J. Physiol. (1971), **212**, *pp.* 411–430
With 7 text-figures
Printed in Great Britain

THE ROLE OF SODIUM AND CALCIUM IONS IN THE HYPOTHALAMUS IN THE CONTROL OF BODY TEMPERATURE OF THE UNANAESTHETIZED CAT

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(*Received 3 July 1970*)

SUMMARY

1. Isolated regions of the anterior, posterior and other areas of the hypothalamus of the unanaesthetized cat were perfused by means of push-pull cannulae lowered through permanently implanted guide tubes. Each site was perfused for a 30 min interval at a rate of 50 μl./min. Concentrations of sodium, calcium, potassium and magnesium ions in the perfusate were altered selectively.

2. Sodium ions in a concentration which varied from 13·6 to 68·0 mM in excess of the level in extracellular fluid caused a steep rise in the temperature of the cat when the solution was perfused at sites located within the posterior hypothalamic area. Shivering, vasoconstriction, and piloerection accompanied the increase in temperature. When the chloride was replaced in the perfusate by the toluene-*p*-sulphonate salt of sodium, the hyperthermia was equally intense. Solutions containing excess sodium ions perfused within the anterior and other hypothalamic areas produced either a slight fall or rise in temperature as well as other physiological changes.

3. Calcium ions in a concentration which varied from 2·6 to 10·4 mM in excess of the physiological level perfused at the same sites within the posterior region of the hypothalamus produced a sharp fall in body temperature, which was accompanied by vasodilatation and a decrease in the activity of the cat. When solutions containing excess calcium were perfused in the anterior and other hypothalamic areas, no consistent change in temperature occurred.

4. Potassium or magnesium ions in concentrations which varied from

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two to ten times the level in extracellular fluid had virtually no effect on the temperature of the cat when they were perfused in the anterior, posterior or other areas of the hypothalamus.

5. We conclude that the constancy in the ratio between sodium and calcium ions in the posterior hypothalamus may be the inherent mechanism by which the set-point for body temperature is determined.

INTRODUCTION

Ever since the early work of Schütz (1916), it has been known that a cation injected directly into the brain substance may affect body temperature. For instance, magnesium or calcium injected in milligram quantities into the tuberal area of a cat caused a fall in body temperature, whereas potassium, sodium, or barium given at the same site produced a rise (Hasama, 1930). More recently, Cooper, Cranston & Honour (1965) found that when potassium was micro-injected into the anterior hypothalamus of the rabbit, an intense hyperthermia developed.

When a solution of isotonic sodium chloride is perfused through the cerebral ventricles of the unanaesthetized cat, shivering and a rise in temperature occur (Feldberg, Myers & Veale, 1970). By adding calcium in a normal physiological concentration to the saline solution, the hyperthermia is blocked. From these results, it was suggested that the calcium level in the hypothalamus may be the physiological basis of the set-point. A major question raised by these findings is whether the sodium ions in the hypothalamus play an active role in the genesis of hyperthermia. Or is the lack of calcium ions in this structure solely responsible for heat production? Furthermore, if the set-point for body temperature is determined by an ionic mechanism, what is its precise anatomical locus?

In the present experiments, isolated regions within the hypothalamus of the unanaesthetized cat were perfused by means of a push-pull cannula modified after Gaddum (1961). The results suggest that the posterior area of the hypothalamus could contain an ionic mechanism for the set-point. Within this region, sodium and calcium ions appear to be of equal importance in maintaining the reference temperature.

METHODS

Each of sixteen cats weighing from 2·6 to 3·5 kg was anaesthetized with pentobarbitone sodium (33 mg/kg) injected intraperitoneally. Following surgical procedures described earlier (Myers, 1967), an array of four guide tubes was implanted stereotaxically under rigid aseptic precautions. Each guide tube was cut from 17-gauge stainless-steel tubing and fitted with an indwelling stylet of corresponding length. The tubes were positioned 4 mm above the rostral, caudal or other areas of

the hypothalamus and permanently affixed to the calvarium by cranioplast cement, which was packed around the tubes and stainless-steel anchor screws. A polystyrene pedestal was screwed to the skull and capped so that a sterile preparation could be maintained for the duration of the experiments.

Push-pull perfusions

Before an experiment began, the base-line temperature of the cat was recorded by means of a flexible YSI thermistor probe inserted into the colon to a depth of 10 cm, and held in place by adhesive tape wrapped gently around the base of the tail.

To perfuse an isolated region of the hypothalamus, the concentric cannulae of the push-pull assembly (Myers, 1970*a*) were lowered through each guide tube. The outer or pull cannula was made of 20-gauge stainless-steel needle tubing, and the inner or push cannula from 27-gauge tubing. The push cannula was passed through the rubber diaphragm of the cannula cap and was extended one mm beyond the tip of the pull cannula. When bromophenol blue dye was perfused with the tips in this position, a sphere of tissue one mm in diameter was stained blue. Each cannula was connected by polyethylene tubing to a calibrated push and a pull syringe, mounted on a multi-channel infusion withdrawal pump (Harvard Apparatus, Co.). The depth of the penetration of the push-pull cannulae into brain tissue could be varied simply by altering the length of a polyethylene spacer placed over the push cannula.

Ion-exchange water which was glass distilled was used to prepare a perfusion solution which was rendered pyrogen-free by standard heating methods or by passing the solution through a sterile millipore filter. The control perfusate was a modified Krebs solution of the following constituents: Na 143·0 mM; K 5·87 mM; Ca 2·6 mM; Cl 128·2 mM; glucose 5·6 mM; Mg 1·2 mM; SO_4 1·2 mM; H_2PO_4 1·2 mM; HCO_3 25·0 mM. Sucrose solutions were prepared in concentrations of 9·25 % (270·4 mM) and 18·5 % (540·8 mM) using the same sterile procedures. When a 0·9 % sodium chloride solution was used, the sodium was considered to be 13·6 mM in excess of the value of extracellular fluid (Davson, 1967). There is evidence for extracellular fluid in the brain (van Harreveld & Crowell, 1964), and it is assumed that the ionic concentration in Krebs solution is reasonably similar to that of this fluid (Woodbury, 1965). In several control experiments, a four-cation solution was perfused which contained the chloride salts of: Na 145·0 mM; Ca 1·3 mM; K 3·5 mM and Mg 1·0 mM. In the experiments in which sodium ion concentrations in the Krebs solution were increased, sodium toluene-*p*-sulphonate was used according to the method of Bülbring & Kuriyama (1963) in order to avoid other changes in ionic composition.

Before an experiment, the cat was fed its daily ration of cat food and was then removed from its cage. It was restrained if necessary as gently as possible, and during a perfusion showed no untoward signs of discomfort or disturbance. The room temperature varied between 23 and 24° C.

At the conclusion of each series of experiments, the placements of the cannulae were verified according to standard histological procedures. The cat was killed by an overdose of intraperitoneal pentobarbitone sodium, and 10 % formalin was perfused through the thoracic aorta after the heart was clamped. The brain was washed and blocked, and sections were taken at 24 micra on a freezing microtome and stained for cells and fibres following a method modified after Klüver–Barrera (1953).

RESULTS

When an isolated region of the hypothalamus of the unanaesthetized cat was perfused with a solution in which the ionic constituents differed from the values found in extracellular fluid (Davson, 1967), changes in

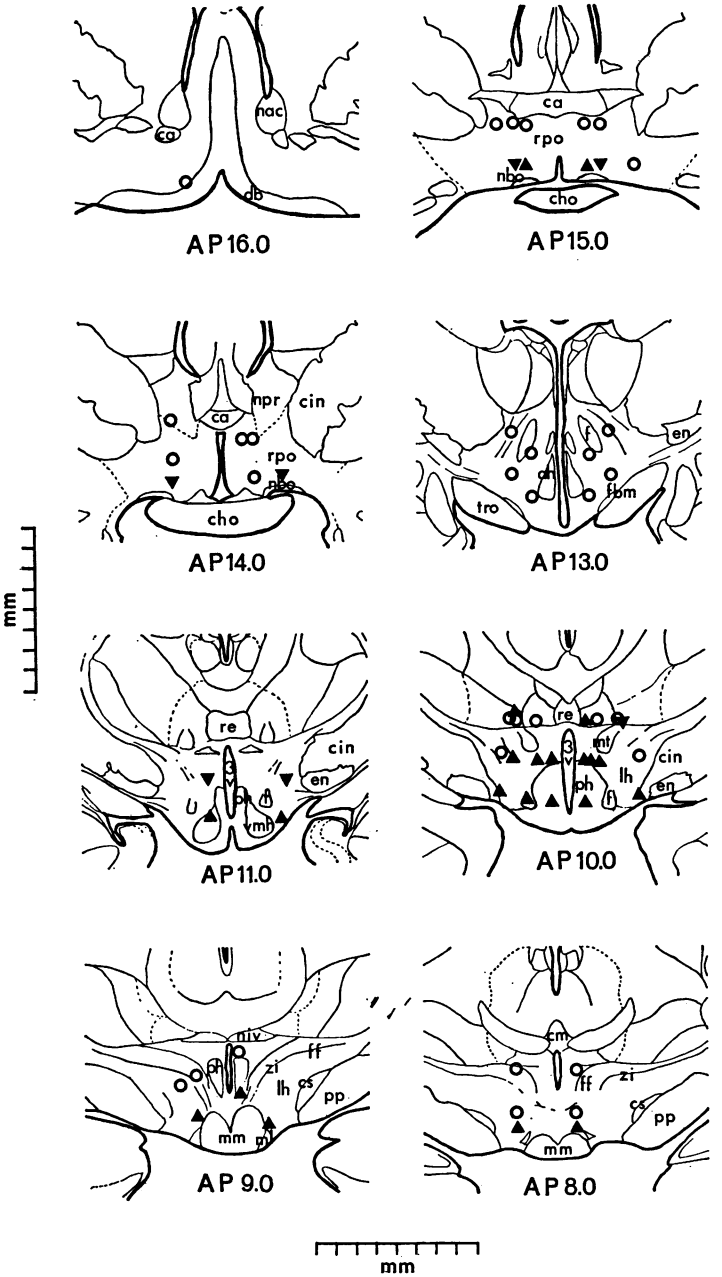

Fig. 1. For legend see opposite page.

temperature occurred. However, a hyper- or hypothermic response was produced only when the ratio of sodium and calcium ions was changed. Histological examinations of sixty-three loci within the hypothalamus of the cat revealed a distinct distribution of specific anatomical sites which were sensitive to alterations in the ratio of these two ions.

'Mapping' of sodium and calcium sensitive sites

An increase in the concentration of sodium from 13·6 to 68 mM above the physiological level produced a steep rise in temperature (▲), hypothermia (▼), or no change in temperature (○). Fig. 1 presents an anatomical 'mapping' in coronal planes which extend from the mammillary region at AP 8.0 to the area of the diagonal band of Broca at AP 16.0, but excluding AP 12.0. A site was categorized as being sensitive to an ion if a temperature change of 0·4° C or more occurred in either direction within the first hour after a perfusion was begun.

In one region of the hypothalamus, an elevated sodium ion concentration always evoked a significant rise in the temperature of the cat. If calcium was absent from the perfusion fluid, the temperature increased during the perfusion. This hyperthermia was evoked when sodium ions were perfused at a number of sites, shown in Fig. 1, found to be located predominantly in the coronal planes of AP 9.0, AP 10.0 and AP 11.0. On the other hand, at several sites within the perifornical, anterior and lateral pre-optic areas, excess sodium ions produced a hypothermic response; however, the fall in temperature was almost always less than 0·5° C and occurred only at four out of twenty-six sites located in planes AP 13.0

Legend to Fig. 1.

Fig. 1. Anatomical 'mapping' at eight coronal (AP) planes of sites within the hypothalamus of the cat at which sodium ions were perfused by means of push-pull cannulae. Sites are shown at which sodium in a concentration 13·6–68·0 mM above that of extracellular fluid produced hyperthermia (▲), hypothermia (▼), or no change in temperature (○). The criterion for a change in temperature was a rise or fall of 0·4° C or more. The scales are in mm and each AP level represents the distance in mm rostral to stereotaxic zero. ah—anterior hypothalamic area; ca—anterior commissure; cho—optic chiasm; cin—internal capsule; cm—central medial nucleus of the thalamus; cs—subthalamic nucleus; db—diagonal band of Broca; en—endopeduncular nucleus; f—fornix; fbm—medial forebrain bundle; ff—fields of Forel; lh—lateral hypothalamic area; ml—lateral mammillary body; mm—medial mammillary body; mt—mammillothalamic tract; nac—nucleus accumbens of the septum; nbo—supraoptic nucleus; niv—interventricular nucleus of the thalamus; npr—prothalamic nucleus; ph—posterior hypothalamic area; pp—cerebral peduncle; re—nucleus reuniens of the thalamus; rpo—preoptic area; tro—optic tract; vmh—ventromedial nucleus; zi—zona incerta; 3v—third ventricle.

through AP 16.0. Other behavioural and physiological responses were usually associated with the hypothermia which included tachypnea, feeding, increased motor activity, persistent vocal responses, circling, searching behaviour, sniffing and washing. Table 1 gives the mean change in temperature following the perfusion of different areas of the hypothalamus with sodium ions in different concentrations.

TABLE 1. Average maximal change from base-line temperature of unanaesthetized cats during the first 60 min of repeated push-pull perfusions of the anterior, posterior and other hypothalamic areas. The millimolar values represent the ion concentrations above those in a standard Krebs solution. The control perfusates consisted of isotonic sucrose; double isotonic sucrose; Krebs solution; Krebs solution with double the weight of each salt; and a solution containing Na, Ca, K and Mg in physiological concentrations

Ion	Concentration (mM)	Perfusions	Mean change in ° C and S.E.	
	Posterior hypothalamic area			
Na	13·6	$n = 10$	+0·55	±0·05
Na	34·0–68·0	$n = 8$	+0·64	±0·10
Ca	2·6	$n = 4$	−0·48	±0·02
Ca	10·4	$n = 11$	−0·66	±0·11
K	4·7–47·0	$n = 9$	−0·03	±0·08
Mg	1·2–12·0	$n = 4$	0·00	±0·11
Controls	—	$n = 10$	+0·01	±0·01
	Anterior hypothalamic area			
Na	13·6–68·0	$n = 8$	−0·36	±0·19
Ca	2·6–10·4	$n = 6$	+0·12	±0·23
K	4·7–23·5	$n = 3$	−0·13	±0·30
Mg	1·2–6·0	$n = 3$	−0·01	±0·33
Controls	—	$n = 5$	+0·18	±0·08
	Other areas of the hypothalamus			
Na	13·6–68·0	$n = 35$	−0·07	±0·06
Ca	2·6–10·4	$n = 17$	−0·39	±0·09
K	4·7–47·0	$n = 5$	−0·08	±0·07
Mg	1·2–12·0	$n = 3$	0·00	±0·04
Controls	—	$n = 11$	−0·16	±0·07

An anatomical 'mapping' is presented in Fig. 2 of those sites at which calcium ions, 2·6 or 10·4 mM in excess of the extracellular concentration, raised the body temperature (▲), lowered it (▼) or had no effect (○). Nearly all of the sites are the same as those at which the sodium was also elevated; hence the map extends from the coronal plane AP 8.0 to AP 16.0 and excludes AP 12.0. A fall in temperature was produced by the perfusion of calcium ions at all sites in the posterior hypothalamic area dorsal

to the mammillary bodies, and also in the region of the ventromedial nucleus at the coronal planes AP 9.0, AP 10.0 and AP 11.0. A hypothermic response also occurred when calcium ions were perfused at sites adjacent to the posterior hypothalamic area (Fig. 2). At AP planes located more rostrally, calcium produced either a fall or rise in temperature, or no change at all.

As shown in Table 1, the intensity of the hypothermia was much greater when calcium was perfused in the posterior hypothalamic area. In fact, in the anterior hypothalamus, the perfusion with excess calcium ions usually evoked a slight but insignificant rise with a mean of 0·12° C for six experiments. Other physiological and behavioural changes accompanied the alterations in temperature produced by calcium ions, which include feeding behaviour, sedation, sleep, a decline in respiratory rate and vasodilatation.

Effects of different ion concentrations on the posterior hypothalamus

The perfusion of sites in the hypothalamus (Fig. 3, inset) with Krebs and other control solutions produced no significant alteration in the temperature of the cat. Usually, whilst the animal was being held, temperature increased 0·1–0·3° C, and then fell to a corresponding extent when the animal was returned to its cage following the perfusion. Table 1 gives the mean changes in temperature for the control perfusions.

The records of colonic temperature are illustrated in Fig. 3 for a cat in which the posterior hypothalamus at two bilateral sites was perfused for a 30 min interval with Krebs solution alone (Fig. 3*A*) and Krebs solution containing sodium (Fig. 3*B*) or calcium (Fig. 3*C*) in excess of the normal values in extracellular fluid. When sodium toluene-*p*-sulphonate was added to the Krebs solution to raise the sodium concentration by 34 mM, a rapid rise in temperature of over 1·0° C occurred in less than 1 hr. During the perfusion, the cat shivered vigorously, piloerection occurred, the ear vessels constricted and the animal became active. Temperature began to return to the normal level within 15 min after the perfusion had ended (Fig. 3*B*).

When the concentration of calcium ions in the perfusion fluid was increased to 10·4 mM in excess of the physiological value, the temperature fell precipitously by more than 1·0° C. During this decline, the ear vessels of the cat became dilatated, the respirations slowed and frequently the animal showed signs of sedation and drowsiness. The hypothermia, illustrated in Fig. 3*C*, persisted for more than 1 hr after the termination of the perfusion, after which temperature returned to normal base-line level.

In order to examine the independent effects of sodium and calcium ions on the hypothalamus of the cat, each in the absence of the other, isotonic

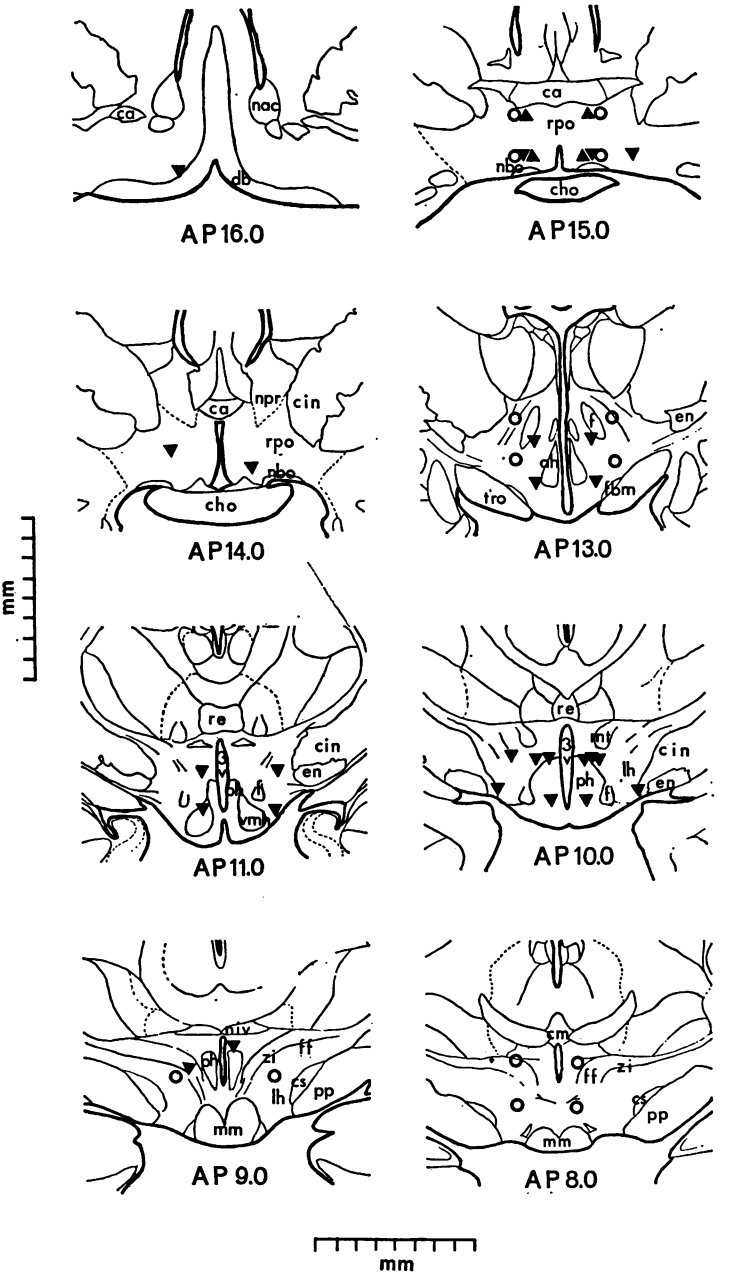

Fig. 2. For legend see opposite page.

sucrose was used as the perfusion medium. By this method, the isotonicity of extracellular fluid within the posterior hypothalamus was maintained, and at the same time the concentrations of the two ions could be varied separately. Fig. 4 illustrates the results of three 30 min bilateral perfusions at two sites within the posterior hypothalamus of one cat. A 9·25 % sucrose solution caused no change in temperature other than that produced by the experimental procedure itself (Fig. 1*A*). However, 34 mM sodium in the form of the toluene-*p*-sulphonate salt added to the 9·25 % sucrose perfusate evoked a sharp rise in temperature of nearly 1·0° C. The hyperthermic response, shown in Fig. 4*B*, was accompanied by vigorous shivering and vasoconstriction. As soon as the perfusion was terminated, the temperature of the cat began to return to the base-line level. When 2·6 mM calcium was added to the isotonic sucrose solution and perfused at the same loci, the temperature of the cat declined. This is shown in Fig. 4*C*. The temperature did not begin to return to the normal level until after an hour had elapsed following the perfusion.

Generally, as shown in Table 1, the magnitude of the hyper- or hypothermia produced by sodium or calcium, respectively, was dependent on the millimolar concentration of these ions in the solution perfused within the posterior hypothalamus. Furthermore, a sucrose solution twice its isotonic concentration (18·5 %) or a Krebs solution with the concentration of each of the ionic constituents doubled (630 mM) had no observable effect on temperature, when either solution was perfused through circumscribed areas of the hypothalamus. As long as the concentrations of sodium and calcium ions in the perfusion fluid were in a ratio equal to that found in extracellular fluid or were absent altogether, no change in temperature occurred. Fig. 5*A* illustrates this finding, since sodium and calcium were the only cations in the perfusate at concentrations of 143·0 and 2·6 mM, respectively. Again, the slight deviations in temperature of from 0·1 to 0·3° C, which occurred during the interval of perfusion, were similar to those observed when Krebs solution was used as a control perfusate. Other essential cations, potassium and magnesium, also failed to alter the temperature of the cat when they were elevated by as much as five to ten times

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Legend to Fig. 2.

Fig. 2. Anatomical 'mapping' at eight coronal (AP) planes within the hypothalamus of the cat at which calcium ions were perfused by means of push-pull cannulae. Sites are indicated at which calcium ions 2·6 to 10·4 mM above the concentration of extracellular fluid produced hypothermia (▼), hyperthermia (▲) or no change in temperature (○). The scales are in mm and the anatomical abbreviations are the same as in Fig. 1.

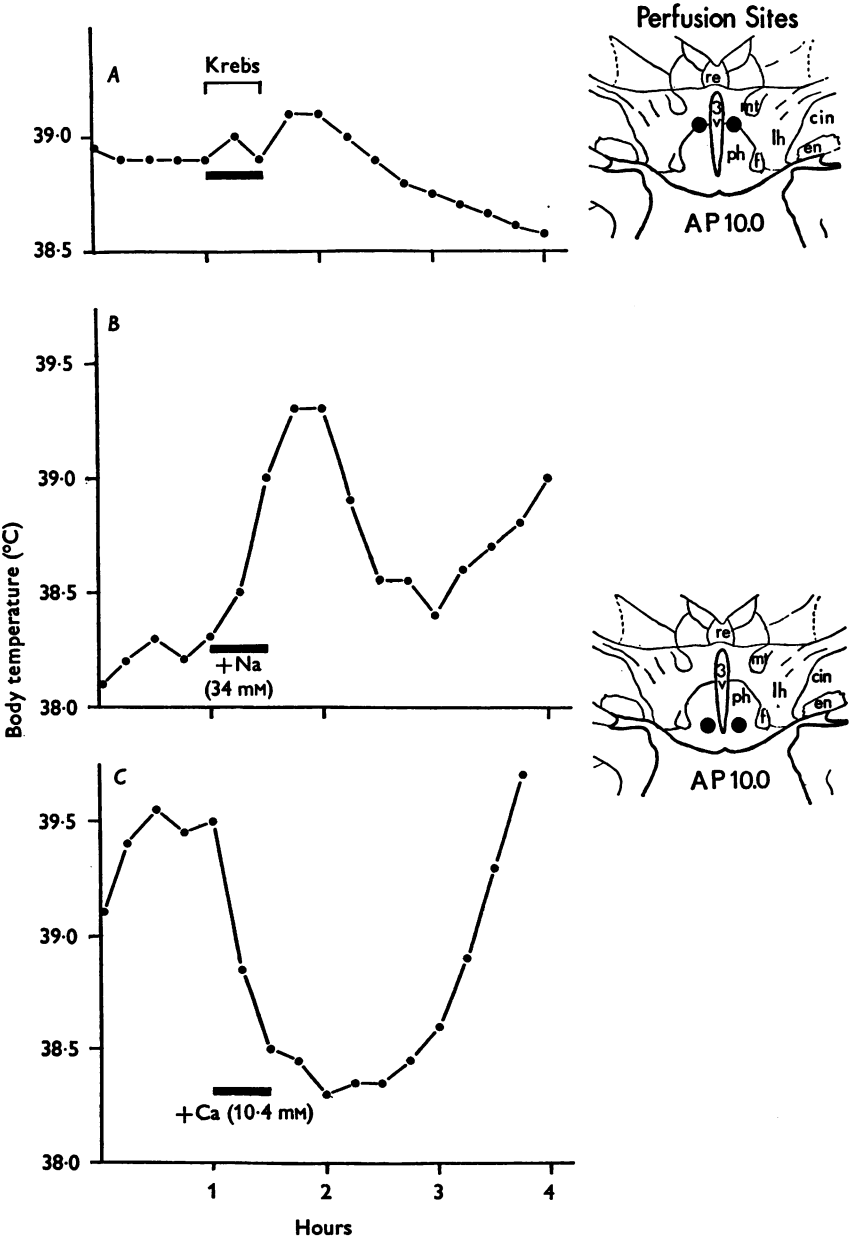

Fig. 3. Temperature records of an unanaesthetized cat in response to the local perfusions for 30 min of: *A*, Krebs solution alone; *B*, Krebs solution plus 34 mM excess sodium; and *C*, Krebs solution plus 10·4 mM excess calcium. The sites of the bilateral perfusions are indicated by the filled circles (●) in the inset.

their normal physiological concentrations. Fig. 5*B* and *C* also shows the record of body temperature obtained from two cats in which sites in the posterior hypothalamic area were perfused with 23·5 mM potassium and 9·7 mM magnesium, respectively.

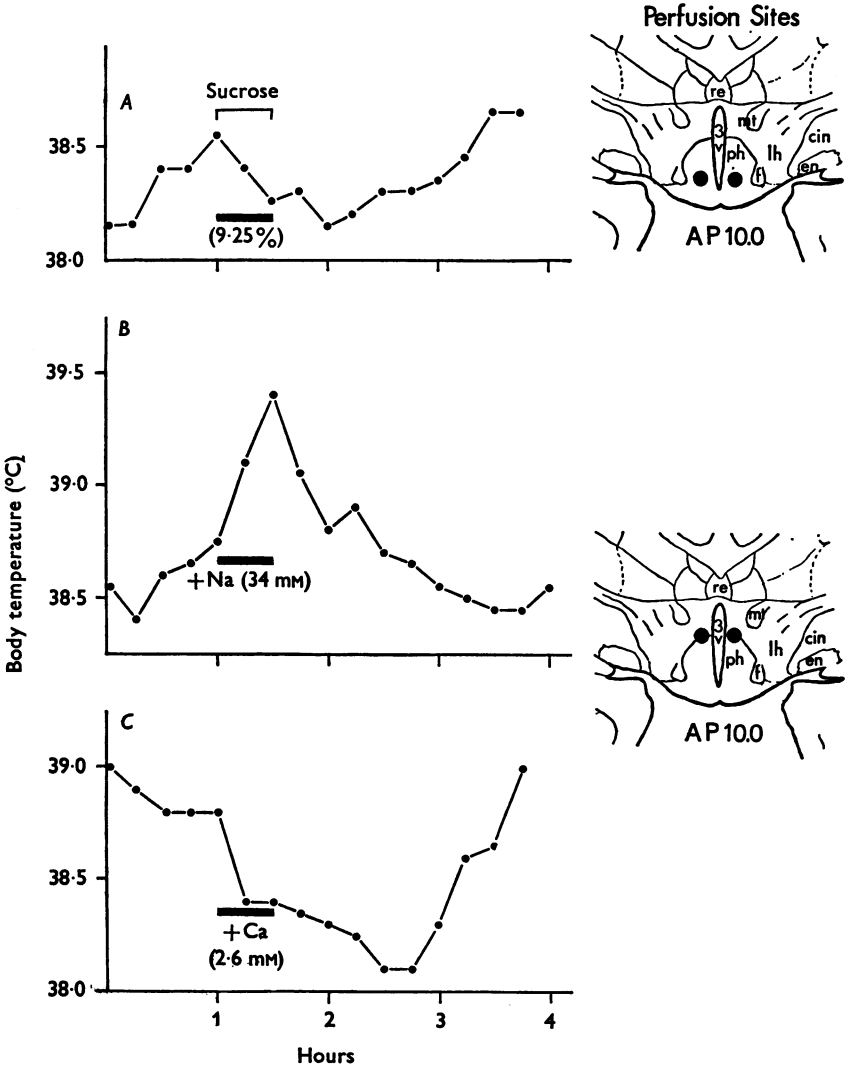

Fig. 4. Temperature records of an unanaesthetized cat in response to the local perfusions for 30 min of: *A*, the sucrose solution alone; *B*, sucrose solution plus 34 mM sodium; and *C*, sucrose solution plus 2·6 mM calcium. The bilateral perfusions are indicated by the filled circles (●) in the insets. The sites for *B* and *C* are the same.

Effects of ions on other hypothalamic areas

When sodium, calcium, potassium or magnesium ions were perfused locally within the anterior, pre-optic region or other areas of the hypothalamus, in excess of their normal physiological concentration, usually no significant change in the temperature of the animal occurred. Fig. 6 presents the temperature records of four cats in which these ions were per-

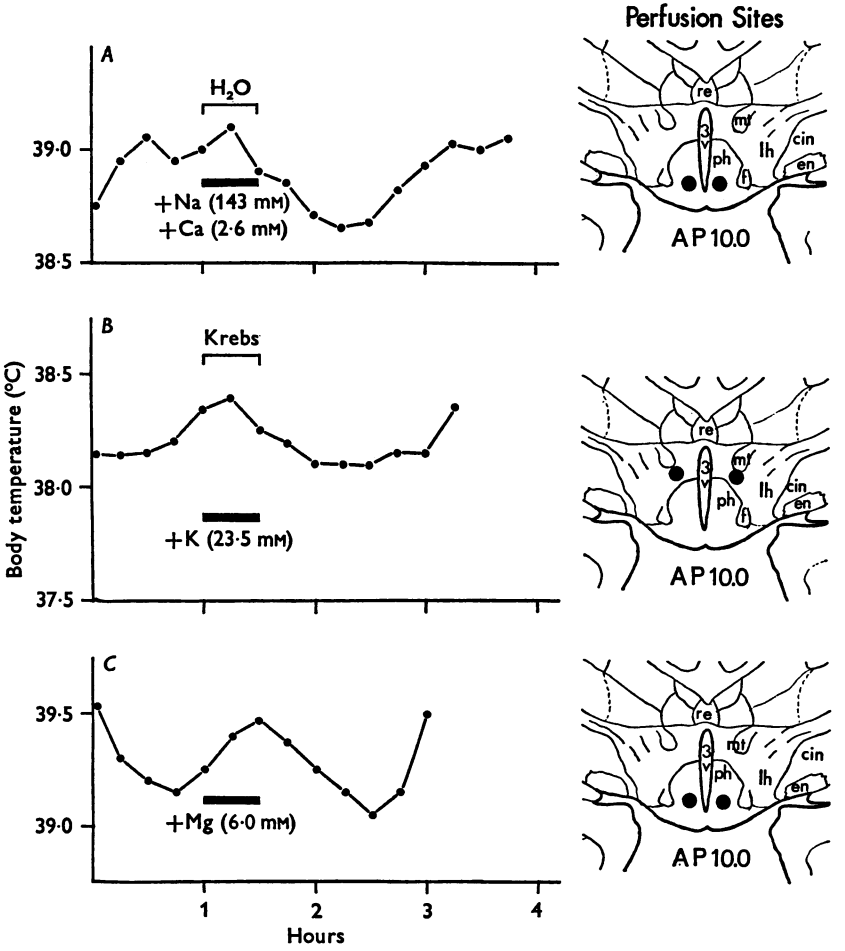

Fig. 5. Temperature records of two unanaesthetized cats in response to the local perfusions for 30 min of: *A*, a solution containing sodium and calcium in concentrations equal to those of extracellular fluid; *B*, Krebs solution plus 23·5 mM excess potassium; and *C*, Krebs solution plus 9·7 mM excess magnesium. The sites of the bilateral perfusions are indicated by the filled circles (●) in the insets. Records *A* and *C* were obtained from the same cat.

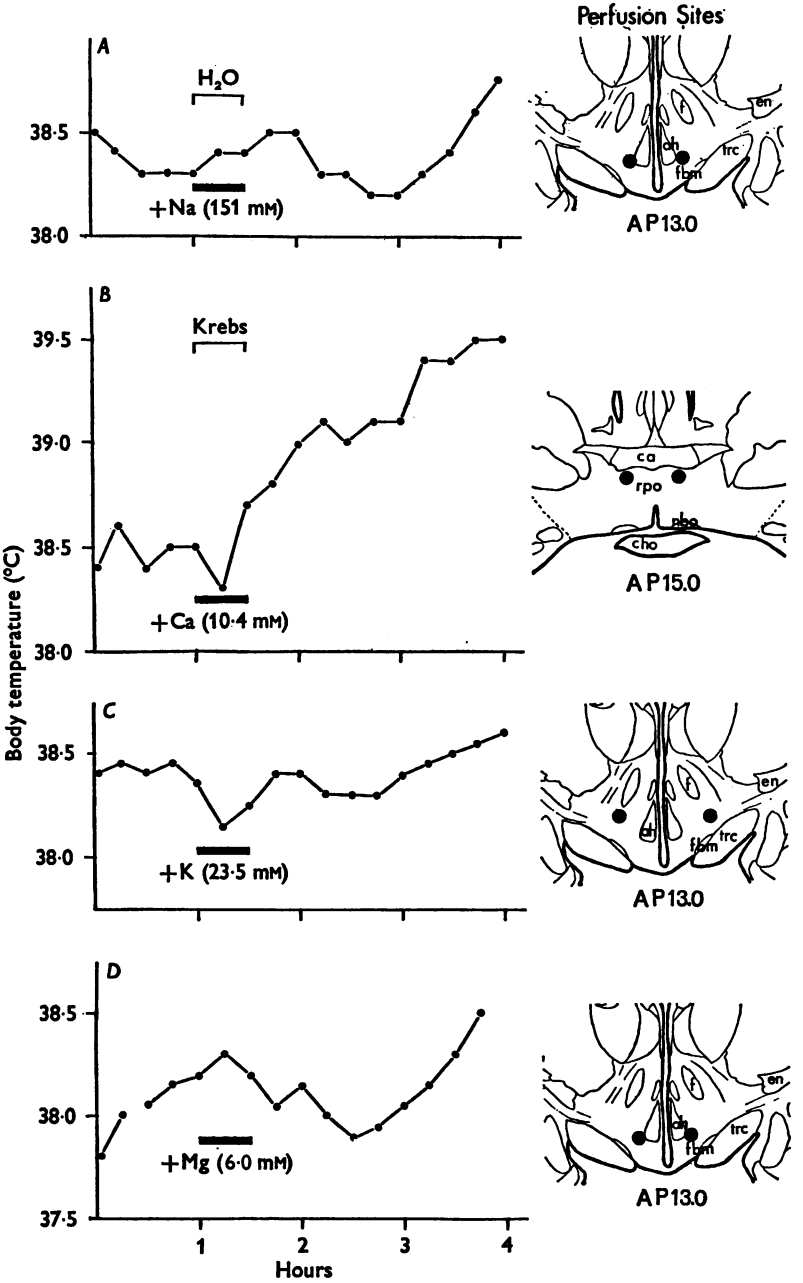

Fig. 6. Temperature records of three unanaesthetized cats in response to the local perfusions for 30 min in the rostral hypothalamus of: *A*, sodium chloride solution containing 151·0 mM sodium; *B*, Krebs solution plus 10·4 mM excess calcium; *C*, Krebs solution plus 23·5 mM excess potassium; and *D*, Krebs solution plus 9·7 mM excess magnesium. The sites of the bilateral perfusions are indicated by the filled circles (●) in the insets. Records *A* and *D* were obtained from the same cat.

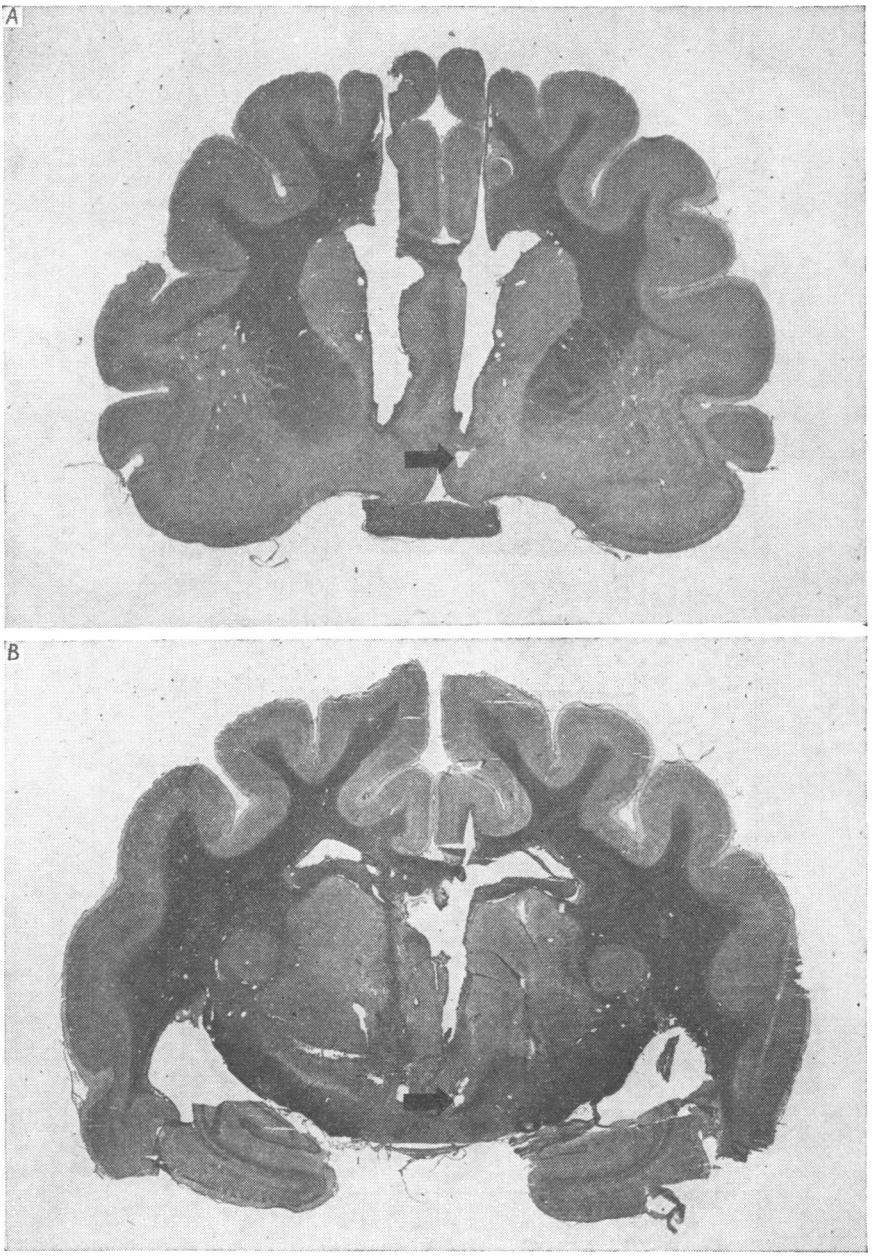

Fig. 7. Representative histological sections, taken in the coronal plane, are shown for two cats. The lesions produced at the tips of the push-pull cannulae are located in the anterior (*A*) and posterior (*B*) areas of the hypothalamus, as indicated by the arrows.

fused at sites in planes AP 13.0 and AP 15.0 indicated in each inset. It is interesting that a hyperthermic response can occur during the perfusion of calcium ions in the anterior hypothalamus, and may continue even after the perfusion is terminated. An example of such a response is illustrated in Fig. 6*B*. This finding corresponds to the finding of Feldberg and his co-workers, who reported that in the unanaesthetized cat the perfusion of the cerebral ventricles with different solutions often produced a long-lasting after rise in temperature (Feldberg *et al.* 1970).

A lesion was created by repeated perfusions at the tips of the push-pull cannulae which was surprisingly no larger than that produced by a micro-injection. The reason for this is that the inflow and outflow rates were identical, and the perfusate was drawn off at precisely the same rate as it was pumped in. Fig. 7 shows two representative histological sections illustrating the lesions produced by repeated perfusions of the anterior (Fig. 7*A*) and posterior (Fig. 7*B*) hypothalamus.

DISCUSSION

The temporary alteration in the balance between sodium and calcium ion concentration by perfusion within different parts of the hypothalamus produces a number of physiological changes. Depending upon the site of perfusion, either of the ions seems to possess an excitatory or inhibitory action on those regions involved in the control of body temperature. The most significant and constant finding is that the shift in the ratio between sodium and calcium concentrations in the posterior area of the hypothalamus causes an immediate hyper- or hypothermia during the interval of perfusion.

Earlier, Feldberg *et al.* (1970) suggested that the calcium levels in the hypothalamus may be the physiological basis of the set-point for body temperature. This suggestion was based on experiments in which the omission of calcium from the solution perfused from the lateral ventricle to the cisterna magna resulted in a rise in the temperature of the unanaesthetized cat. As a result of the present experiments, an ionic basis for the set-point of body temperature receives further support, for the following reasons.

Changes in sodium concentration were as effective as those in calcium concentration in altering the temperature of the cat. When isotonic sucrose was perfused within discrete areas of the posterior hypothalamus, the temperature of the animal did not change. However, when as little as one fourth of the normal physiological concentration of sodium was added to this inert perfusion fluid, the temperature of the cat rose sharply. In the same way, calcium added to the sucrose solution in the absence of sodium

produced a pronounced fall in temperature when the calcium ions were perfused within the posterior region of the hypothalamus.

The caudal region of the hypothalamus has been regarded traditionally as the heat maintenance 'centre' (Benzinger, 1969). A lesion in this area may cause a mammal to become poikilothermic (Ranson, 1940), and an ablation can even prevent the development of a fever following the intravenous injection of a bacterial pyrogen (Thompson, Hammel & Hardy, 1959). Anatomically, the anterior hypothalamic, pre-optic area, which contains thermosensitive cells (Nakayama, Eisenman & Hardy, 1961; Hardy, Hellon & Sutherland, 1964; Hellon, 1967) is considered to be the site of the thermostat involved in the control of body temperature. More recently, some thermosensitive neurones have been found in an area extending from the posterior hypothalamus to the mesencephalon (Nakayama & Hardy, 1969; Cabanac & Hardy, 1969). From the monoamine theory of thermoregulation (Feldberg & Myers, 1964) heat production would be activated by the release of 5-HT within the anterior hypothalamus when an animal is cold (Myers, Kawa & Beleslin, 1969), whereas noradrenaline is released to inhibit the heat production pathway when the animal is warm (Myers, 1969; Myers & Yaksh, 1969). The release of these substances within the thermostat are presumed to occur in response to thermal stimuli, and help to regulate temperature around a given set-point which has been determined inherently (Myers & Veale, 1970). Thus, it is envisioned that an efferent impulse arising from the anterior region is transmitted through the caudal hypothalamus to activate heat production or dissipation around the postulated ionic set-point located there.

The ratio of the concentration of sodium to calcium ions rather than their absolute concentration, seems to determine the level around which the animal's body temperature is regulated. In support of this, when the concentration of all ions was doubled in the Krebs solution which perfused the posterior hypothalamus, the temperature of the cat was not affected. Similarly, when both ions were absent altogether from the perfusion medium, the animal's body temperature again remained stable, at least for the relatively short duration of the perfusion. It would seem that the greatest changes in temperature occur not as a result of the absence in extracellular fluid of one ion or the other, but rather if the balance between the two ions is disturbed. Our hypothesis is that the constancy of the concentration of these ionic constituents within the diencephalon is a principal factor in the steady-state activity of the cells in the posterior hypothalamus mediating temperature responses (Myers & Yaksh, 1969). That is, the external ionic milieu (van Harreveld & Crowell, 1964; Woodbury, 1965) in this region determines the discharge rate of the neurones of the posterior

hypothalamus, and the constancy in the activity of the cells delegated to activating heat production is that which maintains the body temperature at or about 37° C. The possibility does, of course, exist that although the firing rate of the nerve cells may be increased by one ion and lowered by the other, when the concentrations of the ions are altered artificially, this may not occur naturally within the brain stem of the cat.

The balance between sodium and calcium could also play an important part in the development of a fever due to a bacterial pyrogen. It has been suggested that a pyrogen may cause an upward shift in the temperature set-point (Fox & MacPherson, 1954; Cooper, Cranston & Snell, 1964; Wit & Wang, 1968). If this is the case, then a disequilibrium in the ratio of sodium to calcium might occur following the systemic invasion of a bacterial pyrogen. Recently, Skarnes showed that an endotoxin exerts a transitory reduction in the levels of ionized calcium in the plasma (Skarnes, 1970). A slight change in the concentration of serum calcium ions also could be reflected within the posterior hypothalamus and this would shift the set-point to cause a rise in temperature. The direct action of a pyrogen on the cells of the anterior hypothalamus (Myers, 1971; Cooper, Cranston & Honour, 1967; Cabanac, Stolwijk & Hardy, 1968; Eisenman, 1969) would be sufficient to augment even further the elevated set-point.

Although patients with a chronic hypernatremia do not ordinarily exhibit the clinical signs of a febrile condition (Cooper, 1970), an alteration in the ratio of ions in the posterior region may perhaps be only transitory in nature. In several recent experiments with cats, we have found that after ^{45}Ca had been equilibrated in body fluids following its systemic injection, the levels of ^{45}Ca in the posterior hypothalamus declined for a period of 60–90 min following the intraventricular administration of typhoid vaccine. Within the anterior hypothalamus, the tendency of this labelled calcium was to rise, whereas in the mesencephalic reticular formation, ^{45}Ca levels remained relatively constant (Veale, 1970; R. D. Myers, W. L. Veale and M. Tytell, unpublished observations).

It is not clear why the other ions, potassium and magnesium, did not produce any change in body temperature when they were perfused within different areas of the hypothalamus. Earlier, Cooper *et al.* (1965) had found that micro-litre injections of potassium ions produced a sharp rise in temperature. The discrepancy in these results may rest simply in the difference between the two procedures, since the push-pull perfusion results only in an exchange of a fluid at a given site rather than in the addition of a volume of fluid following a micro-injection. There is little distortion or disturbance to the tissue because the effluent collected is identical to the volume infused.

The concept that the constant ratio between the concentrations of

sodium and calcium ions could determine the temperature set-point corresponds well with the amine theory of thermoregulation (Feldberg & Myers, 1964; Myers, 1970*b*) and provides a physiological explanation for other phenomena. For instance, when the content of 5-HT or noradrenaline is lowered in the brain stem, normal temperature regulation is not affected significantly unless the animal is exposed to a thermal stressor or given endotoxin (Reid, Volicer, Smookler, Beaven & Brodie, 1968; Giarman, Tanaka, Mooney & Atkins, 1968). This could be explained by the fact that the set-point is not influenced by the reduction of either of these amines, and hence the constancy in the activity of the cells in the posterior hypothalamus sustains the normal temperature of the animal.

Thus, we conclude that as long as the inherent and constant ratio between sodium and calcium concentrations is maintained within the posterior hypothalamus, the set-point is established at a level around which body temperature is regulated. Of course, this hypothesis requires for its proof evidence that changes in the concentration of these ions, such as those postulated to occur during a pyrogen-induced fever, do in fact take place. Further, it will be necessary to show that during thermoregulatory changes around a set-point, the actual concentration of the ions, when measured directly, remains unchanged.

This research was supported in part by U.S.A. Office of Naval Research Contract N00014-67-A-0226-0003 and National Science Foundation Grant GB 7906. We are indebted to P. Curzon for his valuable technical assistance.

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