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Passive Transfer of an Appetite Suppressant Factor[1] (39913)

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Introduction. In 1959, Hervey (1) reported a series of studies in rats that suggested the presence of a humoral factor, circulating in the hyperphagic state, which supresses appetite. Rats were rendered hyperphagic by destruction of the ventral medial nucleus (VMN) of the hypothalamus, the so-called "satiety center," and were parabiosed to normal rats. While the VMN-lesioned rats gained weight, their parabiosed partners ate less and lost weight. Hervey postulated that the lesioned animals were producing a humoral substance that traversed the parabiotic connection to the intact animals' bloodstream and caused a decrease in food consumption.

In an experiment similar to Hervey's, Davis *et al.* (2) induced hypophagia in normal, food-deprived rats by transfusing them with blood from normal, satiated nonobese rats. These results suggested a similar "feedback" mechanism operative in intact animals under physiological conditions. Marshall and Barnett (3) demonstrated that mice injected with gold thioglucose (GTG) developed hypothalamic lesions in the VMN, inducing hyperphagia and obesity. Unlike electrically lesioned animals, GTG-induced lesions appear to affect food intake with minimal impairment of other hypothalamic functions (4, 5), as the anatomical extent of these lesions in mice appears extremely localized (6, 7).

In the present study, we examined further the possibility of passive transfer of an appetite suppressant factor with the serum from GTG-hyperphagic obese mice as well as from a human subject with hypothalamic obesity.

Materials and methods. Swiss–Webster female mice, obtained from a local source, were fed standard laboratory chow and were maintained under constant environmental temperature and a 12-hr dark, 12-hr light cycle. Gold thioglucose was obtained from Sigma Chemical Company, St. Louis, Missouri. Serum glucose concentrations were measured by the photometric technique of Dubowski (8). Serum concentrations of insulin and glucagon were quantitated by radioimmunoassay (9, 10). Statistical differences between groups were analyzed by Student's *t* test.

"Obese" serum preparation. Four hundred female mice (20- to 30-g body wt) were injected with a single intraperitoneal (ip) dose (800 mg/kg) of GTG. This dose was lethal in 20% of the animals. The survivors demonstrated marked hyperphagia and attained maximal weight gain after 6 weeks, at which time they were sacrificed by exsanguination. The serum was pooled and stored at −10°, and is subsequently referred to as "obese" pooled serum. Histologic preparation of the hypothalamic region was performed in 10 animals that verified the site and extent of the chemical lesion.

"Passive" transfer of the appetite supressant. During the first of the experiment, three groups of mice (10 animals per group) received 0.025-ml ip injections (four times daily) of either obese pooled serum (Group I), normal mouse serum (Group II), or physiological saline (Group III). The injections were administered over an initial 2-week period, followed by 5 weeks of no treatment. A second 2-week injection period was followed by a final 3-week period of no treatment. All animals were given access to the same quantity of food and water, and weights were recorded daily.

Characterization of hypothalamic obesity in a human subject. N.H. is a 27-year-old black male, who sustained blunt head

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trauma causing a skull fracture and a frontal-parietal-temporal subdural hematoma requiring surgical evacuation and producing a right, central, seventh cranial nerve deficit and quadriparesis. During rehabilitation, the patient developed a voracious appetite, increasing his weight from 145 to 172 lb in an interval of 1 month. Although his weight could be stabilized with caloric restriction, when allowed free access to food he demonstrated an additional 16-lb weight gain over a 10-day period. A calorie count conducted during this period was compatible with the hyperphagia-induced weight gain, rather than secondary to decreased physical activity. Endocrine testing was performed while the patient was maintained on no medications. A 5-hr glucose tolerance test (GTT) showed mild chemical diabetes with absent growth hormone (GH) response. An insulin tolerance test demontrated an absent GH response with a borderline cortisol response (Table I). Serum T_3 resin uptake was 33.9% (normal, 42–62%), with serum thyroxine of 3.7 μg% (normal range, 4.7–11.7 μg%) and thyroid stimulating hormone (TSH) of 2.6 μU/ml (normal, less than 10 μU/ml). TRH (300 μg) stimulation showed a normal response (Table I). Baseline serum testosterone was 183 ng% (normal ≥ 300 ng%). Baseline luteinizing hormone (LH) and follicle stimulating hormone (FSH) were normal, but following clomiphene citrate there appeared to be a blunted response (Table I). A 3-g overnight metyrapone test was normal (11-desoxycortisol rose to 12.8 μg%; normal, >7 μg%), and ACTH (RIA) levels increased to 130 pg/ml after a baseline of 14 pg/ml (normal, 15–70 pg/ml). An 18-hr water deprivation test showed a borderline repsonse, with urinary osmolality rising from 155 to 574 mOsm/kg, plasma osmolality rising from 296 to 298 mOsm/kg, and arginine vasopressin levels (RIA) (11) increasing from 1.2 to 2.6 μU/ml. These results suggested hypothalamic involvement, with impairment of TSH and GH release and possibly including LH, FSH, and vasopressin.

Passive transfer of human obese serum. Fasting serum from the patient was collected, pooled, and injected ip into a series of normal mice (Group IV) in doses of 0.25 ml, twice a day for 2 weeks. A control group of 15 normal mice (Group V) was injected with a similar amount of pooled normal human serum.

TABLE I. ORAL GLUCOSE TOLERANCE TEST (100 g), INSULIN TOLERANCE TEST (0.1 units/kg REGULAR U-100 INSULIN INTRAVENOUS BOLUS AFTER A 12-hr FAST), THYROTROPHIN RELEASING HORMONE (TRH) STIMULATION TEST, AND CLOMIPHENE CITRATE (CLOMID) (150 mg/day × 4 days) STIMULATION IN PATIENT WITH HYPOTHALAMIC OBESITY.

5-hr Oral glucose tolerance test

Time (hr)	Glucose (mg%)	Insulin (μU/ml)	HGH (ng/ml)
0	93	13.2	<2.2
1	200	175	<2.2
2	195	173	<2.2
3	120	65.5	<2.2
4	90	25.5	<2.2
5	80	12.5	<2.2

Insulin tolerance test

Time (min)	Glucose (mg%)	Cortisol (μg%)	HGH (ng/ml)
0	112	10.3	<2.2
15	68	8.5	<2.2
30	58	10.5	<2.2
60	75	16.7	<2.2
90	77	12.2	<2.2
120	88	8.6	<2.2

TRH stimulation test		Clomiphene test		
Time (min)	TSH (μU/ml)	Time	LH (mIU/ml)	FSH (mIU/ml)
0	1.5	Baseline	9.7	2.9
15	12.3	Day 3	8.9	<1.6
30	12.5	Day 4	8.2	<1.6
45	9.4			
75	7.0			
90	5.7			

Results. Obesity induction. A 20% mortality was observed after the GTG injections of 800 mg/kg body weight, correlating well with previously reported experiments (11). Maximal weight gain was achieved after 40 days, with a mean increase of 41.2 ± 2.3 (SEM) g in the GTG-injected mice (Group I) as opposed to 7.2 ± 0.3 g in the control group ($P < 0.001$) (Fig. 1). The animals were sacrificed at this time and their serum was pooled. Although individual serum samples were not analyzed, within the pool of obese mouse serum, immunoreactive glucagon was 266 pg/ml, insulin 21 μU/ml, and glucose 236 mg%, as compared to the

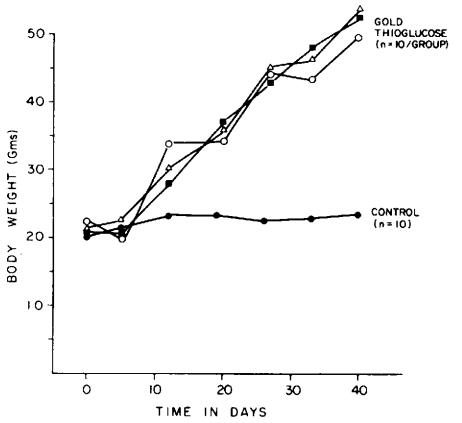

FIG. 1. The weight gain in the groups of normal mice (10 mice per group) injected with gold thioglucose (800 mg/kg) is illustrated by the dashed lines. Body weight showed a constant increase over the 40-day period which was singificantly greater than that of saline-injected controls, illustrated by the solid line ($P < 0.001$). Although 400 mice were injected with gold thioglucose, the results are shown only for 30 mice for the sake of clarity. Each point represents the mean.

pool of normal mouse serum with values of 112 pg/ml, 14 μU/ml, and 166 mg%, respectively.

Passive transfer of appetite suppressant. As illustrated in Fig. 2, at the end of the first 2-week period of injections, the obese serum-injected Group I lost 1.9 ± 0.2 (SEM) g body weight, while Group II gained 0.5 ± 0.5 g and Group III lost only 0.1 ± 0.4 g (Group I vs II, I vs III, $P < 0.01$). This occurred despite a larger initial body weight in Group I. During the second period (5 weeks off treatment), all groups showed similar weight gains. Group I mice appeared to regain enough weight to achieve body weights similar to those of the control groups by the end of the 5-week period. At the end of the third period (2 weeks of obese serum injections), Group I showed a loss of 1.0 ± 014 g, while Groups II and III increased body weight by 0.1 ± 0.4 and 0.4 ± 0.2 g, respectively (Group I vs II, I vs III, $P < 0.1$).

Passive transfer of human obese serum. After 2 weeks of injections, Group IV (human obese serum-injected mice) lost 2.6 ± 0.5 g, while Group V (control) lost only 1.0 ± 0.3 g ($P < 0.02$) (Fig. 3).

Discussion. Control of food intake is presumably regulated by many factors, and recent work has suggested modulation of this process by humoral substances. Changes in circulating metabolic fuels—glucose, lipids, and proteins—have been forwarded as substrates stimulating a hypothalamic feeding center or suppressing a hypothalamic satiety center.

The "glucostat" theory (13) implies that increased glucose utilization is the stimulus for cessation of eating, although hypophagia is not always demonstrable following experimental induction of hyperglycemia. Insulin-induced hypoglycemia (14) or administration of 2-deoxy-D-glucose, a nonmetabolized analog (15), however, usually produces hyperphagia; and intracellular hypoglycemia increases the electrical activity of the VMN (16). In the present experiment, despite slight differences in glucose and insulin concentrations between the obese and normal mouse serum pools, it is unlikely that the amounts injected would significantly alter circulating humoral levels.

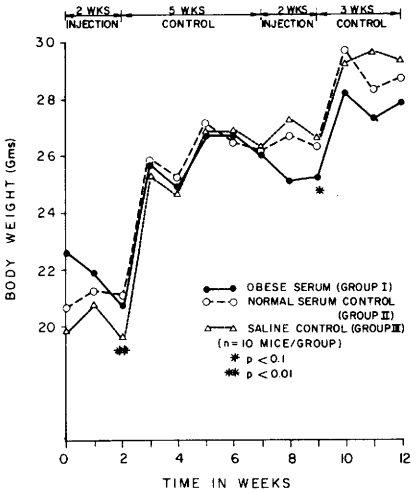

FIG. 2. Changes in body weight for mice in Groups I, II, and III. Group I mice are illustrated by the solid line, and received "obese" serum injections. Group II mice are illustrated by the dashed lines, and received normal mouse serum injections. Group III mice are illustrated by the interrupted lines, and received normal saline injections. Each group received two 2-week series of injections, followed by a control period of no treatment. Weight loss occurred in Group I after the initial 2-week period of injection ($P < 0.01$) and again after the second 2-week period ($P < 0.1$). Each point represents the mean of a group of 10 animals.

The "lipostat" theory (17, 18) states that increased fat stores accumulated during the period of rapid weight gain are associated with an inbalance between food intake and energy expenditure, and circulating lipid metabolites may alter the sensitivity of the VMN. If lesioned obese animals are starved, they will again exhibit hyperphagia, but only until they reach their prestarvation weight and, presumably, their previous amount of depot fat. Hervey's parabiosed rat study, with one partner becoming obese and the other thin, may fit into this lipostat model, with the excess fat of the lesioned rat suppressing food intake of his partner. In addition, if both animals have an intact VMN, the depot fat is reduced to about 50% of normal, suggesting that the VMN of each animal is responding to the total lipid metabolites in the combined vascular bed. In an experiment similar to Hervey's, Han *et al.* (19) failed to observe hypophagia in the normal parabiosed rat, but he also failed to allow obesity to develop in the lesioned animal, thus supporting the premise that enlarged fat pools are in some way essential.

The identity of the postulated metabolite which signals the VMN as to the status of the organism's adiposity is currently unknown. Various lipid metabolites, including nonesterified fatty acids (18), as well as circulating hormones, including glucocorticoids and insulin, have been suggested, but never documented (20). The present study suggests that such a humoral substance may be passively transferred to normal recipient mice via extremely small quantities of pooled obese serum. As the pooled serum was subjected to repeated thawing and refreezing during the injection periods, it also appears that such a substance is chemically rather stable. Unfortunately, exhaustive hormonal analysis of the pooled obese serum was hampered by its limited quantity.

The present studies document a significant weight reduction in the animals treated with obese serum during the initial injection period, despite a larger initial body weight in this group of mice (Group I). Unfortunately, the amount of food consumed by these animals was not quantitated, although the decrease in weight of Group I suggests

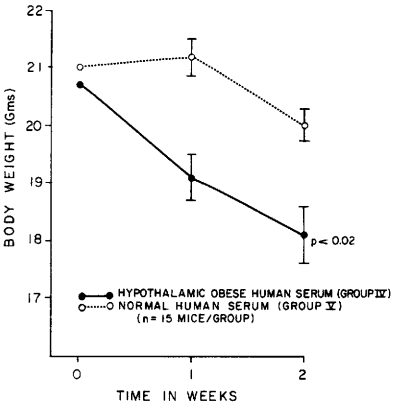

FIG. 3. The changes in body weight in the mice injected with serum from the patient with hypothalamic obesity (Group IV) are illustrated by the solid line, and those in the mice injected with normal human serum (Group V) by the dashed line. Each point represents the mean ± SEM. By the end of 2 weeks of daily injections, Group IV mice lost significantly more weight ($P < 0.02$).

the development of hypophagia. It is unclear why the second 2-week injection period produced a weight loss that approached, but did not clearly attain, statistical significance ($P < 0.1$). Perhaps the continued defrosting of the obese serum in some way altered the postulated humoral substance. The possibility also exists that a critical body mass is optimal for induction of weight reduction following obese serum injection: Older animals with a greater body weight may be more resistant or might require a larger dose of serum.

The observation with serum from a patient with documented hypothalamic obesity extends the original postulate of a humoral appetite suppressant factor to the human model. Unfortunately, documentation of the extent of anatomical brain damage in this patient will have to await a future date. Although serum insulin levels were elevated in this individual, circulating cortisol, growth hormone, and thyroxine were not. The slight decrease in weight in the mice injected with normal human serum (Group IV) may well be secondary to the irritative effects of large amounts of this foreign substance.

Summary. Hyperphagia and subsequent obesity were induced in mice by gold thio-

glucose, and pooled serum was injected into other mice (Group I). Control mice were injected with normal mouse serum (Group II) or saline (Group III). The injections were administered during an initial 2-week period, followed by 5 weeks of no treatment, a second 2-week injection period, and a final 3-week period off treatment. After the first period, Group I lost significantly more than Groups II and III ($P < 0.01$). Weight gains of equal magnitude were observed in all groups during the second period (off treatment). During the last period of injections, Group I lost weight, while Groups II and III gained, but the difference did not reach statistical significance. A similar study was undertaken using serum from a human subject with documented hypothalamic obesity. At the end of 2 weeks, injected mice lost significantly more weight than controls ($P < 0.02$). These results suggest the presence of a humoral substance in the serum of hypothalamic obese mice and in the human counterpart which may be passively transferred to normal mice and suppress food intake.

1. Hervey, G. R., J. Physiol. **145,** 336 (1959).
2. Davis, J., Gallagher, R., and Landove, R., Science **156,** 1247 (1967).
3. Marshall, N. B., and Barnett, J., Proc. Soc. Exp. Biol. Med. **90,** 240 (1955).
4. Ingram, W. R., Clin. Sym. **8,** 117 (1956).
5. Katsuki, S. Y., Diabetes **11,** 209 (1962).
6. Baile, C. A., Herrera, M. G., and Mayer, J., Amer. J. Physiol. **218**(3), 857 (1970).
7. Mayer, J., and Arees, E., Fed. Proc. **27**(6), 345 (1968).
8. Dubowski, K. M., Clin. Chem. **8,** 215 (1962).
9. Frohman, L. A., and Bernardis, L. L., Endocrinology **82,** 1125 (1968).
10. Frohman, L. A., and Bernardis, L. L., Amer. J. Physiol. **221**(6), 1596 (1971).
11. Skowsky, W. R., Rosenbloom, A. A., and Fisher, D. A., J. Clin. Endocrinol. Metab. **38,** 278 (1974).
12. Debons, A. F., Silver, L., *et al.*, Amer. J. Physiol. **202**(4), 743 (1962).
13. Mayer, J., Ann. N. Y. Acad. Sci. **63,** 15 (1955).
14. Booth, D. A., and Brookover, T., Physiol. Behav. **3,** 439 (1968).
15. Likuski, H. J., Debons, A. F., and Cloutier, R. J., Amer. J. Physiol. **212,** 669 (1967).
16. Desiraju, T., Banerjee, M. G., and Anand, B. K., Physiol. Behav. **3,** 757 (1968).
17. Kennedy, G. C., Proc. Roy. Soc. Biol. **140,** 549 (1953).
18. Kennedy, G. C., Brit. Med. Bull. **22,** 216 (1966).
19. Han, P. W., Mu, J. Y., and Leprovsky, S., Amer. J. Physiol. **205,** 1139 (1963).
20. Hervey, G. R., Nature (London) **222,** 629 (1969).

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