Pflügers Arch. 321, 56–66 (1970)

The Stimulation of Hypothalamic Neurones by Changes in Ambient Temperature

R. F. Hellon

From National Institute for Medical Research, Mill Hill, London, NW 7, England

Received June 8, 1970

Summary. 1. Unit activity has been recorded from the antero-medial hypothalamic area of sedated rabbits while ambient and hypothalamic temperatures were independently varied.

2. Out of 202 neurones tested, 19 were found which responded in less than 1 min to rapid changes in ambient temperature of $\pm 15°$ C. Thirteen neurones responded only to cooling, two only to warming and four to both warming and cooling.

3. For a given direction of temperature change, some units showed an accelerated firing rate while others were slowed.

4. It was possible to test 8 of these cells which responded to ambient temperature changes for their sensitivity to hypothalamic temperature. Six of them were found to have this additional temperature response.

5. The results support recent hypotheses of temperature regulation in that they show there is a convergence of thermal information from peripheral and central receptors at the level of the anterior hypothalamus.

Key-Words: Hypothalamic Neurones — Environmental Temperature — Temperature Sensitivity — Temperature Regulation.

Schlüsselwörter: Hypothalamische Neuronen — Umgebungstemperatur — Temperaturempfindlichkeit — Temperaturregulation.

There is now ample evidence to show that the pre-optic and anterior hypothalamic regions of the brain are specifically sensitive to small changes of temperature. Local heating in the hypothalamus of unanaesthetized animals activates heat loss mechanisms which result in a fall in body temperature, while local cooling has the reverse action. This type of experiment has now been carried out on dogs, cats, rabbits goats and oxen. Similar reactions can also be produced by selective heating or cooling of the spinal cord.

The heat-sensitive neurones which are presumed to be responsible for these reactions have been studied by the technique of unit recording in the hypothalamus of cats (Nakayama, Hammel, Hardy and Eisenman, 1963; Eisenman and Jackson, 1967), dogs (Hardy, Hellon and Sutherland, 1964) and rabbits (Cabanac, Stolwijk and Hardy, 1968; Hellon, 1967). These experiments showed that about 10 % of the neurones

close to the midline in the preoptic and anterior hypothalamic regions had a marked sensitivity to local temperature, while the other 90% failed to respond. The firing rate of these sensitive cells was either positively or negatively correlated with temperature. For many cells the correlation was linear, but in some cases above or below a certain temperature they ceased to be strongly thermosensitive.

An outstanding problem in connection with these thermal sensors concerns their role in normal temperature regulation. As was first shown by Forster and Ferguson (1952) in cats and confirmed by Bligh (1959) in sheep, Findlay and Ingram (1961) in calves and Hammel (1965) in dogs, there is generally no detectable change in hypothalamic temperature during the initiation and maintenance of vigorous regulatory responses to hot or cold environments. Recent hypotheses of temperature regulation (Hammel, 1965; Stolwijk and Hardy, 1966; Benzinger, 1969) suggest that afferent impulses from the skin are an important input to the hypothalamic temperature controller, but there was no neurophysiological evidence for this until the recent work of Wit and Wang (1968). They described neurones in the cat's anterior hypothalamus which were excited first by heating the skin and then later by the passive rise in deep body temperature. This secondary response was considered to be due to an increase in hypothalamic temperature.

In the present experiments it was possible to raise or lower both ambient and hypothalamic temperatures independently and to record their separate effects on the firing rate of neurones in the rabbit hypothalamus. A brief account of some of the results has already been published (Hellon, 1969).

Methods

New Zealand white rabbits were used, mostly female and weighing between 2.7 and 4.2 kg.

Those techniques which have been described in detail previously (Hellon, 1967) need only be summarized here. About 5 days before an experiment an operation was performed under barbiturate anaesthesia. The animals were fitted with a skull plate which served as a base for a small hydraulic microdriver and also carried two thermode tubes on each side of the midline for changing brain temperature. A craniotomy was made and temporarily sealed.

At the start of an experiment a small dose of urethane (400 mg/kg i. p.) was given as a sedative, since it was found that the rabbits became restive when exposed to a cold environment. The animals were placed in a restrainer and positioned in a small open-circuit wind tunnel so as to face the air stream. Thus the head and ears were the main areas exposed to the air movement which had a velocity of 50 cm/sec. The tunnel was equipped to enable air temperature to be changed to any point within the range 10° C to 40° C and it was possible to complete any temperature change in 30 secs. Before entering the tunnel, room air passed first through a car radiator (Morris 1100 model) through which alcohol at 0—2° C was continuously circulated. This reduced the air temperature to 10° C. Immediately

beyond the radiator was a wire grid heater energized from a variable autotransformer. This enabled the pre-cooled air to be raised to the desired temperature, which was monitored by an exposed thermistor bead mounted in front of the rabbit's nose.

Extra-cellular action potentials in the anterior hypothalamus were recorded with glass micropipettes having a tip diameter of 1—2 μm, a shank diameter of 60 μm and a shaft of 0.5 mm. The potentials were amplified by a transistor circuit (Narth, 1969) mounted close by the animal and then further amplified by an oscilloscope to be recorded and analysed as already described (Hellon, 1967). Brain temperature was recorded with a thermistor which was advanced symmetrically with the electrode in the opposite hemisphere.

The electrodes were filled with dye solutions to mark the tip position at the end of a penetration. In the early experiments methyl blue was used but the marks left by this dye, although seen on the tissue block, generally disappeared during sectioning and mounting. Much more consistent results were obtained when a 2% solution of pontamine sky blue 6BX was used in 0.5 M sodium acetate. The solution has a pH of 7.7 and the electrodes had a resistance of about 2 MΩ. By passing currents of 10 μA for 2 min with the tip negative, dense blue marks were made which were about 30 μm in diameter. These marks were easily seen on the microtome and also when the sections (30 μm thick and stained with phloxin) were enlarged in a projector.

Results

The wind tunnel temperature was kept at 25° C during the search for spontaneously active neurones. Once a neurone had been located, its activity was recorded at this temperature for several minutes. The tunnel temperature was then lowered to 10° C, raised to 40° C and returned to 25° C. During this time there were only neglible changes in hypothalamic temperature. If contact with the cell was still maintained, brain temperature was then changed cyclically by 1—2° C. The duration of recording from single neurones in this sedated preparation did not usually exceed 30 min and was often shorter. For this reason it was necessary to make rapid changes in external temperature and complete the observations quickly.

Ambient Temperature Responses. A total of 202 neurones has been tested for responses to ambient temperature and 19 of them showed that they were sensitive to alteration of this temperature. The neurones showed a variety of responses to peripheral cooling and warming and the results are summarized in Table 1. This shows that four cells responded to both warming and cooling, but most of them only changed their firing rate when the wind tunnel temperature was raised or lowered and in this group a response to cooling alone was the most common.

The results plotted in Fig. 1 are from a neurone responding to both cooling and warming. The first period of cooling reduced the firing rate almost to zero. On heating the wind tunnel to 40° C there was an increase in rate to about twice that at 25° C. The second period of cooling had a

Table 1. *The number of neurones responding to ambient cooling and warming*

Responding only to cooling		Responding only to warming		Responding to both cooling and warming
Slowed	Accelerated	Slowed	Accelerated	
6	7	1	1	4

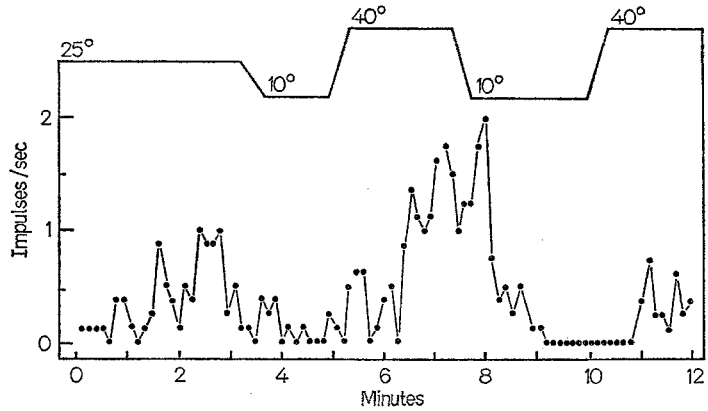

Fig. 1. Firing rate of a neurone responding to ambient temperature. Rate counted over 8 sec intervals. Ambient temperature shown by top of frame

more pronounced effect and prevented the cell from firing for nearly 2 min. Further heating was attempted just before contact with the cell was lost. Each time the temperature was changed there was a delay of 30—60 sec between the completion of the temperature change and the response of the neurone.

Fig. 2 shows the behaviour of a unit responding specifically to ambient warming. At 25° C the firing rate showed occasional increases and when the temperature was lowered to 10° C there was no clear change in this rate. With return of the temperature to 25° C there were two outbursts, but at 40° C there was a marked increase in rate, interrupted by a brief return to the initial rate. The rate subsided abruptly at the end of the heating period. Analysis of variance shows that the firing rate during the period at 40° was significantly higher than at 25° or 10° ($P < 0.001$). It appears that the responses began during the 30 sec period needed to change the wind tunnel temperature.

The results from a neurone which was accelerated by external cooling are illustrated in Fig. 3. Each of the two periods at 10° C was accompanied by a prompt increase of firing rate, while the rate at 40° C was similar

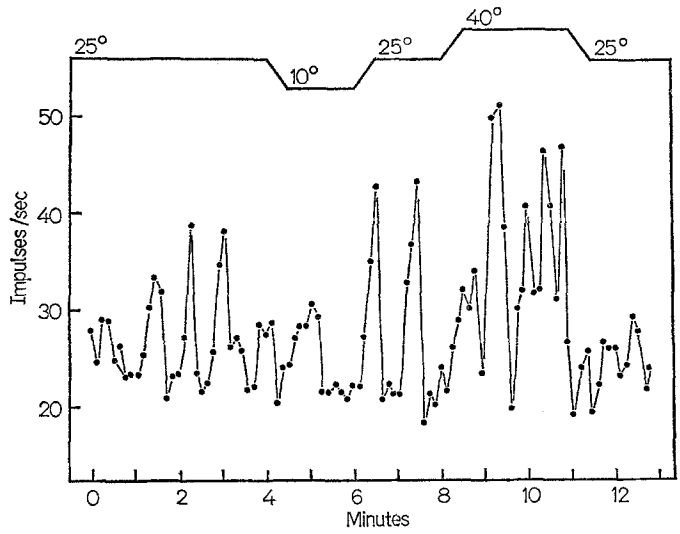

Fig. 2. Firing rate of a neurone excited by ambient heating. Rate counted over 8 sec intervals

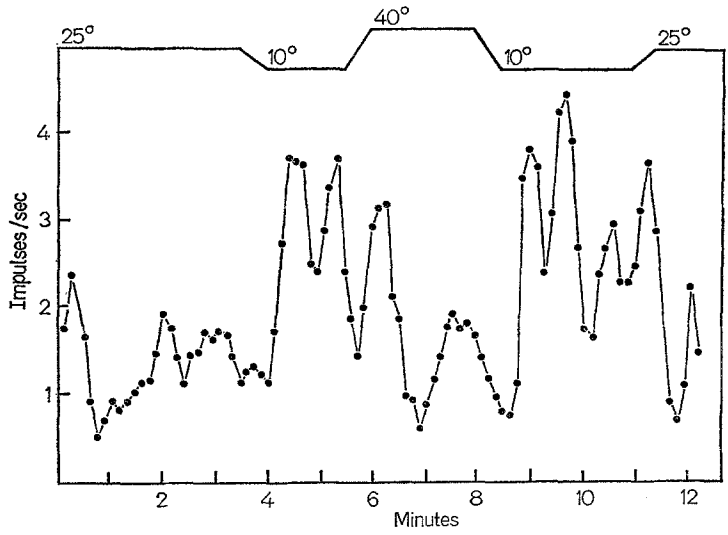

Fig. 3. Firing rate of a neurone excited by ambient cooling. Rate counted over 8 sec intervals

to that at 25° C. The opposite type of response to cooling is shown in Fig. 4. Here the firing rate was reduced from about 2.5/sec before cooling to 1/sec during cooling. As in Fig. 3, heating to 40° C had no apparent effect.

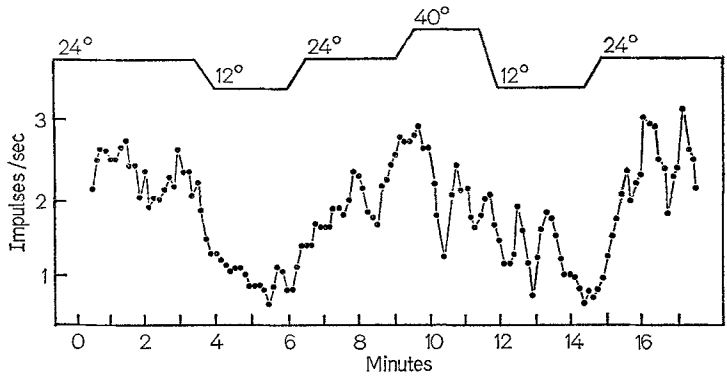

Fig. 4. Firing rate of a neurone slowed by ambient cooling. Rate counted over 8 sec intervals

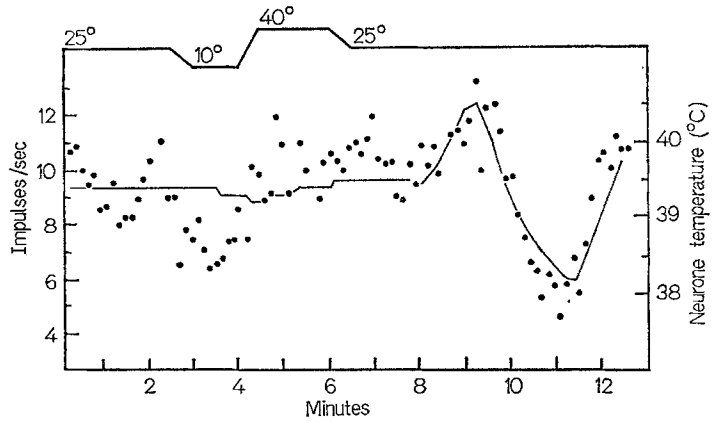

Fig. 5. Firing rate (8 sec intervals) of a neurone slowed by ambient cooling and also responding to hypothalamic temperature (solid line and right-hand ordinate)

If contact with a neurone remained when testing for ambient sensitivity was finished, brain temperature was raised and lowered by changing the thermode water temperature; this was possible with eight cells. In six of these firing rate was found to be a function of both ambient and local temperatures and of the other two, one was sensitive only to ambient temperature and the other only responded to brain temperature. The data in Fig. 5 are from one unit which was slowed by peripheral cooling, unaffected by peripheral warming and then, when brain temperature was displaced, it responded to local heating and cooling. Results from a neurone with a more complex response are plotted in Fig. 6. When the tunnel temperature was reduced to 10° C, there was a delay of about

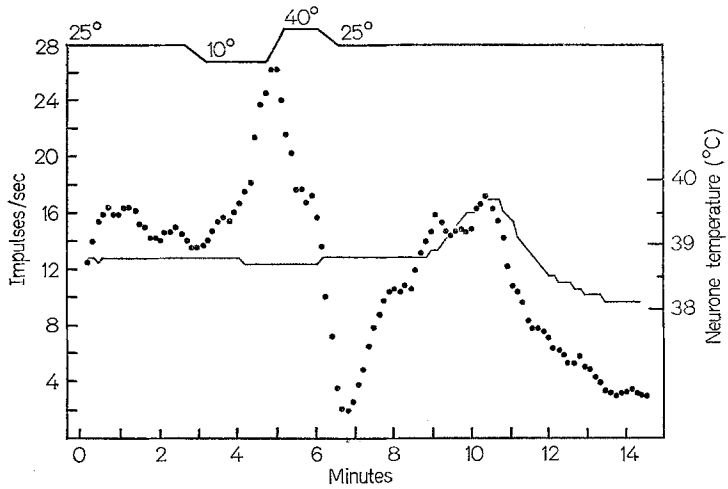

Fig. 6. Firing rate (8 sec intervals) of a neurone excited by ambient cooling, slowed by ambient heating and also responding to hypothalamic temperature (solid line and right-hand ordinate)

Table 2. *Summary of the changes in firing rate to ambient and hypothalamic temperature changes. — Denotes decrease; + denotes increase; O denotes no response*

Unit No.	Ambient temperature		Hypothalamic temperature	
	Cool	Warm	Cool	Warm
66/1	—	O	O	+
68/11	O	+	O	+
68/15 (Fig. 6)	+	—	—	O
71/14 (Fig. 5)	—	O	—	+
81/11	—	O	—	O
87/5	+	O	+	O

1 min before the firing rate increased sharply. Heating caused a dramatic reduction in rate and on returning to 25° C there was a slower acceleration to the initial rate. When brain temperature was raised and lowered, the activity followed the temperature although the response to heating was not marked.

The responses of all six neurones showing this dual sensitivity are summarized in Table 2. Each of the units showed a different response pattern. However, with the exception of unit 68/15 (Fig. 6), all the cells were affected in the same general direction by ambient and brain temperature; that is to say if ambient cooling was excitatory, then brain cooling was also excitatory. In their responses to brain temperature five of the

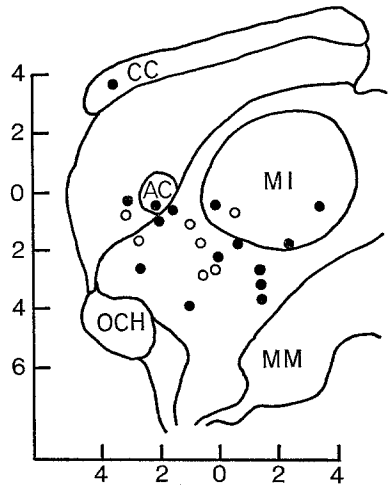

Fig. 7. Positions of temperature-sensitive neurones projected on a parasagittal section of rabbit brain. Scales in mm. Open symbols indicate neurones driven by ambient and hypothalamic temperatures; filled symbols indicate neurones driven by ambient temperature. *CC*, corpus callosum; *AC*, anterior commissure; *MI*, massa intermedia; *OCH*, optic chiasma; *MM*, nucleus mammillaris medialis

neurones responded to either warming or cooling and would be classified as type C (Hellon, 1967) while one of them responded to both warming and cooling and hence belongs to type A.

The positions of all the neurones are given in Fig. 7, which shows that recordings were made from the level of the optic chiasma rostrally to just behind the mammillary bodies caudally. All the positions were 1.5 mm or less from the midline. Four of the neurones were in the massa intermedia of the thalamus, but most of the others were more rostral and ventral in the hypothalamus itself. There seems to be no particular position for the units which were sensitive to local as well ambient temperatures.

Discussion

This paper describes some preliminary experiments which were designed to elucidate the peripheral inputs to the hypothalamic temperature controller. They show that information concerning warming or cooling of the surroundings is relayed to the hypothalamus before there is any change in hypothalamic temperature.

There have been two previous attempts to record signals in the anterior hypothalamus when skin temperature was changed. One was in anaesthetized dogs (Hardy, Hellon and Sutherland, 1964) and the

other was in the encéphale isolé preparation (Murakami, Stolwijk and Hardy, 1967). In neither of these studies was it possible to demonstrate any change in hypothalamic firing rate to peripheral stimulation. The first positive evidence is provided by the experiments of Wit and Wang (1968) who recorded more laterally than Hardy et al. or Murakami et al. while heating the whole animal (cat) with infrared lamps. They found five units 3—5 mm from the midline which increased their activity within 3—6 min after the lamps were turned on. The delay in the present experiments between the beginning of a temperature change in the wind tunnel and a change in firing rate of the units was shorter and varied from a few seconds to 1.5 min. The reason for this variation in the delay is uncertain at the moment but clearly it will depend on a number of factors including the sensitivity of the receptor, the thickness of overlying fur and the change or rate of change of temperature at the site. None of these factors is yet known nor is there any information about the pathways or degree of neuronal processing between the periphery and the hypothalamus. However, since the face and ears were the main regions exposed to the air stream it is likely that the peripheral receptors were located on these areas. So far no temperature receptors have been reported in the rabbit ear (Brown, Iggo and Miller, 1967), but on the nose of the cat Hensel has recently described a high concentration of warm and cold receptors (Hensel and Huopaniemi, 1969; Hensel and Kenshalo, 1969; Hensel and Wurster, 1969). It is not clear if the nose was heated by Wit and Wang.

The unit responses showed that while most neurones responded only to external warming or cooling a few were found which were sensitive to both warming and cooling. More recordings will be needed before conclusions can be made about the proportions of the various types of response.

The fact that neurones have been located which are stimulated by ambient temperature does not necessarily indicate that these neurones are concerned with temperature regulation. For example in the lateral and posterior hypothalamus of rats Cross and Silver (1963) have found units which were accelerated or inhibited when the skin was cooled or pinched. It is the observation that cells which responded to ambient temperature also responded to hypothalamic temperature which makes it more probable that these neurones are involved in temperature control. This convergence of peripheral and local temperature signals on the same neurone was first observed by Wit and Wang (1968). By heating their cats with lamps, they stimulated first the peripheral receptors and then after a delay of about 30 min, brain temperature began to rise. Five neurones (Type II) were found which responded in a additive fashion to these successive stimuli—firing rate increased within 3—6 min of turning

on the lamps and then rose again as brain temperature increased. The present experiments confirm that similar cells exist in the rabbit, but, because there was independent control of ambient and brain temperatures, they reveal a greater complexity of behaviour than Wit and Wang were able to show.

Hammel (1965) has suggested a neuronal model in which all the central thermoreceptors receive inputs from warm and cold peripheral receptors and that this input results in a shift in "set-point" when the ambient temperature changes. The present results are consistent with this model although at the moment there is no way of establishing whether a particular central neurone is an actual thermoreceptor or is being synaptically driven by such a receptor. However, in the cat Wit and Wang (1968) found "pure" central thermoreceptors which were not affected by peripheral warm stimuli.

It will now be important to discover how peripheral and local temperatures interact on the same hypothalamic neurone; in the present experiments their effects have been only examined separately because the stability of recording did not allow longer observations. The control equations of Stolwijk and Hardy (1966) suggest that altering ambient temperature might change the sensitivity of neurones to hypothalamic temperature. On the other hand, Hammel's model (1965) implies that there would be no change in sensitivity to local temperature but that the position of the firing rate/temperature curve might shift.

Addendum. In this paper reference should have been made to the work of Nakayama and Hardy (1969). Using shaved rabbits immersed in a water bath, they were able to show that many neurones in the midbrain reticular formation were activated by skin cooling. Some of these cells were also sensitive to changes in local brain stem temperature.

References

Benzinger, T. H.: Physiol. Rev. **49**, 671—759 (1969).
Bligh, J.: J. Physiol. (Lond.) **146**, 142—151 (1959).
Brown, A. G., Iggo, A., Miller, S.: Exp. Neurol. **18**, 338—349 (1967).
Cabanac, M., Stolwijk, J. A. J., Hardy, J. D.: J. appl. Physiol. **24**, 645—652 (1968).
Cross, B. A., Silver, I. A.: Exp. Neurol. **7**, 375—393 (1963).
Eisenman, J. S., Jackson, D. C.: Exp. Neurol. **19**, 33—45 (1967).
Findlay, J. D., Ingram, D. L.: J. Physiol. (Lond.) **155**, 72—85 (1961).
Forster, R. E., Ferguson, T. B.: Amer. J. Physiol. **169**, 255—269 (1952).
Hammel, H. T.: Neurones and temperature regulation. In: Physiological Controls and Regulations, ed. W. S. Yamamoto and J. R. Brobeck. London: Saunders 1965.
Hardy, J. D., Hellon, R. F., Sutherland, K.: J. Physiol. (Lond.) **175**, 242—253 (1964).
Hellon, R. F.: J. Physiol. (Lond.) **193**, 381—395 (1967).
— Experientia (Basel) **25**, 610 (1969).

Hensel, H., Huopaniemi, T.: Pflügers Arch. **309**, 1—10 (1969).
— Kenshalo, D. R.: J. Physiol. (Lond.) **204**, 99—112 (1969).
— Wurster, R. D.: Pflügers Arch. **313**, 153—155 (1969).
Murakami, N., Stolwijk, J. A. J., Hardy, J. D.: Amer. J. Physiol. **213**, 1015—1024 (1967).
Nakayama, T., Hammel, H. T., Hardy, J. D., Eisenman, J. S.: Amer. J. Physiol. **204**, 1122—1126 (1963).
— Hardy, J. D.: J. appl. Physiol. **27**, 848—857 (1969).
Narth, C. S.: J. Physiol. (Lond.) **200**, 102P (1969).
Stolwijk, J. A. J., Hardy, J. D.: Pflügers Arch. ges. Physiol. **291**, 129—162 (1966).
Wit, A., Wang, S. C.: Amer. J. Physiol. **215**, 1151—1159 (1968).

Dr. R. F. Hellon
National Institute for Medical Research
Mill Hill
London NW 7, England