



Fig. 2. Incorporation of leucine- H^3 into a protein from plasma perfusing isolated rat livers in vitro, which protein was precipitated with a rabbit antiserum specifically directed against rat α_2 (acute phase)-globulin. dpm, Disintegrations per minute.

lation counter (Nuclear-Chicago), and the specific activities were calculated from the protein measurements.

Since no α_2 (acute phase)-globulin could be detected by immunoelectrophoretic analysis of plasma from control perfusions, carried out with liver and whole blood obtained from normal rats, an equal volume of non-radioactive acute-phase rat serum was added to such plasma to provide carrier acute-phase globulin, and the immune precipitates were isolated in a similar manner; this procedure also enabled assessment of the nonspecific absorption of radioactive material by immune precipitates.

There was a delay of 90 minutes before appreciable amounts of radioactivity were incorporated (Fig. 2); next came a rapid and progressive increase, in the specific activity of immune precipitates from "inflammatory" liver perfusates, during the remaining 90 minutes of perfusion. This sequence sharply contrasted with the absence of incorporation of leucine- H^3 into immune precipitates when blood from injured animals was circulated in the apparatus for a similar period but without a liver. These data indicate that whole blood per se cannot incorporate appreciable amounts of radioactivity. Immune precipitates isolated from plasma perfusates from normal perfusions (whole blood and liver from normal rats), to which carrier acute-phase plasma had been added, proved to contain small but significant amounts of radioactivity after 45 minutes; this ra-

dioactivity represents either synthesis of trace amounts of α_2 (acute phase)-globulin, that cannot be detected without addition of carrier, or adsorption of some other leucine-containing plasma protein that had been synthesized during the perfusion.

Other methods of producing injury to rats—such as subcutaneous implantation of sterile polyvinyl sponges or injection of air—yielded similar results, indicating that synthesis of α_2 (acute phase)-globulin is not dependent on liver injury produced by turpentine.

We conclude that the liver is a source of the α_2 (acute phase)-globulin and that the appearance of this protein in the serum reflects *de novo* synthesis by the liver rather than release of preformed and stored protein. Since injury peripheral to the liver is ultimately responsible for the appearance of the globulin in the serum, these data accord with the proposal that hepatic synthesis of this serum component is stimulated by factors released into the blood from injured or necrotic tissues.

Note added in proof: Synthesis of α_2 (acute phase)-globulin by liver-cell cultures has just been described (8).

EDWARD J. SARCIONE

Department of Medicine, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14203

ARTHUR E. BOGDEN

Mason Research Institute, 20 Howard Street, Worcester, Massachusetts 01608

References and Notes

1. G. H. Beaton, A. E. Selby, M. J. Veen, A. M. Wright, *J. Biol. Chem.* **236**, 2005 (1961); J. M. Fine, G. A. Boffa, F. Zajdela, *Compt. Rend.* **255**, 1045 (1962); A. E. Bogden and G. A. Neville, *Proc. Amer. Assoc. Cancer Res.* **3**, 305 (1962); W. G. Heim and P. H. Lane, *Nature* **203**, 1077 (1964).
2. H. E. Weimer and D. C. Benjamin, *Am. J. Physiol.* **209**, 736 (1965).
3. E. J. Sarcione, *Arch. Biochem. Biophys.* **100**, 516 (1963).
4. S. Krauss and E. J. Sarcione, *Biochim. Biophys. Acta* **90**, 301 (1964).
5. From the New England Nuclear Corp.
6. A. E. Bogden, W. E. Woodward, M. Gray, *Proc. Amer. Assoc. Cancer Res.* **5**, 6 (1964).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
8. D. C. Benjamin and H. E. Weimer, *Nature* **209**, 1032 (1966).
9. Supported by NIH grant A-4961. We thank Angela Longo and Marian Leahy for technical assistance.

25 March 1966

Toxic Impurities in Nalgene Filter Units

Abstract. Sterile disposable filter units made of Nalgene contain an impurity which inhibits the growth of the protozoan, *Leishmania tarentolae*, in a defined medium.

Sterile disposable filter units made of Nalgene impart a toxic impurity to the filtrate. Others have also found easily extractable substances from plastic materials (1, 2). Table 1 shows increasing inhibition of growth of the hemoflagellate, *Leishmania tarentolae*, when increasing amounts of filtered, redistilled water (filtered in 30-ml quantities) were substituted for normal redistilled water in preparing the defined medium (3). Inhibition was essentially complete with 0.2 ml of filtered water per 3 ml culture. The pH of the water was unaffected by filtration, and no fluorescence was observed

when the filtered water was repeatedly spotted onto filter paper and examined under ultraviolet light (1). The cells also did not divide if a piece of the sterile membrane were put into the culture at the time of inoculation or if the culture medium were merely passed into the bottom receptacle through the side arm and removed.

Leishmania tarentolea (and *L. donovani*) do grow, however, on blood agar with Nalgene-filtered Locke's solution as the overlay. This detoxifying effect is possibly a result of chelation or adsorption of the toxic material by the high protein content of the medium.

It is possibly pertinent that redistilled water foams after passage through the filter, even after removal of the filter membrane. Four different lots of the filters exhibited this phenomenon.

LARRY SIMPSON

Rockefeller University, New York

References

1. H. A. Kordan, *Science* **149**, 1382 (1965).
2. J. Autian, *J. Pharm. Sci.* **53**, 1289 (1964).
3. W. Trager, *J. Protozool.* **4**, 269 (1957).

21 March 1966

Table 1. Inhibition of growth by substitution of varying amounts of filtered water for normal redistilled water in defined medium.

| Filtered water per 3 ml of culture (ml) | Inhibition after 6 days (%) |
|--|--------------------------------|
| 0 | 0 |
| 0.1 | 62 |
| .2 | 97 |
| .3 | 97 |
| .4 | 98 |
| 1.0 | 98 |