excretion during an ingestion period of 16 days was 8.3 per cent. of the intake.

When vitamin A in halibut liver oil was fed at a level of 23.7 mgm. per day (about 76,000 I.U.), the excretory rate in 6 subjects averaged 20.6 μgm. (about 66 I.U.) per gram feces. The total excretion in two subjects during ingestion periods of 8 and 16 days was 3.97 and 2.74 per cent. of the intake. At still higher intake levels the excreted fraction rose sharply. Conversely, when the daily intake was reduced to about 25,000 I.U., the excretory rate in one subject fell to about 1/10 of its previous value, and a second subject excreted no measurable amount of vitamin A at all. In no case could we obtain a test for vitamin A from the feces of subjects on unrestricted, unsupplemented diets. It appears from these experiments that, unlike carotene or xanthophyll, vitamin A is not excreted until the intake reaches a threshold value, well above all ordinary dietary levels; and that above this the fraction excreted rises with the intake.

De has performed experiments similar to these in the rat.[4] He found the excretion of vitamin A in halibut liver oil to be 3 to 5 per cent. over a wide range of intake levels. The average excretion of carotene in oil was 42.5 per cent., or only about two thirds of that in our experiments on human subjects.

In bioassay experiments on rats, vitamin A is almost exactly twice as potent as an equal weight of β-carotene.[5] If one assumes that the fractions of vitamin A and carotene not excreted are absorbed—the excretion in any case sets an upper limit to the absorption—such apparent absorptions explain adequately the relative potencies of these substances in the rat.[4] By a parallel argument, due to its low apparent absorption, carotene in human bioassay should be at best only about 40 per cent. as effective as an equal weight of vitamin A; and should possess only about two thirds the potency now assigned to it on the basis of rat assays. Some confirmation of this conclusion has already appeared in human bioassay experiments.[6]

It is not now known whether β-carotene is converted to vitamin A *in vivo* by symmetrical cleavage, according to the equation

$$C_{40}H_{56} + 2\,H_2O \longrightarrow 2\,C_{20}H_{30}O \qquad (1);$$

or by stepwise degradation to yield a single molecule of vitamin A. In the former instance carotene and vitamin A should be about equally potent *in vivo;* in the latter, carotene should be only about half as potent as the vitamin. The fact that in rats the different potencies of these substances are explained by their differential absorptions implies that *following absorption* they are about equally effective, and is strong presumptive evidence for the operation of equation (1). The present experiments indicate a similar possibility in man.

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[5] T. H. Mead, S. W. Underhill and K. H. Coward, *Biochem. Jour.*, 33: 589, 1939.
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CORRELATION OF ACTIVITY PER UNIT WEIGHT OF TOBACCO-MOSAIC VIRUS WITH AGE OF LESION

IT has been assumed by investigators working with plant viruses that each virus particle attains its full biological activity as soon as it is formed and that any increase in activity is due to an increase in the number of these infectious units. This would infer that all virus samples prepared under identical conditions should have the same activity per unit weight of virus regardless of the source of the sample. Examination of the literature reveals little experimental evidence in support of this assumption. What evidence there is has been derived from activity measurements of crude plant juice. Such measurements have given no information regarding the possible presence of infectious particles of different sizes and weights.

The development of the ultracentrifuge for virus isolation and purification, together with improvements in the local lesion method for measuring the activity of tobacco-mosaic virus, has made it possible to study virus samples containing known weights of virus protein. This technique has been applied to a study of the virus content and relative activity of preparations extracted at various intervals after inoculation. It was soon found[1] that under nitrogen-deficient conditions there was a falling off in activity per unit weight of virus. This finding led to a critical examination of the biological activity of virus in newly formed lesions.

Turkish tobacco plants (*Nicotiana tabacum* L.) were grown in nutrient sand cultures and supplied a complete nutrient solution. When about 6 inches tall, the plants were inoculated by rubbing over the entire upper surface of one mature leaf on each plant with a suspension of tobacco-mosaic virus (*Marmor tabaci* H.). At 5-day intervals, inoculated leaves from representative plants were harvested, frozen and then minced. The cold juice was cleared of insoluble materials by low-speed centrifugation and then ultracentrifuged. The sediment was suspended in phosphate buffer, cleared by low-speed centrifugation and again ultracentrifuged for 1 hour. Virus-protein content was calculated from the nitrogen content of the suspension of the sediment from the second ultra-

[1] E. L. Spencer, *Plant Physiol.*, 16: 229, 1941.

centrifugation. The biological activity per unit weight of virus protein in this suspension was assayed on bean plants (*Phaseolus vulgaris* L. var. Early Golden Cluster) by the local lesion method, as modified by Spencer and Price.[2] The results were as follows: The content of virus protein in the inoculated leaf continued to increase for as long as 20 days after inoculation. This was a point of immediate interest, as it had been generally supposed that the virus reached its maximum concentration in the inoculated leaf within 7 to 10 days following inoculation. From the activity measurements, it was further found that the activity of the virus protein 5 days after inoculation was only about 25 per cent. of that of virus protein isolated 15 days later. Between the 5th and 10th days, the activity of this material almost doubled, and the increase continued until a maximum was reached about 20 days after inoculation. Therefore, not only the amount of virus protein but also the activity per unit weight of the material increased up to 20 days. This would indicate that virus in young lesions was lower in activity than that from somewhat older lesions. Subsequently, it was found that virus protein from the inoculated leaf was more active than that isolated at the same time from the top of the plant. This result might have been expected in view of the previous experiment, since it can be assumed that part of the virus lesions in the inoculated leaf were formed earlier than any of those in the top of the plant and therefore were somewhat older.

In regard to the characteristics of virus from young and old lesions, preliminary analyses with the ultracentrifuge have shown that a preparation from the old lesions had only one component. However, a sample of virus from young lesions prepared at the same time appeared to be made up of two components, one of which was about double the length of that of the other as determined by the sedimentation constant.

Experiments also indicate that nitrogen may be an important factor in increasing the activity per unit weight of virus *in vivo*. When nitrogen was withheld 10 days after inoculation, virus protein in the inoculated leaf continued to form at about the normal rate for a limited period, but the activity of this material, on a weight basis, remained fairly constant at the level reached about the time when nitrogen was last added. The activity at this point was about one half that ultimately displayed by virus protein from normal nitrogen-fed plants. Preliminary experiments have indicated that it may even be possible to increase this unit activity *in vitro* by supplying the virus with suitable forms of nitrogen.

The observations clearly show that virus in young lesions displays on a unit weight basis only a fraction of its potential biological activity and that such virus may vary somewhat in size and shape from virus isolated from older lesions.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MEASUREMENT OF RESPIRATION AND GLYCOLYSIS OF A SINGLE SAMPLE OF TISSUE IN SERUM

WITH the development of a sensitive method of colorimetric analysis for lactic acid[1] and the "neutralized" serum technique for the manometric measurement of oxygen consumption of tissues suspended in serum,[2] it has become possible to determine in an ordinary Warburg vessel the rates of respiration and glycolysis[3] of a single sample of tissue suspended in serum. Respiration is determined manometrically and glycolysis chemically. At the beginning of the experimental period, a sample of approximately 0.2 cc of serum is tipped from the main vessel onto a few crystals of sodium fluoride in the side-well. The fluoride prevents glycolysis in the few cells which may enter with the serum. The tissue slices are prevented from being tipped in by first inclining the vessel in the opposite direction, allowing the slices to settle and decanting the serum. At the end of the experimental period, a 0.1 cc sample of this serum is analyzed for the initial lactic acid concentration. The serum remaining in the side-well is returned and mixed with the contents of the main vessel and a 0.1 cc sample of this serum is analyzed for the final lactic acid concentration. The amount of lactic acid produced by the tissue during the experimental period is given by the formula

$$X_{Lac} = (C - C_o)(V_o - V_1), \text{ where}$$

X_{Lac} = mg. of lactic acid formed during experimental period.
C = Lactic acid concentration in mg./cc. at end of experimental period.
C_o = Lactic acid concentration in mg./cc. at beginning of experimental period.
V_o = Volume of serum originally placed in vessel (usually 2 cc.).
V_1 = Volume of serum removed from side-well (usually 0.1 cc.).

[2] E. L. Spencer and W. C. Price, *Amer. Jour. Bot.*, 28: (in press), 1941.

[1] S. B. Barker and Wm. H. Summerson, *Jour. Biol. Chem.*, 138: 535, 1941.

[2] J. MacLeod and C. P. Rhoads, *Proc. Soc. Exper. Biol.*, 41: 268, 1939. A. Canzanelli and D. Rapport, *Amer. Jour. Physiol.*, 127: 296, 1939. C. O. Warren, *Amer. Jour. Physiol.*, 128: 455, 1940. D. G. Friend and A. B. Hastings, *Proc. Soc. Exper. Biol.*, 45: 137, 1940.

[3] Used in the restricted sense of meaning lactic acid formation.