steroids, endogenous or exogenous. The most reasonable interpretation of these findings is that SC-5233 and, presumably, SC-8109 act as antagonists to aldosterone and other sodium-retaining steroids. It is suggested that the mechanism through which these sodium-losing steroids act is that of competition with aldosteronelike steroids for a crucial locus of action within the renal tubular cells. A similar mechanism has been postulated previously to explain the sodium loss which has occasionally been seen during treatment of patients with supraphysiologic amounts of cortisone (*6*) and progesterone (*7*).

GRANT W. LIDDLE
Department of Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee

References and Notes

1. J. A. Luetscher, Jr., *Recent Progr. in Hormone Research* 12, 175 (1956); F. C. Bartter, *Metabolism* 5, 369 (1956).
2. L. Wilkins and R. A. Lewis, Transactions of 17th Meeting of Conference on Metabolic Aspects of Convalescence, New York (Josiah Macy, Jr., Foundation, New York, 1948), p. 168; J. W. Jailer, *J. Clin. Endocrinol.* 11, 798 (1951).
3. This study was supported in part by grants-in-aid from the National Cancer Institute (CY-3107) and from the John A. Hartford Foundation. SC-5233 and SC-8109 were generously provided by G. D. Searle and Company. Donald Island rendered invaluable technical assistance.
4. G. W. Liddle *et al.*, *J. Clin. Invest.* 34, 1410 (1955).
5. C. M. Kagawa *et al.*, *Science*, this issue.
6. G. W. Thorn *et al.*, *Trans. Assoc. Am. Physicians* 62, 233 (1949).
7. R. L. Landau *et al.*, *J. Clin. Endocrinol. and Metabolism* 15, 1194 (1955).

19 August 1957

Effect of Citrovorum Factor and Peptones on Mouse Leukemia Cells L-5178 in Tissue Culture

A medium has been devised (*1*) which permits the continuous reproduction of mouse leukemia cells (L-5178, a lymphocytic neoplasm of DBA/2 mice, *2*) in culture in the complete absence of nonleukemic cells (*3*). After at least 150 successive generations *in vitro*, the cells have continued to grow in suspension (rather than on the glass surface), and have retained their round-cell character, as well as their capacity to induce fatal leukemia in DBA/2 mice. In addition to serum and other ingredients usually encountered in culture media, certain peptones are required for the continued multiplication of these cells. Data presented in 1928 by Baker and Carrel and extended by Willmer and Kendal (*4*) indicated that various peptones can stimulate the multiplication of mammalian and avian cells in tissue culture. More recently, Waymouth has reported that peptone, in the presence of albumin, replaces serum for the multiplication of strain L mouse cells (*5*). However, the factors in peptone which support cell multiplication have not been identified. Previous studies of the growth responses of *Lactobacillus leichmannii* (ATCC 7830) and *Pedicoccus cerevisiae* [*Leuconostoc citrovorum* (ATCC 8081)] to peptonelike materials and the relationship of the responses to vitamin B_{12}, citrovorum factor, and thymidine have been described (*6, 7*).

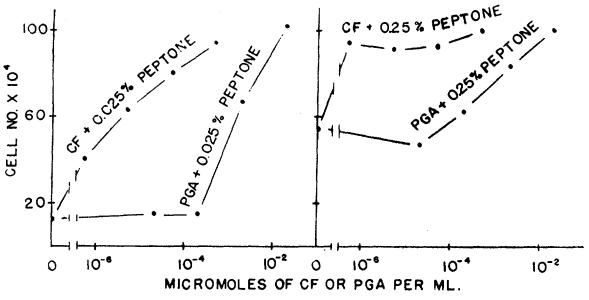

Fig. 1. Reduction of the effect of peptone by citrovorum factor (CF) and the relative activity of pteroylglutamic acid (PGA) and CF in support of cell multiplication. The cells (2.0×10^5) were incubated in 1 ml of medium (*1*) for 48 hours; the extent of cell reproduction was determined by hemocytometer counts.

Experiments (*8*) directed toward the isolation of the active factors in the peptone used (*9*) have demonstrated that synthetic 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid (citrovorum factor; CF; folinic acid; leucovorin) partially replaces peptone in the nutrition of these leukemic cells and that the capacity of the cells to obtain functional derivatives from folic acid (pteroylglutamic acid; PGA) is very much less than their capacity to obtain them from citrovorum factor.

The partial replacement of peptone by leucovorin in the nutrition of the neoplastic cells is shown in Fig. 1. The data indicate that, in the presence of 0.025 percent peptone, a stimulatory effect of leucovorin was clearly demonstrable, while at a ten-fold higher level of peptone, very much less leucovorin was required to attain maximal growth. In this 48-hour experiment, at the lower level of peptone, 3.7×10^{-6} μmole of the active form of leucovorin per milliliter (*10*) permitted half-maximal growth, while a 400-fold higher concentration of pteroylglutamic acid (1.5×10^{-3} μmole/ml) was required to obtain a similar effect. When cultures were carried for a minimum of ten progressive generations, in triplicate, on limiting levels of the two coenzyme-precursors, the doubling-time was less than maximal and remained constant, and, in addition, equivalent rates of multiplication were obtained with active leucovorin in a concentration 1/5000 that of pteroylglutamic acid. The fact that the doubling-time remained constant indicates that, in the presence of either pteridine derivative, certain components of the peptone are no longer required for the nutrition of the cells. During continuous culture in media containing a much higher level of peptone, 0.5 percent, but only 2.2×10^{-5} μmole of pteroylglutamic acid per milliliter, a generation time of less than 22 hours (average, 24 hours) was rarely obtained. In contrast, generation times of 14 to 22 hours (average, 18 hours) were obtained with optimal amounts of active leucovorin (2×10^{-5} μmole/ml) or with very high levels of pteroylglutamic acid (2×10^{-2} μmole/ml) in the presence of low levels of peptone (0.06 percent).

The high levels of folic acid, as compared with the levels of its tetrahydro derivative, which are required to support cell multiplication indicate a very limited capacity of the leukemic cells under these conditions to synthesize coenzymes from pteroylglutamic acid (*11*). The absolute requirement of certain other cell lines for this vitamin is very much less than it is for L-5178, for example, HeLa and L-strain (*12*), a finding which suggests that these cells efficiently convert folic acid to coenzyme forms. Such an interpretation is supported by the fact that citrovorum factor is only 20 to 30 times as effective as pteroylglutamic acid for L-strain cells (*13*). Although the requirement for folic acid of sarcoma 180 cells in tissue culture resembles that of both L-strain and HeLa cells, our colleague, Richard Schindler, has found that this requirement is met (in Eagle's medium, *12*) by active leucovorin in an amount approximately 1/200 that of pteroylglutamic acid.

It is well known that various neoplastic cell lines, *in vivo*, exhibit widely variable sensitivity to the chemotherapeutic action of A-methopterin, an agent which inhibits the enzymic conversion of folic acid to tetrahydro derivatives. It is suggested that the effectiveness of A-methopterin as an inhibitor of the reproduction of various types of cells is markedly influenced not only by the extracellular supply of tetrahydro derivatives of folic acid, but also by the enzymic capacity of such cells to convert folic acid-like compounds to coenzymically active, tetrahydro forms (*14*).

GLENN A. FISCHER
ARNOLD D. WELCH
Department of Pharmacology, School of Medicine, Yale University, New Haven, Connecticut

References and Notes

1. Each 100 ml of medium contained vitamins, glucose, cystine, histidine, and glycine at the levels employed by W. Scherrer [*Am. J. Pathol.* 29, 113 (1953)] and, in addition, acid-hydrolyzed casein (N. B. Co.) (25 mg), undialyzed horse serum (10 ml), peptone (60 mg), glutamine (15 mg), hypoxanthine (2.5 mg), tryptophan (0.25 mg), glutathione (0.15 mg), sodium ascorbate (0.175 mg), and, unless otherwise stated, pteroylglutamic acid (1 mg). Stock cultures were carried by daily transplantation of about 4×10^6 cells in 10 ml of the freshly prepared medium. Serum was dialyzed with stirring for 60 hours against nine changes, each of 10 volumes of distilled water.
2. Kindly supplied by Lloyd W. Law of the National Cancer Institute, Bethesda, Md.
3. These results were described briefly at the meetings of the American Association for Cancer Research, Chicago, Ill., April, 1957 [*Proc. Am. Assoc. Cancer Research* 2, 201 (1957)].
4. L. E. Baker and A. Carrel, *J. Exptl. Med.* 48, 533 (1928); E. N. Willmer and L. P. Kendal, *J. Exptl. Biol.* 9, 149 (1932).
5. C. Waymouth, *J. Natl. Cancer Inst.* 17, 315 (1956).
6. A. D. Welch and M. F. Wilson, *Arch. Biochem.* 22, 486 (1949).
7. C. A. Nichol and A. D. Welch, *Federation Proc.* 9, 367 (1950).
8. In collaboration with R. E. Handschumacher.
9. In most of these experiments, the peptone used was that supplied by the Walker Laboratories, to whom our thanks are due.
10. This calculation recognizes that only one enantiomorph of the racemic, synthetic citrovorum factor, that is, leucovorin (for the supply of which we are indebted to the Lederle Laboratories Division of the American Cyanamid Company), is available for biological utilization.
11. These results were not materially affected by the use of dialyzed horse serum at the 6-percent level in place of the 10-percent undialyzed horse serum of the basal medium. However, increasing the level of dialyzed or undialyzed serum promoted growth when either PGA or CF was limiting.
12. H. Eagle, *J. Exptl. Med.* 102, 595 (1955).
13. H. Eagle, *Proc. Soc. Exptl. Biol. Med.* 91, 358 (1956).

14. A generous grant from the Jane Coffin Childs Memorial Fund for Cancer Research permitted the equipment of a laboratory for tissue culture studies and contributed to the costs of the research. This program was also contributed to by grants from the American Cancer Society and the U.S. Public Health Service. One of us (G.A.F.) is grateful for a special fellowship from a fund generously provided by the Squibb Institute for Medical Research.

25 July 1957

Self-Regulation of Protein Synthesis in Acetabularia

The regulation of normal and abnormal growth has recently attracted extensive studies in numerous areas. The interaction of intracellular growth-promoting substances and extracellular growth-inhibiting substances has been postulated (*1*). In the present study, we have concentrated on the intracellular regulation and limitation of protein synthesis.

We utilized a large unicellular alga, *Acetabularia crenulata* (*2*), cultured in sea water according to the method of Haemmerling (*3*). The unique size of the cell (3 to 4 cm) and nucleus (200 μ) allows an easy preparation of anuclear fragments of sizes varying from 0.5 to 40 mm. Because of the cylindrical shape of the stalks, these algae offer an excellent material for the investigation of the relationship between the rate of growth, as expressed by the rate of protein synthesis, and the relative surface (area/volume) of the cell.

Individual cells, measuring 21 to 23 mm in length, were enucleated by removal of rhizoids. Some of the resulting stalks, 19.3 ± 0.2 mm in length and 0.5 mm in diameter, were then analyzed for nitrogen by the method of Johnson (*4*) after the nonprotein nitrogen had been removed with 10-percent trichloroacetic acid. The average value of protein nitrogen was 4.8 μg per stalk with a variation of 0.2 μg as estimated from three samples, each of which consisted of 12 stalks. Other stalks were cut transversely, some in halves, others into quarters, and still others into eighths. Approximately 12 percent of the stalks partially lost their cytoplasm during cutting and were discarded. After 15 days, all fragments that had come from a single stalk were analyzed together for protein nitrogen. The amount of protein synthesized by each fragmented stalk was calculated from the difference between the protein nitrogen content of the fragmented stalks at the end of the 15-day period and that of the unsegmented stalks analyzed at the begining of the experiment. Fourteen samples were used for each value represented in Fig. 1. Variations among the samples were within 10 percent.

The surface area and volume of each stalk were calculated on the basis of the assumption that the stalk was cylindrical in shape. Since the stalk was cut transversely, the total surface area was increased only at the cut ends. The relative increase in surface area due to cutting was expressed as a percentage of the total surface area of the uncut stalk.

Figure 1 shows that the amount of protein synthesized during the 15-day period per stalk (total synthesis), as well as the amount of protein formed expressed as a percentage of the original protein content (relative synthesis), increases with the number of fragments into which the stalk has been cut. On the other hand, the relative increase in surface area due to cutting of stalks shows only a small rise as the number of fragments increases. These findings indicate that the increase in protein synthesis cannot be satisfactorily explained on the basis of a higher absorption rate of nutrients resulting from an increase in surface area after cutting.

To ascertain the influence of initial length and protein content of stalks on the rate of protein synthesis, 130 cells were cut at various distances from the growing tips of the stalks to provide anuclear fragments of various lengths, each of which contained one intact end and one cut end. Twenty of them were analyzed immediately for protein nitrogen, and the others were grown in the standard medium for 15 days. In this experiment the differences in surface area of stalks of different lengths are due to the size of the lateral walls. Thus, all stalks were subjected to a similar injury at the cut ends. The volume of each fragment varies directly as its length under these conditions. The difference in relative surface area is expressed as the excess of the area/volume ratio of any stalk above that of the longest (36 mm).

As can be seen in Fig. 2, the total protein synthesis per stalk, as well as the

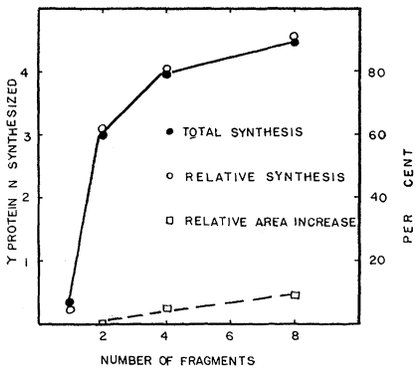

Fig. 1. Effect on protein synthesis of cutting anuclear stalks into a number of fragments. The ordinate to the left represents micrograms of protein nitrogen synthesized per stalk. The term *total synthesis* refers to the absolute value of protein N synthesized, while *relative synthesis* expresses the same value as a percentage of the original protein N content of the stalk. *Relative area increase* represents the increase of surface area due to cutting, expressed as a percentage of an unsegmented stalk.

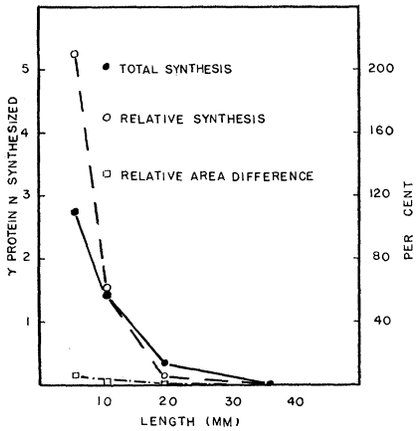

Fig. 2. Relationship between the length of anuclear stalks and the rate of protein synthesis. The ordinates are the same as those in Fig. 1. The term *relative area difference* refers to the excess of area/volume ratio of any stalk over that of the longest stalk (36 cm), expressed as a percentage of the latter.