(*15*); (iii) inhibition of cyclic nucleotide phosphodiesterases by metabolites (*16*); and (iv) conversion of adenosine-related metabolites to *S*-adenosyl homocysteine derivatives with consequent alteration of phosphodiesterase or adenylate cyclase (*17*). Alternative (i) is particularly attractive since it explains the basis for successful isolation of adenylate cyclase–deficient variants of Y1 adrenal (*12*) and GH pituitary cells (*8*) as 8-bromo cyclic AMP–resistant.

We cannot entirely exclude the possibility that AMP and cyclic AMP derivatives may act by a common pathway unrelated to cylcic AMP action. Variants that are 8-bromo cyclic AMP–resistant and protein kinase– and adenylate cyclase–deficient might have a more primary lesion in a cyclic AMP–independent pathway [such as methylation; see (*17*)]. However, our results do indicate that cyclic AMP derivatives inhibit growth by a novel pathway that does not require an intact 3′,5′ phosphodiester linkage.

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Somatomedin B: Mitogenic Activity Derived from Contaminant Epidermal Growth Factor

Abstract. *The mitogenic effect of somatomedin B on human cultured glial cells was neutralized by the addition of antibodies to mouse epidermal growth factor. Somatomedin B contained epidermal growth factor–like activity, competing for binding to the epidermal growth factor receptor. It is concluded that contaminating epidermal growth factor may explain the entire mitogenic activity of somatomedin B.*

It was previously demonstrated in this laboratory that a polypeptide fraction derived from a somatomedin-enriched extract of Cohn fraction IV from human plasma had mitogenic effects on human quiescent glial cells in vitro (*1*). Charge and size fractionation indicated that the glial-cell stimulating activity was distinct from the activity promoting incorporation of $^{35}SO_4$ into cartilage chondroitin sulfate, present in the same preparation. These two activities were denoted somatomedin B (SM-B) and A (SM-A), respectively (*2*). No apparent relation seems to exist between SM-A and B (*3*) and, in fact, it has not been unequivocally demonstrated that SM-B conforms to the definition of a true somatomedin (*4*). SM-B has been purified to apparent homogeneity and its amino acid sequence has been determined (*5*). Highly purified preparations of SM-B show remarkably low specific mitogenic activity; in cultures of serum-deprived glial cells about 10 μg of SM-B per milliliter are required to produce an increase in DNA synthesis equivalent to that induced by 1 percent human serum (*6*). Authentic growth factors such as epidermal growth factor (EGF) or platelet-derived growth factor (PDGF) are regularly three to four orders of magnitude more potent in the same system (*7, 8*). Trace contaminants (< 0.1 percent) of such factors may therefore have contributed to the observed biological effects, as suggested previously (*9*). Since EGF has physicochemical properties similar to those of SM-B it might appear as a contaminant in preparations of the latter. Experiments were therefore undertaken to investigate the involvement of EGF in the mitogenic activity of SM-B. It is demonstrated that most or all of the stimulatory effect of SM-B on glial cells may be ascribed to contaminant EGF.

Rabbit antibodies to pure mouse EGF (mEGF) were prepared as described (*10*). Exposure of SM-B to these antibodies, followed by absorption of the antigen-antibody complexes to Protein A Sepharose (*11*), markedly reduced the mitogenic activity of the resulting solu-

Table 1. Mitogenic activity of SM-B, hEGF, and PDGF after exposure to rabbit antibodies to mEGF. Fifty microliters of SM-B (*6*) (2 mg/ml), partially purified hEGF (*12*) (1 μg/ml), or partially purified PDGF (*8*) (1 μg/ml) in buffer containing 0.15*M* NaCl and 0.01*M* phosphate, *p*H 7.0, was mixed either with antibodies to mEGF (250 μg in 50 μl of the same buffer) prepared by passage of the rabbit immune serum through a column of Protein A Sepharose (*11*) or with plain buffer. After incubation at 22°C for 1 hour and at 4°C overnight, 100 μl of Protein A Sepharose was added. Tubes were kept for 1 hour at 22°C with occasional gentle mixing and then centrifuged. Portions of supernatants were assayed for multiplication stimulating activity, determined by the increase in [^{3}H]thymidine incorporation into DNA of serum-deprived glial cells as described (*8*), at a final concentration of 2 percent (by volume). The biological activity is recorded as the percentage of that given by 1 percent serum; in the presence of 1 percent human serum 60 to 70 percent of the cells were stimulated, as measured by autoradiography.

Growth factor preparation	Addition of antibodies to mEGF	Stimulatory activity (%)
SM-B	–	95
SM-B	+	29
hEGF	–	98
hEGF	+	40
PDGF	–	118
PDGF	+	119
None	–	0
None	+	34
Human serum (1 percent)	–	100

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tion (Table 1). At comparable doses, the reduction was approximately equal to that obtained with a preparation of human EGF (hEGF) (*12*) isolated from human urine. In contrast, the biological activity of PDGF (*8*) remained constant under the same experimental conditions (Table 1). The finding that antibodies to EGF specifically removed the mitogenic activity from SM-B preparations is compatible with the view that the biological activity resided in EGF or material immunologically cross-reacting with EGF. Considering the high molar ratio of SM-B to EGF antibody (12:1) used in this experiment, it is unlikely that the decrease in mitogenic activity was due to precipitation of the SM-B molecule itself. The presence of EGF-like material in the preparation of SM-B was further indicated by its reactivity with the EGF receptor. The preparation of SM-B competed with ^{125}I-labeled EGF for binding to cultured glial cells, 10 μg of SM-B containing the equivalent of about 2 ng of EGF (not shown). If one assumes that this material has the same mitogenic properties as authentic EGF its presence is thus sufficient to explain the entire stimulatory activity of SM-B. The degree of contamination (about 0.02 percent EGF in the SM-B preparation) is, however, so small that it would escape detection by conventional chemical methods.

An enhancing effect of SM-B on protein synthesis (incorporation of leucine) in vivo has been reported (*13*). In view of the present findings it is possible that this activity should not be ascribed to SM-B itself; the effects of EGF in vivo are well documented in other systems (*14*).

In conclusion, the biological activity by which SM-B was originally defined, that is, stimulation of DNA synthesis in human cultured glial cells (*2*), seems to reside in EGF, a minor contaminant of the preparation; the major component of SM-B is not a growth factor in this system. Therefore, although circulating levels of SM-B, as determined by radioimmunoassay, may vary with the growth hormone status of the individual (*15*) there is not sufficient evidence to justify the term somatomedin for this substance.

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Ethanol Reveals Novel Mercury Detoxification Step in Tissues

Abstract. *Volatile mercury was produced de novo by mouse tissue homogenates that contained mercuric ions. Ethanol stimulated the release of tissue mercury into the vapor phase, and the mechanism appears to be an inhibition of reoxidation of volatile mercury. Components responsible for mercury volatilization are heat-labile. The highest volatilizing activity in the liver is associated with the soluble fraction obtained after centrifugation at 105,000g.*

Biotransformation of mercurials, leading to volatilization of the elemental vapor (Hg^0), occurs in microorganisms. In mercury fungicide- or drug-resistant prokaryotes, volatilization of this metal is a detoxifying mechanism (*1*). In contrast to microbial systems, mammalian mechanisms for transforming mercury into volatile products have been largely unexplored. This pathway in higher animals was first proposed by Clarkson and Rothstein (*2*) upon detection of volatilized mercury from rats injected with $HgCl_2$; additional evidence came from a study showing mercury exhalation in human volunteers who had been exposed to mercury vapor (*3*). Studies of laboratory animals have demonstrated the existence of a metabolic route for de novo Hg^0 generation and the ability of ethanol to enhance exhalation of the metal (*4, 5*). None of these studies have characterized the tissue site or sites or the biochemical nature of the interaction between ethanol and the Hg^0-generating pathway. We describe the ethanol-stimulated conversion of Hg^{2+} to volatile mercury by mouse tissues in vitro, the subcellular fractions involved, and a plausible mechanism for the ethanol effect.

Mercury volatilizing activity (MVA) in CBA/J mouse liver and kidney homogenates or fractions was estimated by the release of radioactive mercury from the tissue suspensions into the vapor phase during 30-minute incubations (*6*). The ^{203}Hg-labeled (New England Nuclear) $HgCl_2$ had been equilibrated with excess L-cysteine (Sigma) to ensure that all mercury added to tissues was in the Hg^{2+} form and attached to thiol groups, namely, mercury-cysteine complex. The final mercury concentration before MVA assays was 0.16 μ*M* in all tissue preparations.

Liver and kidney homogenates incubated in the presence of the mercury-cysteine complex and alcohol showed ethanol-dependent increases in the amount of radioactivity released into the vapor phase (Fig. 1). Increasing initial ethanol concentrations above 100 mg/dl elicited no further enhancement of MVA in kidney homogenates. With liver samples, incremental increases in MVA were obtained with ethanol concentrations of up to about 400 mg/dl. Maximal volatilizations in 30 minutes averaged 0.12 and 0.19 percent of the added mercury for kidney and liver homogenates, respectively.

We determined that treating liver homogenates with heat (74°C water bath for 10 to 15 minutes) eliminated MVA regardless of the presence of ethanol. Residual mercury volatilizations in these samples amounted to about 8 pg of Hg in 30 minutes or 0.008 percent of the mercury added to the suspensions. Heat inactivation is usually evidence of heat lability of the component or components necessary for the process. With mercury, however, heat denaturation may create additional binding sites (that is, by

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