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.

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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

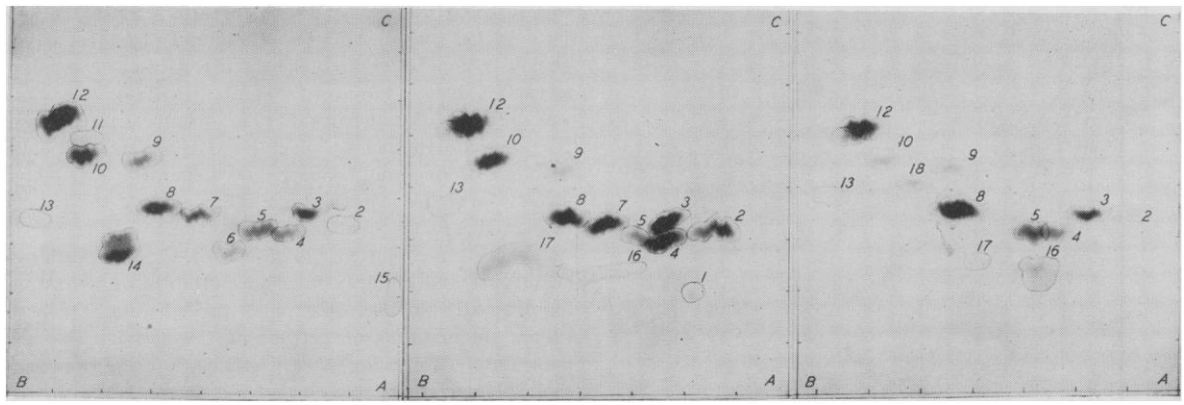

FIG. 1. Paper partition chromatograms of hydrolyzates of tobacco mosaic virus protein; left, pancreatic digest; middle, acid hydrolyzate; right, alkaline hydrolyzate. A–B, water-saturated phenol. A–C, *n*-Butanol-acetic acid-water. Spots: 1, cystine; 2, aspartic acid; 3, glutamic acid; 4, serine; 5, glycine; 6, asparagine; 7, threonine; 8, alanine; 9, tyrosine; 10, valine; 11, tryptophane; 12, leucine, isoleucine, and phenylalanine; 13, proline; 14, a peptide; 15, probably residual protein; 16, lysine; 17, arginine; 18, α-amino-*n*-butyric acid, believed to be an artifact probably originating from threonine.

glass tubes. These were autoclaved for 6 hr at 15 lb pressure. Excess HCl was eliminated by evaporation, and the $Ba(OH)_2$ by precipitation with H_2SO_4.

Each sample was chromatographed routinely in three solvent pairs. As the first solvent, water-saturated phenol was used invariably for all two-dimensional papers. For the run in the second direction, two solvents were employed, either 2–4 lutidine (1 vol) thoroughly shaken in water (1 vol), or freshly prepared *n*-butanol-acetic acid-water. Spots were revealed by spraying the dried sheets with 0.1% ninhydrin in 95% ethanol.

A chromatogram of the pancreatin digest is shown in Fig. 1 (left); the control gave only one faint spot in the position normally occupied by alanine. Asparagine, spot 6, was first tentatively identified by position and by its characteristic rusty color with ninhydrin. Positive identification was later achieved by demonstrating that the asparagine spot was intensified on both phenol/lutidine and phenol/butanol-acetic acid chromatograms by addition of authentic asparagine to the spot at the origin.

It was further shown that acid hydrolysis of the eluate from a cutout of the suspected asparagine spot resulted in its disappearance, with concomitant appearance of an aspartic acid spot. In Fig. 1 (left), the relatively weak spot due to aspartic acid (No. 2) compared to asparagine (No. 6) suggests that the virus protein contains more asparagine than aspartic acid. In the acid hydrolyzate (middle) the aspartic acid spot (No. 2) has been suggested by the conversion of asparagine to aspartic acid.

A spot in the position usually occupied by glutamine was found in chromatograms of aliquots taken during the early stages (before the 7th day) of incubation. Since confirmatory tests were not made, position alone may not be a sufficient criterion for identification; there remains the possibility that the spot may have been due to the presence of a peptide in the incomplete hydrolyzate. On the other hand, glutamine, being a labile compound, may have been destroyed during the prolonged incubation.

All of the amino acids previously reported to be constituents of tobacco mosaic virus protein have been identified by paper partition chromatography. In addition, the presence of one amide, asparagine, has been demonstrated and the probable occurrence of a second, glutamine, has been suggested.

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Effect of Hyaluronidase on the Subcutaneous Absorption of Electrolytes in Humans[1]

Gilbert B. Forbes, Robert W. Deisher, Anne M. Perley, and Alexis F. Hartmann

Department of Pediatrics,
Washington University School of Medicine,
St. Louis

Although observations have been made in animals on the effect of testicular hyaluronidase on the subcutaneous absorption of isotonic saline (*7*), diodrast (*9*), diphtheria antitoxin (*6*), and plasma proteins tagged with radioiodine (*2*), studies on humans have been limited to measurement of the speed at which large subcutaneous infusions can be given. Whereas direct observation of the behavior of intradermal wheals (*5, 8*) is possible in humans, exact data on subcutaneous injections are more difficult to obtain. Since hyaluronidase is currently being used to facilitate the subcutaneous administration of

[1] This investigation was aided by a grant from the Children's Research Foundation.