Synthesis of Alpha$_2$ (Acute Phase) Globulin by Fetal and Neonatal Rat Liver *in Vitro** (34130)

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An immunologically distinct serum macroglobulin, designated alpha$_2$ (acute phase) globulin, can be detected in fetal, neonatal, pregnant, injured, and tumor-bearing rats, but cannot be detected in normal adult rats (1–4). The liver of adult injured and tumor-bearing rats is known to synthesize this alpha$_2$ (acute phase) globulin (5–7). The transient appearance of this plasma protein suggests the operation of a "switch-off-on" type of control mechanism. For this reason, hepatic synthesis of this globulin was chosen as a model system to study regulation of plasma protein biosynthesis. Since plasma alpha$_2$ (acute phase) globulin can be detected in both fetal and pregnant rats (8), it cannot be readily determined whether it is derived from the fetus, the pregnant host, the placenta, or from two or all of these sources. The present study provides evidence that fetal and neonatal rat liver can synthesize this globulin.

Livers were obtained from Charles River CD rats, as follows: fetal and pregnant rats, at 17–19 days of gestation; neonatal rats 6–8 days old, normal adult male rats weighing 300–350 g. Livers from individual litters of fetal and neonatal rats were pooled. Liver slices (300–350 mg) were incubated in Krebs-Ringer's phosphate buffer, pH 7.4, containing 3 μCi of reconstituted ^{14}C-protein hydrolyzate (Schwarz Bioresearch), for 3 hr at 37° with shaking in air. After incubation, the slice-buffer mixture was frozen and thawed once, then homogenized. The homogenate was brought to a final concentration of 1% sodium deoxycholate. An equal volume of pooled serum from injured adult rats was then added, to provide carrier acute phase globulin. (Injury was produced by the subcutaneous injection of 0.5 ml of turpentine 48 hr before bleeding.) The homogenate was centrifuged and the clear supernatant fluid 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 by covering the bag with powdered sucrose. Radioactive labeling of alpha$_2$ (acute phase) globulin was detected by radioautography of immunoelectrophoretic patterns as described by Hochwald *et al.* (9). Immunoelectrophoresis was performed by the method of Scheidegger (10) using 1.0% Noble agar and 0.1 *M* Veronal buffer pH 8.6. The monospecific rabbit antiserum reagent used has been described previously (6). The antiserum reagent was specific for rat alpha$_2$ (acute phase) globulin present in injured adult rats and did not react with any serum proteins present in normal adult rats. After immunoelectrophoresis the slides were washed for 24 hr in isotonic saline and distilled water, dried, and then placed against sheets of Kodak no-screen industrial type X-ray film and exposed for 4–6 weeks.

The immunologic relationship between alpha$_2$ (acute phase) globulin present in sera of fetal, neonatal, pregnant, and injured rats was examined by immunodiffusion in agar against the absorbed monospecific rabbit antiserum reagent described above. Injury was produced in adult rats by three different methods and in each case blood was obtained 48 hr after injury: (1) subcutaneous injection of 0.5 ml turpentine, (2) cutting open the abdominal wall (4.0-cm incision), then

* Assisted by Grant CA 11098 from the National Institutes of Health, U. S. Public Health Service.

closing the wound with clamps, (3) intraperitoneal injection of cadmium sulfate (1 mg/kg body weight), administered in 1.0 ml of sterile isotonic saline.

Radioautography of these immunoelectrophoretic patterns (Fig. 1) showed that liver from fetal and neonatal rats incorporated ^{14}C-amino acids into alpha$_2$ (acute phase) globulin. Comparison of the labeling intensities suggests that synthesis of this globulin by fetal liver is more active than by neonatal liver. This is in agreement with the relative concentrations of serum alpha$_2$ (acute phase) globulin found in such rats *in vivo* (8). Under identical experimental conditions, liver from pregnant rats and from normal male adult rats did not produce detectable labeling of this acute phase globulin. This indicates that the observed labeling of alpha$_2$ (acute phase) globulin was not a result of nonspecific binding of radioactive amino acids to the precipitin arc or due to liver injury which unavoidably occurs during the slicing and incubation procedures. The lack of detectable synthesis of this globulin by liver slices from normal adult rats correlates well with its absence in the sera of such animals. These findings appear to indicate that in fetal and neonatal rats, the animal's own liver is the source of the alpha$_2$ (acute phase) globulin found in the serum.

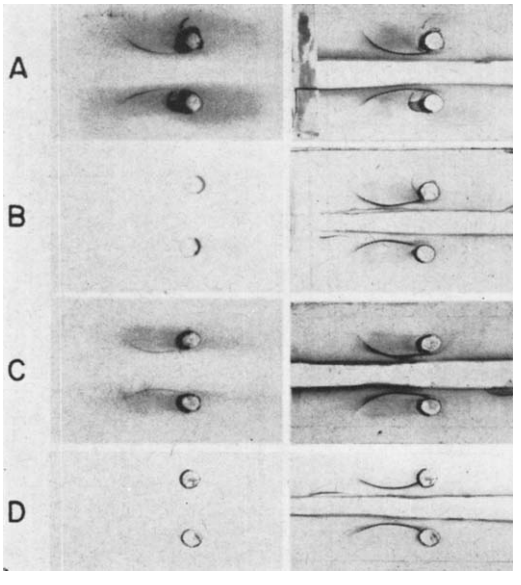

FIG. 1. Immunoelectrophoretic (right side) and radioautographic patterns (left side) obtained by incubating liver slices with ^{14}C-amino acids *in vitro*. Serum from injured rats was added to provide carrier acute phase protein. The precipitin lines were developed using rabbit antiserum reagent to rat alpha$_2$ (acute phase) globulin. A. Pooled 17–19-day fetal livers; B. Pregnant host liver; C. Pooled 6–8-day-old neonatal livers; D. Normal adult male liver.

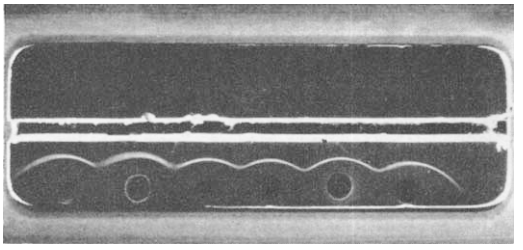

FIG. 2. Double diffusion patterns in agar demonstrating the immunologic identity of alpha$_2$ (acute phase) globulin present in different rat sera. The trough contained rabbit antiserum reagent against rat alpha$_2$ (acute phase) globulin. The wells from left to right contained pooled sera from: fetal rats 17–19 days old; adult rats 48 hr after injury produced by subcutaneous injection of 0.5 ml turpentine; pregnant rats after 17–19 days' gestation; adult rats 48 hr after injury produced by abdominal surgical incision; neonatal rats 6–8 days' old; adult rats 48 hr after injury produced by intraperitoneal injection of cadmium sulfate (1 mg/kg body weight), administered in 1.0 ml of sterile isotonic saline. All sera were used undiluted.

The origin of the small amount of serum alpha$_2$ (acute phase) globulin present in pregnant adult rats remains an open question. Liver slices from such rats apparently cannot synthesize detectable amounts of this protein. It could, therefore, be derived either from the fetal circulation or the placenta. Of interest in this connection is evidence that fetal rat liver and placenta can synthesize an alpha$_2$ protein (11) which may well be identical to the serum alpha$_2$ (acute phase) globulin.

Alpha$_2$ (acute phase) globulin present in sera of fetal, neonatal, pregnant, and injured adult rats all gave reactions of immunologic identity (Fig. 2). Previous studies have demonstrated that liver from injured adult rats can also synthesize this acute phase glob-

ulin (1, 11). Taken together, this information is in accord with the conclusion that the presence of alpha$_2$ (acute phase) globulin in injured adult rats reflects hepatic synthesis of a normal fetal-specific globuin rather than an "abnormal" injury-specific protein. These data further suggest that the DNA cistronic region coding for this protein is present and is "switched-on" in fetal and neonatal rat liver cells, is "switched-off" or repressed in normal adult liver and is again "switched-on" or derepressed in liver of injured adult rats.

Summary. A plasma protein, designated alpha$_2$ (acute phase) globulin, present in fetal, neonatal, pregnant, and injured adult rats, but not detected in normal adult rats all gave reactions of immunologic identity. Incubation of fetal and neonatal rat liver slices resulted in incorporation of ^{14}C-labeled amino acids into this alpha$_2$ (acute phase) globulin. Under identical experimental conditions, incubation of liver slices from pregnant and normal adult rats did not produce detectable labeling of this plasma protein. These findings suggest that (1) fetal and neonatal liver is the source of the plasma alpha$_2$ (acute phase) globulin detected in such animals; (2) the presence of alpha$_2$ (acute phase) globulin in injured adult rats reflects hepatic synthesis of a normal fetal-specific globulin rather than an "abnormal" injury-specific protein; (3) the DNA cistronic region coding for this globulin is "switched-on" in fetal and neonatal rat liver, is "switched-off" or repressed in normal adult liver, and is again "switched-on" or derepressed in liver of injured adult rats.

1. Boffa, G. A., Nadal, A. C., Zajdela, F., and Fine, J. M., Nature **203**, 1182 (1964).

2. Heim, W. G. and Lane, P. H., Nature **203**, 1077 (1964).

3. Weimer, H. E. and Benjamin, D. C., Am. J. Physiol. **209**, 736 (1965).

4. Stanislawski-Birencwajg, M., Uriel, J., and Grabar, P., Cancer Res. **27**, 1990 (1967).

5. Benjamin, D. C. and Weimer, H. E., Nature **209**, 1032 (1966).

6. Sarcione, E. J. and Bogden, A. E., Science **153**, 547 (1966).

7. Sarcione, E. J., Cancer Res. **27**, 2025 (1967).

8. Weimer, H. E., Humelbaugh, C., and Roberts, D. M., Am. J. Physiol. **213**, 418 (1967).

9. Hochwald, G. M., Thorbecke, G. J., and Asofsky, R., J. Exptl. Med. **114**, 459 (1961).

10. Scheidegger, J. J., Intern. Arch. Allergy **7**, 103 (1955).

11. Gitlin, D. and Boesman, M., J. Clin. Invest. **46**, 1010 (1967).

Received Feb. 12, 1969. P.S.E.B.M., 1969, Vol. 131.