availability. Our findings on the comparative rates of oxidation of linoleic acid-1-C^{14} by the fed normal and diabetic rats are compatible with the observations of Peifer and Holman (17), that the alloxan-diabetic rat is more rapidly depleted of essential fatty acids than is the normal rat.

Summary. 1. Oxidation of linoleic and linolenic acids, both randomly labeled with C^{14}, to CO_2 was compared in a) fasted and glucose-fed normal rats; b) normal and diabetic fed rats; and c) insulin-treated and untreated, fed diabetic rats. 2. Administration of glucose spared oxidation of both linoleic and linolenic acids. 3. Oxidation of linoleic acid by *ad lib.*-fed diabetic rat exceeded that by similarly fed normal rat. 4. Insulin spared oxidation of polyunsaturated fatty acids in the diabetic rat.

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Conversion of Serum Proteins into Tissue Proteins.* (25120)

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Although elimination of isotopically labeled plasma proteins from the circulation has been investigated by numerous authors(1-9), very little is known on their conversion into tissue proteins. The purpose of the experiments described here was to gain more insight into this process. Whipple and his coworkers(1,10) in their classical work with C^{14}-lysine containing plasma proteins found incorporation of C^{14}-lysine into the tissue proteins. It is not possible, however, in experiments with proteins containing a single labeled amino acid to decide whether incorporation into tissue protein involves transfer of large peptide fragments, or whether total breakdown to the amino acid stage is a prerequisite for incorporation. External labels such as I^{131} are use-

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less in experiments of this type since they are not utilized in formation of tissue proteins. More information can be obtained when the injected plasma proteins contain 2 or more labeled amino acids. Obviously the ratio of the 2 labels remains unchanged when large peptide fragments are transferred from plasma protein to tissue protein. Conversely, a drastic change in the ratio of the 2 labels would result when breakdown to small fragments were followed by incorporation of the fragments at different rates. In our experiments we injected rats with rat serum proteins biosynthetically labeled by C^{14}- and S^{35}-amino acids.

Methods. Preparation of doubly labeled rat serum proteins. A C^{14}-amino acid mixture was obtained by hydrolysing the proteins of a *Chlorella* culture grown‡ on $C^{14}O_2$. After removal of humin by centrifuging the aqueous solution was purified by chromatography on a Dowex-50 column to remove any carbohydrate that might have been present. The preparation of S^{35}-yeast protein and its hydrolysis has been described(11). Approximately 6.4×10^7 counts per minute (CPM) of the C^{14}-amino acid mixture and 1.5×10^8 CPM of the S^{35}-amino acid mixture were combined and injected intraperitoneally into a rat of 106 g weight which had been starved 12 hours prior to injection. The animal was exsanguinated by heart puncture after 5 hours. After dilution of the serum with 3 volumes of 0.9% saline the globulins were salted out by 50% saturation with ammonium sulfate at pH 8.6 in the presence of 0.4% cysteine hydrochloride. They were electrophoretically free of albumins and contained 4800 CPM/mg of C^{14} and 10,500 CPM/mg of S^{35}. The proteins of the supernatant albumin fraction were salted out by saturation with ammonium sulfate, dissolved in and dialyzed against distilled water. They contained 2940 CPM/mg of C^{14} and 9020 CPM/mg of S^{35}. Electrophoresis revealed the presence of 10-20% globulin. Rat serum albumin containing S^{35} and I^{131} was prepared by the method used for preparation of rabbit serum albumin-S^{35},I^{131} (11). *Injection of rats and analysis of organ proteins.* Rats weighing 100 to 180 g were injected into their femoral veins with the radioactive proteins. Rats Nos. 1, 2 and 3 received 9.2, 17.7 and 29.6 mg serum albumin-S^{35},C^{14}; rats Nos. 4 and 5 were injected with 7.5 and 19.7 mg of serum globulin-S^{36},C^{14}. Control rats were injected with rat serum albumin-S^{35},I^{131}. The animals were exsanguinated by cardiac puncture, the organs rinsed with saline solution, homogenized in saline and precipitated with trichloracetic acid. Powders of the serum and tissue proteins were prepared and counted as described earlier (11). Hair was washed with water, acetone and ether. The activity due to each isotope in doubly labeled material was calculated from the decay rate(12). Sulfur content of the serum and tissue proteins was determined‡ by the Denis-Benedict method as modified by Waelsch and Klepetar(13).

Results. Following injection of rat serum albumin-S^{35},C^{14} into rats, the 2 isotopes disappeared from the serum at a similar rate as shown by Fig. 1. In the tissue proteins, relative specific activities of S^{35} and C^{14} increased during the first few days from 0 to approximately 0.5, then decreased slowly. Since $S^{35}{}_T/C^{14}{}_T$, the ratio of the 2 isotopes in the tissue proteins, depends on $S^{35}{}_P/C^{14}{}_P$, their ratio in the injected plasma proteins, we express our results as quotients, Q_i of the isotope ratios (Table I):

$$(1) \qquad Q_i = \frac{S^{35}{}_T/C^{14}{}_T}{S^{35}{}_P/C^{14}{}_P} = \frac{S^{35}{}_T \times C^{14}{}_P}{S^{35}{}_P \times C^{14}{}_T}.$$

The activities $S^{35}{}_T$, $S^{35}{}_P$, $C^{14}{}_T$, and $C^{14}{}_P$ were measured in CPM per mg of protein. According to Table I the values of Q_i vary from 0.9 to 4.6 after injection of the doubly labeled rat serum albumin, and from 1.1 to 6.7 after injection of the doubly labeled globulin. The highest values of Q_i were found in the hair.

Discussion. If the conversion of plasma proteins into tissue proteins involved merely the transfer of large peptide fragments from the injected protein to tissue proteins, the ratio $S^{35}{}_T/C^{14}{}_T$ in the tissue protein would be equal to the ratio $S^{35}{}_P/C^{14}{}_P$ of the injected

‡ We are indebted to Drs. Sam Hood and Gunter Zweig of Kettering Inst., Yellow Springs, O., for growing *Chorella* in C^{14}-carbonate which we received from Oak Ridge National Labs. We are also grateful to Frank Zaboretzky for carrying out the sulfur analyses.

TABLE I. Relative S^{35}/C^{14} Ratios (Q_i)* and Corrected S^{35}/C^{14} Ratios (Q_c)* in Rat Organ Proteins after Injection of Rat Serum Albumin-S^{35}, C^{14} (RtSA) and Rat Serum Globulin-S^{35}, C^{14} (RtSG).

Rat No.		SF-1		SF-3		SF-4		SF-5	
Injected		RtSA (1.4 % S)		RtSA (1.4 % S)		RtSG (0.91 % S)		RtSG (0.91 % S)	
Killed after (days)		3		9		3		9	
Protein	S %	Q_i	Q_c	Q_i	Q_c	Q_i	Q_c	Q_i	Q_c
Serum†	1.1	1.19		1.15		.89		1.00	
Lungs	.97	1.10	1.59	1.26	1.59	1.55	1.44	1.34	1.25
Heart	1.0	.87	1.22	1.07	1.50	1.27	1.14	1.60	1.44
Muscle‡	.97	1.29	1.85	1.17	1.69	1.15	1.07	1.44	1.33
Spleen	.88	1.26	2.02	.96	1.53	1.26	1.29	1.40	1.43
Hair	3.8	3.95	1.44	4.63	1.71	6.77	1.60	5.90	1.40
Liver	.97	1.18	1.70	1.14	1.65	1.97	1.82	2.14	1.98

* Q_i = cpm S^{35}/cpm C^{14} in tissue protein divided by cpm S^{35}/cpm C^{14} in inj. protein; $Q_c = Q_i \times$ % S in inj. protein divided by % S in tissue protein.

† Q_c values for serum cannot be calculated since the serum contains the inj. as well as newly formed protein(11,14).

‡ From the shoulder region.

proteins and Q_i (in equation 1) would be 1.0. The increase of Q_i to values as high as 6.7 demonstrates clearly that the transfer of large peptide fragments cannot be the general metabolic pathway. The high values of S^{35}/C^{14} in the hair protein indicated a relation to the high sulfur content of hair keratin. We determined, therefore, the percentage of total sulfur in the injected plasma proteins (S_P) and in the analyzed tisue proteins (S_T), respectively. Substituting in equation 1 the corrected S^{35}-activities (CPM per mg of protein sulfur) for the measured S^{35}-activities (CPM per mg of protein) we obtain a "corrected quotient of the isotope ratios" Q_c (equation 2). Since the carbon content of all proteins is close to 50-55%, the C^{14}-activities per mg of protein carbon must be proportional to the C^{14}-activities per mg of protein; hence no correction is necessary for the C^{14}-activities.

(2) $Q_c = Q_i \times (S_P/S_T)$

The mean value of Q_c in tissue proteins of rats injected with serum albumin-S^{35}, C^{14} is 1.64 ± 0.20; in animals injected with rat serum globulin Q_c is 1.43 ± 0.25. Hence our results are represented by equation 3:

$$(3) \qquad \frac{S^{35}{}_T}{C^{14}{}_T} = Q_c \frac{S^{35}{}_P}{C^{14}{}_P} \times \frac{S_T}{S_P}.$$

Equation 3 demonstrates first that $S^{35}{}_T/C^{14}{}_T$ in proteins of the tissues is proportional to $S^{35}{}_P/C^{14}{}_P$ in the injected proteins. While this is not surprising, the equation also shows that $S^{35}{}_T/C^{14}{}_T$ is proportional to S_T, the percentage of sulfur in the examined tissue protein, but inversely proportional to S_P, the total content of sulfur in the injected protein. This suggests strongly breakdown of the injected serum proteins to the amino acid stage, dilution of the amino acid pool by both non-radioactive and radioactive amino acids of the injected plasma proteins, and incorporation of free amino acids at a rate proportional to the sulfur and carbon content of the newly formed tissue proteins.

The factor Q_c may vary for different amino acid mixtures and may also depend on the animal species. Its magnitude of approximately 1.5 reveals an impoverishment of C^{14} relative to S^{35} during the conversion of plasma protein into tissue protein. As Penn *et al.*(15) have shown, this may be due to the preferential utilization of essential amino acids in the biosynthesis of new proteins. A considerable portion of the dispensable C^{14}-amino acids is converted into lipids, carbohydrates and other metabolites which are not formed from cystine and methionine.

Our view that utilization of injected plasma proteins involves their breakdown to the amino acid level is further supported by comparing the metabolic fate of serum albumin-S^{35},I^{131} with that of serum albumin-S^{35},C^{14}. Although the elimination of S^{35} from the serum and its incorporation into the tissue proteins is very similar in both proteins (Fig. 1), the metabolic fate of I^{131} which is not utilized for protein synthesis in the analyzed organs is quite different from that of S^{35} and C^{14}. The

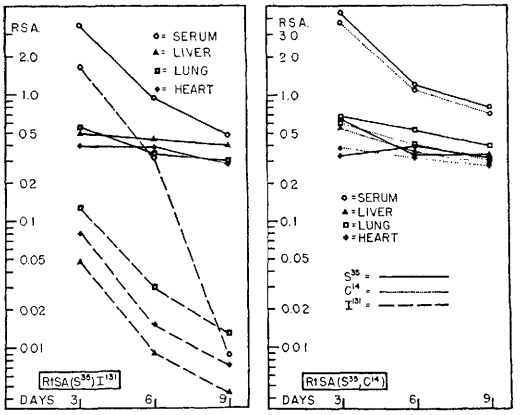

FIG. 1. Relative specific activities (RSA) of protein-bound S^{35}, C^{14} and I^{131} in tissues of rats, 3 to 9 days after intrav. inj. of doubly labeled rat serum albumin. Left: Inj. of rats #6, 7, and 8 with rat serum albumin-S^{35}, I^{131}. Right: Inj. of rats #1, 2, and 3 with rat serum albumin-C^{14}, S^{35}. The relative specific activity is defined as cpm in 100 mg protein divided by cpm inj./g body wt.

quotient Q_i (equation 1), which is equal to $(S^{35}{}_T/I^{131}{}_T)/(S^{35}{}_P/I^{131}{}_P)$ and which in rabbits injected with rabbit serum albumin-S^{35}, I^{131} in 9 days increases to values from 3 to 14 (11), approaches in rats injected with rat serum albumin-S^{35},I^{131} values from 50 to 100 (Figure 1). These high values reflect the higher metabolic rate of rat serum proteins as compared with rabbit serum proteins.

Summary. 1. Incorporation of S^{35} and C^{14} from injected doubly labeled rat serum albumin or globulin into the tissue proteins of rats was investigated. The ratio S^{35}/C^{14} in tissue proteins varies very little during 9 days after injection of the doubly labeled serum proteins. 2. The ratio S^{35}/C^{14} in tisues is approximately equal to $(S^{35}{}_P/C^{14}{}_P) \times (S_T/S_P) \times Q_c$ where the first term is the ratio of the 2 isotopes in the injected plasma protein, S_T and S_P the per cent of total sulfur in the analyzed tissue protein and the injected plasma protein, respectively, and Q_c is approximately 1.5. 3. The increase in S^{35}/C^{14} ratio ($Q_c > 1$) indicates impoverishment of C^{14} relative to S^{35} and is attributed to utilization of C^{14}-amino acids for other metabolic pathways. 4. In rats injected with serum albumin-S^{35}, I^{131} the ratio S^{35}/I^{131} increases during 9 days from 50 to 100 times. 5. Our results indicate that conversion of plasma proteins into tissue proteins involves breakdown to amino acids or very small peptide fragments. There is no indication for the transfer of large peptide fragments from plasma protein to tissue protein molecules.

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