

## Regulation of Feeding in Leeches

*Hirudo medicinalis* (L.) feeding through an artificial membrane can imbibe 6- to 7-fold its own weight of blood during a single meal. We have reported previously that this leech will also imbibe a solution of glucose (1 mg/ml) in 0.15 M NaCl<sup>1</sup>, but in this case the intake is less than half that of blood. Further investigations (to be published) have shown that several amino acids can also elicit feeding response in *H. medicinalis*. In particular, L-arginine in 0.15 M NaCl causes the same average weight gain as whole blood. Whilst the rate of feeding on glucose- or arginine-containing solutions is the same, the sucking of the former is terminated much earlier. The average time of feeding on arginine solutions was 21 min and that on glucose solutions 8.3 min.

This marked difference raised questions regarding feeding control in the leech. In some blood sucking animals, e.g. *Rhodnius prolixus*, ingestion is normally terminated by nervous information as to the size of the abdomen<sup>2</sup>. To test if the same mechanism operates in *H. medicinalis*, we allowed leeches to feed on arginine solution and 2 min after commencement of feeding cut the rear end of the leech, just above the anal sphincter. This treatment did not interrupt feeding but caused the ingested solution to leak out without stretching the gut wall. Leeches thus treated fed more than twice as long as controls, i.e. 45 min, and passed through their gut 13-fold their own weight of solution. The same operation performed on leeches imbibing solutions containing glucose produced only slightly longer feeding period, 11.3 min on the average. It is thus obvious that the mechanism which terminates feeding on arginine differs from that which terminates glucose intake. The former is related to the volume of solution held in the

gut and indicates that the monitor is some kind of a stretch receptor. On the other hand, there seems to be adaptation of the taste receptor to glucose before information regarding the volume ingested exerts inhibition.

When shortly after the commencement of feeding the glucose or arginine solutions imbibed were replaced by 0.15 M NaCl alone, the leeches stopped sucking after 1-2 min. This time is probably required to wash out the chemoreceptors. This shows that the maintenance of feeding is dependent on continuous sensory input from the oral chemoreceptors, as in the case of *Phormia regina* described by DETHIER<sup>3</sup>.

**Résumé.** Des essais de nutrition ont montré que la sangsue *Hirudo medicinalis* qui boit le sang absorbe aussi des solutions de l'arginine-L, ou de glucose dans 0.15 M de NaCl. La durée de nutrition avec l'arginine-L dépend de la quantité bue, tandis qu'avec la glucose la nutrition se termine plutôt, probablement à cause d'une adaptation des chémo-récepteurs.

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<sup>1</sup> R. GALUN and S. H. KINDLER, Comp. Biochem. Physiol. 17, 69 (1966).

<sup>2</sup> S. H. P. MADRELL, Nature 198, 210 (1963).

<sup>3</sup> V. G. DETHIER, in Symp. No. 3, R. Ent. Soc., London, p. 46 (1966).

## Prenatal Development in the Rat Following Administration of Cyclamate, Saccharin and Sucrose

Artificial sweetening agents are becoming increasingly important as food additives to restrict the intake of calories derived from sugar. Apart from saccharin, cyclamate (sodium or calcium cyclohexyl sulphamate, introduced in 1950) is widely consumed in various countries and its safety in use has been substantiated by a large number of investigations<sup>1,2</sup>. Isolated reports on animal experiments, however, have hinted at possible embryotoxic effects of artificial sweeteners<sup>3,4</sup>, or have suggested that feeding rats a diet containing 5 and 10% of cyclamate calcium may reduce the growth rate of weanlings<sup>5</sup>.

In order to investigate whether cyclamate and saccharin have, in fact, any effect on embryonal and foetal development, a detailed study was carried out in the pregnant rat. The doses administered were related to the 'sweetening power'<sup>1,6</sup> of these products and the results were compared with rats treated with sucrose.

Female rats of known fertility of an albino (Wistar-derived) strain were mated with proven males. Cyclamate sodium, saccharin sodium and sucrose (puriss. Merck) were given daily by stomach tube from the sixth to the fifteenth day of pregnancy (day 0 being the day on which spermatozoa were found in the vaginal smear). The dose levels used are indicated in Table I. Tap water served as solvent. The amount of liquid administered was 5 ml/kg in the groups medicated with cyclamate and saccharin and 20 ml/kg in the group to which sucrose was given. In the latter instance, a comparable control group received

the same amount of fluid. The dams were autopsied on the twenty-first day of pregnancy and the foetuses delivered by Caesarean section. The foetuses were carefully inspected macroscopically and weighed. In order to indicate the spontaneous incidence of anomalies in the rats used, the results were compared with those obtained in a cumulative control group of 363 dams.

For visualization of the skeletal elements of the foetuses the method of Alizarin Red S staining was used<sup>7</sup>. The criteria for assessment of development of the skeleton were absence of ossification and the occurrence of anomalies. The results were expressed as percentages of the total number of foetuses examined.

As regards the litter size, the resorption rate, and the mean weight of foetuses, there were no significant differences ( $p \leq 0.05$ ) between the treated groups and the controls (Table I). No malformations occurred in the former. Assessment of the developing skeleton did not

<sup>1</sup> Food Additives and Contaminants Committee Report on Cyclamates, Ministry of Agriculture, Fisheries and Food, London, 1966.

<sup>2</sup> G. BUNGARD, Der Deutsche Apotheker 20, No. 4 (1968).

<sup>3</sup> R. TANAKA, Jap. J. Publ. Hlth 11, 909 (1964).

<sup>4</sup> R. TANAKA, J. Iwate Med. Assoc. 16, 330 (1964).

<sup>5</sup> P. O. NEES and P. H. DERSE, Nature 213, 1191 (1967).

<sup>6</sup> G. BUNGARD, Der Deutsche Apotheker 19, No. 9 (1967).

<sup>7</sup> A. B. DAWSON, Stain Technol. 7, 123 (1926).