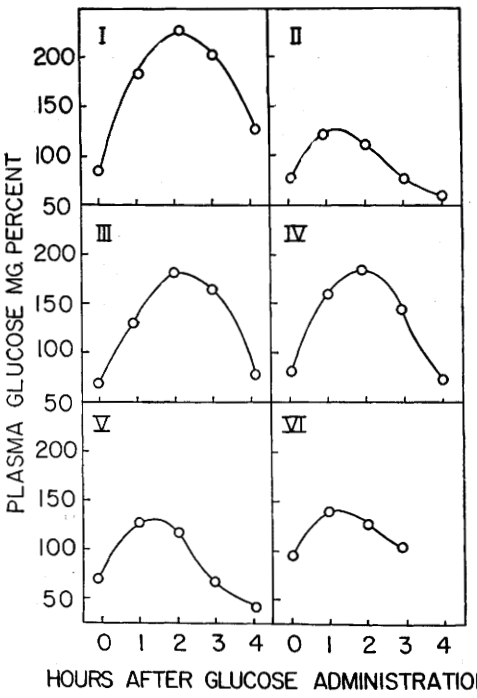

FIG. 1. Glucose tolerance tests of dogs after feeding of diets containing glucose, fructose, or galactose.

as the sole carbohydrate for 7 days produced a glycemic response to the glucose test meal similar to that observed after feeding diet B for 7 days (curve VI). In the galactose-fed dogs, the average plasma glucose level reached a maximum of 140 mg % 1 hour after administration of the glucose test meal, and returned to the initial level in 3 hours.

Summary. 1. Our earlier observation in the rat, that the feeding of fructose as sole carbohydrate results in a considerable loss in the animal's capacity to dispose of ingested glucose, is confirmed in another species, the dog. It is also shown that galactose is not the equal of fructose in bringing about impairment in glucose-disposal mechanism. 2. Feeding of a single meal containing 18 g of glucose/kg body weight failed to restore to normal the glucose-disposal mechanism once it was impaired by fructose feeding. A normal capacity for glucose disposal was observed after the dog had ingested 3 glucose-containing meals.

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Attempted Fractionation of Purified Bovine Growth Hormone.* (22234)

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By any of 3 isolative procedures(1-3) growth hormone (GH) can be obtained from bovine anterior pituitary glands as an apparently homogeneous protein of molecular weight about 47,000. The activity of such preparations does not depend on the presence of alanine and phenylalanine at the N-terminal ends of the 2 peptide chains(4), nor is the activity lost after partial digestion of the free-carboxyl end of the molecule with carboxypeptidase(5-7). It may be noted also that material with growth-promoting activity can, according to an unconfirmed claim (8), pass through a dialysis membrane.

This paper reports briefly an exploration of

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the possibility that a hypothetical "active constituent" might be leached out from the bovine protein GH preparation by fractional extraction with organic solvents. The results tend to support the view of Li and his colleagues(7,9-12) that the hormonal activity is a property of the bovine GH protein as isolated, and that, if the biological activity can be associated with material of lower molecular weight, this must be obtained by partial degradation of the parent molecule.

Methods. The bovine GH preparations were isolated by the method of Wilhelmi, Fishman and Russell(2), modified by conducting the initial extractions at pH 5.5 with 0.3 M KCl, and by collecting the crude GH fraction over the ethanol concentration range 10 to 30% (v/v), beginning at pH 8.5. The purified final products had essentially unit activity (10 μg/day injected for 10 days into 100 g hypophysectomized rats produced a gain in weight of 10 g). *Bioassays.* Growth-promoting potency was determined by the 10-day test(13) in 100 g male hypophysectomized rats, obtained from Hormone Assay, Inc., Chicago. In a series of 2-dose assays, the values obtained for the response (gain in weight) in g/$\log_{10}$ μg of hormone did not vary significantly, the mean value being 16.4 (with variance 0.75). Three to 5 animals were used for each dose of standard and unknown. The 10-day incremnts in width of the tibial epiphyseal cartilage were also observed routinely. These were variable, but roughly paralleled the weight increments. *Fractional extraction of bovine GH.* The usual procedure was to treat GH with ether-miscible mixtures consisting of a "non-polar" solvent admixed with a "polar" solvent in which GH was soluble (glacial acetic acid, pyridine, phenol, formic acid, or diethyl formamide, containing a small proportion of water). Most of the mixtures contained 5% (v/v) of water, but essentially similar results were obtained in a few experiments in which the solvents were almost anhydrous. Addition of thiodiglycol or phenol as antioxidant appeared to offer no advantage. Columns 5 or 7 mm in diameter and about 3 cm in height were set up with the slurry, obtained by addition of the "non-polar" solvent (or of a mixture rich in this solvent), at –10° or 0°C, to the dry-GH admixed with 10 parts of Supercel. In subsequent operations the temperature was usually kept below 2°C. The column was percolated with the solvent mixture at a restricted rate, the mixture being replaced progressively by one richer in the more "polar" constituent if addition of ether to the effluent did not yield or no longer yielded a precipitate. The precipitates obtained from the effluents by the addition of one volume of peroxide-free ether, followed if necessary by a trace of solid potassium acetate, were centrifuged, thoroughly washed with acetone, and finally taken up in water, made slightly alkaline with sodium or ammonium hydroxide, and lyophilized. The addition of ether to the effluents left in solution only a trace of nitrogenous material which was devoid of growth-promoting activity when tested after volatilization of the solvent at a low temperature—a procedure which substantially inactivated GH in control experiments. It was found, however that volatilization of the solvent system, ammoniacal *n*-propanol-water, caused little inactivation of GH. Accordingly, this isolative procedure was employed in fractional extractions performed with aqueous propanol systems.

In several instances, fractional extractions or acetone-dried or lyophilized powders of beef anterior pituitaries and whole pig pituitaries were carried out.

Results. Experiments on purified GH were conducted with 21 different solvent systems, and on dry gland powders with 4 systems. A representative sample of potency determinations of some of the more active fractions is presented in Table I. None of the products obtained in any experiment was significantly more active than purified bovine GH. Fractional extraction typically gave poor recoveries of activity, which was usually spread over several fractions. The behavior of acetone-dried or lyophilized gland powders was similar to that of purified GH. (The ethyl-acetate-formic acid system used with the beef gland powder was particularly effective in furnishing active fractions free of pigments, these being removed in the initial stages of

TABLE I. Representative Fractional Extractions.†

Exp. No.	Material in column	Components of solvent mixture*	% (v/v) of first component	Effluent vol (ml)	Yield of product (%)	Growth potency relative to purified GH
		With 5% (v/v) water				
20	Beef ant. pituitary, acetone powder, 4 g	Acetone-acetic acid	90	74	0	—
			80	57	.5	.4
			80-70	69	9.8	.5
			60-50	81	2.3	.5
			40-30	50	4.0	.3
61	Pig pituitary acetone powder, 3 g	Ethyl-acetate-formic acid	90	115	0	—
			85	120	1.9	.1 *
			78	100	3.0	.05*
			70	70	5.4	.2 *
			63	18	6.4	.1 *
55a	Purified GH, 80 mg, with Supercell	*Idem*	90	12	10	.3
			78-65	42	0	—
			60	26	12	1.4
			55	29	33	.9
			50	15	5	.5
62a	*Idem*	Methyl-formate-formic acid	90	20	1	.2
			78	14	0	—
			70	13	5	.6
			65	21	40	.5

* Adrenals enlarged (ca. 50%) at end of test.

† Other systems used were as follows ("non-polar" constituent in italics): *ether*-phenol; -acetone-phenol-water (containing ammonia); -water (containing hydrochloric acid); -ethyl-acetate; -pyridine; -phenol-dichloroacetic acid (aqueous, 0.2%); *ethanol* (containing ammonia); *n-propanol* (containing ammonia); -phenol-ammonium formate; -ammonium flavianate; -dichloroacetic acid (aqueous, 1%); *ethyl acetate*-acetic acid; -phenol; -pyridine; *dioxane*-phenol; -pyridine. All mixtures contained water, 5%, v/v.

the extraction.) Different runs with the same system varied unpredictably in the relative yields of material obtained from successive effluents, in the ease with which the precipitates could be re-dissolved in dilute alkali, and in the stage at which flow of solvent was arrested because of gelation of the column. When successive fractions from a single run were separately re-run, characteristic differences in the extraction pattern were observed, but in no case was complete recovery of material or activity achieved. Active material was extracted from purified GH only with acidic systems. No material was extracted when the "polar" constituent was diethylformamide (with or without dichloroacetic acid), or pyridine, although GH dissolved in pyridine could be recovered in good yield and activity by precipitation with acetone. No advantage resulted from the use of GH-flavianate in place of GH itself, or from the use of ether-immiscible solvent mixtures in which the "polar" constituent was water with additions such as ammonia, ammonia and urea, ammonium formate, flavianic acid or hydrochloric acid. Finally it may be mentioned that brief dialysis of GH against a solution of 6 M urea in glycine buffer, pH 9.5, or hydrochloric acid, pH 2.0 or 3.3, gave no evidence of dialyzable activity; the remaining protein had always lost some activity. No activity was found in the "extract" obtained by treating GH with liquid ammonia.

The possibility was considered that an "active constituent" separated from GH-protein might, if it were a much smaller molecule, show poor apparent activity in the bioassay because of excessively rapid absorption from the injection site, or because of rapid excretion. In contrast with ACTH, GH is inactive when injected as a suspension in beeswax-arachis oil, but aqueous suspensions (pH 8) adsorbed on aluminum phosphate were found to be as active as aqueous solutions. In no case was the activity of fractions obtained from GH found to be enhanced by the use of aluminum phosphate.

Discussion. The experiments with dry

pituitary gland powders indicate that fractional extraction with organic solvents would offer no advantages over present methods of extracting growth hormone. In one of the best experiments, Exp. 20 (Table I), the yield of active material, 30 to 50% as potent as the purified GH used as a standard, was 34 g/kg of fresh anterior lobes, representing very likely all of the growth-promoting activity originally present. The difficulties are that the fractionation cannot be repeated consistently, and that the products are poorly soluble and are likely to prove intractable to further purification. The pig pituitary fractions were evidently rich in ACTH, as indicated by adrenal enlargement in all of the test animals. An accurate estimate of the growth-promoting properties of these pig fractions is therefore impossible to obtain. Even if these fractions were relatively rich in growth-promoting activity, the indicated order of contamination with ACTH is a decided obstacle to further purification.

It was not possible to show that an "active constituent" could be extracted from purified bovine GH preparations. Such active fractions as were obtained—by procedures which invariably depressed the solubility and inactivated the bulk of the starting material—were essentially similar to the original GH by several criteria, most significantly perhaps in being poorly soluble in water in the pH range 6-7. No differences from the original GH were evident on examination of a few of the fractions for amino-acid composition (by paper chromatography of hydrolysates), for amide nitrogen content, or for the nature and content of free amino groups (by the fluorodinitrobenzene method). It would appear, therefore, allowing the reservation that the bioassay might falsely underestimate a highly active constituent of low molecular weight, that, in agreement with Pierce(14) the biological activity is a property of the protein as isolated, and as defined by the carefully detailed studies of Li *et al.*(7,9-12).

Summary. Purified bovine growth hormone, extracted fractionally with a wide variety of organic solvent mixtures, does not yield more active constituents than the protein starting material, nor do the products differ from it in other properties.

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