# Labeled Methionine as an Indicator of Protein formation in Children with Lipoid Nephrosis.* (18128)

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Hypoproteinemia and proteinuria are constant findings in patients presenting the nephrotic syndrome. Whether the proteinuria is the sole factor responsible for the hypoproteinemia or whether a faulty mechanism of protein synthesis is also involved is a question that has evoked considerable speculation(1). The amount of protein excreted daily by these patients may be as much as 30 g but is usually not more than 5 to 10 g(2,3). It has been estimated that normal man on an adequate diet should be capable of forming 55 g of protein per day in excess of metabolic needs(4). From these considerations it would appear, *a priori,* that a faulty protein metabolism might well be implicated as a contributing cause of the hypoproteinemia in lipoid nephrosis. However, there is insufficient evidence available to prove this point unequivocally.

The demonstration that methionine labeled with radioactive sulfur is incorporated into body proteins and that the rate of incorporation may be taken as an indicator of the rate of protein synthesis(5,6) provided the opportunity for a new approach to the question of the existence of an impaired mechanism of serum protein synthesis in the nephrotic patient. In the present study the rate of serum protein formation by nephrotic patients, as determined by this method, was compared with that of control subjects of a corresponding age group.

*Materials and methods.* The experimental subjects employed in these studies were 4 children whose illness was diagnosed as lipoid nephrosis on the basis of their having anasarca, ascites, albuminuria, hypoproteinemia and hyperlipidemia without arterial hypertension or hematuria. The control subjects were 2 children who were free from proteinuria and any demonstrable metabolic abnormalities related to serum protein formation. One of them, K. B., a 6-year-old girl, was physically normal but was subject to convulsive seizures of post-traumatic origin; the other, L. S., was a mentally retarded but physically healthy boy 1½ years of age.

Methionine, labeled with $S^{35}$ was administered intravenously in 20 ml of 0.85% NaCl solution. Since the same lot of radioactive methionine had to be used over a period of several months the absolute amount injected (4.5 to 24 mg) varied according to the radioactivity as determined at the time of each experiment. Blood specimens were obtained for analysis 5 hours and again 24 hours later. Total serum protein sulfur content and radioactivity were determined by the methods employed by Tarver and Schmidt(5) with minor modifications, including the employment of a "thick-layer" counting technic.

*Results and discussion.* The results are expressed in terms of the "biological concentration coefficient" (B.C.C.), as recently proposed by Schulman *et al.*(7,8), an expres-

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TABLE I. Effects of Injected Radioactive Methionine in Nephrotic and Control Subjects.

| Subject | K.B. | L.S. | J.M. | R.M. | D.F. | P.S. |
|---|---|---|---|---|---|---|
| Diagnosis | Control | Control | Nephrosis | Nephrosis | Nephrosis | Nephrosis |
| Age (yr) | 6 | 1.5 | 2 | 3.5 | 7 | 3.5 |
| Sex | F | M | F | M | M | M |
| Wt in kg | 20.60 | 7.10 | 17.85 | 15.25 | 27.40 | 23.10 |
| Total serum protein (g per 100 ml) | 7.10 | 4.94 | 3.47 | 3.44 | 3.00 | 2.86 |
| Dose of radioactivity inj. (counts per min.) | 618,670 | 618,670 | 618,670 | 643,150 | 311,780 | 477,750 |
| | | | 5 hr after inj. | | | |
| Counts per min. in 5 ml serum | 142.2 | 327.0 | 389.0 | 273.0 | 56.4 | 217.0 |
| Millimoles sulfur in 5 ml serum | 0.148 | 0.126 | 0.067 | 0.071 | 0.065 | 0.063 |
| BCC* | 3199 | 2978 | 16,752 | 9,117 | 7,625 | 16,654 |
| | | | 24 hr after inj. | | | |
| Counts per min. in 5 ml serum | 127.9 | 251.4 | 269.7 | 223.7 | — | 216.5 |
| Millimoles sulfur in 5 ml serum | 0.139 | 0.104 | 0.057 | 0.069 | — | 0.064 |
| BCC* | 3,063 | 2,774 | 13,642 | 7,687 | — | 16,356 |

* Biological concentration coefficient (B.C.C.) $= \frac{(\text{c.p.m./millimol}) \times 100}{\text{c.p.m. administered/g body wt}}$.

sion permitting meaningful comparisons of the rate of incorporation of radioactive material into the serum proteins of individuals differing in weights and receiving varying dosages of radioactivity. The data obtained are summarized in Table I. From these data it may be seen that the B.C.C. values of the two control subjects are in remarkably close agreement, although the patients differ considerably in age, weight, and total serum protein concentration. In striking contrast to these values are the distinctly higher ones exhibited by each of the four nephrotic patients, values ranging from 2.5 to 5.5 times those of the control subjects.

It would appear from these results that the rate of incorporation of methionine into the serum proteins of nephrotic patients is greater rather than less than that of control subjects. The significance of this surprising finding is not immediately apparent. Superficially it would appear to indicate that there is no inadequacy of protein formation in nephrotic patients. However, it may be inferred that, whereas only the normal rate of production of serum proteins has been measured in the control subjects, under the conditions of these experiments the maximal rate of their production has been measured in the nephrotic patients because of the stimulus afforded to such production by the inordinately low levels of protein in their serums. It must be considered also that the body proteins are in a state of dynamic equilibrium. Therefore, an increased concentration of radioactive materials in the serum protein need not necessarily reflect an increased rate of serum protein formation but might indicate a shift to the right of the equilibrium expressed by the equation, tissue protein $\rightleftharpoons$ plasma protein. Furthermore, it seems possible that the presence of edema might cause spuriously high values of B.C.C. but it hardly seems probable that such errors could be of an order of magnitude that would explain the disparity observed between the nephrotic patients and the control subjects. Further investigations will be necessary to clarify these points.

*Summary.* 1. The rate of serum protein formation as indicated by the incorporation of methionine labeled with $S^{35}$ was determined in 4 patients with lipoid nephrosis and in two

control subjects. 2. The rate of serum protein formation thus determined would appear to be greater in the nephrotic patients than in the control subjects.

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## Effects of Hypo- and Hyperthyroidism in Rats and Mice on Ovarian Response to Equine Gonadotrophin.* (18129)

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The effects of thiouracil and thyroprotein on the response of the testes and seminal vesicles of young rats and mice to a constant dose of pregnant mares' serum has recently been reported(1). It was found that thiouracil increased the gonadal response to PMS in rats but decreased it in mice. Thyroprotein decreased the gonadal response to PMS in rats but increased it in mice. Sex differences have been found in the normal thyroid secretion rate of male and female rats and mice(2,3). Other differences between the sexes may also modify the end-organ responses of animals to hormonal stimuli. It was considered of interest, therefore, to determine whether the gonadal response of female rats and mice to PMS would be altered in the same directions by thiouracil or thyroprotein as previously found in males of the two species.

*Methods.* Young female albino rats (Michigan State College strain) and mice (Rockland strain) were used in these experiments. The rats averaged 40 to 50 g in weight at the beginning and 80 to 100 g at the end of the experiments. The mice weighed 8 to 12 g at the beginning and 17 to 21 g at the end of each experiment. The animals were housed in an air-conditioned room with a mean temperature of 74°F. Hyperthyroidism was induced in the rats and mice by incorporating thyroprotein‡ in the ration in concentrations of 0.02 to 1.28%, and feeding for 10 days. In one series of rats hyperthyroidism was produced by injecting d, l-thyroxine subcutaneously for 10 days in doses equivalent to 1.5 to 12 times their approximate normal thyroid secretion rate. Hypothyroidism was induced in the rats and mice respectively by feeding 0.1% and 0.2% thiouracil. The degree of hypothyroidism was controlled by feeding the thiouracil for 4 to 20 day periods. The animals which were to receive thiouracil for the longer periods were started at lower body weights than the controls in order to reach approximately the same body weights at the end of each experiment.

During the last 4 days of each experimental period, the rats were each injected subcutaneously with a constant dose of one Cortland-Nelson unit (20 I.U.) of pregnant mares' serum ("Gonadogen")§ and each mouse with one half of this dose. On the day of autopsy, the ovaries were removed and weighed on a Roller-Smith balance. The weights of the ovaries were calculated on a 100 g body weight basis.

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