enzyme (or homogenate) of mouse liver, 2 degradation products A and B, which were identified as 6-hydroxykynurenic acid and 4,6-dihydroxyquinoline respectively (VI and VII) by comparing with the corresponding synthetic samples on paper chromatograms in their Rf's, their colours of fluorescence under UV-light with their characteristic colour change when exposed to ammonia, and the colours given by spraying Pauli-Monda reagent (Table). As the mere incubation of non-hydroxylated kynurenine gave neither VI nor VII, the authors consider that the reaction in the scheme is one of the best ways for identifying a metabolite with 5-hydroxykynurenine[5]. The extent of 5-hydroxylation of kynurenine roughly estimated by fluorometric method in chicken amounted to ca. 0.5%. As controls, the urine of chickens and mice not treated with kynurenine was collected and worked up in the same way as described above but contained only a trace of 5-hydroxykynurenine or none.

Attempts to purify the 5-hydroxykynurenine spots isolated from the original paper chromatogram by repeated developments on filter paper for identification purposes, resulted very often in obtaining only spots showing UV-absorption spectra deviating from the typical one, presumably owing to some oxidation even when the synthetic 5-hydroxykynurenine was used as the test material and taking precautions such as preventing oxidation. Therefore, the present authors prefer the enzymatic identification as described above to the optical method.

This detection of 5-hydroxykynurenine never means its artificial formation from kynurenine in the isolation procedure, for the same treatment of urine as described above after the addition of kynurenine has never led to the detection of 5-hydroxykynurenine.

The above results show for the first time that kynurenine is converted in animals to 5-hydroxykynurenine, and thus the origin of 5-hydroxykynurenine and a new route in the tryptophan metabolism through 5-hydroxykynurenine suggested by MAKINO and TAKAHASHI[6,7] 12 years ago are now established (scheme).

Formerly H. TAKAHASHI told one of the authors (K.M.) personally that he once detected 6-hydroxykynurenic acid in the urine of a pregnant woman[8]. ROY and PRICE[9] reported the isolation of a small amount of the same acid from pig urine[9]. Recently KIDO et al.[10,11] detected IV, VI and VII in the urine of chickens and human beings administered tryptophan. These findings all coincide with our present scheme.

Zusammenfassung. 5-Oxykynuramin (Vorläufer von Mausamin aus Maushirn) wurde aus Urin von Huhn und Maus nach Verabreichung von Kynurenin durch Kombination der Ionen-Austausch-Chromatographie mit Papier-Chromatographie isoliert. Durch Vergleich mit dem synthetischen Präparat wurde es papierchromatographisch und durch Spaltung mittels Mäuseleberhomogenat in 4,6-Dioxychinolin und 6-Oxykynurensäure identifiziert.

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Development of Hyperphagia in Female Rats with Ventromedial Hypothalamic Lesions Placed at Four Different Ages[1]

Most studies on the hypothalamic control of food intake and the factors influencing it have been conducted on young-adult and adult rats[2] but few have investigated food intake patterns in weanling rats[3–5]. In the latter studies it was observed that young growing rats with ventromedial lesions did not show the hyperphagia observed in similarly treated adult rats. This may be related not only to metabolic differences between fast-growing young rats and adult animals but also to a differential response of the satiety mechanisms to destructive lesions.

Food intake is generally expressed in g/day, kilocalories/day or grams relative to body weight. The present study was undertaken to not only investigate postoperative food intake patterns in rats with ventromedial hypothalamic lesions placed at 4 different ages but also to relate this parameter to 'metabolic size' (KLEIBER unit, i.e. $kg^{3/4}$)[6]. The duration of the experiment was chosen to be 6 weeks since during this time all animals were probably still in their dynamic state of obesity[7].

Female Holtzman rats were divided into 8 groups and treated in the following manner: Groups 1, 3, 5 and 7 received bilateral electrolytic lesions in the ventromedial hypothalamic area when 27, 59, 75 and 140 days old, respectively. Groups 2, 4, 6 and 8 served as corresponding controls. The lesions were placed with a Horsley-Clarke stereotaxic instrument using an enamel-coated stainless steel electrode of 0.37 mm diameter. An anodal current of 1.5 mA was used in the youngest rats (Group 1) while for the older animals 2.0 mA were used; the current was allowed to flow for 10 sec. The coordinates were obtained from previously established charts[8]. The animals were housed in individual cages in a room kept at 25 °C with 12 h light and 12 h dark and given a synthetic diet (4.2 Cal/g) and tap water ad libitum. Food intake was meas-

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ured for 2 days weekly and was expressed in g/day, g/day/100 g body weight and g/day/KLEIBER units[6]. Due to a mishap, no values are available for the 5 week measurement of group 1. Body weight was measured weekly with the rats under light ether anesthesia. The periods of food intake measurements were timed so that they fell between the periods of body weight measurements. After 6 weeks the animals were sacrificed by decapitation and the brains were treated as described previously[4]. The localization of the lesions was determined using the atlas of DEGROOT[9].

(a) *Food intake in g/day:* Figure 1 shows that during the first post-operative week the youngest rats with lesions ate less than their intact counterparts; rats operated on during early (group 3) and late (group 5) adolescence ate as much as their intact controls. The only rats with lesions that showed significant hyperphagia were those in which the operation had been performed during adulthood (group 7).

Comparison of lesioned rats with each other indicated that the youngest rats ate less than the older animals. Furthermore, the youngest rats increased their food intake during the second week and attained then a level which was maintained throughout the remainder of the experiment and the rats of groups 3 and 5 ate from the second week on much more than their controls. The pattern in these two groups of rats was similar but the rats of group 7 showed a decline of food intake toward the end of the experiment.

Among the intact controls, the youngest rats (group 2) ate during the first week less than the older controls but their intake was essentially similar to that of the other controls thereafter.

(b) *Food intake per 100 g of body weight:* The general pattern during the first week resembled that observed when food intake was expressed in g/day inasmuch as the rats of group 1 ate less than their intact controls (group 2). Groups 3 and 5 ate essentially as much as their controls and the animals of group 7 showed a food intake greater than that of their controls (Figure 2). In contrast to the food intake expressed in g/day (Figure 1) the rats of group 1 ate more per 100 g of body weight than some of the groups of older rats with lesions during the second (group 5), third (group 7) and sixth (group 5 and 7) week.

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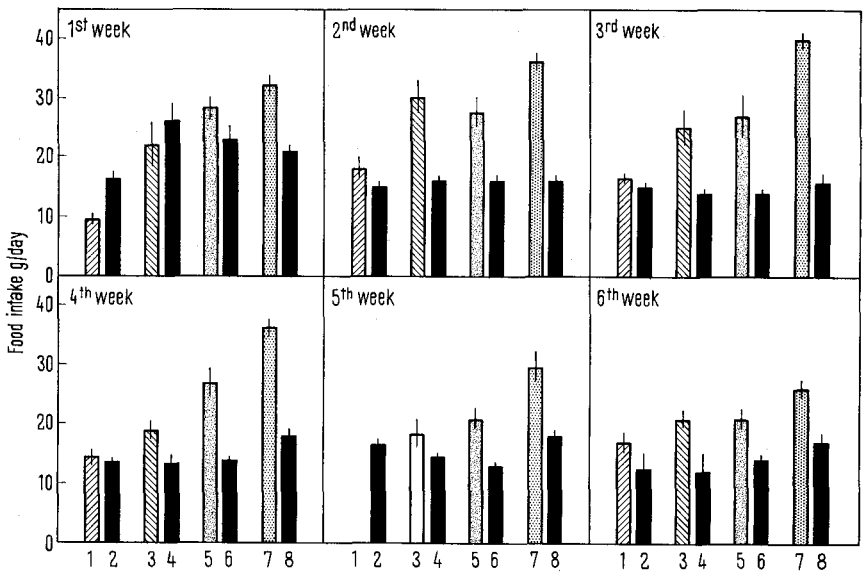

Fig. 1. Food intake in g/day in rats with ventromedial hypothalamic lesions placed at the age of 27 days (group 1), 59 days (group 3), 75 days (group 5) and 140 days (group 7) and of their corresponding intact controls (groups 2, 4, 6, and 8 respectively). Figures at bottom of graph designate group number. Significance of comparisons between means: First week: 1 vs 2 $p < 0.001$, 7 vs 8 $p < 0.001$, 2 vs 4 $p < 0.02$, 2 vs 6 $p < 0.05$, 2 vs 8 $p < 0.02$; Second week: 1 vs 3 $p < 0.01$, 1 vs 5 $p < 0.01$, 1 vs 7 $p < 0.02$; Third week: 1 vs 5 $p < 0.01$, 1 vs 7 $p < 0.001$; Fourth week: 1 vs 5 $p < 0.01$, 1 vs 7 $p < 0.001$.

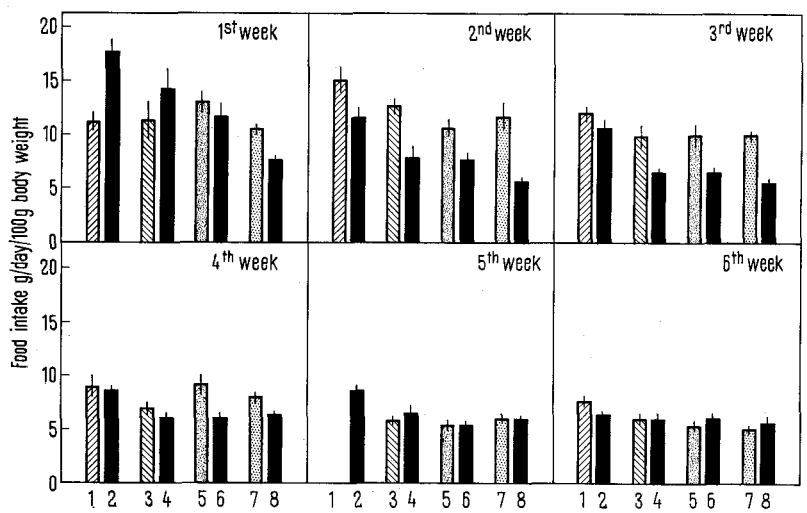

Fig. 2. Food intake relative to body weight of rats with hypothalamic lesions and their intact controls. For grouping see Figure 1. First week: 1 vs 2 $p < 0.001$, 7 vs 8 $p < 0.01$, 2 vs 6 $p < 0.01$, 2 vs 8 $p < 0.001$; Second week: 1 vs 5 $p < 0.02$, 2 vs 4 $p < 0.02$, 2 vs 6 $p < 0.02$, 2 vs 8 $p < 0.001$; Third week: 1 vs 7 $p < 0.01$, 2 vs 4, 6, 8 $p < 0.001$; Fourth week: 2 vs 4, 6, 8 $p < 0.001$; Fifth week: 2 vs 4 $p < 0.05$, 2 vs 6 $p < 0.001$, 2 vs 8 $p < 0.001$; Sixth week: 1 vs 5 $p < 0.01$, 1 vs 7 $p < 0.001$.

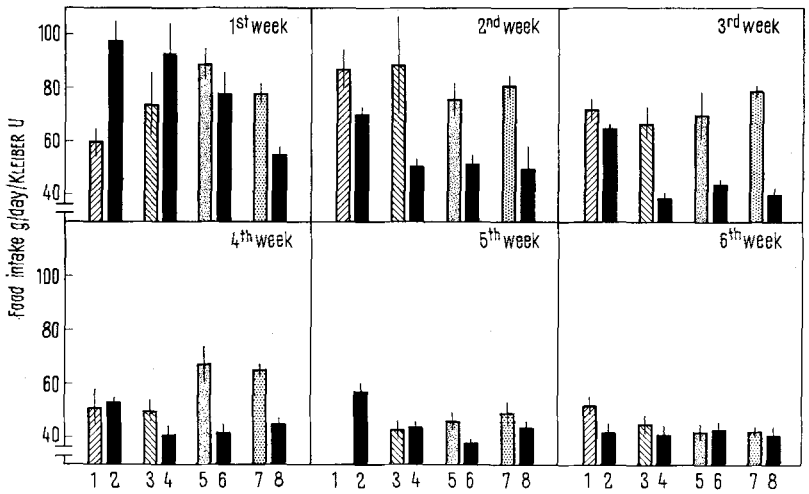

Fig. 3. Food intake relative to 'metabolic size' of rats with ventromedial hypothalamic lesions and of their intact controls. For grouping see Figure 1. First week: 1 vs 5 $p < 0.01$, 1 vs 7 $p < 0.02$, 8 vs 2 $p < 0.001$, 8 vs 4 $p < 0.02$, 8 vs 6 $p < 0.02$; Sixth week: 1 vs 7 $p < 0.05$.

The youngest controls (group 2) ate more than the older controls up to the fifth week of the experiment.

(c) *Food intake relative to metabolic size:* During the first week following the operation the food intake of the lesioned rats was similar to that when food consumption was expressed in g/day, except that there was no significant difference between group 1 and 3 (Figure 3). During the subsequent weeks the food intake of group 1 remained in the same range as that of the older lesioned rats until the sixth week when it exceeded that of group 7.

The intake of the intact controls resembled that expressed relative to body weight. It is in strong contrast, however, to the food intake expressed in g/day. During the first week the oldest controls (group 8) consumed less than all of the other groups of intact rats.

(d) *Body weight:* The Table shows that group 1 had small but significant weight gains when compared with the intact controls. Groups 3, 5, and 7 showed progressively greater weight gains.

Normal weanling rats have been reported to eat twice as much in relation to their body weight as do adult rats. However, when ventromedial lesions are placed in weanling rats hyperphagia so characteristic for older rats with lesions is not evident[3]. The present findings are thus in accord with previous reports[2-5].

A finding of some interest is the difference in food intake in g/day during the first week following hypothalamic lesions between lesioned weanling and lesioned adult rats, an observation reported previously[3]. It appears that, after autonomic stability is regained with time, food intake begins to rise; this seems to occur slower in younger and faster in older rats and is evidenced by significant hypophagia in the lesioned weanlings and significant hyperphagia in lesioned older rats. This may be a manifestation of the trauma of the electrocoagulation perhaps by spreading of current into neighboring neurons, thus causing temporary functional disruption[10] particularly in the youngest rats.

When food intake is expressed relative to body weight and relative to 'metabolic size' intact rats showed during the third week of the experiment a drop that was much less pronounced in the lesioned animals. Thus, during the first 6 weeks of the dynamic phase of obesity[7] the food intake of the operated rats was higher than that of their intact controls.

It is of interest that the rats of groups 3 and 5 showed parallel food intake patterns throughout the experiment for all 3 parameters of food intake. The similarity between these 2 groups and the dissimilarity between them and the youngest and oldest lesioned rats is noteworthy because it is not paralleled by the food intake of their intact controls in comparison with those of the youngest and oldest controls.

Body weight and age at operation and sacrifice, respectively, of rats with ventromedial hypothalamic lesions and of their intact controls

Group	N	Operation		Sacrifice	
		Age (days)	Weight (g)	Age (days)	Weight (g)
1	10	27	70 ± 2[a]	71	231 ± 9[b]
2	12		72 ± 2	71	192 ± 5
3	4	59	178 ± 2	102	359 ± 28
4	5		172 ± 2	102	230 ± 3
5	6	75	193 ± 3	118	390 ± 11
6	7		185 ± 11	118	231 ± 4
7	5	140	274 ± 3	182	524 ± 19
8	6		271 ± 6	182	288 ± 12

[a] Mean ± S.E.M. [b] Group 1 vs group 2 $p < 0.01$; group 3 vs group 4 $p < 0.01$; group 5 vs group 6 $p < 0.001$; group 7 vs group 8 $p < 0.001$.

Zusammenfassung. Ventromedial-hypothalamische Läsionen führen bei nicht mehr säugenden weiblichen Ratten zu temporärer Hypophagie, bei älteren Tieren zu Hyperphagie. In den folgenden Wochen wird eine autonome Stabilität und normale Futteraufnahme wieder erreicht. Bezogen auf das Körpergewicht assen die nicht mehr säugenden Ratten weniger als gleich alte Kontrolltiere, mehr jedoch als ältere lädierte Ratten.

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