plasma was quite variable, but higher-than-normal values were encountered in many cases (Fig. 2). The production of small amounts of parathyroid hormone may be a common characteristic of these tumors, although clinical hyperparathyroidism, with its attendant hypercalcemia, hypercalciuria, and hypophosphatemia, develops only relatively rarely.

In the absence of a standard preparation of the human hormone, accurate values for absolute concentrations cannot be presented. However, based on the assumption that human PTH reacts no more strongly than bovine PTH in competing against bovine $I^{131}$-PTH for antibodies to the bovine hormone, it can be stated that the standard plasma used in this study contains at least 60 m$\mu$g of parathyroid hormone per milliliter. Plasmas used as standards can be stockpiled and stored frozen and can eventually be standardized against purified human PTH when it becomes available, at which time absolute values can be assigned to all plasmas that have been assayed with reference to a standard plasma (*8*).

SOLOMAN A. BERSON
ROSALYN S. YALOW

*Radioisotope Service,*
*Veterans Administration Hospital,*
*Bronx, New York 10468*

### References and Notes

1. A. B. Gutman, T. L. Tyson, E. B. Gutman, *Arch. Intern. Med.* **57**, 379 (1936); see also B. F. Bower and G. S. Gordon, *Ann. Rev. Med.* **16**, 83 (1965).
2. A. H. Tashjian, L. Levine, P. L. Munson, *J. Exp. Med.* **119**, 467 (1964); P. L. Munson, A. H. Tashjian, L. Levine, *Cancer Res.* **25**, 1062 (1965); see also B. F. Bower and G. S. Gordon, *Ann. Rev. Med.* **16**, 83 (1965).
3. S. A. Berson, R. S. Yalow, G. D. Aurbach, J. T. Potts, Jr., *Proc. Nat. Acad. Sci. U.S.* **49**, 613 (1963).
4. L. M. Sherwood, J. T. Potts, Jr., R. A. Melick, G. D. Aurbach, The Endocrine Society, Program of the 48th Meeting, No. 6, June 1966.
5. Curves resulting from B/F plotted as a function of the PTH in the standard plasma, in several other plasmas from uremic patients, and in three crude extracts of human parathyroid adenomata were essentially the same when the concentrations were adjusted to fit the curve at one point.
6. Case records of the Massachusetts General Hospital No. 29-1963, *New England J. Med.* **268**, 943 (1963). Tertiary hyperparathyroidism in our patient was characterized by the development of multiple autonomously functioning adenomata and by hypercalcemia which followed a long period of secondary hyperparathyroidism associated with chronic uremia and hypocalcemia.
7. By the addition of appropriate amounts of plasma (from hypoparathyroid patients) previously found to be devoid of detectable hormone, the total concentration of plasma in standard solutions was kept the same as that of plasma used for assay of unknowns (one part in five or one part in two and one-half parts of 0.01*M* veronal buffer).
8. We thank Drs. G. Aurbach and J. T. Potts, Jr., Dr. H. Rasmussen, Dr. C. Arnaud, and Mr. C. Hawker for supplies of bovine parathyroid hormone used in this study.

10 August 1966

## Lipolysis in Homogenates of Adipose Tissue: An Inhibitor Found in Fat from Obese Rats

Abstract. *The presence of a lipid-bound inhibitor in adipose tissue of rats with hypothalamic obesity may explain the failure of the tissue to release fatty acids on epinephrine stimulation. Aqueous extracts of tissue from obese animals showed no deficiency of lipase activity, but when whole homogenates of epididymal fat from lean and obese animals were mixed, 25 percent tissue from obese animals reduced by 73 percent the release expected from tissue of lean controls.*

Excess fat accumulation can be induced in experimental animals by placement of bilateral electrolytic lesions in the ventromedial nuclei of the hypothalamus. Such lesions apparently affect appetite control; this type of obesity has, therefore, been termed regulatory obesity (*1*). Several metabolic alterations have occurred in rats so treated (*2, 3*). Prominent among these are a change (virtually complete within 3 weeks of treatment) in the fatty acid composition of depot fat, and impairment of release of free fatty acids by isolated slices of epididymal fat. Impairment precedes both the change in tissue composition and accumulation of bulk fat. We have studied the mechanism of the impairment and have found an inhibitor of lipolysis.

Male albino rats were prepared as described by Kennedy and Mitra (*4*). A control of the same age was kept for each animal with brain lesions. Epididymal fat tissue was taken either by biopsy, while the animal was under ether anesthesia, or after the animal was killed. Two assays for lipolytic activity were used. Assay A was similar to that described by Vaughan (*5*), in that the animal's own fat served both as substrate and as enzyme source; after removal from the animal, paired slices of fat pad weighing about 100 mg were incubated for 1 hour in 1.0 ml of Krebs-Ringer bicarbonate buffer, *p*H 7.2, containing 4 percent bovine serum albumin of low fatty acid content. Epinephrine (0.5 $\mu$g) was added to one of each pair of tubes, and incubation was continued for 5 minutes. The fat was then transferred to 2.5 ml of fresh medium and homogenized. One milliliter of homogenate was incubated in a tube for 1 hour at 37°C; an equal portion was pipetted into a second tube containing 10 ml of Dole extraction mixture (*6*) but was not incubated. After incubation of the first tube, the same volume of Dole mixture was added and the fatty acids were isolated and titrated (*7*). The amount of fatty acids released during the experimental period was taken to be the difference between the amounts in the incubated tube and the tube that was not incubated. Release due to epinephrine was computed as the excess of fatty acid appearing during the experimental period in tubes containing homogenates of stimulated tissue.

Assay B was designed to measure extractible lipolytic activity; an artificial substrate was used. One gram of epididymal fat was homogenized with 3 ml of 0.01*M* phosphate buffer, *p*H 7.2. The fat cake was removed by a 10-minute centrifugation (10,000*g*, 3°C), and the aqueous fraction was then filtered, centrifuged, and separated from residual fat. The substrate was triolein (99 percent pure, Hormel), prepared as a 0.02*M* suspension in 10 percent gum arabic with an ultrasonic probe (Branson). The incubation mixture contained in a total volume of 2 ml: 0.3 ml of extract of adipose tissue, 2.0 $\mu$mole of triolein, 0.1 mmole of $MgCl_2$, 0.012 mmole of phosphate buffer (*p*H 7.2), and 5 percent bovine serum albumin of low fatty acid content. Incubation at 37°C lasted 1 hour. The reaction was stopped with 10 ml of Dole extraction mixture, and 20 $\mu$g of heneicosanoic acid (a 21 carbon saturated acid obtained from Hormel Foundation) was added as an internal standard. The fatty acids were then isolated by alkaline extraction, methylated with freshly distilled diazomethane (*8*), and measured by gas-liquid chromatography. Oleic acid was released during the experiment, and the quantity was taken as the difference between the amounts of methyl oleate recovered from the incubated tube and from the tube that was not incubated.

In comparing five lean and five obese animals by assay A, we found that fat from lean animals released a mean of $4.77 \pm 1.02$ $\mu$mole of free fatty acid because of stimulation with epinephrine per gram of tissue; none was released from tissue of the obese animals. In fact, this tissue usually removed small amounts from the incubation system (Table 1). Assay B was used to determine whether the inactivity of the tissue from the obese animals was due to a deficiency of lipase similar to that noted by Lochaya *et al.* (*9*) in adipose tissue of genetically obese mice or whether it

Table 1. Release of free fatty acids from homogenates of adipose tissue. Figures for free fatty acid release represent the means ± standard errors of five or more separate experiments. All values for inhibition are calculated from the changes in free fatty acid release due to epinephrine. The difference between free fatty acids released from tissues of lean and obese animals is taken as 100 percent; values for inhibition in mixed tissue experiments and those with $PGE_1$ are relative to this total difference.

| Weight of fat (mg) | | FFA release ($\mu$mole/g per hour) | | Inhibition (%) |
|---|---|---|---|---|
| Lean animal | Obese animal | Unstimulated | Change due to epinephrine | |
| | | *Unmixed tissue* | | |
| 100 | | 4.96 ± 0.63 | 4.77 ± 1.02 | 0 |
| | 100 | 2.29 ± 0.44 | −1.08 ± 0.70 | 100 |
| | | *Mixed tissue* | | |
| 50 | 50 | 4.08 ± 0.47 | −0.98 ± 0.40 | 98 |
| 75 | 25 | 4.12 ± 0.83 | 0.50 ± 0.32 | 73 |
| | | *Prostaglandin $E_1$, 0.1 $\mu$g/ml added* | | |
| 100 | | 2.19 ± 0.71 | 0.46 ± 0.32 | 74 |

was a characteristic of the endogenous substrate. Lipolytic activity extractible from adipose tissue of the animals with hypothalamic obesity was greater than that recovered from lean controls of the same age. While aqueous extracts of tissue from four lean animals yielded 52.4 ± 11.1 m$\mu$mole of oleic acid per mg protein, those from tissue of four obese rats yielded 148.8 ± 38.6 m$\mu$mole of free fatty acid from the triolein substrate. Recombination of aqueous extracts with the fat cakes from which they had been separated gave results essentially similar to those of assay A (Table 1).

The presence of an adequate lipase in extracts of adipose tissue from treated animals suggests that there may be a fat-bound inhibitor of lipolysis in the lipid fraction. Therefore, we used assay A to study mixtures of fat from lean and obese animals. Before the tissue was homogenized, weighed portions of epinephrine-stimulated fat from a lean and an obese animal were mixed. The homogenate was then prepared and incubated (Table 1). The adipose tissue from obese animals had a marked inhibitory effect on the epinephrine-stimulated release of free fatty acids from tissue of lean animals. Even when only 25 percent of the tissue mixture was from an obese animal, the inhibitory effect was present.

In a previous communication, we have emphasized the importance of depression of free fatty acid release for the development and maintenance of hypothalamic obesity in the rat (*10*). The present study gives information concerning the mechanism of this depression. Using the assay where adipose tissue acts as both enzyme source and substrate, it appeared that the depot fat of obese animals was deficient in lipase. When, on the other hand, lipolytic activity was measured with an aqueous extract of fat tissue as enzyme source and purified triglyceride as substrate, adequate enzymatic activity was found in the tissue extract of obese animals. This type of preparation leaves a large part of the lipolytic activity in the fat cake, but there is now no satisfactory method of separating the bulk of the enzyme from the fat. It is unlikely, however, that a tissue poor in lipase would simultaneously bind the enzyme weakly and thus yield extracts with normal lipolytic activity. Instead, the experiments on mixtures suggest that the tissue of the obese animal contains a potent inhibitor of lipolysis. The inhibitor appears to be fat-bound since its effect disappears when the endogenous fat cake is removed from aqueous extracts of tissue and replaced with pure triglyceride. Preliminary studies substituting organic solvent extracts (80 percent ethanol, followed by petroleum ether) for the homogenates of tissue from obese animals were carried out according to the design of Table 1. These confirmed the lipid nature of the inhibitor and indicated that it is not a lipoprotein. It has been shown (*11*) that free fatty acids themselves are inhibitors of lipolysis. The measurements of free fatty acids in the tubes that were not incubated (assay A) demonstrated that the tissues of the obese animals studied have contained no more of these substances than tissues of lean controls and, therefore, could not be the suppressing factor.

Because the inhibitory effect of fat from obese animals is most marked on epinephrine-stimulated tissue, the suppressor could be operating at the level of the cyclic 3′, 5′-adenosinemonophosphate activating system. The prostaglandins constitute a class of inhibitors having these properties. They are fat soluble, and inhibit epinephrine-stimulated fatty acid release from adipose tissue (*12*). In addition, material with some of the biological properties of prostaglandin $E_1$ ($PGE_1$) has been recovered from epididymal fat pads of intact rats (*13*). Addition of $PGE_1$ (0.1 $\mu$g/ml) (*14*) to a homogenate of adipose tissue from lean animals (assay A) depressed fatty acid release in a manner similar to that seen when tissue from obese animals was added (Table 1).

These studies suggest that the depression of free fatty acid release from adipose tissue of rats with hypothalamic obesity is due to the presence of an inhibitor. This substance, because of its apparent fat solubility and effect on the epinephrine-sensitive lipase system, may be similar to one of the prostaglandins.

Study of inhibitor content of adipose tissue from animals with obesity resulting from force feeding, or from hormonal or genetic influences, will be necessary to determine whether its accumulation is peculiar to the obesity of hypothalamic injury. It is possible that an unusual accumulation of inhibitor substance may be a common feature of several forms of obesity because depressed fatty acid release is not limited to the hypothalamic type (*3*).

HERBERT A. HAESSLER
*Science and Engineering Institute, Waltham, Massachusetts* and *Harvard Medical School, Boston, Massachusetts*
JOHN D. CRAWFORD
*Department of Pediatrics, Harvard Medical School*

### References and Notes

1. J. Mayer, *Physiol. Rev.* **33**, 472 (1953).
2. S. E. Starr, J. D. Crawford, H. A. Haessler, *Metabol.* **15**, 39 (1966).
3. H. A. Haessler and J. D. Crawford, *Ann. N.Y. Acad. Sci.* **131**, 476 (1965).
4. G. C. Kennedy and J. Mitra, *J. Physiol.* **166**, 395 (1963).
5. M. Vaughan, J. R. Berger, D. Steinberg, *J. Biol. Chem.* **239**, 401 (1964).
6. V. P. Dole, *J. Clin. Invest.* **35**, 150 (1956).
7. J. D. Schnatz, *J. Lipid Res.* **5**, 483 (1964).
8. S. R. Lipsky and R. A. Landowne, in *Methods in Enzymology*, S. P. Colwick and N. O. Kaplan, Eds. (Academic Press, New York, 1963), vol. 6, pp. 513–537.
9. S. Lochaya, J. C. Hamilton, J. Mayer, *Nature* **197**, 182 (1963).
10. H. A. Haessler and J. D. Crawford, *J. Clin. Invest.* **45**, 1010 (1966).
11. P. R. Bally, H. Kappeler, E. R. Froesch, A. Labhart, *Ann. N.Y. Acad. Sci.* **131**, 143 (1965); M. Rodbell, *ibid.*, p. 302.
12. D. Steinberg, M. Vaughan, P. J. Nestel, O. Strand, S. Bergstrom, *J. Clin. Invest.* **43**, 1533 (1964).
13. J. E. Shaw, *Federation Proc.* **25**, 770 (1966).
14. We thank Dr. J. W. Hinman for supplying $PGE_1$.

6 July 1966