Although there was no difference in the maximum size of neurons in the ganglia of experimentals and controls, there apparently was a change in cell size distribution, with the antiserum-treated ganglia having a greater proportion of smaller cells than the controls. Perhaps the antiserum interfered to some extent with the growth processes of the neuroblasts. It is also possible that the increased number of small cells was due to degenerative processes taking place in some neuroblasts, since many of these small cells were pyknotic.

The NGF and its antiserum operate extensively over phylogenetic lines. NGF has been detected in fishes[14], amphibians[15], and birds[16], as well as reptiles and mammals, and NGF has comparable effects in these various classes of vertebrates. Similarly, antiserum produced by a cow against mouse NGF will cross-react with snake venom NGF, as seen by complement fixation[17]. BURDMAN and GOLDSTEIN[18] found partial cross-reactivity of goat anti-mouse NGF with NGF from children with neuroblastoma, a tumor of neural crest origin. Furthermore, it has been shown that bovine antiserum against mouse NGF will inactivate NGF from the axial regions of the chick, tadpole, and goldfish, indicating immunological similarity of the molecules[15]. This extensive cross-reactivity of the antiserum is sufficient to cause biological effects across phylogenetic lines, extending now to the amphibia as well as the other classes of vertebrates.

Résumé. Des injections d'un sérum spécifique contre le facteur de croissance nerveuse (anti-NGF) provoquent une réduction de la taille des ganglions rachidiens de la salamandre *Ambystoma* par suite de la réduction de la masse cellulaire du ganglion. Il y a aussi un plus grand nombre de «petites» cellules (d'un diamètre nucléaire de moins de 6,25 μ) dans les ganglions des animaux utilisés.

JUDITH S. WEIS[19]

Department of Zoology and Physiology of the Rutgers University, State University of New Jersey, Newark (New Jersey 07102, USA), 25 August 1969.

[14] J. S. WEIS, Experientia *24*, 736 (1968).
[15] M. WINICK and R. GREENBERG, Pediatrics *35*, 221 (1965).
[16] E. D. BUEKER, I. SCHENKEIN and J. BANE, Cancer Res. *20*, 1220 (1960).
[17] A. ZANINI, P. ANGELETTI and R. LEVI-MONTALCINI, Proc. natn. Acad. Sci. *61*, 835 (1968).
[18] J. BURDMAN and M. GOLDSTEIN, J. natn. Cancer Inst. *33*, 123 (1964).
[19] I should like to express my thanks to Dr. ISAAC SCHENKEIN for supplying the antiserum preparation, and to Mr. EDWARD KALMAR for technical assistance. This research was supported by a grant from the Rutgers University Research Council.

Effect of a Growth-Promoting Factor from Calf Muscles on the Weight Gain of Hypophysectomized Rats

SALMON and DAUGHADAY[1] proposed the hypothesis that the action of growth hormone on cartilage may be mediated by a component of serum called sulfation factor. Hypophysectomy causes a marked decrease in the sulfation activity of serum; this activity can by restored by injections of growth hormone, but not by adding the hormone to the serum in vitro. In a study of various tissues it was found that tissue extracts exhibit different degrees of sulfation activity. High activities were measured in extracts of rat skeletal muscles[2]. Previous reports on sulfation activity were based on results obtained in vitro. Here we report a method for extraction and separation of the active material from calf muscles. We also investigated the effect of the separated material on the growth hormone action in hypophysectomized rats.

The material used was 500 g of fresh calf meat obtained directly from the slaughterhouse. The muscles were incubated in 2000 ml of sterilized distilled water for 3 h at 37 °C. After incubation the muscles were discarded and the fluid filtered through a sterile Zeiss EKS filter. The filtrate was passed through a Dowex-50W-X2(H) resin, mesh size 100–200, pH 6.0, which was prepared according to the method originally described by BOUCHER et al.[3] for the extraction of angiotensin from plasma. The active material was eluted from the column as angiotensin is eluted using this method, and the eluate was lyophylized. The residue was dissolved in sterile distilled water, filtered again through a sterile Zeiss EKS filter, and diluted to 25 ml with water. This solution was then passed through a Sephadex G-25 (Pharmacia, superfine grade) column. The size of the column was 100×2.5 cm, and the Sephadex was equilibrated with 0.05 *M* *Tris*mabuffer (Sigma), pH 7.4. Three separate chromatographies were performed by applying a volume of 6 ml on the column in 2 instances and 4.45 ml of the solution containing the active material in one instance. In each separation the column was eluted at a flow rate of 30 ml/h. The volume of one fraction was 10 ml. The total amount of the gel filtrated solution (22.45 ml) corresponded to 329 g of muscles. The biological activities of the incubation fluid, the solution obtained after Dowex and the Sephadex fractions were tested in vitro by measuring the incorporation of ^{35}S-labelled sulfate in the pelvis of chick embryos[2]. The results obtained by separation on Sephadex G-25, and the sulfation activity determinations are shown in the Figure.

The Figure shows 3 different separations. The separation is fairly well reproduced as can be seen from the curves. The corresponding fractions from 3 separations were pooled, and the biological activity of the pooled fractions was tested. The bars show the biological activity. 2 of the 3 peaks of the biological activity are well expressed – one corresponding to fraction 34, and one to fraction 40. In a preliminary experiment these 2 fractions, together with human growth hormone (HGH), were injected into hypophysectomized rats. Each group consisted of 5 rats; the animals were injected each day for 6 days. The

[1] W. D. SALMON JR. and W. H. DAUGHADAY, J. Lab. clin. Med. *49*, 825 (1957).
[2] K. HALL and M. BOŽOVIĆ, *Hormone and Metabolic Research*, in press.
[3] R. BOUCHER, R. VEYRAT, J. DE CHAMPLAIN and J. GENEST, Can. med. Ass. J. *90*, 194 (1964).

Effect of a growth-promoting factor from calf muscles on the weight gain of hypophysectomized rats treated with HGH

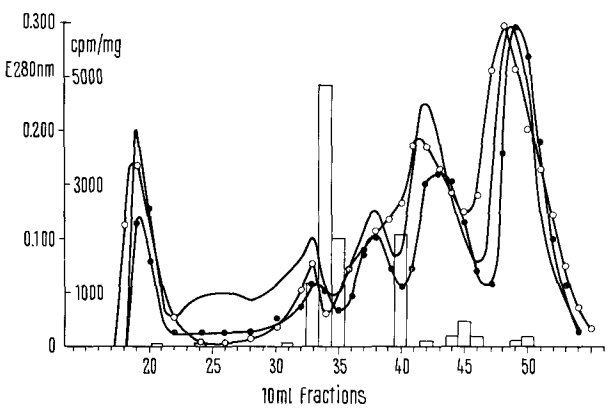
No. of rats	S.c. injections each day for 6 days	Mean body weight ± SE (g)		Mean gain (g) ± SE	P
		1st day	7th day		
14	0.020 mg HGH in 0.5 ml	123.5 + 1.5	131.5 + 2.3	8.0 ± 1.6	<0.001
13	0.020 mg HGH + fr. 40 in 0.5 ml	122.0 ± 1.7	139.7 ± 1.6	16.8 ± 1.5	
	P	0.8	<0.01		

animals in the first group were treated daily with 0.02 mg HGH; in the second group active material from fraction 34, and in the third group fraction 40, was added to the HGH. In this experiment fraction 34 partially inhibited and fraction 40 potentiated the effect of the growth hormone. The following is a detailed description of the experiment in which a submaximal dose of HGH was injected together with the active material from fraction 40.

As was said before 3 fractions 40 from 3 different separations were pooled and lyophylized. The residue was dissolved in 3 ml sterile distilled water. The solutions for injections were made by using 6 mg of HGH prepared at AB Kabi (Stockholm, Sweden) according to the method of Ruus et al.[4]. The content of HGH in this batch was determined by radio-immunologic assay, measurement of extinction and by a biological weight-gain test. The 6 mg of HGH were dissolved in 150 ml of 0.9% NaCl. To 75 ml of this solution 2.3 ml of the solution obtained by pooling 3 fractions 40 were added, and the same volume of water was added to the remaining 75 ml. The animals were given s.c. injections of 0.5 ml of the respective solutions every day for 6 days. This meant that in one group each animal received 0.02 mg of the HGH daily, and in the second group the same dose of HGH together with the active material, which corresponds to 10.08 g of calf muscles. In the experiment 27 female hypophysectomized rats purchased at Hormone Assay Laboratories (Chicago) were used. The body weight of the rats on the day of operation were 125–150 g, and the injections started 14 days after surgery, when the body weight was stabilized. The mean body weight on the first and seventh day, together with the gain in body weight, are shown in the Table.

The mean body weight of both groups were well matched before the experiment was begun. In both groups the body weight increased. The mean gain in body weight was significantly higher in the group treated with the active material from calf muscles plus HGH. This is also evidenced by the fact that the mean body weight in this group was significantly higher on the seventh day than in the group treated with HGH alone. The percentage of water in muscles of animals was 75.1 in the group treated with HGH and 75.6 in the second group.

We conclude that the active material obtained from calf muscles has a growth promoting activity in vivo when injected, together with HGH, into hypophysectomized rats. The active material does not seem to be species specific. From the gel filtration data it is supposed that the growth-promoting factor from calf muscles is a substance or mixture of substances of low molecular weight, presumably polypeptides. Based on the readings of extinctions at 280 nm, 20–40 ng of a polypeptide material was injected into each animal daily. This indicated that the growth-promoting material has a high biological activity per unit of weight. From the present study nothing can be said about the mechanism of action of the growth-promoting factor from calf muscles. There have been reports that speak favourably of the hypothesis the growth hormone stimulates amino acid transport by that influencing the synthesis of peptides involved in the membrane transport mechanism[5]. It is not known if the growth-promoting factor from muscles and the sulfation factor in plasma are identical. We extracted a material showing sulfation activity from plasma and subjected it and the growth-promoting material to high-voltage electrophoresis. The sulfation activity of both materials was found in approximately the same location.



Sephadex G-25 chromatography of the active material from calf muscles. Curves represent the separations and the biological activities of the pooled fractions are represented by bars. Details of the procedure are described in the text.

Zusammenfassung. Aus Kalbsmuskel wurde nach Sephadex G-25 Chromatographie jene Fraktion gereinigt, die als Sulfationsfaktor bekannt ist und den ^{35}S-Sulfateinbau im embryonalen Hühnerknorpel in vitro steigert. Der Faktor kann bei hypophysektomierten Ratten die Wirkung von submaximalen Dosen des menschlichen Wachstumshormons erhöhen.

Milica Božović, H. Boström, K. Uthne, K. Berntsen and L. Božović

King Gustav Vth's Research Institute, Department of Clinical Physiology, Karolinska Hospital, and AB KABI, Research Department, S-10401 Stockholm (Sweden), 18 August 1969.

[4] P. Ruus, H. R. Fevuld and C. A. Gemzell, Biochem. biophys. Acta *74*, 525 (1963).

[5] L. J. Kostyo, Ann. N.Y. Acad. Sci. *148*, 389 (1968).