

Fig. 1. Daily variation in the number of mitotic figures per crypt. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

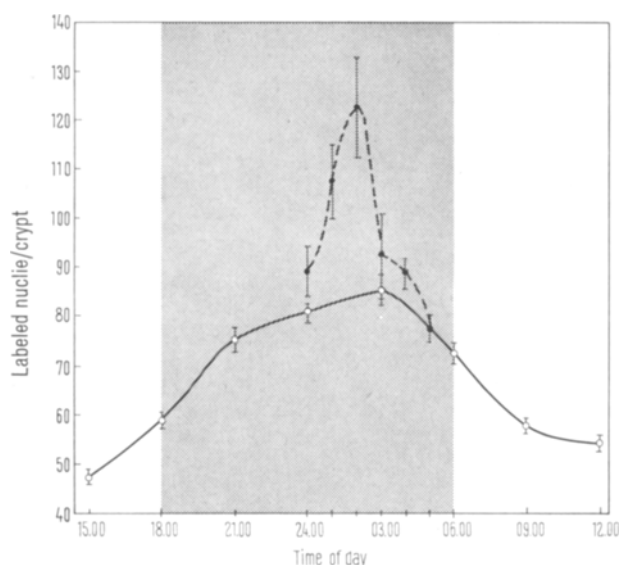


Fig. 2. Variation in the number of cells synthesizing DNA (S-cells) as a function of time of day. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

thetic phase of the cell cycle. This cohort would then be expected to peak in mitosis after $0.5 T_S + T_{G_2} + 0.5 T_M$ has elapsed. Suggestive evidence for this hypothesis was obtained in our previous study. However, when hourly intervals are tested no difference was noted between the peak in mitosis and the peak in DNA synthesis. This argues against the proposed mechanism occurring in the gut.

GELFANT⁹ has demonstrated in the mouse epidermis 2 distinct populations of cells which are blocked in G_1

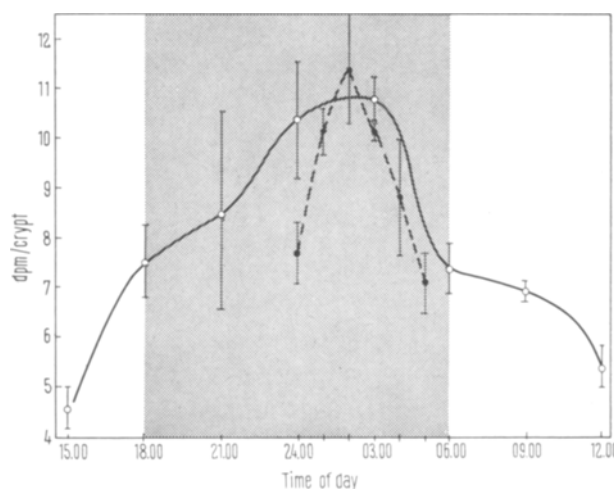


Fig. 3. Diurnal fluctuation in dpm per crypt. Open circles are previously reported results. Shaded area represents the dark cycle in the animal rooms.

and G_2 , and which can be triggered into S or M respectively by appropriate stimuli. This theory fits our results; but studies of LESHER¹⁰ would seem to argue against its occurrence in the intestine because: 1. The percent labeled mitosis curve reaches 100% in 1–2 h after ³HTdR injection, and 2. after 4 injections of ³HTdR each separated by 2 h, the entire proliferative compartment is labeled.

A more recent study of PEDERSON and GELFANT¹¹ had demonstrated a population of G_1 and G_2 cells in the intestine and kidney which are non-cycling. Release of the block could cause a wave of cells into S and M resulting in the simultaneous peaks noted, however, it is extremely doubtful that the small number (4%) of these non-cycling cells could account for the large daily variation noted.

Zusammenfassung. Im Tagesrhythmus der Proliferationsfähigkeit von Darmepithelien wurde in drei verschiedenen Parametern um 02.00 Uhr ein Maximum gefunden. Mögliche Mechanismen werden erörtert.

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⁹ S. GELFANT, International Society for Cell Biology (Academic Press, New York 1963), vol. 2, p. 299.

¹⁰ S. LESHER and JANIE BAUMAN, in *Normal and Malignant Cell Growth* (Eds. R. J. M. FRY, M. L. GRIEM and W. H. KIRSTIN; Springer-Verlag, New York 1969), p. 49.

¹¹ T. PEDERSON and S. GELFANT, *Expl Cell Res.* 59, 32 (1970).

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Sex and Age Differences in Hyperthermia Response to Ether Anesthesia in Weanling Rats with Ventromedial Hypothalamic Lesions¹

The role of the hypothalamus in temperature regulation has been known since the early work of ISENSCHMID and KREHL², who demonstrated that lesions in the posterior hypothalamus prevented appropriate homeostatic re-

¹ This investigation was supported by U.S.P.H.S. Grants No. HE 06 975 and No. HD 03331, N.I.H.

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sponses to cooling. Destruction of the anterior hypothalamus, on the other hand, was shown to lead to an impairment of the animal's ability to counteract over-heating³.

The association of the ventromedial hypothalamic area with feeding mechanisms and of feeding with changes in body temperature⁴ has led to studies in temperature regulation following lesions in the ventromedial hypothalamus. Thus, MAYER and GREENBERG⁵ have demonstrated that light ether anesthesia in mature female hypothalamic-hyperphagic rats resulted in an increased colonic temperature even in a normal environmental temperature; non-hyperphagic rats with ventromedial hypothalamic lesions and control animals did not show this response, neither in the fed nor in the 24 hour-fasted state.

While the above studies were carried out in the mature rat, comparable investigations have not been performed in the weanling rat with ventromedial hypothalamic lesions. The latter animals are of particular interest since they are obese despite normal body weight gains and normophagia⁶⁻⁸, show reduced linear growth^{7,8}, decreased pituitary and plasma growth hormone levels and hyperinsulinemia⁹, hypertriglyceridemia¹⁰ and hypercholesterolemia¹¹. These animals show also an increased incorporation of glucose-U-C¹⁴ into adipose tissue and oxidation to CO₂ as well as increased incorporation of palmitate-1-C¹⁴ into adipose tissue¹².

Since the hyperthermia during ether anesthesia had originally been demonstrated in mature rats with ventromedial hypothalamic lesions, it was of interest to study the response not only in the weanling female rat but also to investigate the possibility of a sex difference.

Weanling male and female Holtzman rats received bilateral electrolytic lesions in the ventromedial hypothalamus at the age of 27 days, using a direct anodal current of 1.5 mAmp that flowed for 10 sec. A length of stainless steel wire of 0.38 mm diameter that was spar varnish-coated except for the tip served as electrode. Sham-operated animals of the same age were used as controls. The rats were accommodated in individual cages in a temperature (23°C) and light cycle (06.00–18.00 h

light, 18.00–06.00 h dark) controlled room. They were fasted for 24 h after the operation and then given Teklad Lab Chow and tap water ad libitum. Food intake was measured throughout the experiment and recorded weekly, as were the obesity index^{7,13} and the colonic temperature. The latter was measured with a Yellow Springs Company telethermometer, a vaseline-lubricated probe being inserted into the rectum for a distance of 5 cm. All measurements were made under light ether anesthesia and during the same time of the day. After 6 weeks, the rats were killed and the brains were treated in a standard manner¹⁴ for the histological analysis of the lesions, using the atlas of DE GROOT¹⁵. Rats with asymmetrical lesions and those that encroached on the base of the brain were excluded from the final statistical analysis.

1. Male rats. Table I shows that ether-anesthetized weanling rats with ventromedial hypothalamic lesions (VMNL) showed significant hyperthermia during the latter part of the 6-week experimental period. Although food intake was normal during 5 out of 6 weekly measure-

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⁹ L. A. FROHMAN and L. L. BERNARDIS, *Endocrinology* 82, 1125 (1968).

¹⁰ L. A. FROHMAN, L. L. BERNARDIS, J. D. SCHNATZ and L. BUREK, *Am. J. Physiol.* 216, 1496 (1969).

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Table I. Comparisons between VMNL and SCNL rats for each weekly measurement

Group ^a	Male weanling rats						
	No.	1 ^b	2	3	4	5	6
Colonic temperature (°C)							
VMNL	6	36.4 ^c	37.3	37.3	38.2 ^f	38.2	37.8 ^e
		0.27	0.12	0.20	0.08	0.11	0.08
SCNL	6	36.7	37.1	37.1	37.6	37.7	37.5
		0.19	0.15	0.16	0.12	0.17	0.11
Food intake (g/day)							
VMNL	6	11.3	16.1 ^e	19.3	16.8	19.1	20.6
		1.12	0.92	1.01	1.57	1.57	3.33
SCNL	6	11.3	13.8	17.9	16.2	17.8	22.2
		0.38	0.38	0.63	0.64	0.53	2.16
Obesity index ^d							
VMNL	6	318 ^f	324 ^g	329 ^g	333 ^g	336 ^g	361 ^g
		2	2	7	6	7	2
SCNL	6	307	301	304	308	310	312
		2	4	1	2	2	2

^a VMNL, rats with ventromedial hypothalamic lesions; SCNL, sham-operated controls. ^b Weekly measurements. ^c Mean \pm S.E.M.

^d Cube root of body weight divided by naso-anal length times 1000.

^e $p < 0.05$. ^f $p < 0.01$. ^g $p < 0.001$.

Table II. Comparisons between VMNL and SCNL rats for each weekly measurement

Group ^a	Female weanling rats						
	No.	1 ^b	2	3	4	5	6
Colonic temperature (°C)							
VMNL	10	36.3 ^c	36.7	37.4	37.4	36.8	37.1
		0.27	0.21	0.15	0.13		
SCNL	12	36.4	36.6	37.1	36.9	37.2	37.4
		0.20	0.24	0.18	0.30	0.17	0.14
Food intake (g/day)							
VMNL	10	9.5 ^e	17.7	16.5	14.6	—	16.9
		0.93	1.83	0.87	1.00		1.41
SCNL	12	16.3	14.7	15.1	13.5	16.4	12.6
		1.36	0.87	0.66	0.58	0.99	2.64
Obesity index ^d							
VMNL	10	327 ^g	336 ^g	335 ^g	341 ^g	347 ^g	349 ^g
		3	3	6	3	7	3
SCNL	12	312	308	303	301	304	298
		2	2	4	2	2	2

^a VMNL, rats with ventromedial hypothalamic lesions; SCNL, sham-operated controls. ^b Weekly measurements. ^c Mean \pm S.E.M.

^d Cube root of body weight divided by naso-anal length times 1000.

^e $p < 0.05$. ^f $p < 0.01$. ^g $p < 0.001$.

ments, the obesity index was greatly elevated throughout the experiment.

2. Female rats. Table II indicates that ether anesthesia did not significantly alter the colonic temperature in female VMNL rats. Similar to the male VMNL rat, however, were the normophagia and the greatly elevated obesity index.

The data indicate an age difference in the hyperthermic response in female VMNL rats and a sex difference in weanling VMNL animals.

The age difference is probably related to the interference by the lesion at an early age with the 'setting' of the central temperature control mechanism(s); weanling VMNL rats would then be unable to respond with hyperthermia as do mature female VMNL rats⁵. It might also be related to the lack of hyperphagia in the weanling VMNL rat, since MAYER and GREENBERG⁵ were unable to find hyperthermia in normophagic mature female VMNL rats. Weanling VMNL animals, however, are consistently normophagic^{7-10, 16}.

The sex difference in the hyperthermic response in the weanling rat could possibly be related to a different sensitivity of the central regulatory mechanism(s) to the lesioning procedure. This appears in keeping with the report by THOMPSON and STEVENSON¹⁷ that the exercised mature female rat can tolerate a higher temperature before commencing vasodilatation and is able to regulate core temperature at a higher level than the mature male rat

as exercise continues. The data show finally that the hyperthermic response (male VMNL rats) or the lack thereof (female VMNL rats) is not related to food intake and the obesity status of the animals⁵.

Zusammenfassung. Entwöhnte junge weibliche Ratten mit ventromedialen hypothalamischen Läsionen zeigten keine Hyperthermie, wie sie bei geschlechtsreifen weiblichen Ratten bei Äthernarkose beobachtet wird. In männlichen entwöhnten, jungen Ratten mit Läsionen war jedoch eine signifikante Hyperthermie nachweisbar, welche in beiden Geschlechtern von der Futtermenge und dem Ausmass der hypothalamischen Fettsucht unabhängig war. Läsionen im ventromedialen Hypothalamus der weiblichen jungen Ratte scheinen einen zentralen Mechanismus zu beeinflussen, welcher es ihnen nicht ermöglicht, auf Äthernarkose mit Hyperthermie zu reagieren.

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¹⁷ G. E. THOMPSON and J. A. F. STEVENSON, Can. J. Physiol. Pharmacol. 43, 437 (1965).

Calcium Evoked Release of 5-Hydroxytryptamine from the Brain of the Unanesthetized Cat¹

The ionic milieu of neurones in the nervous system is an important determinant of transmitter activity at synapses². It is known, for instance, that Ca ions can affect the release from nerve tissue of certain substances such as acetylcholine³, noradrenaline⁴ or γ -amino-butyric acid⁵. However, little is known about the role of Ca ions in the release of 5-hydroxytryptamine (5-HT) particularly within the intact brain. We now present evidence that the level of Ca ions in extracellular fluid can influence markedly the release of 5-HT from sites scattered throughout the hypothalamus of the unanesthetized cat, and that the neural activity of this amine could depend upon the levels of extracellular Ca within the brain-stem.

Materials and method. In cats of either sex, modified push-pull cannulae⁶ were implanted into the rostral, caudal and lateral areas of the hypothalamus according to methods described earlier⁷. 5-7 days later, a 1 mm sphere of tissue at the tips of the cannulae was perfused with Krebs-Henseleit solution prepared with glass-distilled, ion-exchange water. Pyrogen-free glassware, syringes and tubing were used in all experiments, and the rate of perfusion was 50 μ l per min during a 30 min interval. Samples were accepted for assay only if the volume of the effluent matched that of the inflow, and if the perfusate was clear and devoid of tissue fragments. During the course of a perfusion, the animal was held gently and showed no untoward signs of disturbance or discomfort. The effluents were collected on ice and if not tested on the same day, were kept at -10°C until assayed. At the conclusion of each series of experiments, the perfusion site was verified following standard histological procedures.

The content of 5-HT in each 30 min sample was determined by the sensitive assay method of VANE⁸, the isolated rat stomach fundus strip. The contractile activity of a perfusate was considered to be due to 5-HT only if: a) the

contraction produced by a sample was of a similar shape and magnitude to that caused by 5-HT; and b) it was abolished by either methysergide or brom-lysergic acid added to the bath in doses of 10 to 20 μ g. Values for 5-HT were calculated in terms of the creatinine phosphate salt.

Results and discussion. At 19 sites in the hypothalamus extending from the anterior region, between the anterior commissure and optic chiasm, to the posterior area dorsal to the corpora mammillaria, the resting release of 5-HT per 30 min interval varied in the control perfusions from 0.1 to 2.0 ng. However, when Ca was present in the Krebs-Henseleit perfusate in 2.6 or 10.4 mM in excess of the normal mM value of extracellular fluid, the level of 5-HT in the perfusate varied between 0.5 and 3.6 ng per 30 min interval. The average resting output was 0.56 ± 0.11 ng per 30 min in those 19 sites at which 5-HT was released.

Three distinct types of loci could be identified. The Table presents the mean values of 5-HT released from: 1. those sites at which Ca evoked a significant increase in the liberation of 5-HT from the hypothalamus; 2. those at which little change in 5-HT output was observed; and 3. those sites at which the release of 5-HT declined. Of

¹ This work was supported in part by National Science Foundation Grant No. GB 7906 and US Office of Naval Research Contract No. N00014-67-A-0226-0003. We thank P. CURZON for his valuable technical assistance.

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