Inhibition of growth* of cultured mammalian cells by liver extracts

In spite of the genotypic capabilities of at least some of their cells, in adult animals, cell multiplication is largely suppressed and occurs only at a rate sufficient to replace dead ones. Two obvious mechanisms may be invoked to explain this steady state: the existence of one or more growth-limiting factors for each cell type (a series of chemostat-like controls) and the synthesis of growth inhibitors to restrain themselves or other cells.

Because of the advantages they provide over the whole animal, evidence for the existence of growth inhibitors was sought with cultured cells. To this end, the effects were studied of various mature rabbit organ extracts and of serum upon the multiplication of liver and kidney cells freshly cultured from 4-week old rabbits.

Serum (up to 50 %) and extracts of kidney, spleen, and lung (about 0.5 mg protein/ml) were not inhibitory. Liver extracts, on the other hand, not only depressed or completely prevented the growth of the liver and kidney cells but of an established line of fibroblast-like mammalian cells (AMK 2-2) as well. Measured after 4 or 5 days of incubation, cell yields with each of the cultures were depressed about 50 % with 0.02 mg liver protein/ml and growth was completely prevented when 0.09 mg protein was added/ml of growth medium.

The inhibitory effect of liver preparations appears to result from their content of arginase which depletes the medium of an amino acid essential for growth. The evidence in support of this contention is as follows: (a) the inhibitor is destroyed by heat (100°, 2 min); (b) it appears to be present only in liver; and (c) of the compounds tested, including all the amino acids (0.003 *M* except for tryptophan and methionine (each 0.001 *M*)) and vitamins of the basal growth medium[2], only arginine, citrulline, and ornithine prevented the inhibitory action of liver extracts. Thus, with the AMK cells, in the presence of 0.09 mg liver protein/ml, no inhibition of growth occurred upon the addition of $5 \cdot 10^{-3}$ *M* L-arginine, $2 \cdot 10^{-4}$ *M* L-citrulline, or $7 \cdot 10^{-3}$ *M* L-ornithine. Similar results were obtained with liver cells** except that a higher concentration of citrulline ($3 \cdot 10^{-3}$ *M*) was required for a maximal effect. Whether ornithine serves as a precursor of arginine or merely inhibits arginase is not known. No evidence could be found for the presence of additional growth inhibitors in liver extracts.

While they shed no light on growth controls *in vivo*, these results indicate one mechanism of toxicity of crude biologic preparations for cultured animal cells. In addition, they suggest that caution should be used in interpreting the results of growth-inhibitor experiments involving the injection of organ extracts into animals.

This investigation was supported by a research grant from the National Institutes of Health, U.S. Public Health Service.

Department of Microbiology, School of Medicine,
University of Pittsburgh, Pittsburgh, Pa. (U.S.A.)

IRVING LIEBERMAN
PETER OVE

[1] R. B. L. GWATKIN, J. E. TILL, G. F. WHITMORE, L. SIMINOVITCH AND A. F. GRAHAM, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 451.
[2] I. LIEBERMAN AND P. OVE, *J. Exptl. Med.*, 108 (1958) 631.

* Growth is used to describe an increase in cell numbers.
** With kidney cells, the three amino acids were tested at only one level.

Received July 11th, 1959