ALBUMIN METABOLISM IN TROPICAL SPRUE

BY S. K. VAISH, M. IGNATTUS, AND S. J. BAKER

(From the Wellcome Research Unit, Christian Medical College Hospital, Vellore, South India)

In tropical sprue it is common to find hypoproteinaemia and hypoalbuminaemia (Lopez, Milanes, Spies, Toea, Aramburu, and Lopez, 1949; Baker, 1957). Theoretically this hypoproteinaemia can be due to decreased intestinal absorption of dietary protein, or to decreased production, increased degradation, or excessive loss of body protein. The introduction of isotopically labelled albumin provided a means for studying albumin metabolism in the body (London, 1950; Sterling, 1951). This material has also been used by some workers to study protein excretion into the lumen of the intestinal tract (Citrin, Stirling, and Halstead, 1957; Steinfield, Davidson, and Gordon, 1957). However, because the protein excreted into the lumen is digested and the label is split off and reabsorbed, it is not possible to use this method for measuring the amount of protein excreted. Gordon (1959) introduced $^{131}$I labelled polyvinylpyrrolidone ($^{131}$I P.V.P.) as a substitute for labelled albumin. The $^{131}$I P.V.P. was not digested or reabsorbed in the intestine, and therefore the amount excreted into the intestinal tract could be measured. However, with this material it was not possible to study albumin metabolism in the body. Jeejeebhoy and Coghill (1961) introduced a technique of administering $^{131}$I labelled albumin, and feeding an ion-exchange resin to bind any $^{131}$I liberated from protein breakdown in the intestine. These investigators suggested that this provided a means of measuring intestinal losses, and at the same time studying albumin metabolism.

The investigations reported in the present paper were undertaken to study albumin metabolism in subjects with tropical sprue, using $^{131}$I labelled albumin and administering ion-exchange resin by mouth.

Materials and Methods

Nineteen patients suffering from tropical sprue and ten healthy control subjects of similar socio-economic status, but with no evident gastrointestinal disease, were studied. All the patients had steatorrhoea, radiological changes in the intestine, and other evidence of malabsorption (Baker, 1957). The patients tended to improve while in hospital, even in the absence of specific therapy, and in four of the subjects the fat excretion had returned to normal.

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at the time of the study (Table I). All the controls had normal fat balances, a normal D-xylose excretion, and normal radiological findings on barium-meal examination.

Highly purified human serum albumin (Behringwerke) was obtained in a freeze-dried state. $^{131}$I, free of reducing agent, was obtained from the Indian Atomic Energy Establishment. Radio-iodinated human serum albumin (labelled albumin) was prepared by the method of McFarlane (1956 and 1958). The resulting iodinated protein solution contained an average of one atom of iodine per molecule of protein. The non-precipitable radioactivity in each batch, using trichloroacetic acid, was less than 2 per cent. of the total. To minimize radiation damage to the protein the labelled material was diluted with unlabelled albumin to give a final concentration of approximately 1 $\mu$g/mg. of albumin. Some of each batch of $^{131}$I labelled albumin was used in studies on the control group in order to ensure that no significant denaturation of the protein had occurred.

The patients were given Lugol's iodine 0-6 ml. three times a day orally, for two days before and during the test, to block the thyroid uptake of radio-iodine. Amberlite resin 'IRA 400' was given in a dose of 3 g. four-hourly immediately preceding and during the whole period of the test. Twenty-five to 30 $\mu$g. of labelled albumin were injected intravenously in each patient. A sample was taken from another vein, 10 minutes after the injection, to determine the initial plasma activity. Subsequent samples were taken at 24-hour intervals thereafter for the duration of the test, and the plasma radioactivity measured. Urine and faeces were collected separately, care being taken not to contaminate faeces with urine, and the radioactivity measured in successive 24-hour collections for up to two weeks. Blood radioactivity was measured in a sodium iodide crystal well scintillation counter, and stool and urine activity in a ring counter with eight Geiger tubes arranged in parallel.

The serum albumin concentration was determined by the biuret method after precipitation of the globulins with 22-5 per cent. sodium sulphate. Daily 24-hour stool-fat excretion was estimated by the method of van de Kamer, Huinink, and Weyers (1949). Xylose absorption was studied by feeding 5 g. of D-xylose to the fasting subject, and estimating the amount excreted in the urine during the subsequent five hours. Jejunal biopsies were performed with the multiple retrieving intestinal biopsy tube (Baker and Hughes, 1960).

Definitions and Calculations

Plasma volume. The plasma volume was calculated from the formula:

$$\text{Plasma volume} = \frac{\text{Injected radioactivity (mc.)}}{\text{Initial plasma radioactivity (mc./ml.)}}$$

Total plasma albumin. The total plasma albumin (P.A.) was calculated from the formula:

$$\text{P.A.} = (\text{plasma volume}) \times (\text{serum albumin concentration})$$
| Age Sex | Weight | Pooled fat | Pooled fat | Ex Vivo | Ratio of extrarenal to intrarenal glomerular filtration rate | Mean corrected clearance of 125I-albumin | T.E.A. | 'Endogenous' dependence | 'Energetic' dependence | Total | dependence
|---------|--------|------------|------------|---------|---------------------------------------------------------|--------------------------------------|--------|------------------------|------------------------|--------|-----------------
| Controls
| 1. | 55 M | 47.0 | 3.5 | 32.0 | 5.8 | 3.8 | 0.52 | 9.50 | 1.31 | 137 | 222.9 | 244.8 | 4.5 | 5.1 | 66.4 | 124.0 | 168.4 | 197
| 2. | 28 M | 49.0 | 6.0 | 57.4 | 4.5 | 5.4 | 0.24 | 9.60 | 1.31 | 136 | 240.2 | 225.6 | 5.0 | 4.5 | 66.6 | 170.0 | 166.6 | 240
| 3. | 54 M | 48.0 | 5.5 | 40.5 | 5.0 | 5.8 | 0.27 | 9.60 | 1.77 | 152 | 257.9 | 233.4 | 5.5 | 5.2 | 57.2 | 131.0 | 168.2 | 168
| 4. | 25 M | 52.0 | 1.5 | 42.0 | 4.0 | 5.8 | 0.14 | 10.40 | 1.74 | 124 | 234.9 | 255.3 | 4.9 | 4.7 | 71.0 | 217.0 | 314.1 | 208
| 5. | 60 M | 43.0 | 3.0 | 28.4 | 3.0 | 5.7 | 0.23 | 7.23 | 3.12 | 100 | 213.5 | 242.5 | 5.1 | 5.4 | 38.5 | 143.5 | 182
| 6. | 37 M | 47.0 | 4.0 | 38.4 | 3.0 | 5.3 | 0.34 | 9.69 | 5.09 | 218 | 290.5 | 275.2 | 4.9 | 5.1 | 20.5 | 165.0 | 187.0 | 183
| 7. | 60 M | 49.0 | 2.5 | 26.9 | 2.5 | 5.7 | 0.35 | 1.13 | 1.65 | 178 | 230.0 | 233.5 | 4.0 | 4.7 | 16.5 | 105.0 | 121
| 8. | 53 M | 41.0 | 4.0 | 28.4 | 4.0 | 5.7 | 0.60 | 7.43 | 1.94 | 218 | 196.4 | 212.6 | 5.9 | 6.2 | 28.7 | 109.0 | 137.0 | 153
| 9. | 55 F | 25.0 | 4.0 | 28.4 | 4.0 | 5.7 | 0.60 | 7.43 | 1.94 | 218 | 196.4 | 212.6 | 5.9 | 6.2 | 28.7 | 109.0 | 137.0 | 153
| 10. | 50 F | 44.0 | 2.9 | 25.5 | 3.0 | 5.7 | 0.35 | 1.13 | 1.65 | 178 | 230.0 | 233.5 | 4.0 | 4.7 | 16.5 | 105.0 | 121

** = mean pooled fat excursion during first week of the study.  
T.E.A. = total exchangeable albumin.  
\* = ten days during the week of the study.
'Half-life' of labelled albumin. The plasma radioactivity at any particular time was expressed as a percentage of the initial plasma radioactivity. These values, plotted on semi-logarithmic paper, formed the 'plasma decay curve' (Fig. 1). The 'half-life' of the labelled albumin in the plasma was obtained by determining the slope of this plasma decay curve after the initial period of rapid decline (Sterling, 1951) and determining by inspection the number of days taken for the plasma activity to be reduced by 50 per cent.

![Graph showing plasma decay curves](image)

**Fig. 1.** Typical plasma decay curves:
- Control 4.
- Patient 13.

*Faecal excretion of $^{131}$I.* The 24-hour faecal excretion of radioactivity was expressed as a percentage of the original dose of radioactivity administered. The amount of label excreted into the intestinal tract in a given time will be proportional to the plasma concentration during that time. Since the plasma concentration declines with the passage of time, faecal excretion will also similarly decline. The percentage faecal excretion of label in any particular day was therefore corrected so that it represented the amount which would have been excreted if the plasma activity had been at the initial level (100 per cent.) throughout. This was done by dividing the total stool radioactivity by the geometrical mean of the plasma activity during the preceding 24 hours and multiplying by 100. The 'mean daily faecal excretion' was calculated by obtaining the mean of the corrected percentage daily excretion for the first six days of the test.

*Exogenous degradation rate of plasma albumin.* Any plasma albumin 'excreted' into the intestine is normally broken down by intestinal enzymes. The amount of albumin lost into the intestine per day has been termed the 'exogenous degradation rate' of plasma albumin. If it is assumed that the contribution of $^{131}$I due to salivary iodide excretion or passage of 'free' $^{131}$I across

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1 'free' is used here to denote $^{131}$I not bound to protein and may represent several compounds such as iodide, monooiodotyrosine, &c.
the mucosa is negligible, then the 'exogenous degradation rate' of the plasma albumin may be calculated from the formula:

\[
\text{Exogenous degradation rate} = \frac{\text{Mean daily faecal excretion of } ^{131}\text{I}}{\times \text{P.A. } \times 1,000 \text{ mg./kg./day}} \times 100 \times W
\]

where \( W \) is the weight of the subject in kilograms.

**Urinary excretion of \(^{131}\text{I}\.** The excretion of \(^{131}\text{I}\. was measured in successive 24-hour collections of urine, and expressed as a percentage of the radioactivity administered. The excretion in any particular day was corrected so that it represented the amount which would have been excreted if the plasma activity had been at the initial level throughout (100 per cent.\. This was done by the same method as was used for correcting the faecal excretion.

The urinary losses in the first day may include some \(^{131}\text{I}\. liberated from rapidly destroyed albumin molecules, and in accordance with the practice of some other investigators these have been ignored (Veal and Vetter, 1958). The mean daily loss for days two to six inclusive has been called 'the' mean corrected urinary loss (M.C.U.L.).

In the absence of albuminuria, the urinary excretion of \(^{131}\text{I}\. results from the degradation of the labelled albumin (McFarlane, 1963). In this study the urinary \(^{131}\text{I}\. excretion therefore represents the amount of albumin catabolized in the body, less any \(^{131}\text{I}\. which enters the intestine where it is bound by the ion-exchange resin.

**The amount of labelled albumin retained in the body.** The amount of labelled albumin retained in the body on any particular day was calculated by subtracting the sum of the corrected percentage excretion in the urine and stools from 100 per cent.

**Pool distribution of labelled albumin. Method 1:** If the labelled albumin is considered to be distributed in two pools—the 'intravascular' and 'extravascular'—there will be a theoretical time when the concentration of the labelled albumin in the extravascular pool reaches a maximum. At this time the rate of entry of labelled molecules into the extravascular pool from the intravascular pool will be the same as the rate of exit of labelled molecules from the extravascular pool into the intravascular pool, and the two pools will momentarily be in equilibrium. The time at which this occurs is termed the 'equilibrium time'. At this time the ratio of the radioactivity in the intravascular and extravascular pools will be a measure of the relative amounts of albumin in the two pools (Campbell, Cuthbertson, Mathews, and McFarlane, 1956). The plasma decay curve and the amount of labelled albumin retained in the body on successive days were plotted on semi-logarithmic paper. By subtraction, the amount of radioactivity in the extravascular pool could also be plotted. The time when the radioactivity in the extravascular pool reached a maximum was then determined by inspection, and the relative amounts of labelled albumin in the intravascular and extravascular pools at equilibrium time were determined from the graph (Fig. 2).
Method 2: It has been shown by Berson, Yalow, Schreiber, and Post (1953) that after some days the relative amounts of labelled albumin in the intra- and extravascular pools appear to remain constant. Thus if the ratio of the percentage radioactivity in the plasma over the per cent. radioactivity retained in the body is plotted on successive days, the graph will approach towards a straight line. The level of this line may then be taken as an indication of the percentage of the label in the intravascular pool.

![Diagram](image)

**Fig. 2.** Patient 10. Estimation of equilibrium time. Equilibrium time is the point at which the radioactivity in the extravascular pool is a maximum, i.e. 2.8 days.

- ○ Plasma decay curve.
- □ Amount of radioactivity retained in the body.
- ● Amount of radioactivity in extravascular pool.

*Total exchangeable albumin.* The total exchangeable albumin (T.E.A.) may be estimated by the formula:

\[
\text{T.E.A.} = \frac{\% \text{ Radioactivity retained in body} \times \text{P.A.}}{\% \text{ Intravascular radioactivity}}
\]

The T.E.A. was estimated in two different ways using the two different methods for obtaining the pool distribution mentioned above.

1. **Endogenous degradation rate** of plasma albumin. *Method 1:* The ‘endogenous degradation rate’ of plasma albumin, or the amount of albumin broken down per day, exclusive of faecal losses, was calculated as follows:

   \[
   \text{‘Endogenous degradation rate’} = \frac{\text{P.A.} \times (\text{M.C.U.L.}) \times 1,000 \text{mg./kg./day}}{100 \times W}
   \]

2. **Method 2:** It has been shown by Berson, Yalow, Schreiber, and Post (1953) that after some days the ratio of the amount of radioactivity excreted in the urine over the amount retained in the body becomes a constant (C), and is a measure of the rate of endogenous degradation of body albumin. Then

   \[
   \text{‘Endogenous degradation rate’} = \frac{\text{T.E.A.} \times C \times 1,000 \text{mg./kg./day}}{W}
   \]
'Total degradation rate' of plasma albumin. The total amount of body albumin degraded per day was obtained by adding together the 'exogenous' and 'endogenous' degradation rates.

Results

The detailed individual results are set out in Table I.

*Plasma albumin concentration.* The plasma albumin in the control group varied from 3-3 to 5-0 g./100 ml. with a mean of 3-84 g./100 ml. (S.E. 0-15).

In the sprue patients, the plasma albumin varied from 1-8 to 3-7 g./100 ml. with a mean of 3-04 g./100 ml. (S.E. 0-14) (Fig. 3). The difference between the groups is significant at the 0-1 per cent. level ($t = 3-91$).

![Graph](image.png)

**Fig. 3.** Plasma albumin levels in grammes per 100 millilitres in control subjects and patients with sprue.

'Half-life' of labelled albumin. Initially in all subjects the plasma radioactivity declined rapidly, but subsequently the rate of decline decreased. Typical results for a control subject and a patient are shown in Fig. 1. The 'half-life' of labelled albumin in the control subjects varied from 16-0 to 21-0 days with a mean of 18-7 days (S.E. 0-62).

In the patients with sprue the 'half-life' varied from 6-4 to 20-2 days with a mean of 9-46 days (S.E. 0-80). The difference between these two is significant at the 0-1 per cent. level ($t = 9-96$).

*Loss of label into the gastrointestinal tract.* The daily loss of label in the stools of the control subjects varied from 1-3 per cent. to 3-4 per cent. with a mean of 2-3 per cent. (S.E. 0-26). In the subjects with sprue the daily loss varied from 1-8 per cent. to 10-1 per cent. with a mean of 4-6 per cent. (S.E. 0-49) (Fig. 4). The difference between the two groups is significant at the 0-1 per cent. level ($t = 4-40$).

The relationship between the percentage daily excretion of label in the stools and the faecal-fat excretion in the sprue subjects is shown in Fig. 5. Although the points show a wide scatter, there is a positive correlation between the two,
significant at the 5 per cent. level (r = 0.46). There was, however, no detectable correlation between the loss of label in the stools and the percentage xylose excretion, or between the loss of label and the plasma albumin concentration.

Jejunal biopsies were done in all the sprue cases, but there was no correlation between either the dissecting microscopic appearance (Baker, Ignatius, Mathan, Vaish, and Chacko, 1982) or the conventional histological appearance of the biopsy specimens and the amount of label excreted into the intestine per day.

![Graph](image)

**Fig. 4.** Faecal excretion of 3H label, per cent. of administered dose per day, in control subjects and patients with sprue.

![Graph](image)

**Fig. 5.** Relationship in patients with sprue, between daily faecal-fat excretion and mean daily faecal excretion of 3H label expressed as per cent. of administered dose (r = 0.46).

'Exogenous degradation rate.' The 'exogenous degradation rate' in the controls varied from 16·5 to 71·0 mg/kg/day with a mean of 43·2 mg/kg/day (S.E. 6·5). In the subjects with sprue the rate varied from 21·2 to 117·2 mg/kg/day with a mean of 74·8 mg/kg/day (S.E. 6·6). The difference between the two is significant at the 1 per cent. level (t = 3·42).

Ratio of extravascular to intravascular pool. The ratio of the amount of labelled albumin in the extravascular pool to the amount in the intravascular pool in the control group varied from 1·21 to 2·12 with a mean of 1·70 (S.E. 0·10) when calculated by method 1, and from 1·20 to 2·13 with a mean of 1·71 (S.E. 0·11) when calculated by method 2.

In the sprue patients the ratio of the amount of labelled albumin in the extravascular pool to the amount in the intravascular pool varied from 0·29 to 2·31 with a mean of 1·08 (S.E. 0·12) by method 1, and from 0·29 to 2·28 with a mean 1·09 (S.E. 0·12) by method 2. The difference between the two groups by either method is significant at 0·1 per cent. level (t = 4·01 (method 1); and t = 3·90 (method 2)).

Total exchangeable albumin. The total exchangeable albumin in the control group varied from 196·4 to 280·5 g. with a mean of 235·2 (S.E. 8·50) when calculated by method 1, and from 212·6 to 292·2 g. with a mean of 239·8 (S.E. 7·13) when calculated by method 2.
In the patients with sprue the total exchangeable albumin varied from 63.7 g. to 296.9 g. with a mean of 142.6 g. (S.E. 18.5) when calculated by method 1, and from 68.8 g. to 297.3 g. with a mean of 148.1 g. (S.E. 15.5) when calculated by method 2. The difference between the two groups by either method is significant at 0.1 per cent. level \( t = 4.55 \) (method 1); \( t = 5.37 \) (method 2).

**Total exchangeable albumin/kg. body-weight.** The total exchangeable albumin/kg. body-weight in the control group varied from 3.9 to 5.5 g. with a mean of 4.36 g. (S.E. 0.15) when calculated by method 1, and from 4.2 to 5.7 g. with a mean of 4.96 g. (S.E. 0.14) when calculated by method 2.

**Fig. 6.** Total exchangeable albumin expressed as grammes per kilogram of body-weight in control subjects, and in patients with sprue.

**Fig. 7.** Relationship between total exchangeable albumin expressed in grammes per kilogram of body-weight and the mean daily faecal excretion of \(^{13}I\) expressed as per cent. of administered dose.

- **O = Controls.**
- **● = Patients.**

In sprue patients the total exchangeable albumin/kg. body-weight varied from 2.3 to 5.4 g. with a mean of 3.41 g. (S.E. 0.25) when calculated by method 1 (Fig. 6), and from 2.1 to 5.6 g. with a mean of 3.43 g. (S.E. 0.24) when calculated by method 2.

The difference between the two groups by either method is significant at 0.1 per cent. level \( t = 5.05 \) (method 1); \( t = 5.48 \) (method 2).

The relationship between the T.E.A./kg. and the faecal excretion of label is shown in Fig. 7. It will be seen that there was no correlation between the two (controls \( r = 0.26 \); patients \( r = 0.25 \)).

**Mean corrected urinary losses.** The mean corrected urinary losses (M.C.U.L.) in the controls ranged from 5.70 per cent. to 10.40 per cent. with a mean of 8.33 per cent. (S.E. 0.56). In the sprue subjects the range was from 4.16 per cent. to 10.60 per cent. with a mean of 7.44 per cent. (S.E. 0.74). The difference between the mean losses in the two groups is not statistically significant \( t = 1.31 \). There was no correlation between the M.C.U.L. and the faecal fat, the xylose excretion, or the plasma albumin concentration.
"Endogenous degradation rate." The 'endogenous degradation rate' in the control group varied from 102 to 217 mg./kg./day with a mean of 145·2 mg./kg./day (S.E. 10·8) when calculated by method 1, and from 107 to 214 mg./kg./day with a mean of 148·0 mg./kg./day (S.E. 10·3) when calculated by method 2.

In the sprue patients the 'endogenous degradation rate' varied from 39 to 276 mg./kg./day with a mean of 157·7 mg./kg./day (S.E. 14·5) when calculated by method 1 (Fig. 8), and from 35 to 265 mg./kg./day with a mean of 156·5 mg./kg./day (S.E. 14·5) when calculated by method 2.

![Graph showing endogenous degradation rate in controls and patients.](image)

![Graph showing relationship between endogenous degradation rate and plasma albumin.](image)

The difference between the means in controls and the patients by either methods 1 or 2 is not significant \(t = 0·53\) (method 1); \(t = 0·48\) (method 2). However, the values in four of the patients fall above the normal range and three fall below it.

The relationship between plasma albumin concentration and the endogenous albumin degradation rate in the patients is shown in Fig. 9. There is a positive correlation between the two values significant at the 1 per cent. level \(r = 0·62\).

No such correlation could be demonstrated in the control subjects \(r = 0·013\).

There was no correlation between the endogenous degradation rate and the T.E.A. or the T.E.A./kg. nor was there any demonstrable relationship between the degradation rate and the faecal fat excretion, or the xylose excretion.

"Total degradation rate." The 'total degradation rate' in the controls ranged from 119 to 288 mg./kg./day with a mean of 191·6 mg./kg./day (S.E. 15·0) and in the patients from 30 to 371 with a mean of 232·7 mg./kg./day (S.E. 19·0). The difference between these means does not attain statistical significance \(t = 1·69\).
Discussion

Methodology

$^{131}I$ label. The value of $^{131}I$ in labelling proteins for use in metabolic studies has been questioned (Goldsworthy and Volwiler, 1957). However, studies in animals have shown that the behaviour of chemically labelled $^{131}I$-albumin is practically identical with that of biosynthetically prepared albumin labelled with $^{14}C$ (Campbell, Cuthbertson, Mathews, and McFarlane, 1956; Cohen, Holloway, Mathews, and McFarlane, 1956; McFarlane, 1957). Further, in a human subject with analbuminaemia, it was shown that unlabelled and $^{131}I$-labelled albumin behaved similarly (Bennhold and Kallee, 1959; Freeman, Mathews, McFarlane, Bennhold, and Kallee, 1959). Therefore, with proper care in preparation and labelling of the albumin, a satisfactory product for clinical studies can be obtained.

Calculations

The validity of the calculations involved in arriving at the various parameters used in this and similar studies is based on a number of assumptions, some of which are difficult to prove. It is assumed that the patient is in a 'steady state' with respect to albumin metabolism, and that the rates of synthesis, degradation, and intestinal loss do not change from day to day. This is probably a valid assumption in the case of the controls, but whether or not it is true of the patients with sprue is open to some doubt. In order to keep as near as possible to a steady state, no therapy, apart from non-specific measures to control the diarrhoea, was given during the course of the study.

The 'half-life' of the labelled albumin circulating in the plasma has been assumed to follow a simple exponential decay (Sterling, 1951). However, it has been shown that if the observations are carried out accurately for a sufficient period of time, the rate of decay is not a simple exponential one, but is compounded of several different rates (Berson, Yalow, Schreiber, and Post, 1953). In this study the observations were not carried out for more than two weeks, and the 'half-life' was derived by fitting the best line to the plasma decay curves after the initial period of rapid decline. Although this figure may be an artificial concept, it does provide a convenient method of comparing one lot of $^{131}I$-labelled albumin with another in normal subjects. The figure obtained in our study for the 'half-life' of the labelled albumin in normal subjects was similar to that obtained by other workers (Table II). This suggests that there was little significant denaturation of the labelled albumin, and that the product was satisfactory for metabolic studies.

The distribution of labelled albumin in the body has been thought of as being in two major pools, the 'intravascular' and 'extravascular' (Sterling, 1951), and on the basis of this assumption it has been possible to calculate the equilibrium time, and the relative amount of label in the two pools, and hence the total exchangeable albumin. This assumption is not strictly valid. Cohen, Freeman, and McFarlane (1961) have shown, for instance, that there are at least
two extravascular pools—a rapidly exchanging and a slowly exchanging pool—and it may in fact be that there are even more pools than this (Reeve and Bailey, 1962). The validity of the assumption (Berson, Yalow, Schreiber, and Post, 1953) that after the initial period of equilibration the specific activity of the total exchangeable albumin pool is uniform is also doubtful. It has, however, been shown that, in normal subjects, the various methods of analysis give similar results (Cohen, Freeman, and McFarlane, 1961). In the present study, also, the two different methods of analysis used to determine the pool ratios,

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the total exchangeable albumin, and the degradation rates gave comparable results both in normal subjects and in the patients with sprue. The measurement of urinary $^{311}$I excretion has been taken as a measure of ‘endogenous catabolism’ of the labelled albumin. This assumes that the catabolic process occurs at a site closely related to the extravascular pool. McFarlane (1963) has demonstrated in rabbits that this assumption is justified.

**Separation of exogenous and endogenous catabolism**

The reliability of the method of Jeejeebhoy and Coghill (1961) employed in this study to obtain separate estimates of excretion of protein into the gut (‘exogenous catabolism’) and breakdown of albumin in other sites (‘endogenous catabolism’) is open to question. If adequate amounts of resin are fed, reabsorption of the majority of the label entering the intestine will be prevented. The label, however, may enter either as ‘free’ iodide resulting from the endogenous catabolism, or as the labelled protein itself, and the resin technique does not enable any distinction to be made between these two forms of iodide excretion.

Jeejeebhoy and Coghill (1961) and Jeejeebhoy (1962) reported patients with an increased catabolic rate of labelled protein, who did not have an increased excretion of $^{131}$I in the stool when fed amberlite resin, suggesting that protein degradation in extra-intestinal sites made little contribution to the fecal $^{131}$I excretion. Jones and Morgan (1963), on the other hand, using the technique in only three subjects, claimed that there was a correlation between the mean fecal excretion of $^{131}$I and the mean urinary excretion, and suggested that in normal subjects the $^{311}$I appearing in the stools was due largely to the ‘free’ $^{131}$I excreted in the saliva and gastric juice, rather than to protein loss into the intestine.
In the ten normal subjects in this study there was no detectable correlation between the mean daily corrected urinary and faecal losses of $^{131}$I, nor was there any correlation between the 'endogenous' and 'exogenous' degradation rates.

In the 10 patients with sprue there was a positive correlation significant at the 5 per cent. level ($r = 0.55$) between the mean daily corrected faecal and urinary losses (Fig. 10). A similar correlation was also observed between the rates of 'exogenous' and 'endogenous' catabolism. This raises the question as to whether the increased faecal excretion of $^{131}$I observed in the sprue group really represents the passage of labelled albumin into the intestine, or whether it is the result wholly or partly of increased endogenous catabolism, with subsequent passage of 'free' $^{131}$I into the intestine. As there is no significant difference between the mean corrected urinary losses in the control group and the patient group, it seems unlikely that increased endogenous catabolism in the latter could have accounted for all the increased faecal losses observed, since this would imply that all the extra $^{131}$I liberated by any increased endogenous catabolism had been excreted into the gut and not in the urine. It is therefore probable that the increased faecal excretion of $^{131}$I in the patients does in fact represent a true increase in protein loss into the intestine. The calculations made in obtaining the results reported above were based on this assumption. However, since the precise fraction of faecal $^{131}$I which comes from the direct passage of 'free' $^{131}$I into the intestine cannot be determined, the values obtained for the 'exogenous' degradation can only be regarded as an approximation to the true state of affairs. Nevertheless, since $^{131}$I P.V.P. does not permit of a study of protein metabolism, and since $^{131}$I labelling of serum albumin appears to denature the protein (Vaish, 1962), the present technique is the best method at present available for obtaining an indication of the relative contributions of 'exogenous' and 'endogenous' albumin catabolism to total body catabolism.

**Results**

The results obtained in this study in the 10 control subjects are similar to those obtained by investigators in other parts of the world (Table II).
The mean serum albumin concentration in the patients with sprue (3.94 g./100 ml.) was significantly lower than that in the normal controls (3.84 g./100 ml.). There was an even greater difference between the mean total exchangeable albumin in the two groups (142-6 g., as compared with 235-2 g.). Some of this difference may be accounted for by the difference in mean body-weight, since most of the sprue patients had lost weight. However, when calculated as total exchangeable albumin per kg. body-weight there was still a big difference between the means of the two groups (3.41 g./kg. in the patients as compared with 4.86 g./kg. in the controls). These figures show more clearly than simple serum albumin estimations the extent of body albumin depletion which may occur in tropical sprue. A study of the pool distribution ratios shows that in the sprue patients the albumin in the extravascular pool was reduced to a greater extent than that in the intravascular pool. It should be noted, however, that there were five cases in which the values for total exchangeable albumin fall within the normal range (Fig. 6).

There was a marked difference between the mean faecal excretion of \(^{131}\text{I}\) in the control subjects (2.3 per cent.) and in the patients with sprue (4.6 per cent.). However, in seven patients the excretion was within the normal range (Fig. 4). As pointed out above this increased excretion of \(^{131}\text{I}\) is considered to represent an increased passage of labelled albumin into the intestine. Subjects with non-tropical sprue have been shown by several authors to have an increased loss of 'albumin' into the intestinal tract (Parkins, 1960; Dawson, Williams, and Williams, 1961; London, Bamforth, and Creamer, 1961; Jeejeebhoy and Coghhill, 1961). Similarly Rubini, Sheehy, Meroney, and Louro (1961) using \(^{251}\text{P}\)-labelled protein and \(^{35}\text{I}\)-labelled P.V.P. demonstrated an increased excretion of 'protein' into the intestinal tract in eight cases of tropical sprue in Puerto Rico.

The mechanism by which protein passes into the intestinal lumen is not clear. In intestinal lymphangiectasia (Waldmann, 1961) increased protein loss may result from rupture of dilated lymphatics, but no such dilatation has been observed in patients with tropical sprue. The increased loss is presumably related in some way to the intestinal damage which is responsible for the production of the various absorptive defects. In this respect it is interesting to note that in the patients studied here some correlation, though significant only at the 5 per cent. level, was found between the amount of label excreted in the stools and the degree of steatorrhoea. However, there was no correlation between the amount of label excreted and the xylose absorption, or the jejunal biopsy findings. Parkins (1960) was similarly unable to demonstrate any relationship between the \(^{35}\text{I}\)-P.V.P. loss into the intestine and the histological appearance of the jejunum in cases of non-tropical sprue.

Increased loss of protein into the intestine in sprue patients may be one possible cause for the reduction in total body albumin. However, the lack of any correlation between the amount of label lost in the stools or the amount of 'exogenous albumin catabolism' and the total exchangeable albumin suggests that other factors must also be involved acting in conjunction with the increased
intestinal losses. A lack of correlation between faecal losses and hypoalbuminaemia was also found by Parkins (1960) in cases of non-tropical sprue, although Gordon (1959) using $^{131}$I P.V.P. in cases of 'exudative enteropathy' suggested such a relationship.

### Table III

<table>
<thead>
<tr>
<th>Group</th>
<th>Exogenous catabolism</th>
<th>Endogenous catabolism</th>
<th>Number with reduced T.E.A.</th>
<th>Number with normal T.E.A.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Increased</td>
<td>Increased</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Group 2</td>
<td>Increased</td>
<td>Normal</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Group 3</td>
<td>Normal</td>
<td>Normal</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Group 4</td>
<td>Normal</td>
<td>Low</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

The sprue cases may be subdivided into four groups according to the values obtained for exogenous and endogenous catabolic rates (Table III). It will be seen that four cases had both catabolic rates increased, eight had an increased exogenous and a normal endogenous catabolic rate, four had both catabolic rates within the normal range, and three had normal exogenous catabolic rates but a lowered endogenous catabolic rate. It will also be seen that of the five patients with normal total exchangeable albumin two were in Group 1, two were in Group 2, and one in Group 4. In the four cases in Groups 1 and 2 with normal body albumin in spite of excessive catabolism, either the excessive catabolism had not been established long enough to reduce the body albumin, or, as seems more probable in view of the long duration of the patients' symptoms (three to seven months), albumin synthesis was increased sufficiently to fully compensate for the increased losses. The six cases in Groups 3 and 4, where body albumin was reduced but total catabolic rates were normal or low, must either represent the end result of previous hypercatabolism which had depleted the body of protein, or a decreased rate of synthesis of albumin, or a combination of both of these. If the patients are in a steady state then the rate of protein synthesis must equal the total catabolic rate, and as such, only cases in Group 4 can be considered to have had hypoalbuminemia at the time of study (Joejeehboy, 1964); however, it is possible that cases in Group 3 had had a decreased rate of synthesis previously which had returned to normal.

The rate of albumin synthesis will depend on the availability of dietary protein and on the integrity of the albumin forming mechanism in the liver. In some cases of sprue, appetite and hence protein intake is reduced. It has also been shown in non-tropical sprue that $^{15}$N-labelled protein absorption is decreased (Crane and Neuberger, 1960), and the same is probably true of tropical sprue. In some subjects with long-standing tropical sprue liver-function tests are disturbed, and liver biopsies show a marked fatty infiltration (Baker, 1962), so it is possible that albumin synthesis by the liver may also be defective even in the presence of adequate diet and absorption.
The precise roles of increased exogenous catabolism, increased endogenous catabolism, and decreased synthesis in the hypoalbuminemia of sprue need further elucidation. Longitudinal study of a series of cases might provide useful information in this respect.

The observation that in the sprue patients the 'endogenous degradation rate' of albumin is closely related to the plasma albumin concentration (Fig. 9) suggests that in conditions of hypoalbuminemia the plasma albumin concentration determines the rate of albumin catabolism. If the data from both the control and patient group are combined, and the mean catabolic rate determined at different levels of serum albumin, a curve can be drawn as shown in Fig. 11, which is suggestive of the kinetics of an enzyme-substrate reaction. The absolute amount of degradation would appear to depend directly on the amount of albumin presented to the degradation site(s) per unit time, i.e. on the plasma albumin concentration. Then as the plasma albumin concentration increases the optimal rate of degradation appears to be reached and beyond this no further increase in catabolic rate occurs. This appears to be one way in which the body compensates for a lowering of the serum albumin concentration.

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Summary

Using $^{131}$I labelled human albumin, and feeding an ion exchange resin by mouth, albumin metabolism has been studied in 10 control subjects and in 19 subjects with tropical sprue.

Mean serum albumin, total exchangeable albumin, and total exchangeable albumin per kilogram body-weight were all considerably reduced in the patients with sprue.

The means of the corrected urinary excretion of the $^{131}$I label and the rate of 'endogenous albumin degradation' were similar in the two groups. The sprue patients, as a group, had an increased loss of the $^{131}$I label into the intestine, and an increased 'exogenous albumin degradation' rate. This increase was not related to the xylose absorption test or to the biopsy findings. There was, however, a positive correlation with the degree of steatorrhoea.

There was no correlation between the amount of intestinal loss of 'albumin' and the degree of hypoalbuminaemia. The body depletion of albumin in tropical sprue may also be contributed to by diminished protein intake, defective absorption of dietary proteins, or diminished albumin production by the liver.

In the patient group there was a positive correlation between plasma albumin concentration and the endogenous degradation rate—the higher the albumin level, the higher the degradation rate. No such correlation was observed in the controls.

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