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STUDIES IN METHIONINE METABOLISM. III. THE FATE OF INTRAVENOUSLY ADMINISTERED S^{35}-LABELED-METHIONINE IN NORMAL ADULT MALES, IN PATIENTS WITH CHRONIC HEPATIC DISEASE, "IDIOPATHIC" HYPOPROTEINEMIA AND CUSHING'S SYNDROME

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STUDIES IN METHIONINE METABOLISM. III. THE FATE OF INTRAVENOUSLY ADMINISTERED S^{35}-LABELED-METHIONINE IN NORMAL ADULT MALES, IN PATIENTS WITH CHRONIC HEPATIC DISEASE, "IDIOPATHIC" HYPOPROTEINEMIA AND CUSHING'S SYNDROME [1]

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In previous reports from this laboratory it has been shown that S^{35}-labeled-methionine can be used for the evaluation of certain phases of protein metabolism in the human subject (1, 2a).

Measurement of nonisotopic D- and L-methionine in plasma and urine following infusion of DL-methionine has also provided information concerning normal and abnormal metabolism of this material (2b).

In the present study, tracer amounts of S^{35}-labeled-methionine have been administered to three normal adults, and to five patients—three with active chronic liver damage, viral and non-viral; one with "idiopathic" hypoproteinemia of more than four years' duration; and one with severe, progressive Cushing's syndrome of more than two years' duration. All of these individuals were on absolutely or relatively constant food intake preceding and during much of the period of S^{35}-labeled-methionine study. Several were on long-term balance studies, the results of which will be considered elsewhere.

METHODS

The methionine used in these studies was synthetic DL-methionine labeled with S^{35}, a pure negative beta emitter of low energy (0.17 MEV) with a half-life of 87.1 days. The labeled amino acid was dissolved in distilled water, sterilized, and injected intravenously.

The quantities of S^{35} and of total methionine received by each individual are shown in Table I.

TABLE I

Dosage of S^{35}-labeled-methionine in all individuals studied

Patient	Age	Sex	Diagnosis	* Microcuries of S^{35}	Milligrams of DL-methionine	Volume of solution injected
BAB	20	M	Normal	50	157.00	10.00 cc.
KUY	26	M	Normal	25	18.90	4.72 cc.
MOS	19	M	Normal	50	42.00	10.46 cc.
PAR	49	M	Cirrhosis	100	156.00	7.80 cc.
CUM	25	M	Chronic viral hepatitis	50	34.50	16.60 cc.
HOL	22	M	Chronic liver damage—etiology unknown	50	20.70	9.31 cc.
ZIM	39	F	Cushing's syndrome	25	9.14	4.00 cc.
MAT Study No. 1	43	M	Hypoproteinemia, "idiopathic"	50	157.00	10.00 cc.
MAT Study No. 2	43	M	Hypoproteinemia, "idiopathic"	25	24.50	6.10 cc.

* One microcurie $\equiv 3.77 \times 10^5$ counts per minute under these experimental conditions.

All patients were in the fasting state for 12 hours before and five hours after administration of the methionine. Blood specimens were obtained at three-, five-, eight-, 12-, and 24-hour intervals postmethionine during the first day, daily thereafter for the following week in the majority, and at fairly regular intervals thereafter for many weeks. Fractional urine specimens were obtained the first day, then specimens for 24-hour to 72-hour periods thereafter as shown. Stools when obtained were collected in six-day periods for 12 or more days.

The blood was fractionated according to the following outline:

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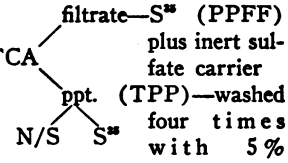
[2] Senior Research Fellow, U. S. Public Health Service, 1947–48; Schering Fellow in Endocrinology, 1948–49.

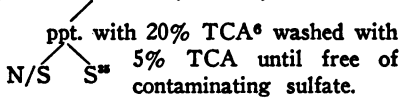
[3] Some of these and related data were submitted in partial fulfillment of the requirements for a degree of Master of Arts in Chemistry.

[4] Lt. (j.g.), M.C., U.S.N.R.

1. *Whole Blood*—1 cc. hemolyzed with 5 cc. water.
   Precipitated with 5 cc. of 20% TCA.[5]
   Filtered and washed with 5% TCA four times, washings added to filtrate.
   Filtrate (WBPFF) for S^{35} (plus inert sulfate carrier).
2. *Plasma*



   B. 2 cc. plasma plus 38 cc. of Na_2SO_4 (23%) incubated for three to 24 hours at 37.5°C. (Howe method)—20 cc. filtrate (albumin)



3. *RBC*—1 cc. triple-washed packed RBC (centrifuged for 15 min. × 2,500 RPM)—hemolyzed with 5 cc. water.
   Ppt. with 5 cc. 20%TCA, washed four times with 5% TCA.
   N/S S^{35} (RBCP)
4. WBPFF (S^{35}) minus PPFF (S^{35}) equals RBCPFF (S^{35}) (corrected for hematocrit)
5. TPP (N/S and S^{35}) minus Albumin (N/S and S^{35}) equals Globulin (N/S and S^{35}).
6. In studies on Patient MAT, globulin resuspended in saline, precipitated with 20% TCA, and washed with 5% TCA until free of contaminating sulfate.

The urine was analyzed for total (inorganic and ethereal) sulfate, and S^{35} activity, using the method of Fiske (3). Urine and stool were assayed for total sulfur and S^{35} activity by initial oxidation with Pirie's reagent (4). The sulfate formed by the oxidation was then determined as above by following the method of Fiske. The urinary organic sulfur was calculated as the difference between the total sulfur and sulfate sulfur.

Nitrogen was determined by the micro-Kjeldahl method of Kirk (5) and albumin-globulin separation, as previously noted, was initially carried out by sulfate precipitation and later by phosphate precipitation.

Counting

1. *Preparation of samples for counting:*

After precipitation of the sulfate with benzidine hydrochloride, the material was allowed to remain in the refrigerator for a period of at least two hours. The cold material was then filtered through a filtration apparatus similar to that used by Tarver and Schmidt (6). Instead of using an alundum plate for support of the filter paper, a 100-mesh stainless steel screen was substituted, which increased the filtration speed and gave a more uniform mat. In addition, it was found that Munktells No. OK filter paper was the only entirely satisfactory paper that could be used. The filtration tower used had an internal diameter of 22 mm., giving an area of material to be counted of 3.8 $cm.^2$ The precipitation flask and precipitant were washed four times with a total of 12 to 16 cc. of 95% acetone, and the filter tower was removed. The filter paper and precipitate were dried under an infrared lamp, preparatory to counting. In the quantitative handling of the material, it was necessary to wash the material adhering to the filtration tower back into the original precipitation flask with boiling hot distilled water. This was then titrated. After the precipitate on the filter paper had been counted, the filter paper and precipitate were introduced into the same flask. The total sulfate present was titrated. From these data the S^{35} content of the total precipitated material was calculated and reported in terms of specific activity (plasma) or per cent of the administered dose (urine and stool).

Blanks were titrated throughout to correct for the acidity remaining in the filter paper from the acid benzidine dihydrochloride.

2. *Counting procedure:*

The material on the filter paper was counted directly by the use of a thin window Geiger tube (1.5 $mg./cm.^2$) and an Autoscaler counting unit. Each sample was counted for a sufficient length of time to give a statistical accuracy of under 5%. Each count was corrected for background, coincidence, decay, and self-absorption—using the formula of Henriques, Kistiakowsky *et al.* (7) for the latter correction. Mass absorption and geometry corrections were not made, for the same tube was used throughout, and the distance from sample to window was kept constant.

CLINICAL SUMMARY OF PATIENTS

BAB, 20-year-old male (normal control), completely convalescent from acute viral hepatitis clinically and chemically. Prior to the study he had had 50 days of full activity. His last abnormal finding (a positive cephalin flocculation) was noted 13 weeks before the study was begun. Plasma albumin was 5.43 gm./100 cc.; plasma globulin, 2.43 gm./100 cc.

MOS, 19-year-old male (normal control), completely convalescent from viral hepatitis.

KUY, 26-year-old male (normal control), no recent disease.

[5] *Abbreviations used:*
WBPFF —whole blood protein-free filtrate.
PPFF —plasma protein-free filtrate.
TPP —total plasma protein.
RBCP —red blood cell protein.
TCA —trichloracetic acid.
RBCPFF—red blood cell protein-free filtrate.

[6] Although the TCA-precipitated albumin was washed thoroughly to remove sulfate derived from Na_2SO_4, some of the data suggested that the washing was not always complete. In subsequent studies, separation of albumin and globulin was carried out in a 2.2 M potassium phosphate buffer at pH 6.5.

PAR, a 49-year-old male (chronic liver damage, non-viral), clinical, histological (liver biopsy), and chemical findings were characteristic of chronic, moderately active, regenerative liver damage, at the time of this study. (Albumin 4.63 gm.; globulin 4.07 gm./100 cc.; cephalin cholesterol flocculation 3 plus; bromsulfalein retention 0% (5 mg./kg. × 45 min.); total serum bilirubin .29 mg./100 cc.; glycogen storage test plus 28. (Normal 40 and above.)

MAT, a 43-year-old male ("idiopathic" hypoproteinemia), admitted one year before the present study with an extreme degree of ascites and anasarca, associated with marked hypoalbuminemia, and normal or slightly decreased serum globulin. Explcratory laparotomy revealed a normal liver and spleen and no obstruction to the thoracic duct. Renal function was normal. With plasma and albumin infusions, his edema disappeared rapidly and his general health improved. At no time did his serum albumin become normal. During the first year of study, withdrawal of plasma or albumin therapy resulted in a gradual return to his initial state. Subsequently it was found that a very high protein intake would maintain his serum albumin at a level of about 2 gm. At this level he manifested only moderate peripheral edema.

Balance studies, using (*a*) a high protein diet, (*b*) a diet in which the protein equivalent was derived from oral hydrolysate (Amigen), and (*c*) a diet in which the protein equivalent was derived from intravenous hydrolysate (Amigen), failed to supply the answer to the mechanism of the hypoproteinemia. The only positive statement permissible was, that of all agents observed, preformed serum protein alone produced rapid (although temporary) improvement in his disease, so presumably the basic defect related to impaired anabolism or abnormal catabolism of serum albumin.

Except for the continuously low serum albumin (albumin 1.90 gm./100 cc.: globulin 1.63 gm./100 cc., when the present study was begun), his other laboratory findings were normal.

ZIM, a 39-year-old female (Cushing's syndrome). This woman, after a long period of misdiagnosis, was finally correctly evaluated by Dr. Minnie B. Goldberg of San Francisco, who kindly permitted this study. ZIM had Cushing's syndrome, with all of the usual clinical and chemical findings, of approximately two years' duration, referable to a large (280 gm.) tumor of the left adrenal cortex. The study to be described was carried out a few days before the removal of the tumor; as a result, only a three-day evaluation was possible.

The patient died of pulmonary edema five days after operation. S^{35} content of material obtained at operation and at autopsy is discussed elsewhere (8).

CUM, 25-year-old male (hepatitis, chronic, viral, active), had a severe attack of acute hepatitis in August 1946, from which he has never fully recovered chemically, clinically, or histologically (9, 10). During the period of observation he was not on a balance regimen but did receive a relatively constant, high protein intake. Serum albumin was 3.68, serum globulin 3.30 gm./100 cc. at the time of the study.

HOL, a 22-year-old male (liver damage, chronic, etiology unknown), at the time of this study was convalescing from an earlier hemorrhage referable to bleeding from esophageal varices. He had a definitely enlarged liver and spleen, and had significant bromsulfalein retention immediately prior to this study. At autopsy (he died as the result of a spleno-renal shunt procedure), he was found to have relatively slight histologic evidence of liver damage, but did have considerable splenic enlargement and fibrosis. The only known etiologic factor in this man was alcoholism, which at his age was of itself probably insufficient to account for his pathology.

The patient was on the same high protein intake as CUM above. At the time of the study his serum albumin was 4.26 gm., serum globulin 2.7 gm., cephalin cholesterol flocculation, 0 in 24 hours, total serum bilirubin 0.80 mg./100 cc., thymol turbidity 1.9 units and bromsulfalein retention 8.5% (5 mg./kg. ×45 min.).

RESULTS OF THE S^{35}-LABELED-METHIONINE STUDIES

As noted under "METHODS," the Howe method (11) was used in the early studies for fractionations of the albumin and globulin fractions of the plasma protein. In later studies, the phosphate method was used (12). Because of the unreliability of the data obtained with the Howe procedure (due to contamination with sulfate from the sodium sulfate used for precipitation), only S^{35} content of total plasma protein will be considered at this time. The S^{35} studies, in relation to red cell proteins, will also be considered in another report.

Metabolism of S^{35}-Labeled-Methionine in Normal Controls

In Figure 1 are shown the data obtained in the three normal individuals during the first 24–28 hours following the administration of the tracer dose of S^{35}-labeled-methionine. It is apparent that:

1. Maximal incorporation of S^{35} into plasma protein was attained at eight hours in all three controls. There was little change during the following 16 hours. The actual amounts of S^{35} present during this period were strikingly similar in all three individuals.

2. The excretion of urinary organic sulfur in the two controls, in whom quantitative urine collections were obtained, is nearly complete at the end of eight hours. As will be noted later, D-methionine probably accounts for most of this organic sulfur.

3. The excretion of urinary total sulfate occurs at a rather constant rate during the first 24 hours in both of the individuals in whom quantitative urine collections were obtained.

In Figure 2 are shown the S^{35} findings during a period of many days following administration of the S^{35}-labeled-methionine. It appears that:

1. The rates of disappearance of S^{35} from plasma protein in all three normal individuals are comparable. The rate of disappearance over the first four days may be somewhat more rapid than that which occurs over the subsequent days and weeks. Measurable amounts of activity are still present at the end of eight weeks.

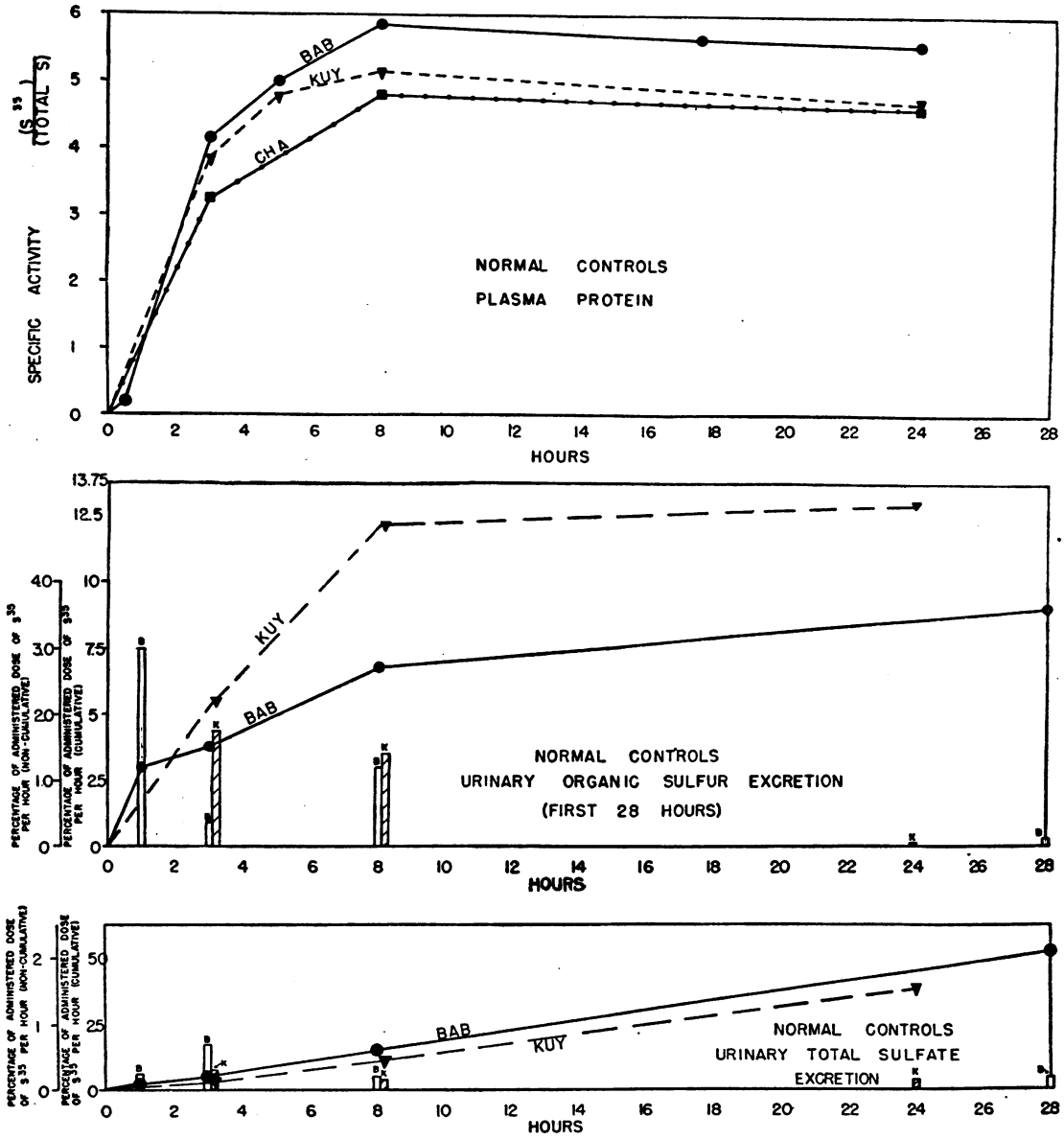


FIG. 1. S^{35} DATA OBTAINED IN NORMAL INDIVIDUALS

In the urinary organic and inorganic sulfur graphs, the columns represent the actual amount excreted at a given time, and the line graphs represent the cumulative excretion up to that time. It is apparent that maximum incorporation of S^{35} into plasma protein has occurred during the first eight hours, and that total catabolism of administered S^{35}-labeled-methionine (as represented by the excretion of S^{35}-labeled-sulfate) occurs at a constant rate over the first 28 hours.

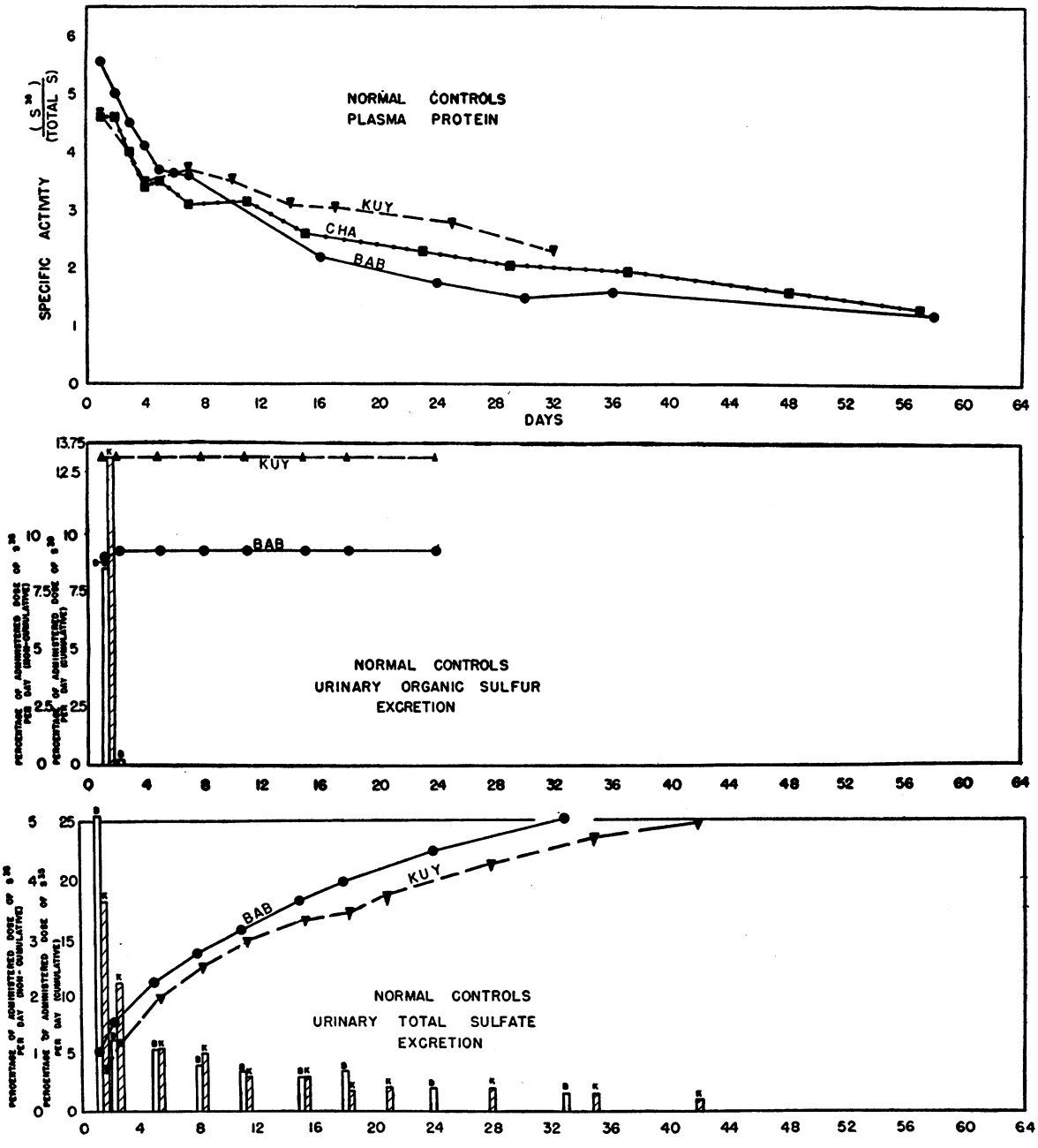


FIG. 2. S^{35} DATA IN NORMAL CONTROLS OVER A PERIOD OF SEVERAL WEEKS FOLLOWING THE ADMINISTRATION OF S^{35}-LABELED-METHIONINE

As in Figure 1, the urinary data are so graphed that the columns represent excretion at a given time and the line graphs represent cumulative excretion up to that time. It is apparent that all three normal controls metabolize S^{35}-labeled-methionine in a comparable fashion.

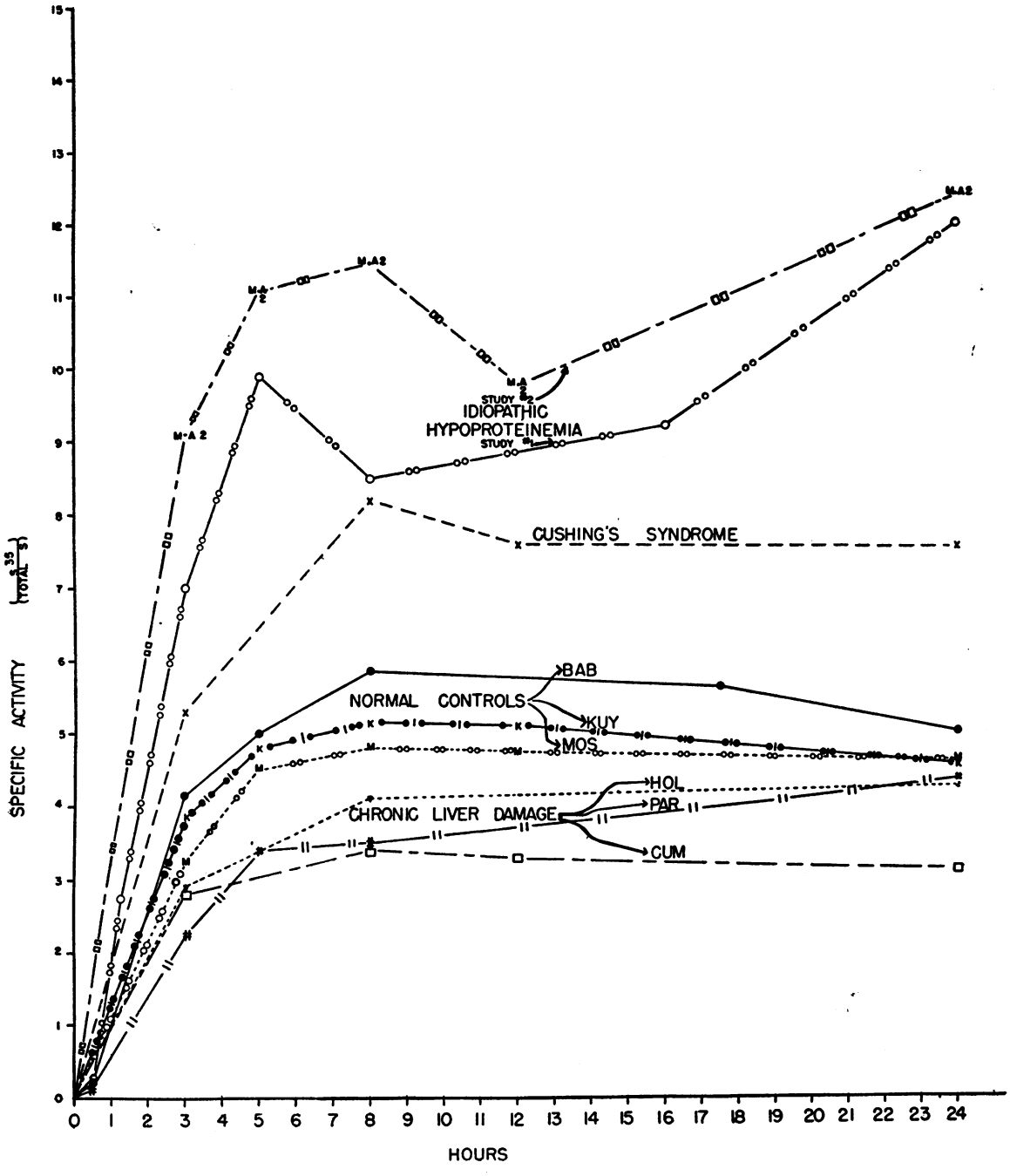


FIG. 3. INCORPORATION OF S^{35} INTO PLASMA PROTEIN DURING THE FIRST 24 HOURS FOLLOWING THE ADMINISTRATION OF S^{35}-LABELED-METHIONINE

$$\left(\text{Specific activity} = \frac{\text{Corrected cts. per min.}}{\text{mg.} \times 100} \div \frac{\text{Dose in microcuries}}{50}\right)$$

2. Negligible amounts of S^{35}-labeled-organic-sulfur are found in the urine after the first 48 hours.

3. The rates of excretion of S^{35}-labeled-sulfate in the urine in the two individuals so studied are highly comparable. As one would expect, the actual daily excretion of urinary inorganic S^{35} becomes progressively less over a period of 44 days.

Metabolism of S^{35}-Labeled-Methionine in Patients with Metabolic Abnormalities

Incorporation of S^{35} into plasma protein during the first 24 hours (Figure 3)

Examination of Figure 3 reveals the following:

1. In three patients with chronic liver damage, it appears that the amount of S^{35} incorporated into plasma protein during the first 24 hours is significantly less than that occurring in normal individuals.

2. The rate of incorporation of S^{35} into plasma protein in a patient with Cushing's syndrome is considerably greater than that which occurs in the normal controls (this same finding has recently been noted in other patients with Cushing's syndrome) (8).

3. The rate of incorporation of S^{35} into plasma protein in a patient with "idiopathic" hypoproteinemia appears to be in excess of that found in the normal controls. A duplicate study performed in this patient one year after the initial study gave quite comparable findings. It should be noted that this patient had a total serum protein which was less than half the normal, and that consequently, the administered dose of S^{35}-labeled-methionine was greater in relation to the total plasma protein mass, than was the case in the other individuals studied. The implications of this will be discussed (see below).

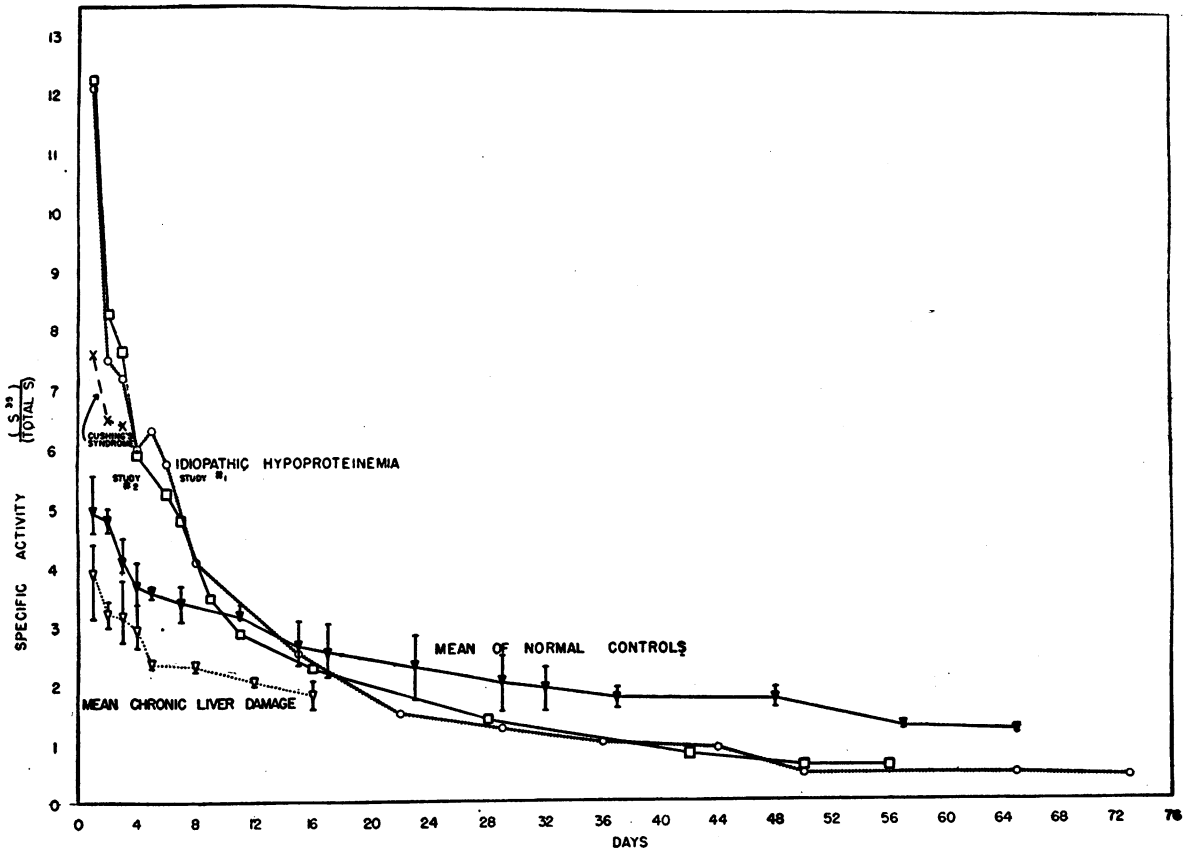


FIG. 4. RATE OF DISAPPEARANCE OF S^{35}-LABELED-METHIONINE FROM THE PLASMA PROTEIN AFTER THE INITIAL 24-HOUR PERIOD

The initial values represent the concentration of S^{35} in plasma protein at the end of the first 24 hours following the administration of S^{35}-labeled-methionine.

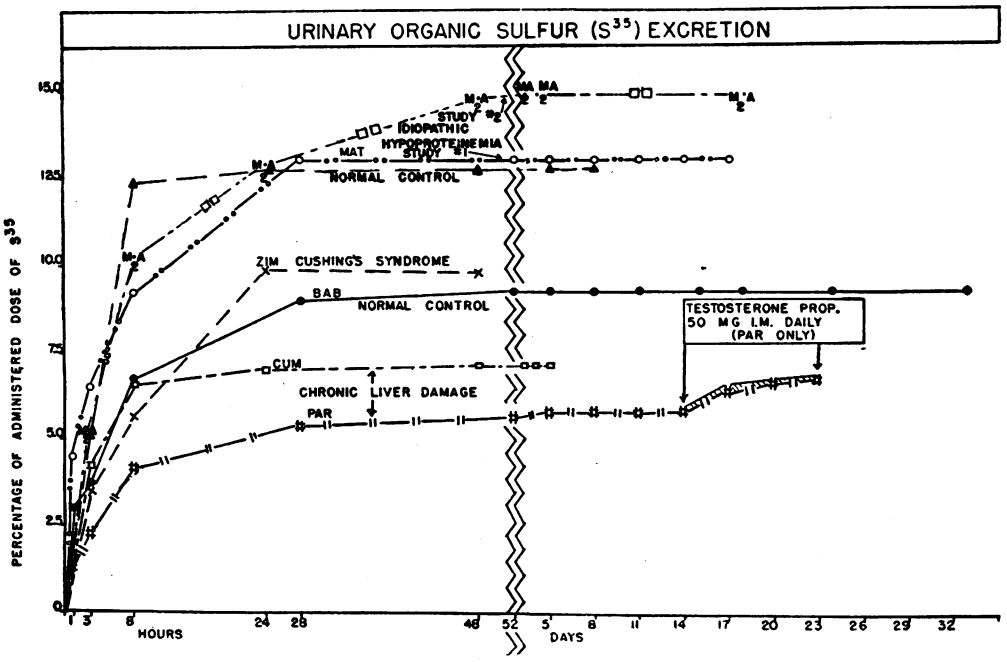


FIG. 5. EXCRETION OF S^{35}-LABELED-ORGANIC-SULFUR, FOLLOWING INTRAVENOUS ADMINISTRATION OF S^{35}-LABELED-METHIONINE (CUMULATIVE CHART)
Most of the organic S^{35} excreted during the first 48 hours is probably D-methionine.

Disappearance of S^{35} from plasma protein after the initial 24-hour period

The data presented in Figure 4 represent plasma protein S^{35} concentrations in the same individuals considered in Figure 3, subsequent to the initial 24-hour period. It appears that:

1. The *rate of disappearance* of S^{35} from the total plasma protein in patients with chronic liver damage does not differ greatly from that noted in the normal controls. The *actual* plasma S^{35} concentration is at all times less in these individuals than in the normal controls. This latter observation is attributable to the initially slower rate of incorporation of S^{35} into plasma protein in patients with chronic liver damage as compared to normal controls (see above).

2. This portion of the study in the patient with Cushing's syndrome was too short to permit of interpretation. The patient was operated (for the removal of an adrenal tumor) at the end of four days, and the study was discontinued for that reason. Longer studies in other patients are under way.

3. The *rate of disappearance* of S^{35} from the plasma protein in the patient with "idiopathic" hypoproteinemia occurred at a vastly greater rate than was the case in the normal individual. The S^{35} content of plasma protein at the end of 24 hours after methionine administration in this patient was approximately two and one-half times that noted in the normal. At the end of three weeks, the S^{35} content of plasma protein in this patient was approximately two-thirds that noted in the normal.

Urinary excretion of S^{35}-labeled-organic sulfur (Figure 5)

In all individuals studied, including the normal controls, the urinary excretion of S^{35}-labeled-organic sulfur occurred most rapidly during the first eight hours following the administration of the labeled methionine and had almost ceased after the second day. In previously reported studies with non-isotopic DL-methionine (2b), it was found that a large portion of administered D-methionine was excreted in the urine over this same period, whereas extremely little L-methionine was excreted at any time. The material which comes through in the urine as S^{35}-labeled-organic sulfur has yet to be identified, but in view of the foregoing, it is probable that most of the urinary

isotopic organic sulfur is D-methionine. Furthermore, in the work just mentioned, using non-labeled methionine, it has been found (with one exception) that the urinary excretion of D-methionine appears to bear no relation to pathological states; viz., there is as great a variability in normal individuals as there is in individuals with pathological entities.

In Patient PAR, one of the men with chronic liver damage, it will be noted that when testosterone propionate was administered (14 days after the initial administration of the labeled methionine), there was an immediate resumption of excretion of labeled organic sulfur in the urine. *This occurred at the same time that the patient went into strongly positive nitrogen and sulfur balance in response to the anabolic effect of the testosterone.* The possible interpretation of this finding is discussed later (see below).

Urinary excretion of S^{35}-labeled-total-sulfate

Urinary total sulfate serves as an index of catabolism of the administered S^{35}-labeled-methionine. In Figure 6 are shown the sulfate data in the patients described above. One notes that:

1. In the normal controls less than 5% of the administered dose of S^{35}-labeled-methionine is catabolized and excreted during the first 24 hours. Thereafter the rate of catabolism and excretion of the administered methionine progressively decreases, so that by the end of the fifth week the normal individual has catabolized and excreted approximately 25% of the administered dose of S^{35}-labeled-methionine. If one adds to this the approximately 10% of the administered S^{35} which was excreted as organic sulfur, it appears that at the end of five weeks, about one third of the original S^{35} has been excreted in the urine, plus a small amount in the stool. If one adds to the excretion figures the loss through decay of the S^{35}, it is apparent that approximately 50% of the original amount of S^{35} is still present in the body at the end of five weeks.

2. In the patients with chronic liver damage, approximately 10 % of the administered dose has been catabolized and excreted at the end of the first 24 hours (twice that of the normals). By the end of the second week, the one patient with chronic liver damage in whom urinary inorganic sulfate data are available for that period of time,

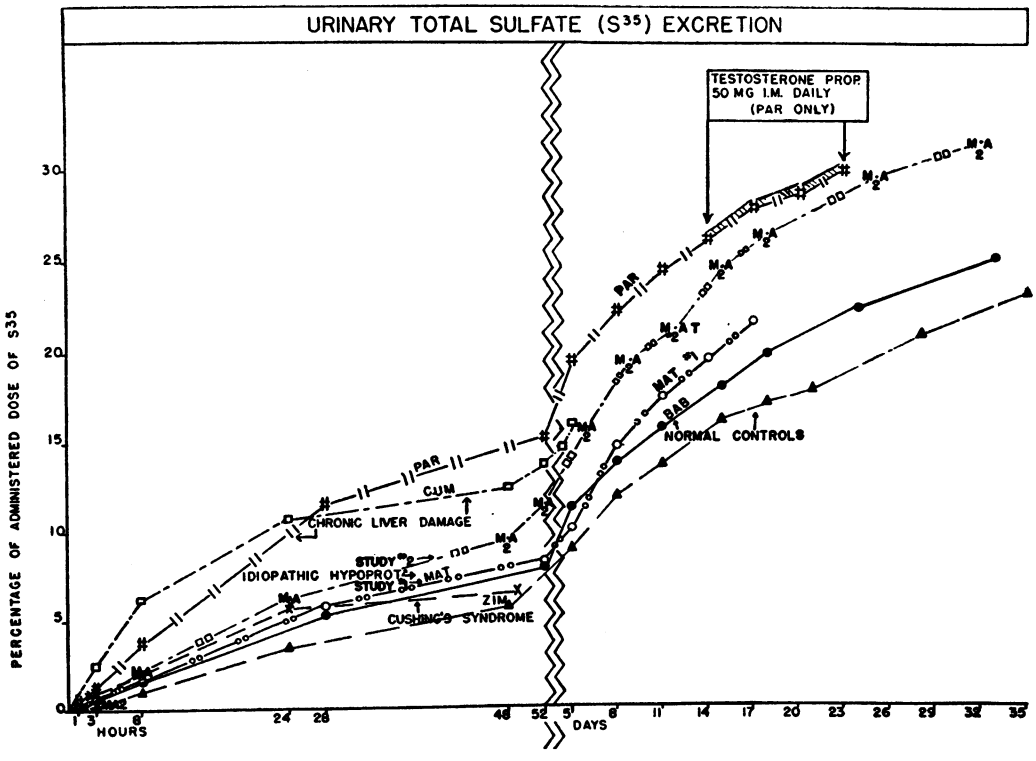


FIG. 6. EXCRETION OF S^{35}-LABELED-TOTAL-SULFATE IN THE URINE

had catabolized and excreted approximately 28% of the original amount S^{35}-labeled-methionine as compared to catabolism and excretion of approximately 18% in the normal controls.

3. The patient with "idiopathic" hypoproteinemia, during the first 24 hours metabolized and excreted approximately 6% of the administered dose of S^{35}. By the 32nd day, he had catabolized and excreted approximately 32% of the administered dose as compared to a figure of 23% in the normal controls.

4. The shortness of this portion of the study on the patient with Cushing's syndrome makes impossible legitimate interpretation of data.

S^{35} content of protein-free filtrate of plasma

In all patients studied, a rapid fall occurred over the first three hours. Thereafter, the S^{35} disappeared from the plasma protein-free filtrate at a gradually decreasing rate. Measurable amounts were still present at the end of 24 hours in all patients studied, but negligible amounts were present at the end of 48 hours and thereafter. Testosterone propionate administration produced no change in the S^{35} content of the plasma protein-free filtrate in Patient PAR.

S^{35} content of the stool (Table II)

It is apparent that small but appreciable amounts of the administered S^{35} are excreted into the stool. It is of interest that the patient with "idiopathic" hypoproteinemia had the greatest amount of any of the three individuals whose fecal S^{35} has been quantitated. Considerably more data will have to be obtained before one can determine whether this finding has any significance.

In vitro *incorporation of S^{35} into plasma protein*

Incubation of plasma with S^{35}-labeled-methionine (600 counts/cc. of plasma/minute) at 37.5° C., followed by precipitation and washing of the plasma protein as outlined under "METHODS," resulted in complete lack of incorporation of S^{35} in the plasma protein at intervals of three, eight, 24, 48, and 72 hours.

TABLE II

Fecal excretion of S^{35} in three of the individuals studied

	BAB (Normal)	PAR (Cirrhosis)	MAT "Idiopathic" hypoproteinemia
Period 1 (six days)	0.91%	1.53%	1.98%
Period 2 (six days)	0.45%	0.43%	1.20%
Period 3 (six days)	0.47%	0.21%	0.75%
Period 4 (six days)	0.32%	0.17%	

DISCUSSION

S^{35}-labeled-methionine represents a research tool which can be used safely in the human subject for the evaluation of protein metabolism in general, and sulfur metabolism in particular. S^{35} appears to have many of the advantages of isotopic carbon and to be free of the serious disadvantage represented by the 5,000-year half life of C^{14}. Further, S^{35} has certain specific metabolic advantages that C^{14} does not possess.

The data presented above indicate that S^{35} quantitation, when carried out properly, yields reproducible and comparable results in normal individuals. Considerable deviations from the normal are observed in individuals with specific metabolic abnormalities. In the following pages will be presented those interpretations of the data which, to us, appear justified.

Evaluation of S^{35} Data

By definition, the biological incorporation of sulfur or any other element into protein tissue represents *anabolism.* During the first eight hours following the administration of S^{35}-labeled-methionine, there is a steadily rising titre of plasma-protein-S^{35}; in other words, the concentration of plasma-protein-S^{35}-precursor is such that more S^{35} goes into than comes out of plasma protein. During the period eight to 24 hours a near equilibrium is achieved, *i.e.,* a plateau is approached. Thereafter, the rate of loss of S^{35} from plasma protein (*i.e.,* its dilution by non-isotopic sulfur) exceeds the rate of incorporation, and the plasma protein concentration of S^{35} falls. It will be recalled that the S^{35} concentration in the plasma protein-free filtrate reached levels too low to permit of measurement within 48 hours after S^{35} administration. At this time one may assume that all S^{35} which has not been excreted is incorporated in various organs and tissues—and that available precursor of S^{35}-labeled-plasma-protein approaches zero. Hence the decreasing concentration of S^{35} in plasma protein after the initial 48-hour period may be regarded as an index of the rate of catabolism of plasma protein. Certainly this is true in a comparative sense (*i.e.,* normal *vs.* abnormal), and perhaps in an absolute sense. The *absolute*

interpretation of the "anabolic limb" of the curve (*i.e.*, the first eight hours) is not permissible at the present time. Demonstration of a constant mathematical relationship between a specific precursor and plasma-protein-S^{35} may make such interpretation possible. Until such information is available, one is justified in speaking of *relative* rates of anabolism, *i.e.*, normal *vs.* abnormal, under standard conditions.[7]

Metabolic evaluation of patients with liver damage

Anabolism of plasma protein appears to be diminished in all such individuals studied. The increase in $S^{35}O_4$ excretion in these patients during the first 48 hours can be attributed to catabolism of that portion of the isotopic methionine which is not incorporated into protoplasm.

Consideration of the metabolic defect in a patient with "idiopathic" hypoproteinemia

Diminished plasma protein could result from a number of possible causes—impaired food intake, impaired absorption, excess urinary excretion of protein or amino acids, diminished protein anabolism, or increased protein catabolism. All except the last two possibilities had been eliminated in Patient MAT by other metabolic studies. For the following reasons we believe that one may safely conclude that in MAT the basic defect is one of hypercatabolism:

1. His rate of incorporation of S^{35} into plasma protein is at least as fast as normal, or faster. The data as graphed (Figure 3) indicate a rate of incorporation more than twice normal. It will be recalled that MAT's plasma protein was approximately half normal. If the amount of S^{35} incorporated into plasma protein bears a direct linear relationship to the total plasma protein content, all the S^{35}-plasma-protein figures in this man should be divided by 2 to make them comparable with the normal control. Since the total muscle mass in this man (in terms of physical findings and creatinine excretion) approached the normal, and since less than 10% of the administered S^{35}-labeled-methionine normally appears in plasma protein, it is probable that a figure of considerably less than 2 should be used for such correction. In any event there is no evidence of impaired plasma protein anabolism, as compared to the normal.

2. The rate of disappearance of S^{35} from plasma protein, regardless of any correction figure, is greatly in excess of the normal.

3. The urinary excretion of $S^{35}O_4$ is well in excess of the normal. This increase in the rate of excretion appears after the first 24 hours and is progressive (Figure 6). It may well be that the physiologic abnormality in this man is analogous to that occurring in patients with hemolytic anemia—*i.e.*, hypercatabolism, not compensated by hyperanabolism.

The protein metabolic defect in Cushing's syndrome

Diminished protein mass is an impressive part of Cushing's syndrome. Albright has raised the question as to the mechanism and has presented the evidence for and against the concept of "antianabolism" (13).

If the rate of anabolism of plasma proteins in this disease is at all representative of the metabolic defect in the fixed tissue proteins, one may conclude that the protein metabolic defect is not one of antianabolism, and hence is presumably one of hypercatabolism. Additional evidence in support of this concept is presented elsewhere (8).

Metabolic effects of testosterone propionate

Testosterone propionate administered to Patient PAR produced a protein anabolic effect, *i.e.*, his urinary nitrogen, sulfur, potassium, and phosphorus excretions diminished. Further, there appeared to be a transfer of S^{35} from tissue into plasma protein (Figure 7).

The rate of urinary $S^{35}O_4$ excretion was altered very little (Figure 6), but immediate resumption of urinary excretion of organic sulfur occurred (Figure 5). This latter finding was most unex-

[7] We believe the term "turnover" can be misleading in the evaluation of metabolic data obtained with the use of isotopes. "Turnover" by definition implies metabolic equilibrium, *i.e.*, anabolism = catabolism. It is obvious that such a concept is untenable during periods of growth (hyperanabolism), or senescence (relative hypercatabolism). We think it is desirable to use the terms "anabolism" and "catabolism" rather than "turnover," so long as one bears in mind that, at least at the present time, these terms represent comparative rather than absolute values.

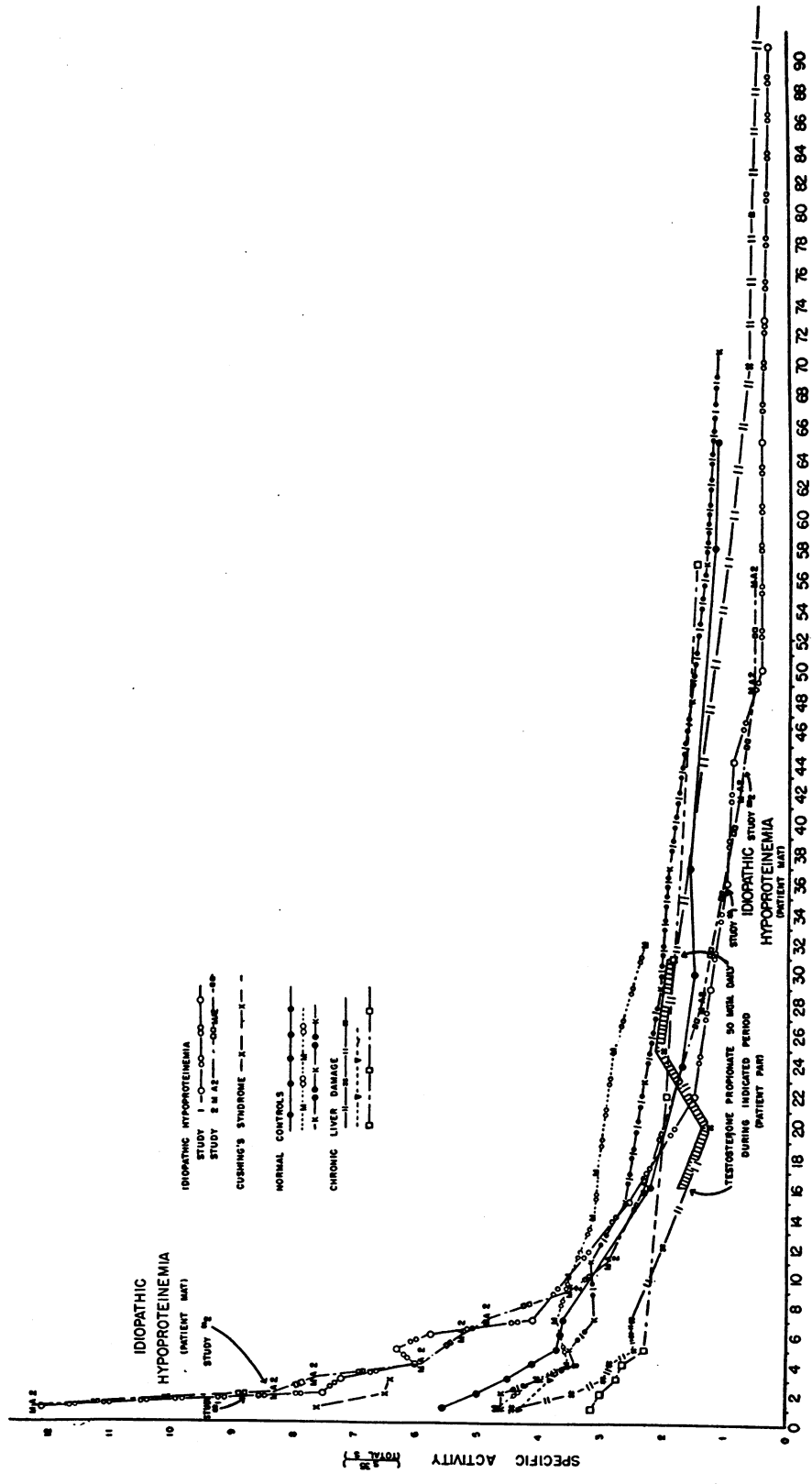


FIG. 7. EFFECT OF TESTOSTERONE PROPIONATE UPON PLASMA PROTEIN FORMATION IN A PATIENT (PAR) WITH CHRONIC LIVER DAMAGE

pected. Until the nature of the organic sulfur has been determined its significance must remain obscure. It seems probable that testosterone stimulates the anabolism of specific tissues, and that other tissues are broken down to supply "raw materials." Since the renal threshold for L-methionine is very high, it is probable that some other sulfur-containing compound is responsible for the urinary organic S^{35}.

SUMMARY

S^{35}-labeled-methionine can be used safely in the human subject for investigative purposes.

The incorporation of this material into plasma proteins in normal individuals occurs at a predictable rate. This is equally true of its later disappearance from plasma proteins and its rate of excretion as S^{35}-labeled-sulfate in the urine.

In patients with chronic liver disease, with "idiopathic" hypoproteinemia, and with Cushing's syndrome, significant deviations from the normal pattern are observed. The findings suggest that there is no impairment of protein anabolism in patients with Cushing's disease; that there is a significant defect in protein anabolism in the presence of liver disease; and that in one variety of "idiopathic" hypoproteinemia a very excessive rate of catabolism of plasma protein occurs.

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Because of erroneous initial backscatter measurements, all *excretion* figures included in this paper must be multiplied by a factor of 1.18.

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