(8/32) of all sibling mixtures studied by one-way testing have not stimulated, no case of nonstimulation has been observed in one-way testing of 52 mixtures of cells from unrelated individuals. These data are in agreement with our earlier data obtained by use of two-way stimulation and with the two-way data of Bain *et al.* (*3*). In one-way studies one would expect that 25 percent or more of sibling mixtures would not stimulate if the genotype at only one locus controlled the presence of factor(s) responsible for stimulation, regardless of the number of different alleles distributed in the general population, since a maximum of four alleles can be represented in a two-generation family at any one locus. If one uses the definition of zero stimulation expressed in this report, the simplest hypothesis—but not the only one—compatible with the present data is that stimulation in the MLC test results from difference between the stimulating cells and the responding cells controlled by the genotype at a single locus (*5*).

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12. Supported by the Damon Runyon Memorial Fund for Cancer Research (grant DRG886) and the Wisconsin Heart Association, and by grants to the University of Wisconsin Medical School from NIH, the American Cancer Society, and Graduate School Research Funds. F.H.B. is an advanced research fellow of the American Heart Association. We thank Oliver Smithies for suggestions and stimulating discussions. Paper 1079 from the Laboratory of Genetics, University of Wisconsin.

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Hepatic Synthesis of Alpha$_2$ (Acute Phase)-Globulin of Rat Plasma

Abstract. *Synthesis of plasma alpha$_2$ (acute phase)-globulin was demonstrated in isolated perfused rat liver obtained from animals showing acute inflammatory reaction to injury. These findings indicate that the liver is a source of the globulin and that appearance of this protein in the serum results from* de novo *synthesis by the liver rather than from release of performed and stored globulin.*

An alpha globulin, referred to as "slow" α_2-globulin because it migrates with the mobility of an α_2-globulin on paper electrophoresis but more slowly than β-globulin on vertical starch-gel electrophoresis, has been detected in the serum of animals under widely varying diverse pathological and experimental conditions, including acute inflammatory reaction to injury (*1*). This serum protein has also been designated α_2 (acute phase)-globulin (*2*). Observations that this singular α-globulin cannot be detected in the serum of normal adult animals have stimulated curiosity as to its origin. Because isolated perfused rat liver has been successfully employed to demonstrate hepatic synthesis of serum orosomucoid (*3*) and haptoglobulin (*4*), it appeared that the same system might serve to determine whether the liver is a site of synthesis of this acute-phase globulin. We have demonstrated synthesis of α_2 (acute phase)-globulin in plasma with isolated perfused rat liver that had been obtained from animals showing acute inflammatory reaction to injury.

The rat-liver perfusion system has been detailed (*3*). The donors of liver and blood were (fed) males of the Sprague-Dawley strain weighing 350 to 400 g. Acute inflammation was produced by daily subcutaneous injection of 1.0 ml of commercial-grade steam-distilled wood turpentine for 3 days before livers and whole blood were taken from the injected animals. In control experiments comparable amounts of "inflammatory" blood and radioactivity were circulated without the presence of the liver. Synthesis by normal livers was studied with liver and whole blood obtained from normal rats. At zero time, 100 μc of DL-(4,5-H^3)leucine (5.45 c/mmole) (*5*) was added to the blood perfusing the livers, and the plasma was sampled at intervals during 3 hours of perfusion.

To obtain specific antiserum, rabbits were immunized at 10 to 14 day intervals with an acute-phase α_2-globulin fraction of rat serum, obtained by chromatography on diethylaminoethyl cellulose (*6*); Freund's complete adjuvant was used, and the rabbits were bled 3 weeks after the last injection. Specific antiserum reagent was prepared by completely absorbing the rabbit antiserums with normal rat serum, thus removing all antibodies against components of normal rat serum. Figure 1 shows the results of immunoelectrophoresis with unabsorbed and absorbed rabbit antiserum against acute-phase rat serum as the antigen; the only precipitin activity remaining after absorption is the antirat α_2 (acute phase)-globulin.

Samples of plasma perfusate were obtained at various intervals, frozen and thawed, heated for 30 minutes at 56°C, and centrifuged, and the clear plasma was dialyzed against isotonic saline for 48 hours at 4°C. An equal volume of the absorbed rabbit antiserums was added to dialyzed rat plasma, and the tubes were incubated for 2 hours at 37°C and left for 3 days at 5°C. The resulting immune precipitates were collected and washed three times with cold saline; they were then dissolved in 0.5 ml of 0.1*N* sodium hydroxide, and protein measurements were made on 0.1-ml portions of this solution by the method of Lowry *et al.* (*7*). The radioactivity in a remaining 0.2-ml portion of the solution was measured with a liquid-scintil-

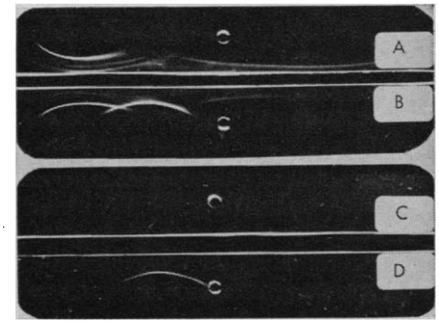

Fig. 1. Agar-gel immunoelectrophoretic patterns of normal and "acute phase" rat serums, illustrating the specificity of the rabbit α_2(acute phase)-globulin antiserums. Wells *A* and *C*, normal rat serum; *B* and *D*, "acute phase" rat serum containing α_2(acute phase)-globulin. Upper channel, unabsorbed rabbit antiserum prepared against a diethylaminoethyl-cellulose fraction of "acute phase" rat serum; lower channel, the same rabbit antiserum absorbed by normal rat serum.

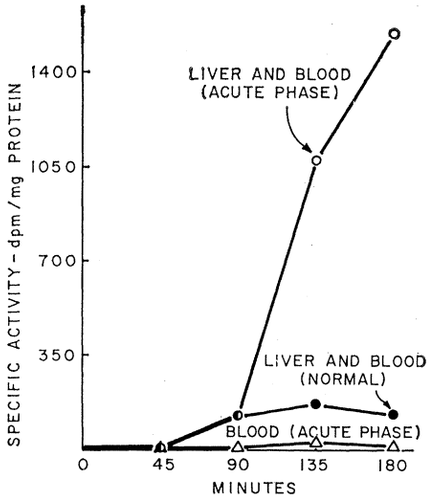

Fig. 2. Incorporation of leucine-H^3 into a protein from plasma perfusing isolated rat livers in vitro, which protein was precipitated with a rabbit antiserum specifically directed against rat α_2(acute phase)-globulin. *dpm*, Disintegrations per minute.

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 nonradioactive 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 radioactivity 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*).

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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 *p*H 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.

Table 1. Inhibition of growth by substition 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

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.

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