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Hyperphagia in Ruminants Induced by a Depressant

Abstract. *Attempts at causing ventromedial hyperphagia in ruminants have been hitherto unsuccessful. In our experiments perfusion of the ventriculocisternal system with pentobarbital caused marked hyperphagia. This suggests that the ventromedial hypothalamic area is functioning in ruminants, probably as in monogastric animals, by inhibiting the lateral area.*

Central organization of the regulation of food intake of ruminants is little understood; it is not necessarily identical or even closely similar to that of monogastric animals. While presence of a feeding center similar to the one found in monogastric animals is inferred from the demonstration (*1*) that in goats and sheep stimulation of the lateral area of the hypothalamus causes indiscriminate feeding and drinking behavior, existence of such a symmetrical ventromedial satiety center in ruminants has not been established. In monogastric animals, the ventromedial area is concerned not only with satiety but also with gastric contractions (*2*) and gastric juice secretion (*3*); anatomical and functional differences in the gastrointestinal tract of polygastric animals make such a role difficult to interpret. In monogastric animals, circulating glucose and fatty acids represent the major components of available energy, and the ventromedial satiety area is known to contain glucoreceptors (*4*). In ruminants the major sources of available energy are volatile fatty acids, but monitoring glucose utilization may not monitor volatile fatty-acid utilization.

Furthermore, in their natural habitat, the feed of mature ruminants has such low digestible caloric density and the process of microbial degradation and rumination is so slow and cumbersome (mean ruminal retention time is approximately 60 hours) that it appeared possible that no ventromedial satiety mechanism operated. Even in monogastric animals this mechanism does not operate during periods of active growth or during lactation (*5*). In a recent publication the inability to produce hyperphagia through hypothalamic lesions (though the ventromedial area was not located) is reported and this failure is ascribed to lack of distensibility of the gastrointestinal tract (*6*).

We reasoned that if the ventromedial area functions as a satiety center, its temporary inactivation should appear as a release of inhibition on the feeding mechanism. Since it has been shown that injection of procaine in the ventromedial area of the rat (*7*) or injection of pentobarbitone sodium in the lateral ventricles of the cat (*8*) causes transient hyperphagia, we examined the effect of pentobarbital sodium perfusion in cerebrospinal fluid of goats.

Two goats were prepared with left ventricular and cisterna magna guide tubes (*9*). They were fed a concentrated feed (less than 10 percent crude fiber) and about 300 g of alfalfa hay daily. Several of the pentobarbital perfusions followed a 2-hour control perfusion during which the goats ate until apparently satiated. Other perfusions were conducted following a 16-hour ad libitum feeding after the ventricular and cisternal probes were placed in the goats, and after the animals received fresh feed again and were allowed to eat at will for 1 hour.

Animals were placed in a chute with a sling to help support them in the event sleep or ataxia was induced. Ventricular pressure was monitored. Perfusion rate varied between 0.5 and 1.0 ml per minute; 1 to 5 mg of pentobarbital sodium per milliliter of perfusion fluid [a synthetic cerebral spinal fluid (*9*)] was used.

Goats were not aware of the starting or stopping of the perfusion but they often appeared somewhat uneasy for about 1 minute, 4 or 5 minutes after the perfusion began. They then began to nibble at the concentrated feed and to increase their rate of intake which at times could be characterized only as voracious. In fact, the animals ate until their flanks were so distended at the end of an induced feeding that they appeared to be suffering from bloat. The rate of intake depended on rate of perfusion, concentration of pentobarbital sodium, the stage at which perfusion was stopped, and the rate of onset of drowsiness and sleep. Table 1 presents the feed-intake data. The goats ate for varying lengths of time; a maximum induced feeding of over 900 g was eaten in less than an hour (they usually ate about 500 g of food in the first hour following a 48-hour fast). The quantity of pentobarbital sodium perfused, shown in Table 1, is the quantity actually injected in the animal for periods lasting from 4 to 15 minutes. During the longer intervals some of the pento-

Table 1. Duration of perfusion and amount of feed consumption of satiated goats with the ventriculocisternal system perfused with pentobarbital sodium solution.

Goat	Date	Perfusion		Pentobarbital perfused (mg)	Interval during which feed was eaten (min)	Feed eaten (g)
		Time started	Duration (min)			
Hercules	8/24	1:00 p.m.	6	6	5	145
Zeus	8/26	12:15 p.m.	8	13	15	582
		1:35 p.m.	6	9	20	276
		2:25 p.m.	6	9	10	98
Hercules	9/8	8:30 a.m.	15	22	15	240
Zeus	9/8	12:30 p.m.	5	10	12	260
		2:20 p.m.	9	22	15	240
Hercules	9/15	10:25 a.m.	4	10	20	221
		11:05 a.m.	15	15	20	174
Zeus	9/15	4:00 p.m.	4	10	29	476
		4:37 p.m.	9	41	34	632
	9/18	12:42 p.m.	15	68	48	922
		2:09 p.m.	15	68	27	460

barbital was probably appearing in the perfusate, as the volume of the ventricules is 8 to 12 ml (*9*).

The goats eventually became ataxic and the ataxia in varying degrees persisted for at least an hour after perfusion. Often after the animals had eaten appreciable amounts of feed, they would consume 500 to 1000 ml of water. When water was offered ad libitum during perfusion, the goats would always eat before drinking. Apparently hyperdipsia is secondary to hyperphagia, and the thirst centers were probably not directly affected. Usually the goats became drowsy and dozed occasionally; they could be awakened with varying degrees of difficulty, but would invariably start to eat until they dozed again. Unlike the cats of Feldberg's experiment (*8*), the goats did not eat while recovering from induced sleep.

Since pentobarbital sodium is definitely an inhibitory agent of neural activity, it is anticipated that the hypothalamic ventromedial nuclei (satiety center) are affected. If this is the case and hyperphagia is due to increased hypothalamic lateral-area (feeding center) activity, then in the normal animal some factor or factors, that is, intermediary metabolites, possibly stimulate activity in the satiety center which maintains a balance with the feeding-center activity.

Stimuli that cause increased lateral-area activity intitiate feed intake (*1*). The fact that, in these experiments, feed intake was induced in apparently satiated goats by perfusing the ventriculocisternal system with a pentobarbital solution suggests that in normal, satiated ruminants, as in monogastric animals, the ventromedial-nuclei activity inhibits lateral-area activity and, therefore, promotes satiety. It is not apparent from previous work (*10*) what, if any, receptors may exist in these nuclei that make possible the energy-intake regulation. Perhaps they are only part of a central control with afferent neural communication from receptors in the ruminal area, which, if present, may perceive distention, changes in volatile fatty-acid concentration, and other parameters associated with satiety and hunger.

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Serum Concentration: Effects on Cycle and X-ray Sensitivity of Mammalian Cells

Abstract. *If the content of serum in the culture medium of exponentially growing Chinese hamster cells is below optimum (15 percent), the doubling time and the resistance to x-irradiation of the cells are increased. In synchronously dividing populations the increase in doubling time is primarily caused by increase in duration of the postmitotic (G_1) phase of the cells; this phase is relatively radiation resistant. The response of the cells growing synchronously is related quantitatively to the response of the cells dividing randomly.*

That the nutritional environment strongly influences the rate of cell growth in culture is widely recognized (*1*). Less well known is the effect of nutritional environment on x-ray sensitivity and on the component phases of the cell cycle. This report deals with the effects of varying serum concentrations in the growth medium in monolayer cultures. In a subline of Chinese hamster ovary cells recently subcloned in this laboratory, the doubling time can be predictably controlled within certain limits by varying the amount of fetal bovine serum (FBS) added to the growth medium [Eagle's minimal essential medium (MEM) plus antibiotics]. Doubling times, as obtained during logarithmic growth from daily cell counts (Coulter counter), compared to the percentage of serum supplement are presented in Fig. 1. If the medium completely lacks serum, the cell number increases by about 50 percent during the first few hours after serum deprivation, and then the cells cease growth entirely. In marked contrast, mouse L cells show only a very limited dependence on the amount of FBS available (Fig. 1). For Chinese hamster cells, supplementing the medium with more than 15 percent of serum favors growth little if at all. Medium containing 15 percent of serum is used as reference and is referred to as standard medium.

In one test of the effect of serum concentration on x-ray sensitivity of the Chinese hamster cells (Fig. 2, closed circles), cells were grown in stock bottles for at least 2 weeks on media containing different amounts of FBS. At a stage of growth when previous experiments had shown the cells to be in ex-

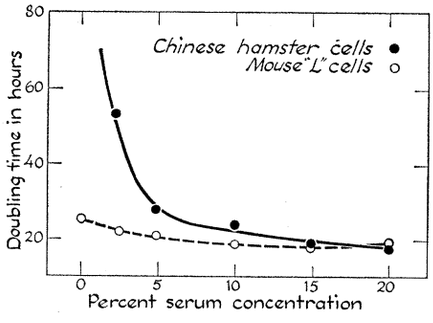

Fig. 1. Mean doubling time of cells during exponential portion of the growth curve. Cells were grown on glass, treated with trypsin (.05 percent, 300:1) and counted on Coulter counter. Without FBS, L cells show short-term (4 to 6 days) growth only. Standard deviations do not exceed ± 5 percent of the mean values.