The incorporation of reserpine differed markedly from that of guanethidine in that it was completely independent of temperature, the uptake being about the same at 0.23 and 37°C. The incorporation of guanethidine was temperature dependent, being 10% at 0°C and 35% at 23°C of the uptake at 37°C. The incorporation of neither drug was inhibited by NaCN at 10^{-5} *M* of FCCP at 3×10^{-6} *M*. Incubation of mast cells with 5-HT added 30 min before the addition of reserpine did not affect the uptake of reserpine even at a 5-HT concentration of 2.5×10^{-5} *M*. The incorporation of guanethidine was reduced to about 50% of the control level by 5-HT at 6×10^{-6} *M* added 15 min before the addition of guanethidine.

The intracellular location of both drugs seems to be mainly granular, judging from the assay of nuclear and granular fractions collected as described above after incubation with drugs. The percentual distribution of 5-HT and guanethidine and 5-HT and reserpine was almost identical (Table I). The subcellular location of 5-HT and histamine is mainly granular[16,21,22]. Taking 5-HT as a granular marker, this leads to the conclusion that reserpine and guanethidine are almost exclusively located in the amine storing granules in mast cells.

Both drugs seem to become incorporated into mast cells independently of each other since reserpine at 5×10^{-6} *M* did not affect the uptake of guanethidine. Neither did guanethidine at 5×10^{-5} *M* affect the uptake of reserpine (Table II).

These preliminary results indicating incorporation of both drugs into mast cells, where the drugs become bound to the amine storing gramules, are in close correspondence with earlier observations[15-17] showing that both drugs tested interfere with 5-HT kinetics in mast cells. Experiments designed to reveal the exact mechanisms underlying these effects and to explain the difference in action of reserpine and guanethidine on 5-HT kinetics in mast cells and simple neuronal models are in progress.

Zusammenfassung. Isolierte peritoneale Mastzellen der Ratte wurden in KRG-Puffer mit Reserpin und Guanethidin inkubiert. Beide Substanzen scheinen unabhängig voneinander aufgenommen zu werden, da die gleichzeitige Inkubation mit Reserpin und Guanethidin die Aufnahme im Vergleich mit den Kontrollen nicht herabsetzt.

J. GRIPENBERG and S.-E. JANSSON

Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 B, Helsinki, (Finland),
26 March 1971.

[21] I. L. THON and B. UVNÄS, Acta physiol. scand. *67*, 455 (1966).
[22] D. LAGUNOFF, M. T. PHILLIPS, O. A. ISERI and E. P. BENDITT, Lab. Invest. *13*, 1331 (1964).

Hyperthermic Effect of Disodium Edetate Injected into the Lateral Cerebral Ventricle of the Unanesthetized Cat

FELDBERG et al.[1] reported that perfusion of physiological NaCl solution through the cerebral ventricular system of unanesthetized cats resulted in the rapid development of high fevers, whereas body temperature was not altered if the solution also contained a physiological concentration of calcium ion. A later report[2] extended these observations to the unanesthetized rabbit and also demonstrated that increasing calcium ion concentrations above those normally present in cerebrospinal fluid (CSF) caused hypothermia in some animals and antagonized the pyrogenic effect of leukocytic pyrogen. Similar effects have also been produced in unanesthetized cats by perfusion in the posterior hypothalamus[3]. No change in temperature was produced, however, provided the relative concentrations of sodium and calcium ions in the perfusing fluid were kept the same as those in extracellular fluid. The authors suggested that the balance between sodium and calcium ions in the hypothalamus may be responsible for determining the set point of the thermoregulatory thermostat[1-3] and that pyrogens may act by altering this balance[2]. The purpose of the present experiments was to determine the effect on body temperature of calcium ion binding in CSF by the chelating agent disodium edetate (Na_2EDTA).

Methods and materials. Cats, weighing between 2.4 and 5.0 kg, were prepared with lateral cerebral ventricular cannulas, jugular venous catheters and retroperitoneal thermocouples as in previous experiments[4]. Body temperature was recorded automatically on a multipoint recorder at intervals of 3 min during the 1st h after each test injection and at least every 15 min thereafter until recovery. The average of temperature readings 0, 15 and 30 min before ventricular injection was used as the baseline from which changes were measured. Environmental temperature was maintained at 75 ± 2°F. Ventricular injections (all 0.10 ml in volume) were made at the same time of day in each cat, usually at daily intervals. Antipyretics were administered i.v., 30 min before ventricular injections of Na_2EDTA, and were flushed in with 1.0 ml of saline solution. Cannulas and catheters were also flushed 3–4 h before tests. A Harvard syringe pump was used for infusions into the ventricular cannulas.

Commercial, nonpyrogenic saline solution was used for all solutions, control injections and flushes. All containers, syringes and needles were either of the commercial, nonpyrogenic, disposable type or were sterilized in dry heat at over 200°C for at least 2 h. Stock solutions of $Na_2EDTA \cdot 2H_2O$ and of calcium disodium edetate ($CaNa_2EDTA$) were stored at 4°C until needed. Fresh solutions of sodium salicylate (100 mg/ml) and acetaminophen (10 mg/ml) were prepared for each injection.

Results. Dose-related hyperthermic responses were produced by intraventricular injections of Na_2EDTA. Figure 1 shows responses to various doses in one of the cats. A 200 µg dose was effective in all cats. Tremor of the ears usually developed within 30 sec, followed by a fine

[1] W. FELDBERG, R. D. MYERS and W. L. VEALE, J. Physiol., Lond. *207*, 403 (1970).
[2] W. FELDBERG and P. N. SAXENA, J. Physiol., Lond. *211*, 245 (1970).
[3] R. D. MYERS and W. L. VEALE, J. Physiol., Lond. *212*, 411 (1971).

tremor over the body and then shivering beginning 3–5 min after injection. A rise in temperature was usually apparent within 3 min. Cessation of shivering and development of marked tachypnea (80–170 respirations/min), with or without panting, coincided with attainment of maximal hyperthermia and the beginning of recovery. Recovery of body temperature began abruptly, resulting in a characteristic spiking without any appreciable plateau phase. Hyperthermia was enhanced and prolonged either by repeated injections at the time recovery was beginning or by infusion following a priming injection. An example is shown in Figure 2 in which the responses of a cat to 1, 2 and 3 successive injections of 200 µg are plotted. Termination of the rise after single injections, therefore, was apparently not due to decreasing sensitivity to the drug or to attainment of a maximal response, but rather to the short duration of drug action. Tolerance to the hyperthermic response did not develop with 10 or more daily injections of a given dose of Na_2EDTA in the 3 cats so tested. Hyperthermia was related neither to the acidity of the Na_2EDTA solutions (pH about 4.7) nor to the molecule per se, but was apparently due to its chelating action. Intraventricular injection of NaCl solution adjusted with HCl to pH 3.6 did not cause any change in temperature and neither did injections of up to 1 mg $CaNa_2EDTA$, which can not chelate calcium ions. Figure 3 shows the means and ranges of responses of 6 cats to 250 µg Na_2EDTA and to 1 mg $CaNa_2EDTA$. 1 mg/kg Na_2EDTA administered i.v. did not alter body temperature.

The hyperthermic effect of Na_2EDTA was blocked by pentobarbital anesthesia. However, neither of the antipyretics, acetaminophen (10 or 25 mg/kg) and sodium salicylate (40 or 100 mg/kg), antagonized this effect of Na_2EDTA.

In addition to the effects on thermoregulatory mechanisms, a number of behavioral and other effects were observed following ventricular injection of Na_2EDTA. After single injections of the lowest doses, vocalization, grooming and mydriasis were observed. Increased locomotor activity, salivation and piloerection were common after intermediate doses. Recovery from these effects coincided with recovery from hyperthermia. With still higher doses (up to 750 µg), repeated injections or infusions, the effects were enhanced and associated with increasing excitability and even convulsions, leading to death in 1 animal. Recovery with these higher doses was prolonged with evidence of sympathetic stimulation, hyperexcitability and aggressive behavior lasting for 2–3 days.

Discussion. It has been shown that alterations in body temperature of unanesthetized animals can be produced by perfusing the cerebral ventricular system or the posterior hypothalamus with solutions containing unphysiologic sodium to calcium ion ratios[1-3]. The results of the present experiments with Na_2EDTA are compatible with the previous observations that increasing the sodium to calcium ion concentration ratio causes hyperthermia. Na_2EDTA, which reduces the availability of calcium ions, caused rapid development of hyperthermia, while control injections of $CaNa_2EDTA$ did not affect body temperature. It is highly unlikely that the hyperthermic responses were due to contamination by agents such as bacterial endotoxins or leukocytic pyrogen since the onset of responses to these pyrogens injected intraventricularly are slower, and the duration of action is considerably longer than after Na_2EDTA[5,6]. In fact, leukocytic

[4] W. G. Clark and J. S. Page, J. Bact. *96*, 1940 (1968).
[5] D. L. Jackson, J. Neurophysiol. *30*, 586 (1967).
[6] U. K. Sheth and H. L. Borison, J. Pharmac. exp. Ther. *130*, 411 (1960).

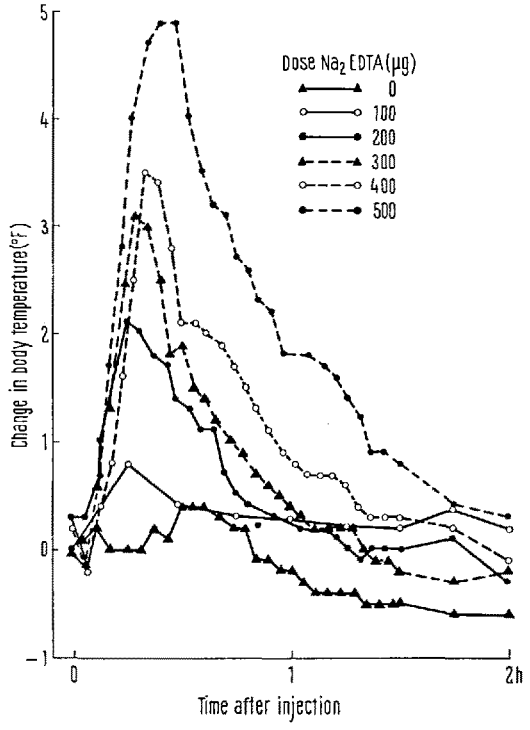

Fig. 1. Changes in temperature produced in 1 cat by intraventricular injections of various doses of Na_2EDTA or saline solution alone as indicated in the key.

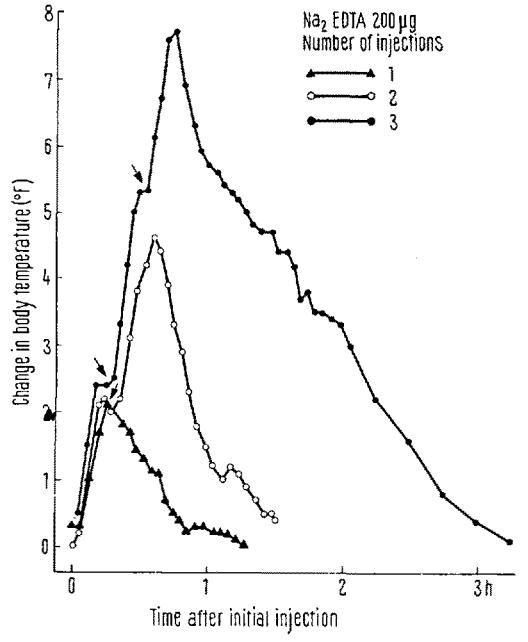

Fig. 2. Hyperthermic responses of 1 cat to 1 or more intraventricular injections of Na_2EDTA. Arrows indicate the time of successive injections in each series.

pyrogen, a substance likely to be the cause of a variety of experimental and pathologic fevers, when injected directly into sensitive areas of the hypothalamus still causes fevers with longer latencies and durations[5,7].

The present study doesn't specifically bear on the question of whether fluctuations of sodium-calcium ion balance within the CNS play a physiologic role in thermoregulation. It does suggest that the changes in calcium ion concentration caused by Na_2EDTA act downstream from the site at which pyrogens act since acetaminophen and sodium salicylate in doses which effectively antagonize the pyrogenic actions of endotoxin[8] and leukocytic pyrogen in cats after either i.v. or intraventricular administration[9,10], were ineffective in antagonizing the action of Na_2EDTA. An action downstream from the thermostat is also indicated by the findings[3] that only perfusions of the posterior hypothalamus with varied salt concentrations consistently altered temperature. Perfusion of the anterior hypothalamus, a region which is clearly involved in normal thermoregulation and which is assumed to be the site of the major central control mechanisms in models of temperature regulation[11], produced inconsistent responses. In favor of an action on the thermostat, however, is the observation that the responses to Na_2EDTA appeared to result from coordinated effector activity similar to that seen after pyrogens[12]. Thus, shivering was present during the development of the hyperthermia without significant antagonism by heat loss mechanisms. As the peak was reached, shivering was replaced by tachypnea and panting.

Although not studied extensively, Na_2EDTA seemed to differ from pyrogens which act directly or indirectly on the central nervous system in that no apparent ceiling to its effect was reached when repeated injections or infusions were made. After a maximally pyrogenic dose of bacterial endotoxin or staphylococcal enterotoxin is determined, increasing the dose 50–100 times does not increase the height of the fever[10].

The behavioral effects seen after Na_2EDTA were considerably different from those reported after perfusion of the ventricular system of cats with NaCl solutions for 30 min[1]. The obvious distress, sympathetic activity and excitability produced by Na_2EDTA were not reported to occur with the perfusions, even with comparable increases in temperature. However, longer perfusions in the rabbit[2] did cause restlessness, struggling and even convulsions. Perhaps longer perfusions in the cat would have produced behavior more like that seen after Na_2EDTA[13].

Conclusions. Chelation of calcium ions by Na_2EDTA injected intraventricularly in unanesthetized cats caused a hyperthermic response characterized by a very rapid onset and brief duration. The response could be blocked by pentobarbital anesthesia but not by the antipyretics, acetaminophen and sodium salicylate. The results favor an action downstream from the site at which pyrogens act.

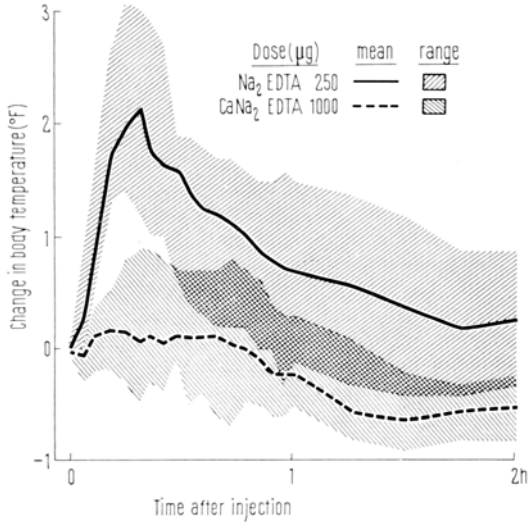

Fig. 3. Responses of 6 cats to intraventricular injections of Na_2EDTA and $CaNa_2EDTA$.

Résumé. La chélation des ions de calcium par l'EDTA Na_2 injecté dans le ventricule cérébral des chats non anesthésiés a produit une réaction hyperthermique caractérisée par une installation rapide et une durée brève. Cette réaction peut être bloquée par l'anesthésie au pentobarbital, mais non pas par les antipyrétiques, le N-acétyl-paraaminophénol ou le salicylate de sodium. Les résultats indiquent une action plus tardive dans la chaîne des événements que celle des pyrogènes.

W.G. CLARK

Department of Pharmacology, The University of Texas Southwestern Medical School at Dallas, Dallas (Texas 75235, USA), 14 June 1971.

[7] K. E. COOPER, W. I. CRANSTON and A. J. HONOUR, J. Physiol., Lond. *191*, 325 (1967).
[8] W. G. CLARK, J. Pharmac. exp. Ther. *175*, 469 (1970).
[9] W. G. CLARK and S. G. MOYER, Fedn. Proc. *30*, 563 (1971).
[10] W. G. CLARK, unpublished.
[11] H. T. HAMMEL, Ann. Rev. Physiol. *30*, 641 (1968).
[12] H. L. BORISON and W. G. CLARK, Adv. Pharmac. *5*, 129 (1967).
[13] Supported by USPHS Grant No. NS08618. I wish to thank Dr. J. M. LIPTON for critically reading the manuscript.

Electronmicroscope Studies of Crayfish Setae (*Austropotamobius pallipes*)

Although the setae of decapods are conspicuous, and occur in great profusion, little is known of their anatomy. A study of the setae present on the crayfish *Austropotamobius pallipes* at the optical level, failed to resolve some aspects of their anatomy[1], particularly the nature of the setal tips and outgrowths of the setal wall.

The presence of an apical pore on certain decapod setae has been suggested by some authors[2], but never figured. Stereoscan electronmicroscope studies on the setae of *A. pallipes* show conclusively that an apical pore is present (Figures 1a, b and c). The exact position of the pore differs from one seta to another but has a constant position for a given setal variety. A great variety of apices occurred amongst the different setae (Figures 1a–d), the pore rarely being terminal in position but often set someway behind the tip (Figures 1a + c). None of the many and varied outgrowths from the setal wall appeared to bear pores, and no pores were detected over the shaft surface.

Figure 1a shows the apical region of a serrate seta taken from the dactylopodite of the third maxilliped, note the apical pore just behind the smoothly pointed tip. The