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Metabolic Effects of the Major Component of Bovine Growth Hormone

By N. I. SWISLOCKI, M. SONENBERG and M. KIKUTANI*
Division of Endocrinology, Sloan-Kettering Institute for Cancer Research, New York, N.Y. 10021, U.S.A.

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Bovine growth hormone, subjected to DEAE-cellulose chromatography, yielded one major and several minor components. The various chromatographic fractions of bovine growth hormone were compared with the parent material for their ability to promote hormone effects *in vivo* and *in vitro*. The major component of bovine growth hormone was homogeneous by acrylamide-gel electrophoresis, rechromatography and sedimentation equilibrium. Its amino acid composition was similar to that of the parent hormone. The major component possessed all the qualitative activities present in the original heterogeneous material, including promotion of acute hypoglycaemia and hypolipaemia. In studies *in vitro* in adipose-tissue segments the major component of the hormone increased entry of glucose and its oxidation to CO_2, conversion of glucose into glyceride glycerol, release of glycerol and incorporation of histidine into adipose-tissue protein. Other chromatographic fractions of bovine growth hormone were not homogeneous and possessed some but not all of the metabolic activities attributed to the hormone preparations or its major component. Thus, the metabolic effects obtained with bovine growth-hormone preparations *in vivo* and *in vitro* can be obtained with the major homogeneous component of the hormone. This observation precludes the possibility that the metabolic effects obtained with bovine growth-hormone preparations are due to the combined actions of a number of components found therein.

Bovine growth-hormone preparations that are generally used for metabolic studies are heterogeneous on zone electrophoresis (Barrett, Friesen & Astwood, 1962; Ferguson & Wallace, 1963; Free & Sonenberg, 1966) and may be contaminated to a minor extent with other pituitary hormones. It may well have been that some of the metabolic effects observed with these hormone preparations were due to contaminants (Jungas & Ball, 1960; Leboeuf & Cahill, 1961; Christophe, 1963; Hamid, Rubinstein, Ferguson & Beck, 1965; Landau, Bartsch & Williams, 1966). Others (Goodman, 1965; Fain, Kovacev & Scow, 1965) have attributed several commonly observed metabolic effects of bovine growth hormone to the hormone present in heterogeneous preparations.

Recent successes in separating a homogeneous fraction from a bovine growth-hormone preparation (Free & Sonenberg, 1966; Sonenberg, Kikutani, Free, Nadler & Dellacha, 1968) provided the opportunity to determine whether the metabolic effects observed both *in vivo* (Swislocki & Szego, 1965; Swislocki, 1968*a*) and *in vitro* (Hamid *et al.* 1965; Goodman, 1965; Fain *et al.* 1965; Swislocki, 1968*b*, 1970) were due to a single species of protein. Our results obtained with a homogeneous bovine growth hormone demonstrate that the metabolic effects of the hormone are not due to the combined action of the several components present in most bovine growth-hormone preparations but reside in a single species of protein. A preliminary account of this work has been presented (Swislocki & Sonenberg, 1969).

* Present address: Biochemistry Laboratory, Faculty of Pharmacy, University of Nagasaki, Nagasaki, Japan.

EXPERIMENTAL

Fractionation of bovine growth hormone. Bovine growth hormone was prepared by the methods of Dellacha & Sonenberg (1964) and Wallis & Dixon (1966) by ion-exchange chromatography on DEAE-cellulose (Cellex-D) obtained from Calbiochem, Los Angeles, Calif., U.S.A. All other reagents employed were of analytical grade. DEAE-cellulose was equilibrated with 0.01 M-NH_4HCO_3 buffer, pH 8.4, by repeated suspensions and decantations. Bovine growth hormone in 0.01 M-NH_4HCO_3 was applied to the column (2.5 cm × 20 cm) and developed first with starting buffer. Stepwise elution was performed with NH_4HCO_3 solutions as indicated in Fig. 1. A flow rate of 25 ml/h was maintained. Protein concentration in

10ml samples of eluate was measured by u.v. absorption at 275nm. The pooled fractions were freeze-dried directly.

Amino acid analyses. Protein samples were hydrolysed in 6M-HCl at 110°C under reduced pressure for 24 and 72h. Samples of hydrolysates were analysed on an amino acid analyser by the technique of Spackman, Stein & Moore (1958). Corrections have been applied for amino acids destroyed by extrapolation to zero time.

Determination of molecular weight. The molecular weight of the major component was determined by sedimentation equilibrium (Yphantis, 1964) at 4–10°C in a Spinco model E ultracentrifuge equipped with u.v. optics and an automatic scanner. Three simultaneous determinations were made in an Yphantis six-hole centrepiece in which three holes contain the solution and the other three the respective solvent. Molecular weights were calculated as described by Sonenberg *et al.* (1968).

Disc electrophoresis. Analytical electrophoresis on polyacrylamide gel was performed by the disc-electrophoretic method of Ornstein (1964) and Davis (1964). Electrophoresis was performed at pH9.5 in 7.5% (w/v) polyacrylamide gel. Protein components were stained with 0.5% Amidoschwarz in 7% (v/v) acetic acid.

Bioassay. Growth-hormone activity of the original bovine growth-hormone preparation, as well as of the fractions derived therefrom, was determined in hypophysectomized rats by the method of Marx, Simpson & Evans (1942), in which the weight gain for 10 days was recorded. Animals received either the international standard or the bovine growth-hormone fractions at a daily dose of 2.5 or 10μg per rat respectively.

Metabolic studies. Male CD hypophysectomized rats (150–170g) were obtained from Charles River Breeding Farms, Wilmington, Mass., U.S.A., and used 6–9 days after surgery. Rats were maintained on laboratory chow and starved for 24h before the experiment. For studies *in vivo* rats were bled under sodium pentobarbital anaesthesia (4mg/100g body weight) (Swislocki & Szego, 1965; Swislocki, 1968*a*). Plasma free fatty acids were determined by the method of Dole (1956) and glucose by glucose oxidase with the Glucostat reagent obtained from Worthington Biochemical Corp., Freehold, N.J., U.S.A.

Studies *in vitro* were carried out on fat-pad segments that were distributed so that in all experiments one portion of the tissue from each rat always served as the control. Glycerol was determined by the method of Wieland (1963) and distribution of label in neutral lipid derived from [^{14}C]glucose by the method of Rodbell (1964). Incubation procedures and radioactivity-counting methods have been described previously (Swislocki, 1968*b*, 1969, 1970). Statistical evaluation of the metabolic results was performed as indicated in the text either with the Fisher *t*-test or with the method of paired analysis (Snedecor, 1948).

RESULTS

Fractionation of bovine growth hormone. As Fig. 1 shows, chromatography of bovine growth hormone on DEAE-cellulose by stepwise elution with ammonium hydrogen carbonate at pH8.4 yielded a number of peaks, with most of the material being eluted in the first peak. A large number of these fractions were investigated by polyacrylamide-gel electrophoresis for the distribution of several components of bovine growth hormone; fractions 11, 21 and 35 (shaded as shown in the elution pattern) were selected and were examined for growth-promoting activity and their metabolic effectiveness *in vivo* and *in vitro*.

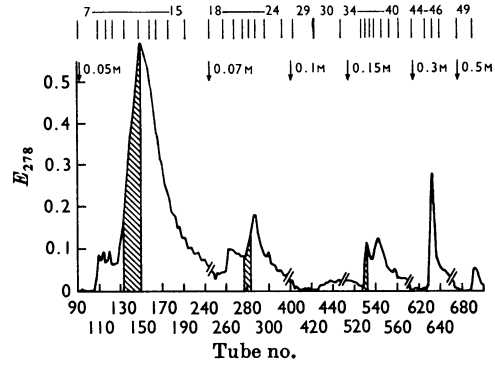

Fig. 1. Chromatography of bovine growth hormone on DEAE-cellulose with discontinuous increases in the concentration of eluting solutions of NH_4HCO_3. Pooled fractions were freeze-dried directly. Shaded regions correspond to fractions 11, 21 and 35. Fraction numbers are indicated by the numerals at the top of the figure. Arrows indicate different concentrations of NH_4HCO_3. Tube size was 10ml.

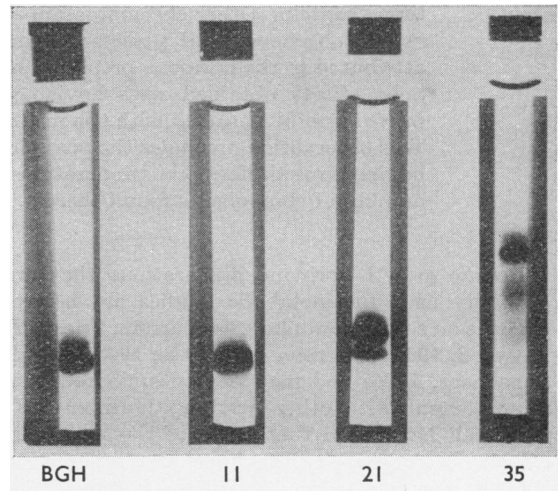

Fig. 2. Disc electrophoresis of parent bovine growth hormone (BGH) and fractions 11, 21 and 35 derived therefrom by DEAE-cellulose chromatography. Migration is from bottom to top, i.e. cathode to anode.

Fig. 2 shows the polyacrylamide gels after electrophoresis at pH9.5 of several fractions of bovine growth hormone. Parent bovine growth hormone

Table 1. *Amino acid composition of fraction* 11, *the major component of bovine growth hormone, and the parent bovine growth hormone*

The values were calculated for mol.wt. 20200. The composition of bovine growth hormone is from Sonenberg *et al.* (1968).

Amino acid	Composition (mol of amino acid/mol) Fraction 11	Bovine growth hormone
Lys	11.0	10.1
His	3.3	2.9
Arg	13.1	11.4
Asp	14.2	14.6
Thr	10.6	10.9
Ser	11.0	11.3
Glu	22.1	21.1
Pro	6.5	5.9
Gly	10.0	9.2
Ala	13.0	13.1
½Cys	4.8	4.4
Val	7.1	6.2
Met	3.4	4.2
Ile	7.0	6.3
Leu	24.2	23.5
Tyr	5.4	5.8
Phe	11.7	11.2

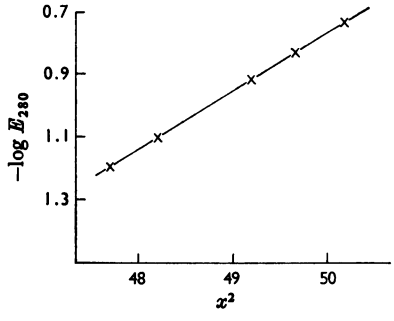

Fig. 3. Plot of the log of concentration (E_{280}) against square of the distance (x) from the centre of rotation derived from sedimentation equilibrium at 20000 rev./min of the major component of bovine growth hormone (0.1 mg/ml) in bicarbonate buffer, pH 9.5.

contained one major band, one minor band and traces of other more rapidly migrating bands. Fraction 11, obtained from the leading edge of the first eluted peak (Fig. 1), is identical with the component 1 described in an earlier report from this laboratory (Free & Sonenberg, 1966). Fraction 21 contains components 2 and 3. Fraction 35 contains components 3, 4 and 5, as well as a number of bands not observed in native bovine growth hormone, where their presence in minute amounts was too low to be detected. Their appearance in the higher-numbered fractions is due to their concentration after chromatography.

The major components eluted with 0.05, 0.07 and 0.15M-ammonium hydrogen carbonate (fractions 11, 21 and 35, respectively) contained similar growth-promoting activities, 1.56 ± 0.55, 1.44 ± 0.40 and 1.07 ± 0.19 international units/mg respectively, on the basis of weight gain and parallel slopes. The peaks eluted with higher concentrations of ammonium hydrogen carbonate contained little or no growth-promoting activity.

The amino acid composition of fraction 11, the major component of bovine growth hormone, is presented in Table 1. It is not significantly different in amino acid composition from the parent material. Fig. 3 shows that the major component of bovine growth hormone (fraction 11) is homogeneous as determined by sedimentation equilibrium. The calculated molecular weight is 20200, which is in agreement with the value of 21000 reported for the parent bovine growth hormone (Sonenberg *et al.* 1968).

Metabolic effects. (*a*) *In vitro.* The several fractions of bovine growth hormone were evaluated for their ability to stimulate metabolic activity in rat adipose tissue *in vitro* (Table 2). They elicited similar qualitative effects on uptake and oxidation of glucose, and release of glycerol, as did the parent bovine growth hormone. All the fractions tested, like the parent hormone, stimulated conversion of glucose into total neutral lipid, with most of the label being incorporated into the glyceride glycerol moiety and not into fatty acid (Table 3).

Fraction 11 of bovine growth hormone increased linearly release of glycerol by fat-pads of hypophysectomized rats (Fig. 4). The amount of glycerol released is not different from control amounts at 1h but rises to concentrations of statistical significance at 2 and 3h. This 1h time-lag coincides with the elevated plasma free fatty acid response to bovine growth hormone *in vivo* (Swislocki & Szego, 1965), and is indicative of the secondary nature of the hyperlipaemic response to bovine growth hormone.

Fraction 11 is also able to effect incorporation of [^{14}C]histidine into protein of adipose tissue (Swislocki, 1968*b*). The lowest concentration tested (0.05 μg/ml of medium) was effective in stimulating protein synthesis (Table 4). The specific radioactivity of protein due to incorporation of [^{14}C]-histidine was increased by 25% over control values.

As Fig. 5 shows, increasing concentrations of fraction 11 elicit increasing metabolic responses by adipose tissue. Oxidation of glucose and its conversion into lipid and glyceride glycerol, as well as release of glycerol, were stimulated to a statistically significant degree by 0.1 μg of fraction 11. At 1 μg/ml uptake of glucose likewise became significant. The responsiveness of the tissue seems to

Table 2. *Effect of various fractions of bovine growth hormone on uptake of [^{14}C]glucose and its oxidation to carbon dioxide and release of glycerol by segments of epididymal adipose tissue*

Portions of epididymal fat-pad were incubated in 1 ml. of Krebs–Ringer bicarbonate buffer containing 1 mg of [^{14}C]glucose/ml. Initial specific radioactivity of the glucose in the medium was 0.2 μCi/ml. The bovine growth-hormone fractions were added at 25 μg/25 μl of saline per flask. Saline alone was added to the flasks of the control group. Flasks were gassed with $O_2 + CO_2$ (95 : 5), capped and shaken for 3 h at 37°C in a Dubnoff incubator. Each value ± S.E.M. was obtained from a minimum of six determinations. *P* values were calculated by the Fisher *t*-test.

		Uptake of [^{14}C]glucose		Oxidation to $^{14}CO_2$		Glycerol released	
Additions	Dose (μg/ml)	(c.p.m./mg of tissue)	*P*	(c.p.m./mg of tissue)	*P*	(μmol/g of tissue)	*P*
None		308±67		19.7± 2.5		1.3±0.28	
Bovine growth hormone	5	525±79	<0.05	75.8± 9.8	<0.001	6.2±1.03	<0.001
Bovine growth hormone	25	585±78	<0.02	106.0±12.8	<0.001	8.4±1.44	<0.001
None		264±23		24.4± 1.7		1.5±0.18	
Fraction 11	25	383±29	<0.02	75.5±12.7	<0.01	4.0±0.20	<0.001
Fraction 21	25	470±31	<0.01	127.2±14.0	<0.001	7.2±1.26	<0.001
None		106±26		32.6± 5.7		1.9±0.28	
Fraction 35	25	375±35	<0.01	150.7±34.3	<0.01	8.9±1.00	<0.001

Table 3. *Effect of various fractions of bovine growth hormone on the incorporation of [^{14}C]glucose into lipid of epididymal adipose tissue*

The experimental details are as given in Table 2. N.S., not significant.

		Radioactivity					
		Total lipid		Fatty acid		Glyceride glycerol	
Addition	Dose (μg/ml)	(c.p.m./mg of tissue)	*P*	(c.p.m./mg of tissue)	*P*	(c.p.m./mg of tissue)	*P*
None		36.6± 3.6		2.8±1.1		33.7± 2.9	
Bovine growth hormone	5	129.3±20.4	<0.01	3.8±1.1	N.S.	125.6±19.6	<0.001
Bovine growth hormone	25	162.9±30.0	<0.01	3.3±1.0	N.S.	159.7±29.4	<0.01
None		32.7± 3.6		2.6±1.0		37.3± 4.1	
Fraction 11	25	96.0± 6.3	<0.001	3.3±0.8	N.S.	100.9± 8.0	<0.001
Fraction 21	25	162.6±15.0	<0.001	2.7±0.6	N.S.	176.5±13.1	<0.001
None		32.1± 1.8		0.9±0.3		31.4± 1.8	
Fraction 35	25	154.5±15.9	<0.001	1.0±0.4	N.S.	153.6±15.9	<0.001

be limiting at a dose of 5 μg of the major component, since increasing the dose to 25 μg did not markedly enhance the magnitude of the various responses.

(*b*) *In vivo*. As described earlier (Swislocki & Szego, 1965; Swislocki, 1968*a*) we observed that 1 mg of bovine growth hormone elicits dramatic decreases in plasma glucose and free fatty acid concentrations 30 min after treatment of either hypophysectomized or pancreatectomized–hypophysectomized rats that had been starved for 24 h. Since these reports have been variously subjected to the criticism that the acute insulin-like response of bovine growth hormone could be due to contaminants present in bovine growth hormone it was decided to test the ability of the hormone fractions to effect both hypoglycaemia and hypolipaemia at 30 min. As Table 5 shows, when plasma glucose and free fatty acids were determined under similar experimental conditions of treatment and sampling, fraction 11 was the only fraction tested that had both the qualitative and quantitative activity *in vivo* that was present in bovine growth hormone. Fraction 21 possessed only hypoglycaemic activity, as the decrease in free fatty acid concentration was not statistically significant. Fraction 35, as well as subsequent fractions not shown here, were ineffective in evoking a decline in either plasma glucose or free fatty acids.

DISCUSSION

Fraction 11, which at pH 9.5 is the component of bovine growth hormone migrating most slowly to the anode, represents the major species of protein in both the bovine growth hormone (Dellacha & Sonenberg, 1964) used in this study and in an NIH bovine growth-hormone preparation (Free & Sonenberg, 1966). It is the only fraction of those tested that promotes both hypolipaemia and hypoglycaemia *in vivo* under conditions where such effects are observed with the original material. In addition, *in vitro*, like the parent hormone preparation, it enhances the entry of glucose into fat-pads and its oxidation to CO_2, incorporation of glucose label into glyceride glycerol of tissue lipid, release of glycerol by adipose-tissue segments, and incorporation of histidine into adipose-tissue protein.

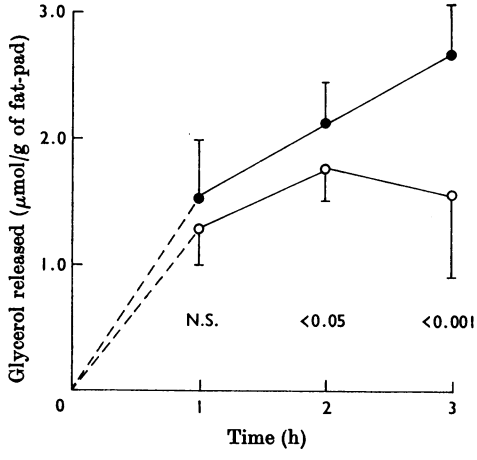

Fig. 4. Effects of the major component of bovine growth hormone on release of glycerol by adipose tissue of hypophysectomized rats. Portions (90–100 mg) of epididymal fat-pad were incubated in beakers in 2 ml of Krebs–Ringer bicarbonate buffer containing 2 mg of glucose. Fraction 11 (25 μg), the major component of bovine growth hormone, was dissolved and added to each of six samples in 25 μl of the buffer. Buffer alone was added to control flasks. The samples were gassed with O_2+CO_2 (95:5) throughout the incubation period. Samples of the media were taken at indicated intervals for determination of glycerol. ●, Flasks to which the major component of bovine growth hormone was added; ○, controls. Points indicate mean± S.E.M. *P* values were calculated by the Fisher *t*-test, and are indicated on the figure. N.S., not significant.

As demonstrated above, the major component of bovine growth hormone is effective in promoting several metabolic effects in adipose tissue of hypophysectomized rats at concentrations of 0.05–25 μg/ml. These amounts of hormone are in the range of effectiveness of commonly available bovine growth-hormone preparations. However, our results differ in part from those reported earlier in which heterogeneous growth-hormone preparations were used. Fain *et al.* (1965) were unable to demonstrate an effect of bovine growth hormone (1 μg/ml) on release of glycerol during a 4h incubation of adipocytes isolated from normal rats unless dexamethasone (0.016 μg/ml) was also present in the incubation medium. In the presence of dexamethasone bovine growth hormone stimulated lipolysis after a lag period of 2h (Fain *et al.* 1965). In our hands, the major component of bovine growth hormone at 1 μg/ml, acting alone, stimulated lipolysis at 3h. The effect of the major component of bovine growth hormone became manifest only after a 1h lag period. Our experimental design differed in part from that of Fain *et al.* (1965) in that in the present study tissues of hypophysectomized rats were used to evaluate the homogeneous major component of bovine growth hormone as purified herein, whereas in their experiments, a heterogeneous bovine growth-hormone preparation (NIH-GH-B3) was examined for its metabolic effects in adipocytes derived from normal rats.

Table 4. *Effect of the major component of bovine growth hormone on incorporation of histidine into adipose-tissue protein*

Portions of epididymal fat-pad were incubated in 2 ml of Krebs–Ringer bicarbonate buffer containing 1 mg of glucose and [2-^{14}C]histidine (0.1 μCi/ml of incubation medium). Flasks were gassed with O_2+CO_2 (95:5), capped and shaken for 3h at 37°C in a Dubnoff incubator. Each value was obtained from six determinations. *P* values were calculated by Snedecor's (1948) method of paired analysis.

Additions	Dose (μg/ml)	Histidine incorporation (c.p.m./μg of protein)	*P*
None		12.9±0.92	
Fraction 11	0.05	14.9±1.33	<0.05
Fraction 11	0.50	14.4±0.94	<0.05
None		11.9±0.92	
Fraction 11	2.5	15.3±0.98	<0.001
Fraction 11	12.5	14.9±0.96	<0.001

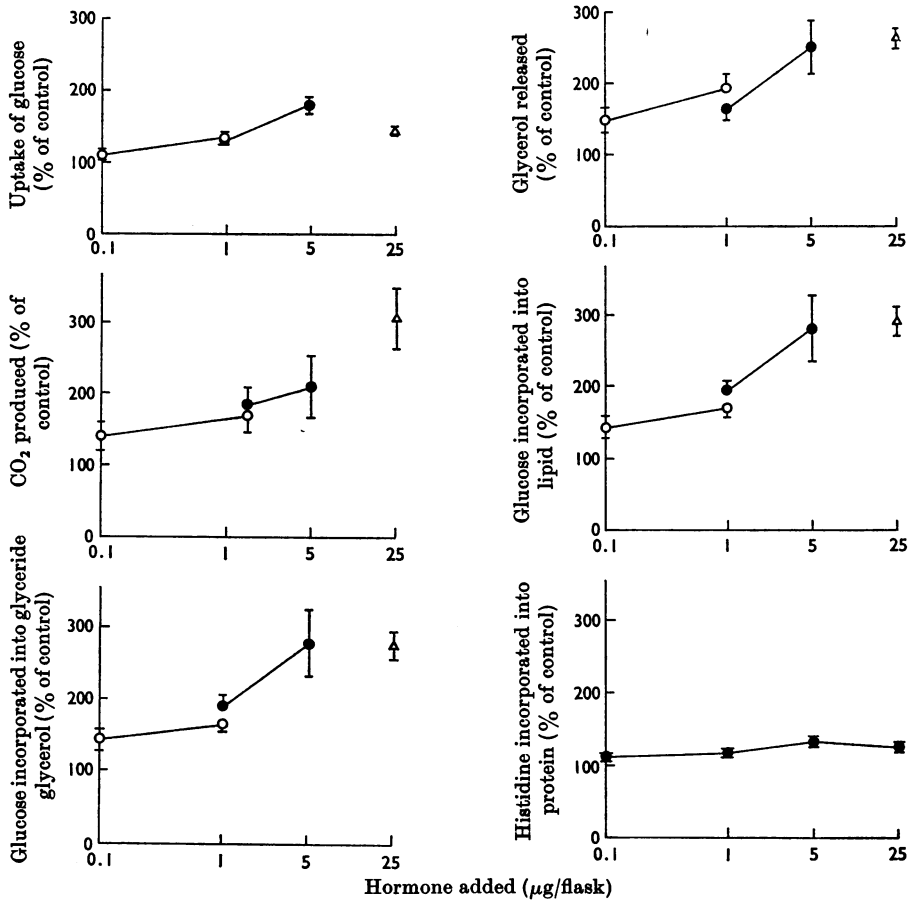

Fig. 5. log(dose)–response relationship of the major component of bovine growth hormone on several parameters of bovine growth-hormone action *in vitro*. Results are reported as a percentage of the control value set at 100 for each experiment. ○, ● and △ represent results of different experiments. The effects of 1 μg of the major component were evaluated in two separate experiments. In each experiment six samples of adipose tissue from different rats were used. Incubation conditions are as described in the legends to Tables 2 and 4. *P* values were calculated by the method of paired analysis (Snedecor, 1948).

Table 5. *Effect of various fractions of bovine growth hormone on plasma glucose and free fatty acids*

Male hypophysectomized rats (six per group) were injected intraperitoneally at zero time with 1 mg of bovine growth hormone/ml of saline (pH 9.9) and of various fractions of bovine growth hormone, and bled 30 min thereafter (Swislocki & Szego, 1965). The control animals received the saline alone. *P* values were calculated by the Fisher *t*-test. All fractions were compared with saline-treated controls. Results are given as mean ± S.E.M.

	Plasma glucose		Plasma free fatty acids	
Treatment	(mg/100 ml)	*P*	(μequiv./l)	*P*
Saline	91 ± 7.4		306 ± 28	
Bovine growth hormone	63 ± 7.2	<0.05	213 ± 17	<0.02
Fraction 11	55 ± 4.2	<0.01	192 ± 20	<0.01
Fraction 21	51 ± 5.4	<0.01	235 ± 20	N.S.
Fraction 35	81 ± 3.9	N.S.	258 ± 17	N.S.

Goodman (1965) demonstrated metabolic effects *in vitro* of 0.01 μg of hormone under incubation conditions where gelatin was added to the incubation medium to prevent adsorption of the hormone to the glass of the incubation vessels. Moreover, his studies were carried out on rat adipose tissue obtained from hypophysectomized rats that were fed, up to the time of the experiment, on a high-carbohydrate 'fat-free' diet rich in sucrose, to sensitize the tissues to bovine growth hormone. Under these conditions bovine growth hormone increased incorporation of glucose carbon into fatty acids; no results were given on distribution of label in glyceride glycerol (Goodman, 1965). In our experiments on adipose tissue obtained from hypophysectomized rats fed on a laboratory-chow diet, which was withdrawn 24h before the experiment, the major component of bovine growth hormone was effective at 0.05–0.1 μg. In our hands glucose label is predominantly incorporated into glyceride glycerol and not into fatty acid (Table 3). We have observed a similar disposition of glucose carbon by adipose tissue incubated with bovine growth hormone modified by limited proteolysis (Swislocki, 1969). Thus, the different nutritional status of the donor hypophysectomized rats used in Goodman's (1965) laboratory and ours may be the basis for the qualitative differences in distribution of glucose carbon in glyceride glycerol and fatty acid.

Our observations confirm and extend the findings of Manchester & Wallis (1963) who resolved bovine growth hormone into fractions of different growth-promoting potency that possessed activity *in vitro*. As reported here all fractions tested had activity *in vitro* but only one, the major component, was able to elicit the acute metabolic responses *in vivo* that are associated with bovine growth-hormone action (Swislocki & Szego, 1965; Swislocki, 1968*a*). even though all fractions contained growth-promoting activity.

The inability of fractions 21 and 35 to effect concomitant declines to values of statistical significance in plasma free fatty acid and glucose concentrations may be due, at least in part, to the dose used and time of sampling. We have previously demonstrated that the manifestation of the acute hypoglycaemic and hypolipaemic effects of bovine growth hormone in hypophysectomized rats is dependent not only on the relative dose of hormone but also on the method of preparation of the hormone and the time after treatment at which plasma samples are taken (Swislocki, 1968*a*). The different sensitivities of the hypoglycaemic and hypolipaemic responses to these fractions may be attributed to thyrotrophin and prolactin, which as contaminants in the parent material may be hyperglycaemic or hyperlipaemic themselves. Prolactin has been demonstrated in fraction 35 by immunoelectrophoresis and immunodiffusion (Sundaram & Sonenberg, 1969). Thyrotrophin can be eluted at high salt concentrations (Sonenberg *et al.* 1968) (see the Experimental section). These contaminants may have been present in the original material in concentrations too low to be inhibitory. However, after chromatography and concentration the amount of contaminating material may have been sufficiently increased to mask partially the effects of bovine growth hormone *in vivo*. Thyrotrophin increases plasma free fatty acid concentrations (Rudman, Brown & Malkin, 1963), whereas bovine growth hormone has the opposite effects at the early time-interval studied (Swislocki & Szego, 1965; Swislocki, 1968*a*). The effectiveness of fractions 21 and 35 *in vitro* on glucose metabolism is apparent, since various contaminants found in bovine growth-hormone preparations themselves promote the entry of glucose into adipose tissue. Thus, whereas thyrotrophin and prolactin appear to antagonize the action of bovine growth hormone *in vivo*, they will augment or mimic its effect on glucose metabolism *in vitro*.

It appears that the spectrum of acute metabolic effects observed *in vivo* after administration of bovine growth hormone to rats or its addition to rat tissue resides in a single species of protein as determined by rechromatography (Free & Sonenberg, 1966), polyacrylamide-gel electrophoresis and sedimentation equilibrium. The major component of bovine growth hormone, fraction 11 of the present study, which corresponds to component 1 of Free & Sonenberg (1966), was devoid of the faster electrophoretic components of bovine growth hormone. The latter were found in increasing amounts in fractions 21 and 35, and in subsequent fractions. Component 2 (Free & Sonenberg, 1966) was located in fractions 21 and 35 and, although it differs from component 1 only in numbers of amide groups, displays the same growth-promoting activity. Whether these components are different growth hormones of different metabolic activity remains to be seen. A correlation has been found between the number of amide groups and biological activity for a number of proteins (Robinson, McKerrow & Cary, 1970).

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REFERENCES

Barrett, R. J., Friesen, H. & Astwood, E. B. (1962). *J. biol. Chem.* **237**, 432.

Christophe, J. (1963). *Proc. 7th int. Cong. Biochemical Problems of Lipids*, pp. 373–384. Amsterdam: Elsevier Publishing Co.

Davis, B. J. (1964). *Ann. N.Y. Acad. Sci.* **121**, 404.
Dellacha, J. M. & Sonenberg, M. (1964). *J. biol. Chem.* **239**, 1515.
Dole, V. P. (1956). *J. clin. Invest.* **35**, 150.
Fain, J. N., Kovacev, V. P. & Scow, R. O. (1965). *J. biol. Chem.* **240**, 3522.
Ferguson, K. A. & Wallace, A. L. C. (1963). *Recent Prog. Horm. Res.* **19**, 1.
Free, C. A. & Sonenberg, M. (1966). *J. biol. Chem.* **241**, 5076.
Goodman, H. M. (1965). *Endocrinology*, **76**, 216.
Hamid, M. A., Rubinstein, D., Ferguson, K. A. & Beck, J. C. (1965). *Biochim. biophys. Acta*, **100**, 179.
Jungas, R. L. & Ball, E. G. (1960). *J. biol. Chem.* **235**, 1894.
Landau, B. R., Bartsch, G. E. & Williams, H. R. (1966). *J. biol. Chem.* **241**, 750.
Leboeuf, B. & Cahill, G. F., jun. (1961). *J. biol. Chem.* **236**, 41.
Manchester, K. L. & Wallis, M. (1963). *Nature, Lond.*, **200**, 888.
Marx, W., Simpson, M. E. & Evans, H. M. (1942). *Endocrinology*, **30**, 1.
Ornstein, L. (1964). *Ann. N.Y. Acad. Sci.* **121**, 321.
Robinson, A. B., McKerrow, J. H. & Cary, P. (1970). *Proc. natn. Acad. Sci. U.S.A.* **66**, 753.
Rodbell, M. (1964). *J. biol. Chem.* **239**, 375.
Rudman, D., Brown, S. J. & Malkin, M. F. (1963). *Endocrinology*, **72**, 527.
Snedecor, G. W. (1948). In *Statistical Methods*, p. 43. Ames, Iowa: Iowa State College Press.
Sonenberg, M., Kikutani, M., Free, C. A. Nadler, A. C. & Dellacha, J. M. (1968). *Ann. N.Y. Acad. Sci.* **148**, 532.
Spackman, D. H., Stein, W. H. & Moore, S. (1958). *Analyt. Chem.* **30**, 1190.
Sundaram, K. & Sonenberg, M. (1969). *J. Endocr.* **44**, 517.
Swislocki, N. I. (1968*a*). *Metabolism*, **17**, 174.
Swislocki, N. I. (1968*b*). *Biochim. biophys. Acta*, **169**, 556.
Swislocki, N. I. (1969). *Metabolism*, **18**, 895.
Swislocki, N. I. (1970). *Biochim. biophys. Acta*, **201**, 242.
Swislocki, N. I. & Sonenberg, M. (1969). *Fedn Proc. Fedn Am. Socs exp. Biol.* **28**, 572.
Swislocki, N. I. & Szego, C. M. (1965). *Endocrinology*, **76**, 665.
Wallis, M. & Dixon, H. B. F. (1966). *Biochem. J.* **100**, 593.
Wieland, O. (1963). In *Methods of Enzymatic Analysis*, p. 211. Ed. by Bergmeyer, H. U. New York: Academic Press Inc.
Yphantis, D. (1964). *Biochemistry, Easton*, **3**, 297.