A PARTIAL FRACTIONATION OF PITUITARY GROWTH HORMONE

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It has been reported to us that if solutions of pituitary growth hormone are ultrafiltered at an alkaline pH, a small quantity of peptide material having insulin-like properties can be shown to be present in the ultrafiltrate (R. H. SMITH, unpublished work). SMITH concentrated the material, but did not free it from salt; acid hydrolysis showed it to have an amino-acid composition not very different from growth hormone itself. When amounts containing 30–40 μg N were injected daily into female rats of constant weight, no induction of growth could be observed, but the material increased the glucose uptake of rat diaphragms when tested according to the technique of OTTAWAY[1].

Our interest in the insulin-like property of growth hormone has led us to confirm and extend these findings, especially in view of earlier indications that some dissociation of the growth hormone took place on solution in alkali[2,3]. We have tested the effect of the ultrafiltrate and of the residual protein on the isolated rat diaphragm and have also investigated their effect on blood sugar when injected into rabbits.

METHODS

Growth hormone was prepared from beef anterior pituitaries by a modification of the method of WILHELMI, FISHMAN AND RUSSELL[4]. It was tested for biological activity by its effect on the gain in weight of plateaued female rats, and was found homogeneous in the Tiselius electrophoresis apparatus.

Small batches (50–100 mg) of the growth hormone were dissolved in freshly prepared ice-cold 0.01 *N* NaOH, at a concentration of 1 mg protein/ml. The solution was filtered under pressure in a 9 cm Seitz filtration vessel at 1° C, and the filtrate collected in 10 ml fractions. The membrane was a sheet of cellophane, supported on a gauze pad; it was washed *in situ* with water and 0.01 *N* NaOH until the washings no longer had a significant absorption at 240 mμ. It was found most convenient to attach the receiving flask to the water pump, and to apply positive pressure of about 16 lb./sq. in. to the filter vessel from a gas cylinder. Nitrogen was used for this purpose, as exclusion of air from the alkaline solution appeared to retard decomposition of the protein, as judged by its solubility characteristics after recovery. Complete filtration took 2–3 days; the residual protein was extracted from the vessel with 0.01 *N* NaOH, dialyzed against distilled water, and freeze-dried. The filtrate was neutralised and stored either at 0° or at —25° C, until required.

The methods used for testing the activity of the preparations on isolated rat diaphragm were as previously described[5]. The ultrafiltrate was prepared for use by diluting it with an equal volume of modified STADIE medium[6] containing twice the usual concentration of all the constituents. This solution was diluted further with medium as required.

Male hooded Norway rats of an M.R.C. strain, and male albino rats bred in this Department, were used. They were kept on a restricted diet sufficient just to maintain their weight (150–200 g) for at least a fortnight before use. The rabbits were a group of mixed breeds which had become accustomed to the withdrawal of blood samples from the marginal ear vein. Highly excitable animals were rejected. All the rabbits were fasted for 24 hours before the test. Blood sugar was estimated by the method of Hagedorn and Jensen.

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RESULTS

Recovery of protein from the filter vessel was poor, usually only about 50%. This was partly due to unavoidable losses in the freeze-dryer, but most of the unrecovered protein remained firmly attached to the cellophane membrane. When an atmosphere of N_2 had been maintained in the vessel during filtration, the recovered residue was almost completely soluble in 0.01*N* NaOH, but insoluble at pH 7 in the absence of salt. Because so little material was available, it was not examined electrophoretically. One batch was tested for growth-promoting activity in plateaued female rats, and was found to have about 80% of the activity of the original material.

The ultrafiltrate was regularly tested for gross contamination with protein, by the addition of trichloroacetic acid. The average N content was 5 μg/ml equivalent to 30 μg/ml of peptide or 3% of the original protein.

Effects on glucose uptake by diaphragm

Several preparations of ultrafiltrate and of residual protein were tested for their effects on the glucose utilisation of rat diaphragm. The results for one preparation are given in Table I, and other results are illustrated in Fig. 1.

It is evident that the ultrafiltrate has quite a strong insulin-like activity which is presumably due to the peptide material which it contains. Particularly noteworthy is the low concentration of peptide which is necessary to produce this effect, for the material illustrated in Table I 3 μg/ml, as compared to 100 μg/ml for recrystallized growth hormone. Untreated hormone produces inhibition of glucose uptake at low concentrations, and stimulation of uptake at higher concentrations[3] (Fig. 1). There was no evidence of inhibition of glucose uptake by ultrafiltrate at dilutions greater than those which produced an insulin-like effect. In one experiment a 1:2 dilution of ultrafiltrate inhibited glucose uptake, but this was not found with the other preparations.

Fig. 1 shows that the residual protein had lost most of its insulin-like activity, but still retained some or all of its inhibitory activity. Since the shape of the curve was different from that characteristic of the untreated protein, it was impossible to estimate the extent to which the hormone had been inactivated by treatment.

TABLE I

EFFECT OF GROWTH HORMONE ULTRAFILTRATE ON GLUCOSE UPTAKE OF RAT DIAPHRAGM

The ultrafiltrate was made isotonic with rat plasma as described under METHODS and diluted again as shown in the first column. The diaphragms were incubated for 90′ in medium containing 2 mg/ml glucose in an atmosphere of O_2. Control uptake is that of diaphragms incubated in glucose-containing medium only.

Final dilution of ultrafiltrate	*Estimated concn. of peptide*	*No. of expts.*	*Control uptake mg/g/h*	*Treated uptake mg/g/h*	*Effect*
1:10	3 μg/ml	9	3.16 ± 0.21	3.84 ± 0.23	+0.68 ± 0.17
1:50	0.6 μg/ml	2	2.92	2.91	+0.01
1:200	0.15 μg/ml	6	3.34 ± 0.13	3.16 ± 0.23	−0.18 ± 0.15

Test of significance:
For 1:10 dilution $t = 4.0$, $P = 0.004$.

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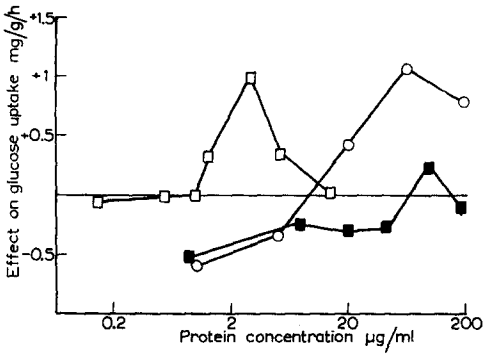

Fig. 1.

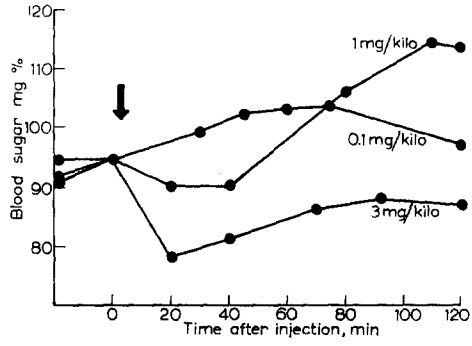

Fig. 2.

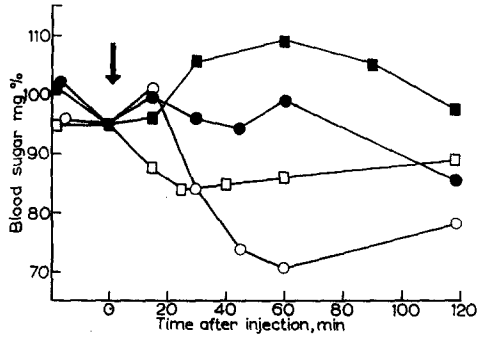

Fig. 3.

Fig. 1. Effect on glucose uptake by rat diaphragm of ultrafiltrates and residues of pituitary growth hormone. The abscissa give the change in uptake produced by the fraction when compared with the uptake of a control muscle. The points are the means of all the experiments from four preparations. ○—○ purified growth hormone; ■—■ residue from filtration; □—□ ultrafiltrate.

Fig. 2. Effect of growth hormone on the blood sugar of fasted rabbits. The hormone was injected subcutaneously into 3 groups of rabbits in the amounts shown in the figure. For comparison, the mean blood sugar of each group immediately before injection has been adjusted to 95 mg %.

Fig. 3. Effect on the blood sugar of fasted rabbits of the subcutaneous injection of 100 µg residue or approx. 3 µg ultrafiltrate peptide from growth hormone. For comparison, the actual values have been adjusted so that the mean blood sugar of each group immediately before injection (marked by arrow) is 95 mg %. The effect produced by the injection of 4 µg (0.1 unit) insulin is also shown. Controls were injected with 1 ml saline. ●—●, control; ○—○, insulin; ■—■, residue; □—□, ultrafiltrate.

Effects on rabbit blood sugar

The effect of subcutaneous injection of purified growth hormone on the blood sugar of fasting rabbits[7] is shown in Fig. 2. A diphasic curve is evidenced, a slight hypoglycaemia is followed by a slight but lasting hyperglycaemia. The initial hypoglycaemia becomes more pronounced as the dose of growth hormone is increased. Similar effects have been noted by GROSS AND MÄUSER[8] and by RABEN[9].

Subcutaneous injection of residue (100 µg/kilo) and of ultrafiltrate (0.1 ml/kilo = 3 µg peptide/kilo) into fasting rabbits caused a relatively long-lasting hyperglycaemia and hypoglycaemia respectively. The hypoglycaemic effect of the ultrafiltrate was manifest within 15 minutes of injection, but hyperglycaemia was not observable until 30 minutes after injection of the residue. The blood sugar of animals treated with residue was still significantly greater than that of the controls 60 minutes after injection and remained elevated 2 hours after injection (Fig. 3). No trace of a diplasic blood sugar curve was seen with either group; if these experiments alone were taken into consideration it would seem that a complete separation of the two activities of growth hormone protein had occurred.

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TABLE II

EFFECT OF INJECTION OF ULTRAFILTRATE AND OF NON-FILTRABLE GROWTH HORMONE ON THE BLOOD SUGAR OF RABBITS

The animals were fasted for 20 hours before a subcutaneous injection of the material dissolved in 1 ml of physiological saline. Blood samples were taken from the ear vein.

	No. of animals	*Time after injection (min)*						
		0	*15*	*30*	*45*	*60*	*90*	*120*
		Mean blood sugar						
Residue	8	94 ± 5.2	95 ± 10.6	104.5 ± 5.7	107.5 ± 5.7	108 ± 6.8	108 ± 8.3	99 ± 5.6
Ultrafiltrate	8	102 ± 5.5	94.5 ± 7.0	91 ± 8.9	92 ± 7.0	93 ± 7.4	—	96 ± 7.2
Controls	6	94 ± 3.3	99 ± 7.4	95 ± 2.7	93 ± 7.1	98 ± 9.5	—	89.5 ± 2.7

Tests of significance:

45 min after injection
Residue against filtrate $t = 2.40$, $P = 0.03$.
Residue against control $= 2.46$, $P = 0.03$.

60 min after injection
Residue against filtrate $t = 2.11$, $P = 0.05$.

DISCUSSION

Not enough peptide was collected in these experiments to enable us to test it for growth-promoting activity. SMITH, however, was able to prepare several milligrams, which he found was inactive in the tibia test on hypophysectomised rats, or in the weight-increase test in normal female rats. Thus our results, together with those of SMITH, appear to establish the existence of an ultrafiltrable fragment of growth hormone which has insulin-like, but not growth-promoting properties. The possible dissociation of such a fragment when growth hormone is dissolved in alkaline solutions has been inferred from earlier experiments in this laboratory[2,3]. It is probable that material having insulin-like properties can be separated from pituitary proteins in other ways than the one which we have described. RABEN[9] has reported effects on blood sugar of rabbits and mice produced by oxycel preparations of growth hormone which agree well with those that have been described in this paper. GROSS AND MÄUSER[8] have found that the injection of a proprietary preparation into human beings and into rats is followed by hypo- or hyperglycaemia, depending on the dosage. The relationship between effect on blood sugar level and dosage which they found is not the same as that which we have found to be characteristic of purified growth hormone.

That such an active peptide can be separated from growth hormone does not mean that it is necessarily a component of the hormone as it circulates in the blood. The fact that the protein remaining after ultrafiltration retains a good deal of its growth-promoting activity, while the ultrafiltrate has, according to SMITH, no such activity, itself suggests that the association may be physico-chemical rather than

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physiological. The only evidence we have at the moment bearing on this problem is that in the many samples of growth hormone which we have now examined in this laboratory, insulin-like activity is always shown *in vitro* at concentrations of 100–200 μg/ml, and maximum inhibition of glucose utilisation at concentrations of 5–10 μg/ml growth hormone. The ratio of the two concentrations does not vary, as it might be expected to do with a purely fortuitous association of two proteins. We hope to present further evidence on this point in a later paper. The peptide showing insulin-like activity must, in any case, have come from anterior pituitary tissue, and this is itself of great interest.

It was not to be expected that the method used here for filtration would effect a complete separation of peptide from the residual protein. Protein must be concentrated in the filter vessel as filtration proceeds and this must tend to prevent dissociation, apart from the fact that the protein was not washed with further 0.01 *M* NaOH. Nevertheless, the results show that the protein has lost a good deal of its insulin-like activity, but it still retains hyperglycaemic activity. The mechanism by which this hyperglycaemia is produced is not proved. The residual protein will inhibit the glucose uptake of rat diaphragm, and causes hyperglycaemia in fasted rabbits. This suggests that the effect is directly on peripheral tissue. On the other hand, stimulation of glucagon secretion has not been ruled out by experiment, and mediation of the effect is to some extent suggested by the delay, after injection, before it can be observed.

SUMMARY

1. Pituitary growth hormone has been subjected to ultrafiltration at pH 12. The filtrate contained nitrogenous material amounting to about 3 % of the original protein. It was presumed to be a peptide but was not isolated.

2. The filtrate increased the glucose utilisation of rat diaphragm when it was added to the incubation medium, and caused a slight hypoglycaemia in fasting rabbits.

3. The residual protein, which had retained its growth-promoting activity, was found to have less insulin-like activity than normal when tested on isolated diaphragm, and to cause a slight but long-lasting hyperglycaemia in fasting rabbits.

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