a motor-driven glass homogenizer in four volumes of 0.1*M* phosphate buffer, *p*H 7.0; all operations were carried out at 4° ± 2°C.

The supernatant from centrifugation at 75,000*g* was subjected to successive ammonium sulfate fractionations. The only fraction showing significant response to sweetness was the one containing material soluble in 20-percent ammonium sulfate but insoluble in 40-percent ammonium sulfate (40-percent fraction), and the data now reported are results obtained with this material. In no instance was the response time-dependent.

Except for the study of effects of *p*H, all assays were carried out in 0.05*M* sodium phosphate buffer at *p*H 7.0. For the *p*H variations, the following buffers were used, all at 0.1 ionic strength: formate, *p*H 3.6; acetate, *p*H 4.4; formate, *p*H 4.9; acetate, *p*H 5.4; phosphate, *p*H 6.0; acetate, *p*H 6.1; phosphate, *p*H 6.7; tris, *p*H 7.1; phosphate, *p*H 7.6; tris, *p*H 8.6 and 9.6.

Equation 2 proved to be adequate to describe the data obtained with all test compounds. Figure 1 shows data obtained with fructose, which are typical of the results obtained. Values of R_m and K, determined experimentally, are shown in Table 1, along with the values of $\triangle F$ calculated from the values of K; the table also includes the relative sweetnesses of the sugars, as determined in vivo by others for the dog *(8)* and for humans *(9)*.

The 40-percent fraction from bovine taste buds appears to interact with sugars, forming complexes whose strengths parallel the sweetnesses of the tastes of the sugars (see Table 1). Physicochemical studies, to be reported elsewhere, show this material to be proteinaceous and nearly homogeneous. Sugar-protein interactions have been previously observed, but only at sugar concentrations far higher than we used. Thus it appears unlikely that the interactions observed are nonspecific, a conclusion supported by our observation that most of the protein extracted from bovine taste buds, which appears in the other fractions in our procedure, shows no evidence of formation of complexes with comparable sugar concentrations.

Beidler's *(2)* "fundamental taste equation," originally used to describe interactions of salts with taste buds, described the interaction of sugars with the 40-percent fraction most adequately *(10)*. The values calculated for $\triangle F$ suggest weak interactions (the figures of 1 to 2 kcal/mole are generally accepted as being the approximate strength for a hydrogen bond) rather than chemical reactions; in accord with this is our failure to observe any time-dependency for the changes in spectrum or refractive index. Beidler *(2)* and Evans and Mellon *(11)*, who used mammalian material and blowfly salt receptors, respectively, concluded that the response of taste receptors to salts entailed weak interactions.

Since our material was obtained by pooling papillae from several tongues, our values represent averages from individuals and can probably be used for comparison with results of in vivo studies. Such a comparison (Table 1) shows that the strengths of the complexes parallel the relative sweetnesses of the sugars; the value for K is used in ranking, since it describes the relation between degree of binding and concentration.

Since the response to sweet-tasting compounds in vivo is virtually independent of *p*H above *p*H 4 to 5 *(3)*, it was of interest to compare the *p*H dependence of the interaction between a sugar and our protein fraction. Figure 2 shows that the interaction of fructose with our material is virtually independent of *p*H between *p*H 9.6 and about *p*H 5.5, and that it falls off dramatically at lower *p*H.

Although it is difficult to prove conclusively that the material prepared by our procedure is in fact the component of the taste bud that is normally responsible for the initial interaction with sweet compounds, the following points suggest that it is:

1) It is derived from bovine taste buds as a fraction having an extraordinary tendency to complex sweet-tasting compounds.

2) The relative strengths of the complexes between this fraction and sugars parallel the relative sweetnesses of the sugars.

3) The *p*H-dependence of formation of complexes between this fraction and sugars parallels the *p*H dependence of the sweet tastes of sugars.

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Parathyroid Hormone in Plasma in Adenomatous Hyperparathyroidism, Uremia, and Bronchogenic Carcinoma

Abstract. *The concentration of parathyroid hormone (measured by radioimmunoassay) in plasma of patients with severe chronic uremia is frequently much higher than it is in the majority of cases having adenomatous hyperparathyroidism. Higher-than-normal concentrations of parathyroid hormone in plasma are found in a significant percentage of unselected patients with bronchogenic carcinoma.*

Enlargement of the parathyroid glands is a frequent consequence of chronic renal disease associated with hypocalcemia. Primary hyperparathyroidism may be caused by functioning tumors of the parathyroid glands or by the ectopic production of hormone by tumors other than those of the parathyroid glands, particularly bronchogenic carcinoma *(1)*. Recently, Tashjian *et al.* *(2)* have extracted parathyroid hormone from such tumors. However,

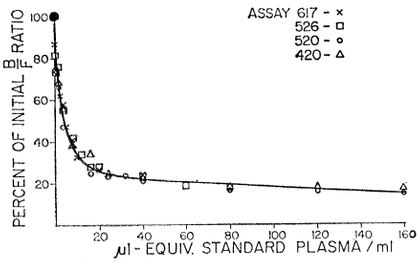

Fig. 1. The initial B/F ratio is the ratio observed in hypoparathyroid plasma without added standard. This ratio varies somewhat from assay to assay because of differences in the dilution of antiserum and the quantities of the bovine I^{131}-PTH used in the individual assays. However the percentage decrease in the ratio (B/F) for a given increment in concentration of standard plasma remained fairly constant among the different assays.

parathyroid hormone (PTH) in human plasma in these or other conditions has not yet been measured, except in a few patients with adenomatous hyperparathyroidism (*3*) and one with nonparathyroid tumor (*4*). In this one patient, the serum concentration of calcium was more than 20 mg/100 ml (*4*).

Parathyroid hormone was measured by radioimmunoassay (*3*). The method is based on the ability of unlabeled hormone in human plasma to inhibit, competitively, the binding of bovine PTH labeled with I^{131} to antibodies against bovine PTH. A convenient measure of the inhibition is the decrease in the ratio (B/F) of I^{131}-PTH bound to the antibody (B) to unbound ("free") I^{131}-PTH (F). Hormone concentration in an unknown sample is determined from the ratio observed in the sample referred to a standard curve on which B/F is plotted as a function of the hormone concentration in standard solutions. Since a suitably purified and bioassayed preparation of human PTH is not presently available, and since endogenous plasma hormone as well as crude preparations of human PTH do not react precisely as those of purified bovine PTH do, we used plasma hormone as the standard (*5*), selecting plasma which contained a very high concentration of hormone from a patient with tertiary hyperparathyroidism (*6*). Dilutions of the standard plasma over the range of one part in 5000 to one in 20 or from one part in 2500 to one in 10 provided a set of standard solutions (*7*). Standard curves from different assays were fairly reproducible when the percentage of the initial B/F (the ratio observed in plasma from a hypoparathyroid patient with no added standard) was plotted as a function of the concentration in microliters of standard plasma per milliliter (Fig. 1). Ratios in plasmas were referred to the standard curve of the same assay. Concentrations of parathyroid hormone were then expressed as microliter-equivalents (μl-equiv) of the standard plasma per milliliter, 1000 μl-equiv/ml being the hormonal concentration in the standard plasma.

Concentrations of PTH in plasma of patients with chronic uremia were frequently much higher than those in many patients with proven parathyroid adenoma (Fig. 2). Since the concentrations of other hormones (insulin and growth hormone), as judged by radioimmunoassay, were not high, one can assume that plasmas from uremic patients, which contained high concentrations of parathyroid hormone, did not have nonspecific factors that affected the reaction of antigen and antibody. Although almost all of the uremic patients had hypocalcemia, there was no strong correlation of the concentration of parathyroid hormone with the degree of hypocalcemia; rather, the severity of the uremia seemed to be the more significant factor. However, the concentrations of PTH in the plasmas of two uremic patients given calcium intravenously decreased 75 and 80 percent, respectively, within 1 hour. Although the precise onset of the uremia could not be documented in most cases, there was a rough correlation between the duration and the severity of the disease. The degree of parathyroid hyperplasia may be generally related to the duration of uremia. When ethylenediaminetetraacetic acid (EDTA) was injected into normal subjects, the concentration of serum calcium was acutely reduced to values as low as or lower than those in many of the uremic subjects. Although an increase, of a significant percentage, in the concentration of PTH in plasma then occurred (Fig. 3), the absolute increment in concentration was small compared to the concentration in plasma from patients with chronic uremia.

In patients with bronchogenic carcinoma, without uremia, and with normal concentrations of calcium in their serums, the concentration of PTH in the

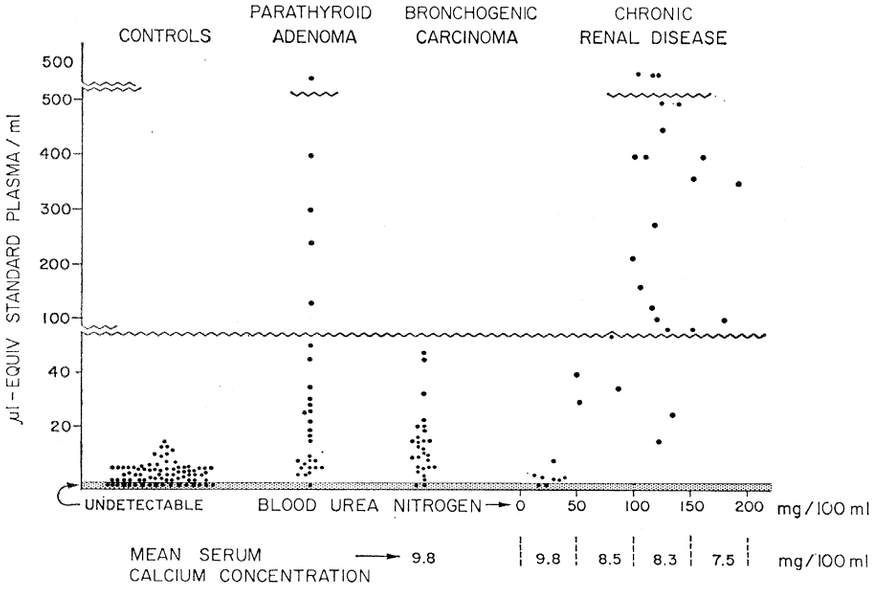

Fig. 2. Concentrations (expressed in microliter-equivalents of the standard plasma per milliliter) of PTH in plasma from patients in various conditions. Cases in which the ratio was not significantly lower than that observed in hypoparathyroid plasma are plotted as undetectable.

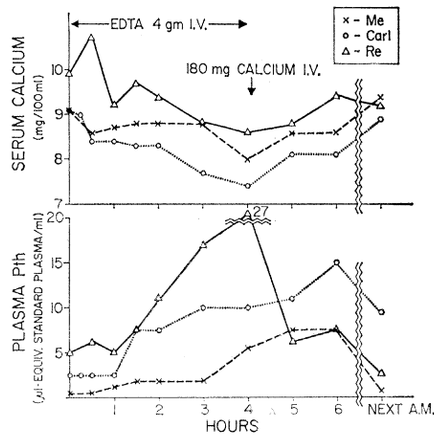

Fig. 3. Concentrations of PTH in plasma from three subjects first given EDTA intravenously, then given calcium.

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 mμg 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*).

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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.

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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 μg) 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 μmole 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 μg 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 ± 1.02 μmole 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