SYNTHESIS OF ALL PLASMA PROTEIN FRACTIONS EXCEPT GAMMA GLOBULINS BY THE LIVER*, ‡

The Use of Zone Electrophoresis and Lysine-ε-C^{14} to Define the Plasma Proteins Synthesized by the Isolated Perfused Liver

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A recent report (1) presented direct evidence for the dominant role of the liver in the biosynthesis of the plasma proteins as manifest in a study of the isolated perfused rat liver. The development of the technique of preparative zone electrophoresis (2) has made possible a more exact definition of the qualitative and quantitative nature of the C^{14}-labeled plasma proteins produced by the liver from mixtures of amino acids containing lysine-ε-C^{14} or histidine-2-C^{14}. In brief, the normal perfused rat liver produces plasma proteins with mobilities corresponding to those of all plasma protein fractions except the gamma globulins.

Methods

The plasma specimens studied in these experiments were obtained from seven rat liver perfusions carried out using methods described in a previous report (1). In each case the liver was perfused for 6 hours after the addition of a nutritionally complete amino acid mixture containing 4 to 8 μc. of C^{14} as lysine-ε-C^{14} to the perfusing blood. A large sample of plasma obtained from the blood at the end of the perfusion was frozen and preserved at −30°C. for periods of 2 to 18 months before study. The plasma was then allowed to thaw at room temperature and centrifuged for a short time to remove small amounts of fibrin.

For the purpose of comparison, normal adult male Wistar strain rats maintained on a stock diet of Purina checkers and weighing 300 to 350 gm. were injected intraperitoneally with 6.7 to 13.4 mg. of DL-lysine-ε-C^{14} (7 to 14 μc.) or 10.6 mg. of L-histidine-2-C^{14}·HCl (25 μc.), and allowed to remain without food for 6 hours. At this time they were exsanguinated by cardiac puncture. Heparin (liquaemin Roche), 1 mg. per 10 ml. of blood, was used as anticoagulant. The plasma was removed after centrifugation, stored frozen, and allowed to thaw at room temperature before electrophoresis.

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The procedure of preparative zone electrophoresis used was essentially as described by Kunkel and Slater (2) with potato starch as a supporting medium, except that the plasma specimens were not dialyzed against veronal buffer before the electrophoretic separation. The electrophoretic separations were carried out in a cold room (3–5°C.) at 400 to 500 volts, 24 to 32 milliamperes, for 24 to 36 hours. Typically with 2 to 5 ml. of plasma set up in a starch plaque 0.8 to 1.0 cm. thick, 9 cm. wide, and 28 cm. long, the albumin fraction migrated 11 to 15 cm. from the origin toward the anode while the gamma globulin moved 5 to 8 cm. from the origin toward the cathode, as a result of electroosmosis.

After electrophoresis was completed, the plasma was separated into 20 to 25 fractions by cutting the starch plaque into segments 1 cm. wide. The protein was eluted from each fraction by suspension in and mixing with 5 ml. of 0.9 per cent sodium chloride, and the starch separated by filtration on a sintered glass funnel of medium porosity. The starch was washed twice on the funnel each time with about 2 ml. of saline.

The protein in the combined filtrates of each fraction was then precipitated with trichloracetic acid (final concentration 6 per cent) in the presence of carrier DL-lysine or L-histidine, and after centrifugation, washed twice more with trichloracetic acid containing DL-lysine or L-histidine. Finally the protein precipitates were dissolved in the smallest volume of 0.1 N NaOH necessary to give a clear solution, which was made up to a known volume (2 to 8 ml.). Duplicate aliquots of each fraction were removed for protein analysis either by micro-Kjeldahl analysis or by a modification of the Kingsbury, Clark sulfosalicylic acid turbidity method (3). It was found that the results obtained with the latter method as arbitrarily standardized against crystalline egg albumin showed agreement with the Kjeldahl method for the alpha globulins, beta globulins, and gamma globulins with deviations generally not exceeding plus or minus 20 per cent. However in the determination of the protein content of the albumin fractions by the turbidimetric method, the results were as much as 80 per cent higher than the Kjeldahl values. In later experiments we have used the Folin reagent as modified by Lowry (4). Although this method standardized against egg albumin gives results in good agreement with the Kjeldahl method for the alpha, beta, and gamma globulins of rat plasma, the albumin values are as much as 25 per cent low. The plasma proteins remaining in each fraction were converted to carbon dioxide and the gas assayed for radioactivity by the method of Bale (5), which entails the use of an ionization chamber coupled with a dynamic vane electrometer.

By totaling the protein and radioactivity contents of all the fractions it became apparent that 20 to 25 per cent of the protein and radioactivity were retained by the waxed paper wrapping used in the electrophoretic procedure and particularly by the starch from which the protein could not be quantitatively eluted even with large volumes of alkaline (0.1 N NaOH) 0.9 per cent saline. In several experiments the proteins were precipitated from the large volumes of wash solution, the amount of protein precipitated measured as above, and its radioactivity measured. The resulting calculated specific activity approximated the mean value for the specific activity of all the protein fractions. This result presumably is in accord with the view that under the conditions of the electrophoretic separation, the starch does not preferentially adsorb any particular fraction of the proteins. The failure to recover quantitatively the proteins from the starch used in electrophoresis in no way detracts from the validity of the conclusions which may properly be drawn from the specific activity data.

EXPERIMENTAL RESULTS

For the purpose of reference and comparison, Fig. 1 presents the results of the electrophoretic separation of 2.0 ml. of pooled plasma obtained from intact normal male rats 6 hours after the injection of DL-lysine-ϵ-C^{14}. The electrophoretic pattern obtained is qualitatively analogous to those patterns obtained

from studies of rat plasma in free electrophoresis (6). As was to be expected, all of the plasma protein fractions contained C^{14} activity. It is noteworthy that the specific activity curve shows the alpha globulins to have a specific C^{14} activity at least twice that of any other fraction. The plasma protein specimens were obtained from blood drawn at a time when the total plasma protein

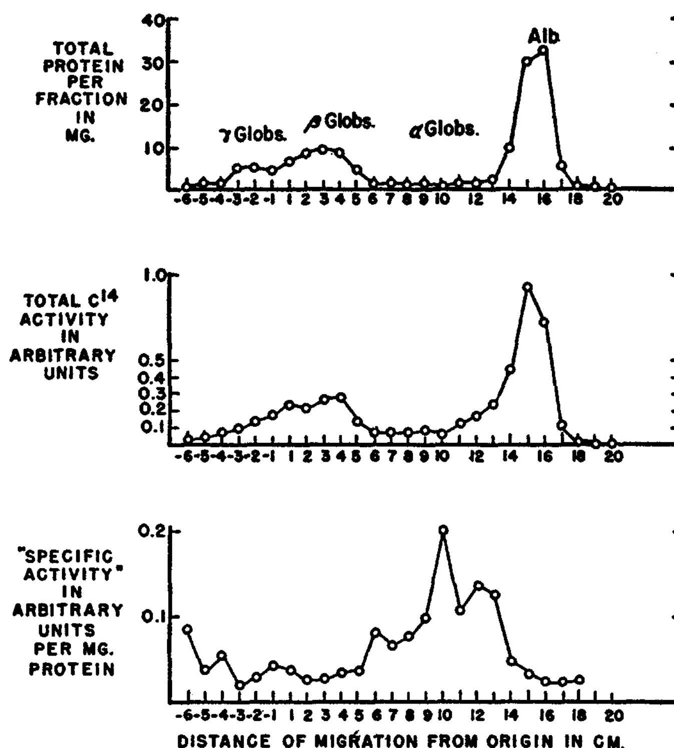

FIG. 1. Electrophoretic separation of plasma protein fractions of plasma from normal intact rats given DL-lysine-ε-C^{14}. Zone electrophoresis of 3 ml. donor rat plasma obtained from rats 6 hours after intraperitoneal injection of DL-lysine-ε-C^{14} (6.8 mg., about 7.3 μc.). "Specific activity" referred to here and in Fig. 2 is obtained by dividing the total C^{14} activity in a given fraction by the total estimated weight of protein in milligrams. Each arbitrary unit of C^{14} activity is equal to about 2.0×10^4 disintegrations per minute.

activity is maximal, and in the normal intact rats used there was no reason to presume an abnormally large incorporation into the alpha globulins. Since the alpha globulins do not have a much larger content of lysine in comparison with the other plasma protein fractions, and since the concentration of alpha globulins in the plasma remains low, the above results may be taken to indicate that normally the alpha globulins are turned over at a more rapid rate than any of the other plasma protein fractions. Because closely similar results were ob-

tained from electrophoretic study of whole plasma obtained from normal intact rats injected with histidine-2-C^{14}, the data are not presented here.

The results of the electrophoretic separation and C^{14} assay of the labeled plasma proteins obtained at the close of 6 hours' perfusion of the isolated rat

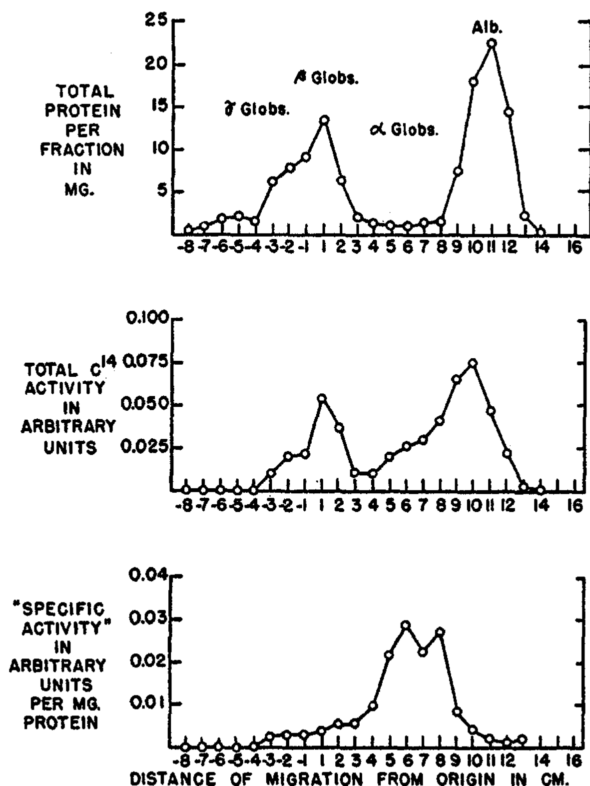

FIG. 2. Electrophoretic separation of C^{14}-labeled plasma proteins from isolated perfused liver. Zone electrophoresis of 5 ml. plasma obtained from blood 6 hours after onset of perfusion of normal liver with 192 ml. of heparinized rat blood containing 327 mg. of a complete amino acid mixture (1) and 6.76 mg. of DL-lysine-ε-C^{14}, 7.3 μc. Protein content of fractions determined by method of reference (3).

liver with blood containing a complete amino acid mixture (1) and DL-lysine-ε-C^{14} are exemplified by Fig. 2. It is at once apparent that all of the plasma protein fractions except the gamma globulins contain appreciable radioactivity. Again it is of interest to note that the alpha globulins have a higher specific activity than the albumin or the beta globulins. This result is in keeping with the above stated view that the normal animal produces the alpha globulins more rapidly than albumin or the beta globulins, and by the same token, that

the normal turnover of the alpha globulins occurs more rapidly than that of the other plasma protein fractions produced by the perfused liver. Except for the gamma globulins the curve of total C^{14} incorporation for the plasma proteins produced by the isolated perfused liver is similar in the gross to the corresponding curve for the plasma proteins of the intact animal.

DISCUSSION

The electrophoretic separation of the C^{14}-labeled plasma proteins produced by the isolated perfused liver from perfused amino acids including lysine-ϵ-C^{14} makes more definitive the previous demonstration (1) of the dominant role of the liver in the biosynthesis of the plasma proteins. This, taken together with the observations on the minor role of the non-hepatic tissues (7), should leave little doubt concerning the function of the liver in the synthesis of all the plasma albumin and virtually all the alpha globulins as well as the beta globulins including fibrinogen.

If the liver produces all of the plasma protein fractions except the gamma globulins, it may be anticipated that the normal production of the gamma globulins is primarily a function of the non-hepatic tissues. This is exactly what was found when surviving eviscerated rats were injected intravenously with DL-lysine-ϵ-C^{14} and the plasma proteins separated electrophoretically (7).

If we may presume that the liver of other mammalian species produces plasma proteins in a manner at least qualitatively similar to that of the rat liver, we are now able to offer a firm experimental demonstration of the long suspected role of the liver (8) as the sole source of plasma albumin. The dominant if not exclusive role of the liver in the biosynthesis of the alpha and beta globulins is somewhat surprising yet it is in harmony with the observed failure of some cirrhotic mammals to respond normally to acute infections with an increased α_2 globulin peak as noted in electrophoretic patterns (9). Furthermore, to the extent that the mucoproteins of the plasma may be reckoned with the alpha globulins (10) at least during electrophoresis at pH 8.5, the observed lowering of plasma mucoprotein levels with serious liver disease (11) may be understood.

Along with the marked diminution of the plasma albumin content, perhaps the most striking abnormality of the plasma proteins observed in chronic liver disease is the prominent increase in the gamma globulin fraction especially as estimated from electrophoretic patterns (12). Observations of the turnover time of I^{131}-labeled normal gamma globulins in human beings with cirrhosis reveal that the gamma globulins are turned over more rapidly in cirrhotic than in normal human beings (13, 14). It appears likely that the gamma globulins accumulate in cirrhotic persons because of enhanced production rather than diminished removal. The enhanced production of antibodies in response

to the administration of diphtheria toxoid noted in chronic hepatic disease (15), and in acute hepatitis (16) as compared with the production in normal individuals supports this view.

There are at least three factors which may favor excessive gamma globulin production in chronic hepatic disease. The first depends on the degree of metabolic derangement with respect to the liver's normal ability to remove from the blood and concentrate amino acids, as well as to oxidatively destroy excess amino acid substrate brought to the liver and not used for protein synthesis by the liver. The second depends on the degree of portal obstruction and relates to the anatomic diversion of amino acid substrate in portal vein blood from the liver *via* collaterals to non-hepatic tissues. The third factor is concerned with the ability of the non-hepatic tissues to produce in the main only gamma globulins with comparatively small but detectable amounts of beta globulins and traces of alpha globulins (7). To these factors must be added the increased potential capacity to produce gamma globulins as manifest by increased numbers of plasma cells (17) as reviewed by Kolouch *et al.* (18).

To the extent that the antibody proteins are regarded as gamma globulins, it may be concluded that antibodies are not produced by the liver but by the extrahepatic tissues. Our experimental results do not permit a precise conclusion but are compatible with the view that the antibody gamma globulins are elaborated by radiosensitive cells of the hematopoietic system. The plasma cells and the lymphocytes including the morphologically ill defined round cells have all been proposed (19) as the cells of the reticuloendothelial system which are primarily concerned in antibody gamma globulin production.

Thus, although massive dose (LD_{100} to LD_{50}) whole body radiation of the rat and rabbit profoundly depress or completely eliminate the production of specific antibodies (20), we have found that such irradiation depresses only the incorporation of C^{14}-labeled lysine into the gamma globulins. The synthesis of the other plasma protein fractions as estimated by the incorporation of C^{14}-labeled lysine into the plasma proteins by the massively irradiated intact rat and rabbit or by the isolated perfused livers from the irradiated rat is not depressed. On the contrary, the synthesis of plasma fibrinogen and the alpha globulins appear to be enhanced (21). Nor is there solid experimental support for the notion that the reticuloendothelium of the liver is either radiosensitive or directly concerned in the final elaboration of antibodies. Yet some workers (22) have by broad inference ascribed an antibody-producing function to the liver solely on the basis of the localization of I^{131}-labeled antigen in the liver cell microsomal fraction.

A preliminary report of *in vitro* experiments describes (23) synthesis of antibody proteins by liver slices, as estimated by the incorporation of C^{14}-labeled glycine into proteins precipitable with a specific antigen. Yet tissue culture of liver from immunized rabbits (24, 25) has failed to produce detectable

antibody. Unpublished data from spleen perfusion experiments carried out in our laboratory are in agreement with all the above reports concerning the ability of the spleen from immunized rabbits to elaborate specific antibody proteins *in vitro*.

SUMMARY

Lysine-ε-C^{14}-labeled plasma proteins produced by the normal rat and the isolated perfused rat liver have been fractionated by preparative zone electrophoresis. The isolated perfused liver incorporates lysine-ε-C^{14} into the plasma albumin, alpha globulin, and beta globulin (including fibrinogen) fractions. No significant C^{14} incorporation into the trichloracetic acid-precipitable proteins of the gamma globulin fraction was observed.

Presumptive evidence indicates that the alpha globulins turn over more rapidly than any other major plasma protein fraction.

The increased production of gamma globulins in liver disease is discussed.

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