pregnant mare's urine. P. G. Weil, University Clinic, Royal Victoria Hospital, Montreal, is pursuing biochemical studies on the metabolism of progesterone, investigating the sterol metabolism in the toxemias of pregnancy, and studying a new sterol, with reference to the adrenal cortex.

The trustees were pleased to note that medical literature of last year contained over thirty reports of research carried out with the assistance of the Banting Research Foundation. The indication many of these reports gave to the effect that medical science is slowly making inroads upon some of man's most stubborn ills, should, in the opinion of the trustees, be a source of satisfaction to those who showed their appreciation of Sir Frederick Banting's researches by endowing a foundation to allow him and others to continue to advance the state of medical knowledge.

V. E. HENDERSON,
A. W. HAM,
Honorary Secretaries

SPECIAL ARTICLES

THE OCCURRENCE OF GAMMA TOCOPHEROL IN CORN EMBRYO OIL[1]

OLCOTT and Emerson[2] showed that the tocopherols have strong antioxidant powers, and concluded that they represent at least a large part of the antioxidants in wheat-germ and cotton-seed oils. There seems to be no relation between the vitamin and antioxidant activities of these substances, since alpha, which is the most potent as the vitamin, is the weakest antioxidant. Gamma, which is approximately equal in vitamin potency to beta, is definitely a more powerful antioxidant.

It seemed interesting to determine if the antioxidant properties of other vegetable oils might be due to the presence of tocopherols, and those oils with less vitamin potency might contain the less vitamin-potent beta or gamma. Accordingly we investigated corn oil, since Mattill and Crawford[3] had shown it to be rich in antioxidants.

Freshly pressed, unrefined corn oil[4] was assayed for vitamin E. A single dose of 4 gm enabled all four test rats to cast good litters, but at 2 gm only resorptions resulted. Three kilograms of the oil was saponified, the non-saponifiable fraction distributed between high boiling petroleum ether and 92 per cent. methanol, and then dry methanol, and the methanol solution was concentrated, chilled to free it as much as possible of sterols, and finally the oily residue distilled in a molecular still, as previously described for palm oil[5]. The fraction distilling between 120–140°, which contained the bulk of the vitamin, weighed 5.65 gm. Fed at a level of 15 mg, three resorptions and one litter resulted, but at 45 mg all four rats fed had litters. Karrer and Keller[6] measured by titration with gold chloride the tocopherol content of a non-saponifiable fraction of corn oil, freed from most of the sterols, and found it to be 0.2 per cent. Assuming the critical level of gamma tocopherol to be 6 mg, Karrer and Keller's measurement would appear to be in reasonable agreement with the results of our feeding tests.

The concentrate was treated with cyanic acid in benzene, as previously described. The only tocopherol which could be isolated was gamma, whose allophanate, mp. 137–140°, gave no depression on admixture with gamma tocopheryl allophanate previously obtained from cotton-seed oil. The yield was about 700 mg.

The allophanate was saponified, and the free tocopherol fed at levels of 3 to 6 mg. Of four rats fed 3 mg one had a litter and three resorbed, while of five rats receiving 6 mg, two had litters and three resorbed.

The gamma allophanate, on admixture with beta allophanate, mp 143–6°, melted at 130–5°. This, together with the complete difference in the habit and appearance of the two allophanates, would seem to leave little reason to doubt their non-identity. On the other hand, the admixture of alpha tocopheryl allophanate mp 158–60° lowers the melting point of gamma only two or three degrees, which makes it very difficult to be certain that a preparation of gamma is not contaminated with alpha. However, the absence of any considerable amounts of alpha from corn oil greatly facilitates the preparation of gamma in a comparatively pure form.

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[1] Aided by grants from the Department of Agriculture, University of California and by Merck and Company, Inc., Rahway, N. J. Assistance was rendered by the Works Progress Administration, Project No. 10482 A-5.

[2] H. S. Olcott and O. H. Emerson, *Jour. Am. Chem. Soc.*, 59: 1008, 1937.

[3] H. A. Mattill and Blanche Crawford, *Jour. Ind. Eng. Chem.*, 22: 341, 1930.

[4] The corn oil was kindly supplied by the Miner Millard Milling Co., Wilkes-Barre, Pa.

[5] O. H. Emerson, G. A. Emerson, Ali Mohammad and H. M. Evans, *Jour. Biol. Chem.*, 122: 99, 1937.

THE QUANTITATIVE DETERMINATION OF VITAMIN C IN MILK

RECENT studies[1, 2, 3, 4] have shown that there are a number of important factors which may influence the

[6] P. Karrer and H. Keller, *Helv. Chim. Acta*, 21: 1161, 1938.

[1] P. F. Sharp, *Jour. Dairy Science*, 21: 85, 1938.

[2] S. K. Kon and M. B. Watson, *Jour. Soc. Chem. Ind.*, 55: 508, 1936.

results obtained in the determination of vitamin C in milk by titration with 2,6-dichlorobenzenoneindophenol. Such variables as temperature, light, method of standardizing the dye (2,6-dichlorobenzenoneindophenol), stability of the dye, contact of the milk with oxygen, catalytic elements present and methods of precipitating milk protein have been outstanding in these reports.

The present work was undertaken to simplify and to make reproducible a quantitative procedure for determining the total amount of vitamin C in milk as it comes from the dairy cow.

Kon and Watson[5] have shown that ascorbic acid (vitamin C) in milk as it leaves the cow's teat is in the reduced form. In this form the vitamin C content of milk may be determined quantitatively by direct titration with 2,6-dichlorobenzenoneindophenol. Should the milk, however, in the process of collection, be exposed even very briefly to contact with air, catalytic metals, light or heat, an unavoidable loss of ascorbic acid occurs due to oxidation. In order to eliminate the effect of these factors in collecting samples directly from cows of the college herd, a special apparatus was devised (See Fig. 1).

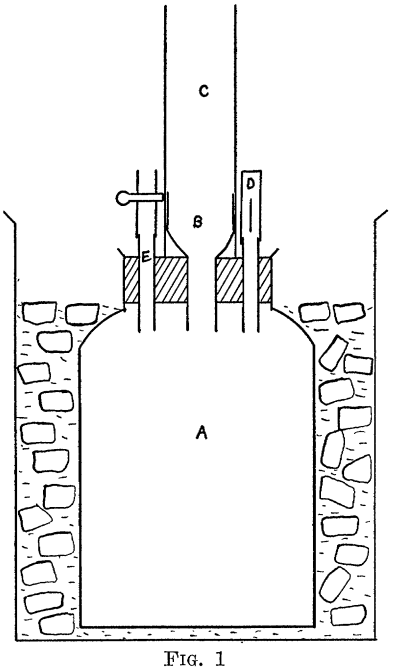

FIG. 1

A 500 ml dark-glass bottle (A) was placed inside a 2-liter beaker and surrounded with ice and water. This was done a short time prior to milking in order that the bottle might become thoroughly chilled. The bottle was fitted with a 3-holed rubber stopper. Through one hole of this stopper projected a glass adapter (B) having a funnel stem. A dark rubber tube (C), thin enough to be quite pliable and about one inch in diameter, was stretched over the adapter and pulled down to the rubber stopper. The upper part of this tube extended about three inches above the top of the adapter and was the part which was slipped over the teat of the cow during milking. A tube fitted with a Bunsen valve (D) passed through another hole in the stopper. Another glass tube (E) passed through the third hole and was provided with a short length of rubber tubing so that it could be closed off with a pinch clamp. The size of this tube was such as to permit the insertion of a 10 ml bulb pipette for removal of samples. Before milking, the air in the dark bottle was expelled through the Bunsen valve by closing all outlets with pinch clamps and flushing with carbon dioxide from a pressure tank.

To determine if the ascorbic acid of milk obtained in the above apparatus was completely in the reduced form, two samples were removed and titrated immediately, while other samples were subjected to treatment with hydrogen sulfide and then titrated after removing excess hydrogen sulfide with carbon dioxide. Typical results are given in Tables 1 and 2.

TABLE 1
TITRATION OF UNTREATED MILK SAMPLES

Ml milk	Ml dye	Mg ascorbic acid per aliquot	Mg ascorbic acid per liter
10.00	1.90	0.2280	22.80
10.00	1.91	0.2292	22.92
	Average ..	0.2286	22.86

TABLE 2
TITRATION OF HYDROGEN SULFIDE TREATED SAMPLES

Ml milk	Ml dye	Length of treatment in minutes	Mg ascorbic acid per aliquot	Mg ascorbic acid per liter
10.00	1.91	10	0.2292	22.92
10.00	1.91	10	0.2292	22.92
		Average	0.2292	22.92
10.00	1.92	30	0.2304	23.04
10.00	1.91	30	0.2292	22.92
		Average	0.2298	22.98
10.00	1.91	100	0.2292	22.92

The instability of 2,6-dichlorobenzenoneindophenol solutions reported by some workers was satisfactorily overcome by preliminary extraction of the dye with anhydrous ethyl ether. Solutions of dye, thus purified, and standardized according to the methods of Menaker and Guerrant,[6] remained perfectly stable and gave sharp end points over a period of 21 days.

Metaphosphoric acid was found by Fujita and

[3] R. R. Musulin and C. G. King, *Jour. Biol. Chem.*, 116: 409, 1936.

[4] C. H. Whitnah, W. H. Riddell and W. J. Caulfield, *Jour. Dairy Science*, 19: 373, 1936.

[5] S. K. Kon and M. B. Watson, *Biochem. Jour.*, 31: 223, 1937.

[6] M. H. Menaker and N. B. Guerrant, *Jour. Ind. Eng. Chem.*, Anal. Ed., 10: 25, 1938.

Iwatake,[7] Lyman, Schultze and King[8] and others to protect vitamin C in solution against oxidation. This property, combined with the protein-precipitating ability of metaphosphoric acid, was made use of in the present work in preparing milk samples for titration. To eliminate the uncertain protein-precipitating power encountered with ordinary metaphosphoric acid solutions, the required volume of a stable sodium metaphosphate solution, prepared by the method of Briggs,[9] was acidified just before each titration and added to the milk. Using 10 ml of 10 per cent. sodium metaphosphate solution, the addition of 0.6 ml of concentrated hydrochloric acid was found to bring the pH of the solution to a point (pH 2.5–3.0) where immediate and complete flocculation of protein resulted upon addition of the metaphosphate solution to 10 ml of milk.

Titrations were made in the presence of precipitated milk protein. By repeated centrifuging and washing of the milk protein followed by separate titrations of combined centrifugate and protein residue, it was found that slightly higher ascorbic acid values were obtained in titrations of milk in the presence of precipitated protein. However, upon the addition of pure ascorbic acid to the protein residues and repetition of the centrifuging procedures and titrations, it was shown that the slightly higher apparent values obtained in the presence of milk protein were due to adsorption of negatively charged dye by positively charged protein. No adsorption of ascorbic acid by the milk protein could be demonstrated.

The new apparatus and improved technique provides a simple but reliable method of obtaining and determining vitamin C of milk in its naturally occurring form. The improvements mentioned should be of value in following fluctuations of vitamin C in milk at different stages of lactation, at various seasons of the years, during feeding experiments and under numerous other conditions.

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A PLANT GROWTH INHIBITOR

DURING the course of physiological investigations on the plant hormone relationships in radish, strain French Breakfast, an ether extraction was made of 4.935 grams fresh weight of cotyledons from seven-day-old seedlings. These plants had been grown in the open in rich, loamy soil. The extraction was carried out according to the simplified auxin extraction method of Van Overbeek.[1] On testing the extract by the Avena test (Went and Thimann[2]) positive curvatures of from 17 to 23 degrees were found instead of the usual negative ones. (If the substance being tested is growth-promoting then the Avena plant will grow more rapidly on the side on which the substance is applied. Thus, because of this unsymmetrical growth, the plant will become curved in a direction away from the side on which the substance is applied. This is known as a negative curvature. If, however, the material causes an inhibition of growth then the plant will likewise grow unsymmetrically, but now the resulting curvature will be in a direction toward the side of application of the substance. This is known as a positive curvature.)

The relation between the concentration of the extract and degrees of positive curvature was investigated. In determining the amount of positive curvature 48 Avena plants were used at each dilution value. The inhibitor, extracted as above, was taken up in 1½ per cent. agar and cut into blocks $1.6 \times 2 \times 2$ mm for application to the test plants. The standard Avena technique for auxin determination was used except that the curvature—positive in this case—was measured 150 minutes after applying the inhibitor instead of after 90 minutes, as is customary when testing growth-promoting substances. The results are seen in Fig. 1.

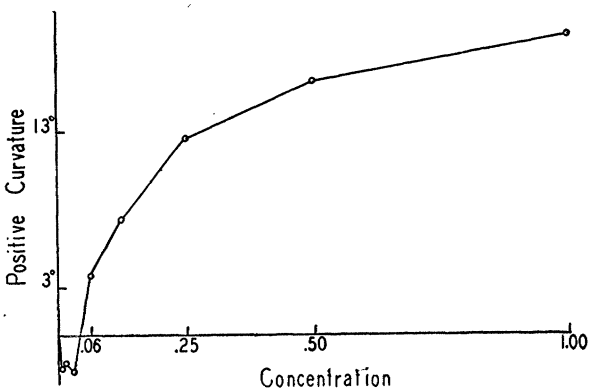

FIG. 1. Relation between positive curvature of Avena plants and two-fold dilutions of inhibitor substance.

This graph shows that positive curvatures between 3 to 13 degrees are proportional to the concentration of the inhibitor.

Using the method given by Schneider and Went[3] a Photokymograph test was made of the reaction time of the coleoptile to growth inhibitor. The results are pre-

[7] A. Fujita and D. Iwatake, *Biochem. Zeits.*, 277: 293, 1935.
[8] C. M. Lyman, M. O. Schultze and C. G. King, *Jour. Biol. Chem.*, 118: 757, 1937.
[9] D. Briggs, *Proc. Soc. Exp. Biol. and Med.*, 37: 634, 1938.

[1] J. Van Overbeek, *Proc. Nat. Acad. Sci.*, 24: 42, 1938.
[2] F. W. Went and K. V. Thimann, "Phytohormones," Macmillan Company, New York, 1937.
[3] C. L. Schneider and F. W. Went, *Bot. Gaz.*, 99: 470, 1938.