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

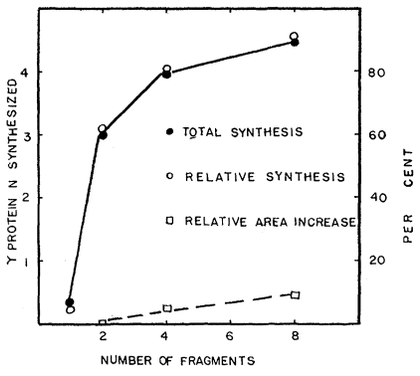

Fig. 1. Effect on protein synthesis of cutting anuclear stalks into a number of fragments. The ordinate to the left represents micrograms of protein nitrogen synthesized per stalk. The term *total synthesis* refers to the absolute value of protein N synthesized, while *relative synthesis* expresses the 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.

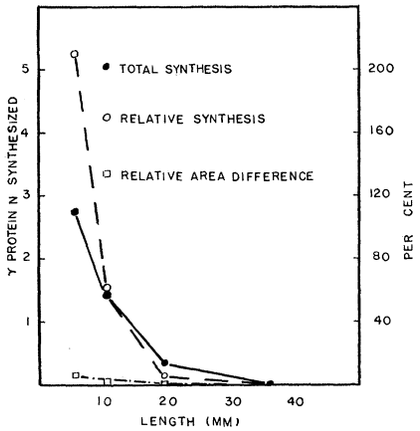

Fig. 2. Relationship between the length of anuclear stalks and the rate of protein synthesis. The ordinates are the same as those in Fig. 1. The term *relative area difference* refers to the excess of area/volume ratio of any stalk over that of the longest stalk (36 cm), expressed as a percentage of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

relative protein synthesis, varies inversely as the length of the stalks at the beginning of the experiment. The difference in relative area, on the other hand, does not show any significant variation among stalks that differ greatly in length. This offers even more convincing evidence that an increase in surface area does not play a significant role in the higher rate of protein synthesis in the shorter stalks. Moreover, since all stalks have been subjected to a similar amount of injury, the operative procedure can be eliminated as a factor in the phenomenon.

These experiments are considered to reveal additional evidence of the independence of the cytoplasmic protein synthesis from the presence of a nucleus as described by Brachet and co-workers (*5*) and Stich and Plaut (*6*). The cytoplasm and not the nucleus must be regarded as playing an essential role in determining the amount of synthesized proteins. The simplest interpretation of our results would be made by assuming an intracellular inhibitory effect which increases with cell growth and which is reversible if the cytoplasm is divided into smaller units. The higher activity of smaller cytoplasmic fragments may explain the surprising results obtained by Brachet (*5*) and Beth (*7*) that cytoplasmic fragments synthesize proteins and differentiate at a faster rate if the nucleus containing rhizoid is removed.

HANS F. STICH*
AMARA KITIYAKARA
Department of Pathology, Medical School, University of Wisconsin, Madison

References and Notes

1. P. Weiss, *Biological Specificity and Growth* (Princeton Univ. Press, Princeton, N.J., 1954); S. M. Rose, *Am. Naturalist* 86, 337 (1952); L. Barth, *Analysis of Development* (Saunders, Philadelphia, Pa., 1955), p. 664.
2. We are greatly indebted to J. Haemmerling and J. Brachet for supplying us with *Acetabularia*.
3. J. Haemmerling, *Arch. Protistenk.* 97, 7 (1944).
4. M. Johnson, *J. Biol. Chem.* 137, 575 (1941).
5. J. Brachet, H. Chantrenne, F. Vanderhaeghe, *Biochim. et Biophys. Acta* 18, 544 (1955); J. Brachet and H. Chantrenne, *Cold Spring Harbor Symposia Quant. Biol.* 11, 329 (1956).
6. H. Stich and W. Plaut, *J. Biophys. Biochem. Cytol.*, in press.
7. K. Beth, *Z. Naturforsch.* 8b, 771 (1953).

* Present address: Saskatchewan Research Unit, National Cancer Institute of Canada, University of Saskatchewan, Saskatoon.

9 September 1957

Production of Tolerance to Psychosis-Producing Doses of Lysergic Acid Diethylamide

It has been shown that 2 mg of crude beef brain extract per milliliter blocks the usual effect of 2 μg of lysergic acid diethylamide (LSD-25) per milliliter in the outside liquid on the Siamese fighting fish (*1*). This report (*2*) describes a study of a blocking effect that is probably produced by another mechanism: the development of tolerance to LSD-25 in man (*3*) by the prior administration for a period of days of a compound similar to LSD-25, 1-methyl lysergic acid diethylamide (MLD-41) (*4*).

Table 1. Comparison of production of tolerance to lysergic acid diethylamide (LSD-25) by 1-methyl lysergic acid diethylamide (MLD-41) and 2-bromo lysergic acid diethylamide (BOL-148).

Date of experiment	Total preparatory dose of MLD-41 (μg)	Pretreatment period	Total preparatory dose of BOL-148 (μg)	LSD-25 (μg)	Responses (No.)
29 March 1957	0		0	50	35
12 April 1957	1100	7 to 12 April	0	80	0
10 May 1957	700	4 to 10 May	0	100	7
7 June 1957	0	1 to 7 June	1450	50	14
21 June 1957	0	16 to 21 June	1000	50	21

1-Methyl lysergic acid diethylamide produces in man and the Siamese fighting fish reactions that are essentially indistinguishable from those produced by LSD-25, but there are higher reaction thresholds. In the fish, MLD-41 is about one-tenth as effective as LSD-25; it is approximately one-third as effective in man, as judged by our questionnaire technique. The questionnaire consists of a first part containing 47 questions and a second part containing nine reactions, which are rated both by the subject and by the observer. Positive responses to the questionnaire are added irrespective of the intensity of the response. Thus, in Table 1 the total of positive responses to the questionnaire refers to the sum of both parts of this questionnaire (*5*).

The effect of MLD-41 on man was obtained by giving it to a group of five nonpsychotic test subjects who have been used in the study of LSD-25 and its derivatives for the past 3 years. Both LSD-25 and MLD-41 were administered orally in distilled water or tap water with no essential differences observed between the two. Development of tolerance to LSD-25 was achieved by administering MLD-41 for 5 or 6 days in increasing doses, starting with 100 μg on the first day and reaching 350 μg on the fifth day. Since the threshold to MLD-41 is approximately 70 μg orally, tolerance to MLD-41 itself was developed rapidly. It appears that approximately 1000 μg of MLD-41 administered in this way protects against approximately 80 to 100 μg of LSD-25 taken orally 8 hours after the last dose of MLD-41.

Table 1 illustrates a typical series of experiments on one of our subjects. Although 2-bromo lysergic acid diethylamide (BOL-148) produces some tolerance to LSD-25, its effect for equal weights is much less, approximately one-third of that of MLD-41. The highest doses of LSD-25 varied from 1.1 to 1.6 μg/kg of body weight. These doses invariably produced a severe typical LSD-25 reaction in our test group. Note in Table 1 that, whereas 50 μg of LSD-25 in this subject, without pretreatment by MLD-41, produces 35 positive responses to the questionnaire, there were no positive responses to the questionnaire following a 50 μg dose of LSD-25 when this subject had been pretreated for 5 days with 1100 μg of MLD-41.

A similar experiment in which BOL-148 was substituted for MLD-41 resulted in 21 positive responses to the questionnaire (21 June 1957). The 21 positive responses obtained represent the equivalent of at least a 25-μg response to the LSD-25 administered. The subject himself estimated that he experienced a 35-μg LSD response.

The fact that a substance like MLD-41, which is less toxic than LSD-25, can produce a marked tolerance to LSD-25 lends hope to the possibility that if the schizophrenias are produced by a disturbance in biochemical mechanisms analogous to that resulting from the administration of mescaline, LSD-25, and similar substances, there is good reason to believe that comparatively nontoxic molecules might be administered to produce a similar tolerance to the chemicals that originate the schizophrenic state.

H. A. ABRAMSON, B. SKLAROFSKY,
M. O. BARON, N. FREMONT-SMITH
Biological Laboratory, Cold Spring Harbor, New York, and *Research Division, State Hospital, Central Islip, New York*

References and Notes

1. H. A. Abramson *et al.*, *Science* 125, 397 (1957); *A.M.A. Arch. Neurol. Psychiat.* 77, 439 (1957).
2. This investigation has been aided in part by grants from the Josiah Macy, Jr. Foundation, New York, N.Y., and the Foundation for Research in Pulmonary Disease, New York, N.Y.
3. H. A. Abramson *et al.*, *J. Psychol.* 41, 81 (1956).
4. We are indebted to Sandoz Pharmaceuticals for the supplies of MLD-41 and BOL-148.
5. H. A. Abramson *et al.*, *J. Psychol.* 39, 3 (1955).

27 August 1957