mononuclear leukocytes into chemotactically responsive cells in vivo. The inhibitor does not, however, have the properties of a chemotactic factor inactivator such as the products of higher molecular weights (> 68,000) described by Bronza and Ward (*12*). It is possible, though, that the factor specified herein is similar to the low-molecular-weight, anti-inflammatory products described by Graham and Graham and by Fauve *et al.* (*13*).

The biological significance in tumorigenesis of a factor or factors contained in neoplasms which depress macrophage function remains to be determined. The inhibitory activity could, however, retard the ability of the immune system to rapidly mobilize sufficient numbers of macrophages to a developing tumor site to produce destruction of the neoplasm.

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6. The neoplastic cell lines were supplied by Drs. D. B. Amos and L. Gooding.
7. Two days after the intraperitoneal injection of 35 μg of purified phytohemagglutinin (Burroughs Wellcome), the mice were killed; the peritoneal cavities were exposed by abdominal incision and then lavaged vigorously with 10 ml of Gey's balanced salt solution containing 2 percent bovalbumin (Flow Laboratories), 0.01*M* HEPES buffer, *p*H 7.0 (Gey's BSS), and 10 units of heparin per milliliter to remove the accumulated inflammatory cells. The exudates from individual mice were centrifuged at 300*g* (4°C for 10 minutes) and resuspended in 1 ml of Gey's balanced salt solution; the total number and type of accumulated cells were determined (*5*).
8. In vitro chemotaxis was measured with peritoneal macrophages from normal mice that had been injected with phytohemagglutinin 48 hours earlier. The extensively washed cells were standardized to contain 2.2×10^6 macrophages per milliliter in medium RPMI 1640, and 0.4 ml of this suspension was placed in the upper compartment of a modified Boyden chamber. The cells were separated from the chemotactic stimulus, endotoxin-activated mouse serum, or medium alone by a 5.0-μm polycarbonate (Nuclepore) filter. All assays were performed in triplicate, and the chambers containing cells and stimulants were incubated for 4 hours in humidified air at 37°C. Chemotaxis was quantified by counting and averaging the number of macrophages per field in 20 (oil immersion) fields (×1540) that had migrated completely through the filter [R. Snyderman, M. C. Pike, D. McCarley, L. Lang, *Infect. Immun.* **11**, 488 (1975)].
9. Polymorphonuclear leukocytes were obtained from the peritoneal cavities of C3H/HeJ mice that had been injected with 75 μg of *Salmonella typhosa* 0901 endotoxin (Difco) 18 hours previously. The extensively washed PMN's (3.0×10^6 per milliliter of RPMI 1640) were placed in the upper compartment of a modified Boyden chamber and separated from the chemotactic stimulus, endotoxin-activated mouse serum, or medium alone by a 5.0-μm nitrocellulose (Millipore) filter. After incubation for 3 hours in humidified air, the filters were processed as described [R. Snyderman, J. K. Phillips, S. E. Mergenhagen, *J. Exp. Med.* **134**, 1131 (1971)].
10. The supernatant (3.0 ml) of sonicated hepatoma 129 cells was applied to a Sephadex G-50 (Pharmacia) column (2.5 by 55 cm) and eluted with RPMI 1640, *p*H 7.0, containing 0.01*M* HEPES buffer. Fractions were tested for inhibitory activity of macrophage chemotactic responsiveness.
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Growth Promotion by Homocysteic Acid

Abstract. *Homocysteic acid, $HO_3SCH_2CH_2CHNH_2CO_2H$, promotes growth of hypophysectomized rats, assayed by observation of increased thickness of epiphyseal cartilage of the tibia and by observation of tail growth. Doses of homocysteic acid as low as 1 microgram per day for 4 days in the tibia assay and 2.5 milligrams per kilogram per day for 5 weeks in the tail assay were effective in promoting growth. Serum somatomedin activity, determined by the porcine cartilage disk assay, was also increased by homocysteic acid. These findings relate an area of sulfur amino acid metabolism to the physiological action of growth hormone, accelerated growth in homocystinuria, initiation of arteriosclerosis, and control of cellular growth.*

Abnormalities of homocystine metabolism are associated with accelerated skeletal growth in individuals with homocystinuria (*1, 2*). Myointimal hyperplasia is observed both in individuals with homocystinuria (*3*) and in animals given homocystine derivatives (*4, 5*). We investigated the chemical nature of the homocystine derivative that promotes skeletal growth and hyperplasia of myointimal cells by assaying several sulfur amino acids for growth response in hypophysectomized rats. We found that homocysteic acid, the sulfonic acid derivative of homocysteine, promotes growth of hypophysectomized rats and that the serum from these animals contains somatomedin activity similar to that of normal rats. Thus the biological effects of homocysteic acid are similar to those produced by pituitary growth hormone in hypophysectomized rats. We believe these findings support the previous suggestion that homocystine derivatives are important in the physiological action of growth hormone and the initiation of arteriosclerosis (*6*).

Growth hormone activity was assayed by observation of tail growth in hypophysectomized rats given thyroxine, accord-

Table 1. Growth hormone activity of homocysteic acid (tail assay). Hypophysectomized rats (Charles River), tail length 135 to 150 mm, were injected 6 days per week with 6 μg of L-thyroxine subcutaneously and with sulfur amino acids in 0.01*M* sodium citrate, *p*H 8.9, intraperitoneally (*7*). Purified porcine growth hormone (Calbiochem) was used as a positive control. The last two groups were modified from data of de Groot (*7*). The *P* values were calculated for differences from the control group, by the paired *t*-test. Completeness of hypophysectomy was verified by dissection of the sella turcica.

Compound	Daily dose (mg/kg)	Animals		Days	Tail growth (cm)	*P*
		Number	Survivors			
None		20	14	33–37	0.9 ± 0.05*	
Homocysteic acid	2.5	11	5	35	2.0 ± 0.05	<.01
	20	10	4	33	1.9 ± 0.22	<.01
	80	18	8	33	2.5 ± 0.42	<.01
Homocystine	10	9	6	35	1.4 ± 0.28	>.1
Homolanthionine sulfone†	0.5	14	9	35	0.7 ± 0.06	>.8
Homolanthionine sulfone and sulfoxides†	0.5	14	13	35	0.6 ± 0.12	>.6
Growth hormone	1.0	10	4	37	2.0 ± 0.03	<.01
Growth hormone (*7*)	1.0	23	16	36	1.8	
None (*7*)		44	35	36	1.1	

*Means ± S.E.M. †Homolanthionine sulfoxide and homolanthionine sulfone were synthesized in our laboratory (*10*).

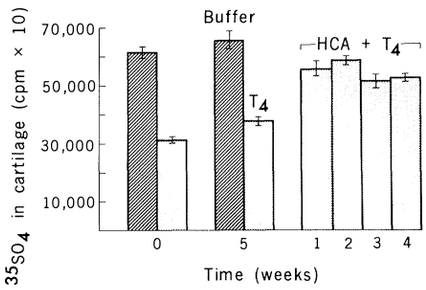

Fig. 1. Porcine disk assay for somatomedin activity. Groups of hypophysectomized rats were treated for 1 to 4 weeks with 6 μg of L-thyroxine (T4) per day and 10 mg of homocysteic acid (HCA) per kilogram per day. Serum, pooled from three animals, was incubated with porcine cartilage disks in tris amino acid buffer with $^{35}SO_4$. The disks were washed, then weighed and the radioactivity was determined by counting. Results are expressed as average number of counts of ^{35}S per minute ± the S.E.M. for six pairs of cartilage discs. Diagonal shading, normal animals; dotted shading, hypophysectomized animals.

ing to the method of de Groot (*7*). Hypophysectomized females weighing 80 to 90 g and with tail lengths of 135 to 150 mm were used. Tail length was measured twice weekly for 5 weeks, and the difference between initial and final tail length was determined. Growth hormone activity was also assayed by observation of the thickness of the epiphyseal cartilage plate of tibias from hypophysectomized rats (*8*). Groups of 6 to 8 female rats were used for each dose. The rats were hypophysectomized at 26 days of age. After a 12-day postoperative interval, the rats were injected intraperitoneally daily for 4 days and killed 24 hours after the last injection. The tibias were split in the sagittal plane and stained with 2 percent silver nitrate. The thickness of the epiphyseal cartilage plate was estimated by measuring its width under the dissecting microscope with a micrometer eyepiece. In both growth hormone assays, data from animals found to have pituitary remnants by dissection of the sella turcica were excluded.

Serums of normal or hypophysectomized rats were assayed for somatomedin activity (*9*). Six pairs of porcine cartilage disks from a 3-month-old pig were used for each sample of serum assayed. Average disk weight was 0.71 mg ± 0.01 S.E.M. Cartilage disks were incubated with $^{35}SO_4$ in tris–amino acid buffer, *p*H 7.4, at a final serum concentration of 40 percent. Each sample consisted of serum pooled from three animals. After incubation and washing, each pair of disks was dissolved in 0.2 ml of 5*M* KOH, and the ^{35}S was counted, with 10 ml of a mixture of toluene and methanol (1 : 1, by volume) containing 0.5 percent PPO. The statistical significance of differences was evaluated by calculation of *P* values from the paired *t*-test, with 0 week for untreated animals and 5 weeks for treated animals as controls. The differences shown in Fig. 1 between normal and hypophysectomized rats, both untreated and treated with 6 μg of thyroxine per day, and the differences between untreated hypophysectomized rats and those that were treated with homocysteic acid at 10 mg/kg per day and thyroxine for 1 to 4 weeks were all significant ($P < .005$).

Homocysteic acid in doses of 2.5 to 80 mg/kg per day promoted tail growth of thyroxine-treated hypophysectomized rats as effectively as an optimum dose (*7*) of purified growth hormone (Table 1). The differences in tail growth between controls and animals treated with homocysteic acid were statistically significant. No attempt was made to determine the minimum effective dose of homocysteic acid in the tail assay. Neither homocystine, homolanthionine sulfoxide, nor homolanthionine sulfone promoted tail growth.

Homocysteic acid increased the thickness of the epiphyseal cartilage plate of tibias from hypophysectomized rats. The dose response to homocysteic acid over the range of 4 to 4000 μg (total dose) was linear, when plotted semilogarithmically (Fig. 2). The dose response to purified porcine growth hormone was also linear over the range of 80 to 600 μg, similar to the results of Geschwind and Li (*8*). The differences in epiphyseal width between controls and animals treated with homocysteic acid were statistically significant (Table 2).

Serums from hypophysectomized rats

Table 2. Growth hormone activity of homocysteic acid (tibia assay). Hypophysectomized rats were injected for 4 days with homocysteic acid in normal saline or growth hormone in sodium citrate buffer, *p*H 8.9. Data for epiphyseal cartilage width are given as means ± S.E.M. The *P* values are calculated for differences from control group by the paired *t*-test. Completeness of hypophysectomy was verified by dissection of the sella turcica.

Dose (μg/4 days)	Animals (No.)	Cartilage width (μm)	*P*
	Growth hormone		
None	6	113.8 ± 5.8	
80	7	122.7 ± 7.6	>.2
160	7	161.0 ± 4.8	<.001
300	7	177.0 ± 6.7	<.001
600	6	191.3 ± 9.9	<.001
	Homocysteic acid		
None	7	137.0 ± 7.6	
4	6	158.3 ± 8.0	<.1
40	6	165.8 ± 8.9	<.05
400	5	172.2 ± 4.2	<.005
4000	7	179.3 ± 6.3	<.005

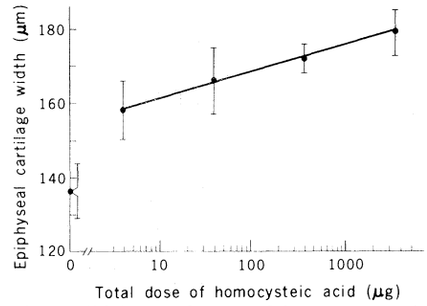

Fig. 2. Tibial assay for growth hormone activity. Groups of six to eight hypophysectomized rats were treated for 4 days with 1 to 1000 μg of homocysteic acid per day. After the tibias were split sagittally and stained, the epiphyseal cartilage width was measured. Results are expressed as average width in micrometers ± S.E.M.

treated daily with homocysteic acid at 10 mg/kg and 6 μg of L-thyroxine for periods of 1 to 4 weeks showed increased somatomedin activity ($P < .005$) compared to serums from hypophysectomized rats treated only with thyroxine (Fig. 1). Somatomedin activity of serums from animals treated with homocysteic acid for 1 to 2 weeks was not significantly different from that in serums from normal rats ($P > .2$). However, somatomedin activity during the third and fourth weeks was slightly lower than that in serums from normal rats ($P < .05$). When homocysteic acid was added directly to the somatomedin assay, either with or without normal serum, there was no effect on somatomedin activity over a wide concentration range (0.01 to 100 mg/liter).

We believe that homocysteic acid is the first example of a low-molecular-weight compound with physiological effects similar to those of pituitary growth hormone. Two separate assays, involving observation of tail growth and increased thickness of the tibial epiphysis, show that the growth promoting activity of homocysteic acid on the cartilage of hypophysectomized rats is similar to the effect of pituitary growth hormone. Our results also show that administration of homocysteic acid increases the serum somatomedin activity of hypophysectomized rats. Since homocysteic acid has no direct effect on the somatomedin assay either with or without normal serum, the stimulatory effect of homocysteic acid on tail growth and epiphyseal cartilage of hypophysectomized rats probably involves increased synthesis or release of endogenous somatomedin. These observations support the hypothesis that somatomedin is required to mediate the action of pituitary growth hormone on cartilage by increasing the sulfation of cartilage matrix (*11*).

The sulfonic acid group of homocysteic acid appears to be necessary for physiological activity since homocystine, homolanthionine sulfoxide, and homolanthionine sulfone have no growth hormone activity when tested by the tail growth assay. Previous work has shown that homocysteic acid is a precursor of phosphoadenosine phosphosulfate, the coenzyme necessary for sulfate ester synthesis (*6*).

The growth promoting effect of homocysteic acid supports the validity of previous suggestions that homocystine derivatives initiate arteriosclerosis in individuals with homocystinuria (*3*) and in animals given homocystine thiolactone (*4*, *5*). The finding also explains why individuals with homocystinuria have accelerated skeletal growth (*2*), since homocysteic acid is a known metabolic product of homocysteine (*6*) and is present in the urines of patients with homocystinuria (*12*). The increased sulfate binding and the abnormalities of cellular growth observed in cell cultures from individuals with homocystinuria (*13*) may possibly be related to the cellular effects resulting from the metabolism of bound forms of homocysteic acid.

The findings of our study are of importance because they establish a relation between an area of sulfur amino acid metabolism and the physiological action of growth hormone.

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Peripheral Blood Elements Found in an Egyptian Mummy: A Three-Dimensional View

Abstract. *Intact peripheral blood elements were found within an intracranial mass, possibly either an antemortem subdural hematoma or a postmortem blood clot, removed from a 2200-year-old Egyptian female mummy. Surface topographies of neutrophils and lymphocytes were similar. Some erythrocytes partially retained their biconcave disk shape; others were spherical. Individual platelets exhibited pseudopodia.*

Tissues of mummies have been studied by light microscopy to determine whether they contained intact elements of peripheral blood (*1*). Preserved erythrocytes and probable autolyzed leukocytes were observed in a thoracic vein of a 2000-year-old mummy (*2*). To date, none of the other peripheral blood elements, such as intact leukocytes or platelets, have been found in these mummified tissues.

An autopsy was performed on a 2200-year-old Egyptian female mummy, Pum III (*3*), and a brown mass (22 cm in diameter and 1 cm thick) that adhered to the occipital region of the skull was removed for subsequent study. Presumed to be brain tissue, the mass was placed in 10 percent formalin. After 4 days, it was dehydrated and embedded in paraffin, and sections 6 μm thick were cut from several portions of the mass. No recognizable brain structures were found by our light microscope examination of the sections after they were stained with hematoxylin and eosin; however, we did observe a group of preserved peripheral blood elements. Each of the leukocytes present was identified as either a granulocyte or a mononuclear leukocyte according to its nuclear and cytoplasmic characteristics.

Two tissue sections containing the focalized group of peripheral blood elements were prepared for further study in the scanning electron microscope. The cover slip was removed after the microscope slide had been immersed in xylene for 24 hours. The adherent section was exposed to a change of xylene to remove any remaining traces of mounting medium. Tissue sections were then coated by the sputtering technique with a thin layer of gold, and coated samples were viewed

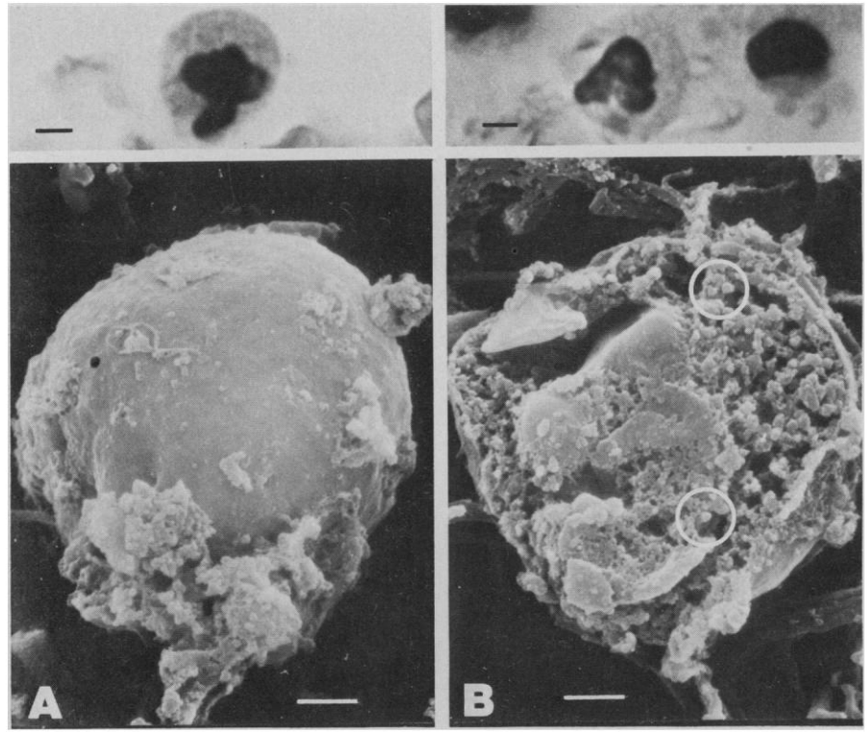

Fig. 1 (A) Scanning electron micrograph (scale equals 1 μm) of the exterior surface of the same intact neutrophil depicted at the top as it appeared by light microscopy (scale, 2 μm). (B) Interior of the neutrophil (scale, 1 μm) depicted at the top (scale, 2 μm). Note the presence of small, round structures (circles) compatible in morphology and size with specific granules of the neutrophil.