# Thyroactive Iodinated Protein in Protein-Depletion of Rats for Use in Protein Assays. (18492)

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Since the development by Cannon and his associates(1,2), of the use of the hypoproteinemic rat in studies on the dietary utilization of proteins and of amino acids, the general technic has been employed in many laboratories for assaying the plasma protein-regenerating ability of proteins and of amino acid mixtures. However, the method has the disadvantage that a relatively long time, usually about 3 months, is required for the preparation of the animals for purposes of assay. In view of the accelerating effects of thyroid hormone on protein metabolism, which have been well described by various investigators(3), the feeding of one of the thyroactive substances, such as an iodinated protein, would appear to be a useful means of speeding up the depletion process in the preparation of test rats. Data presented here were obtained from some preliminary and exploratory trials made to test this possibility and indicate that the use of iodinated casein for this purpose is practical.

Observations were made on the changes in plasma proteins in rats fed for different periods of time on a basal low-protein diet and in rats fed the same basal diet supplemented with 0.1% and 0.15% of iodinated casein.* Further observations were made after a period of repletion, on one of the groups of animals which had been depleted with iodinated casein. Male albino rats of approximately 200 g weight were used in all groups. The animals were housed individually and fed the diets *ad libitum*. The diets used are indicated in Table I. The iodinated casein added to diets 2 and 3 was mixed directly with the other ingredients. Blood samples of 0.3 to 0.4 ml amounts were obtained from the rats by heart puncture and mixed with a small amount of purified heparin. The blood was centrifuged and total protein determined on the clear plasma, using the falling drop technic of Barbour and Hamilton(4).

TABLE I. Composition of Diets Used.

| Ingredients | Diets 1 % | 2 % | 3 % | 4 % |
|---|---|---|---|---|
| Equal parts sucrose and dextrinized starch | 83.8 | 83.7 | 83.65 | 61.8 |
| Agar | 5 | 5 | 5 | 5 |
| Lard | 4 | 4 | 4 | 4 |
| Casein | — | — | — | 22 |
| Salt mixture | 4 | 4 | 4 | 4 |
| Liver conc. | 0.2 | 0.2 | 0.2 | 0.2 |
| Irradiated yeast | 3 | 3 | 3 | 3 |
| Iodinated casein | — | 0.1 | 0.15 | — |

Additional vitamins were included, per k, in all diets as follows: Thiamine 1.08 mg, riboflavin 1.63 mg, niacin 2.64 mg, pyridoxine 1.20 mg, calcium pantothenate 2.55 mg, 90% beta carotene mixture 20 mg, alpha tocopherol 24 mg.

The results are summarized in Table II. These data show the effect of iodinated casein in reducing the time necessary for depletion. Gross appearance and weight changes of the animals corresponded to the changes in plasma protein shown by the various groups in the table.

Rats in Group 5, after having plasma proteins depleted by 3 weeks feeding on diet 2 to a level 1.3 g below that of the controls, were then repleted by 7 days of feeding 1.8 g per day of crude casein in addition to the basal low protein diet. These animals gained about 4 g per day in weight and the regenera-

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* Iodinated casein (Protamone) labeled to contain activity equal to 3% thyroxine obtained from the Cerophyl Laboratories, Kansas City, Mo.

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TABLE II. Effect of Thyroprotein on Plasma Protein.

| Animals | | Diet | | Time on treatment, weeks | Plasma protein | |
|---|---|---|---|---|---|---|
| Group No. | Total No. | No. | Thyroprotein level, % | | Mean, % | S.E. of mean |
| 1 | 6 | 4 | 0 | 16 | 7.26 | .25 |
| 2 | 5 | 1 | 0 | 4 | 6.08 | .02 |
| 3 | 5 | 1 | 0 | 10 | 5.15 | .15 |
| 4 | 4 | 2 | .1 | 2 | 5.89 | .06 |
| 5 | 4 | 2 | .1 | 3 | 5.92 | .14 |
| 6 | 4 | 2 | .1 | 4 | 5.32 | .16 |
| 7 | 3 | 2 | .1 | 6 | 4.94 | .38 |
| 8 | 4 | 3 | .15 | 4 | 4.46 | .21 |
| 9* | 4 | 1† | 0 | 1 | 7.12 | .46 |

* These were depleted animals from Group No. 5.

† For a repletion period of 1 wk they were fed, in addition to Diet 1, enough casein to supply .24 g N/day.

tion of plasma protein occurred equally as rapidly as in other experiments in which rats were similarly repleted after bring depleted without thyroactive protein.

This modification of the Cannon technic has been applied to mixed feeds containing both animal and vegetable proteins. In this assay trial, nitrogen intake was kept constant. Commercial casein and blood fibrin, as standards representing good quality protein, raised the plasma protein 1.10 g and 1.29 g respectively. A mixed feed which had given questionable results with other animal species raised the plasma protein only .59 g. So far as the method was concerned the only effect of iodinated casein appeared to be to speed up reduction of body weight and plasma protein.

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## Comparison of Cardiac Action of Bufalin, Cinobufotalin, and Telocinobufagin with Cinobufagin. (18493)

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Ch'an Su is a commercial preparation of the Chinese toad venom employed in medicine for centuries. Previous investigations in this laboratory(1-3) resulted in the isolation of two digitalis-like principles, cinobufagin and cinobufotoxin. The source of Ch'an Su was proved to be the parotoid secretion of *Bufo bufo gargarizans*(4). The chemical structure of cinobufagin was elucidated by Tschesche and Offe(6) and Jensen(6). From a supply of Ch'an Su from this laboratory Meyer(7) in T. Reichstein's laboratory, succeeded in isolating 6 well-characterized substances, all having a digitalis-like action. They are cinobufagin, bufalin, bufotalin, cinobufotalin, gamabufotalin (gamabufagin), and telocinobufagin. Bufalin, cinobufotalin, and gamabufotalin were previously isolated by Kotake and Kuwada(8). Bufotalin is identical with

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