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Influence of Reticulo Endothelial Blockade on Turnover Rate of Homologous Plasma Proteins in Mice. (24376)

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(Introduced by B. W. Zweifach)
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Studies on formation of plasma proteins have established that parenchymal cells of liver are the main site of synthesis of serum albumin, α and β globulin and fibrinogen(1) and that part of the β globulin and all of the γ globulin is formed by basophilic cells (plasma cells) in lymphoid tissue and bone marrow(2,3,4). However, the cells primarily responsible for the catabolism of plasma proteins have not been identified. Cells of the reticulo endothelial system, particularly those localized in liver and spleen, are specialized to phagocytize different substances like carbon particles(5,6), bacteria, saccharated iron oxide(6), and cholesterol(7). Previous experiments have shown that heat denatured serum proteins(8), or proteins denatured by other methods, are taken up by the reticulo endothelial cells. It was demonstrated that such proteins can compete with clearance of carbon particles from blood by these cells(9). Possibly cells of the reticulo endothelial system also have a function in catabolism of normal, circulating serum proteins. Therefore, it was decided to study whether an impairment of the clearing function of the reticulo endothelial system by blockade would affect the turnover rate of S^{35} labelled homologous plasma proteins.

Materials and methods. Mice were used because turnover rate of their serum proteins is so rapid that, in a comparatively short time, these experiments could be carried out through several serum protein half lifes. The use of internally S^{35} labelled proteins seemed advisable, since externally labelled iodinated proteins may contain fractions that are slightly denatured and, therefore, handled differently by the animals. *R.E.S. blockade.* During experiments, blockade of R.E.S. was maintained by intravenous injections of 1 ml/100 g of a 25% solution of thorium oxide (thorotrast*) every 48 hours. In the first part of the experiments, effectiveness of such a blockade was investigated in about 20 animals. Carbon clearance rates were determined in groups of mice every 24 hours during 5 days of the experiment. Extent of blockade was investigated by measuring rate of clearance from blood of a colloidal carbon suspension (C11-1431 a†, particle size 250 Å), prepared as described previously(5). In these mice a dose of 16 mg carbon/100 g of body weight was used. Blood samples of 0.025 ml were taken from the retro-orbital venous plexus at various time intervals after intravenous injection of carbon. Carbon concentration in the samples was measured spectrophotometrically at 675 mμ wave length. It has been shown that rate of phagocytosis of a carbon suspension as related to time follows an exponential equation $K = \frac{C_o - C_t}{t}$, where K is a measure of phagocytic activity for the injected dose. Clearance curves were drawn for each animal and values for K calculated according to this equation. Mean values for K in control animals and in blocked animals at specific times are recorded in Fig. 1. Values of K in blocked animals are much smaller than those observed in control mice. This shows that an effective blockade of the R.E.S. has been successfully maintained during the experiment, when turn-

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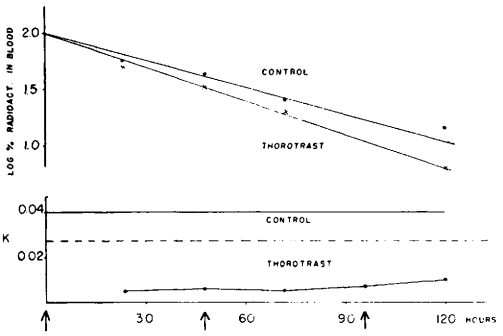

FIG. 1. Effect of blockade on turnover rate of internally S^{35} labelled plasma protein. Arrows indicate thorotrast injections. Upper part of Fig. represents plasma protein turnover in thorotrast blocked and contol mice. Lower part shows carbon clearance rates (K) in groups of mice receiving the same thorotrast treatment. Phagocytic indices K are recorded for the dose of 16 mg carbon/100 g. Broken horizontal line indicates lowest value obtained in control group.

over of plasma protein was measured. *Turnover rates of plasma proteins.* Two mice were injected with 180 mg methionine containing 1.5 mc of S^{35}. Four injections were given intraperitoneally in 20 hours. The mice were bled 8 hours after last injection and heparinized plasma harvested. A volume of 0.05 ml of plasma, diluted to 0.25 ml and containing 100,000 c.p.m., was injected intravenously into each of 10 mice. Five mice were used as controls and another 5 received a blockade treatment similar to the one described above. In all animals daily blood samples were drawn, starting 24 hours after intravenous administration of S^{35} labelled plasma proteins. The first thorotrast injections were given immediately after first blood samples had been obtained. Thorotrast injections were repeated every 48 hours for a total of 3 doses. *Sampling technics.* Mice were bled from the retro-orbital venous plexus with a calibrated glass pipet previously heparinized and dried. Blood samples of 0.05 ml were obtained and centrifuged in capillary tubes. After separation of cells and plasma by centrifugation, 0.01 ml plasma samples were plated, usually in duplicates, in steel planchets. After drying at 96°C they were counted in a Geiger Müller counter. For each animal radioactivity was expressed as percentage of radioactivity that existed in plasma when the first sample was taken, the latter being considered as 100%. Mean values for each group of 5 animals are plotted in Fig. 1. After the last plasma samples were taken, control and blocked mice were injected with 16 mg of carbon/100 g body weight and rates of carbon clearance determined. The mean value for K, calculated from these clearance curves, is recorded in Fig. 1 as the K value at 120 hours after first thorotrast injection.

Results. The results in Fig. 1 show that injection of thorotrast every 48 hours maintains an effective reduction of ability of R.E.S. to clear colloidal carbon particles from blood. While values for K in control animals varied from 0.027 to 0.070, those for the blocked animals varied from 0.004 to 0.009 and at end of experiment from 0.007 to 0.016.

Yet the turnover rate of homologous plasma protein was not decreased but, instead, was either normal or somewhat faster in the blocked than in the control mice. The value obtained in the control mice for the half life of total plasma protein was 37 hours, as compared to 30 hours in the thorotrast treated animals.

Treatment with thorotrast caused other changes that should be mentioned. The mice lost, on the average, 4 g body weight in the 6 days. Liver size increased from an average of 1.4 to 2.0 g and spleen size from 190 to 440 mg. This illustrates the considerable proliferative response observed when attempts are made to block the R.E.S.

Discussion. The 37 hours half life observed for total plasma proteins in normal mice is in complete agreement with what has been described for I^{131} labelled homologous serum proteins in mice by Dixon *et al.*(10,11). The observation that blocked mice showed a half life of 30 hours indicates that repeated injections of colloidal particles, which reduce clearing ability of R.E.S., do not reduce rate of plasma protein turnover but rather seem to cause an increase of this rate. These results are in agreement with the observations of Freeman *et al.* in carbon blocked rats(12). It is possible that increased catabolism, evidenced by loss of weight, may be responsible for the somewhat shorter half life of plasma proteins in blocked animals. The extent to which a possible enhanced excretion of pro-

teins in urine could be a factor has not been investigated.

It appears, then, that denatured and non-denatured serum proteins are not removed by the same mechanism, since rate of clearance of denatured proteins is very much altered by previous blockade of the R.E.S.(13,14).

Our data do not exclude that R.E.S. plays a part in turnover of plasma protein, but merely indicate that, if it does so, it is not through the same mechanism by which it usually handles colloidal suspensions. It is likely, however, from the work of Gordon(13), with perfusion of isolated livers from rats, that normal serum proteins are not predominantly broken down in the liver where the most active part of R.E.S. is found(6).

It seems, therefore, that other cells or possibly all body cells(16) are involved in this process. The observations of Coons(15) showing localization of normal foreign serum proteins in the parenchymal cells of organs like liver, kidneys and adrenals and in vascular endothelium, are in favor of this interpretation, though, of course, the fact that cells take up protein may not mean that they catabolise it.

Summary. 1) It was shown that 3 intravenous injections of thorotrast every 48 hours could induce an effective reticulo endothelial blockade in mice over 1 week. 2) S^{35} labelled mouse plasma proteins were transferred intravenously to 2 groups of 5 normal mice and 5 mice which then received such blockade treatment. 3) The average half life of labelled plasma proteins was 37 hours in the normal and 30 hours in the blocked mice. 4) The significance of these findings, as related to a possible function of the reticulo endothelial system in catabolism of plasma proteins, is discussed.

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Blood Leucocyte Response in Rats Fed a Magnesium Deficient Diet.* (24377)

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The magnesium deficiency syndrome in experimental animals is well known and reproducible(1,2,3,4). In the course of experiments designed to study the effects of magnesium deficiency, marked changes in blood leucocyte counts were observed which to our knowledge have not been previously reported. A general leucocytosis, which reached a peak

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