TABLE 1

RATE OF HYDROLYSIS OF SYNTHETIC PEPTIDES BY CARBOXYPEPTIDASE

| Substrate | Enzyme concn. $10^{-4}$ mg N/ml | Time min | Hydrolysis % | Velocity constant, $K$* $10^{-3}$ $min^{-1}$ |
|---|---|---|---|---|
| Carbobenzoxyglycylphenylalanine (I) | 1.5 | 70 | 27 | 1.9 |
| | | 140 | 50 | 2.1 |
| | 2.7 | 30 | 17 | 2.6 |
| | | 60 | 46 | 4.4 |
| | | 130 | 63 | 3.3 |
| Carbobenzoxyglycyl-β-2-thienylalanine (II) | 1.5 | 70 | 13 | 0.9 |
| | | 140 | 19 | 0.6 |
| | | 255 | 31 | 0.6 |
| Carbobenzoxyglycyl-β-1-naphthylalanine (III) | 2.7 | 30 | 0 | |
| | | 60 | 2 | |
| | | 130 | 2 | |
| | 7.0 | 25 | 0 | |
| | | 135 | 2 | |
| Carbobenzoxyglycyl-β-2-naphthylalanine (IV) | 3.4 | 30 | 1 | |
| | | 90 | 6 | |
| | | 8 hr | 11 | |
| | 13.6 | 23 | 2 | |
| | | 70 | 9 | |
| Carbobenzoxyglycyl-p-methylphenylalanine (V) | 2.7 | 30 | 17 | 2.6 |
| | | 60 | 25 | 2.0 |
| | | 130 | 52 | 2.4 |

* $K = \frac{1}{\text{min}} \log_{10} \frac{100}{100 - \%\ \text{hydrolysis}}$.

TABLE 2

INHIBITION OF HYDROLYSIS OF CARBOBENZOXYGLYCYL-PHENYLALANINE BY CARBOBENZOXYGLYCYL-β-2-NAPHTHYLALANINE

| Substrate | Enzyme concn. $10^{-4}$ mg N/ml | Time min | Hydrolysis % |
|---|---|---|---|
| Carbobenzoxyglycylphenylalanine | 13.6 | 23 | 63 |
| | | 70 | 73 |
| | | 17 hr | 79 |
| Carbobenzoxyglycylphenylalanine plus carbobenzoxyglycyl-β-2-naphthylalanine | 13.6 | 23 | 15 |
| | | 70 | 54 |
| | | 17 hr | 75 |
| Carbobenzoxyglycyl-β-2-naphthylalanine | 13.6 | 23 | 2 |
| | | 70 | 9 |


### References

1. ANSON, M. L. *J. gen. Physiol.*, 1936, **20**, 663.
2. DU VIGNEAUD, V. *et al.* *J. biol. Chem.*, 1945, **159**, 385.
3. FERGER, M. F. and DU VIGNEAUD, V. *J. biol. Chem.*, 1949, **179**, 61.
4. GRASSMAN, W. and HEYDE, W. *Z. physiol. Chem.*, 1929, **183**, 32.
5. NEURATH, H., ELKINS, E., and KAUFMAN, S. *J. biol. Chem.*, 1947, **170**, 221.
6. STAHMANN, M. A., FRUTON, J. S., and BERGMANN, M. *J. biol. Chem.*, 1946, **164**, 753.


# Uptake of Radioactive Iodine by the Thyroids of Underfed Rats[1, 2]


**Joseph Meites and L. F. Wolterink[3]**

***Department of Physiology and Pharmacology, Michigan State College, East Lansing***


Thyroid activity in rats is depressed during starvation, an effect which has been attributed to a decrease in thyrotrophic function by the anterior pituitary (*3*, *4*). From a quantitative aspect, a recent report from this laboratory on the effects of thiouracil on the thyroids of starved rats and mice indicated that the decrease in thyroid activity may be directly proportional to the reduced body weight of these animals (*1*). In other words, thyroid activity appeared to remain unchanged in starved rats when computed on a body weight basis.

We decided to test this finding further by administering radioactive iodine to starved rats and comparing their thyroid uptake with that of controls fed ad libitum.

TABLE 1

EFFECTS OF UNDERFEEDING ON SURVIVAL, GROWTH, AND THYROID WEIGHTS OF RATS

| Group | Orig. No. per group | Final No. per group | Avg orig. body wt in g | Avg final body wt in g | Avg thyroid wt in mg | Avg thyroid wt/100-g body wt in mg |
|---|---|---|---|---|---|---|
| Controls, fed ad lib. .... | 10 | 10 | 147.0 | 169.5 | 13.79 | 8.13 ±* 0.29 |
| Fed ¾ ad lib. . | 10 | 10 | 146.6 | 148.5 | 10.34 | 7.00 ± 0.42 |
| Fed ½ ad lib. . | 10 | 10 | 145.5 | 126.6 | 8.97 | 7.07 ± 0.55 |
| Fed ¼ ad lib. . | 10 | 8 | 145.4 | 111.0 | 8.60 | 7.71 ± 0.23 |
| No feed ..... | 10 | 4 | 146.0 | 87.0 | 7.20 | 8.24 ± 0.52 |

* Standard error of mean.

Fifty young female rats of the Sherman strain, weighing approximately 145 g each, were divided into five groups of ten each and were started on ad libitum, ¾, ½, ¼, and no-feed regimens. The ¾, ½, and ¼ feed-allowance levels were computed from the daily ad libitum feed consumption of the control group. The ration consisted of ground Purina Laboratory Chow. All rats were maintained in an air-conditioned room at a temperature of 75° F.

The unfed group was sacrificed at the end of 7 days, and the other four groups at the end of 14 days. Eight hours prior to sacrifice, each rat was injected intraperitoneally with 0.2 ml of carrier-free $I^{131}$ (radioactive) estimated to contain approximately 2 μc. The thyroid of each sacrificed rat was removed, immediately weighed on a Roller-Smith balance and placed on the center of a


[1] Published with the approval of the Director of the Michigan Agricultural Experiment Station as Journal Article No. 1071.

[2] This study was aided in part by a grant from the U. S. Atomic Energy Commission.

[3] The authors wish to express their thanks to J. O. Reed and C. C. Lee for technical assistance and statistical analysis of the data.

small copper disk for counting. After allowing the thyroids to dry thoroughly, each was counted separately with a thin mica (1.55 mg/cm$^2$) end window counter.

The data given in Table 1 show the expected effects of underfeeding on loss in body weight, the most severe weight losses occurring on the ¼ and no-feed regimens. In these two groups, only eight and four rats, respectively, survived out of an original number of ten each. Thyroid weight was reduced in all the underfed groups, but remained relatively unchanged on the basis of a 100-g body weight. This confirms a previous report from this laboratory on the effects of underfeeding on thyroid weight in rats (*2*).

The amounts of radioactive iodine taken up by the thyroids of each group of rats are given in Table 2. It

TABLE 2

EFFECTS OF UNDERFEEDING ON UPTAKE OF $I^{131}$ BY THE THYROIDS

| Group | Avg uptake of $I^{131}$ per thyroid % | Radioactivity in counts per sec: Avg No. counts per thyroid | Avg No. counts per mg thyroid | Avg No. counts per thyroid / 100-g body wt |
|---|---|---|---|---|
| Controls fed ad lib. ..... | 7.9 | 20.05 ± 1.52* | 1.50 ± 0.17* | 12.01 ± 1.13* |
| Fed ¾ ad lib. . | 6.1 | 16.71 ± 1.64 | 1.62 ± 0.15 | 11.28 ± 1.07 |
| Fed ½ ad lib. . | 5.0 | 13.26 ± 1.32 | 1.51 ± 0.14 | 10.52 ± 1.03 |
| Fed ¼ ad lib. . | 4.1 | 10.51 ± 1.00 | 1.28 ± 0.18 | 9.75 ± 1.27 |
| No feed .... | 3.2 | 8.92 ± 0.39 | 1.30 ± 0.08 | 10.23 ± 0.57 |

* Standard error of mean.

can be seen that the greatest amount of iodine, 7.9%, was taken up by the thyroids of the group fed ad libitum and progressively smaller amounts were taken up by the thyroids of the underfed groups. This was further shown in the actual number of counts obtained from the thyroids of each group of rats. The average number of counts per mg of thyroid tissue remained the same in all groups, indicating that underfeeding did not affect the concentration of iodine within the thyroids. On the basis of 100-g body weight, the counts per thyroid were similar in all the groups, showing that the amount of radioactive iodine taken up by each of the underfed groups was directly proportional to body weight.

It is well known that the thyroids of animals on diets deficient in iodine but otherwise adequate show a greater affinity for administered iodine than animals on iodine-adequate diets. To what extent the rats in this experiment were deficient in iodine as a result of underfeeding is unknown. However, these data support the conclusion that the primary effect of underfeeding is to reduce thyroid activity, and this effect is sufficient to overcome any increased affinity for iodine the thyroids may possess as a result of a possible deficiency in iodine intake.

These data are believed to constitute further proof of the hypothesis that the reduction in thyroid activity during starvation in rats is directly proportional to the reduced body weight of these animals. Whether these data would apply to rats during longer periods of starvation, or to rats of other age groups, cannot be answered at the present time.

### References

1. MEITES, J. and AGRAWALA, I. P. *Endrocrinology,* 1949, **45**, 148.
2. MEITES, J. and REED, J. O. *Proc. Soc. exp. Biol. Med.,* 1949, **70**, 513.
3. MULINOS, M. G. and POMERANTZ, L. J. *Nutrition,* 1940, **19**, 493.
4. STEPHENS, D. J. and ALLEN, W. M. *Endocrinology,* 1941, **28**, 580.

## Amide Constituents of Tobacco Mosaic Virus Protein

**William Stepka and William N. Takahashi**

***Division of Plant Nutrition and Division of Plant Pathology, University of California, Berkeley***

The possible presence of amides in tobacco mosaic virus protein has received little attention, although considerable work (*2–8*) has been done to identify its constituent amino acids. Based on the rate of ammonia formation, a value for amide nitrogen of 1.9% was calculated by Ross (*7*). No attempt, however, was made by him to characterize this fraction further.

The work reported here was undertaken mainly to establish the presence or absence, and if present, the nature of the amides occurring in tobacco mosaic virus protein. At the same time the work of others, reporting amino acids to be components of the virus, was confirmed.

The analytical method of paper partition chromatography (*1*) was used and found to be particularly suitable for this work. Only a brief description of methods is given here. The technical aspects of the problem will be treated elsewhere in more detail.

The tobacco mosaic virus used in these experiments was prepared from leaves of greenhouse-grown Turkish tobacco plants which had been infected with the virus for 20 days. The method of purification consisted of 3 cycles of alternate low speed and high speed centrifugations. The final preparation had a bluish-white opalescence and electron micrographs showed inappreciable impurities.

The purified virus was subjected to enzymatic hydrolysis, since amides are known to be converted to their respective amino acids by the common methods of chemical hydrolysis. Pancreatin was added to heat-denatured virus protein, the pH was adjusted to 8.0, and the preparation was then incubated at 33° C. Appropriate controls of enzyme added to water were carried simultaneously. The course of hydrolysis was followed by withdrawing aliquots at intervals and testing them by paper chromatography. After the 10th day of incubation, no further changes were observed. The preparations were then heated to coagulate the enzyme and the resultant suspensions were cleared by filtration.

The acid—HCl—and alkaline—$Ba(OH)_2$—hydrolyses were carried out in an atmosphere of nitrogen in sealed