Synthesis and Secretion of α_2- (Acute Phase) Globulin by the Isolated Perfused Liver from Injured Adult Rats*

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ABSTRACT: Synthesis and secretion of α_2- (acute phase) globulin was studied using the isolated perfused liver from normal and injured adult rats. Perfused livers from injured rats incorporated ten times more [^{3}H]leucine into immunochemically isolated plasma α_2- (acute phase) globulin than did perfused livers from normal adult rats. The presence of radioactivity in this plasma acute phase globulin was confirmed by immunoelectrophoresis and radioautography. It is concluded: (a) that the appearance of α_2- (acute phase) globulin in the plasma after host injury *in vivo* is due to *de novo* synthesis and secretion by the host liver rather than to the release of preformed and stored globulin; (b) the isolated perfused liver from injured adult rats *in vitro* is a suitable experimental system to study the mechanisms regulating the transient synthesis of this acute phase plasma protein. Taken together, these data and previously reported evidence that fetal liver can also synthesize this acute phase globulin are consistent with the conclusion that the presence of α_2- (acute phase) globulin in the plasma of injured adult rats reflects hepatic synthesis of a fetal-specific globulin not normally detected in adult rats, rather than synthesis of an "abnormal" injury-specific protein.

The transient appearance and disappearance of this plasma acute phase globulin in rats *in vivo* further suggests that selective fetal gene sites can be reversibly "switched-on" and "switched-off" in adult liver.

Among the qualitative changes known to occur in man and animals after host injury is the appearance of immunologically distinct plasma proteins such as C-reactive protein in man and α_2- (acute phase) globulin in rats. α_2- (Acute phase) globulin is a carbohydrate-containing macroglobulin that can be detected in fetal, neonatal, pregnant, tumor-bearing, and injured adult rats, but cannot be detected in normal adult rats (Boffa *et al.*, 1964; Heim and Lane, 1964; Weimer and Benjamin, 1965; Bogden *et al.*, 1967; Stanislawski-Birencwajg *et al.*, 1967). The liver is known to synthesize this acute phase plasma protein (Benjamin and Weimer, 1966; Sarcione, 1967; Sarcione and Bohne, 1969). Since the transient appearance of this plasma protein under diverse physiological and pathological conditions suggests the operation of a "switch-off," "switch-on" type of control mechanism, synthesis of this acute phase globulin was chosen as a model system to investigate the mechanisms involved in regulation of mammalian gene expression and protein synthesis.

The present investigation was undertaken to demonstrate the suitability of this isolated perfused liver from injured adult rats to study synthesis and secretion of plasma α_2- (acute phase) globulin and to define the properties of this biosynthetic system for future studies. Preliminary findings have been reported previously (Sarcione and Bogden, 1966).

Materials and Methods

Animals and Liver Perfusions. The isolated perfused liver technique used was that developed by Miller *et al.* (1951) and details of the procedure used in this laboratory have been described previously (Sarcione, 1962). Liver and blood donors were adult Charles River CD rats weighing 350–450 g which were fasted overnight before use. Two perfusions were performed with both liver and blood obtained from injured adult rats (Figure 2, open circles). The liver weights for experiments 1 and 2 were 17.3 and 14.7 g, respectively. Injury to rats was produced by subcutaneous injection of 1.0 ml of commercial grade steam-distilled wood turpentine and the rats were used 48 hr later. Two perfusions were performed with both liver and blood obtained from normal adult rats (Figure 2, closed circles). The liver weights for experiments 3 and 4 were 15.9 and 13.1 g, respectively. Normal rats used as liver and blood donors were screened before use for the presence of plasma α_2- (acute phase) globulin and discarded if this protein was detected. Isolated livers were perfused with diluted heparinized whole rat blood (70 ml of whole blood:28 ml of Ringer's solution) to which was added 25,000 units of penicillin, 6.25 mg of streptomycin sulfate, 2.5 mg of polymixin B sulfate, and 32 mg of an amino acid mixture (TC amino acids, Hela, dried, DIFCO). After an initial 20-min period of perfusion for the system to equilibrate, 100 μCi of DL-[4,5-^{3}H]leucine (5.45 Ci/mmole, New England Nuclear Corp.) was added to the perfusing blood. Blood perfusate (3.0 ml) was sampled at 45-min intervals during 3 hr. In experiments 5 and 6 (Figure 2, triangles), 98 ml of diluted heparinized whole blood obtained from injured adult rats, containing the same additives and amounts of leucine radioactivity, was circulated in the perfusion apparatus without the liver.

Plasma and Liver Total Free Amino Acids. A minor lobe was removed from each liver before perfusion, homogenized in isotonic saline (1 g/13 ml), and then centrifuged. Plasma samples were obtained from the pooled heparinized whole blood used for perfusion before the mixture of amino acids was added. Both liver supernatant fractions and plasma

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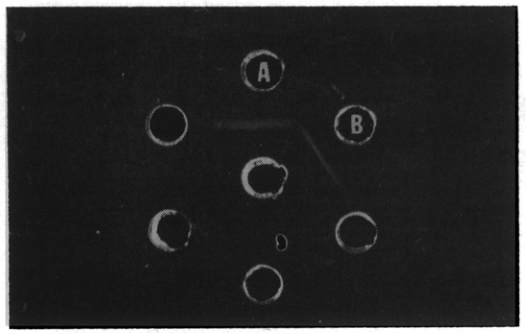

FIGURE 1: Double-diffusion patterns in agar of monospecific rabbit antiserum reagent (center well) against α_2- (acute phase) globulin present in the plasma of injured adult rats (A) and acute phase globulin present in the purified plasma fraction used as antigen to prepare the antiserum reagent (B).

samples were deproteinized with zinc hydroxide as described by Somogyi (1945) and the total amino acid concentration measured in these protein-free filtrates by the ninhydrin method of Moore and Stein (1948).

Antiserum Reagent to α_2- (Acute Phase) Globulin. This was prepared in rabbits as described previously (Sarcione, 1967) except that the α_2-globulin preparation used as antigen was obtained from the sera of rats injured by the subcutaneous injection of turpentine as described above. The rabbit antiserum reagent was shown to be specific for α_2- (acute phase) globulin present in the sera of injured adult rats and did not react with any normal adult plasma proteins (Sarcione and Bogden, 1966).

Immunochemical Isolation of α_2- (Acute Phase) Globulin. The immunochemical isolation of α_2- (acute phase) globulin from plasma perfusates was standardized as follows. To plasma perfusate samples from the normal liver–normal blood perfusion system, an equal volume of pooled nonlabeled plasma from injured adult rats was added to provide carrier acute phase globulin. This was necessary because α_2- (acute phase) globulin cannot be detected in normal adult rat plasma. An equal volume of pooled plasma from normal adult rats was added to perfusate samples from the injured rat liver perfusions. Plasma samples were frozen and thawed once, then heparin and fibrinogen were removed by addition of protamine sulfate and thrombin (as described by Miller *et al.*, 1964). After centrifugation to remove fibrin clots, the supernatant fraction was dialyzed for 48 hr against three changes of isotonic saline containing a mixture of unlabeled amino acids. The dialyzed material was concentrated to one-fifth its original volume for immunoelectrophoresis and to the original plasma volume for isolation of immune precipitates. Concentration was achieved by covering the dialysis bag with powdered sucrose. The ratio of antiserum to plasma required for maximum precipitation of α_2- (acute phase) globulin from dialyzed plasma perfusates was determined in a preliminary titration as described by Gordon and Koj (1968). A ratio of antiserum reagent to plasma perfusate of 1:1 was found to precipitate 88–90% of the α_2- (acute phase) globulin present and this ratio was used in all experiments. The tubes were incubated for 2 hr at 37°, stored at 5° for 24 hr, then centrifuged. Precipitates were washed three times with cold isotonic saline, then dissolved in 0.5 mg of 1.0 N sodium hydroxide. Protein measurements were performed on 0.1-ml portions of this solution by the method of Lowry *et al.* (1951). The radioactivity in the remaining 0.2-ml portion of the solution was measured in a liquid scintillation counter (Nuclear-Chicago) and the specific radioactivities were calculated from the protein measurements.

Immunoelectrophoresis and Radioautography. Radioactive labeling of α_2- (acute phase) globulin in concentrated plasma samples was detected by radioautography of immunoelectrophoretic patterns as described by Hochwald *et al.* (1961). Immunoelectrophoresis was performed by the method of Scheidegger (1955), using 1.0% Nobel agar and 0.1 M Veronal buffer, pH 8.6. After immunoelectrophoresis, the slides were washed for 24 hr in changes of isotonic saline and distilled water, dried, and then placed against sheets of Kodak No-Screen industrial type X-ray film and exposed for 8 weeks.

Isolation of Albumin. Paper electrophoresis was carried out in a Durrum-type cell (Spinco) with barbital buffer, pH 8.6, ionic strength 0.075. The albumin area of the heated unstained strips was located by cutting 0.8-cm strips from each of the papers and staining with bromophenol blue. The unstained paper was then aligned with the stained strip and the albumin area cut from the paper and counted in a liquid scintillation counter. The paper was removed from the counting vial, air-dried, and placed on 0.6 ml of 0.1 N sodium hydroxide for 30 min at 90° to dissolve the albumin. The specific activities were calculated from the protein measurement made on aliquots of this solution by the method of Lowry *et al.* (1951).

Results

Figure 1 shows that the monospecific rabbit antiserum reagent used in this study gave reactions of immunologic identity when diffused in agar against both the α_2- (acute phase) globulin present in the plasma of injured adult rats (A), and that present in the purified fraction used as antigen (B).

Figure 2 shows the time course of incorporation of [^{3}H]-leucine into α_2- (acute phase) globulin (left panel) and into albumin (right panel) obtained from plasma perfusing isolated rat livers *in vitro*. When livers were perfused for 3 hr with both liver and whole blood obtained from injured adult rats (open circles, experiments 1 and 2), the specific activity of immunochemically isolated plasma α_2- (acute phase) globulin increased rapidly and progressively, after an initial delay of 45 min, and was linear between 45 and 180 min. In contrast, perfusion of a duplicate pair of livers and whole blood obtained from normal adult rats (closed circles, experiments 3 and 4) resulted in the appearance of only slight, though gradually increasing amounts of radioactivity in this plasma acute phase globulin. The observation that circulation of whole blood from injured rats without the liver for 3 hr (triangles, experiments 5 and 6) did not result in incorporation of [^{3}H]leucine into α_2- (acute phase) globulin indicates that the formed elements in blood were not the source of this acute phase protein. These data further indicate that a significant amount of free [^{3}H]leucine was not adsorbed to α_2- (acute phase) globulin during the immunochemical isolation procedure used.

Incorporation of [^{3}H]leucine into plasma albumin during these same liver perfusions also proceeded rapidly (Figure

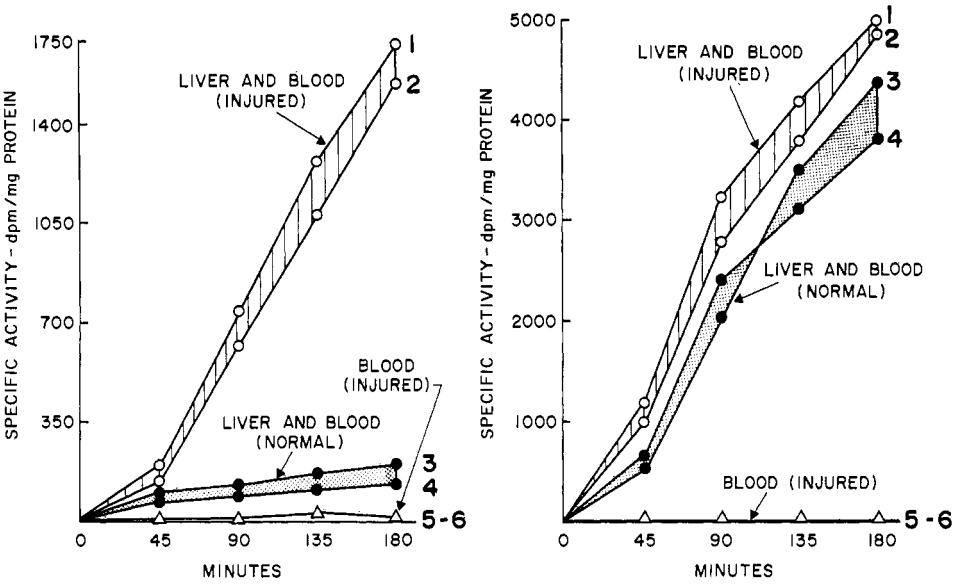

FIGURE 2: Time course of incorporation of [^{3}H]leucine into plasma α_2- (acute phase) globulin (left panel) and plasma albumin (right panel) obtained during perfusion of duplicate pairs of livers and whole blood from injured adult rats (experiments 1 and 2, open circles); liver and whole blood from normal adult rats (experiments 3 and 4, closed circles); and during circulation of whole blood from injured adult rats circulated without the liver (experiments 5 and 6, triangles). DL-[4,5-^{3}H]Leucine (100 μCi) was added to the perfusion blood at zero time. α_2- (Acute phase) globulin was isolated immunochemically from dialyzed plasma perfusates; the specific activity is that of the total immune precipitate protein. Albumin was isolated by preparative paper electrophoresis. The shading indicates the range of values for duplicate perfusions of each type.

2, right panel). Throughout these liver perfusions, the specific activity of albumin obtained from plasma perfusing livers and blood from injured rats was slightly but consistently higher (open circles, experiments 1 and 2) than that obtained after perfusion of liver and blood from normal rats (closed circles, experiments 3 and 4). As would be expected, circulation of whole blood from injured rats without the liver (triangles, experiments 5 and 6) did not result in incorporation of radioactivity into albumin. The initial total free amino acid levels measured in liver and plasma of each of these experiments described in Figure 2 were: (1) 50.4 μmoles/g and 5.6 mmoles/l.; (2) 57.6 μmoles/g and 6.8 mmoles/l.; (3) 54.0 μmoles/g and 6.4 mmoles/l.; (4) 61.2 μmoles/g and 7.6 mmoles/l. The initial and final blood hematocrit values for these perfusions were: (1) 35.0–33.5%; (2) 34.0–32.5%; (3) 34.0–32.5%; (4) 33.5–32.0%.

Immunoelectrophoresis of concentrated plasma perfusates obtained after 3 hr from each of these perfusions described in Figure 2 using monospecific rabbit antiserum reagent to rat α_2- (acute phase) globulin resulted in a single precipitin arc in the slow α_2 region (Figure 3, right panel). Radioautography of these immunoelectrophoretic patterns (Figure 3, left panel) showed that only perfusion of both liver and blood from injured adult rats (A, experiments 1 and 2) resulted in a positive radioautograph. Neither plasma perfusing liver and blood from normal adult rats (B, experiments 3 and 4) nor plasma from injured adult rats circulated without the liver (C, experiments 5 and 6) produced detectable labeling of these precipitin arcs. This latter finding indicates that the radioautographic labeling of α_2- (acute phase) globulin is not a result of nonspecific binding or radioactive amino acid to the precipitin arc.

Discussion

The results obtained in this study indicate that the isolated perfused liver system from injured adult rats is a suitable experimental system to study mechanisms involved in regulation of synthesis and secretion of plasma α_2- (acute phase) globulin. Perfused livers from injured rats incorporated and secreted ten times more [^{3}H]leucine into comparable amounts of α_2- (acute phase) globulin isolated immunochemically from plasma perfusates than did perfused livers from normal adult rats. Since the presence of radioactivity in plasma perfusate α_2- (acute phase) globulin was confirmed by immunoelectrophoresis and radioautography, it is concluded that the appearance of this acute phase plasma protein after injury *in vivo* is due to stimulation of *de novo* synthesis and secretion by the host liver rather than release of preformed and stored globulin.

Under comparable experimental conditions, perfusion of isolated livers from normal adult rats for 3 hr resulted in only slight but gradually increasing amounts of radioactivity measured in perfusate α_2. However, immunoelectrophoresis and radioautography of 3-hr perfusates did not result in a positive radioautograph. Li *et al.* (1970) reported that the specific activity of L-[1-^{14}C]leucine added to isolated perfused rat liver perfusate at zero time declined exponentially to 4% of initial values after 6 hr, and the concentration perfusate total free leucine increased fivefold. On the other hand, the specific activity of perfusate prothrombin-bound leucine increased linearly after 2 hr in these liver perfusions. Although the specific activity of perfusate [^{3}H]leucine was not measured in the present studies, perfusion of livers from both normal and injured rats resulted in rapid and progressive

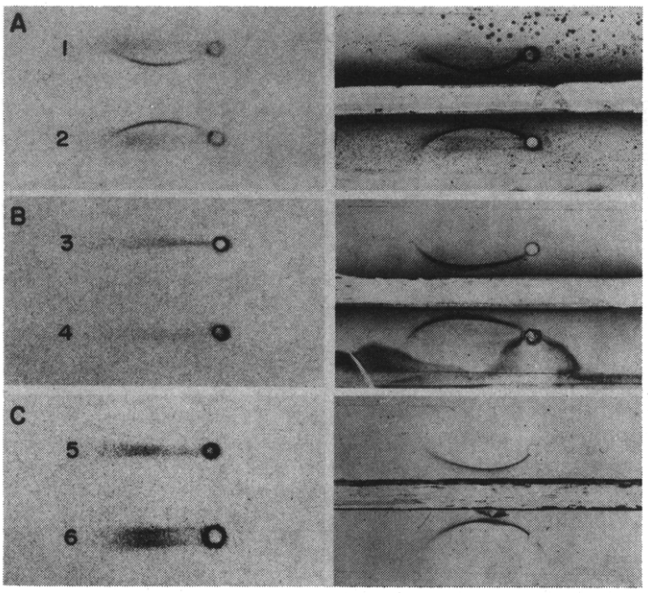

FIGURE 3: Radioautographs (left panel) of immunoelectrophoretic patterns (right panel) of plasma perfusing: liver and blood from injured adult rats (A, experiments 1 and 2); liver and blood from normal adult rats (B, experiments 3 and 4) and after circulation of whole blood from injured adult rats without the liver (C, experiments 5 and 6). Plasma perfusate was sampled after 3 hr. Plasma from injured rats was added to perfusate samples from normal liver and blood perfusions (B, experiments 3 and 4) to provide carrier acute phase globulin. The anode was to the left and the precipitin arcs were obtained with rabbit antiserum reagent to rat α_2- (acute phase) globulin.

incorporation of [^{3}H]leucine into plasma albumin at nearly comparable rates. This finding is consistent with the conclusion that the small amount of [^{3}H]leucine incorporated into plasma α_2 by perfused livers from normal rats cannot be attributed to either changes in [^{3}H]leucine specific activity occurring during liver perfusion or to inability of these perfused normal livers to synthesize and secrete plasma protein. α_2- (Acute phase) globulin can be detected in intact rats as early as 8 hr after injury (Weimer and Benjamin, 1965). It is, therefore, reasonable to assume that the unavoidable injury involved during surgical removal of liver from normal rats could stimulate synthesis and secretion of trace amounts of this protein during 3-hr perfusions. This could explain the slight but gradually increasing amounts of radioactivity measured in perfusate acute phase globulin during 3-hr perfusions of normal livers but not detected by immunoelectrophoresis and radioautography. In accord with this assumption, John and Miller (1969) showed that plasma perfusate levels of α_2- (acute phase) globulin increased rapidly after 8 hr during prolonged perfusions of normal rat livers supplemented with amino acids and hormones. On the other hand, the inability to detect α_2- (acute phase) globulin in concentrated extracts of either liver or serum from normal intact adult rats in this laboratory suggests that the liver in such animals *in vivo* does not normally synthesize and secrete this acute phase protein.

In a previous report (Sarcione and Bohne, 1969) fetal and neonatal rat liver slices, but not normal adult rat liver, were shown to synthesize α_2- (acute phase) globulin giving reactions of immunologic identity with that present in the plasma of injured adult rats. Taken together, these data are in accord with the conclusion that the presence of α_2- (acute phase) globulin in the plasma of injured adult rats reflects hepatic synthesis of a normal fetal-specific globulin rather than an abnormal injury-specific protein. This conclusion suggests that host injury stimulates or activates the biochemical expression of fetal genetic information in adult liver. The transient appearance and disappearance of this plasma acute phase globulin in injured adult rats *in vivo* further suggests that selective fetal gene sites can be reversibly "switched-on" and "switched-off" in adult liver.

References

Benjamin, D. C., and Weimer, H. E. (1966), *Nature 209*, 1032.
Boffa, G. A., Nadal, C., Zajdela, F., and Fine, J. M. (1964), *Nature 203*, 1182.
Bogden, A. E., Neville, G. A., Woodward, W. E., and Gray, M. (1964), *Proc. Amer. Ass. Cancer Res. 5*, 6.
Gordon, A. H., and Koj, A. (1968), *Brit. J. Exp. Pathol. 49*, 436.
Heim, W. G., and Lane, P. H. (1964), *Nature 203*, 1077.
Hochwald, G. M., Thorbecke, G. J., and Asofsky, R. (1961), *J. Exp. Med. 114*, 459.
John, D. W., and Miller, L. L. (1969), *J. Biol. Chem. 244*, 6134.
Li, L. F., Block, J., Kipfer, R. K., and Olson, R. E. (1970), *Proc. Soc. Exp. Biol. Med. 133*, 168.
Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F. (1951), *J. Exp. Med. 94*, 431.
Miller, L. L., Hanavan, H. R., Titthasiri, N., and Chowdhury, A. (1964), *Advan. Chem. Ser. 44*, 17.
Moore, S., and Stein, W. H. (1948), *J. Biol. Chem. 176*, 367.
Sarcione, E. J. (1962), *Biochemistry 1*, 1132.
Sarcione, E. J. (1967), *Cancer Res. 27*, 2025.
Sarcione, E. J., and Bodgen, A. E. (1966), *Science 153*, 547.
Sarcione, E. J., and Bohne, M. (1969), *Proc. Soc. Exp. Biol. Med. 131*, 1454.
Scheidegger, J. J. (1955), *Int. Arch. Allergy Appl. Immunol. 7*, 103.
Somogyi, M. (1945), *J. Biol. Chem. 160*, 69.
Stanislawski-Birencwajg, M., Ureil, J., and Grabar, P. (1967), *Cancer Res. 27*, 1990.
Weimer, H. E., and Benjamin, D. C. (1965), *Amer. J. Physiol. 209*, 736.