mains apparent irrespective of whether peptidase activity is calculated on the basis of nitrogen content of tissue, wet or dry weight of tissue or weight of the animals. It is of interest that, judging from the amount of hydrolysis occurring in 18 hours when the liberation of 25 μM/ml presumably indicates complete splitting of 1 peptide bond, lung and kidney extracts and homogenates were capable of hydrolyzing considerable quantities of dipeptide formed from the initial cleavage of LGG and GGG. This finding is supported by the fact that of all the tissues studied, *cobalt-activated* GGase activity is highest in lung and kidney.

Discussion. The hypothesis that the protein catabolic action of ACTH may be reflected, at least in part, in increased catheptic activity is not supported by this study. However, it may be that such changes in proteolytic enzyme activity, if they do exist, may be discernible only in viable tissue. This possibility is being explored using a procedure(6) which permits quantitative measurement of GGase activity in the surviving rat diaphragm.

Summary. The administration of ACTH to fasted rats has no appreciable effect on LGGase, GGGase and cobalt-activated GGase activity of crude extracts of liver, muscle, lung, spleen, kidney or skin.

The technical assistance of Mrs. Catherine Towbin and Mr. James F. O'Neill is gratefully acknowledged.

6. Schwartz, T. B., Engel, F. L., and Towbin, C. C., *Fed. Proc.*, 1951, v10, 123.

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Comparative Utilization of Carotene Administered Orally and Parenterally. (18866)

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The problem of utilization of carotene when administered parenterally to rats was shown by Tomarelli *et al.*(1) to be primarily a matter of the solvent used and the state of dispersion of the carotene. When carotene was solubilized in water by the use of a surface active agent (Tween 80), these workers found that one intramuscular injection of 0.5 mg carotene produced as good growth response in vit. A-deficient rats as did the same dosage given orally. In contrast, when carotene was dissolved in sesame oil and injected, the growth response and survival time were markedly reduced. We have confirmed these findings and have carried out experiments to determine the relative utilization of aqueous carotene when given orally and parenterally.

Methods. Male, weanling rats were caged individually and fed a purified vit. A-free diet composed of purified casein* 22%, salts(2) 4%, cottonseed oil (Wesson) 2%, vitamin-mix in casein 2%, and sucrose 70%. The vitamin-mix contained per 100 g: Thiamine hydrochloride 25 mg, riboflavin 50 mg, pyridoxine hydrochloride 25 mg, calcium pantothenate 250 mg, niacin 100 mg, folic acid 10 mg, biotin 10 mg, 2-methyl-1,4-naphthoquinone 25 mg, inositol 5 mg, p-aminobenzoic acid 50 mg, crystalline vit. B_{12} 0.005 mg, and choline chloride 5 g. In addition, 2 mg dl-α-tocopherol and 150 U.S.P. units of vit. D were added per 100 g ration. The food intake was restricted during the depletion period in an attempt to produce uniform weight gains. Purified, all-trans B-carotene(3) and crystalline vit. A acetate† were dissolved in water, using 20% Tween 40, or in cottonseed oil, by

1. Tomarelli, R. M., Charney, J., and Barnhart, F. W., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 108.

* General Biochemicals, Inc.

2. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, v138, 459.

3. Bieri, J. G., *J. Nutrition*, 1951, v44, 327.

the procedure described previously(3). dl-α-tocopherol was added to the supplements so that the concentration was 0.3%. The preparations were stored under nitrogen at 7°C. After 19 days, when the animals had ceased to gain for three consecutive days, and many had developed xerophthalmia, they were divided into groups of similar average weights. The ration, from which the α-tocopherol was now removed (except for the control animals), were given *ad lib*. Supplements of carotene or vit. A in 0.1 ml oil or water (containing 0.3 mg α-tocopherol) were given daily for 28 days. Oral supplements were given by stomach tube using a dull, bent number 16 hypodermic needle. The intramuscular injections were given in the thigh muscle, alternating the legs daily. It was found that the oily preparations frequently leaked from the site of injection, especially after the first week. This was seldom encountered with the aqueous preparations except during the last several days of the experiment. To prevent the oral ingestion of any injected supplement which may have exuded, the animals were inspected within a few minutes after injection, and any leakage was carefully washed off.

Preliminary experiments revealed that over a 21-day period, 10 γ of carotene in Tween injected intramuscularly, daily, produced as good growth as did 1.8 γ orally (average of 4.1 and 3.9 g daily, respectively). A dosage of 5 γ intramuscularly gave a smaller response, 3.3 g daily. When dissolved in oil, 15 γ carotene injected daily failed to maintain weight. In the subsequent experiments, smaller supplements of aqueous carotene were used to determine the effectiveness of low dosages when given parenterally.

Table I shows the various supplements, their route of administration and the survival and growth of the rats for the 28-day period. The daily injection of 1.6 γ (group II) and of 4.2 γ (group III) of carotene solubilized in water produced growth responses of 2.2 g and 3.2 g daily, respectively. When 1.6 γ of aqueous carotene were given orally (IV), the average daily gain was 4.1 g. This is probably the maximum growth obtainable, as shown by the growth of rats in group V receiving 1.5 γ of aqueous vit. A orally. From a comparison of groups III and IV, it is apparent that 4.2 γ of carotene when injected is slightly less active than 1.6 γ orally. Inasmuch as the preliminary experiments showed that 10 γ of carotene injected daily produced maximum growth, while 5 γ did not, it would appear that from 4 to 6 times as much carotene is required parenterally as orally for a similar growth response.

TABLE I. Comparative Utilization of Carotene in Water (20% Tween 40) or Cottonseed Oil by Vit. A-Deficient Rats; 28 days.

Group	Supplement	No. rats, No. survivors	Avg daily wt gain (survivors)
I	0	5/0	—
II	1.6 γ carotene in Tween, IM	9/6	2.2 ± .27*
III	4.2 γ carotene in Tween, IM	10/8	3.2 ± .22
IV	1.6 γ carotene in Tween, oral	9/6	4.1 ± .18
V	1.5 γ vit. A in Tween, oral	10/9	3.9 ± .33
VI	24.6 γ carotene in oil, IM	8/1	1.6

* Standard error $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$.

Further evidence that carotene in oil is not utilizable when injected is shown by group VI, in which all but one rat died before the end of the experiment. At autopsy, these rats had large deposits of oily carotene at the site of injection, as has been reported by other observers(4,5). The death of some of the rats in those groups apparently receiving adequate carotene or vit. A is attributed to the fact that at the beginning of the experiment, some of the animals were in an advanced stage of vit. A-deficiency, and could not respond to the supplement. At the end of the experiment, the surviving animals were sacrificed

† Kindly supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

4. Lease, V. G., Lease, E. J., Steenbock, H., and Baumann, C. A., *J. Lab. and Clin. Med.*, 1942, v27, 502.

5. Sexton, E. D., Mehl, J. W., and Deuel, H. J., *J. Nutrition*, 1946, v31, 299.

TABLE II. Utilization of Carotene in Water (20% Tween 40) Administered Subcutaneously to Vit. A-Deficient Rats; 21 Days.

Group	Supplement	No. rats No. survivors	Avg days survival	Avg daily wt gain
I	0	5/1	11	—
II	.1 ml aqueous 20% Tween 40, oral	6/0	14	—
III	9.6 γ carotene in Tween, subcut.	5/5	—	4.7

and the livers and kidneys analyzed for vit. A and carotene. Small amounts of carotene (6-9 γ, total) were found in the livers of the group receiving 4.2 γ carotene by injection, and only traces of the pigment were detected in the group injected wth 1.6 γ carotene. Neither vit. A nor carotene was found in the kidneys of these two groups. The tissues of the other groups contained no detectable vit. A or carotene. Examination of the thigh muscles of the rats receiving injections of aqueous carotene revealed no apparent atrophy. The muscle appeared to be torn, however, presumably due to the repeated insertion of the needle. A light yellow coloration throughout the tissues of the leg indicated that the carotene was not completely absorbed.

A second experiment was performed to determine if aqueous carotene when injected subcutaneously would also be effective in curing vit. A-deficiency. Three groups of vit. A-deficient rats were placed on the vit. A-free ration described above. Group I, the controls, received vit. E in the food, while groups II and III received this vitamin in the supplement. Group II was given daily 0.1 ml of 20% Tween 40 in water, orally, while group III received 9.6 γ of carotene in 0.1 ml of water (20% Tween 40) subcutaneously. The results in Table II show that the Tween solution (group II) had no appreciable effect on the survival of vit. A-deficient rats, and that subcutaneous, aqueous carotene (group III) is well utilized.

Summary. Carotene solubilized in water by the use of Tween 40 will support growth in vit. A-deficient rats when as little as 1.6 γ is injected intramuscularly daily. For maximum growth, approximately 4 to 6 times as much carotene is required parenterally as orally. Aqueous carotene is also well utilized when given subcutaneously. Carotene dissolved in cottonseed oil and administered parenterally is essentially not utilizable.

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Developmental Abnormalities in the Chick Embryo Following Infection with Newcastle Disease Virus. (18867)

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The report of Gregg(1) that maternal rubella may act as a teratogenic agent in human subjects, has focused interest on the possible role of virus infections in the production of congenital abnormalities. In efforts to pursue the study of teratogenicity of viruses, the following laboratory experiments were planned to observe the effects of viral agents on the developing chick embryo. Initial investigations using fowl pox virus were inconclusive. However, Newcastle disease virus produced a high percentage of developmental defects, particularly in the lens of the eye and in the auditory sac.

Methods and materials. The Newcastle disease virus was supplied by Dr. F. R. Beaudette.* Fertile eggs from New Hampshire

1. Gregg, N. McA., Trans. Ophthalmological Soc. of Australia, 1941, v3, 35.