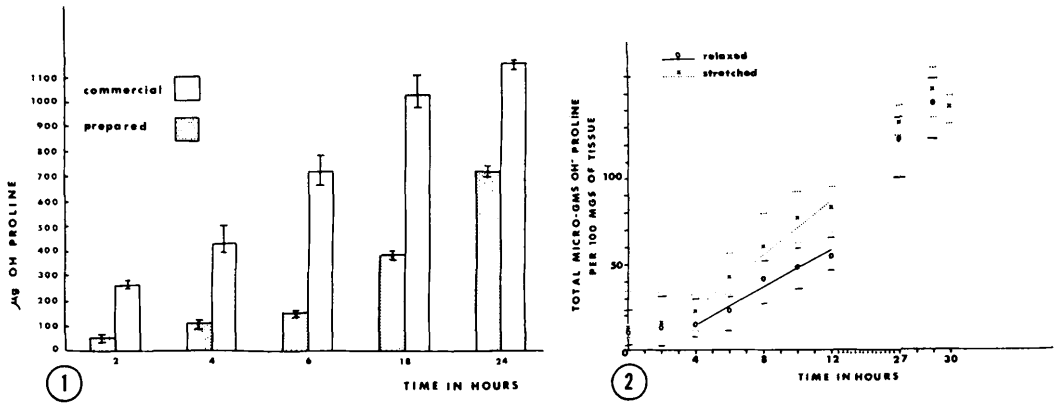

FIG. 1. Comparative release of hydroxy proline from prepared collagen and commercial undenatured collagen on incubation with collagenase.
FIG. 2. Hydroxy proline release from stretched and relaxed bovine tendon.

g weight, which produced the tension. The silk of the control collagen loop was tied loosely to suspend it in a relaxed position. Twenty-five milliliters of a solution containing 200 μg per ml of collagenase (Cal Biochem) adjusted to pH 7 Tris buffer, was added to each tube and incubated at 35°C. To measure the breakdown of collagen 1 ml samples of the enzyme solution were withdrawn periodically and their content of hydroxy proline determined by the method of Leach(7), using a Bausch and Lomb colorimeter with a 560 mμ filter. The results of 5 pairs of runs are given in Fig. 2. In all instances there was more rapid breakdown of the stretched tendons. The hydroxy proline release from the stretched tendons during the first 12 hours was significantly ($t = 4.65$) greater than from the relaxed ones, but after 48 hours this difference had disappeared.

Our findings justify suggesting that traumatic occlusion may speed up the destruction of the collagenous supporting structures of the tooth by making them more susceptible to the action of enzymes. Possibly like reactions occur in other arthropathies.

1. Lunseth, John H., Science, 1963, v141, 438.
2. Chiricuta, I., Mustea, I., Ind Usoara (Bucharest), 1960, v7, 8, Chem. Abs., 1961, v55, 19289.
3. Rigby, B. J., Biochem. Biophys. Acta, 1964, v79, 634.
4. Sizer, I. W., Enzymologia, 1949, v13, 293.
5. Einbinder, J., Schubert, M., J. Biol. Chem., 1951, v188, 335.
6. Mandl, I., *et al.*, Arch. Biochem. & Biophys., 1958, v74, 465.
7. Leach, A. A., Biochem. J., 1960, v74, 70.

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Hypergammaglobulinemia in Experimental Liver Injury. (32506)

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Chronic liver injury in both experimental animals and man is characterized by a significant increase in serum gamma globulin. The degree of hypergammaglobulinemia is related to the number of gamma globulin producing cells in the bone marrow(1), lymph nodes(2) and liver(3). It has been suggested that reactivity of these cells is a major determinant of the level of serum gamma globulin, however, the relationship of fixed cells in the liver to gamma globulin production has not been established. The present investigation

was undertaken to examine further the ability of the isolated perfused normal and diseased liver to produce gamma globulin. Observations were made on the influence of experimental chronic liver injury on serum protein patterns and incorporation of C^{14} lysine into gamma globulin.

Materials and methods. Male Sprague-Dawley rats weighing 125 to 150 g were maintained on Purina Chow and given 0.2 ml of CCl_4 subcutaneously 3 times a week for periods of 3 to 6 months. At monthly intervals, 48 hours after receipt of the last dose of CCl_4 serum protein electrophoresis and incorporation of tritiated thymidine (H_3T) into hepatic DNA was studied in randomly selected experimental and litter mate normal control animals. Four hours after intraperitoneal injection of 1 μc per gram weight of H_3T, 5 ml of blood was obtained by cardiac puncture under ether anesthesia for serum protein electrophoresis and the liver was removed for morphologic studies. An autoradiographic technique was used to assess to degree and localization of hepatic DNA synthesis(4).

The livers from 6 healthy control animals and 6 animals with CCl_4-induced cirrhosis were perfused using a modified technique of Miller(5) to study the incorporation of C^{14} lysine into gamma globulin. The perfusate consisted of 180 ml of heparinized rat blood, 4 cc of a mixture containing all of the essential and nonessential amino acids, and 10 μc of C^{14} lysine (specific activity 222 mc/nm.* An aliquot of the perfusate was removed, centrifuged, frozen and stored. Goat serum which contained antibody against rat gamma globulin† was used to precipitate gamma globulin in the perfusate. This material did not contain any detectable antibodies against beta globulin. The perfusate was diluted 1:16 with 0.1 N EDTA to prevent precipitation of complement and equal amounts of anti-gamma globulin added. After standing in the refrigerator overnight, the precipitate was removed by centrifugation and washed 8 times until radioactivity not incorporated into gamma globulin was eliminated. The precipitate was dissolved in 0.1 N NaOH, the volume brough to 2 ml and radioactivity determined in 1 cc aliquots using the Packard Tricarb Liquid Scintillation Counter.

Results. Histologic sections revealed normal liver in litter mate control animals, moderate active cirrhosis in animals receiving CCl_4 for 3 months and severe active cirrhosis in those given CCl_4 for 6 months. Autoradiographs showed 30 to 40 labeled nuclei per 100,000 liver cells in the normal liver and 7,000 to 10,000 labeled nuclei per 100,000 liver cells in animals with active cirrhosis. A 4:1 ratio of labeled mesenchymal to liver cells was present in the normal liver; this ratio was 8:1 to 12:1 with active cirrhosis. Labeled mesenchymal cells included Kupffer cells, fibroblasts, plasmacytoid cells and undifferentiated mesenchymal cells.

Serum gamma globulin in normal control animals ranged between 0.9 and 1.2 g per cent. A progressive rise in serum gamma globulin and a decrease in serum albumin was noted with chronic liver injury. An absolute increase in total protein was noted; the mean level of serum gamma globulin was 2.5 g per cent ± 0.45 S.D. after administering CCl_4 for 3 months and 3.2 g per cent ± 0.30 S.D. after 6 months of CCl_4. Autoradiographs showed labeled C^{14} lysine in mesenchymal cells of both the normal and cirrhotic liver. There was a 3-fold greater incorporation of C^{14} lysine into serum gamma globulin by the

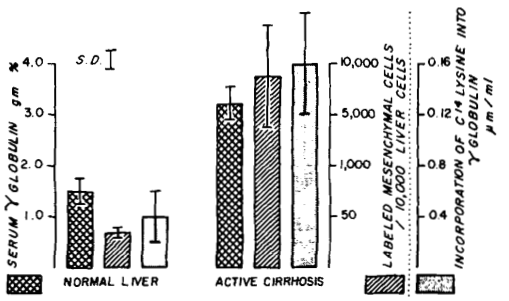

FIG. 1. Comparison of serum gamma globulin, labeled mesenchymal cells and incorporation of C^{14} lysine into gamma globulin in animals with normal liver and active cirrhosis.

* Obtained from New England Nuclear Laboratories.

† Prepared from commercial goat serum containing anti-rat gamma globulin obtained from Hyland Laboratories.

cirrhotic as compared with the normal isolated perfused liver (Fig. 1).

Discussion. Previous studies using the isolated perfused rat liver and zone electrophoresis indicate that this organ is the exclusive site of albumin synthesis and the major site of alpha and beta globulin production(5,6). Protein separation by immunologic techniques in the present study shows that gamma globulin is also synthesized by the isolated perfused normal rat liver. Kinetic studies suggest hypergammaglobulinemia in human liver disease results from increased production of gamma globulin; there is no evidence that decreased catabolism is contributory. Approximately 3% of IgG(7) is catabolized each day, 10 to 15% of IgM(8) and IgA(9) are catabolized daily.

Active cirrhosis induced by CCl_4 is characterized by hyperplasia and increased function of both hepatic and extrahepatic mesenchymal cells(10). The increased population of mesenchymal cells include gamma globulin producing, phagocytic, hemopoietic and fiber-forming cells(11). A cause and effect relationship between gamma globulin production and hepatic mesenchymal cell replication is suggested by (a) the concomitant increase in serum gamma globulin and mesenchymal-plasmacytoid cells in chronic active cirrhosis and (b) increased incorporation of C^{14} lysine into serum gamma globulin by the isolated perfused cirrhotic liver. Further studies are desirable to determine the exact contribution of hepatic and extrahepatic reticuloendothelial cells to individual components of the elevated serum gamma globulin encountered in various pathologic states.

Summary and conclusions. The isolated perfused cirrhotic rat liver which is the site of mesenchymal cell proliferation incorporates 3 times as much C^{14} lysine into serum gamma globulin as the normal liver from litter mate controls. This data is interpreted as further evidence that hypergammaglobulinemia in active cirrhosis results partly from persistent increase of gamma globulin producing mesenchymal cells in the liver.

1. Jarrold, T., Vilter, R. W., J. Clin. Invest., 1949, v28, 286.
2. Glagov, S., Kent, G., Popper, H., Arch. Path., 1959, v67, 9.
3. Cohen, S., Ohta, G., Singer, E. J., Popper, H., J. Exp. Med., 1960, v111, 285.
4. Leevy, C. M., George, W. S., Deysine, M., Gnassi, A. M., Exp. & Molec. Path., 1962, v1, 457.
5. Miller, L. L., Bale, W. F., J. Exp. Med., 1954, v99, 125.
6. Miller, L. L., Bly, C. G., Bale, W. F., ibid., 1954, v99, 133.
7. Solomon, A., Waldmann, T. A., Fahey, J. L., J. Lab. Clin. Med., 1963, v62, 1.
8. Barth, W. F., Wochner, R. D., Waldmann, T. A., Fahey, J. L., J. Clin. Invest., 1964, v43, 1036.
9. Solomon, A., Tomasi, T. B., Jr., Clin. Res., 1964, v12, 452.
10. Paumgartner, G., Longueville, J., Leevy, C. M., Determinants of phagocytosis in liver injury, Proc. Internat. Assn. for Study of Liver, in press.
11. Schaffner, F., Barka, T., Popper, H., Exp. & Molec. Path., 1963, v2, 419.

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Separation of Anticomplementary Material and Plasminogen from a Cytotoxic Factor Active Against Ehrlich Ascites Cells in Cohn Fractions I-III by Fluorocarbon and n-Butanol.* (32507)

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It has been previously shown that normal human serum possesses a complement-dependent cytotoxic factor (CyF) active against Ehrlich ascites tumor cells(1-7). The CyF,

* Part of this work was done in the Dept. of Exp. Med. and Cancer Research, Hebrew University—Hadassah Medical School, Jerusalem.