3. C. J. Avers, M. W. Rancourt, F. H. Kim, *Proc. Nat. Acad. Sci. U.S.* **54**, 527 (1965).
4. M. S. Burstone, *Enzyme Histochemistry* (Academic Press, New York, 1962).
5. K. Ogawa and R. J. Barrnett, *Nature* **203**, 224 (1964).
6. P. Green, *Amer. J. Bot.* **51**, 334 (1964).
7. F. E. Fritsch, *The Structure and Reproduction of the Algae* (Cambridge Univ. Press, New York, 1935), vol. 1.
8. L. F. Leloir, M. A. Rongine de Fekete, C. E. Cardini, *J. Biol. Chem.* **236**, 636 (1961).
9. This work was supported in part by the NSF grant GB-5461.

30 November 1966

L-Asparaginase: Toxicity to Normal and Leukemic Human Lymphocytes

Abstract. *Quantitative in vitro tests showed that purified preparations of* L-*asparaginase from* Escherichia coli *were more toxic to blood lymphocytes from 12 of 15 patients with chronic lymphocytic leukemia than to lymphocytes from 25 persons with normal hemograms. Incubation for 7 days with 10 units per milliliter killed, on the average, 77 percent of leukemic and 34 percent of normal lymphocytes. The reagent produced appreciable toxicity to leukemic lymphocytes after 2 days of incubation.*

Guinea pig serum has been shown to cause regression of transplantable mouse lymphoma 6C3HED (*1*) and of transplants from spontaneous mouse lymphomas (*2*). Broome (*3*), Old *et al.* (*4*), and Holmquist (*5*) produced evidence that L-asparaginase may be the antilymphoma factor in guinea pig serum. Asparaginase isolated in purified form from guinea pig serum produced regression of 6C3HED (*6*). Mashburn and Wriston (*7*) and Dolowy *et al.* (*8*) demonstrated that L-asparaginase obtained from *Escherichia coli* also produced regression of 6C3HED lymphomas. By quantitative in vitro methods, Schrek (*9*) found that guinea pig serum had a greater cytocidal effect on lymphocytes from patients with chronic lymphocytic leukemia than on lymphocytes from healthy individuals. Our study measured the cytocidal effect of L-asparaginase from *E. coli* on normal and leukemic lymphocytes.

Purified suspensions of blood lymphocytes were prepared by methods described previously (*10*). The medium consisted of equal parts of normal human serum and Fischer's medium No. 147G; both serum and medium contained 10 μg of asparagine per milliliter (*11*). Suspensions with and without L-asparaginase were incubated in test tubes for 2 to 7 days. To obtain counts of viable lymphocytes, 0.2 ml of suspension was placed in a special slide chamber that consisted of two large cover slips (45 by 50 mm) separated by a metal disk (40 by 40 mm by 1 mm in thickness) with a central hole 25 mm in diameter. Cells were examined with an inverted phase contrast microscope and the number of viable lymphocytes in an area 10 by 0.04 mm was counted. Viable lymphocytes were counted in treated and untreated suspensions before and after incubation. Cytologic criteria used for determining viability were developed by studying time-lapse cinemicrographic films (*10, 12*). In brief, a lymphocyte was considered viable if it had a morphologically intact nucleus with a thin nuclear wall and chromatin masses or a nucleolus. Other criteria of viability were elongation or irregularity in the shape of the cell and the presence of a pseudopod or of cytoplasmic projections. A typical dead lymphocyte, in this study, had no nucleus but was round with a thin cellular wall, a few cytoplasmic granules, and an ill-defined clear space. A few dead cells had homogeneously gray pyknotic nuclei without any discernible structure. The percentage of lymphocytes that survived incubation was based on the number of viable lymphocytes in the original suspension before incubation; the percentage killed by the reagent (or percent cytocidal effect) was based on the ratio of the numbers of viable lymphocytes in treated and untreated suspensions after a given period of incubation.

L-Asparaginase from *E. coli* was assayed according to a method described previously (*13*); protein concentration was determined by the method of Lowry *et al.* (*14*). Specific activity (micromoles of NH_3 produced from L-asparagine per hour per milligram of protein) of the preparations used ranged from 295 to 398.

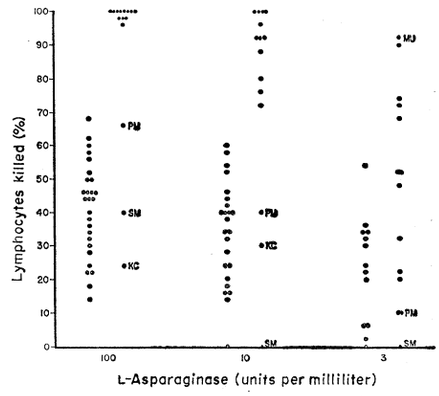

Fig. 1. The percentage of lymphocytes killed in vitro by incubation for 7 days with 3, 10, and 100 units of L-asparaginase per milliliter. This percentage was based on the number of lymphocytes that survived in the control suspensions incubated for 7 days without L-asparaginase. Lymphocytes were derived from 25 persons with normal hemograms (open circles) and from 15 patients with chronic lymphocytic leukemia (closed circles).

Suspensions of blood lymphocytes from 25 persons with normal hemograms and from 15 patients with chronic lymphocytic leukemia were incubated for 2, 5, and 7 days with 1, 3, 10, and 100 units of L-asparaginase per milliliter (see Table 1). At all dosages and time intervals tested, the leukemic lymphocytes were more sensitive than normal lymphocytes. At 10 units per milliliter, the reagent produced only a minimal effect on normal lymphocytes after 5 days of incubation but killed many of the leukemic lymphocytes in 2 days, and most of them in 5 days. This dose (10 units of asparaginase per milliliter) is of particular interest since Dolowy *et al.* (*15*) showed that while

Table 1. Effect of L-asparaginase on blood lymphocytes from persons with normal hemograms and from patients with chronic lymphocytic leukemia. Only the first test on a patient is used in this table.

Incubation time (days)	No. of persons tested	Lymphocytes killed (%) by incubation with L-asparaginase (units/ml)*				Viable lymphocytes (%) in untreated suspension†
		100	10	3	1	
		Normal lymphocytes				
2	7	10.4				87.0
5	14	19.3	13.5			59.4
7	25	41.0	33.9	22.0	8.1	50.7
		Leukemic lymphocytes				
2	12	68.5	18.7			71.1
5	13	85.0	66.5			64.1
7	15	88.0	76.9	45.7	11.5	61.7

* Percentage of lymphocytes killed equals 100(1 — No. viable lymphocytes in treated, incubated suspension/No. viable lymphocytes in untreated, incubated suspension). † Percentage of lymphocytes surviving in untreated, incubated suspension is based on the number of lymphocytes in the original suspension before incubation.

mice with lymphoma were being treated with asparaginase, a concentration of 7 or more units per milliliter was maintained in the blood plasma for 24 hours after one injection of 15 units per gram of body weight.

Lymphocytes from 12 of the 15 patients showed a higher sensitivity to 10 and 100 units of L-asparaginase per milliliter than lymphocytes from any of the 25 control persons (Fig. 1). The sensitivity of lymphocytes from only two patients (SM and KC) was within the range for normal lymphocytes. Repeat tests were done on cells of six patients. Lymphocytes from one of them (PM) had a relatively low sensitivity to 10 and 100 units on the first test (Fig. 1) and in two repeat tests. Another patient (MU) had a high sensitivity to both 3 units and 1 unit on the first test; a repeat test showed a high sensitivity to 3 units but not to 1 unit. In all six patients, the findings on one or two repeat tests were consistent with the findings obtained in the first test.

Human leukemic lymphocytes were found to have a relatively high sensitivity to guinea pig serum in a previous study (*9*) and to a purified preparation of L-asparaginase from *E. coli* in this one. The only known common factor in the two reagents is L-asparaginase and presumably this enzyme has a greater toxicity for leukemic than for normal lymphocytes in vitro.

The difference in sensitivity of normal and leukemic human lymphocytes to L-asparaginase raises the question of the asparagine requirements of cells. The amino acid is not required for in vitro growth of several types of normal and malignant cells of man (*16*) and lower animals (*17*) but is required by cells of Walker rat carcinosarcoma 256 (*18*) and of mouse leukemia L5178Y (*19*). L-Asparaginase is required in the culture medium by cells of mouse lymphoma 6C3HED (*20*), a tumor which regresses in vivo on treatment with guinea pig serum or with L-asparaginase. In contrast, L-asparagine is not necessary for the continuous cultivation of a derived lymphoma 1RG which is resistant to in vivo treatment by guinea pig serum (*20*). These studies indicate that asparagine is not required for culturing many types of normal and tumor cells but is essential for growth of cells of two mouse tumors and one rat tumor. Preliminary work (*21*) showed that C^{14}-asparagine was incorporated by lymphocytes from seven patients with chronic lymphocytic leukemia and from four control persons. The requirements of normal and leukemic human lymphocytes for L-asparagine are not known.

ROBERT SCHREK
Tumor Research Laboratory,
Veterans Administration Hospital,
Hines, Illinois

WILLIAM C. DOLOWY
ROBERT N. AMMERAAL
Medical Research Laboratory,
University of Illinois, Chicago

References and Notes

1. J. G. Kidd, *J. Exp. Med.* **98**, 583 (1953).
2. E. A. Boyse, L. J. Old, E. Stockert, *Nature* **198**, 800 (1963).
3. J. D. Broome, *ibid.* **191**, 1114 (1961).
4. L. J. Old, E. A. Boyse, H. A. Campbell, G. M. Daria, *ibid.* **198**, 801 (1963).
5. N. D. Holmquist, *Proc. Soc. Exp. Biol. Med.* **113**, 444 (1963).
6. J. D. Broome, *J. Nat. Cancer Inst.* **35**, 967 (1965).
7. L. T. Mashburn and J. C. Wriston, Jr., *Arch. Biochem. Biophys.* **105**, 450 (1964).
8. W. C. Dolowy, J. Cornet, D. Henson, R. Ammeraal, *Proc. Soc. Exp. Biol. Med.* **123**, 133 (1966).
9. R. Schrek, *Nature* **182**, 724 (1958).
10. ———, *J. Nat. Cancer Inst.* **33**, 837 (1964).
11. W. H. Stein and S. Moore, *J. Biol. Chem.* **211**, 915 (1954).
12. R. Schrek, *Arch. Pathol.* **66**, 569 (1958); ———, *Cryobiology* **2**, 122 (1965); H. U. Zollinger, *Amer. J. Pathol.* **24**, 1039 (1948).
13. W. C. Dolowy, D. Henson, J. Cornet, H. Sellin, *Cancer*, in press.
14. O. H. Lowry, N. J. Rosebrough, L. A. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
15. W. C. Dolowy, R. Ammeraal, J. Cornet, D. Henson, in preparation.
16. K. A. Piez and H. Eagle, *J. Biol. Chem.* **231**, 533 (1958); H. Eagle, V. I. Oyama, M. Levy, *Arch. Biochem. Biophys.* **67**, 432 (1957).
17. C. Waymouth, *J. Nat. Cancer Inst.* **19**, 495 (1957); T. A. McCoy, M. Maxwell, P. F. Kruse, Jr., *Proc. Soc. Exp. Biol. Med.* **100**, 115 (1959).
18. R. E. Neuman and T. A. McCoy, *Science* **124**, 124 (1956).
19. E. E. Haley, G. A. Fischer, A. D. Welch, *Cancer Res.* **21**, 532 (1961).
20. J. D. Broome, *J. Exp. Med.* **118**, 121 (1963).
21. R. N. Ammeraal, unpublished data.
22. Supported by PHS grants CA-07541 and GRSG-172. We thank J. Teller of Worthington Biochemical Corporation for the donation of L-asparaginase.

28 October 1966

Mammalian Ribosomal Protein: Analysis by Electrophoresis on Polyacrylamide Gel

Abstract. *Ribosomal protein from five mammalian tissues when analyzed by discontinuous electrophoresis on polyacrylamide gel at* pH *4.5 yielded 24 bands. Densitometric tracings indicated that the patterns of the basic ribosomal proteins from the several tissues were qualitatively similar. Protein from* Escherichia coli *ribosomes analyzed at* pH *4.5 gave 29 bands, and the pattern was different from that of mammalian ribosomal protein. No distinct band was found when mammalian ribosomal protein was analyzed at* pH *8.3 (acidic proteins). Ribosomal protein from* Escherichia coli *gave eight bands at* pH *8.3. Thus, the structure of the genes responsible for synthesis of ribosomal protein in several mammalian tissues is the same, and different genes direct synthesis of ribosomal protein in bacteria.*

Whether the cistrons for ribosomal protein are the same in different mammalian tissues has not yet been determined. Although a substantial amount is known about the electrophoretic characteristics of bacterial ribosomal protein (*1–4*), only one report (*5*) of analysis of ribosomal protein from mammalian tissues has appeared.

Ribonucleoprotein particles were isolated from rat liver (*6*), kidney (*6*), skeletal muscle (*7*), and cardiac muscle (*8*); from rabbit reticulocytes (*9*); and from *Escherichia coli* (*3*). The ribosomes were washed with suitable media until the absorbancy ratios, 260/280 mμ and 260/235 mμ, were greater than 1.75 and 1.50. Portions of the ribosomes from skeletal and cardiac muscle, from reticulocytes, and from *E. coli* were assayed (*10*) and found capable of catalyzing the synthesis of protein.

Protein extracted from the ribosomes with 8*M* urea and 4*M* LiCl was subjected to electrophoresis on polyacrylamide gel (*3*). The urea-lithium chloride method for the extraction of ribosomal protein is more reproducible than other methods, provides better separation of the protein from RNA, and gives good recovery (*4*); the protein prepared by this method yields on electrophoresis the largest number of bands (*11*). Usually the concentration of the separation gel was 10 percent, but for the resolution of the mammalian ribosomal protein bands 12 to 14 (compare Fig. 1), it was reduced to 7.5 percent. At the lower concentration the number of bands resolved is greater but many individual bands are less sharply defined. A 10-μl sample containing 50 μg of ribosomal protein was placed on the gel with 1 μl of tracker dye—0.1 percent pyronin Y for runs made at *p*H 4.5, and 0.1 percent bromphenol