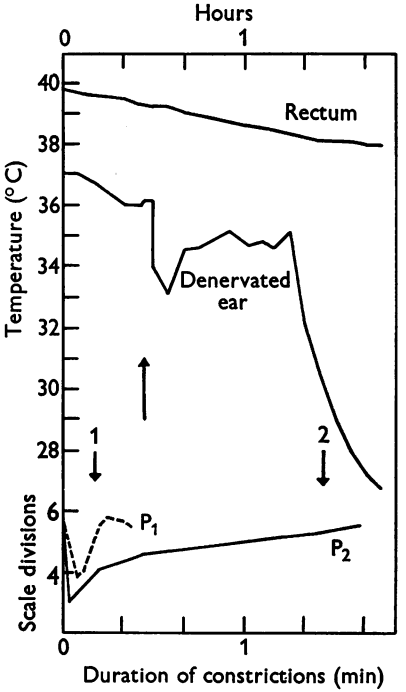

Fig. 5. Rabbit, 2·3 kg, anaesthetized; catheter in femoral artery; heating withdrawn. Chart of rectal and denervated ear temperatures. Room temperature 24–25° C. At upward pointing arrow, *E. coli* 0·1 ml/kg injected. At downward pointing arrows 1 and 2 blood samples drawn from the femoral artery. Beneath are charted details of the constriction of the artery of a test ear by the plasmas, P 1 and P 2, separated from the blood samples. The constriction due to P 2 was closely matched by adrenaline 1 μg/l.

The anaesthetized rabbit requires more warming to maintain its rectal temperature nearly constant than does the unanaesthetized and the rise of rectal temperature following the injection of the vaccine is slower and less in degree. Nevertheless, both the early and late constriction of the denervated ear develop just as they do in the unanaesthetized animal. Moreover, even if heating is withdrawn and the rectal temperature continues to fall after the

injection of the vaccine, the expected reaction of the denervated vessels takes place. For example, in Fig. 5 when the delayed constriction occurs at about 50 min the rectal temperature is a degree lower than at the time of the injection.

Adrenalectomy

After extirpation of the adrenal glands the early constriction develops as usual in the unanaesthetized or anaesthetized rabbit in response to either a first (Figs. 6 and 9) or second dose of the vaccine (Figs. 7 and 10). On the other

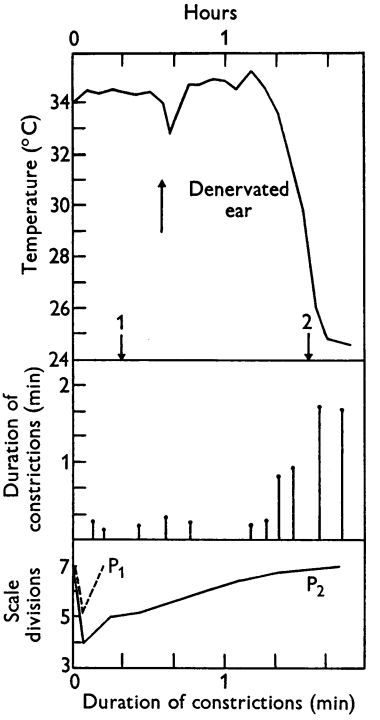

Fig. 6. Rabbit, 1·7 kg; bilateral adrenalectomy 4 days previously; anaesthetized; catheter in femoral artery and connected to test ear of another rabbit. At the upward pointing arrow, *E. coli* 0·1 ml./kg injected. Chart of denervated ear temperature, of the durations of constrictions provoked by blood samples transferred from the donor to the test ear and details of the constrictions caused by the plasmas P 1 and P 2 separated from the blood samples taken at the time of the downward-pointing arrows 1 and 2. Room temperature 24–25° C.

hand, the delayed constriction usually develops more slowly and to a less degree (e.g. Fig. 9), though sometimes, as Fig. 6 shows, the temperature response of the denervated ear may differ but little from that in the animal with intact adrenal glands. However, the arterial constriction of the delayed phase is not accompanied, as it is in rabbits with intact adrenals, by early and distinct paling of the ground tone, and the calibre of the smaller visible vessels does not diminish to the same extent. As the constriction of the central artery

increases, the ground tone of the ear may become a little paler than it was at the start, but the appearance of the ear is different from that of a rabbit given an intravenous infusion of adrenaline and resembles that caused by an infusion of 5-hydroxytryptamine. The rise in blood sugar that normally accompanies the delayed constriction is abolished by adrenalectomy as is shown in Fig. 9. We find that the intravenous infusion of 5-hydroxytryptamine, in a dose (e.g. 90 μg/kg/min for 30 min) sufficient to cause a similar arterial constriction and fall of ear temperature, does not cause hyperglycaemia.

Effect of blood and plasma on a test ear

By the repeated transfer of 0·1 ml. samples of blood from the donor animal to a test ear, the development of the two constrictor phases can be followed in detail during the course of the febrile reaction. An approximate assay of the blood constrictor activity can be made in terms of a known standard. A more accurate assay of the blood constrictor activity at any particular time can be made by withdrawing larger blood samples, separating the plasma and injecting it into a test ear (Armin & Grant, 1955, 1957).

Before the vaccine injection, blood and plasma exert only the slight and transient constrictor effect expected from unanaesthetized resting or anaesthetized animals, the adrenaline equivalent being $<0{\cdot}01$ μg/l.

Early constriction. During the early phase of constriction, blood and plasma from a rabbit with or without adrenal glands exert an increased constrictor effect on the test artery which is the greater, the stronger the constriction shown by the donor's denervated ear. In the usual slight constriction of the early phase (Figs. 4 and 6) the increase is small, hardly more than can be accounted for by variations in the response of the test ear. The equivalent activities are those of approximately adrenaline 0·01 μg/l. or 5-hydroxytryptamine 0·1 μg/l. The increase subsides as the constriction in the donor's ear passes off. With the strong constriction that occurs in response exceptionally to a first dose of the vaccine and always to a later repeated dose, the constrictor equivalent may rise to about adrenaline 1 μg/l. or 5-hydroxytryptamine 10 μg/l. or even higher for a brief period immediately after the injection; the activity may not subside before the late constriction supervenes. In Fig. 7 there is no separation between early and late phases.

Delayed constriction. With the onset of the delayed constriction in the donor, blood and plasma exert an increasing constrictor effect on the test ear which soon comes to a height. Figs. 4 and 6 illustrate these points. In animals with intact adrenals the adrenaline equivalent of the activity is about 1 μg/l. or a little greater. In animals lacking adrenal glands the adrenaline equivalent is sometimes the same but is usually less, nearer 0·1 μg/l. The corresponding 5-hydroxytryptamine equivalents are approximately five to ten times greater than those of adrenaline.

Other observations

The constrictor substances present in the plasma during both phases pass through a filter impermeable to proteins. No difference can be detected by the test ear between the activity of plasma and that of protein-free plasma ultrafiltrates.

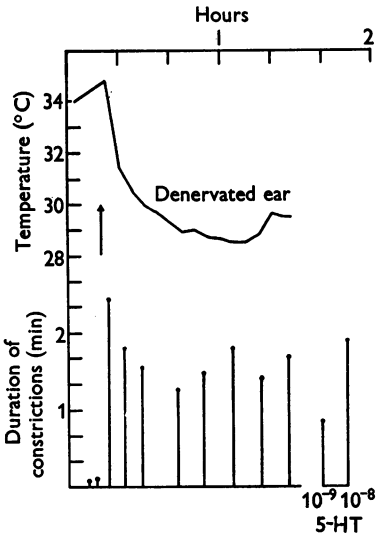

Fig. 7

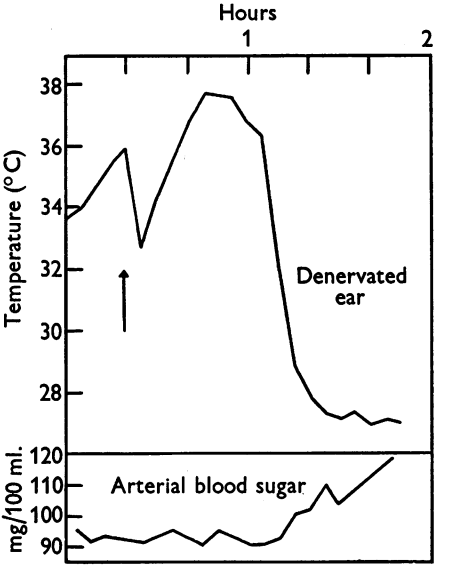

Fig. 8

Fig. 7. Rabbits, 1·8 kg; bilateral adrenalectomy 14 days previously; *E. coli* 0·1 ml./kg 10 days previously; anaesthetized, catheter in femoral artery and connected to the test ear of another rabbit. At arrow, *E. coli* 0·1 ml./kg injected. Room temperature 22·7–24·5° C. Chart shows denervated ear temperature and durations of constrictions provoked in the test ear by blood samples transferred from the donor. For comparison shown on the right are the durations of the constrictions provoked in the test ear by 5-hydroxytryptamine 1 μg/l. and 10 μg/l.

Fig. 8. Rabbit, 3·6 kg; unanaesthetized; fasting 17 hr; catheter in normal ear central artery. Room temperature 23·5–24·3° C. At arrow, 0·1 ml. *E. coli* injected. Chart of the denervated ear temperature and of arterial blood sugar. At the start of the observation the ear vessels, partly constricted by previous activity of the rabbit, gradually relaxed as shown by the initially rising ear temperature.

In rabbits with and without adrenal glands, the plasma of blood drawn as the delayed constriction comes to its height is usually slightly but definitely browner in colour than is the plasma of blood drawn before the vaccine injection. This colour difference is removed by ultrafiltration.

Though by the denervated test ear we can detect these substances in low concentration and assay them in terms of a known standard, we cannot, so far, identify them. The presence of more than one substance in the blood and the relatively low concentrations attained render difficult their separate

identification and assay by the other methods available. Nevertheless, an observation on the effect of plasma extracts on the rat's uterus shows that the substance released during a strong delayed constriction in the absence of the adrenal glands is very probably 5-hydroxytryptamine or a closely related substance. Thus, *E. coli* vaccine, 0·1 ml./kg, was injected into an anaesthetized rabbit whose adrenal glands had been excised 6 days previously. Rectal temperature was maintained about 39° C and room temperature between 20 and

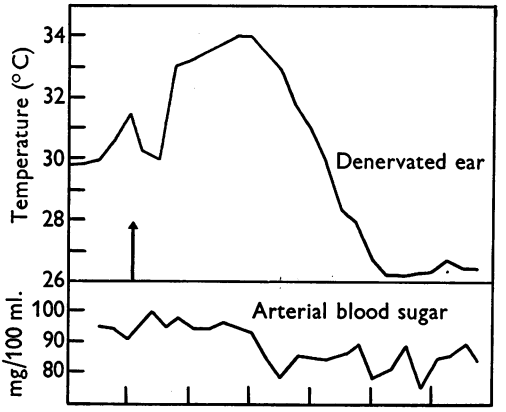

Fig. 9. Rabbit, 1·9 kg; bilateral adrenalectomy 4 days previously; fasting 17 hr; unanaesthetized; catheter in normal ear artery. Room temperature 21·3–22·7° C. Chart of denervated ear temperature and arterial blood sugar. At arrow, *E. coli* 0·1 ml./kg injected. At the start, the ear vessels as in Fig. 8.

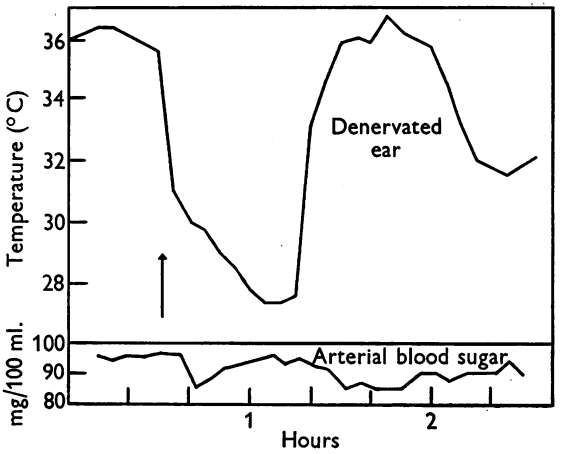

Fig. 10. Rabbit, 3·3 kg; bilateral adrenalectomy 4 days previously; fasting 17 hr; unanaesthetized; catheter in normal ear central artery. Room temperature 22·5–23·5° C. *E. coli* 0·1 ml./kg 7 days previously and again at arrow. Chart of denervated ear temperature and of arterial blood sugar. During the strong constriction after the injection, the denervated central artery showed segmental oscillations in calibre.

22° C. The denervated ear vessels were well relaxed. The vaccine injection was quickly followed by a slight constriction of the denervated ear vessels (ear temperature falling from 37° to 36° C) which passed off in about 10 min. At 47 min after the injection, the denervated ear vessels again began to contract; constriction gradually increased and ear temperature fell steadily from 37° to 24° C over a period of 70 min. As usual, the constriction affected mainly the arteries and the appearance of the ear was like that due to the effect of 5-hydroxytryptamine and not of adrenaline. A blood sample was drawn 10 min before the vaccine injection when the denervated ear vessels were well relaxed and ear temperature only about 2° C below that of the rectum. Another sample was drawn at 95 min after the injection when arterial constriction was pronounced and ear temperature had fallen to about 28° C. From past experience we judged that the constrictor activity of the circulating blood would be about 5-hydroxytryptamine 10 μg/l., and therefore probably assayable on the superfused rat's uterus. Both blood samples after 5 min centrifuging at 3000 rev/min showed a well-marked buffy layer separating the cloudy plasma from the red cells. Further centrifuging for 30 min yielded clear plasmas; that from blood drawn during the delayed constriction was definitely darker in colour than the control plasma. Acetone extracts were made from both plasmas according to the technique of Hardisty & Stacey (1955) and the volumes of the residues were made up to the original plasma volumes by the addition of double-glass-distilled water. Tested on the superfused rat's uterus (Gaddum, 1953) constrictor phase plasma extract caused contractions greater than those of 5-hydroxytryptamine 10 μg/l., less than those of 50 μg/l., and closely matched by those of 20 μg/l. The extract of control plasma contained no measurable amount of 5-hydroxytryptamine, i.e. less than 10 μg/l. The addition of lysergic acid diethylamide to the superfusing fluid to give a concentration of 50 μg/l. abolished the contractions to the plasma extract and to 5-hydroxytryptamine, but did not affect those due to oxytocin.

DISCUSSION

From our general description it will be apparent that the febrile reaction following the intravenous injection of an *E. coli* vaccine into rabbits under our conditions is much the same as that described by other authors, e.g. Grant (1949) who used a typhoid-paratyphoid vaccine and Douglas (1954) who used a pyrogen extracted from *Bacillus proteus*.

So far as we can find, only three previous publications deal with the changes in ear vessels deprived either of their sympathetic nerves or of both sensory and sympathetic nerves. Both types of denervation render the ear vessels abnormally sensitive to constrictor substances (Armin, Grant, Thompson & Tickner, 1953). Douglas (1954), whose figures, like ours, show prompt con-

striction in the normal ear vessels after the pyrogen injection and subsequent relaxation of the vessels about the end of the first hour, found no evidence of constriction in the ear deprived of its sympathetic nerves. Boquet, Delauny, Lehoult & Lebrun (1947), on the other hand, find that there is constriction in the vessels when the superior cervical ganglion is excised. But these latter observations may be left aside since, as Douglas also points out, excision of the superior ganglion alone leaves the ear vessels still supplied with sympathetic fibres passing by way of the vertebral artery from the stellate ganglion. Pinkstone (1935) used a typhoid-paratyphoid vaccine and 'sympathectomized' the rabbit's ear either by removing the stellate ganglion alone or by removing the superior cervical ganglion and severing the auricular nerves. He noted a fall of temperature in the sympathectomized ear in five of nine rabbits. In two of the experiments the temperature of the sympathectomized ear followed closely that of the normal ear after the injection of the vaccine. In three instances there was a distinct decrease in the temperature of the sympathectomized ear, but the decrease came on relatively late in the febrile response. In none of five animals with 'inactivated' adrenals was there an immediate constriction of the sympathectomized vessels after the injection. A delayed constriction occurred in three. On the basis of these observations he suggests that: (1) adrenal stimulation is responsible for the vasoconstriction which occurs promptly after the injection, and (2) the delayed constriction is not the result of liberation of adrenaline into the blood stream but perhaps of some other humoral substance liberated late in the febrile reaction, possibly from the pituitary.

Our results differ from those of Douglas (1954) and Pinkstone (1935) and provide strong evidence for the regular occurrence of two phases of constriction in denervated ear vessels, one early and the other delayed. Both are independent of blood-pressure changes and of the rise of rectal temperature; neither is altered by anaesthetization with pentobarbital sodium and phenobarbital sodium. Both are due to the appearance of constrictor substances in the circulating blood. These substances are filterable through a membrane impermeable to protein.

The nature of these substances is uncertain. However, judging by the appearance of the denervated ear, the level of blood sugar and the effects of adrenalectomy, it seems highly probable that adrenaline is released from the adrenal glands, not as Pinkstone (1935) suggested during the early, but only during the delayed phase. Zwecker & Goodell (1925) also found a rise of blood sugar following the injection of an *E. coli* vaccine into the rabbit, and their data show that this rise is delayed till about the end of the first hour, that is, about the time of our delayed constriction. Later, Evans & Zwecker (1927) showed that this hyperglycaemia is prevented by removing one adrenal and denervating the other. It is clear that adrenaline is not the only substance

released during the delayed phase and that the other substance, not derived from the adrenal glands, does not affect the blood-sugar level or the denervated ear vessels as does adrenaline. It therefore could be 5-hydroxytryptamine. The more specific test of acetone extraction of the plasma and the effect on the rat's uterus show that the substance is very probably 5-hydroxytryptamine or closely related to it. The substance (or substances) responsible for the early constriction is not adrenaline-like. The effects at this time are more consistent with the release of 5-hydroxytryptamine and possibly histamine as well.

We have no evidence to indicate the source of the substances resembling 5-hydroxytryptamine and histamine or the mechanisms by which these and the adrenaline-like one are released. It seems possible, however, that the early phase is a reaction to the injection of foreign protein, like that evoked in the rabbit ear by human plasma (Armin & Grant, 1957) and that the exaggeration of the early phase by a second injection after an interval is the expression of hypersensitivity to the protein induced by the first injection or, in other words, an anaphylactic reaction. If this is so, then a release of 5-hydroxytryptamine and histamine during the early phase is perhaps not unexpected. Humphrey & Jacques (1955) find that *in vitro* both 5-hydroxytryptamine and histamine are released from rabbit platelets by antigen–antibody reactions. Dr S. Udenfriend informs us (personal communication) that he has evidence for the release of both substances into the circulating blood during the anaphylactic reaction in rabbits sensitized to various antigens. It seems likely also that the late phase is due to some factor in the vaccine other than that responsible for the pyrexia or that for the early phase. It has been seen that pyrexia may occur without constriction in the denervated ear and that either constrictor phase may occur without the other.

We do not know the meaning of the darker coloration of the plasma during the delayed constrictor phase. A similar colour change was noted in after-struggle plasma (Armin & Grant, 1955), but that coloration was apparently prevented by adrenalectomy. We have not pursued the matter further.

Our observations also provide possible explanations for the conflicting results of Douglas (1954) and Pinkstone (1935). Douglas used a pyrogen extracted from *B. proteus*. Dr A. A. Miles, who provided the pyrogen, informs us that the method of preparation (acetone extraction and tryptic digestion) would remove the greater part, if not all, of the protein. According to our view, this would remove the factor responsible for the early phase. It might also remove the factor responsible for the delayed phase, since from Zwecker & Goodell's (1925) finding of hyperglycaemia after the injection of a *B. proteus* vaccine, it is very probable that adrenaline at least is released into the circulation.

Several factors may account for Pinkstone's (1935) inconstant results. Thus his doses were smaller than ours and the age of his vaccine is not stated. Again,

it may be that the typhoid-paratyphoid organisms are less effective in provoking release of constrictor substances than is *E. coli* and it may have been that his rabbits were less sensitive than ours. Lastly, Pinkstone (1935) seems to have relied on temperature changes for evidence of vasoconstriction without inspection of the ears. His rabbits were enclosed in a box open only at the top; thus the ears may not have been sufficiently exposed to room air to render temperature changes a good index of vasoconstriction. This is suggested by the relatively small falls of normal ear temperature shown in his figures compared with those of Douglas and ourselves. Constriction in the denervated ear might have been undetected.

It seems, therefore, that whether or not constrictor substances are released into the blood stream during fever depends on the constitution of the pyrogen and that another factor affecting release is the sensitivity of the animal to the pyrogen. The various elements of the pyrogen responsible for the release are unknown, but the protein fraction seems possibly responsible for the early phase of constriction.

What part the released substances play in the fever, or in the illness associated with it is not clear. Since one of the actions of both adrenaline and 5-hydroxytryptamine is vasoconstriction, it seems likely that, by intensifying the constriction brought about through sympathetic nerve stimulation, they will tend to heighten the fever. But this effect is probably small since the substances are present in relatively low concentration and normally innervated vessels are much less sensitive to their action than are denervated vessels. But they have other actions as well and in this connexion the difference in body distribution between adrenaline and 5-hydroxytryptamine should be remembered. Adrenaline is located in the adrenal glands and on release its effects are produced by its reaching the various tissues through the blood stream. 5-Hydroxytryptamine, on the other hand, is widely distributed in the body and large amounts are present for example in the brain, the gut and the platelets. Moreover, since it is known that certain toxins, or pyrogens, have affinities for certain tissues, 5-hydroxytryptamine may be preferentially released from one site rather than another, e.g. from the brain in a neurotropic virus infection, from the gut in an enteric infection. So that one substance, e.g. 5-hydroxytryptamine, might produce a variety of local symptoms depending on the site from which it is released and before it enters the circulation to reach distant tissues.

SUMMARY

1. Intravenous injection of a heat-killed suspension of *E. coli* into a rabbit provokes a febrile reaction which is accompanied by two phases of constriction in the vessels of the denervated ear, one early and the other delayed.

2. Both phases, which are independent of the rise of body temperature, are

due to the appearance of filtrable substances in the circulating blood. The substance in the early phase resembles 5-hydroxytryptamine; those in the delayed phase resemble adrenaline and 5-hydroxytryptamine.

3. The phases are modified when the pyrogen is injected again after an interval. The early phase is exaggerated and is due to the release of substances resembling 5-hydroxytryptamine and possibly histamine. The delayed phase is diminished.

4. The adrenaline-like substance is derived from the adrenal glands; the source of the others is unknown.

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REFERENCES

ARMIN, J. & GRANT, R. T. (1955). Vasoconstrictor activity in the rabbit's blood and plasma. *J. Physiol.* **128**, 511–540.

ARMIN, J. & GRANT, R. T. (1957). Further observations on vasoconstrictor substances in blood of the rabbit and man. *J. Physiol.* **138**, 401–406.

ARMIN, J., GRANT, R. T., THOMPSON, R. H. S. & TICKNER, A. (1953). An explanation for the heightened vascular reactivity of the denervated rabbit's ear. *J. Physiol.* **121**, 603–622.

BOQUET, P., DELAUNY, A., LEHOULT, Y. & LEBRUN, J. (1947). Observations directes de réactions vasculaires cutanées chez le lapin soumis à l'épreuve d'une endotoxin typhique. *C.R. Acad. Sci., Paris*, **225**, 1193–1194.

BOYSE, E. A., MORGAN, R. S., PEARSON, J. B. & WRIGHT, G. P. (1956). The spread of a neurotropic strain of herpes virus in the cerebrospinal axis of rabbits. *Brit. J. exp. Path.* **37**, 333–342.

DOUGLAS, W. W. (1954). The role of local sympathetic innervation in pyrogen-induced vasoconstriction in the rabbit ear. *J. Physiol.* **126**, 319–328.

EVANS, C. L. & ZWECKER, I. T. (1927). On the nature of the hyperglycaemic response to injections of certain killed bacteria. *Brit. J. exp. Path.* **8**, 280–288.

GADDUM, J. H. (1953). The technique of superfusion. *Brit. J. Pharmacol.* **8**, 321–326.

GRANT, R. T. (1935). Further observations on the vessels and nerves of the rabbit's ear, with special reference to the effects of denervation. *Clin. Sci.* **2**, 1–33.

GRANT, R. (1949). Nature of pyrogen fever. *Amer. J. Physiol.* **159**, 511–524.

HARDISTY, R. M. & STACEY, R. S. (1955). 5-Hydroxytryptamine in normal human platelets. *J. Physiol.* **130**, 711–720.

HUMPHREY, J. H. & JACQUES, R. (1955). The release of histamine and 5-hydroxytryptamine (serotonin) from platelets by antigen-antibody reactions (*in vitro*). *J. Physiol.* **128**, 9–27.

KING, E. J. & WOOTTON, I. D. P. (1956). *Micro-analysis in Medical Biochemistry*, 3rd ed. London: J. and A. Churchill.

PERERA, G. (1941). Clinical and physiologic characteristics of chill. *Arch. intern. Med.* **68**, 241–260.

PINKSTONE, J. O. (1935). Peripheral circulation during experimental fever. *Amer. J. Physiol.* **110**, 448–457.

ZWECKER, I. T. & GOODELL, H. (1925). Rapid alterations in the blood sugar level of rabbits as result of intravenous injections of killed bacteria of various types. *J. exp. Med.* **42**, 43–56.