slopes (1.3×19^{-3} min^{-1}) and between plasma and edema fluid slopes (1.9×10^{-3} min^{-1}) were both statistically significant.

Discussion. The impairment of daytime glomerular filtration rates in this series of cases of cirrhosis and congestive heart failure was evident in the decreased rate of plasma clearance of mannitol. In the measurement of the mannitol space by the infusion-slope method, a basic requirement is that mannitol be uniformly cleared from all recesses of the extracellular compartment. The differences in slope values between plasma vs. ascitic fluid, and plasma vs. interstitial edema fluid, indicated that this requirement was not met in the edematous states studied.

It was of particular interest to note that the mannitol was cleared at a more rapid rate from ascitic fluid than from peripheral edema fluid. Other data indicate that mannitol and inulin equilibrate at a faster rate in ascitic fluid than in peripheral edema fluid(13). This conflicts with any *a priori* concept that ascitic fluid is relatively adynamic in contrast to peripheral edema fluid, and is in agreement with recent findings on the rapid exchange of labelled protein in experimental ascites(14).

Conclusions. 1. The plasma clearance of mannitol observed during morning hours was significantly decreased in this series of cases of cirrhosis with ascites and congestive heart failure with edema. 2. Rates of diffusion of mannitol from various recesses of the extracellular compartment were not uniform in two edematous states, namely, cirrhosis with ascites and congestive heart failure with edema. 3. The mannitol method as determined by the infusion-slope procedure cannot be applied to the measurement of the extracellular fluid compartment in certain edematous states.

The authors are indebted to Dr. Smith Freeman for his helpful criticisms in the planning and execution of these studies. The authors also wish to gratefully acknowledge the support and unfailing interest of Dr. Charles B. Puestow, Chief of the Surgical Services and Dr. Lyla A. Baker, Chief of the Medical Services at Hines Hospital.

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Received September 6, 1951. P.S.E.B.M., 1952, v79.

Plasma Protein. IV. Results with Generally Labeled Plasma Protein.* (19287)

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We have reported(1,2) on the metabolism of plasma protein labeled by feeding donor rats serine-β-C^{14} and administering the C^{14}-

* Our thanks are due to the American Cancer Society which, upon recommendation of the Committee on Growth of the National Research Council, provided us with funds which permitted us to continue these investigations.

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labeled protein to recipients. In these experiments it was found that the C^{14}-labeled protein rapidly disappeared from the blood circulation when intravenously injected, and rapidly appeared in the blood circulation when intraperitoneally injected. Metabolism of the protein occurred at such a rate as to give a half life to 3.1 days. The maximum rate of $C^{14}O_2$ production was attained in 2 to 4 hr whether the labeled protein was given intravenously or intraperitoneally. After oral administration of the labeled protein the rate of $C^{14}O_2$ production reached its maximum still earlier in the experiment but its activity fell rather rapidly. The C^{14} also appeared in the tissues, very rapidly in some such as liver, more slowly in others such as muscle.

It was thought advisable to check these results using a different method of labeling the proteins in the donor animals. To this end we have fed the donors killed C^{14}-labeled *Rhodospirillum rubrum* organisms. In these organisms all the amino acids were labeled though perhaps not uniformly. Data concerning their preparation will be given in another communication.‡ The results show that the rate of loss of these two types of labeled protein from the circulation is the same but that there are differences in the rate of appearance of the C^{14} in the respiratory carbon dioxide and in the tissues.

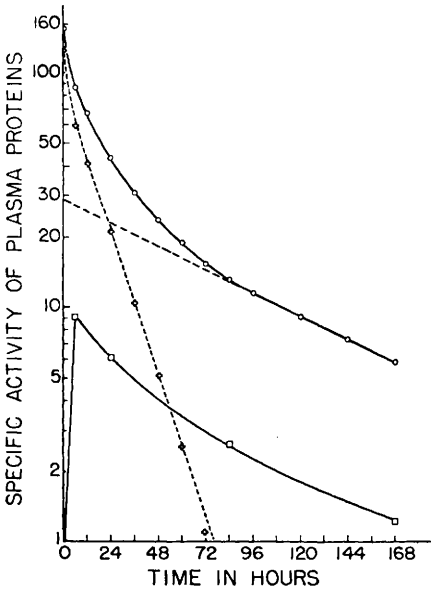

FIG. 1. Semi-log plot of specific activity of plasma protein of Recipients B. Upper unbroken curve (circles) represents average values obtained from 4 rats given plasma protein intravenously and 4 rats given plasma protein intraperitoneally (after 6 hr in the latter); lower unbroken curve (squares) values from 5 rats given plasma protein orally. Regarding broken curves see text.

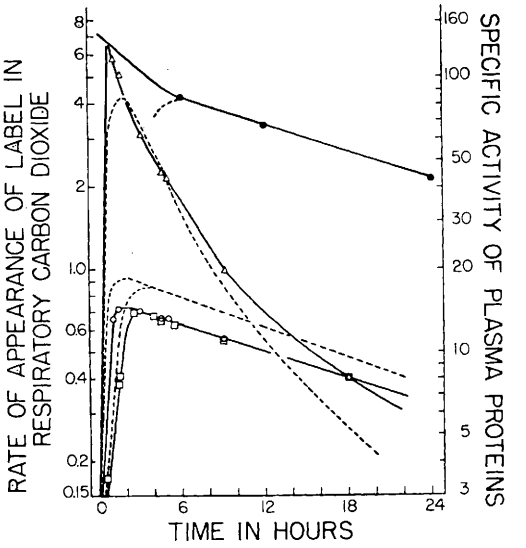

FIG. 2. (a) Semi-log plot of specific activity of plasma protein (of Recipients A or B) vs. time (solid circles). (b) Semi-log plot of rate of appearance of C^{14} in the respiratory CO_2 of Recipients B, expressed as % of dose per hr vs. time (open circles, after intravenous injection; squares, after intraperitoneal injection; triangles, after oral administration). (c) Dotted curves, semi-log plot of rate of appearance of C^{14} in the respiratory CO_2 of Recipients A expressed as above (same curves as Fig. 2, paper I).

Experimental. The animals used weighed between 190 and 229 g and were divided into 3 groups, one from each group being sacrificed 6, 24, 84 and 168 hr after administration of the plasma. The different groups were given the plasma either intravenously, intraperitoneally or orally, and in the same dose namely 1.5 ml containing 110 mg of protein with 10^5 counts per min per mg. The C^{14}-labeled plasma protein was prepared by feeding the washed and killed *Rhodospirillum rubrum* organisms to 9 donor rats which were sacrificed after 8 hr as in the previous experiments

‡ Our thanks are due to Professor H. A. Barker for growing the organisms used. About a quarter or more of the activity is located in the dicarboxylic acids (Unpublished data of Tabachnick, M., and Canellakis, E.).

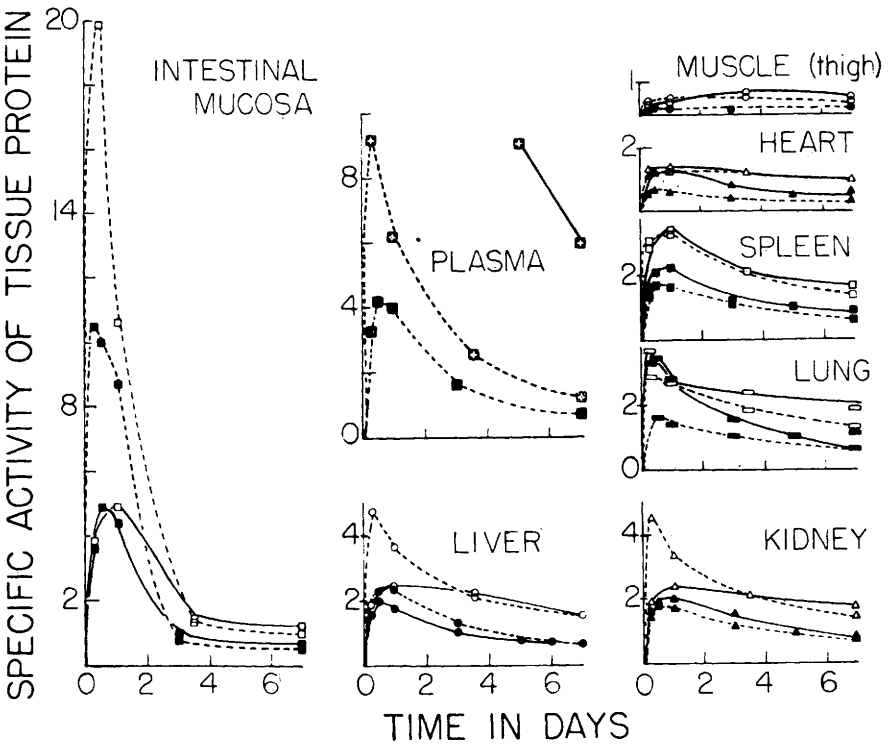

FIG. 3. Specific activities of protein from perfused tissues of Recipients A and B in terms of c.p.m. per mg vs. time in days. Dotted curves after oral administration, solid curves average results following intravenous or intraperitoneal administration. Solid points plasma protein A, open points plasma protein B.

(1). After dialysis of the labeled plasma there was no significant amount of activity in compounds other than protein. Thus 99.8% was trichloroacetic acid insoluble whereas only 0.05% was soluble in the acid; also there was only 0.11% soluble in chloroform. This type of labeled plasma protein will be referred to as 'B', that used in the previous experiments as 'A' *i.e.*, the serine-β-C^{14} labeled plasma protein. All samples of protein, respiratory carbon dioxide, urine and feces collected were subjected to analysis by the procedures previously described(1,2). Specific activities are expressed as counts per min per mg of protein (equals % dose per g protein).

Results and discussion. Loss of label from plasma. The rate of loss of C^{14}-labeled plasma protein from the circulation of animals given the material intravenously is virtually identical with that previously observed in animals injected with labeled plasma protein A. This is shown in Fig. 1 (upper solid curve). Again it appears that more than one process is involved in the loss. The metabolic process which has a half time of 74 hr§ is preceded by a process with a half-time of 11 hr (dotted curve), and by one with a still shorter half-time (not shown). When the same dose of C^{14}-labeled plasma protein is given orally, the amount of C^{14} appearing in the plasma protein of the recipients is rather low, but it will be noticed that it is higher than that previously found with plasma A (Fig. 3). After oral administration the rapid processes

§ From the metabolic half-life of 74 hr we may calculate the rate of synthesis of plasma protein in these 200 g rats as follows: The total plasma protein in the animals is plasma volume x mg protein per ml or 7.5 x 74 = 555 mg. The turnover time = 1.44 x 74 = 107 hr so the amount of protein synthesized is 556 ÷ 107 or 5.2 mg per hr. This represents a minimum rate estimate because no account has been taken of the extra vascular plasma protein. Thus the actual rate is probably 2 to 6 times this value.

found after intravenous administration are not apparent and only the loss of C^{14}-labeled protein from the circulation due to its catabolism is evident. The rate found after 2 days corresponds to that of the slowest process observed following the intravenous injection, and also corresponds with the rate following oral administration of plasma protein A. It is quite understandable that the rates observed for the faster physical processes, should be the same whether labeled plasma A or B is employed, but it does not follow that the rates of the metabolic processes measured should likewise be identical. If plasma protein were to be partially broken down and the peptides or still larger fragments were to be used in resynthesis to any extent then it might be anticipated that different rates would be obtained when different amino acids in the plasma protein were labeled with C^{14}.

It will be noted that there appears to be a more rapid component in the metabolic process, since at the beginning the curve is not linear. The data of Miller and coworkers(3) with dogs and London(5) with human subjects have shown that the half-lives of albumin and globulin are not the same. Consequently a difference is also likely to exist in the rat, so that in a curve for total plasma proteins there will necessarily be a deviation from a straight line in the logarithmic plot even in the part of the curve where catabolism is preponderant. At first slope will be steep due to the contribution of the more rapid process to the rate and later the slope will approach that corresponding to the slower process. It should also be noted that another complication exists because of the unequal specific activities of the albumin and globulin fractions. Similar differences in specific activity have been observed previously(3,4,5) and have been extensively investigated by Yuile and coworkers (6). We do not anticipate that this would greatly affect the results. It is also probable that the rate of loss of albumin and globulin into the intercellular fluid, lymph and cells, proceed at different rates(7). Consequently the more rapid process or processes involved in the loss of plasma protein from the circulation will be complicated in a similar manner to the slower.

When the rate of $C^{14}O_2$ production is examined (Fig. 2) it is seen that it behaves somewhat the same as in the previous experiments(1), which are shown as dotted curves, *i.e.*, the maximum rate of $C^{14}O_2$ production is reached 2 to 3 hr after giving the dose, and then falls off at a rate similar to that observed before. Again there is some delay in attaining the maximum rate after intraperitoneal injection. However, it is obvious that the $C^{14}O_2$ is always less when plasma protein B is used as compared with A *e.g.* in the period 0 to 3 hr the actual figures were for group B (intravenously) 2.1, A 2.7, and for 24 hr B 12.3, whereas A was 15.3%. The animals used were quite comparable so the plasma protein itself was broken down at exactly the same rate in both groups. Even lower rates of $C^{14}O_2$ production have been observed during similar periods in dogs injected with plasma protein labeled by feeding lysine-ϵ-C^{14} to donor animals(5). The difference must be due to differences in the rate of catabolism of the C^{14} label in the free amino acids to carbon dioxide in the several cases.

The difference between plasma proteins A and B is still more obvious when the catabolism of the orally administered material is considered (Fig. 2). With the plasma protein B a very rapid rate of $C^{14}O_2$ production is attained early in the experiments; with A the peak rate is neither so high nor is it attained so rapidly. Consequently there must be some amino acid carbon in the plasma protein B which is metabolized more rapidly than the β-carbon of serine; or possibly the amino acid is released more rapidly from the protein and thus there is a higher concentration of label in the amino acid pool in the one case than in the other. But why the C^{14} from the A protein should be metabolized more rapidly when the protein is given parenterally and more slowly when given orally, as compared with that from the B protein, is not entirely clear. It should, therefore, be emphasized from these results that, although the appearance of $C^{14}O_2$ from C^{14}-labeled plasma protein may serve as an indication of plasma protein breakdown, yet different conclusions may be reached if variously labeled proteins are fed to otherwise identical animals.

Great differences in the amount of C^{14} appearing in the tissue protein are also noted when the results from labeled plasma proteins A and B are compared (Fig. 3). This is particularly true when the proteins are given orally. The labeling of some tissues after protein B may be almost twice as high as after protein A.

It is also noteworthy that following the oral administration of either of the C^{14}-labeled proteins the tissue C^{14} is higher than that attained after parenteral administration. In the latter case part of the activity measured in the tissues must be due to C^{14}-labeled plasma and lymph(2,7). With the protein B lower respiratory $C^{14}O_2$ accompanies the higher tissue C^{14} when the dose is given parenterally. Consequently it is clear from the comparison between these results that little can be deduced concerning the quantities of plasma protein taken up by tissues when the protein is labeled by administering to the donor animals a single labeled amino acid like serine-β-C^{14}. The results obtained at any given time interval will depend on the rate of uptake of the plasma protein by the tissue, its rate of breakdown to free amino acid and on the *rate of catabolism of the particular labeled carbon atom (s) concerned.*

Summary. It is possible to produce generally labeled plasma protein with very high specific activity in rats by feeding them suitable bacteria *(Rhodospirillum rubrum)* cultured in media containing labeled bicarbonate. These bacteria have protein in which all the amino acids present are labeled. The metabolism, in the recipient rats, of the C^{14} in the plasma protein from these donor animals (B) has been investigated and compared with that already described(1,2,7) for plasma protein labeled by feeding donor rats serine-β-C^{14} (A), with the following results: (1) The disappearance of the C^{14}-labeled protein B from the circulation, following its intravenous injection, follows the same curve as that found when protein A was employed. (2) The appearance of $C^{14}O_2$ in the respiratory gases is significantly less in these animals following the parenteral injection, but more following the oral administration than in recipients A. (3) As compared with A, the tissues of recipients B contain more C^{14}-labeled protein no matter which route of administration is employed, and this is particularly true when the C^{14}-labeled protein is given orally.

Our best thanks are due to Miss Ruth Allemann for technical assistance. We are indebted to the U. S. Atomic Energy Commission, Oak Ridge, for supplies of C^{14} used in these studies.

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Received October 23, 1951. P.S.E.B.M., 1951, v79.

Concentration of a Hyperglycemic Factor from Urine of Schizophrenics. (19288)

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The presence of a hyperglycemic factor in the urine of so-called schizophrenic patients has been reported by Meduna and Vaichulis (1). Meduna and McCulloch(2,3) have reclassified these schizophrenics as "oneiro-

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