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NOTES	Plasma Proteins
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DANOR RUMFORD MEMORIAL FUND

for

Cancer Research, Inc

Walter Winchell, Treas.

Application for Institutional Research Grant

for Cancer Research

Renewal Application

Hotel Astor
Broadway at 44th Street
New York 36, New York

1. Applicant (Institution): University of California
Address: Berkeley, California

2. Name and Title of President or Chief Executive Officer:
R. G. Sproul, President

3. Name and Title of Director of Project:
H. Tarver, Associate Professor of Biochemistry

4. Name and Title of Chief Financial Officer:
Mr. James H. Corley, Vice President - Business Affairs
University of California
Address: Berkeley, California

5. Title of Cancer Research Project: Turnover of Plasma Proteins in Human Subjects

6. Funds Requested: Amount \$10,247 for period of one year.

Answer the following on supplemental sheets:

7. Funds from other sources: - page 0

8. Explanation of Project: - page 1 - 12

9. Budget detail: - page 13

10. Anticipated duration: - page 13

11. What provision has been made or contemplated for support of this project upon termination of this grant - page 13.

12. If an application has been filed with any other agency for support of this specific project, please list - page 13.

Authorized Executive: _____

Date July 8, 1953

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Title: _____

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Funds from other sources:

There are no other funds to support this type of project in human subjects.
 In animal work is being currently supported by:

Am. Cancer Society	27,500 (1952-1953)
School of Medicine	31,000 (1952-1953)

Equipment including scintillating counter is available from these sources
 and elsewhere

See next page

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PROJECT REPORT (Oct. 1952 to June 1953)

The problem of the dynamic fate of plasma proteins, especially of the various individual fractions, is of extreme importance if we are to understand some of the basic processes of growth and protein metabolism. Before attacking this problem in disease states, it was deemed necessary to establish both a technic which would be readily interpretable and would "truly" measure protein turnover and to standardize the technic on a large series of normal individuals. With this in mind, two general technics of labeling plasma proteins have been compared in a number of normal individuals. Briefly, to date, these technics consist of:

The simultaneous administration of

- 1) 131 tagged albumin to determine the turnover pattern of an exogeneously labeled protein moiety and
- 2) The administration of S^{35} labeled cystine or methionine to the same individual and following the incorporation and disappearance of the S^{35} labeled albumin and globulin fractions synthesized in vivo from the labeled amino acid precursors.

Methods

Iodination of albumin. Several technics of iodinating human serum albumin were attempted. The actual iodination proved to be a simple matter since the available tyrosine groups of the albumin molecule readily bind iodine in an alkaline solution. However, several technics were employed in an attempt to remove the excessive unbound 131 . These consisted of

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Attempts to remove the material by passing it through an exchange resin column. Although successful at times, the results were rather variable and marked denaturation of the protein was observed on several occasions.

Electrodialysis was attempted but this technic likewise resulted in denaturation.

The final technic was simple dialysis against a phosphate buffer of pH 6.5.

All the 131 labeled albumin used was prepared in our laboratory and had an unbound 131 concentration that did not exceed 2% of the total radioactivity.

The S^{35} labeled amino acids, L-cystine and L-methionine were prepared by allowing microorganisms to grow in S^{35} sulfate-containing media. The amino acids were synthesized into protein by the microorganisms and these proteins were then hydrolyzed with acid and isolated by chemical means.

The 131 was counted by the use of a thin micro-window Gieger-Mueller tube. Since S^{35} was present in these samples, adequate shielding was used which reduced the 131 activity by about 60%. The beta particles of S^{35} were screened to under 5% of the total count. Under the conditions that the plasma was counted the possible S^{35} interference with 131 counting was 2% or less. Duplicate one milliliter plasma samples were dried at room temperature in planchets containing trichloroacetic acid. Within the range of material employed, self absorption corrections were not necessary. Diluted samples of the injected iodinated albumin were used as radioactive standard throughout. All the results have been corrected for physical decay to the time of injection of the material. The S^{35} was determined by methods developed in our laboratory

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in which the protein-bound sulfur is oxidized to sulfate and precipitated as
 benzidine sulfate. In this technic all the 131 is removed. The benzidine
 sulfate is counted in a windowless flow counter. Appropriate corrections for
 self absorption coincidence and decay were made. The sulfur content was
 determined by back-titration of the benzidine sulfate with sodium hydroxide.
 The 131 albumin dosage varied from 10-20 microcuries in each of
 the subjects, the S^{35} dose was 20-40 microcuries.

Studies have been carried out to date on 20 subjects. All subjects
 were normal males ranging in age from 21 to 44. All except one of the subjects
 were medical students or graduate students in the Department of Physiological
 Chemistry. All 20 subjects received 131 albumin. Five received simultaneously
 S^{35} L-cystine and six simultaneously administered S^{35} L-methionine. The sub-
 jects took a diet which was adequate in protein and calories (although no
 attempt at uniformity was made, and 24 hour urine samples were collected upon
 which nitrogen determinations were done to ascertain the approximate protein
 intake. Determinations of hematocrit, plasma albumin and globulin were made
 on all subjects. There was no attempt at regulation of activity nor was the
 thyroid gland blocked with non-radioactive iodine. The dose of 131 labeled
 protein ranged from 1-20 mg, that of the S^{35} cystine from approximately 10-30
 mg, and that of the L-methionine under 1 mg.

Results

The fate of the 131 tagged albumin is well illustrated in Fig. 1.
 This is a graph of three subjects and is typical of almost all patients studied
 who received undenatured 131 albumin. As seen from this semilogarithmic plot
 the observed curve can best be resolved into three components. The rapid

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component is attributed to the distribution of the labeled albumin between the extravascular and intravascular spaces. The third or slow component is felt to be the metabolic component, that is, it represents the metabolic degradation of the iodinated albumin. The intermediate component is rather difficult to explain, but most likely represents an equilibrium of the "exchangeable albumin pool," or an equilibrium between the "extracellular" and "intracellular" albumin components.

From this data employing the metabolic portion of the curve it is possible to determine the half-life which averaged approximately ten days in the group studied to date. The data has not yet been subjected to a statistical analysis for the determination of the deviation from the mean. From these curves, it is possible to calculate the albumin turnover rate as well as the magnitude of the "exchangeable albumin pool", the concentration of extravascular albumin and the plasma volume. These calculations are generally in agreement with those reported by others. Inadvertently, one of the lots of 131 albumin was denatured during the process of removing the unbound 131 . This was discovered after the material had been administered to three subjects. Fig. 2 shows the first 24 hours difference between the non-denatured and the denatured albumin. It is seen that the fall is much more rapid with the denatured material so that at the end of 24 hours the concentration was too low to count. The further fate of this material was not determined. It is exceedingly interesting to note the marked difference between denatured and native material. From this it is certain the presence of denatured albumin would a) be easily determined and, b) would not influence the metabolic curve which begins after the third or fourth day of the experiment for at this period none would be perceptible.

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It should be noted that under the conditions of our experiments the exponential biological degradation did not hold beyond approximately ten days (Fig. 5). After this time the curve began to flatten and in some instances an actual increase of 131 protein concentration was noted. This we feel is due to the appearance of 131 thyroglobulin occurring from the small amount of unbound 131 which would be fixed by the thyroid as well as subsequent fixation of 131 degraded with the albumin taken up by the thyroid and again secreted into the blood stream as 131 bound thyroglobulin.

Fig. 3 and 4 show studies carried out with S^{35} L-cystine over a period of approximately 70 days and S^{35} L-methionine over a period of 35 days respectively. Concerning ourselves with the albumin portion of the curve first, it is seen that over the first 12-15 days of the experiment there has been no significant fall in albumin specific activity. Subsequent to this, the fall is very gradual and does not appear to follow any clear-cut exponential pattern. The same phenomenon can be observed in the case of the L-methionine. In Fig. 4 there has been little significant decrease over a period of approximately 20 days. Subsequent to this the fall is very slow and erratic. These changes are in marked contrast to the 131 albumin pattern. All the studies to date have followed these general patterns with minor variations. From this it would appear that there is a marked difference in the behavior of 131 albumin as contrasted with endogeneously labeled plasma protein synthesized from an amino acid precursor.

In the case of the mixed globulins, the fall is much more rapid initially than that of the albumin but after the eighth to the twelfth day the two parallel one another very closely.

Discussion

The question has been raised as to the validity of the iodinated

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albumin measurement of turnover because it depends upon the underlying assumption of a) lack of denaturation, b) normal metabolic behavior *in vivo*, and c) stability of the 131 tag. From these preliminary studies it would appear at least that part a, namely, the question of whether denaturation would be readily appreciated or perceived, must be answered in the affirmative. That is, denatured 131 labeled albumin behaves entirely differently from that of non-denatured material. However, the question of the stability of the tagging and whether metabolic behavior follows a normal pattern still remains to be answered. It was hoped that the comparison of the 131 technic with that of labeling with amino acids would give a satisfactory answer.

By employing the S^{35} labeled amino acid technic these two objections should be overcome. That is, the label being one natively present would behave in a "normal" manner and secondly, there would be no danger of this material being lost except as it is removed by the normal metabolic processes. However, in order for this technic and its interpretation to be valid, certain assumptions are necessary. The most important assumption is that shortly after the peak specific activity has been reached, the metabolic precursor or precursors of the labeled protein would no longer contain a significant isotope concentration. The previous work has never elucidated whether these assumptions do or do not hold in this type of experimentation. Previous work using N^{15} glycine labeled protein in a normal man and a normal woman were interpreted by London to indicate a half time for serum albumin of 20 days. Our former data had not been carefully worked out and has proven to be quite valueless in attempting to answer this question. On the basis of the results obtained in this series of experiments, it is clear that as far as the albumin moiety is concerned, the flat curve obtained during the period of about the first 10-20 days of the experiment can best be interpreted as demonstrating either continued replacement of a single metabolic

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accounted by a relatively high concentration of isotope or the contributing
 to the albumin fraction from several sources having varying, but always high,
 isotope concentrations. Therefore, this experimental approach would not be
 valid for attempting to measure albumin turnover. This is in marked contrast
 to the animal experiments reported by many investigators. In these experi-
 ments it would appear that a definite exponential function is obtained and
 suggests that the interpretation of this animal data is similar to that for
 the human 131 albumin curves. The same cystine administered intravenously
 to two rats gave the results shown in Fig. 5. Here it is seen that the typi-
 cal exponential curve reported previously in animal studies is being repeated.

As noted, the globulin fraction does show a rapid component ini-
 tially. No critical analysis of this data has yet been attempted. This is a
 part of the current phase of the research program.

Projected Research

It is proposed that in the next phase the problem be investigated
 as follows:

1. The same technics employed with albumin should be applied to
 individual ions. We have begun our studies with 131 gamma globulin and the
 turnover pattern of gamma globulin after the administration of S^{35} L-amino
 acids. We desire to determine a) the metabolic fate of 131 gamma globulin,
 and b) the pattern of behavior of this fraction after labeled amino acid
 administration. In view of the rapid fall of the total globulin fraction
 there is some suggestion that the behavior of the 131 and the S^{35} amino
 acid technics may be quite similar. If they were, this would suggest a
 different metabolic pathway for the synthesis of gamma globulin, possibly
 much more direct and simple than that of the albumin portions. This phase

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of the program is now underway and methods of isolating and iodinating the relatively unstable gamma globulin are being worked out. It is still not decided as to whether a chemical method or starch electrophoresis is the preferred isolation technic for gamma globulin. Subsequently, other of the globulin fractions are to be investigated. Whether this work will be done by the iodination of mixed globulins and their separation after their administration by chemical means or the iodination of the individual globulins is dependent upon technics upon which we are currently working.

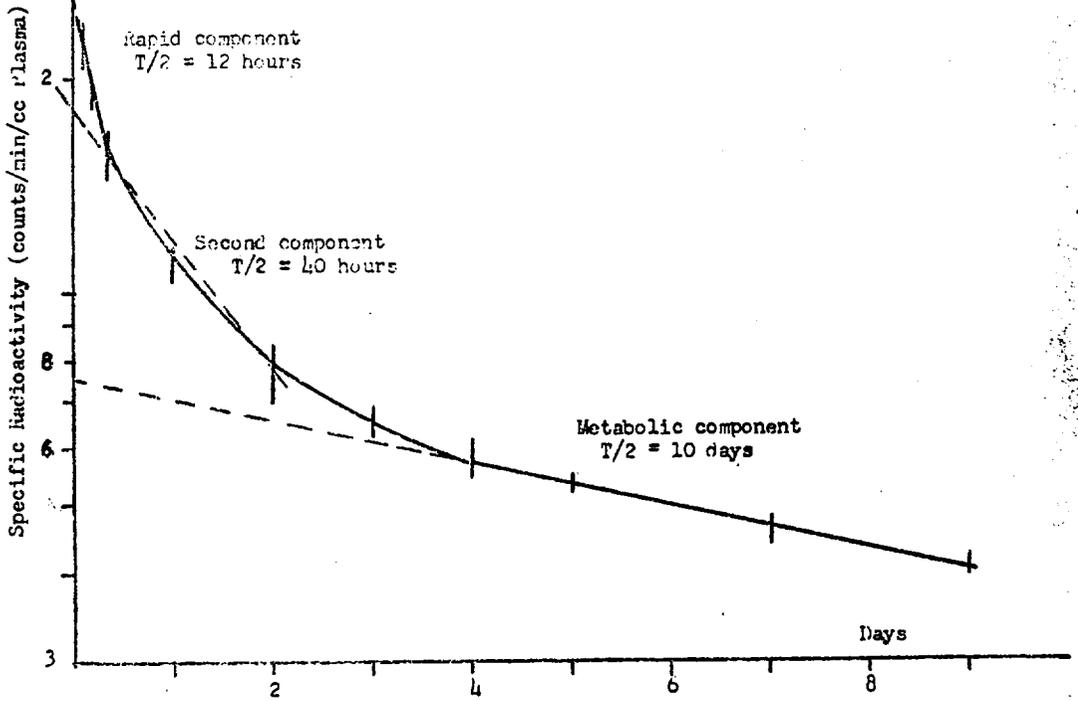
The next phase of the investigation will be concerned with an attempt to answer the questions which were posed originally, namely, whether the 131 albumin enters into the normal metabolic processes and the stability of the 131 tags. This will be answered by administering massive doses of 35 amino acids to suitable hosts and at appropriate times removing blood and isolating the plasma from the donor. Recipients will then be given tracer quantities of this natively labeled plasma protein and each of the fractions will then be separated and their fate determined. After this phase of the work has been completed and the results evaluated, studies are to begin on patients suffering from cancer and other states of altered protein metabolism.

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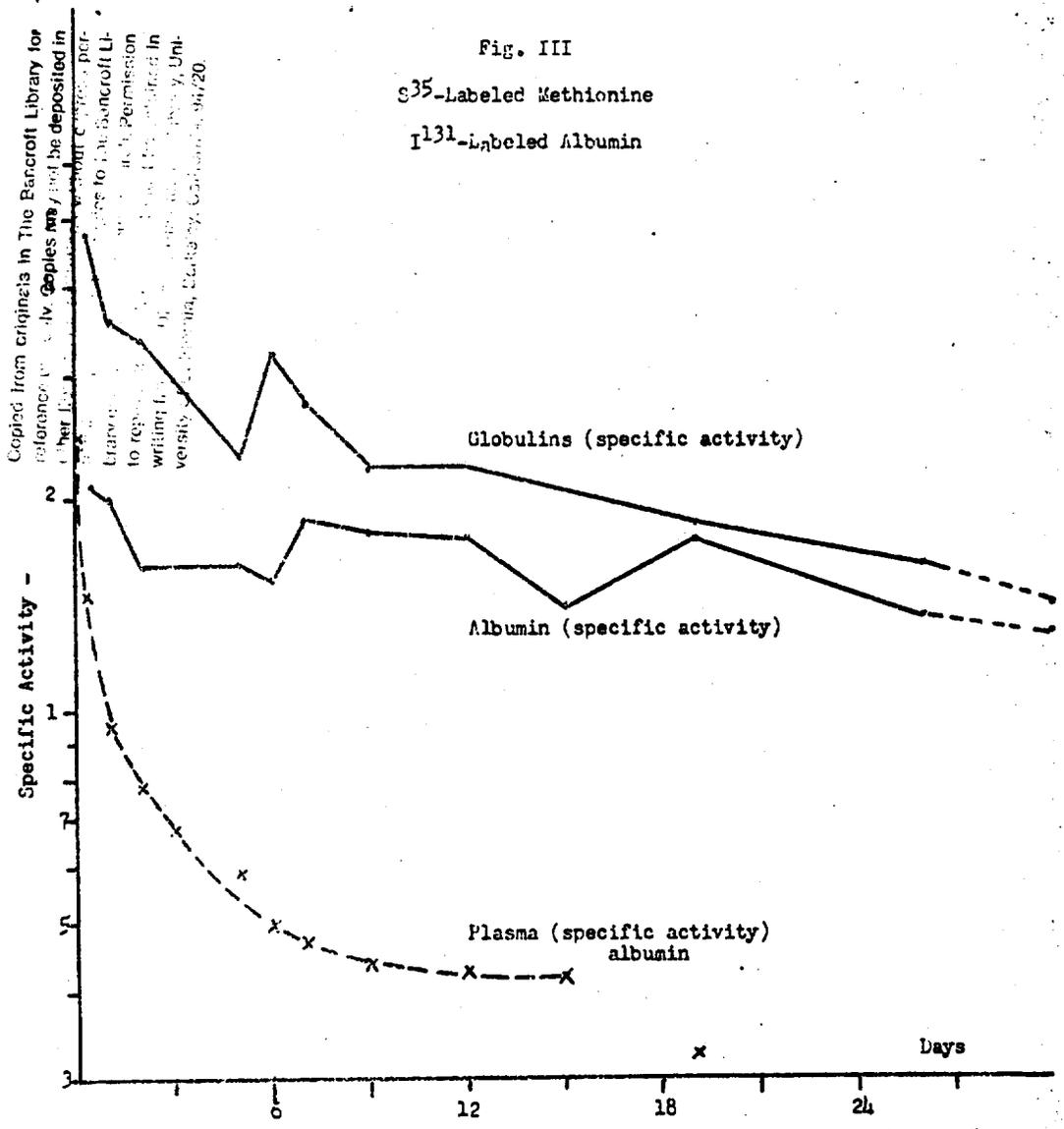
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Fig. I
 131 Labeled Albumin Administered i.v.
 Average of 3 Studies



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Urine	Total Plasma Protein	7.19 gm. %
1st 24 hrs. after administration	Albumin	4.39 gm. %
Excreted 8.9% of dose (all inorganic)	Globulin	2.60 gm. %
Nitrogen 13.4 gm. per day	Hematocrit	0.49

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Budget detail

SALARY		
Dr. Sheldon Margen		\$3,768
Technical Assistant		3,720
SUPPLIES		
Radioactive materials		\$ 400
Expendable supplies		350
EQUIPMENT		
Part cost of automatic counting equipment (Nuclear Corp., Chicago)		\$1,000
TRAVEL		\$ 250
	SUBTOTAL	<u>\$9,488</u>
	INSTITUTIONAL OVERHEAD (8 per cent)	\$ 759
	TOTAL	<u>\$10,247</u>

10. Anticipated duration

It is estimated that the assessment of methods will require another year and a half. Subsequently and simultaneously a survey of diseased states can be made in an additional two years.

11. No provision.

12. No other application to support this type of work in humans.

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WORK SHEET - PROPOSED CONTRACT

Contracting Agency: DAMON HUNTON MEMORIAL FUND Government Number: _____

Type of Contract: Grant-In-Aid Starting Date: 1 year

Campus: San Francisco Termination Date: _____

Department: Biochemistry Maximum Amount: \$10,217

Investigator: Dr. Harold TAYLOR Subject to Sales Tax? _____

Title of Investigation: TURNOVER OF PLASMA PROTEINS IN HUMAN SUBJECTS

Space Requirements: ADEQUATE

Utility Requirements: ADEQUATE

What Activity is Being Displaced by This Research Project? NONE

<u>BUDGET</u>		
Salaries:	<u>Dr. Sheldon Margen</u>	<u>3,768</u>
	<u>Technical Assistant</u>	<u>3,720</u>
General Assistance:	_____	
SERS:	_____	
	<u>Total Salaries and Assistance</u>	<u>7,488</u>
Equipment: (Itemize)	<u>Part Cost of automatic counting equipment</u>	
	<u>(Nuclear Corp., Chicago)</u>	<u>2,000</u>

Supplies: (Itemize)	<u>Radioactive materials</u>	<u>400</u>
	<u>Expendable supplies</u>	<u>350</u>
	<u>TRAVEL</u>	<u>250</u>

	<u>Total Expense and Equipment</u>	<u>2,000</u>
University Overhead:	<u>2% of subtotal</u>	<u>752</u>
	<u>1164666</u>	
IER 123B	<u>TOTAL FOR PROJECT:</u>	<u>\$10,217</u>

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