J. Physiol. (1974), **243**, *pp.* 167–193
With 12 *text-figures*
Printed in Great Britain

FEVER PRODUCED BY ENDOTOXIN INJECTED INTO THE HYPOTHALAMUS OF THE MONKEY AND ITS ANTAGONISM BY SALICYLATE

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(*Received 16 April 1974*)

SUMMARY

1. A suspension of the killed cell bodies of either *E. coli*, *S. dysenteriae* or *S. typhosa* was micro-injected through cannulae implanted chronically at specific sites within the diencephalon and mid-brain of the unanaesthetized monkey. A biphasic, monophasic or an undifferentiated fever could be induced by each type of micro-organism, but the type of response depended solely upon the locus of injection.

2. Although little difference in the potency of the three pyrogens was found, the rise in body temperature was in each instance dependent upon the concentration of the endotoxin. A more intense fever was accompanied by shivering, vasoconstriction of the ear vessels, piloerection and huddling behaviour. Tolerance to the pyrexic effect of repeated injections of endotoxin did not develop.

3. The febrile response having the shortest latency, greatest maximum rise in temperature and largest 10-hr fever index was evoked by micro-injections into the anterior hypothalamic, preoptic area. The incidence of biphasic fevers was also greater after endotoxin was injected into this same region. Endotoxin given similarly in the posterior hypothalamus or in the mesencephalon had either no effect or produced a smaller elevation in temperature after a longer latency. The distance of an injection site from the coronal plane formed by the optic chiasm and anterior commissure correlated significantly with the latency and magnitude of the temperature change as well as the fever index.

4. When given intravenously, endotoxin in a quantity at least 100 times greater was required to evoke a fever similar to that produced when the pyrogen was micro-injected into the anterior hypothalamic, preoptic region. However, a biphasic fever was evoked with a latency of

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from 3 to 15 min when a larger amount of endotoxin was injected intravenously. Tolerance developed rapidly to the febrile effect of endotoxin administered by this route although toxic reactions were not observed.

5. After the fever evoked by the hypothalamic injection of endotoxin had reached a plateau, 300–1200 mg sodium salicylate administered intragastrically produced a dose-dependent fall in temperature, but had no effect on the body temperature of an afebrile monkey.

6. It is concluded that in the rhesus monkey, a bacterial pyrogen can evoke a fever which is mediated entirely by an action on the central nervous system, the principal site being the anterior hypothalamic, preoptic area. The first phase of a biphasic fever caused by bacteria acting either by the central or peripheral route seems to be due either to a direct action of the pyrogen on the cells of the anterior hypothalamus, or to the secondary release within this region of an intermediary thermogenic substance such as 5-hydroxytryptamine or prostaglandin. The finding that sodium salicylate counteracts a centrally evoked fever is not compatible with the hypothesis that an antipyretic exerts its action by preventing a pyrogen that is circulating in the blood stream from entering the central nervous system.

INTRODUCTION

A bacterial pyrogen injected intravenously produces a fever which is characterized by two distinct peaks (Petersdorf & Bennett, 1959; Sheth & Borison, 1960; Tully, Gaines & Tigertt, 1965; Weiss, Laties & Weiss, 1967; van Miert & Atmakusuma, 1970). Although both of these peaks may be mediated by endogenous pyrogen (Atkins, 1960), Bennett, Petersdorf & Keene (1957) suggested that the first peak is caused by a direct action of the injected endotoxin on the central nervous system. The second peak of this biphasic response is thought to be caused by an action upon the hypothalamic thermoregulatory centres of an endogenous pyrogen released from granulocytes in the blood, which are 'activated' by coming in contact with the injected endotoxin (Atkins, 1960; Rašková & Vaněček, 1964).

A bacterial endotoxin injected directly into the cerebrospinal fluid evokes a profound and long-lasting fever in the dog, cat, rabbit or mouse (Bennett *et al.* 1957; Sheth & Borison, 1960; Villablanca & Myers, 1965; du Buy, 1966; Cooper, Cranston & Honour, 1967; Repin & Kratskin, 1967; Cashin & Heading, 1968). Experiments in which bacteria are injected into different areas of the brain-stem of the cat (Villablanca & Myers, 1965) rabbit (Repin & Kratskin, 1967) or rat (Lipton, Welch & Clark, 1973) have demonstrated that the region most sensitive to endotoxin is the anterior hypothalamic, preoptic area (AH/POA).

In preliminary experiments we have found that a bacterial pyrogen applied directly to the hypothalamus evokes a febrile response in a sub-human primate (Myers, Rudy & Yaksh, 1971*a*). Since both the rostral hypothalamus and mesencephalon have been implicated as sites of pyrogen action in other species (Cabanac, Stolwijk & Hardy, 1968), the present experiments were carried out to determine the anatomical regions in the brain stem which are sensitive to endotoxin. Since a salicylate may exert its antipyretic effect by preventing a blood-borne pyrogen from passing into the brain (Cooper, Grundman & Honour, 1968; Adler, Rawlins, Rosendorff & Cranston, 1969), the effect of sodium salicylate on a fever produced by an endotoxin administered centrally (Myers *et al.* 1971*b*) was examined further.

METHODS

Each of thirty male rhesus monkeys (*Macaca mulatta*), weighing 4·5–7·5 kg, was trained to sit in a primate restraining chair for 3–7 days before an experiment. Each animal was maintained on a diet of Purina monkey pellets and fresh fruit, and water was provided *ad lib.* from automatic dispensers. The ambient temperature was kept at 22 –24° C, and the room was illuminated continuously.

Surgical procedures. Each of twenty monkeys was anaesthetized with pentobarbitone sodium (35 mg/kg) injected into the saphenous vein or one of its superficial branches. After the monkey was placed in a stereotaxic instrument, four or eight micro-injection guide tubes were inplanted under aseptic precautions, according to procedures described earlier (Myers, 1971*b*). Each guide was cut to a length of 40 or 50 mm from either 17 or 22 gauge Hypoflex needle tubing and then bevelled to a sharp tip. An indwelling stylet of matching dimension was inserted into each guide tube.

After a trephine opening had been made in the bone, the array of cannulae was lowered to a pre-determined depth so that the tip of each tube rested directly above the intended site of injection within the hypothalamus, mesencephalon or other structure. Stainless-steel anchor screws were inserted into the bone and Cranioplast cement was packed around the array and the screws. To protect the cannulae and maintain a sterile preparation, a polyethylene pedestal with a removable cap was placed over the array and screwed to the skull. So that intracranial temperature could be recorded, a thermistor bead was also positioned against the falx cerebri 7–9 mm below the dura mater as described previously (Myers & Yaksh, 1969). Post-operatively, penicillin was administered for 10 days.

Micro-injection procedure. A Hamilton microlitre syringe mounted on an infusion pump was connected to a 28 gauge injector cannula by a length of fine polyethylene (PE 10) tubing. After a stylet was removed from a guide tube, the injector cannula was lowered beyond the tip of the guide to a predetermined depth ranging from 2 to 10 mm. Then, 0·8–1·2 μl. of a pyrogen or control solution was delivered over 30 sec after which the stylet was replaced. In those monkeys in which the guide tube was 17 gauge, the upper portion of the injector cannula was ensheathed always within a 20 gauge sleeve to ensure a tight fit within the guide. Except in those experiments on the tolerance to pyrogen, a micro-injection at a given locus was repeated only after an interval greater than 72 hr.

Preparation of solutions. Shigella dysenteriae (type SH 16), *Salmonella typhosa* (type 643) and *Escherichia coli* (type W3110) were cultured to a concentration of

$2–5 \times 10^9$ organisms/ml. and then killed by toluene or chloroform bubbling. The cells were separated by centrifugation, washed and re-suspended in an equivalent volume of pyrogen-free saline and stored at 5° C. From the stock suspension, a solution for micro-injection was prepared in pyrogen-free 0·9 % saline in a dilution ranging from 1:2 to 1:100,000. A sample of each stock suspension was plated on nutrient media at periodic intervals, but viable organisms were never detected. The pyrogen-free control solution micro-injected at each site was either 0·9 % saline or a standard artificial cerebrospinal fluid (Myers, 1971*c*) based on electrolyte values for the monkey. Glassware was rendered pyrogen free by heating at 190° C for 2 hr, and polyethylene tubing and injector cannulae were stored in 70 % ethanol and flushed thoroughly with pyrogen-free saline before use.

Recording of physiological responses. Before an experiment began, the monkey's intracranial temperature was recorded for at least 1 hr. When colonic temperature was also measured, a flexible thermistor probe was inserted 10 cm into the colon and taped to the base of the tail. Peripheral vasomotor tone was estimated by the surface temperature of the ear, recorded directly by a YSI disk thermistor affixed to the skin, or by palpation of the pinna. These temperatures were recorded concurrently on a multi-channel potentiometric recorder throughout each experiment.

The respiratory rate, general demeanour of the animal and the occurrence of shivering, piloerection or huddling were also monitored continuously. Each change in the body temperature of the monkey following a micro-injection was quantitated on the basis of latency, maximum rise, and the shape of the curve. In addition, a 10 hr Fever Index (FI_{10}) was calculated by plotting the temperature on lined graph paper and counting the number of squares between the base-line temperature and the curve of the fever for the 10 successive hours following the onset of the hyperthermia.

Histological procedures. At the conclusion of a series of experiments, every micro-injection site in the monkey was verified according to standard histological procedures. After the animal was anaesthetized with pentobarbitone sodium, 1 μl. of 10 % solution of Indian ink was injected at each locus. Then the heart was clamped and the brain was perfused via the thoracic aorta with 0·9 % saline followed by buffered formalin. The brain was removed, washed thoroughly in de-ionized water

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Fig. 1. Composite anatomical maps in the frontal plane of the sites in the brain at which a suspension of 1:2 dilution of *E. coli* was micro-injected in the rhesus monkey. In terms of the onset of fever, an open circle (○) indicates a latency of 30 min or less, a triangle (△), a latency of 31–60 min, and a filled circle (●) a latency greater than 60 min or no effect. Abbreviations are: ac, anterior commissure; ant, anterior hypothalamic area; ca, caudate nucleus; cc, corpus callosum; cp, cerebral peduncle; dm, dorsomedial hypothalamic nucleus; f, fornix; gp, globus pallidus; ic, internal capsule; lat, lateral hypothalamic area; ls, lateral septal nucleus; lv, lateral ventricle; md, medial dorsal nucleus of the thalamus; mm, medial mammillary nucleus; ms, medial septal nucleus; mt, mammillothalamic tract; na, anterior nucleus of the thalamus; nac, nucleus accumbens; oc, optic chiasm; ot, optic tract; pc, nucleus paracentralis; po, preoptic area; pu, putamen; re, reuniens nucleus; rn, reticular nucleus of the thalamus; sn, substantia nigra; st, subthalamic nucleus; va, ventral anterior nucleus of the thalamus; vl, ventral latera lnucleus of the thalamus; vm, ventromedial hypothalamic nucleus; vpl, ventral posterior lateral nucleus of the thalamus; zi, zona incerta; 3v, third ventricle.

and blocked. Sections were cut at 28 μ on a freezing microtome and stained for cell bodies and fibres according to a method modified after Klüver & Barrera (1953).

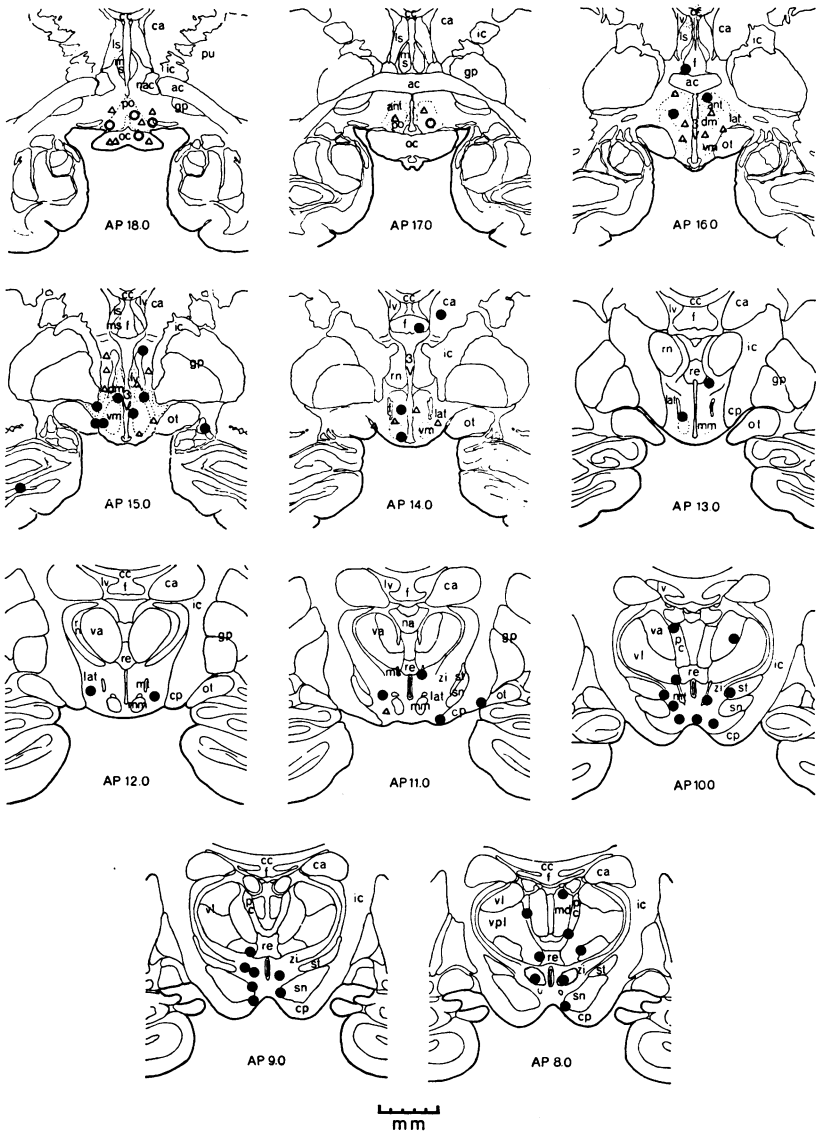

Fig. 1. For legend see facing page.

RESULTS

Fever is produced by a micro-injection into the brain of the rhesus monkey of 0·8–1·2 μl. of a 1:2 dilution of a suspension of *E. coli* or other bacteria. Not only is the febrile response dependent on the site of the injection, but the hyperthermia can be classified on the basis of its shape as monophasic, biphasic or undifferentiated.

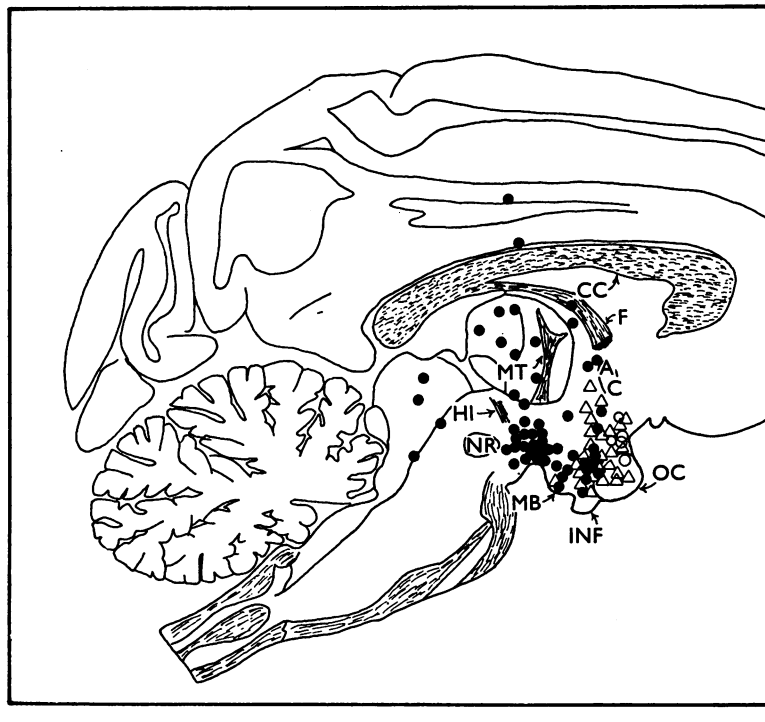

Fig. 2. Composite anatomical map in a parasagittal plane 2·5 mm from mid line of sites in the brain at which a suspension of a 1:2 dilution of *E. coli* was micro-injected. In terms of the onset of a fever, an open circle (○) indicates a latency of 30 min or less, a triangle (△) a latency of 31–60 min, and a filled circle (●) a latency greater than 60 min or no effect. AC, anterior commissure; CC, corpus callosum; F, fornix; HI, habenulo-interpeduncular tract; INF, infundibulum; MB, mammillary body; MT, mammillothalamic tract; NR, red nucleus; OC, optic chiasm.

Anatomical mapping of sites sensitive to endotoxin

In a total of twenty monkeys, eighty-nine loci were tested for their sensitivity to *E. coli* in a 1:2 dilution. In Fig. 1, the anatomical positions of eighty of the sites are plotted in sequential histological maps that include coronal planes AP 18·0 through AP 8·0. The remaining nine sites were in

structures not included on the reconstructions. To illustrate the longitudinal extent of sites responsive to bacterial pyrogen, Fig. 2 portrays the morphological distribution of eighty-six of the loci in a parasagittal plane 2·5 mm from the mid line. In both Figs. 1 and 2, an open circle indicates a latency in the onset of fever of 30 min or less, an open triangle a latency of 31–60 min, and a filled circle a latency of more than 60 min or no effect on temperature. With one exception, a latency of hyperthermia

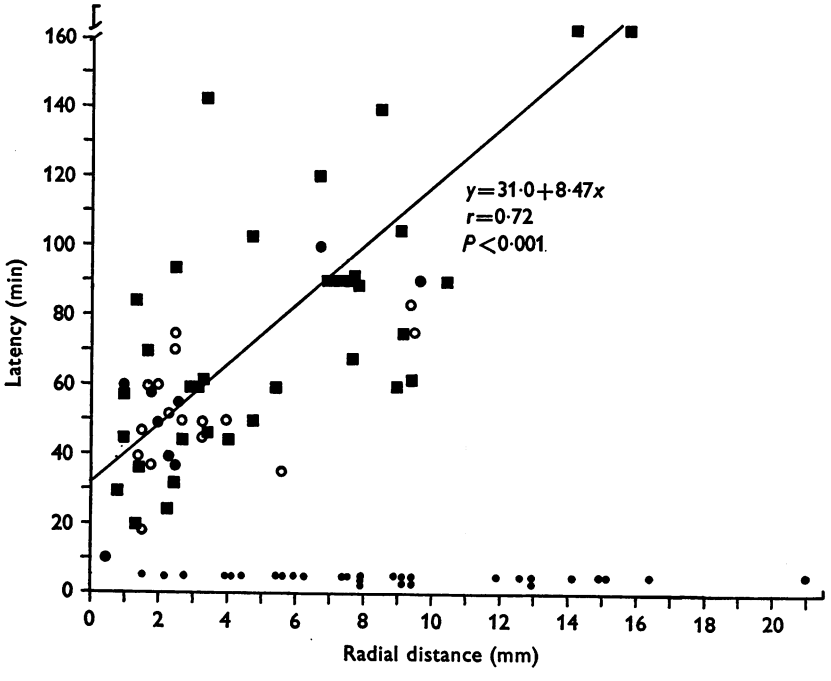

Fig. 3. Relationship between the latency to onset of a fever evoked by microinjection of *E. coli* and the radial distance of the injection site from the locus in the anterior hypothalamus of maximum sensitivity to the pyrogen. The type of febrile response is denoted as follows: (○), biphasic; (●), monophasic; and (■), undifferentiated. The regression line was calculated by the method of least squares, and its equation, as well as the correlation coefficient (r) is given in the upper right. P indicates the degree of significance of r. Small filled circles along the abscissa denote the radial distance of sites at which an injection of endotoxin produced a fever of less than 0·5° C, the latency of which could not be fixed precisely and which was not included in the regression.

of less than 60 min was observed only when an injection was made into the rostral hypothalamus or preoptic region. Although the shortest latencies to endotoxin were noted when the injection was made just caudal to the

preoptic region, the plane of maximum sensitivity to a pyrogen runs in the dorsal-ventral extent.

That the locus most sensitive to bacteria is the AH/POA was deduced on the basis of the intensity of the fever following injections into this region. Therefore, a scatter diagram presented in Fig. 3 was constructed in which each response latency was plotted against the radial distance of

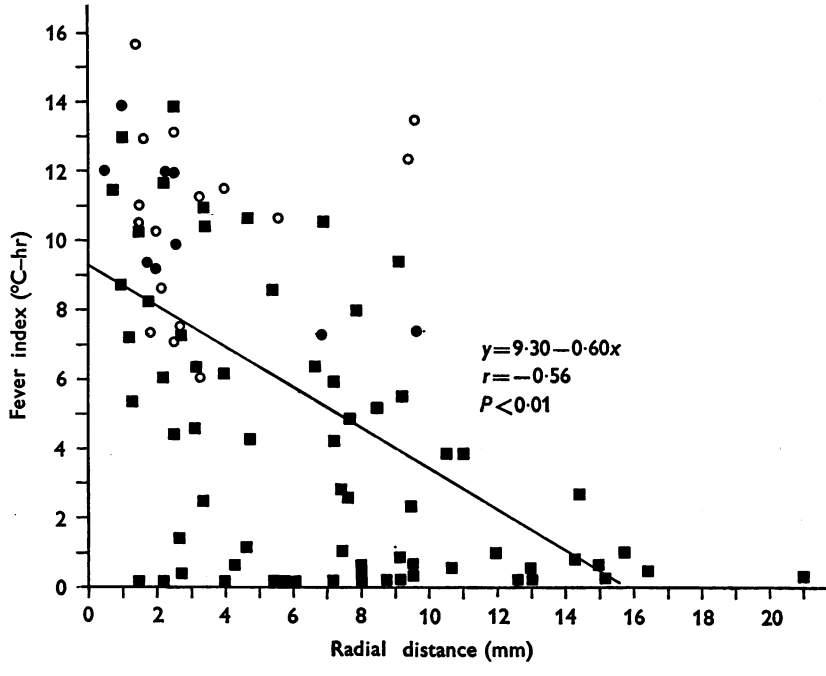

Fig. 4. Relationship between the FI_{10} of each fever evoked by a micro-injection of *E. coli* and the radial distance of the injection site from the postulated site of maximum pyrogen sensitivity. Symbols are the same as in Fig. 3. When a latency could not be determined exactly, the FI_{10} was computed so that the summation of the area under the curve of fever was begun at the time of injection.

the injection site from the apparent focus of sensitivity. The spatial reference from which each radial distance was measured was a point located in the coronal plane containing the anterior commissure, 1·5 mm lateral to the mid line, and equidistant between the anterior commissure and the optic chiasm, i.e. AP, 17·0; Lat, 1·5; Hor, +1·5. Fig. 3 shows the regression line fitted to these data. The positive correlation of 0·72 between latency and the radial distance was significant. Fig. 3 also shows that the majority of the biphasic fevers were evoked in the monkey by pyrogen injected less than 4 mm from the anterior reference point.

A plot of the fever index in terms of the elevation in temperature measured over 10 hr (FI_{10}) versus the radial distance also revealed a significant correlation illustrated in Fig. 4. The FI_{10} decreased as the radial distance increased ($P < 0{\cdot}01$, $r = -0{\cdot}56$, $y = 9{\cdot}30 - 0{\cdot}60x$). The correlation which is not shown between the maximum temperature increase and radial distance also was significant ($P < 0{\cdot}01$, $r = -0{\cdot}48$, $y = 1{\cdot}43 - 0{\cdot}08x$).

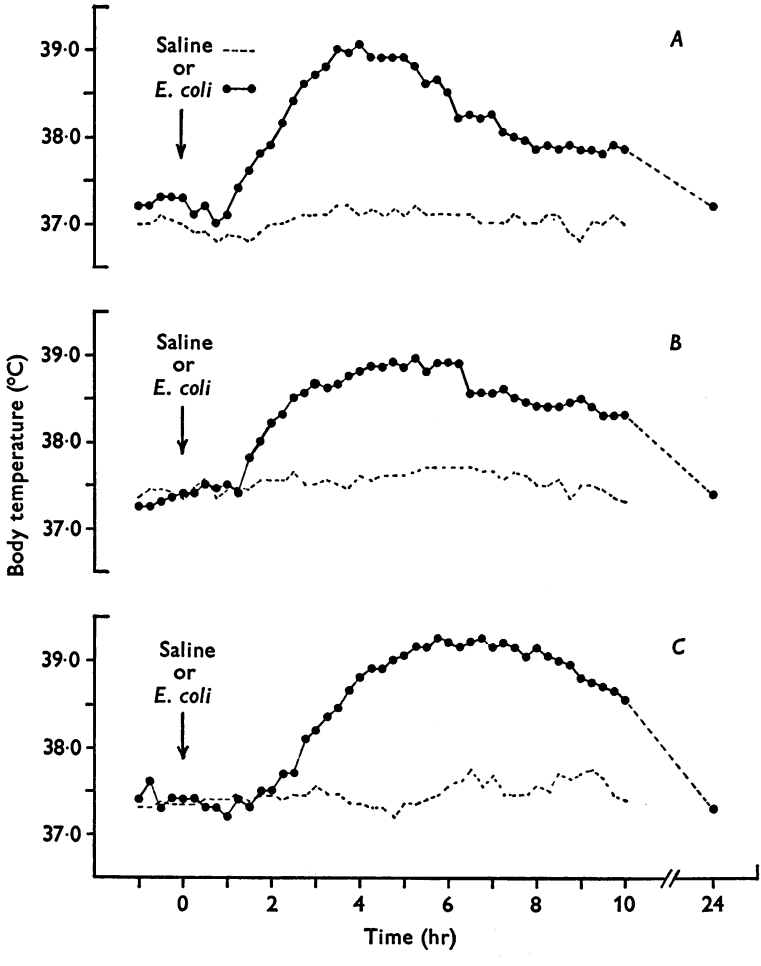

Fig. 5. Three monophasic fevers of varying latency induced by injection of 0·8–1·2 μl. *E. coli* into the hypothalamus of the monkey. Panels *A*, *B* and *C* illustrate responses evoked by injections into three different anterior loci. In each instance, both control and *E. coli* responses were obtained from the same site.

Characteristics of an endotoxin fever

Representative examples of the monophasic fever in three monkeys are presented in Fig. 5. A rapid rise in the temperature of the monkey was usually accompanied by vigorous shivering, huddling, piloerection and constriction of the ear vessels. Shivering became less intense once the animal's temperature had attained the maximum level, but sporadic bursts could still usually be seen. Because of the diurnal variation in the

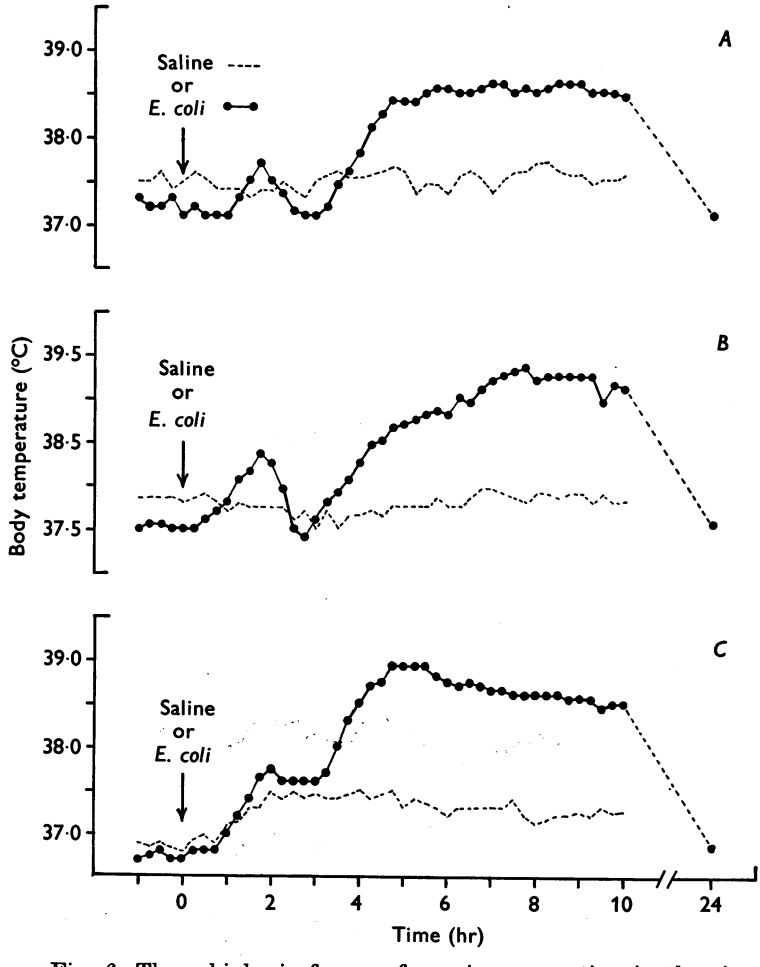

Fig. 6. Three biphasic fevers of varying separation in the time course. At the arrow, injection into the hypothalamus of 0·8–1·2 μl. *E. coli*. Panels *A*, *B* and *C* illustrate responses evoked by injections into three different anterior loci. In each instance, the responses to *E. coli* and the control solution were obtained from the same site.

monkey's temperature, the precise duration of an endotoxin fever was often difficult to ascertain. Nevertheless, the core temperature of the monkey usually returned to the base-line level within 24 hr.

Illustrative examples of the biphasic fever in three monkeys are given in Fig. 6. Ordinarily, the latency of a biphasic fever was shorter and the magnitude was greater than the monophasic or undifferentiated pyrexia. Both the magnitude and duration of the second phase of the biphasic response shown in Fig. 6 were invariably greater than the first. In the brief period of interphase during a biphasic response, the monkey did not shiver and its ear vessels were not constricted. If the results of Fig. 5 are contrasted with those of Fig. 6, it can be seen that the magnitude and duration of a monophasic fever closely resembles the second phase of a biphasic response. A latency of about one hour was similar in both instances.

The average latency and the magnitude of all monophasic, biphasic and undifferentiated types of fever are presented in Table 1. Only those febrile responses evoked by the micro-injection of *E. coli* are included because of the frequency of its use. As indicated in the Table, many responses could not be differentiated unequivocally as either an uninterrupted monophasic rise or a biphasic hyperthermia with two distinct peaks. Usually, the undifferentiated response was not as intense as the others and had a relatively long latency.

Comparison of different endotoxins and control solutions

As shown in Table 2, the hyperthermic responses to *E. coli*, *S. typhosa* and *S. dysenteriae* given at pyrogen-sensitive loci were very similar. When the bacteria were tested at the same fifteen sites, however, the mean FI_{10} of the response to *E. coli* was much greater than that produced by a micro-injection of *S. dysenteriae*. In addition, *S. typhosa* and *S. dysenteriae* evoked the same sort of biphasic, monophasic or undifferentiated fever, after they were micro-injected into the AH/POA.

A micro-injection of 0·9% saline or artificial cerebrospinal fluid usually caused little change in the body temperature of the monkey. When the effect of a 1:2 dilution of *E. coli* was compared to that of either control solution tested at forty-four sites, both the maximum temperature increase and FI_{10} were significantly greater after the endotoxin. These results are shown in Table 2. In a few instances, an endotoxin-like response depicted in Figs. 6*C* and 7*A* occurred following a control injection. However, as illustrated in Fig. 7*A* a second phase did not appear or was of a much reduced magnitude. In two other experiments, a similar response was evoked by a sham injection consisting of the insertion of an empty

TABLE 1. Characteristics of the rise in temperature of at least 0·5° C evoked by a micro-injection of *E. coli* suspension, 1:2 dilution. *n* = number of sites from which the type of fever was elicited; S.E. = standard error of the mean. Since the latency of the second peak could not always precisely be determined, the latencies of the second phase of the fever were estimated by extrapolating the slope of the second peak back to base line

		Mean latency of first peak		Mean magnitude of first peak		Mean latency of second peak		Mean magnitude of second peak	
Fever type	*n*	Min	S.E.	°C	S.E.	Min	S.E.	°C	S.E.
Monophasic	9	60·2	±7·1	1·7	±0·1		—		—
Biphasic	16	49·9	±4·1	0·7	±0·1	129·9	±6·7	1·8	±0·1
Undifferentiated	35	78·6	±7·1	1·2	±0·1		—		—

TABLE 2. Comparison of the febrile response evoked by the intracerebral injection of the control solution and the three pyrogens, each in a 1:2 dilution. Each substance was injected at least once at a given site. S.E. = standard error of the mean; n.s. = not significant. *P* values were computed using a *t* test for paired comparisons

Treatments compared	Sites tested	Mean FI_{10}	S.E.	*P* value	Mean maximum temperature Increase °C	S.E.	*P* value
E. coli	44	8·65	±0·70	<0·01	1·49	±0·09	<0·01
Control		2·90	±0·46		0·57	±0·06	
E. coli	15	5·79	±1·35	<0·05	0·87	±0·19	n.s.
S. dysenteriae		3·03	±0·80		0·59	±0·13	
E. coli	10	4·39	±1·38	n.s.	0·69	±0·18	n.s.
S. typhosa		4·02	±1·25		0·75	±0·19	
S. dysenteriae	7	3·69	±1·80	n.s.	0·66	±0·25	n.s.
S. typhosa		3·67	±1·69		0·64	±0·24	

injector cannula. Removing and replacing the stylet without inserting the injector had no effect.

Occasionally, a precipitous fall, shown in Fig. 7*B*, rather than a rise in body temperature, followed a micro-injection of either endotoxin or saline. This effect was not repeatable nor was it associated with an injection into any specific region of the brain stem.

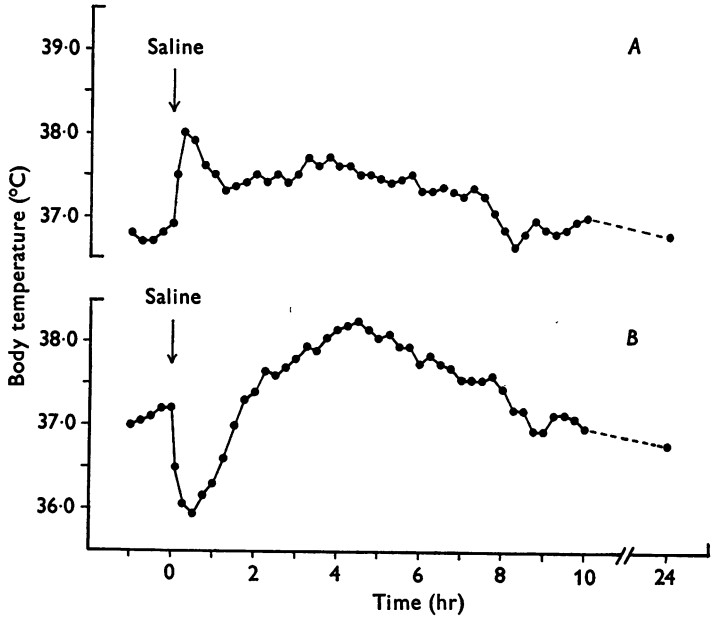

Fig. 7. Effects on body temperature of saline micro-injected into the dorsomedial posterior hypothalamus (*A*) and the preoptic region (*B*).

Endotoxin concentration and fever

In addition to ascertaining the anatomical region sensitive to endotoxin by using a fixed 1:2 dilution of *E. coli*, concentration–effect curves for the three pyrogens were also obtained. Fig. 8 illustrates the dilution–response relationships for three suspensions of endotoxin as well as the *E. coli* solution of the purified cell wall lipopolysaccharide. A dilution of 1:2 or 120 nanograms of the lipopolysaccharide applied to the anterior hypothalamus resulted in a greater FI_{10} in the monkey and, in most cases, a greater maximum temperature increase than the lower concentration. Thus, the standard dilution of 1:2 fell on or near to the linear portion of the concentration–response curve. Fig. 8 also shows that these concentration-effects fall into two distinct sets of parallel slopes. The type of pyrogen would not seem to be a factor in the steepness of the slope since the group of steep curves were obtained from one monkey.

Tolerance to endotoxin

Although tolerance develops after as few as two intravenous injections of an endotoxin made at a 24 hr interval (Beeson, 1947; Bennett *et al.* 1957), the possibility that tolerance might occur after repeated injections of an endotoxin directly into cerebral tissue has not been studied previously.

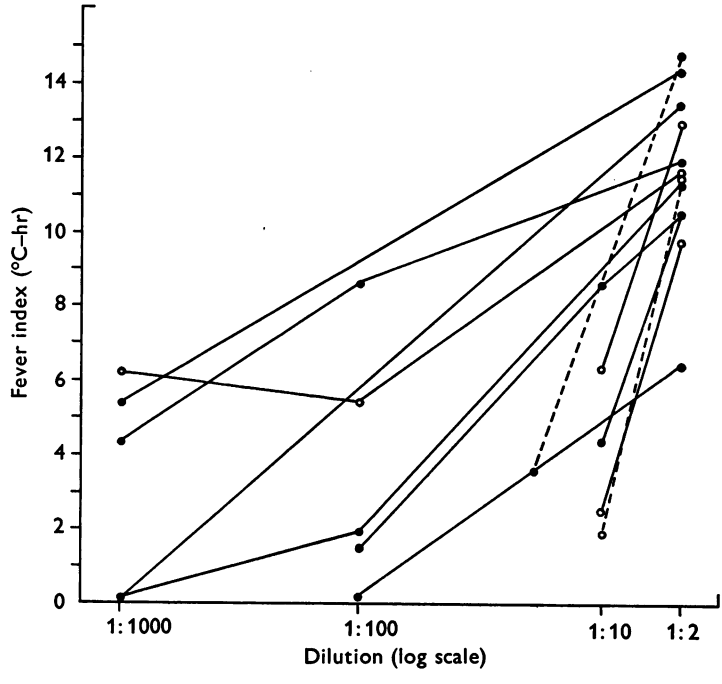

Fig. 8. Relationship between the dilution of endotoxin (abscissa) micro-injected at eleven different sites in the hypothalamus and the FI_{10} (ordinate). The specific endotoxins were suspensions of killed cell bodies of *E. coli* (●—●), *S. dysenteriae* (○—○), *S. typhosa* (○----○) and 120 ng purified *E. coli* cell wall lipopolysaccharide (●----●).

Hypothalamic resistance. To determine whether closely spaced micro-injections at a single locus would produce tolerance, three successive micro-injections of *E. coli* in a 1:2 dilution were made at 48 hr intervals into single loci in six monkeys. In four animals, there was little or no difference in the maximum temperature increase or FI_{10} over the injection sequence. Representative fevers of one monkey are presented in Fig. 9*A*. However, as illustrated in Fig. 9*B*, the second peak of the fever in one animal was greatly diminished after the first micro-injection; in another monkey, a fever was not produced by either the second or third injection.

Although tolerance to pyrogen may have occurred, this seems unlikely because the injection sites in these two monkeys were insensitive to endotoxin when re-tested several weeks later. This could have been due to a local damage or possibly haemorrhage produced at the site of injection by the first micro-injection.

A series of micro-injections were made at closely spaced intervals at alternating sites in two monkeys. By exposing the hypothalamus to an

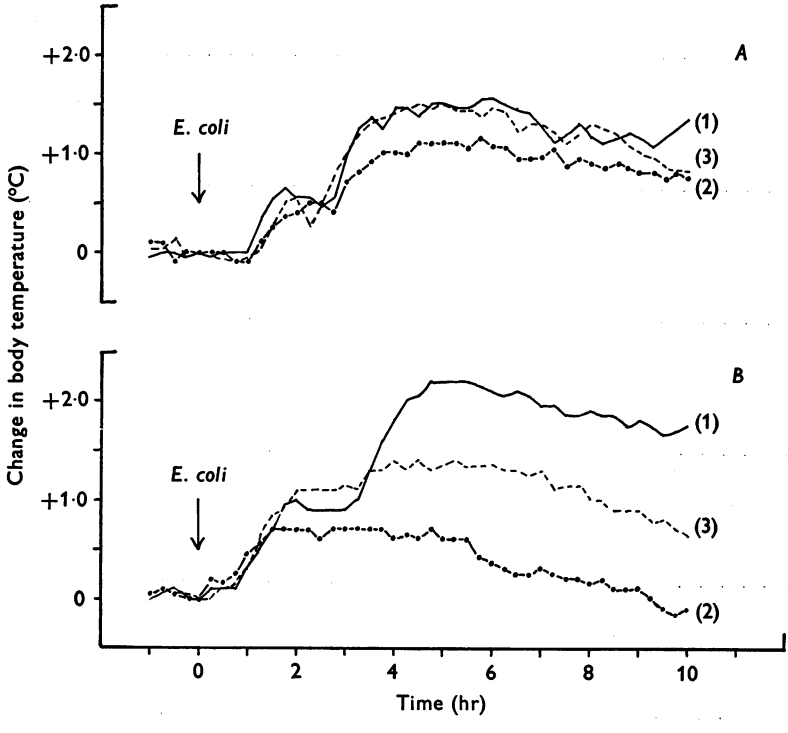

Fig. 9. Lack of tolerance (*A*) and loss of the second fever peak (*B*) in two monkeys which received repeated micro-injections of a 1:2 dilution of *E. coli* at a single hypothalamic locus at 48 hr intervals. The number in each parenthesis indicates the order in which the injections were given.

endotoxin at frequent intervals, a fever of at least 0·9° C resulted in each case. The sequences in which there was no evidence of neural tolerance were: (1) sixteen injections over 34 days at 1–4 day intervals, and (2) twelve injections over 23 days at 1–3 day intervals.

Response to intravenous E. coli. To determine whether tolerance would occur after repeated intravenous injections of the same endotoxin, two non-operated monkeys were given 2 ml. of the undiluted suspension of *E. coli* intravenously over a 20 sec interval every second day until a total

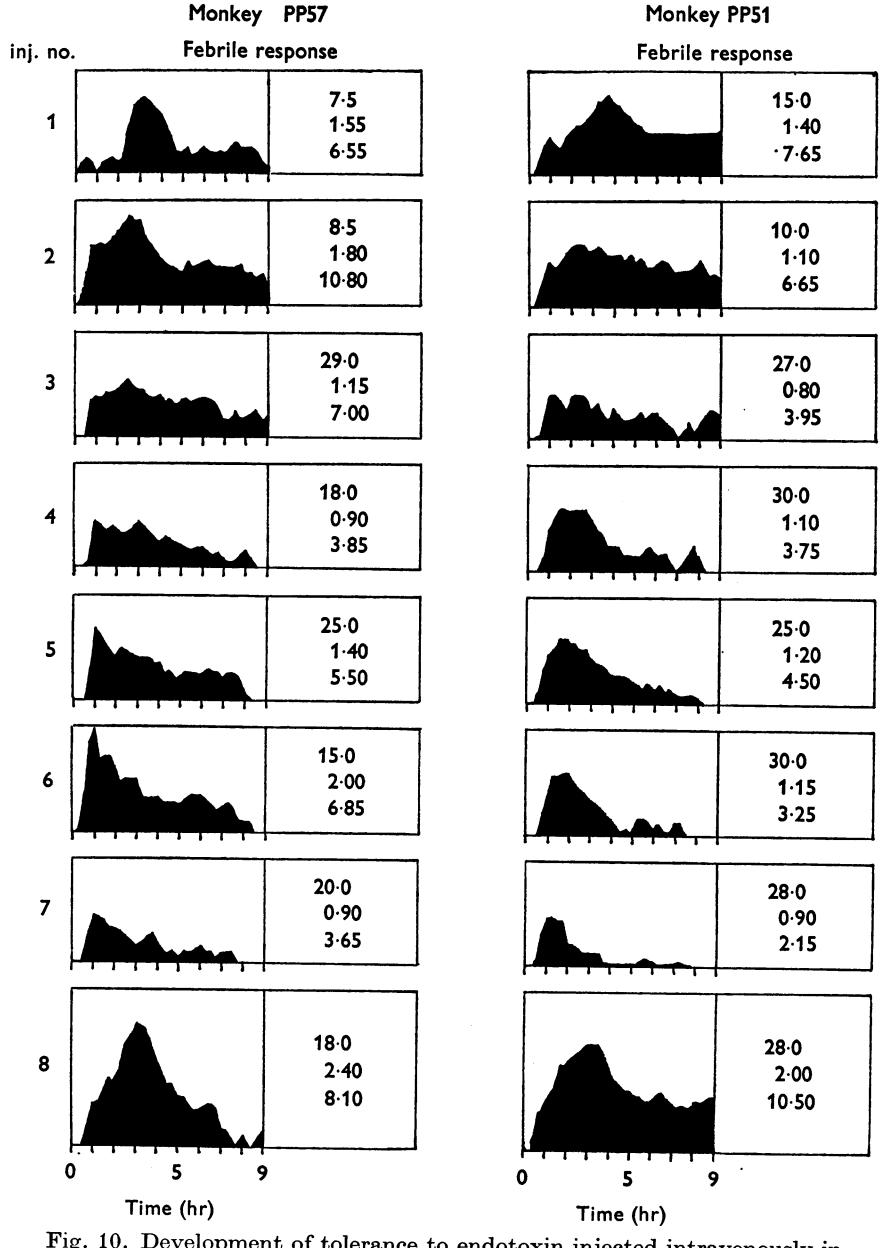

Fig. 10. Development of tolerance to endotoxin injected intravenously in the non-operated monkey. Each of two animals received seven successive intravenous injections of 2 ml. undiluted *E. coli* at 48 hr intervals. A re-test was performed 1 month later (injection no. 8). The numbers to the right of each fever indicate, in descending order: minutes to fever onset; maximum increase in temperature in °C; and FI_{10}.

of seven injections had been made. After a 30-day interval, the monkeys were re-tested with the same dose of endotoxin. Fig. 10 indicates that by the third injection tolerance had begun to develop, which is reflected by a decrease in the magnitude of the fever, its duration, and FI_{10} as well as by the increase in latency. When the two monkeys were re-tested with

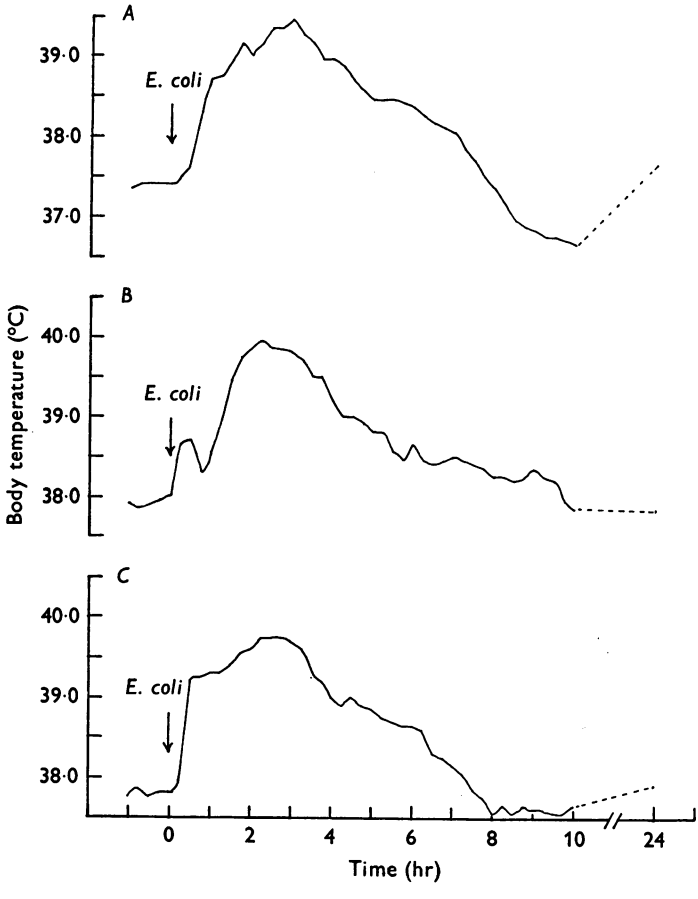

Fig. 11. Febrile responses of three monkeys to an intravenous injection of 2 ml. of an undiluted suspension of *E. coli*.

the last injection, the fever was even more intense than that evoked by the initial injection; however, the time of onset did not return to the pre-injection latency. Tolerance to toxic effects of the pyrogen developed after the second injection, and these reactions were still absent when the animals were challenged with endotoxin 30 days later.

A partial loss of tolerance is suggested by the augmented fever and a reduced latency noted in Fig. 10 for the sixth injection given to monkey

TABLE 3. Effect on body temperature of a suspension of *E. coli* injected intravenously in different dilutions. The volume injected into the saphenous vein or one of its branches was always 2 ml. over a period of 20 sec. Guide cannulae had been implanted in three animals, whereas the other monkeys had not been tested previously

Dilution of *E. coli* injected	*n*	Mean latency in min and range	Mean maximum rise in °C and range	Mean FI_{10} and range
0·0 (saline)	2	—	0·00	0·00
0·0006	2	—	0·00	0·00
0·006	2	40 (39–41)	0·55 (0·5–0·6)	0·85 (0·6–1·1)
0·06	2	38 (37–39)	0·85 (0·4–1·3)	2·00 (0·55–3·45)
1·0	3	10 (6–15)	1·53 (1·4–1·6)	6·32 (4·75–7·65)
1·0	3 (implanted)	6 (3–10)	2·08 (2·0–2·2)	9·08 (8·45–10·10)

TABLE 4. The effect of salicylate given by the intragastric route on body temperature in afebrile monkeys and in those given *E. coli* in the anterior hypothalamus. * = significantly different ($P < 0·05$) from the mean obtained in the afebrile animals; *n* = number of animals in each group; S.E. = standard error of the mean

Condition	Dose (mg)	*n*	Mean fever at time of salicylate		Mean maximum change in temperature	
			°C	S.E.	°C	S.E.
Febrile	300	7	1·51	±0·22	−0·37*	±0·12
	600	9	1·72	±0·14	−0·94*	±0·15
	1200	4	1·08	±0·17	−0·72*	±0·17
Afebrile	300	7	—		+0·05	±0·14
	600	10	—		−0·09	±0·06
	1200	8	—		−0·05	±0·12

PP57. In this one experiment, the injection was made over a 10 sec interval into the cephalic vein rather than the saphenous vein. Thus, the endotoxin could have reached the sensitive site in the hypothalamus more rapidly and in a more concentrated volume.

To find out whether the concentration of endotoxin required to cause a fever is less when applied in the brain than when injected systemically, a suspension of *E. coli* was injected intravenously over 20 sec to non-operated monkeys. The effect of the highest intravenous concentration was also studied in another group of animals in which guide tubes had been implanted previously in the brain. As shown in Table 3, at least 100 times as much endotoxin had to be given intravenously to evoke a fever of approximately the same magnitude as that produced by an injection into the hypothalamus. Indeed, even 2 ml. of undiluted endotoxin injected intravenously was less effective than 1 μl. of a 1:2 dilution given into the AH/POA region, i.e. a 4000-fold difference. It was noted that an intravenous injection was somewhat more potent in an animal bearing the array of guide cannulae.

The rapid onset of fever in three monkeys given 2 ml. of undiluted *E. coli* endotoxin intravenously is illustrated in Fig. 11. A sustained increase in temperature occurred in less than 3 min after the injection (Fig. 11*C*). Unexpectedly, such injections very rarely produced a notable toxic reaction which included transient nausea, vomiting and intermittent contractions of the abdominal musculature characteristic of retching.

Antagonism of endotoxin fever by salicylate

To examine the effect of sodium salicylate on the fever induced centrally, *E. coli* was injected into the rostral hypothalamus of a group of monkeys. After the plateau of the pyrexic response had been reached, 300, 600 or 1200 mg of sodium salicylate, dissolved in 30 ml. distilled water warmed to body temperature, were given in the stomach by way of a naso-pharyngeal tube. A summary of the responses is given in Table 4 which shows that the salicylate produced a clearly defined defervescence, the magnitude of which was dose-related. Although the two higher doses were significantly more effective than the 300 mg dose ($P < 0{\cdot}05$), the difference in the magnitude of the decline in temperature following 600 and 1200 mg salicylate was not significant, whereas the action of 1200 mg was more prolonged. Two examples of the antipyretic effect of salicylate are illustrated in Fig. 12. Usually within 15–30 min after the administration of the antipyretic, vasodilation of the ear vessels occurred and the intensity of shivering was reduced. Since salicylate could produce a hypothermia in the rhesus monkey rather than antipyresis, the experiments were repeated in afebrile animals, which either bore the array of guide cannulae

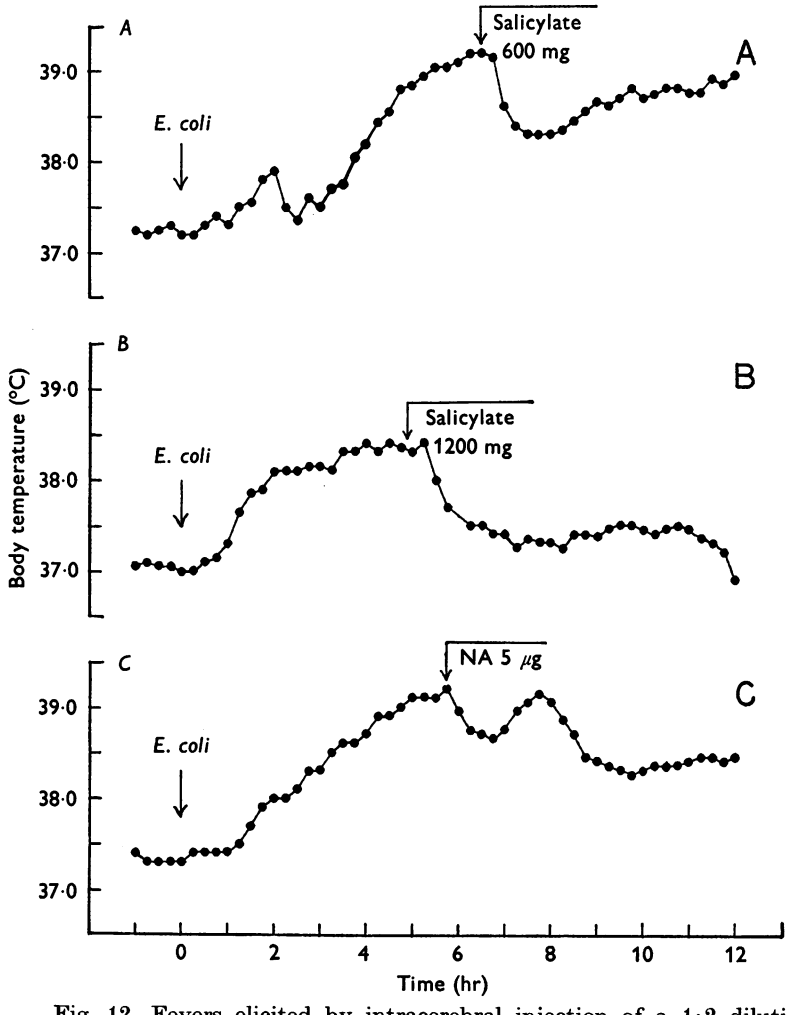

Fig. 12. Fevers elicited by intracerebral injection of a 1:2 dilution of *E. coli* at zero hour. A solution of sodium salicylate was administered in the dose specified (panels *A* and *B*) by naso-gastric tube at the time indicated by the arrows. 5 μg noradrenaline were micro-injected (panel *C*) at the same site as the suspension of *E. coli*.

or were non-operated. As illustrated in Table 4, salicylate in the same doses did not cause a significant fall in body temperature of the monkeys.

DISCUSSION

The present results show that the fever produced in the monkey by different types of bacteria can result from a direct action of each endotoxin on neural tissue. For two reasons it is unlikely that a febrile response

evoked in this way results from a systemic action of the pyrogen due to its leakage from the central nervous system. First, the intravenous concentration of endotoxin required to elicit a fever is substantially higher than the hypothalamic concentration needed to produce a comparable hyperthermia. Second, most micro-injection sites in the diencephalon of the monkey were found to be insensitive to endotoxin, even though all loci were equally accessible to the blood supply by way of local capillary beds.

Anatomical specificity. As in the cat (Villablanca & Myers, 1965) and rabbit (Repin & Kratskin, 1967), the region between the anterior commissure and the optic chiasm apparently is one of the ultimate sites of action of endotoxin. The pyrexia following an injection of bacteria near this region had the shortest latency, highest peak and the largest Fever Index. A fever with a latency of 20 min or less occurred only after an injection of endotoxin into an area located within 1·5 mm of the region lying between the optic chiasm and the decussation of the anterior commissure. Thus, the hyperthermia caused by a micro-injection in structures located elsewhere was due probably to a diffusion or transport of the endotoxin to this anterior centre of maximum sensitivity.

Although a large number of sites in the posterior hypothalamus and upper mid-brain of the rhesus monkey were insensitive to endotoxin, Allen (1965) has demonstrated that in the rabbit the posterior hypothalamus takes up radiolabelled endogenous pyrogen selectively. Rosendorff & Mooney (1971) reported that some sites scattered in the mid-brain as well as in the AH/POA were responsive to leucocyte pyrogen. Other endotoxin-sensitive sites are postulated to lie in the more caudal brainstem (Sheth & Borison, 1960). Further, it is possible that there are species differences in the distribution of pyrogen-sensitive loci in the brain, or that neurones in the mesencephalon of the rabbit are sensitive mainly to leucocyte pyrogen.

Phasic characteristics of fever. When a bacterial pyrogen is administered systemically, a biphasic fever is produced in the cat (Sheth & Borison, 1960; Weiss *et al.* 1967), rabbit (Morgan, 1938; Petersdorf & Bennett, 1959; van Miert & Atmakusuma, 1970), dog (Morgan, 1938; Petersdorf & Bennett, 1959), goat (van Miert & Atmakusuma, 1970) and chimpanzee (Tully *et al.* 1965). Our results with *E. coli* injected intravenously show that the rhesus monkey responds identically. Moreover, the same pyrogen injected into or near the AH/POA of the monkey evokes a biphasic fever which has not been reported previously. Although the similarity of such fevers induced by a pyrogen administered either by the central or peripheral route could mean that the endotoxin acts solely on this part of the diencephalon, this conclusion is not warranted as Atkins

(1960) and Rašková & Vaněček (1964) have established that the second peak of a biphasic fever is due to the release of endogenous pyrogen from leucocytes, or other types of cells, as a consequence of their reaction with circulating endotoxin.

On the other hand, the first peak of a fever could be mediated in one of two ways: by a direct action of endotoxin on elements of the brain, or indirectly through the release of endogenous pyrogen from leucocytes. One argument favouring the indirect mechanism is that pathogenic organisms, unlike the smaller molecules of endogenous pyrogen, do not penetrate the blood-brain barrier readily because of their size. Against this is the finding that bacterial pyrogen has been detected in the cerebrospinal fluid after its intravenous injection (Bennett *et al.* 1957). Furthermore, the very delay in onset of a pathogen-induced fever may well depend on the sluggish rate of passage of the bacteria into the brain (Bennett *et al.* 1957; Clark, 1970). Because of the corresponding latencies of onset of fever in the monkey after the injection of endotoxin either intravenously or into the hypothalamus, our experiments would partially support the view of a direct action of bacteria on the brain. Since there is no evidence that hypothalamic neurones possess the capacity to elaborate endogenous pyrogen, at least the initial peak could originate in this way.

The origin of the second peak of a biphasic fever after endotoxin is applied to the hypothalamus is not understood. The relatively long latency of the second phase, even after an injection directly into the AH/POA, may mean that this portion of the fever is due to a delayed release of an intermediary pyrexic substance rather than to a direct effect of the endopyrogen on thermoregulatory neurones. Cooper *et al.* (1967) propose that a fever induced centrally by endotoxin may be due to the production of endogenous pyrogen by leucocytes that infiltrate into the area of the injection from the systemic circulation, because after an infusion of a bacterial pyrogen into the hypothalamus of the rabbit, a significant number of polymorphonuclear leucocytes were identified at the site of injection. The prolonged action of pyrogen in the monkey could thus be explained by the fact that polymorphonuclear cells, once 'activated', continue to produce leucocyte pyrogen for many hours after the initial activating substance dissipates (Bondy & Bodel, 1971).

Although it is puzzling how a pyrogen-free solution of saline or artificial c.s.f. could produce a fever in one of every ten control injections, an unlikely explanation is that some of these solutions contained contaminants. Perhaps more feasible is the possibility that during the course of an injection the micro-injection needle or the solution itself dislodges necrotic tissue which contains leucocytes at the tip of the guide tube

(Myers, 1974). It is also conceivable that a local mechanical disturbance is responsible for the rise in temperature particularly in the ventral loci that are implicated in the efferent system subserving shivering (Stuart, Kawamura, Hemingway & Price, 1962). Whatever the reason, the necessity of using several control injections and different concentrations in experiments of this kind is emphasized.

Action of salicylate. The finding that salicylate ameliorates in a dose-dependent manner a fever induced by endotoxin given centrally corroborates the earlier results in the rabbit (Cranston, Hellon, Luff, Rawlins & Rosendorff, 1970) and monkey (Myers *et al.* 1971*b*). In contrast to the supposition of Cooper *et al.* (1968), a salicylate can act as an antipyretic by a mechanism other than one which inhibits the passage of a pyrogen from blood into the central nervous system. Since salicylate has no effect on the body temperature of the afebrile monkey and apparently does not suppress the central or peripheral systems underlying thermoregulation, the antipyretic apparently attenuates the pyrogen hyperthermia by its action within the brain. Even more plausible evidence of this is the recent finding that an injection of salicylate into the brain of the rabbit or cat antagonizes a fever evoked by a pyrogen given systemically (Clark & Alderdice, 1972; Cranston, Luff, Rawlins & Rosendorff, 1970; Cranston & Rawlins, 1972). Clark & Coldwell (1972) propose that an antipyretic competes with a pyrogen for receptor sites in the central nervous system.

Hypothalamic mechanism of action of endotoxin. The anterior hypothalamus of the monkey is a highly thermosensitive region which is thought to be essential for the control of body temperature of this species (e.g. Hayward & Baker, 1968; Baker, Stocking & Meehan, 1972; Myers, 1971*a*). When a bacterial pyrogen is given systemically either to the cat (Wit & Wang, 1968; Eisenman, 1969) or rabbit (Cabanac *et al.* 1968), the frequency of firing of neurones in the anterior hypothalamus is altered selectively. Endotoxin given to the monkey would presumably modify the normal function of these thermoregulatory cells in an homologous anterior region. If this is so, then at issue is the generally accepted idea that the fever of a microbial infection represents an acute elevation of the set-point for body temperature (Fox & Macpherson, 1954; Andersen, Hammel & Hardy, 1961), the mechanism of which is postulated to exist within the posterior, not anterior, hypothalamus (Myers & Veale, 1971).

A number of experiments intimate that the ratio of Na^+ to Ca^{2+} ions in the mammillary region of the cat, rabbit or monkey maintains the set temperature of 37° C (see review of Myers, 1974). Of special significance is the fact that the presence of endotoxin in the brain results in a reciprocal shift in the normal efflux of Na^+ and Ca^{2+} ions from the hypothalamus as the ensuing fever develops (Myers & Tytell, 1972). From this it might be

expected that a bacterial pyrogen could greatly disturb both anatomical systems of the hypothalamus: the anterior thermosensitive area and the posterior ion-sensitive region.

Although the cellular mechanism by which the local presence of a lipopolysaccharide in the cells of the hypothalamus stimulates heat production is unknown, there are several alternative explanations. Bacterial endotoxin may release 5-hydroxytryptamine (5-HT) in the hypothalamus (Feldberg & Myers, 1964; Myers, 1971*d*) in a way similar to that which occurs from platelets (Nomura & Takagi, 1972) by way of a plasma releasing factor containing thrombin, prothrombin, thromboplastin or globulin (Nomura, Okada & Takagi, 1972); when 5-HT is injected in the anterior hypothalamus of the monkey, a prolonged hyperthermia arises (Myers & Yaksh, 1969). Or the pyrogen may elevate the regional level of a prostaglandin which when injected in a minute amount into the hypothalamus also elicits a fever in the monkey (Waller & Myers, 1973; Myers, 1974), the cat and other species (Cooper & Veale, 1974; Feldberg & Saxena, 1971; Milton & Wendlandt, 1971). Evidence for the latter view is the increase in release, during a pyrogen fever, of a prostaglandin-like contractile substance into hypothalamic perfusate (Myers, 1971*d*) as well as into the c.s.f. of the cat (Feldberg & Gupta, 1973). It is of special interest that prostaglandin perfused in the hypothalamus may also release [^{3}H]5-HT within this structure (R. D. Myers, M. B. Waller & G. E. Martin, unpublished observation). In turn, this monoamine is known to release acetylcholine from sites all along the heat production pathway in the caudal hypothalamus and mesencephalon (Myers, 1973).

Thus, a provisional sequence of hypothalamic events is as follows. Blood-borne endotoxin could elevate the level of a prostaglandin in the hypothalamus, which would retard the release of noradrenaline in the anterior hypothalamus but would enhance the output of 5-HT. Subsequently, 5-HT would activate the heat-gain pathway which is mediated by acetylcholine (Myers & Waller, 1973). At the same time, the endotoxin in circulating plasma would serve to shift the balance between Na^+ and Ca^{2+} ions in the posterior hypothalamus and thereby alter the discharge pattern of cells in this structure. In 1938, Morgan demonstrated in a classical experiment that *S. typhosa* given intravenously to the rabbit or dog does, in fact, produce acute cytological changes including chromatolysis in the anterior and posterior parts of the hypothalamus as well as in the paraventricular nucleus. This important finding now lends support to the concept that both rostral and caudal hypothalamic regions, so vital to the control of body temperature, are subject to the pathological impact of endotoxin.

This research was supported in part by U.S.A. office of Naval Research Contract N 00014-67-A-0226-0003 and by grant GB 24592 from the National Science Foundation. T. A. R. was a Post-Doctoral Fellow supported by Health Sciences Advancement Award PH 5-S04-FR-6013 from the National Institutes of Health and T. L. Y. was a Pre-Doctoral Fellow in Neurobiology supported by Grant 5-S04-FR-0615 from the National Institute of Mental Health. We are indebted to Dr R. L. Somerville for the cultures of endotoxin.

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