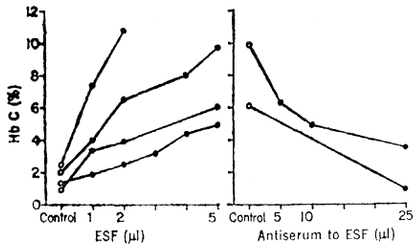

Fig. 3. Relation between ESF dose and percentage of Hb C synthesis in culture and the blocking effect of antiserum to ESF.

cultures, different doses of ESF were added to determine the dose-response relation of ESF to Hb C synthesis. As shown (Fig. 3), there was a direct dose dependence between ESF and Hb C synthesis, which was completely blocked by the simultaneous addition of antiserum capable of neutralizing the biological activity of ESF (*9*).

These studies demonstrate that the switch from Hb A to Hb C synthesis can be induced in suspension cultures of marrow from nonanemic sheep and provide the first in vitro evidence that the switching substance is ESF. Preferential Hb C synthesis resulted from and depended on the dose of added human ESF, an effect that was completely blocked by the addition of neutralizing antiserum. Similar to results for rat marrow cultures, the ESF response in this system is dependent on cell proliferation. Thus, ionizing irradiation in low doses blocks ESF-dependent hemoglobin synthesis.

The high correlation between the percentage of Hb C synthesized in culture and total hemoglobin synthesis in response to ESF (Fig. 1B) points up an important feature that may apply in the intact animal. The marrow response in this system is totally ESF-dependent; since total hemoglobin synthesis and Hb C content are linearly related, the amount of Hb C synthesized in the nonanemic animal may be due to endogenous concentrations of ESF. Thus, any increase above normal in total hemoglobin synthesis predictably should be accompanied by a preferential increase in Hb C synthesis. While these data do not offer information as to the mechanism by which ESF exerts its differential effect, the relatively simple method may lend itself to the elucidation of these mechanisms.

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5. Approximately 20 mg of hemoglobin were applied to 2 by 50 cm columns and eluted with 0.05*M* tris(hydroxymethyl)aminomethane hydrochoride buffer (*p*H gradient, 8.2 to 7.3). Radioactivity was confined completely to the hemoglobin peaks.
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7. An increase in Hb C content was seen whether heme or globin was labeled. However, the ratio of Hb C in stimulated cultures to Hb C in control cultures was uniformly lower when globin was labeled, presumably due to the higher baseline value for Hb C when [^{14}C]leucine was used.
8. Radioactivity of Hb C in basal cultures ranged from 8 to 74 count/min, while that of Hb A ranged from 431 to 2041 count/min. With ESF, Hb C radioactivity was 116 to 1665 count/min, and that of Hb A was 963 to 7192 count/min. The percentage of Hb C was calculated as 100 times the radioactivity in Hb C divided by the sum of radioactivity in Hb C and Hb A.
9. Rabbit antiserum against human urinary ESF was prepared by the method of J. Schooley and J. Garcia [*Blood* **25**, 204 (1965)].
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Somatomedin: Inhibition of Adenylate Cyclase Activity in Subcellular Membranes of Various Tissues

Abstract. *Somatomedin in concentrations between 3 and 20 units per milliliter significantly inhibits the basal activity of adenylate cyclase in crude membrane preparations obtained from homogenates of fat cells, liver, and spleen lymphocytes of the rat, and from chondrocytes and cartilage of chick embryos. The enzyme activity measured in the presence of stimulating hormones (epinephrine, prostaglandin PGE_1, parathyroid hormone) is also inhibited in these preparations by somatomedin. These observations may be relevant in a general way to the mechanism of action of growth-promoting substances and to the processes which normally regulate cell growth.*

Somatomedin is the term which has been proposed (*1*) to designate an apparently unique plasma hormone which is responsible for the activities of "sulfation factor" and "thymidine factor," and which is similar to if not identical with the acid ethanol–soluble component of serum which elicits nonsuppressible insulin-like activity (*2*). Although the mechanism of production and the precise physiological significance of somatomedin are not known, it appears to play an important role in mediating the effects of growth hormone on skeletal as well as nonskeletal tissues. This hormone, whose activity may be attributed to a neutral peptide with a molecular weight of about 8000 (*3*), enhances the rate of incorporation of thymidine into DNA (*4*) and uridine into RNA (*5*), and it stimulates the conversion of proline to hydroxyproline in cartilage (*6*). In addition, somatomedin at physiologic concentrations exhibits striking insulin-like effects (*7*) in adipose tissue which have recently been correlated with specific interactions of this hormone with insulin receptors of plasma membranes. Physiological concentrations of somatomedin effectively compete with [^{125}I]insulin for receptor sites on isolated fat cells, liver membranes, and isolated chondrocytes (*8*). Since the final purification of somatomedin has not yet been achieved, it is not known whether this biological activity can be attributed to a single peptide or to multiple species of closely related peptides. The possible relationship of somatomedin to other insulin-like pleiotypic peptides has been pointed out previously (*8*).

The present report describes a biochemical effect of a partially purified preparation of somatomedin in a subcellular system—the inhibition of adenylate cyclase activity in crude membrane preparations of various tissues. Similar inhibition of adenylate cyclase activity has been described for insulin in membrane preparations of fat cells (*9*) and liver (*9*), in fat cell ghosts (*10*), and in membranes obtained from *Neurospora crassa* (*11*).

Furthermore, other substances possessing potent insulin-like activity, such as the plant lectins concanavalin A and wheat germ agglutinin, are effective inhibitors of adenylate cyclase activity in fat cell membranes (*12*). In view of the analogous anabolic and growth-promoting metabolic properties of insulin and somatomedin, the present studies suggest the existence of common biochemical events which may have important implications on some general processes which regulate cell growth.

Fat cells (*13*), liver (*9*), and spleen lymphocytes (*14*) obtained from 100- to 140-g Sprague-Dawley male rats, and isolated chondrocytes (*15*) or total pelvic rudiments (cartilage) obtained from 15-day-old chick embryos, are homogenized in cold (4°C) 50 m*M* tris·HCl buffer, *p*H 7.6, for 30 seconds (Polytron PT-10 homogenizer, 3000 rev/min). The liver and cartilage homogenates are centrifuged for 10 minutes at 600*g*; these supernatants, and the fat cell and lymphocyte homogenates, are centrifuged (4°C) at 100,000*g* for 15 minutes. The particulate material ("membrane preparation") is suspended in cold 50 m*M* tris·HCl buffer, *p*H 7.6, and assayed for adenylate cyclase activity within 5 minutes (*16*). Adenylate cyclase activity is determined by a modification (*12, 17*) of the technique of Pohl *et al.* (*18*). The incubation mixture consists of 0.1 ml containing 50 m*M* tris · HCl buffer (*p*H 7.6), 7.5 m*M* $MgCl_2$, 1 m*M* ethylenediaminetetraacetic acid, 5.0 m*M* aminophylline, 0.1 percent (weight to volume) albumin, 3 m*M* [α-^{32}P]adenosine triphosphate (1.5 μc), and 100 to 200 μg of membrane protein; 5 m*M* phosphoenolpyruvate and 60 μg of pyruvate kinase per milliliter are used as the adenosine triphosphate (ATP) regenerating system. After incubation at 30°C for 10 minutes, the reaction is stopped by placing the tubes in boiling water for 3 minutes. After cooling, 1.0 ml of a recovery mixture containing cyclic [^{3}H]adenosine monophosphate (AMP) is added to each sample. Cyclic AMP is isolated on a column containing 1 g of alumina (*19*) which is eluted with 2.0 ml of 50 m*M* tris · HCl buffer, *p*H 7.6. All incubations are performed in triplicate.

Somatomedin (*20*) is effective in inhibiting the unstimulated activity of adenylate cyclase in particulate fractions of fat cells, liver, spleen, and cartilage (Fig. 1). At a concentration of 20 units per milliliter, somatomedin

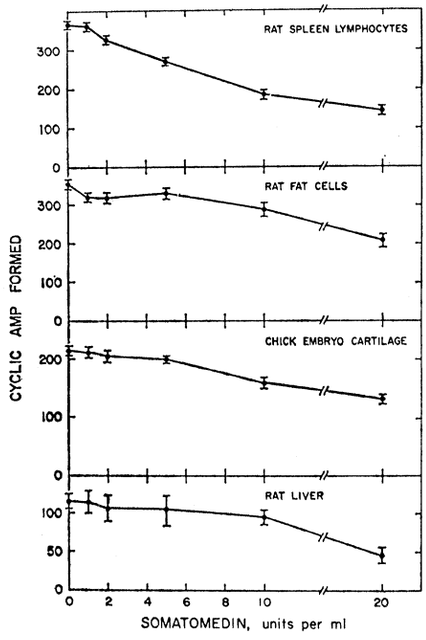

Fig. 1. Effect of somatomedin on adenylate cyclase activity of particulate preparations of rat spleen lymphocytes (1.5 mg of protein per milliliter), fat cells (1.2 mg of protein per milliliter), cartilage (2.0 mg of protein per milliliter), and liver (1.5 mg of protein per milliliter). Cyclic AMP formed is expressed as picomoles of cyclic AMP per milligram of protein per 10 minutes of incubation; average of three replications ± standard error of the mean. Although the effects of somatomedin in some of the above experiments are small, the reproducibility of the inhibition has been confirmed by numerous similar experiments.

causes a 30 to 60 percent inhibition of the basal enzyme activity in all of these preparations. Concentrations above 20 units per milliliter have been tested only on preparations from chick embryo cartilage, where further inhibition is obtained by using 50 units of somatomedin per milliliter (*21*). Concentrations lower than 6 to 8 units per milliliter are effective only in preparations from rat spleen lymphocytes, which appear to be very sensitive to this hormone. This effect of somatomedin on the adenylate cyclase activity of the membrane preparations does not result from an effect of this hormone on phosphodiesterase activity or on the ATP-regenerating system used (*22*).

Somatomedin also inhibits the activity of adenylate cyclase in the presence of hormones which stimulate the activity of the enzyme (Table 1). Complete reversal of the stimulating effect of the added hormone can generally be obtained with 20 units of somatomedin per milliliter when moderately low concentrations of the stimulating hormone are used. This inhibition is independent of the type of stimulating hormone used. In particulate preparations of rat spleen lymphocytes, which again show a great sensitivity to somatomedin, inhibition of adenylate cyclase activity is equally profound whether the enzyme is stimulated with 0.75 μg of L-epinephrine per milliliter (Table 1) or with prostaglandin PGE_1 (not shown). In the preparations of liver and fat

Table 1. Effect of somatomedin (20 units per milliliter) on the basal and stimulated adenylate cyclase activities of particulate fractions from various tissues. Particulate fractions and adenylate cyclase activity are as described in Fig. 1 and in the text.

Tissue	Cyclic AMP production*
Lymphocytes	
No addition	274 ± 25
+ somatomedin (20 unit/ml)	46 ± 10
+ L-epinephrine bitartrate (0.75 μg/ml)	365 ± 17
+ L-epinephrine bitartrate (0.75 μg/ml) + somatomedin (20 unit/ml)	52 ± 3
Fat cells	
No addition	470 ± 5
+ somatomedin (20 unit/ml)	379 ± 6
+ L-epinephrine bitartrate (1 μg/ml)	843 ± 11
+ L-epinephrine bitartrate (1 μg/ml) + somatomedin (20 unit/ml)	540 ± 19
Cartilage	
No addition	195 ± 6
+ somatomedin (20 unit/ml)	110 ± 8
+ parathyroid hormone (20 unit/ml)	229 ± 12†
+ parathyroid hormone (20 unit/ml) + somatomedin (20 unit/ml)	141 ± 8
Liver	
No addition	95 ± 9
+ somatomedin (20 unit/ml)	58 ± 8
+ prostaglandin PGE_2 (5 μg/ml)	182 ± 6
+ prostaglandin PGE_2 (5 μg/ml) + somatomedin (20 unit/ml)	119 ± 13

* Picomoles of cyclic AMP per milligram of protein per 10 minutes of incubation; average of three replications ± standard error of the mean. Results similar to those presented here have been obtained reproducibly in at least three separate experiments. † The enhancement in activity is significant to the *P* value = .025.

cells somatomedin inhibits the activity in the presence of L-epinephrine, and in the liver preparation the effects of prostaglandin PGE_1 are apparently inhibited. In addition, the stimulation of enzyme activity of fat and liver membrane preparations caused by 2.5 m*M* NaF is significantly reversed by 20 units of somatomedin per milliliter (data not shown). Attempts to stimulate adenylate cyclase in particulate fractions of homogenates of chondrocytes and of cartilage with 1 to 50 μg of L-epinephrine per milliliter and with 1 to 50 μg of prostaglandins PGE_1 and PGE_2 per milliliter were not successful. Moderate stimulation is observed with 20 to 50 units of parathyroid hormone per milliliter and with 10 m*M* NaF, and the activity in the presence of parathyroid hormone is inhibited by 10 to 20 units of somatomedin per milliliter (Table 1).

Since the known metabolic effects of somatomedin are anabolic and thus opposed to the known effects of cyclic AMP in tissues, it is reasonable that the biochemical effects of this hormone may be mediated by the inhibition of adenylate cyclase activity demonstrated in this report. It is not yet known whether somatomedin can decrease the intracellular concentrations of cyclic AMP in intact tissues. The concentrations of somatomedin required to inhibit adenylate cyclase in membrane preparations are about ten times higher than those usually found in the blood (*15*) and which are capable of competing with [^{125}I]insulin for binding to receptors (*8*). However, little is known concerning the physiological variations in plasma somatomedin concentration. Furthermore, the in vitro conditions used here may not be optimal for eliciting the somatomedin effect on broken cell preparations. It is well recognized, for example, that hormones (such as, glucagon, adrenocorticotropin, epinephrine) known to act by stimulating adenylate cyclase activity must generally be used at unphysiologically high concentrations to elicit enzymic effects in isolated membrane preparations. In the work reported here, the sensitivity to somatomedin varied considerably between tissues, and the lymphocyte preparation responded to hormone concentrations which are within a reasonable range.

The ability of somatomedin to interact with the same receptor sites as insulin in fat cells, liver, and chondrocytes (*8*) may suggest that the inhibition of adenylate cyclase activity may simply result from interactions of somatomedin with insulin receptor sites. Furthermore, the question is raised whether specific somatomedin receptors exist which are independent of those for insulin, and whether interaction with such receptors leads to the same change in adenylate cyclase activity. It is known that unstimulated circulating lymphocytes of humans have few, if any, insulin receptors compared to the number that these cells acquire during in vitro transformation (*23*). Although little is known about the nature of insulin receptors in rat spleen lymphocytes, it is pertinent that the adenylate cyclase activity of the particulate fraction of homogenates of these cells cannot be affected by 30 to 6000 μunit of insulin per milliliter (*24*). This contrasts sharply with the marked effects of somatomedin in the same tissue preparation, and with the relative ease with which effects of insulin are demonstrable in preparations from other tissues (*9, 24*). These results suggest that rat spleen lymphocytes may have specific somatomedin receptors which are independent of insulin receptors, and that interaction with such receptors leads to inhibition of adenylate cyclase activity. Whether other tissues possess unique receptors for somatomedin, and interpretation of the interaction of somatomedin with insulin receptors, will require further study.

It is notable that certain growth-promoting and anabolic substances, such as insulin, concanavalin A (*12*), and somatomedin exhibit similar kinds of metabolic effects on cells and can directly inhibit the activity of adenylate cyclase in subcellular systems. At least under some circumstances, insulin (*25*) and concanavalin A (*26*) can decrease intracellular levels of cyclic AMP. The possible role of cyclic AMP, or of closely related processes regulating its synthesis or degradation, in mediating the biological effects of such compounds is especially meaningful in view of the increasing evidence in tissue culture cells that cyclic AMP plays a fundamental role in the regulation of cell growth (*27*). Contact inhibition of growth, decreased rates of cell growth, and inhibition of plant lectin transformation of lymphocytes are associated with increased levels of intracellular cyclic AMP or with the addition of substances (for example, prostaglandins, theophylline, and dibutyryl cyclic AMP) which stimulate adenylate cyclase. Rapid rates of cell growth and reversal of contact inhibition appear to be associated with a fall in the intracellular level of cyclic AMP or with the addition of substances (for example, serum, trypsin) which lower the concentrations of this nucleotide. It is possible that the normal in vivo regulation of cell growth or transformation in various tissues by physiologic humoral agents, such as hormones, may be governed at least in part by interactions of a special class of receptors on the cell membrane. Such receptors may be activated specifically and selectively by different hormones in different tissues, but activation may lead to essentially similar kinds of biochemical events, including inhibition of adenylate cyclase activity.

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21. Although the effects of somatomedin on adenylate cyclase activity occur in the membrane preparation of isolated chondrocytes, most of the experiments, including the data presented here, were performed with the particulate fraction of cartilage homogenates.
22. The adenylate cyclase activity of spleen lymphocyte particulate preparations, and its inhibition by somatomedin, are unaltered by the addition of 1 m*M* unlabeled cyclic AMP to the incubation medium. In the absence of an ATP-regenerating system the basal adenylate cyclase activity of these particulate preparations is decreased by about 30 percent, but the inhibition of activity by somatomedin is still clearly discernible.
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6 February 1973

Acetylcholine: Fast Axoplasmic Transport in Insect Chemoreceptor Fibers

Abstract. *Acetylcholine is transported along insect chemoreceptor axons at a rate of 12 to 13 centimeters per day after peripheral uptake of choline. Colchicine, vinblastine sulfate, and cytochalasin B all block transport, but transport continues in axons separated surgically from the cell body. These data from an insect are in accord with many studies on vertebrates which have implicated intracellular microtubules in the transport mechanism. The peripheral uptake of choline and its acetylation and transport to nerve terminals in the brain are consistent with the suggestion that acetylcholine is an antennal sensory transmitter in insects.*

With a single exception (*1*), axoplasmic transport has not been studied in insects, although insect nerves offer a variety of unique morphological adaptations which might serve as useful experimental preparations. A case in point is the insect antennal nerve with its neural organization much reduced in size and morphological complexity as compared with most other neural systems.

The chemoreceptor fibers in the antennal nerves of insects are primary sense cells, that is, the receptor cells located in the antennal sense organs (sensilla) project from the periphery to the central nervous system without fusion and without synaptic connection through interneurons (*2*). Electron micrographs of the two main antennal nerves of the woodroach, *Leucophaea maderae*, show that more than 96 percent of the axons have a diameter of less than 0.5 μm and most lack individual glial sheaths. None of the fibers is myelinated. The small, naked fibers average 0.15 μm in diameter and are contained in fascicles of 25 to 100 axons with a common glial sheath surrounding the whole bundle. The interaxonal separation is about 100 Å, leaving little sodium space between the fibers. With glutaraldehyde-osmium fixation, only three cytoplasmic structures are consistently seen in the axons: the plasma membrane; mitochondria scattered at intervals along each axon; and 2 to 5 microtubules, typically measuring 240 Å in diameter. No neurofilaments have been observed in the small-caliber fibers of woodroach antennal nerves (*3*).

Approximately 60 percent of the antennal nerve fibers in *Leucophaea* originate from receptor cells located in thin-walled sensilla (olfactory sense organs) on the antennal surface, 30 percent from thick-walled sensilla (probable contact chemoreceptor organs or taste hairs), and the remaining 10 percent from mechanoreceptors and other receptor modalities (*3, 4*). Extensive electron microscope studies on the antennae of other insects reveal that the sensory dendrites of thin-walled sensilla are exposed to the external environment through a complex poretubule system which penetrates the cuticle of the sense organ (*5*). Dendrites of the thick-walled chemoreceptive sensilla of the cockroach antenna communicate with the external environment through an opening at the tip of the hairlike sensillum (*4, 6*). The presence of these minute openings in the cuticle of the antennal sense organs suggested that external application of isotopically labeled chemicals might result in the uptake and transport of the isotope by chemoreceptor cells.

Initial attempts to introduce an isotopic label into the cell bodies of the antennal chemoreceptors consisted of immersing the distal tip of the antenna in insect Ringer solution containing 9 μ*M* L-[^{3}H]leucine (0.5 mc/ml) or 28 μ*M* L-[^{3}H]proline (0.31 mc/ml). The average length of the antennae used was 3.5 cm ± 5 percent. Antennae were dipped for 15 minutes (proline) or 30 minutes (leucine) and thoroughly rinsed with unlabeled Ringer solution. Within 30 minutes, approximately 10 percent of the radioactivity was incorporated into a fraction of antennal homogenate which was insoluble in trichloroacetic acid (TCA), indicating that a portion of the labeled amino acids had been incorporated into protein. Tritiated leucine and [^{3}H]proline were recoverable as free amino acids after enzymatic or acid hydrolysis of the TCA-insoluble precipitate.

Attempts were made to demonstrate axoplasmic transport of the polypeptide material toward the brain by sectioning the antenna into 0.5-cm segments after a specific time interval, homogenizing the antennal segments in 0.15*M* phosphate buffer, and counting the isotopic activity in each segment with the liquid