# Fate in Rats of Heterologous Protein Labeled "Internally" by $S^{35}$ and "Externally" by $I^{131}$

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The widespread use of radioactive labels to study protein metabolism prompted us to compare the fate of $I^{131}$ attached as an "external" label to serum albumin with that of $S^{35}$-amino acids incorporated into the same protein molecule as an "internal" label. The doubly labeled protein was prepared by injecting guinea pigs with a yeast hydrolyzate containing $S^{35}$-amino acids (1), sacrificing the animals after 6 to 12 hr, isolating the serum albumin, and labeling it with traces of $I^{131}$ *in vitro* (2). Nine days after the intravenous administration of the doubly labeled guinea-pig serum albumin to rats, the animals were sacrificed. Dry protein powders (3) were prepared from the liver homogenates, and the protein-bound radioactivity was determined repeatedly during a period of several weeks. An aliquot of the injected protein was treated similarly.

The activities resulting from $I^{131}$ and $S^{35}$ were calculated from the decay rate of 30-mg samples using the equations $R_0 = S_0 + I_0$ and $R_t = S_0 \times 0.5^{t/87} + I_0 \times 0.5^{t/8}$, where $R_0$ is the total activity (in counts per minute) and $S_0$ and $I_0$ the activities of $S^{35}$ and $I^{131}$ at zero time, respectively; $R_t$ is the total activity at the time $t$. The results of two such experiments (4) are shown in Table 1.

We express our results by the ratio "percentage of persisting $S^{35}$/percentage of persisting $I^{131}$" in the proteins of the rat liver. Obviously, this ratio remains 1.0 when both isotopes are metabolized at the same rate. According to Table 1, the percentage of persisting $S^{35}$ is, however, 72 or 58 times higher than that of persisting $I^{131}$. This may be caused by (i) partial deiodination of the injected protein and persistence

Table 1. Radioactivity of $I^{131}$ and $S^{35}$ in doubly labeled guinea-pig serum albumin and in the liver homogenate of injected rats. (All activities are expressed as counts per minute on the 6th day after the rats were sacrificed.)

|  | Rat 4-WF | Rat 7-WF |
|---|---|---|
| *Guinea-pig serum albumin* | | |
| Injection (mg) | 10.4 | 8.0 |
| $S^{35}$ activity | 40 $\times 10^3$ | 67 $\times 10^3$ |
| $I^{131}$ activity | 11.2 $\times 10^6$ | 13.3 $\times 10^6$ |
| *Rat liver proteins* | | |
| Dry weight (g) | 1.02 | 1.20 |
| $S^{35}$ (activity) | 0.85 $\times 10^3$ | 1.87 $\times 10^3$ |
| $S^{35}$ (% of injection) | 2.1 | 2.8 |
| $I^{131}$ (activity) | 3.2 $\times 10^3$ | 6.45 $\times 10^3$ |
| $I^{131}$ (% of injection) | 0.029 | 0.048 |
| *Ratio* | | |
| % $S^{35}$/% $I^{131}$ | 72 | 58 |

of the deiodinated product in the liver; (ii) incorporation into the rat liver proteins of $S^{35}$-cystine, $S^{35}$-methionine, or peptides containing these amino acids; or (iii) a combination of these processes. In each of these cases, the percentage of guinea-pig serum albumin persisting in the rat liver will be quite different from the percentages of persisting $I^{131}$ and/or $S^{35}$. If deiodination or loss of iodinated amino acids, as we believe, is the major factor responsible for our results, the amount of guinea-pig protein deposited in the rat liver would be many times higher than that indicated by the low $I^{131}$ content.

### References and Notes

1. R. B. Williams and R. M. C. Dawson, *Biochem. J. (London)* **52**, 314 (1952).
2. S. Warren and F. Dixon, *Am. J. Med. Sci.* **216**, 131 (1948).
3. F. Haurowitz and C. F. Crampton, *J. Immunol.* **68**, 73 (1952).
4. Support of this work by research grants from the U.S. Public Health Service and the American Cancer Society, as well as by contracts between the U.S. Atomic Energy Commission, the Office of Naval Research, and Indiana University, is gratefully acknowledged.

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# Goiter Production and Prevention in Rats

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Laboratory studies of the causes of dietary goiter have been handicapped by the lack of an experimental diet that would rapidly and reproducibly produce goiter in the absence of known goitrogenic chemicals. A low iodide diet is available commercially (1), prepared according to the recommendation of Remington (2). In rats this diet produces goiter very slowly. We have prepared low-iodide diets (3) with casein and sucrose as the basic components. These mixtures were not goitrogenic.

In June 1953 we received a communication from Eartly and Leblond (4) stating that a commercial cereal (5) was low in iodine content and could be used as a diet for rats. For the past 20 mo we have studied iodide metabolism of rats fed this cereal. In this report (6) the cereal is referred to as Cereal-G. Repeated analyses have shown that Cereal-G contained 0.015 to 0.035 µg of iodide per gram (7–11), and it was consistently goitrogenic.

The commercial Remington diet contained 0.08 µg (7) of iodide per gram according to our analyses, although Remington reported that his original mixtures were as low as 0.015 µg of iodide per gram. Our diets of sucrose with 13 and 26 percent casein (3) contained, respectively, 0.03 and 0.05 µg of total iodide per gram (7).

Groups of Long-Evans male rats, 60 to 100 g initial body weight, were fed one of the afore-mentioned diets for 6 wk to 2 yr. All diets were supplemented with distilled water, and the rats were main-

tained in wire-bottomed cages in an isolated, air-conditioned room ventilated with filtered air. At indicated intervals, groups of the animals were sacrificed. Their fresh thyroid glands were removed and weighed.

Table 1 shows that Cereal-G produced thyroids 3 times the normal size within 6 wk. Some of the Remington-fed rats developed slight goiters after 8 mo; however, after 18 to 26 mo, thyroids of the Remington rats were as large as the glands in the rats fed Cereal-G for 12 mo. The final age and body weight of the Remington-fed rats exceeded the age and weight of our other groups. The thyroid weights of the rats fed Cereal-G increased progressively, but their body growth was retarded. Four of the animals, fed Cereal-G for 12 mo, had thyroids that weighed more than 100 mg/100 g of body weight; control values never exceeded 14 mg/100 g.

Large thyroids were obtained by alternating Cereal-G and Cereal-G with casein. The glands in this group averaged more than 11 times normal weight (largest thyroid weighed 492 mg). When these rats were sacrificed, their health, appetite, and fur were in good condition. The mean thyroid size was large in this group; however, the standard deviations were also large. This resulted in no significant difference $(P = > 0.1)$ between the mean thyroid weight of this group and the mean values of the groups fed Remington or Cereal-G for the longest duration.

Cereal-G with 25 percent casein for 8 wk resulted in significant enlargement $(P = 0.01)$ of the thyroid.

After 20 wk of this diet the thyroid size was normal. The casein-sucrose diets did not produce goiter.

The addition of 1.5 µg of NaI to the daily Cereal-G intake practically prevented the goiter, and 3 µg of NaI per day resulted in thyroid weights similar to those of the controls.

These data may be summarized as follows. Cereal-G produced goiters in rats more rapidly than a somewhat similar mixture of grains (Remington). A casein-sucrose diet was less goitrogenic than casein plus Cereal-G, even though both diets had the same total iodine content. Addition of casein to Cereal-G partially prevented goiter and stimulated body growth; withdrawal of casein from this diet produced the largest goiters that we have observed. The goitrogenic effect of Cereal-G was prevented by 3 µg of NaI per day.

These observations suggest the following possible explanations: (i) The fact that Cereal-G is more quickly goitrogenic than Remington diet indicates that there is either a goitrogen among the original ingredients of Cereal-G or an antigoitrogen in the Remington diet; or else the processing of Cereal-G may have increased its goitrogenic properties. (ii) The iodide requirements of rats may be decreased by casein or increased by Cereal-G. (iii) The goitrogenic property of Cereal-G may be due to an ingredient that interferes with production or utilization of the thyroid hormone or to the lack of a substance required to produce thyroid hormone. (iv) The goiter

Table 1. Thyroid weights of rats fed different diets for progressively increased durations.

| Diet | Weeks on diet | No. of rats | Body wt. (g) ± S.D.* | Thyroid wt. (mg) ± S.D.* | Thyroid mg/100gm body wt. ± S.D.* |
|---|---|---|---|---|---|
| *Fed diet less than 4 mo* | | | | | |
| Control (Purina lab. chow) | 8 | 8 | 153 ± 14 | 15 ± 1.7 | 9.8 ± 1.1 |
| Cereal-G | 6 | 9 | 175 ± 33 | 54 ± 18 | 31 ± 10 |
| Cereal-G | 12 | 9 | 202 ± 41 | 60 ± 12 | 29.7 ± 12 |
| Cereal-G + 25% casein | 8 | 14 | 208 ± 43 | 31.4 ± 14 | 15.1 ± 4.1 |
| Cereal-G + NaI (1.5 µg NaI per day) | 8 | 6 | 152 ± 17 | 16 ± 3.6 | 10.5 ± 2.4 |
| Cereal-G + NaI (3.0 µg NaI per day) | 8 | 4 | 155 ± 17 | 15 ± 3.4 | 9.7 ± 2.8 |
| *Fed diet 4 to 8 mo* | | | | | |
| Control (Rockland Farms) | 24 | 9 | 197 ± 44 | 22 ± 4.8 | 11 ± 1.9 |
| 26% casein (with sucrose) | 16 | 5 | 315 ± 38 | 23 ± 4.3 | 7.3 ± 1.3 |
| 13% casein (with sucrose) | 16 | 11 | 260 ± 43 | 18 ± 5.6 | 7.0 ± 1.8 |
| Cereal-G + 25% casein | 20 | 6 | 336 ± 14 | 32 ± 8.8 | 10.7 ± 4.1 |
| Remington | 32 | 15 | 275 ± 18 | 39 ± 19 | 14 ± 7.1 |
| Cereal-G | 32 | 11 | 266 ± 27 | 192 ± 58 | 72 ± 20 |
| *Fed diet 10 to 26 mo* | | | | | |
| Control (Purina lab. chow) | 40–50 | 13 | 362 ± 38 | 27 ± 3.6 | 7.5 ± 0.98 |
| Cereal-G | 50 | 10 | 249 ± 57 | 135 ± 55 | 54.4 ± 22 |
| Cereal-G + 10% casein | 50 | 2 | 370 ± 71 | 61 ± 9 | 17 ± 6 |
| 26% casein (with sucrose) | 100 | 5 | 294 ± 36 | 26 ± 5.2 | 8.7 ± 1.7 |
| Remington | 80–110 | 6 | 438 ± 118 | 231 ± 146 | 52.8 ± 33 |
| Cereal-G 3 mo, then Cereal-G + 25% casein 2 mo, then Cereal-G 6 mo | | 7 | 329 ± 53 | 296 ± 104 | 90.0 ± 53 |

* S.D. means standard deviation calculated from the range [R. B. Dean and W. J. Dixon, *Anal. Chem.* **23**, 636 (1951)].

prevention effect of NaI may demonstrate that Cereal-G is deficient in iodide; however, these data could result if NaI reduced the effectiveness of a goitrogenic mechanism (12) other than iodine deficiency.

### References and Notes

1. Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.
2. R. E. Remington, *J. Nutrition* **13**, 223 (1937). Remington diet No. 347 contained wheat gluten 18 percent, brewer's yeast 2 percent, corn meal (obtained from "iodide-deficient" areas) 78 percent, calcium carbonate 1 percent, sodium chloride 1 percent.
3. Composition: casein (0.11 μg of iodide per gram, vitamin free) (*1*) 13 or 26 percent, sucrose (Domino) 68 or 55 percent, vegetable fat (Crisco) 10 percent, purified salts, and crystalline vitamins.
4. H. Eartly and C. P. Leblond, private communication.
5. Produced by Mead Johnson and Company, Evansville, Ind., and marketed under the trade name Pablum Mixed Cereal. This cereal is stated to contain "wheat meal (farina), oatmeal, yellow corn, wheat germ, tribasic calcium phosphate, powdered alfalfa leaf, dried yeast, sodium chloride, thiamine hydrochloride, riboflavin, and reduced iron." The mixture is "precooked" and dried to a moisture content of 7 percent. This cereal was purchased on the open market, 1-mo supply at a time, from July 1953 through January 1955.
6. This investigation was supported by grants from the U.S. Atomic Energy Commission and U.S. Public Health Service. Such support should not be construed as endorsement of, or concurrence in, the findings and opinions expressed herein, which are solely my own.
7. Analysis for total iodine in dietary materials are subject to many serious errors (*8*). The values reported were the best analytic results obtained by the methods of Chaney (*9*), Barker (*10*), and Zak (*11*).
8. L. Van Middlesworth and J. Truemper, *Federation Proc.* **12**, 147 (1953).
9. A. L. Chaney, *Ind. Eng. Chem., Anal. Ed.* **12**, 179 (1940).
10. S. B. Barker *et al.*, *J. Clin. Invest.* **30**, 55 (1951).
11. B. Zak *et al.*, *Anal. Chem.* **24**, 1345 (1952).
12. L. Van Middlesworth, *Federation Proc.* **11**, 166 (1952).

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## Production of Milky-Disease Spores (*Bacillus popilliae* Dutky and *Bacillus lentimorbus* Dutky) on Artificial Media

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The larvae of two destructive soil-inhabiting insects, the Japanese beetle, *Popillia japonica* Newm., and the European chafer, *Amphimallon majalis* (Razoum.), are susceptible to milky disease (*1*). Dutky (*2*) described two species of spore-forming bacilli, *Bacillus popilliae* and *B. lentimorbus* that cause milky disease. These two species are able to invade the blood of larvae and produce large numbers of spores. After the larvae die, the spores are liberated in the soil, where they may infect other larvae.

To facilitate the natural spread of the disease, it has been feasible to inoculate the soil with milky-disease spore dust produced by a patented process (*3*) described by White and Dutky (*4*). Living larvae are used as the substrate. Dutky (*2, 5*) reported cultivation of both species of bacilli on artificial media in the vegetative state; however, they did not sporulate. The biological control of these insects could be considerably facilitated if a practical method for the production of milky-disease spores on artificial media were developed.

This article (*6*) presents the results of a project that was set up to study the *in vitro* characteristics of these bacteria with the objective of duplicating, as far as possible, the *in vivo* reactions, particularly sporulation.

A detailed study was undertaken of the growth of these organisms in the vegetative state on various types of media (*7*). Particular attention was given to *p*H, oxygen tension, growth factor, and the carbohydrate and nitrogen requirements of the organisms. Excellent vegetative growth was obtained, and spores formed occasionally. However, the spores were not typical of those formed within the living larvae.

Sporulation in a diseased larva occurs after the vegetative cells have become very numerous in the blood. This suggested that the supply of an essential nutrient might become deficient preceding sporulation. A nutrient deficiency might be a factor inducing sporulation (*8*). This possibility was investigated by growing the vegetative cells on a complete medium and then transferring them to a starvation medium on which further growth was impossible. Spores formed that were indistinguishable from those produced within the living larva. Details of the method follow.

An inoculum was prepared by sterilizing the surface of a diseased larva in 0.5-percent sodium hypochlorite solution, puncturing the hemacoele through the dorsal body region, and suspending the spores in sterile water. The spore suspension was heated at 70°C for 15 min. to destroy the vegetative cells. The spores were inoculated onto the surface of petri-dish cultures containing a complete medium made with the following ingredients: tryptone, 5 g; yeast extract, 3 g; $K_2HPO_4$, 3 g; glucose, 1 g; maltose. 1 g; soluble starch, 10 g; agar, 15 g; and distilled water, 1000 ml. The cultures were incubated at 32°C for approximately 4 days in order to insure good growth and germination of all spores.

The cells were then transferred as a paste from the surface of the complete medium to the surface of a starvation medium containing the following ingredients: $(NH_4)_2HPO_4$, 1 g; KCl, 0.2 g; $MgSO_4$, 0.2 g; yeast extract, 0.2 g; agar, 15 g; and distilled water, 1000 ml. Cells were incubated on the starvation medium at 32°, 37°, and 45°C. Although no further growth took place under these conditions, sporulation occurred within 24 to 72 hr at all three temperatures; the highest yield of spores was obtained at 37°C.