AN EFFECT OF LIPID FEEDING UPON VITAMIN C EXCRETION BY THE RAT[1,2]

DURING the past year we have been searching for the biological precursor of vitamin C (ascorbic acid) by a technique that has given clear-cut and consistent results —that is, the results are clearly positive or negative in each case. The earlier published suggestions that mannose and certain other substances might serve as precursors of ascorbic acid could not be verified in our own or other laboratories during the past six years. Meanwhile, identification of the vitamin as a single substance and the development of sensitive, quantitative methods of analysis have led to a greater appreciation of the vitamin's probable wide-spread importance in cellular processes. It is now evident that ascorbic acid can be synthesized by practically all plants and animals, with the exception of guinea pigs, man and the other primates. Therefore, even though only the latter types are subject to scurvy, it is reasonable to assume that the vitamin has definite, essential functions in all the higher plants and animals (the lower, or less highly organized types, have not been investigated extensively). From a chemical point of view the role of the vitamin *in vivo* is essentially unknown, even though many empirical physiological relationships are clearly recognized.

The technique that we have used is essentially as follows. Young or adult albino rats are kept in raised-bottom cages of the usual type, below which a fine screen and funnel serve to collect the urine into small vials. The vials contain enough metaphosphoric acid to provide a final concentration of approximately 3 per cent. The samples are removed and titrated once each day to provide a measure of vitamin excretion. Animals fed a stock diet of Purina chow or Sherman's diet No. 13 show a sharp drop in vitamin C excretion during inanition, or a more gradual drop when placed on a diet of condensed milk. Hence, after a short period of inanition (3 to 4 days) and an additional period (generally 3 to 6 days) on condensed milk, they are in a suitable condition for assay purposes. The animals are then fed the test supplement plus a basal diet of condensed milk.

The common purified foodstuffs such as sugars, proteins and oils do not affect the rate of vitamin C excretion. Oats, oat oil, the unsaponifiable matter from oat oil and halibut liver oil quickly induce a high rate of excretion that may readily exceed 2 mg per day. Comparable results with respect to the time and extent of response are obtained by feeding the pure vitamin. Certain volatile fractions from liver oil and oat oil are especially active, and investigations are being continued for the purpose of checking the identity and structural relationships of the active materials from different sources. The fatty acids and common sterols are inactive.

The results are of interest, not only in relation to the biological synthesis of a vitamin, but also because of the novel effect of a lipid upon the synthesis of a carbohydrate (the vitamin, $C_6H_8O_6$, is essentially a sugar). Although the suggestion of this type of lipid-to-carbohydrate conversion has apparently never been made, there is considerable evidence in the literature to indicate that vitamin C is peculiarly related to the mitochondrial, adreno-cortical and carotenoid lipids.

It is possible that the lipid effect may be exerted through an indirect agency, such as by accelerating a synthesis from other substances, or by serving as a protective agent against tissue destruction. These phases of the problem are being investigated, but the weight of evidence at the present time favors the "precursor" interpretation.

The vitamin excretion has been verified by biological assays with guinea pigs, in addition to chemical titrations, so that there is no question concerning the identity or approximate quantity of the product excreted.

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[1] Research Publication No. 371, from the Department of Chemistry, University of Pittsburgh.

[2] This investigation was made possible by a research grant from the Buhl Foundation.

PLANT VIRUS INHIBITORS PRODUCED BY MICROORGANISMS

IN a recent paper from this laboratory[1] it was shown that many bacteria and fungi were capable of inactivating the virus of ordinary tobacco mosaic, and that the time required for this action depended upon the organism used. It was also suggested at the time that the inactivation of the virus in the cultures was due to decomposition or digestion. The high comparative rate of inactivation of the virus by certain organisms such as *Aerobacter aerogenes* (Kruse) Bergey *et al.* and *Aspergillus niger* Van Tiegham stimulated further investigation of their behavior. It soon became evident that these organisms differed from most microorganisms with respect to type of inactivation in that they were capable of producing a substance (or substances) in culture which when added to an extract of tobacco mosaic is immediately inhibitory to the infectivity of the virus. This substance is not toxic to living matter in the usual sense when used on bacterial, fungal or higher plant cultures, and in this respect, resembles charcoal, Phytolacca juice,[2] dry soil and trypsin, in its action on the virus. Whether or not the substance

[1] J. Johnson and I. A. Hoggan, *Phytopath.*, 27: 1014–1027, 1937.

[2] B. M. Duggar and J. K. Armstrong, *Ann. Mo. Bot. Garden*, 12: 359–366, 1925.

is to be regarded as a virus inactivator or an inhibitor to host infection as reported by Stanley for trypsin[3] has not been fully determined. Since the inhibitory effects on infectivity is instantaneous it suggests that the inactivation of the virus in the instance of the two above organisms grown in culture can not be attributed to decomposition or digestion. Efforts at precipitation and purification have not been successful. The substance may be concentrated appreciably by evaporation of the culture medium, the inactivator being tolerant to drying and to heat, partly withstanding 100° C. for several hours. In a dried broth culture the product is normally active at 0.1 per cent. water solution and retains most of its activity at 0.01 per cent. concentration. A normal concentration will completely inactivate an equal volume of undiluted extract of ordinary tobacco mosaic. It is equally effective on several other plant viruses. The inactivator passes bacterial proof filters but with considerable reduction in potency. The substance retains its activity for months in either the original culture, a heat-sterilized culture or in a state of desiccation. It will also withstand high concentrations of alcohol, chloroform, mercuric chloride and charcoal. The substance differs strikingly from the Phytolacca juice and trypsin inhibitors in several respects, as for example in its ability to tolerate higher temperatures. There is therefore probably no chemical relationship between these peculiar virus inhibitors.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE USE OF PLASTIC MATERIALS FOR OPERATIONS ON AMPHIBIAN EMBRYOS

OPERATIONS on amphibian embryos are usually carried out in black wax dishes, while grafts are held in place by means of glass rod "bridges."[1] When these devices are employed for transplantation experiments on the hind limb of Amblystoma,[2] however, it is usually difficult to hold the embryos firmly since the wax can not be molded about them *in situ;* nor is it always possible to adjust the glass bridges to the proper contact and pressure for good healing.

The following materials and devices have been developed[3] to provide more adequate equipment for operations on Amblystoma embryos. They have been used successfully, with minor improvements, for five seasons and have been found adaptable to a wide variety of experiments with both anurans[4] and urodeles.

A. OPERATING DISHES

Various types of waxes and modeling clays have been tested to find a non-toxic material which can be molded readily and into which pins can be inserted easily and firmly. Most waxes are unsuitable for these purposes, although colorless "Rainbow Wax" (American Art Clay Company) works easily but lacks the firmness of clays. Stone[5] also reports satisfactory results from molding white refined beeswax. On the other hand, almost all clay modeling materials can be molded readily under the solution used for operating and are sufficiently firm, but many of them are toxic, especially those with noticeable odor or color.

To date, uncolored or gray Permoplast Modeling Clay (made of China clay, petrolatum and glycerine—American Art Clay Company, Indianapolis, Ind.) has been found most satisfactory for retaining the embryos without injury and for holding the graft covers securely during the progress of healing.

The operating dish is made by filling a Stender preparation dish of 50 or 60 mm diameter about half full of the modeling material. In performing the operation a depression is made in the plastic material by means of a blunt glass stylus, while the embryo lies alongside for comparison of size and shape. The embryo is then inserted into the cavity and the material pressed around it, or tiny spurs rolled up over it by means of a spear-shaped needle or glass rod. When the embryo is ready to be removed, it may be freed without injury by pushing or scooping away the surrounding material.

B. GRAFT COVERS

Graft covers with pin supports have been devised to hold the transplanted tissue in place during healing. The cover proper is made of some clear transparent plastic sheet material such as du Pont's Lucite, Plastacele or Pyralin of .090 inch to .125 inch thickness. The pin supports are fashioned from small gold-plated safety pins (Fig. 1, A) or large (No. 7) non-corrosive insect pins. The safety pins are preferable, however, since the clasps form convenient "handles."

The graft covers are made by sawing a piece of the sheeting into small rectangular blocks of about $\frac{1}{8}$ inch x $\frac{3}{8}$ inch each. A fine hole is then drilled through each end of the block, using a No. 70 or 71 wire gauge drill,

[3] W. M. Stanley, *Phytopath.*, 24: 1055–1085, 1934.
[1] R. G. Harrison, *Jour. Exp. Zool.*, 32: 6, 1921.
[2] W. A. Stultz, *Jour. Exp. Zool.*, 72: 330, 1936.
[3] W. A. Stultz, *Anat. Rec.*, 64: Suppl. no. 1, 43, 1935.
[4] K. A. Youngstrom, *Jour. Comp. Neur.*, 68: 353, 1938.
[5] L. S. Stone, *Proc. Soc. Exp. Biol. and Med.*, 31: 1084, 1934.