ascorbic acid which has proved itself non-toxic when administered intravenously to either experimental or human subjects and has been used in this clinic for the treatment of secondary anemias.<sup>3</sup> This salt was found by us to have a highly antiscorbutic property when given intravenously, daily, over a period of six days to a patient with severe scurvy. A daily dose of 250 mgm was sufficient to bring the plasma ascorbic acid level from .02 mgm per cent. to 1.2 mgm per cent. and the withdrawal of marked scorbutic symptoms.

With the cooperation of Dr. Alexander and Dr. Townsend, these findings were confirmed in scorbutic guinea pigs and in normal subjects. An interesting feature in the use of the salt, as is especially demonstrated in normal subjects, is the slow rise in the plasma ascorbic acid content as determined by the method of Pijoan and Klemperer,<sup>4</sup> following its intravenous injection as contrasted to the slope of the values obtained after the injection of ascorbic acid. It would appear from these biological tests that the compound of iron ascorbate breaks down slowly. Chemically, after precipitating the ferrous iron by H<sub>o</sub>S as ferrous sulfide and the reduction of the ascorbic acid by H.S. we were able by the method of Emmerie<sup>5</sup> to recover 97 per cent. of the ascorbic acid. This would indicate that in the salt the double bond of the ascorbic acid molecule is still present, which alone would allow for further reduction to ascorbic and titration with 2.6 dichlorophenol indophenol. The salt as synthesized by us and by Messrs. Hoffman-LaRoche contains 20 per cent. iron and in a 1 M. solution is of pH 6.9. At this pH only one of the hydrogens at the double bond could be replaced by iron. In conclusion, this salt is not only successful in bringing ferrous iron into the treatment of secondary anemias but has valuable antiscorbutic properties in which single daily doses produce prolonged and increased plasma ascorbic acid values.

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#### THE MAYNARD PLUM—A CARRIER OF THE PEACH MOSAIC VIRUS

SINCE 1935 plum trees have been suspected of being carriers of the peach mosaic disease in the Palisade district, Colorado, though they show no apparent symptoms of the disease. To investigate this possibility, fresh roots and twigs were taken from six Maynard plum trees growing in an area where heavy losses had been incurred from peach mosaic.

On September 4, 1936, buds from each of these

<sup>4</sup> M. Pijoan and F. Klemperer, *Jour. Clin. Invest.*, 16: 3, 443, May, 1937.

plums were grafted into five one-year-old peach seedling trees, making a total of 30 budded seedling trees. With the beginning of growth in the early spring of 1937, 15 seedling peach trees grafted with buds from parent plum trees Nos. 1, 5 and 6 showed typical symptoms of peach mosaic. The remaining 15 trees grafted with plum buds from parent plum trees Nos. 2, 3 and 4 remained healthy. All buds made growth unions. Twenty-eight seedling peach trees used as control remained healthy. The experiment was conducted in an isolated planting in a remote valley many miles from the mosaic-infected region.

On March 23, 1937, roots collected from the six Maynard plum trees were grafted on roots of 34 two-year-old peach seedling trees. Peach mosaic symptoms were observed on May 15 of the same year on 15 of the 17 peach seedling trees, which were root grafted, using plum trees Nos. 1, 5 and 6 as stock. Two root grafts failed to make growth unions and the peach trees remained normal. Seventeen peach seedling trees root grafted with roots from plum trees Nos. 2, 3 and 4 remained normal also. Thirty-three peach seedling trees used as controls remained free of infection.

From these experiments it appears that plums may be carriers of the peach mosaic virus, though the trees do not show the symptoms evident in the peach.

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## A COMPANION WORD FOR PLANKTON

IN SCIENCE for September 25, 1936, I asked for a new word to rank with plankton, but indicating food that is found in the top layer of mud, feeding perhaps as many forms of life as the ones depending upon plankton.

The numerous answers made too extensive text for publication as a group in the limited space that could be devoted to them, but I have selected the gist of the material for brief presentation. Incidentally, in my original letter I should perhaps have added *Accipenser* and larval *Petromyzon* to the group using this food.

Dr. Wm. Rienhoff, Sr., of Baltimore, Md., suggested either Iloen or Ascion, expressing in slime-imbedded organic particles serving as animal foodstuffs in contrast to plankton, expressing free floating material.

Dr. W. A. Dayton, of the U. S. Forest Service, suggested ''ilyophagous organisms.'' He said the Greeks had a word for mud feeder, ''borborophagous.''

Dr. Carl L. Hubbs, curator of fishes, University of Michigan, suggests "hyperbius."

Dr. Dorothy Cobb Adams, of the Johns Hopkins Hospital, suggested ''limous plankton'' from *limus*—mud or slime.

<sup>&</sup>lt;sup>5</sup> A. Emmerie, Biochem. Jour., 28: 268, 1934.

Dr. W. H. Bradley, senior geologist, U. S. Geological Survey, likes the word "sapropol" previously suggested by H. Potomie in 1908.

Dr. Glover M. Allen, Cambridge, Mass., suggested "ilyonic food."

Dr. Denis L. Fox, Scripps Institution, La Jolla, Calif., likes "ilytrophic food"-from ilytrophon (mud food).

Dr. L. O. Shapolano, of Stanford University, would qualify "benthotic food" as littoral, sub-littoral, profundal and abysmal benthotic. Dr. Wm. Rienhoff, Sr., also suggested the necessity of qualifying the term benthotic according to depth.

Dr. Agnes De Sales, College of Mount St. Joseph on the Ohio, suggests "acropelotic"-akros, top: pelos, mud.

Dr. A. Willey, Mille Isles, Quebec, agrees with Dr. Glover M. Allen-"'ilyonic food" to compare with planktonic food.

My own choice would lie between "ascion," suggested by Dr. Rienhoff, and "sapropol," suggested by Dr. Bradley.

STAMFORD, CONN.

ROBERT T. MORRIS. M.D.

# SPECIAL ARTICLES

### A CRYSTALLINE PROTEIN WITH HIGH LACTOGENIC ACTIVITY\*

DURING the course of chemical studies of anterior lobe fractions of the pituitary gland, it has been possible to isolate in crystalline form a protein having marked prolactin (lactogenic) activity. The method of preparation of the prolactin fraction from the gland is essentially that described by Lyons.<sup>1</sup> The purified prolactin preparations have been obtained in crystalline form from pyridine-acetic acid mixtures, using a procedure which is essentially the same as one employed for the crystallization of insulin.<sup>2</sup> One hundred milligrams of the purified fraction are dissolved in a centrifuge tube by the addition of 2 cc of 13 per cent. acetic acid. The material dissolves slowly. When solution is complete, 2 cc of 10 per cent. pyridine are added and the mixture centrifuged. The supernatant fluid, which is usually slightly turbid, is set aside and the precipitate dissolved in 2 cc of acetic acid and 2 cc of pyridine solution added as before; the mixture is then centrifuged. This procedure is repeated 10 times. The mother liquors are combined, and from this solution a crystalline precipitate can usually be obtained in either one of two ways: (1) The pyridine-acetic acid solution slowly deposits a crystalline material on standing for several days in the ice-box; (2) the pyridine-acetic acid solution is treated carefully with one per cent, ammonium hydroxide solution. The latter is added until a distinct, heavy turbidity results. Any material settling out immediately is centrifuged off and the turbid mother liquor placed in the ice-box over night. Microscopically, the crystals appear for the most part as cylindrical rods of varying length, with the rounded edges usually characteristic of protein crystals. The precipitate may be prepared in dry form by centrifuging, and washing at the centrifuge twice

with 2 cc portions of ice-cold water, followed by washing once with a mixture of equal parts of absolute alcohol and dry ether, and finally washing two times with dry ether. It is dried in a vacuum desiccator over sulfuric acid.

In recrystallization the material may be treated exactly as described above by means of the pyridineacetic acid procedure. The lactogenic activities of the various crystalline fractions and residues were determined by bioassays on one-month-old squabs, using the 2-day "local" test.<sup>3</sup> Some of the data obtained are shown in Table I.

TABLE I Assay of Crystalline Prolactin Preparations by the "Local" or Intradermal Test

Preparation	Crystallized	Extinction point*
A A B B C C C	Once Twice Thrice Once Twice Once Twice	$\begin{array}{c} < 0.25  \text{gamma} \\ < 0.125  \  \   \  \   \\ 0.0625  \  \   \  \   \\ < 0.125  \  \   \\ < 0.125  \  \   \\ < 0.125  \  \  \  \\ 0.10  \  \  \  \  \  \  \\ 0.0625  \  \  \  \  \  \  \  \  \  \  \  \  \$

\* The extinction point is designated as the dosage below which a positive response to the injection can not be detected.

It will be seen from the data in Table I that after two recrystallizations, the preparations attain a fairly constant level at which a positive reaction is still obtained, *i.e.*, between one tenth and one twentieth of a gamma. When tested by the "systemic" test,<sup>4</sup> these preparations were found to have an average minimal effective dose of 0.1 mg. It is interesting to note how closely the results of the bioassays agree with the activities reported by Lyons recently<sup>1</sup> for his purified mammotropic hormone. It is evident that the latter investigator has a preparation of a high degree of purity.

A study of the x-ray diffraction pattern of the once crystallized product was kindly conducted by Professor L. W. McKeehan, director of the Sloane Physics

<sup>\*</sup> This study was made possible by a grant from the Fluid Research Fund of Yale University School of Medicine.

<sup>1</sup> W. R. Lyons, Proc. Soc. Exp. Biol. and Med., 35: 654, 1936-37.

<sup>&</sup>lt;sup>2</sup> V. du Vigneaud, H. Jensen and O. Wintersteiner, Jour. Pharm. Exp. Ther., 32: 367, 1927-28.

<sup>&</sup>lt;sup>3</sup> W. R. Lyons and E. Page, Proc. Soc. Exp. Biol. and

Med., 32: 1049, 1935. 4 O. Riddle, R. W. Bates and S. W. Dykshorn, Am. Jour. Physiol., 105: 191, 1933.