A. P. Orr, *Rapp. Procès-Verbaux Réunions Conseil Perm. Int. Explor. Mer* **153**, 92 (1962); M. Anraku, *J. Oceanogr. Soc. Japan* **20**, 221 (1964)] have confirmed this only for very small volumes. In spite of the increased mortality that corresponds to a decrease in dish size, we have found that the minimum development time for *A. tonsa* has remained unchanged in our 100-, 150-, and 190-mm dishes containing 200, 700, and 1500 ml of sea water, respectively. This suggests that a comparable nutritional status exists among these volumes, but leaves the cause of the increased mortality unexplained. We did not study development time in less than 200 ml.

6. The necessity for employing such a high degree of temperature control has not been established, but experience suggests that reasonable control over sudden increases of temperature is necessary. The temperature of 17°C was chosen because it is suitable for both the copepods and our various algal cultures, and, if temperature control is lost, it allows extra time for corrective action before lethal temperatures are reached. The range of illumination used appears to have no effect on either development or survival; investigations outside of this range are incomplete.
7. Although the concentration limit for satisfactory development with our standard feeding regimen has not been determined, cultures containing 100 or more nauplii per liter will usually show retarded or erratic development followed by high mortality. On one occasion, however, a natural population of *A. tonsa* eggs and nauplii matured and subsequently was maintained for at least a week at a concentration of more than 600 animals in 300 ml of sea water that contained a heavy dinoflagellate bloom. Adult mortality was low and nearly 100 percent mating occurred, but survival of the offspring was negligible.
8. E. J. Zillioux and D. F. Wilson, in preparation.
9. D. R. Heinle, abstract in *Ocean Science and Ocean Engineering 1965* (Proc. meeting Amer. Soc. Limnol. Oceanogr. and Marine Technol. Soc., 1965), vol. 1, p. 98.
10. G. B. Deevey, *Bull. Bingham Oceanogr. Collect.* **17**, 5 (1960).
11. R. J. Conover, *ibid.* **15**, 156 (1956).
12. J. E. G. Raymont and R. S. Miller, *Int. Rev. Ges. Hydrobiol. Hydrogr.* **47**, 169 (1962).

13. We thank Kathy Kirouac for technical assistance during the later phases of this work.

19 November 1965

Antagonism of Purified Asparaginase from Guinea Pig Serum toward Lymphoma

Abstract. *The tumor inhibitory activity of highly purified asparaginase from guinea pig serum toward the Gardner lymphosarcoma in C3H mice was compared with that of the serum itself. The purified enzyme, homogeneous by ultracentrifugation and immunoelectrophoresis, had activity comparable to that of the serum. The serum was also effective in mice which were made immunologically incompetent by radiation with cobalt-60.*

Kidd reported that normal guinea pig serum had inhibitory activity against certain transplantable lymphoma tumors in mice (*1*). Broome (*2*) suggested that the enzyme L-asparaginase (*3*) was the agent in the serum responsible for this effect. Evidence has been obtained that supports this suggestion (*4–7*).

We have recently obtained from guinea pig serum a preparation of L-asparaginase which is homogeneous in the ultracentrifuge under conditions of sedimentation equilibrium (*8*), shows the migration of a single homogeneous peak in an ultracentrifugal sedimentation velocity experiment (Fig. 1), gives a single band upon polyacrylamide gel electrophoresis (Fig. 2), and gives a single arc in immunoelectrophoresis (*9*). We now report the antagonistic effects of this highly purified preparation toward lymphoma and suggest that the evidence is conclusive that the enzyme L-asparaginase is responsible for the inhibitory activity of guinea pig serum.

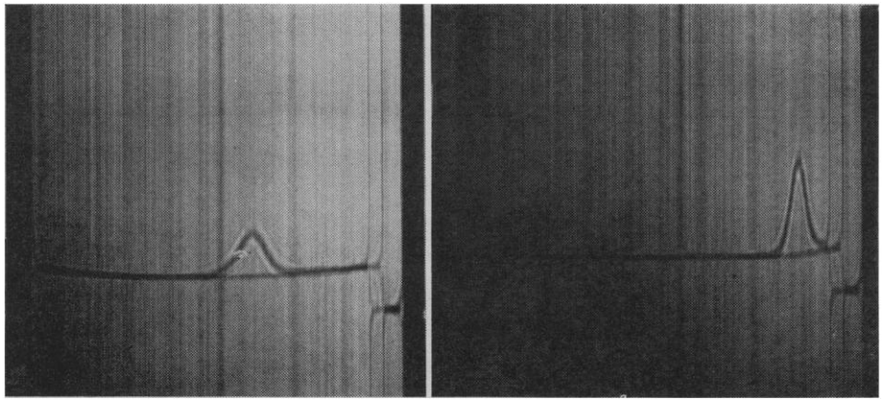

Fig. 1. Schlieren pattern of the descending boundary for guinea pig serum asparaginase in the double-sector cell after approximately 16 minutes (right) and 48 minutes (left) at 59,780 rev/min in the Beckman model E ultracentrifuge; 0.1*M* potassium phosphate buffer, *p*H 7.0, 25°C.

The inhibitory activity of the purified enzyme was tested as follows. Female C3H mice (Jackson Laboratories), weighing 18 to 25 g, were separated randomly into groups of ten animals each, and five were housed in a cage. Selection of treatments and the order of injections were also random. Each animal was given a single subcutaneous injection of 0.2 ml of a suspension of Gardner lymphosarcoma (6C3HED) cells in the right, lower ventral side. The suspension was prepared by briefly homogenizing 10 mg of tissue (wet weight) per milliliter of saline in a small Potter-Elvehjem homogenizer. The tissue was a 1-week-old tumor carried subcutaneously in C3H mice. Immediately after transplanation of the tumor, one group received an intraperitoneal injection of 0.5 ml of saline containing 1 mg of crystalline bovine serum albumin (BSA) per milliliter, another group 0.5 ml of guinea pig serum, and the third 0.3 ml of purified asparaginase solution to which was added BSA (1 mg/ml). Albumin is necessary to protect the enzyme against surface denaturation that occurs when the enzyme is highly purified and in dilute solution. After passage of enzyme solution through the needle used for injection, there was no loss of activity. One experimental group was given purified asparaginase (160 units, 75 μg), and the other was given 90 units in whole guinea pig serum. Under similar conditions, a second injection of 1.0 ml of serum given 12 hours after the first injection invariably protected the animals against appearance of the tumor for at least 30 days; and if more dilute suspensions of cells (2 to 5 mg/ml) were used in transplantation, this dose schedule prevented the appearance of tumors entirely. Tumors which appeared after a long latent period (30 days) remain sensitive to the serum, but Kidd (*10*) has shown that repeated low doses of guinea pig serum can produce sublines of tumors resistant to inhibition by guinea pig serum.

In control animals the tumor appeared within 4 to 6 days as a tiny, circumscribed elevation of the skin at the site of injection; it was revealed as a solid nodule by palpation. The tumors went into a "log" period of rapid growth in 12 to 14 days, and the animals died in 16 to 20 days.

Table 1. Antagonistic effect toward mouse lymphoma of guinea pig serum and purified asparaginase from guinea pig serum. Values are averages of latent periods, survival, and gain in weight of the mice at intervals after transplantation of the tumor.

Latent period (days)	Survival (days)	Wt gain (g) from days:		
		1–7	7–13	13–19
Saline and BSA				
4.6	18.2	2.8	6.4	
Guinea pig serum				
10.8	27.7	2.0	1.6	4.2
Purified asparaginase from serum and BSA				
17.8	33.2	2.5	0.4	0.7

The animals were checked daily for the appearance of tumors, and their weights were recorded every other day (Fig. 3). The purified asparaginase had inhibitory activity comparable to that of the serum (Table 1). In connection with the data on weight gain, we have noticed that guinea pig serum depresses the weight gain of growing mice (about 20 g) for a short period.

The effect of treatment with saline or with serum (0.5 ml) was compared

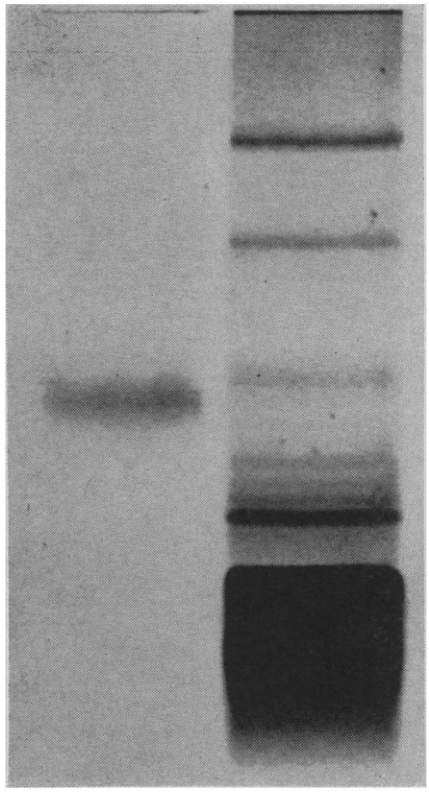

Fig. 2. Polyacrylamide-gel electrophoresis of whole guinea pig serum (right) and purified asparaginase from guinea pig serum (left); 250 volts for 4 hours; *p*H 8.6; in ethylenediaminetetraacetate-boric acid-tris buffer.

in animals that received 900 rad of total-body radiation at the rate of 1000 rad/min from a Co^{60} source 1 day before transplantation of the tumor. The same experimental design was used. All animals treated with saline developed tumors by the 5th day, whereas only 50 percent of the animals treated with serum had tumors by the 9th day, when the animals began to die of radiation sickness. A dose of 900 rad was expected to render the animals immunologically incompetent (*11*), but the serum was as effective with such animals as it was with nonirradiated animals. The serum was also active against tumors in animals receiving 500 rad (a nonlethal dose). The antagonistic effect of the L-asparaginase is independent of any immune phenomenon in the host. Kaufman and Kidd (*12*) were led to much the same conclusion by less direct methods.

The importance of demonstrating correlations between the growth of tumors on the one hand, and unique or highly modified metabolic pathways on the other, is widely recognized, and serves as an impetus for much of the current research on cancer. Other enzymes have been used in attempts to treat cancer (*13*), but none has shown as striking an effect as L-asparaginase. Tumors susceptible to asparaginase—for example, Gardner lymphosarcoma (6C3HED) and leukemia EARAD1; see references *6*, *14*—require asparagine in order to divide normally in tissue culture, and no other enzyme which has a tumor inhibitory effect (for example, xanthine oxidase or ribonuclease; see reference *13*) has been so closely linked to a specific metabolic requirement of the tumor cell.

There are no known metabolic pathways which require asparagine, and if one assumes that asparaginase inhibits the tumor by depriving it of asparagine, the central problem of the role of asparagine still remains. Kapoor and Waygood (*15*) showed that asparagine is more active than glutamine in the synthesis of glycinamide ribonucleotide from phosphoribosylpyrophosphate by wheat embryos. Mashburn and Wriston reported (*16*) that asparaginase from *Escherichia coli*, which also has lymphoma-inhibiting activity (*5*), caused an 80-percent increase in alkaline ribonuclease of an asparaginase-sensitive tumor 2 hours after administration, but had no effect

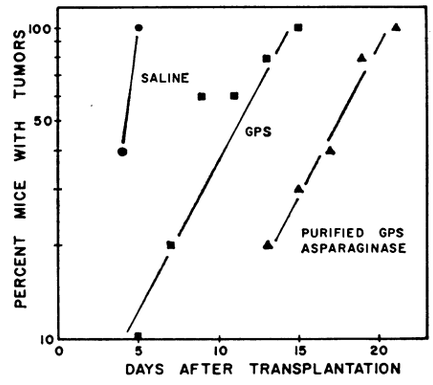

Fig. 3. Effect of asparaginase from guinea pig serum on the time of appearance of the Gardner lymphosarcoma in C3H mice.

on this enzyme in two resistant strains, including one derived from the original Gardner lymphosarcoma. It is not known whether asparaginase is acting indirectly, by way of ribonuclease, for example, or is limiting the availability of asparagine for a novel biosynthetic pathway in the tumors.

TOBIAS O. YELLIN
JOHN C. WRISTON, Jr.
Department of Chemistry,
University of Delaware, Newark

References and Notes

1. J. G. Kidd, *J. Exp. Med.* **98**, 565, 583 (1953).
2. J. D. Broome, *Nature* **191**, 1114 (1961).
3. L-Asparagine amidohydrolase, No. 3.5.1.1 in *Enzyme Nomenclature*. Recommendations of the International Union of Biochemistry (Elsevier, New York, 1965).
4. L. T. Mashburn and J. C. Wriston, Jr., *Biochem. Biophys. Res. Commun.* **12**, 50 (1963).
5. ———, *Arch. Biochem. Biophys.* **105**, 451 (1964).
6. J. D. Broome, *J. Exp. Med.* **118**, 99, 121 (1963).
7. E. A. Boyse, L. J. Old, E. Stockert, *Nature* **196**, 800 (1963); L. J. Old, E. A. Boyse, H. A. Campbell, G. M. Daria, *ibid.*, p. 801; H. M. Suld and P. Herbut, *J. Biol. Chem.* **240**, 2234 (1965).
8. D. A. Yphantis, *Biochemistry* **3**, 297 (1964).
9. Details of the purification procedure, which involves salt fractionation, gel filtration, and chromatography on diethylaminoethyl cellulose and calcium hydroxylapatite, are being prepared for publication.
10. J. G. Kidd, *J. Exp. Med.* **108**, 665 (1958).
11. R. D. Stoner and W. M. Hale, in *Ionizing Radiations and Immune Processes*, C. A. Leone, Ed. (Gordon and Breach, New York, 1962), p. 183.
12. J. L. Kaufman and J. G. Kidd, *Proc. Soc. Exp. Biol. Med.* **91**, 164 (1956).
13. F. Bergel, *Chemistry of Enzymes in Cancer* (Thomas, Springfield, 1961), pp. 60–80.
14. H. A. Campbell, L. J. Old, E. A. Boyse, *Proc. Amer. Assoc. Cancer Res.*, 55th Mtg. (9–11 April 1964), p. 10.
15. M. Kapoor and E. R. Waygood, *Biochem. Biophys. Res. Commun.* **9**, 7 (1962).
16. L. T. Mashburn and J. C. Wriston, Jr., *Fed. Proc.* **24**, 597 (1965).
17. We thank Dr. D. Filmer, Biology Department, Brookhaven National Laboratory, for help with the ultracentrifugation. Supported by grant 06780 from the Nat. Cancer Inst. T.O.Y. is a PHS predoctoral fellow. Submitted as partial fulfillment for the requirements for the Ph.D. degree at the University of Delaware.

27 December 1965