COMPARATIVE STUDIES ON THE TURNOVER OF SERUM ALBUMIN IN NORMAL HUMAN SUBJECTS [1]

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Many tracer studies involving the use of different elements have been carried out on the distribution and metabolism of plasma protein. In human experiments, the isotopic preparations used to date have been amino acids or plasma proteins labeled with N^{15} (1), S^{35} (2), C^{14} (3) and especially with I^{131} (3–6).[2] In general the techniques employed have involved measurements of the rate of loss of a label from proteins although some have considered the incorporation (6). The proteins have been initially labeled by different methods as follows: 1) Labeled amino acid has been administered either orally or parenterally to the subject, resulting in the endogenous labeling of the plasma proteins. The loss of the label, for example from N^{15} or C^{14} labeled glycine or from S^{35}-labeled cystine or methionine, has been followed *in the same subject.* 2) Endogenously labeled homologous plasma protein fractions have been transferred from a donor to a recipient. This will be referred to as the standard method (see under discussion). 3) Plasma protein fractions iodinated with I^{131} *in vitro* have been administered.

Markedly different half-lives for albumin have been reported in some of these studies (2, 3, 5), so in order to evaluate these methods, duplicate studies have been performed in normal subjects involving the use of different pairs of the methods available. One group received labeled amino acids and I^{131}-labeled albumin; a second group received both I^{131} and endogenously labeled albumin simultaneously. The results obtained exhibit marked differences depending upon the type of labeling. The significance of these differences will be dealt with in the discussion.

MATERIALS AND METHODS

1. *Preparation of S^{35}-labeled methionine and cystine:* The S^{35}-labeled methionine and cystine employed were prepared from the yeast *Torulopsis ulitis,* grown in suitable media containing S^{35}-sulfate [3] as the limiting substrate (7–9). At the end of the incubation period the yeast was collected, washed and hydrolyzed with acid. The two labeled L-amino acids were isolated using Dowex 50 columns.

In all experiments where this material was used to follow the endogenously labeled plasma protein in the same subject, the amino acids were injected intravenously. In the case of cystine, the amino acid was prepared and sterilized in the minimum of acid necessary for its dissolution, and immediately before injection it was mixed with sufficient sterilized phosphate buffer to bring the pH to 7.0. Under these conditions there was no precipitation of cystine. The methionine was dissolved as such in pyrogen-free water, sterilized by autoclaving and injected intravenously.

2. *Preparation of S^{35}-labeled plasma and albumin:* The S^{35}-labeled plasma and albumin were prepared in the following manner. A fifty-year old white male, suffering from severe pulmonary emphysema and fibrosis and chronic cardiac failure with a marked secondary polycythemia, was given a mixture of S^{35}-labeled L-cystine and L-methionine orally. Twenty-four hours later 500 ml. of blood were withdrawn. This blood was collected in 80 ml. of ACD solution as anticoagulant. The plasma was separated immediately and half was fractionated according to the Cohn technique.[4] The remaining half of the plasma was used as such. The albumin was isolated for each specimen obtained from the recipient according to the technique outlined below. This was done in order to determine whether any differences might be observable between the albumin obtained by the Cohn fractionation procedure and non-fractionated plasma. However, a paper electrophoretic study of the donor plasma protein revealed an entirely normal distribution pattern.

3. *Preparation of I^{131}-labeled albumin:* In the majority of the experiments reported here the albumin was iodinated according to a slight modification of the method of Pressman and Eisen (10), the I^{131} being distilled into the potassium iodide, borate buffer, protein mixture. Following this technique, between 7 and 11 per cent of the radioactive iodine distilled into the reaction flask became protein bound. The iodinated material was dialyzed against several changes of 0.01 M phosphate buffer at pH 6.5 until the trichloracetic acid-precipitable iodine was 97 per cent or more of the total radioactivity. The albumin solution was then passed through an ultrafine sintered glass filter and stored in the cold. The labeling generally was very light, being only approximately one atom of iodine per 36 molecules of protein.[5] One series of studies was carried out with albumin which was iodinated with ten times the usual amount of iodine. Under these circumstances there was about 0.25 to 0.35 atom of iodine per molecule of albumin. This will be referred to as lightly iodinated albumin.

In order to test whether different methods of iodination give products with altered turnover rates, a series of studies was carried out by iodinating the material according to the method of Berson, Yalow, Schreiber, and Post (5). Although they state that about 25 to 35 per cent of the I^{131} became protein bound, in our hands the yields were much poorer than this.

4. *Electrophoretic studies on iodinated albumin* [6]*:* Several lots of I^{131}-labeled albumin were studied by zone electrophoresis, performed on starch. To 25 mg. of non-iodinated albumin was added 5 mg. of the iodinated preparation and electrophoresis was performed at 4.7 volts per cm. for 16 hours in barbital buffer at pH 8.6 and with an ionic strength of 0.05.

The starch slabs were cut into sections of 5 or 10 mm. wide and each section was assayed for both radioactivity and protein content. It can be seen from Figure 1, that there were differences in the mobility between the major part of the albumin and the radioactivity. Part of the labeled material moved more rapidly than did the albumin as a whole. This is in accord with the findings of Gabrieli, Goulian, Kinersly, and Collet (11).

5. *Preparation of samples for determination of S^{35}:* The separation of the albumin fraction from the rest of the plasma proteins was attained by a two-step phosphate separation, carried out as follows: Three ml. of serum were mixed with 12 ml. of 1.75 M phosphate buffer, pH

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[5] The ordinary procedure of iodination involved the use of 100 mg. of albumin iodinated with the iodine from 0.02 ml. 0.02 M iodide: *i.e.,* micromoles of albumin (M. W. 69,000) $= 1.45$, microatoms of iodine used $= 0.4$; so on the basis of a 10 per cent yield, the actual amount of iodine used was 0.04 microatoms, or approximately 1 atom iodine per 36 moles of albumin. The ratio may be somewhat higher than this if the radioiodine came into equilibrium with the iodide in the system (1 : 24).

[6] Our best thanks are due to George Bagatella for his assistance and advice in carrying out the experiments involving electrophoresis.
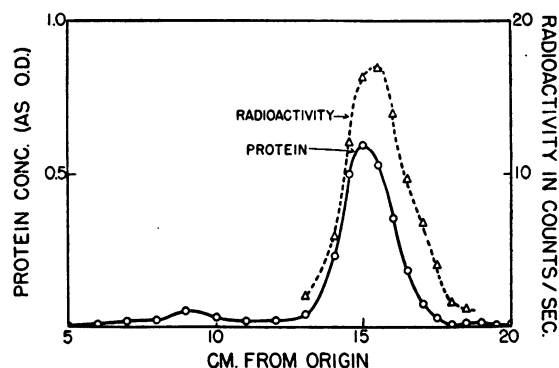

FIG. 1. STUDY OF A MIXTURE OF IODINATED AND NON-IODINATED HUMAN SERUM ALBUMIN BY THE METHOD OF ZONE ELECTROPHORESIS ON STARCH

6.5, and allowed to stand for a minimum of 7 hours. The precipitate of gamma globulin was removed by centrifugation. To 15 ml. of the supernatant were added 45 ml. of 2.6 M phosphate, pH 6.5, and the mixture allowed to stand 12 hours. This resulted in the precipitation of the remainder of the gamma globulin, the mixed alpha and beta globulins and a small fraction of the albumin. The precipitate was filtered off leaving the rest of the albumin in the supernatant. Albumin so prepared was analyzed by free boundary electrophoresis and found to be approximately 95 per cent pure, the chief contaminant being a small amount of alpha globulin.

The albumin was precipitated from the supernatant with trichloracetic acid (TCA) at a final concentration of 10 per cent and washed repeatedly with 5 per cent TCA, until the phosphate contamination became such as not to interfere with the subsequent sulfate analysis. In the case where the endogenously labeled albumin was administered, 1 ml. of total plasma protein was taken for analysis and no separation was carried out.

The protein samples labeled with S^{35} were digested with Pirie's reagent (12), brought to dryness and treated with hydrochloric acid to convert all copper salts into the soluble chloride and precipitated with benzidine dihydrochloride. The benzidine sulfate was then filtered onto Whatman No. 1 filter paper and counted directly on this paper. During the process of digestion any iodide (I^{131}) from the double labeling was lost, since there was no detectable I^{131} in any of the samples after this type of digestion.

After the samples were counted the benzidine sulfate was titrated with standard sodium hydroxide (13).

6. *Preparation of samples for I^{131} determination:* For I^{131} determination 1 or 2 ml. of plasma or serum, in an aluminum dish rimmed with silicone stopcock grease, were thoroughly mixed with 1 ml. of a 1 per cent TCA solution to which a small amount of detergent was added. The solutions were allowed to dry slowly over a hot plate. Under these conditions a smooth uniform layer of protein was easily obtained.

7. *Determination of radioactivities:* Radioactivities were determined in either a thin mica end window Geiger-

Mueller tube, in a gas flow tube, or in the tube with gas flow and a thin pliofilm window described below. Since most of the samples that were counted were double labeled (both I^{131} and S^{35}) it was necessary in this counting procedure to screen out the S^{35}. This was done by the use of a mica shield of sufficient thickness to absorb over 97 per cent of the soft-β radiation from the S^{35}. The possible contribution of the S^{35} to the counting rate was less than 1 per cent of the iodine.

In earlier work the S^{35} samples were counted on a windowless flow counter. However, this method was discarded in favor of a modified flow counter which consisted of a simple bell-type Geiger tube with the opening about 2½ inches in diameter. Over this was stretched a thin pliofilm window about 0.3 mg. per sq. cm. thickness. Because of the permeability of this film to gas, it was necessary to pass the counting gas, helium saturated with ethanol at 0°, through the tube during counting. This tube proved to be very stable, although it cut down counting efficiency by about 50 per cent over the use of a windowless counter. However, the stability of the tube more than made up for the loss of efficiency.

Since all protein samples were prepared in a similar way no correction was necessary for self absorption, but all benzidine sulfate samples were corrected for both physical decay and self-absorption. Counting was carried out long enough to achieve a statistical accuracy ± 5 per cent.

8. *Subjects:* All subjects studied were adult males and with one exception ranged in age from 20 to 30. The remaining subject was 45. They were all ambulatory and at their customary work. The diet was not controlled, but appeared to be adequate. The blood was collected and either allowed to clot or placed into a Wintrobe ammonium-potassium oxalate mixture.

The subjects who received I^{131}-labeled material were divided into two groups; the first group did not receive any iodide to block thyroid uptake of radioiodine; the larger group received ten drops of saturated solution of potassium iodide, twice daily, throughout the duration of the experiment.

9. *Administration of radioactive material:* The I^{131}-labeled albumin was administered intravenously in a dose of 5 to 10 mg. containing approximately 10 to 15 microcuries. Blood samples were taken within 15 min. in all instances, in order to establish the circulating blood volume. Subsequently several specimens were taken during the first day followed by specimens every twenty-four hours for the first week and three times weekly thereafter as long as counting was feasible.

When the S^{35}-labeled cystine or methionine was administered the dose again was given intravenously, amounting to between 20 and 30 microcuries in 5 to 10 mg. of material. Blood samples were taken either twice on the first day or more frequently. Beginning twenty-four hours after the dose, samples were then collected daily for the first week and either two or three times weekly for the next two weeks thereafter. Subsequently samples were taken at varying intervals up to 60 days.

In cases where the S^{35}-labeled plasma or albumin were administered 1.25 to 2 gm. of material containing 5 to 10 microcuries of S^{35} were injected intravenously.

I^{131} and S^{35} were determined on the same samples of blood.

10. *Calculation of results:*

(a) *The plasma volume and albumin in the vascular circulation:* This was calculated directly from the dilution found in the first blood sample; e.g., from the iodine curve of Wil (Figure 4), the total dose of 8.02×10^6 c.p.m. was diluted to 3.10×10^3 c.p.m. per ml. Hence, plasma vol. $= \dfrac{8.02 \times 10^6}{3.10 \times 10^3} = 2,590$ ml. and the rapidly circulating albumin $= 2,590 \times 0.053 = 137$ gm. where the per cent albumin in the phosphate fractionated serum was found to be 5.3 by micro Kjeldahl analysis. Similar values for plasma volume and circulating albumin were found by the standard method.

(b) *Total albumin:* This was also calculated from the dilution using the extrapolation of the metabolic portion of the exponential curve, e.g., in the case of Wil, the second linear portion of the curve being employed. Hence,

total albumin $= 137 \times \dfrac{3.10 \times 10^3}{5.6 \times 10^2} = 760$ gm. This value

does not agree with that given in Table IV from the albumin-S^{35} data (426 gm.) for reasons given in the discussion. From this value of 760 gm. the ratio of total albumin to the albumin in the blood vascular circulation is 5.5.

(c) *Rate of synthesis:* The calculation of the rate of protein synthesis rests on the assumption that all the radioactivity lost from the protein is lost due to net destruction, neither the amino acid nor its label exchanging out of the protein.

The half-life of the protein is determined graphically, in the case of Wil, being 15 days. Then,

Synthetic rate as

$$\text{per cent per day} = \frac{100 \ln 2}{t_{\frac{1}{2}}} = \frac{69.3}{15} = 4.6 \text{ per cent}$$

or,

Synthetic rate as

$$\text{gm. per day} = \frac{4.6}{100} \times 760 = 35 \text{ gm.}$$

This figure should be contrasted with the figure of 12.3 gm. per day given in Table IV. The higher value is in error due to the operation of several factors which are dealt with in the latter part of the discussion.

RESULTS

Experiments with I^{131}-labeled albumin

Figure 2 shows the results of plotting the average values obtained with three normal subjects given I^{131}-albumin. Further details are given in Table I. The curve is essentially the same as those obtained by others (2, 4, 5), and can be resolved into at least two and possibly three com-

TABLE I

Short term studies in which normal human subjects were given human serum albumin very lightly labeled with I^{131}

Subject	Weight kg.	Period followed days	Albumin gm. per cent	Circu- lating albumin gm.	Total albumin gm.	Ratio total to circu- lating albumin	Half- life days	Albumin synthesized	
								gm. per day	gm. per kg. per day
Bon.	71	9	5.22	150	406	2.7	11.0	25.6	0.360
Cre.	68	9	4.26	120	312	2.6	9.0	24.0	0.350
Ele.	74	12	4.98	147	456	3.1	13.0	24.6	0.330
Koe.	77	8	5.05	146	380	2.6	10.0	26.2	0.392
Mas	62	13	4.03	96	211	2.2	9.3	15.8	0.253
Spa	66	12	4.66	123	283	2.3	9.0	22.0	0.330
Tar.	70	9	4.94	130	429	3.3	9.4	31.8	0.454

Mean and standard deviation   0.346+0.054

ponents. The first component is assumed to indicate intravascular, extravascular mixing. The second component, with the half-life of about forty hours, may represent the intracellular, extracellular equilibrium of the I^{131}-albumin, whereas the third portion of the curve represents the metabolic destruction of the I^{131}-albumin. From the extrapolation of the third portion of the curve it is possible to calculate the total albumin as previously indicated.

In one experiment during the preparation of the I^{131}-labeled albumin, the material was inadvertently denatured. Although the denaturation was not sufficient to noticeably change its solubility, the effect upon the metabolic handling of the material was very pronounced as shown in Figure 3. It is seen that the denatured albumin was very rapidly removed from the circulation so that in twenty-four hours there was virtually none demonstrable.

In Figure 4 and Table II are shown the results obtained when samples of albumin iodinated with two different amounts of the halogen were used in comparable studies. Curve with triangle symbols represents the values obtained with the I^{131}-albumin with about one atom of iodine per 36

FIG. 3. SEMI-LOGARITHMIC PLOT SHOWING THE RELATIONSHIP BETWEEN THE SPECIFIC ACTIVITY OF PLASMA PROTEIN AND TIME; (a) FOLLOWING THE INJECTION OF ENDOGENOUSLY LABELED ALBUMIN, (b) FOLLOWING THE INJECTION OF VERY LIGHTLY IODINATED HUMAN SERUM ALBUMIN AND, (c) FOLLOWING THE INJECTION OF DENATURED VERY LIGHTLY IODINATED ALBUMIN
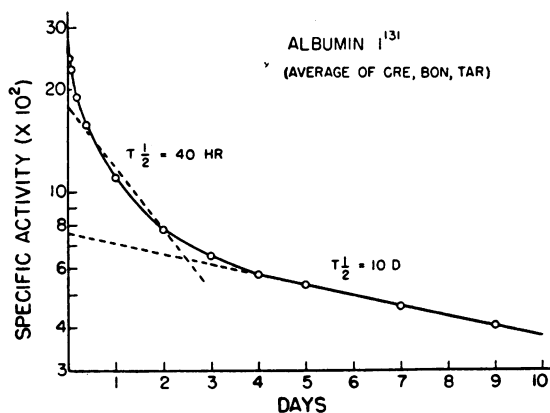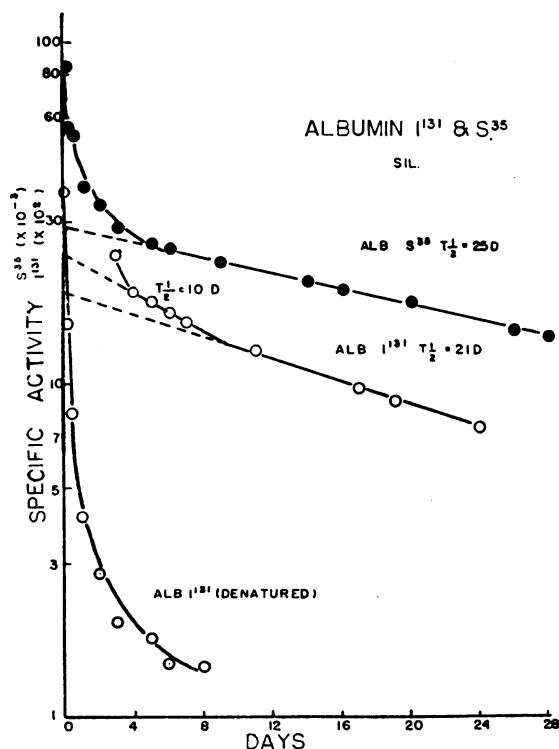
All experiments carried out on the same subject.

FIG. 2. SEMI-LOGARITHMIC PLOT SHOWING THE RELATIONSHIP BETWEEN THE SPECIFIC ACTIVITY OF PLASMA PROTEIN AND TIME FOLLOWING THE INJECTION OF VERY LIGHTLY IODINATED HUMAN SERUM ALBUMIN IN THREE NORMAL HUMAN SUBJECTS

TABLE II
Long term studies in which normal human subjects were given human serum albumin lightly or very lightly labeled with I^{131}

Subject*	Period followed days	Values derived from the fast component of the turnover data						Values derived from the slow component of the turnover data				
		Circulating albumin gm.	Total albumin gm.	Ratio total to circulating albumin	Half-life days	Albumin synthesized gm. per day	Albumin synthesized gm. per kg. per day	Total albumin gm.	Ratio total to circulating albumin	Half-life days	Albumin synthesized gm. per day	Albumin synthesized gm. per kg. per day
Cos.	22	173	450	2.6	8.	28.4	0.383	690	4.0	17	38.9	0.525
Gal.	27	154†	324	2.1	9.8	19.6	0.288	482	3.1	17	22.9	0.337
Mar.	22	125	275	2.2	8.4	19.6	0.258	425	3.4	15	22.5	0.295
Rei	19	145	464	3.2	8.5	34.0	0.500	740	5.1	15	37.9	0.555
Rus	24	168†	385	2.3	8.	21.4	0.268	770	4.6	25	33.4	0.416
Sil	24	172†	388	2.2	10.	17.5	0.227	533	3.1	21	24.4	0.317
Ste	27	120†	305	2.5	8.2	17.9	0.271	466	3.9	18	25.8	0.392
Wil	22	137	410	3.0	8.5	34.6	0.525	755	5.5	15	33.3	0.504

* The weights and per cent albumin in the circulation of the subjects used in these studies are to be found in the other tables.

† Values obtained from S^{35}-labeled protein experiments.

moles of albumin (very lightly iodinated). The other two curves with open circles show the values obtained with albumin iodinated 10 times as heavily (lightly iodinated). This, therefore, would represent about 0.3 atoms of iodine per mole of albumin. As can be seen in the two curves for the first ten-day period there is no noteworthy difference between the results with the albumin iodinated in either of these ways. However, it should be noted that the so-called heavily iodinated material used in these studies is actually lightly iodinated by the generally accepted standards.
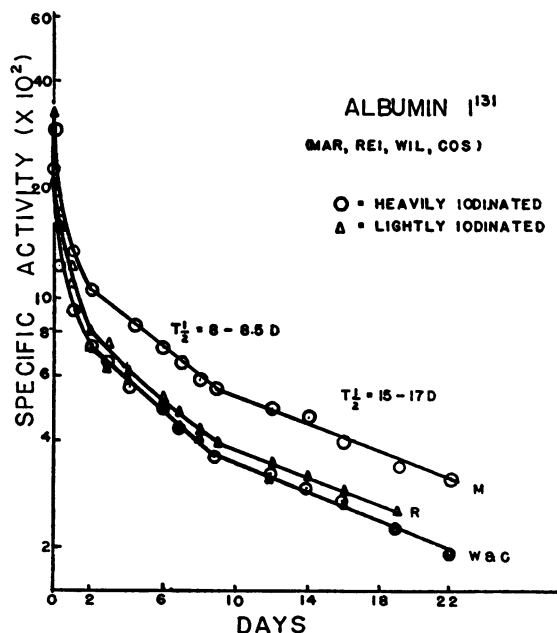

FIG. 4. SEMI-LOGARITHMIC PLOT SHOWING THE RELATIONSHIP BETWEEN SPECIFIC ACTIVITY OF PLASMA PROTEIN AND TIME IN NORMAL SUBJECTS GIVEN HEAVILY IODINATED AND LIGHTLY IODINATED HUMAN SERUM ALBUMIN (LIGHTLY AND VERY LIGHTLY IODINATED PREPARATIONS BY GENERALLY ACCEPTED STANDARDS) (SEE TEXT)
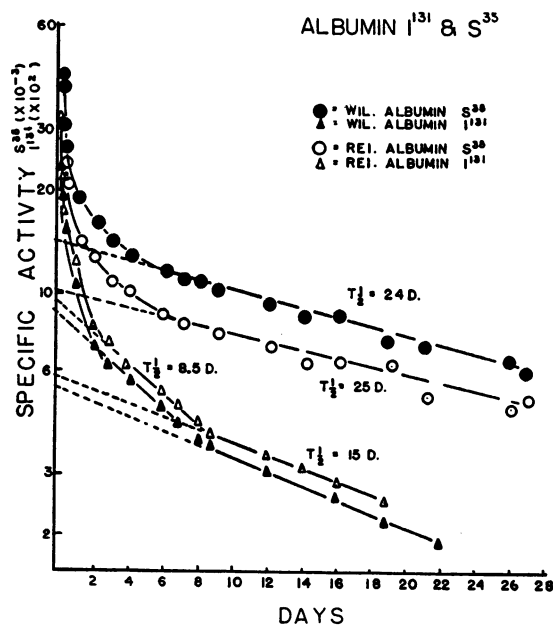

FIG. 5. SEMI-LOGARITHMIC PLOT SHOWING THE RELATIONSHIP BETWEEN SPECIFIC ACTIVITY OF PLASMA PROTEIN AND TIME IN TWO NORMAL SUBJECTS GIVEN BOTH VERY LIGHTLY IODINATED HUMAN SERUM ALBUMIN AND ENDOGENOUSLY LABELED ALBUMIN FOR PURPOSES OF DIRECT COMPARISON
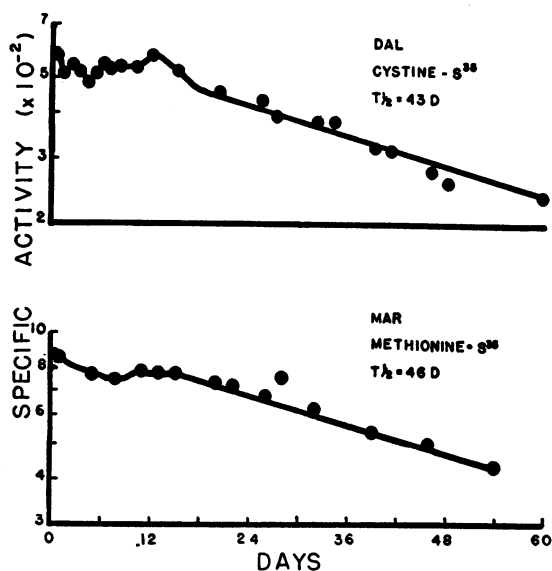
FIG. 6. SEMI-LOGARITHMIC PLOTS SHOWING THE RE-
LATIONSHIP BETWEEN THE SPECIFIC ACTIVITY OF SERUM
ALBUMIN AND TIME IN REPRESENTATIVE NORMAL SUB-
JECTS FOLLOWING THE ADMINISTRATION OF S^{35}-CYSTINE
OR S^{35}-METHIONINE

In Figures 3, 4 and 5 are shown the results
from studies in which the activity from iodinated
albumin was followed for more than 10 days. It
can be seen that about the eighth day, there is a
change in the slope of the curve. The curve which
has been previously interpreted as resulting from
the metabolism of the protein, shows a component
with a longer half-life. These results are similar
to those reported by Berson, Yalow, Schreiber,
and Post (5), but are in conflict with the recent
report of Sterling, Lipsky, and Freedman (14).
It is not clear from our studies whether there are
two or more distinct "metabolic" components.

Studies with S^{35}-labeled amino acids

In Figure 6 and Table III are shown examples
of the data obtained after the injection of cystine-

S^{35} or methionine-S^{35}. It may be noted from both
of these curves that the specific activity of the al-
bumin shows no significant change for a period of
about ten to fourteen days, after which there is a
very slow fall. From these experiments, the bio-
logical half-life of the albumin appears to be in the
neighborhood of thirty-five to fifty days or more.
These results are in substantial agreement with
those reported in the literature (2, 15, 16).

S^{35}-labeled plasma and albumin experiments

In Table IV are presented the data from the
experiments in which S^{35}-labeled "ACD" plasma
was administered intravenously to a recipient and
the plasma albumin isolated. One typical curve is
shown in Figure 3. The albumin-I^{131} behavior
was determined simultaneously, the data being
given in Table II. The latter data lie on a typical
two or three phase exponential curve as was noted
previously with the I^{131} albumin. However, the
data do not show the typical exponential behavior
until approximately the sixth to the eighth day.
Subsequently, the metabolic half-life in these ex-
periments varied from twenty-five to thirty days.

Figure 5 shows two typical curves for the re-
sults obtained when the S^{35}-labeled albumin was
administered. The results given in detail in Table
IV, are identical with those obtained when the S^{35}-
labeled plasma was administered and the albumin
fraction of each specimen isolated. Again the I^{131}-
albumin-curves on the same individuals were de-
termined and are shown on the figure. It can be
seen that there is a marked difference in the slopes
of the S^{35} and I^{131}-albumin curves, although the
slope of the I^{131}-albumin-curve may eventually ap-
proach the S^{35}-albumin-curve.

To summarize, it is clear from the data pre-
sented that the longest half-life determined with
I^{131}-albumin is shorter than that determined with

TABLE III

Studies in which normal human subjects were given S^{35}-labeled amino acids

Subject	Weight kg.	Period followed days	Albumin gm. per cent	Half-life days		Subject	Weight kg.	Period followed days	Albumin gm. per cent	Half-life days
Fin	89	35	4.64	>60		Pea*	69	29	5.28	43
Dal*	71	60	3.99	43		Sch	63.5	34	4.66	>60
Mar	76	54	4.80	46		Spa	66	28	4.66	35
Oco	76	33	4.81	>60		Wil	66	51	5.30	51
Oso	74	40	4.39	44						

* These subjects received S^{35}-cystine. All other subjects received S^{35}-methionine.

TABLE IV

Studies in which normal human subjects were given protein endogenously labeled with S³⁵

Subject	Weight kg.	Period followed days	Albumin gm. per cent	Circulating albumin gm.	Total albumin gm.	Ratio total to circulating albumin	Half-life days	Albumin synthesized	
								gm. per day	gm. per kg. per day
Cos	74	27	5.24	173	485	2.8	26	12.9	0.174
Gal	68	49	5.22	154	462	3.0	30	10.6	0.156
Hor	86	51	5.27	182	546	3.0	27	14.0	0.163
Rei*	68	27	5.10	145	435	3.0	25	12.1	0.178
Rus	80	40	5.35	168	562	3.4	27	14.6	0.183
Sil	77	50	5.37	172	524	3.05	25	14.6	0.189
Ste	66	41	4.91	120	518	4.3	28	12.8	0.214
Wil*	66	39	5.30	137	426	3.1	24	12.3	0.187
						Mean and standard deviation			0.180±0.022

* These subjects received endogenously labeled S³⁵-albumin. All others received whole plasma in which all the proteins were endogenously labeled.

endogenously labeled S³⁵-albumin. Furthermore, if one considers only the first ten to twelve days of the I¹³¹-labeled albumin experiments, the differences between the experimental results and those obtained with the endogenously labeled material are very marked. The rate of metabolism of the I¹³¹-labeled albumin during this phase is 2½ to 3 times more rapid than the rate observed with the endogenously labeled protein. It is only after about the eighth day that there appears to be some slowing of the rate. However, even then the slowest component observed in most instances had a half-life of about fifteen to seventeen days, which still was almost half as long as the average metabolic half-life of the endogenously labeled plasma protein.

DISCUSSION

Concerning the standard for turnover measurements

Assuming the reality of the difference in turnover rates obtained by the three methods used in this investigation, it is necessary to try to arrive at some satisfactory interpretation to account for these differences. It is tempting to assert that the endogenously labeled albumin behaves identically to the natural material. However, this is not necessarily the case for the following reasons: (a) The material was obtained from a pathological subject; hence, it may be an aberrant form of protein (albumin). There is no proof that the albumins from all subjects, normal or pathological, are the same, in fact some investigators have reported significant heterogeneity (17), at least one different

species of albumin (mercaptalbumin) having actually been isolated (18); (b) in processing or in the ageing the material may have undergone some change or it may contain some impurity, radioactive or proteinaceous; (c) the size of the dose may have resulted in some change in the rate of metabolism of the material.

However, it is improbable that any of these factors are of real importance. First, with regard to the source—the turnover rates of internally labeled albumin obtained by other investigators (2) using different donors are essentially the same as those obtained in these studies. Since the subjects used in the studies mentioned were normal, it is evident that the albumins were metabolically similar, and in all probability both were normal.

Second, with regard to the processing—similar results were obtained with unfractionated material. Hence, any change must have resulted from the separation of cells or was due to the ageing. No ageing changes were observed nor are to be anticipated, so this possibility is discounted.

Furthermore, it is unlikely that the protein is contaminated with any significant amount of extraneous radioactivity since the donor was bled 28 hours after giving the radioactive precursor. During this period the precursors and any contaminating material must have fallen to a low level in the plasma sample. If the albumin was contaminated with a small per cent of other labeled protein, since this is also undergoing turnover, it should not have made any great difference in the albumin picture, although others have entertained this possibility (15).

Third, with respect to the dose—if the size of the dose did affect the metabolism, the only logical change to anticipate would be that of a higher rate, *i.e.*, a more rapid catabolism till such time as the extra protein was removed, followed by a lower rate later in the experiment. No such effects were observed with the albumin; the logarithmic plots remained linear, except for the initial days. Differences might have been concealed in the non-linear period, but did not exist elsewhere. In view of these considerations the endogenously labeled albumin will be taken as the standard of reference, and the method involving the administration of such protein will be referred to as such.

The turnover of proteins after endogenous labeling with S^{35}-labeled amino acids

If we turn to the differences between the standard turnovers and those found by the endogenous (cystine or methionine) labeling method, the extension of the half-life must be explained. The only reasonable explanatory basis exists in the re-entry (reutilization) phenomenon. This aspect of the use of isotopes in studies of plasma protein metabolism has been emphasized by McFarlane (18) and Niklas and Poliwoda (16).

In employing the kinetic approach which is used by all recent workers (1) in this field, the tacit assumption is made that after the protein is once labeled no more labeled material becomes incorporated. It is assumed there is no reutilization of either labeled amino acid or any labeled protein fragment of a larger size; the specific activity of any precursor of the protein is, in effect, taken to be zero. Unfortunately, there is no data extant, to our knowledge, relative to the specific activity of the free cystine and methionine, during the period of turnover measurements in human subjects. However, the specific activity of the glycine pool at intervals following the administration of glycine to rats and human subjects has been measured by various workers (20–23). A rapid fall in the activity was observed. Large dilutions of the radioactive material were found. This speaks for the existence of either a large glycine pool, or for the dilution of the endogenous glycine by the dietary glycine.

However, even though dilutions observed are large, according to the work of Berlin, Hewitt, and Lotz (23), they are not sufficient to negate effects in the measurements of the life of the heme in hemoglobin in human subjects following the labeling of the red blood cells by feeding glycine-2-C^{14}. In these experiments estimates of the specific activity of the free glycine available for synthesis were made by determining the activity in urinary hippurate at intervals during the experiment. When these data were applied as a correction to that of the heme there was a significant change in the values.

In spite of this, when Masouredis and Beeckmans (3) measured half-lives of albumin in human subjects by feeding glycine-2-C^{14} values of 28 and 39 days were found in two subjects (one with polycythemia and the other with rheumatic heart disease), values about twice as long as with I^{131}-albumin in the same subjects. Presumably if these values had been corrected for the glycine which was reincorporated the half-lives would have been shorter and thus nearer to the average observed in the present studies, 26 days. Thus the re-entry phenomenon assumes importance in the method of endogenous labeling.

At this point it should be noted that according to other investigators (2) it is possible there is significant re-entry even when the endogenously labeled protein only is injected because tissue protein must become labeled to some extent even under these conditions. This is evident from the animal work of Abdou and Tarver (24, 25).

At any rate it is clear that from a comparison of the results with glycine on the one hand and those with cystine and methionine on the other that the re-entry phenomenon with the non-essential amino acids with a high rate of endogenous formation must be of lesser importance than with the essential sulfur-containing amino acids, the sulfur of which may not be in equilibrium with dietary sulfur. The amino acid pool may be inhomogeneous as is the pool for other amino acids (20) and the dietary amino acid may not be mixed completely with labeled endogenous amino acid arising from albumin breakdown (15).

The method employed to assess the specific activity of the precursor pool in many glycine studies (20–23), suggests that similar methods be used in the cystine studies. It should be possible to assess the variable by employing brombenzene, or some similar aromatic compound, to transfer

cystine into the form of mercapturic acid in the urine in sufficient amount to permit the determination of its specific activity.

The behavior of iodinated protein

It remains to consider the difference in behavior between the endogenous protein and the iodinated protein. The main possibilities in this case may be summarized as follows: 1) The albumin is modified during the process of iodination, and is rendered metabolically more labile due to partial denaturation, or oxidation of sulfhydryl groups or other reactions; 2) the metabolic system can distinguish between the iodinated protein and normal protein; 3) the iodinated protein consists of molecules which are iodinated to different degrees, and hence possess different degrees of metabolic lability; 4) a special type of albumin molecule has been iodinated preferentially; 5) iodine may be lost from or exchanged out of the protein; 6) iodine released from protein may be reincorporated into protein appearing in the plasma.

1) With regard to the first of these possibilities, the amount of iodine used to iodinate the protein was very small in most of the studies presented; namely, 0.03 to 0.3 atom per mole (69,000) of albumin. When such amounts of iodine are used for iodination, it is impossible to detect any significant decrease in the number of thiol groups in the protein by the method of Boyer and Segal (26).[7] In addition, there is no visual evidence of denaturation. Moreover, when the amount of iodine used was increased ten fold, the product yielded approximately the same half-life as the very lightly iodinated material. There is, therefore, no evidence in our data to indicate that the protein suffered any modification beyond iodination. However, for more heavily iodinated proteins differences in metabolism have been detected (5).

The fact that in some of our data and in that published by others (2, 5) there is no stable half-life with the iodinated protein, may be explicable on the basis of partial modification of some of the

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[7] For these tests 0.3 micromole of the albumin preparation were dissolved in 3 ml. of acetate buffer of pH 4.6 and ionic strength 0.2. After adding 3 ml. of the *p*-chloromercuribenzoate reagent there was no difference detectable in the absorbancy readings at 255 millimicrons (Beckman D.U.), between iodinated and untreated samples.

protein. It may equally well be explained as indicated under 3) or 4).

2) Whether the metabolic system can distinguish between the weakly iodinated and normal protein is an open question, the answer to which is probably in the affirmative. No doubt, the iodination of the protein influences the pK's of the various tyrosine residues, and so may very well modify their ability to combine with proteolytic enzymes, and hence may affect the lability of the protein, or may affect the ease with which the protein is denatured.

3) With respect to the possibility that different residues in the protein or proteins are iodinated to different degrees, it is probable that such differences occur when different amounts of iodine and different methods of iodination are employed. It may well be that the proportions existing between mono- and di-iodinated tyrosine, histidine (10, 27) and perhaps other iodinated species vary between different albumin molecules in any one batch as well as between different types of iodinated preparations. The extent to which such differences may exist appears to be in need of investigation, since the different species may be metabolized at different rates.

4) Various workers have reported on special types of albumins. The most noteworthy of these is the mercaptalbumin first described by Hughes (17, 18). But clearly, it is impossible to assess differences in lability of such preparations without, at least, their prior identification and isolation.

5) The possibility that iodine may be exchanged out of the protein needs to be considered in view of the work of Miller, Anderson, Madison, and Salley (28), who showed that under the proper conditions iodine may be exchanged into or out of diiodotyrosine. Were such a reaction to proceed with the albumin-bound iodine *in vivo*, the effect would be to give a spuriously short life. It is equally possible that iodine might be lost from the protein by reduction or some other reaction. Since we have found that the half-life of I^{131}-albumin is not altered by the feeding of extra iodide, exchange reactions may not occur *in vivo* to any significant extent.

6) It is possible that in some of our experiments, iodine from I^{131}-albumin was released and reincorporated into other proteins appearing in the plasma. However, such a phenomenon could only

have led to the finding of a spuriously long half-life for the protein. Since we are concerned with explaining short half-lives, this possibility will not be considered further.

The size of the metabolic pool

In the preceding paragraphs consideration has been given to the importance of the specific activity and the size of the precursor pool, in arriving at an interpretation of the turnover data. It is of equal, if not greater, importance to consider the size and specific activity of the protein pool if any significant interpretations are to be made from turnover data obtained from human subjects with various diseased states in which the rate of metabolism of protein may or may not be deranged. Unfortunately, this aspect of the problem was not considered in the report of previous investigators (13), nor has it been given sufficient weight in many other more recent communications. In this connection it is clear that the endogenously labeled plasma protein method possesses great advantages over the feeding of amino acids insofar as the actual assessment of the condition of the individual is concerned with respect to the rate of protein metabolism. This is because with the endogenously labeled material transferred from donors it is possible to make an assessment of the size of the protein pool. *Without both a knowledge of the pool size and the half-life of the protein it is virtually impossible to arrive at any significant conclusions with regard to the actual rate of metabolism of the protein* (29). For any given half-life of a protein in the organism, the actual rate of replacement, of synthesis and degradation, may have any value depending on whether the protein pool is normal, large or small in size.

It is for this reason that the results with the iodinated protein give a particularly erroneous value for rates of replacement. Since the slope of the metabolic portion of the half-life curve is overestimated, the intercept of the extrapolation of this curve to zero time leads to high values for the zero time specific activity of the protein. Hence, the size of the pool is underestimated. However, a second source of error exists in that the whole iodinated protein curve is generally displaced downward on the vertical axis. Thus, when the turnover rate is calculated from the quo-

tient of pool size and half-life the net result may be in serious error, as illustrated by the calculations in the case of Wil (see under Materials and Methods). In this instance the turnover with the endogenously labeled plasma protein was found to be 12 gm. per day, whereas the iodinated protein gave a value of 35 gm. per day, nearly a three fold difference.

Where ascites are present it is possible to treat the data as if two protein pools exist, one in the body as a whole and one in the ascites, where the two pools have different rates of turnover (30, 31). To some extent the same situation must exist in the body as a whole because of the lack of homogeneity in the protein pool, the lymph albumin no doubt not having the same specific activity as the plasma albumin, for instance.

No elaborate statistical treatment has been applied to the data, since this does not appear to be justified at the present time due both to the paucity of the material, and to the fact that a complete assessment of the sources of error in the experimental work has not been made. Therefore, the average values given in the tables should be treated with caution until such time as further material becomes available. This is particularly true of the estimates of rates of synthesis which, due to the methods involved in their calculation, are prone to subjective errors.

SUMMARY

An attempt has been made to assess, in normal subjects, three methods for the measurement of albumin turnover. The method taken as the *standard of reference* involves the administration of sulfur labeled amino acid to a donor subject and the investigation of the behavior of the labeled protein from the donor in the recipient. This method is compared in the same subjects with methods involving either the injection of iodinated albumin or of S^{35}-methionine or cystine.

In agreement with other workers, the longest half-lives are found with the last method using either S^{35}-methionine or cystine and the shortest half-lives are found with iodinated albumin. However, with the iodinated protein no stable half-life is apparent. There appears to be a continuous slowing in the metabolism of the material so that the half-life of albumin measured by the standard

method involving the use of endogenously labeled protein from a donor was found to be 26.5 days in 8 normal adult male subjects.

It is pointed out that the measurement of pool size is of extreme importance in such studies as these. Only when such measurements are made is it possible to calculate replacement rates (turnover), i.e., actual rates of synthesis and degradation. Erroneous ideas concerning such rates are obtained from measurements with the iodinated albumin. With the standard method the rates in the subjects mentioned are 13 gm. per day from a pool of approximately 500 gm.

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