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THE ROLE OF LOCAL SYMPATHETIC INNERVATION IN PYROGEN-INDUCED VASOCONSTRICTION OCCURRING IN THE RABBIT EAR

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Vasoconstriction in the skin is an early and prominent response to the injection of bacterial pyrogens and is generally thought to be brought about by a sympathetic vasoconstrictor discharge from the central nervous system to the vessels of the skin. This explanation, however, has recently been challenged by Boquet and his co-workers, who conclude that pyrogen-induced vasoconstriction is not dependent upon local activity of sympathetic vasoconstrictor nerves (Boquet, Delauney, Lehoult & Lebrun, 1947), but is due to some circulating constrictor substance, probably adrenaline, which makes its appearance in the blood shortly after injection of pyrogen (Boquet & Izard, 1950). They point out that pyrogens are known to be able to release adrenaline into the blood (von Euler, 1927) and that certain other effects of pyrogen, such as hyperglycaemia (Evans & Zeckwer, 1927), are attributable to circulating adrenaline. Pinkston's (1934) experiments on the rabbit also indicated that pyrogens could, in some instances, induce vasoconstriction after removal of the sympathetic nerves, and suggested to him that some of these effects were due to circulating adrenaline. Unlike Boquet *et al.* (1947), however, he considered the vasoconstrictor action of pyrogen to be due normally to activity in sympathetic vasoconstrictor nerves. In the present paper the vasoconstriction caused by pyrogen in the rabbit ear has been re-examined. No evidence of any vasoconstrictor action of pyrogen has been found other than that mediated by the sympathetic nerves to the ear.

METHODS

Experiments were conducted upon Himalayan and Dutch rabbits, trained so that they would sit quietly throughout an experiment in boxes which held them lightly about the neck but which allowed them free movement of the limbs. Under such conditions their body temperatures

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remained steady: there was no tendency to the 'emotional' hypothermia which is prone to occur in rabbits that are frightened or over-restrained. Rectal temperature was recorded in each animal by an indwelling thermocouple enclosed in a rubber catheter and inserted 6 in. beyond the anus. Ear temperature was taken as an index of vasoconstriction and was measured by a thermocouple attached with collodion and insulated from the surrounding air by a small patch made up of several thicknesses of paper. The junction was placed about 3 cm from the tip of the ear, on its outer surface, and a few mm posterior to the central artery. The majority of experiments were conducted at ambient room temperatures of 18–21° C. In some experiments where vasoconstriction was being sought, a resting state of vasodilation was induced by keeping the rabbit in a room heated to 26–28° C during the entire experiment. Such experiments are described as having been conducted in a warm room.

In some experiments the sympathetic vasoconstrictor supply to the rabbit's ear was interrupted by removal of the superior cervical ganglion or the stellate ganglion, or both. The operations were done under ether anaesthesia 1–6 weeks before pyrogen injection. In other experiments sympathetic activity was interrupted by intravenous injection of the ganglion blocking drug hexamethonium chloride. The superior cervical ganglion was removed by the usual ventral approach and the stellate ganglion by the dorsal extrathoracic approach described by Feldberg (1926).

The pyrogen used was a preparation from *B. proteus* obtained from Dr A. A. Miles of the National Institute for Medical Research, Mill Hill, London.

RESULTS

The effect of pyrogen on ear vessels after removal of the superior cervical ganglion

Boquet *et al.* (1947) concluded that the vasoconstriction produced in rabbits' ears after pyrogen is not the result of vasoconstrictor discharge to the skin, because they found that it persisted after removal of the superior cervical ganglion. This result has been confirmed. In two rabbits, removal of the right superior cervical ganglion caused miosis and dilation of the ear vessels of the same side. The vasodilation diminished rapidly during the following few days, and after 10–11 days there was little difference in temperature between the two ears. At this time the injection of pyrogen caused vasoconstriction in both ears, the response of the operated ear being no less than that of the normal (Fig. 1*a*).

Although this finding confirms Boquet *et al.* (1947), it does not support their conclusion, for the experiments of Fletcher (1898) and Feldberg (1926) showed that removal of the superior cervical ganglion causes only partial sympathetic denervation in the rabbit's ear. Residual sympathetic constrictor activity in the right ear of the two rabbits deprived of their right superior cervical ganglion was indicated by the following findings: first, that the operated ear shared in the emotional flush which could be induced by tying the rabbits back-down to the table; and secondly, that hexamethonium, which has no direct dilator action but which causes flushing by blocking vasoconstrictor activity (Paton & Zaimis, 1951), caused reddening and increased warmth in the operated ear indistinguishable in course and intensity from that which it caused in the normal ear (Fig. 2*a*). It was evident that a week or so after

removal of the superior cervical ganglion, vasoconstrictor tonus was virtually normal. From Fig. 2 it will be seen that the action of hexamethonium was remarkably short-lived.

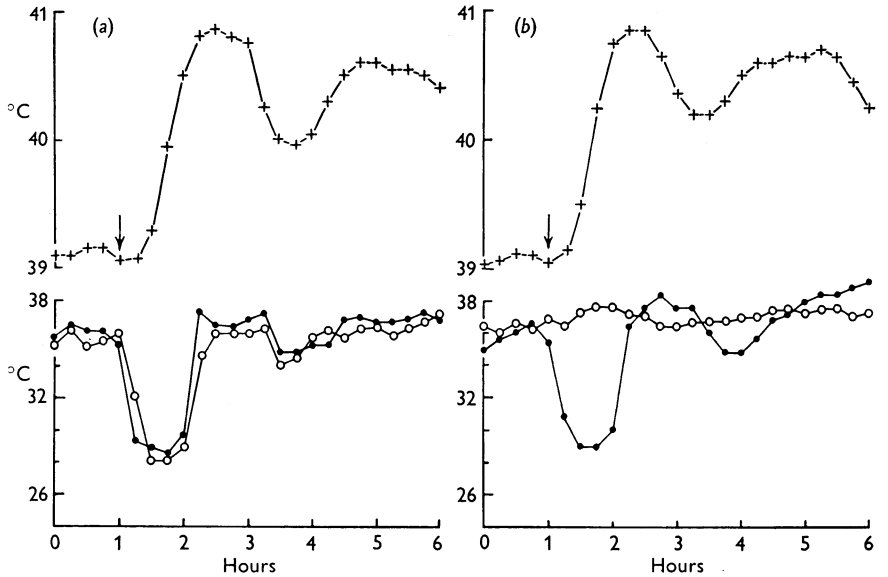

Fig. 1. Responses of a rabbit to intravenous injections of 1 μg/kg *B. proteus* pyrogen (at arrow). The upper trace (+——+) shows the rectal temperature: the lower traces show the temperatures of the left ear (●——●) and the right ear (○——○). The rabbit was in a warm room (28° C). (*a*) Responses obtained after extirpation of the right superior cervical ganglion. (*b*) Responses obtained in the same rabbit after subsequent extirpation of the right stellate ganglion.

The effect of pyrogen on ear vessels after removal of the stellate ganglion

In the two rabbits in which the right superior cervical ganglion had been extirpated, the residual sympathetic innervation was removed by subsequent extirpation of the right stellate ganglion. The right ears immediately became flushed, more so than after the first operation, and this flush persisted. The temperature of these ears was always much higher than that of the unoperated ears on each of the several occasions on which it was measured during the following 6 weeks. At room temperatures of 18–28° C the difference was about 10° C, the operated ear being within 2–4° C of the rectal temperature and the normal ear close to room temperature. The spontaneous fluctuations in vasoconstrictor tone which occur normally—and which also occurred after removal of the superior cervical ganglion alone—were no longer present. Further evidence of the completeness of sympathetic denervation in these two rabbits was obtained by the use of hexamethonium, whose injection no longer caused any increase in the temperature of the operated ear. The effect of

hexamethonium was, in fact, the reverse, for it now induced some cooling of the operated ear (Fig. 2*b*) while warming the normal ear.

Injection of pyrogen into these rabbits gave results different from those previously obtained. Now the ear of the operated side failed to cool at any time during the course of fever, but rather tended to increase in warmth as rectal temperature rose (Fig. 1*b*).

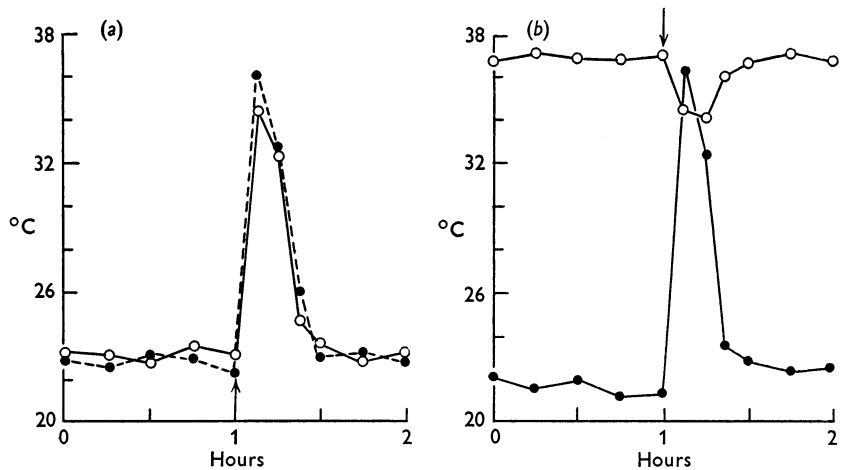

Fig. 2. The same rabbit as in Fig. 1, showing ear temperature responses to hexamethonium (5 mg/kg intravenously at arrow). The response of the left ear is shown thus: ●——●, and the right ear thus: ○——○. (*a*) Responses obtained after extirpation of the right superior cervical ganglion. (*b*) Responses obtained after subsequent removal of the right stellate ganglion.

In two rabbits the effect of pyrogen after stellate ganglionectomy alone was tried. The postoperative course in these animals was similar to that following the second operation in the first two animals. The ear of the operated side showed a steady, intense vasodilation which diminished only slightly over a period of several weeks. Pyrogen injection in these rabbits gave effects in every way comparable with those already obtained after the combined operation and already illustrated in Fig. 1*b*.

It was the practice to induce a steady level of vasodilation by conducting these experiments in a warm room. This technique allowed the onset of vasoconstriction to be readily observed, but it raised some doubt that the effect of pyrogen might be different at more normal ambient temperatures. To test this pyrogen was given to all four rabbits at room temperatures of 18–20° C. In these experiments the denervated ear behaved as it did in the warmed rabbits: no vasoconstriction was observed during the course of fever (Fig. 3). The control vasomotor responses in the normal ears were, of course, less striking, for considerable constrictor tonus was present at such room temperatures.

The experiments so far described have concerned the effect of chronic sympathetic denervation on the response to pyrogen. Experiments on chronically denervated preparations are, however, open to the criticism that the effector mechanism may be abnormal. To meet this objection, experiments

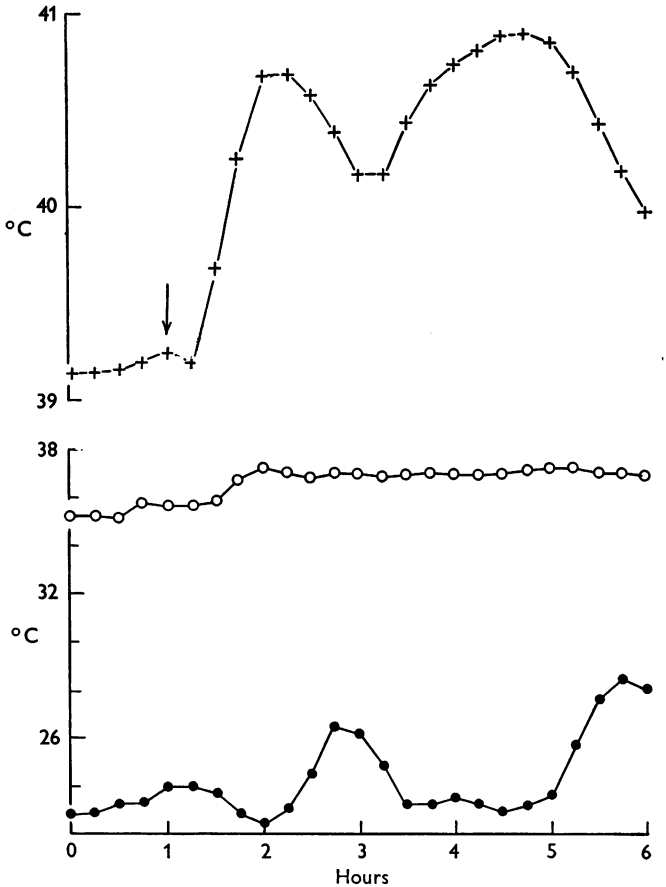

Fig. 3. Another rabbit. Showing the response to 1 μg/kg *B. proteus* pyrogen at a room temperature of 20° C. The upper trace (+——+) shows the rectal temperature. The lower traces show the temperatures of the left (●——●) and the right (○——○) ears. The right stellate ganglion alone had been removed.

were performed in which the effect of pyrogen was tested during acute deprivation of sympathetic nerve influence. Ganglion block by hexamethonium was the technique employed.

It has been mentioned that the vasodilator effect of hexamethonium is transient. Even very large doses (10 mg/kg) given at half-hourly intervals cause a greatly fluctuating response, the effect of one dose being almost over

before that of the next occurs (Fig. 4). This made it difficult to estimate the effect of pyrogen when it was given after hexamethonium. An alternative

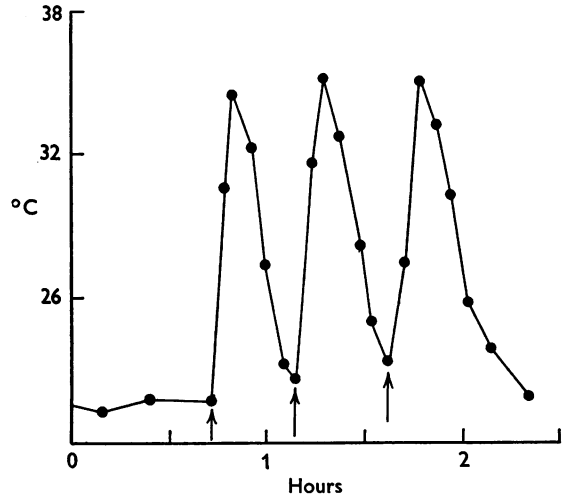

Fig. 4. Temperature responses in a normally innervated rabbit ear caused by injecting intravenously three doses of hexamethonium (10 mg/kg at each of the arrows).

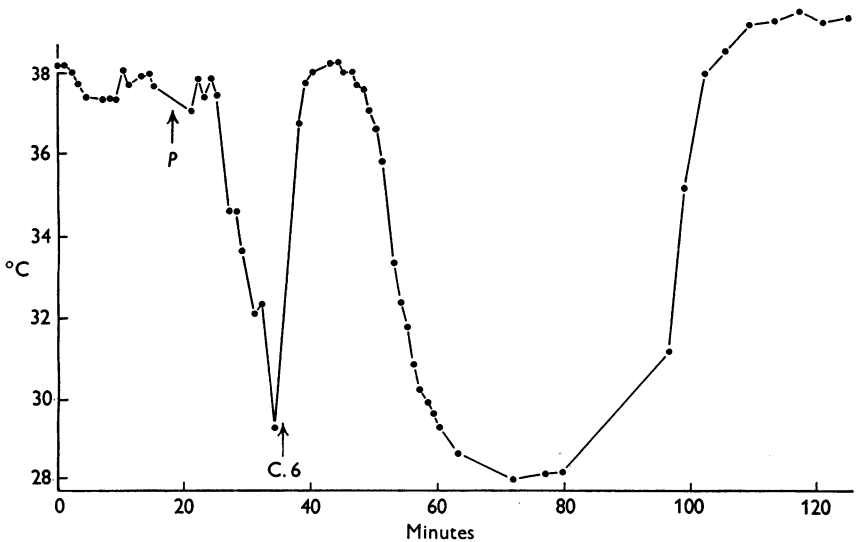

Fig. 5. The effect of a single dose of hexamethonium (10 mg/kg at arrow marked 'C. 6') on the vasoconstriction caused in the normally innervated ear by 1 μg/kg *B. proteus* pyrogen (at arrow '*P*'). The rabbit was in a warm room.

procedure was therefore adopted, i.e. to induce vasoconstriction with pyrogen and then to try the effect of hexamethonium upon it. The experiments, on four fresh rabbits, were conducted in a warm room to induce vasodilation. To

obviate the vasomotor fluctuations which accompany any manipulation of the rabbit, injection of pyrogen and hexamethonium was made by an indwelling catheter placed in the marginal vein of the ear not under observation. After the injection of pyrogen (1 μg/kg) and the establishment of vasoconstriction, hexamethonium was injected either in a single dose or in multiple doses at short intervals. The results were clear-cut. In each rabbit pyrogen caused intense vasoconstriction after a latent period of 7–15 min. The subsequent injection of hexamethonium reversed this effect and restored the ear vessels to a state of vasodilation comparable with that existing before pyrogen injection.

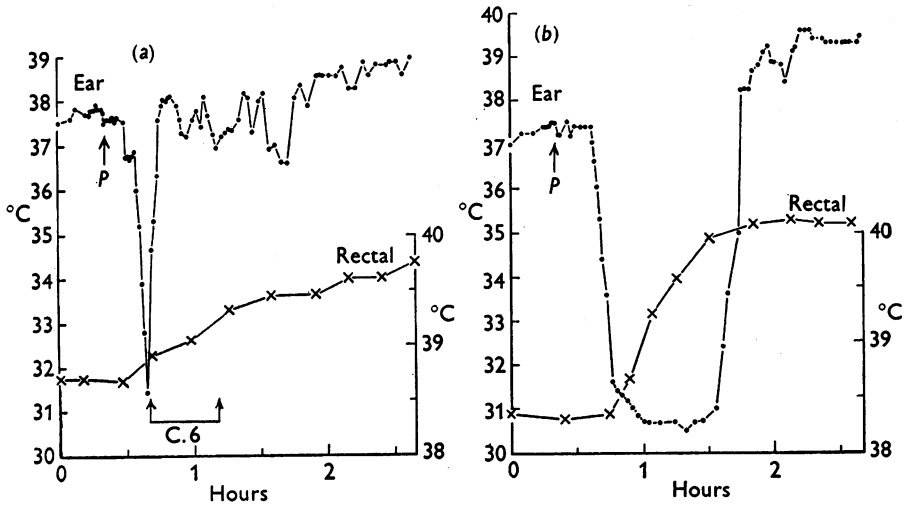

Fig. 6. The effect of repeated injections of hexamethonium on the ear and rectal temperatures of a normal rabbit during pyrogen vasoconstriction. (*a*) At the arrow marked *P*, 1 μg/kg *B. proteus* preparation was injected. During the period marked C. 6, eight injections of 5 mg/kg hexamethonium were given. (*b*) Shows a control response to pyrogen alone (1 μg/kg) obtained 1 week later. In each experiment the rabbit was in a warm room.

A single injection of hexamethonium caused only a transient effect (Fig. 5); but this effect could be obtained at any time during the normal duration of the vasoconstriction (about 1 hr). By giving repeated injections of hexamethonium at short intervals, it was possible to induce vasodilation throughout the period normally occupied by vasoconstriction (Fig. 6). An incidental observation made in each of the two rabbits in which these repeated injections of hexamethonium were given was that the febrile response to pyrogen was less than when no hexamethonium was injected (compare the rectal temperature in Fig. 6*a* with that in Fig. 6*b*).

DISCUSSION

In concluding that the vasoconstriction following an injection of a bacterial pyrogen is independent of local sympathetic nerve activity, Boquet *et al.* (1947) based their opinion on results obtained on rabbits' ears after removal of the superior cervical ganglion. The validity of their conclusion rests, therefore, on the ability of this operation to effect a complete sympathectomy. There is evidence, however, that superior cervical ganglionectomy does not in itself deprive the rabbit ear of sympathetic vasoconstrictor fibres (Fletcher, 1898; Feldberg, 1926) and this evidence has been amply corroborated in the present investigation. A week or so after superior cervical ganglionectomy the ear showed little sign of abnormality. Its temperature was no higher than that of the normal ear and it participated normally in the vasomotor response to different environmental temperatures, to restraint and to hexamethonium. It follows, therefore, that results obtained after this operation do not allow of conclusions concerning the role of sympathetic nerves in the pyrogen response.

On the other hand, results obtained after stellate ganglionectomy which also, of course, deprives the superior cervical ganglion of its central connexions do allow of such conclusions, for after this operation a persistent vasodilation was observed in the ear and none of the several indices of sympathetic innervation were seen. The finding that pyrogen failed to cause vasoconstriction after stellate ganglionectomy shows that the presence of intact sympathetic nerves is necessary for the production of vasoconstriction by pyrogen. The results obtained by Boquet *et al.* which were confirmed in the present experiments, must be attributed to the presence of that part of the sympathetic innervation to the ear which is not interrupted by removal of the superior cervical ganglion.

The absence of the vasoconstrictor effect of pyrogen in chronically denervated ears does not yield conclusive proof that the vasoconstrictor effect is normally mediated by these sympathetic nerves. It could still be argued that pyrogen itself or some substance released by pyrogen into the circulation is the factor responsible for constriction in normal ears, but that sympathectomy so alters the responsiveness of the ear vessels that they fail to react. This could not be true were the putative substance adrenaline or noradrenaline, to which sympathectomy would sensitize the ear vessels, but it might hold for the pyrogen itself or some other released substance, However, these explanations can be excluded by the finding that hexamethonium abolished pyrogen-induced vasoconstriction in the normally innervated ear, for change in responsiveness of the ear vessels would not be expected to occur within a minute or so of the ear being deprived of its sympathetic supply by the ganglion blocking action of the drug.

The present results are not fully in accord with Pinkston's (1934) experience. He found that pyrogen still evoked a vasoconstrictor response in the ears in

five of nine rabbits after sympathectomy performed either by removal of the superior cervical ganglion and section of the remaining sympathetic fibres locally in the ear, or by removal of the stellate ganglion. In two of these the temperature of the sympathectomized ear fell shortly after injection and thereafter followed closely the temperature of the normal ear throughout the course of the fever; in three others the evidence of vasoconstriction was not apparent until 1½ hr or so after the injection, although the unoperated ear had responded with usual promptness. Pinkston ascribed the prompt response to adrenaline released by the pyrogen, but considered the delayed effect to be due to some other humoral agent. He obtained no direct evidence for these beliefs but based them on an experiment on three further rabbits with inactivated adrenals in which pyrogen caused no immediate vasoconstriction in the sympathectomized ear of any, but a delayed vasoconstriction in two. It is not easy to find a satisfactory explanation of this difference in results. The obvious explanation, that Pinkston's sympathectomy was less complete than in the present series, does not appear likely on the evidence available. It is true that he applied no rigorous physiological or pharmacological test for the completeness of sympathectomy, but the persistence of postoperative vasodilation and the absence of spontaneous fluctuations of temperature in the ears of his rabbits suggest that it was in fact complete. It may be that difference in pyrogen dosage explains the divergent results, and that high doses of pyrogen release adrenaline in amounts sufficient to cause vasoconstriction in the denervated ear. But since the normally innervated ear is much less responsive than the denervated ear, this mechanism would be of doubtful significance in the production of vasoconstriction in the normal ear already strongly vasoconstricted through its nerves. Alternatively, it may be that some adrenaline was released in his experiments, not as a result of the pyrogen but from the trauma of injection. This would account for the immediate constrictor response which was abolished by adrenal inactivation. Or again, the delayed response might be due not to active constriction of local blood vessels, but to a fall in systemic blood pressure which pyrogens are known to be capable of causing (Bennett & Beeson, 1950). A fall in systemic blood pressure would similarly account for the paradoxical effect of hexamethonium in the denervated ear, where it caused cooling rather than warming.

A further point arising from the use of hexamethonium is its antipyretic action which was observed in each of the two rabbits in which it was given in multiple doses after pyrogen. The action might be attributed to suppression of vasoconstriction by hexamethonium, for it is well recognized that vasoconstriction normally contributes substantially to the production of fever in the rabbit. But other factors are involved in fever, and it does not follow that in the absence of vasoconstriction fever should be less. For example, the onset of fever was always heralded by moderate shivering, and it might be expected

that the enfeeblement of vasoconstrictor mechanisms in the hexamethonium-injected animals would be compensated for by an increase of shivering. It is difficult to say from observation whether shivering was increased or not after hexamethonium, but it was quite apparent that it was in each instance submaximal, for it did not approach the violence of the efforts made by rabbits in a cold environment (4° C). Perhaps the warm flush of the skin induced by hexamethonium prevented the full development of shivering. This, however, is only one of the ways in which the antipyretic action of hexamethonium might be explained.

SUMMARY

1. Experiments have been carried out to analyse the way in which a bacterial pyrogen causes vasoconstriction in the rabbit ear.

2. In confirmation of work by other authors, it has been found that removal of the superior cervical ganglion does not materially alter the vasoconstrictor response.

3. Evidence is presented that the persistence of the pyrogen effect after this operation is due to the continued presence in the ear of a substantial sympathetic nerve supply.

4. After complete sympathetic denervation, i.e. removal of the stellate ganglion, no vasoconstrictor effect of pyrogen is observed.

5. In normally innervated ears the pyrogen-induced vasoconstriction is abolished by the intravenous injection of hexamethonium.

6. It is concluded that the vasoconstriction which occurs after an injection of bacterial pyrogen in the rabbit ear is effected solely by the sympathetic vasoconstrictor nerves in the ear.

REFERENCES

BENNETT, I. L. & BEESON, P. B. (1950). The properties and biologic effects of bacterial pyrogens. *Medicine, Baltimore*, **29**, 365–400.

BOQUET, P., DELAUNEY, A., LEHOULT, Y. & LEBRUN, J. (1947). Observation directe des réactions vasculaires cutanées chez le lapin soumis à l'épreuve d'une endotoxine typhique. *C.R. Acad. Sci., Paris*, **225**, 1193–1194.

BOQUET, P. & IZARD, Y. (1950). Effect of dibenamine on the vascular response of rabbits to typhoid endotoxin. *Proc. Soc. exp. Biol., N.Y.*, **75**, 254–256.

VON EULER, U. S. (1927). Über Hyperadrenalinämie bei Fieberzuständen. *Pflüg. Arch. ges. Physiol.* **217**, 699–716.

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

FELDBERG, W. (1926). The peripheral innervation of the vessels of the external ear of the rabbit. *J. Physiol.* **61**, 518–529.

FLETCHER, W. M. (1898). The vaso-constrictor fibres of the great auricular nerve in the rabbit. *J. Physiol.* **22**, 259–263.

PATON, W. D. M. & ZAIMIS, E. J. (1951). Paralysis of autonomic ganglia by methonium salts. *Brit. J. Pharmacol.* **6**, 155–168.

PINKSTON, J. O. (1934). Peripheral circulation during experimental fever. *Amer. J. Physiol.* **110**, 448–457.