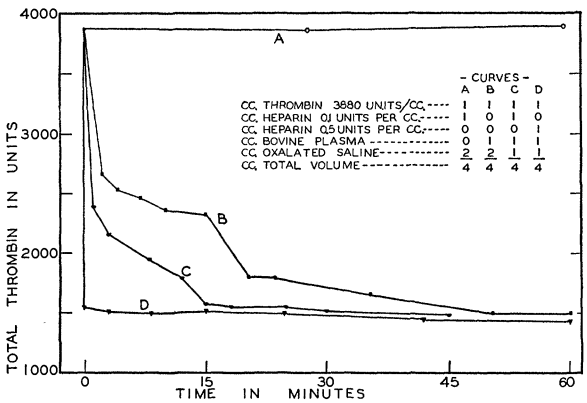

FIG. 1. The thrombin and the heparin were dissolved in oxalated saline (0.075 per cent. $K_2C_2O_4$ + 0.80 per cent. NaCl). Oxalated bovine plasma was obtained from a mixture of 7 parts blood and 1 part 1.85 per cent. $K_2C_2O_4$. The thrombin unit is the amount required to clot 1 cc of purified fibrinogen solution, containing calcium and acacia, in 15 seconds. In these experiments, calcium was omitted in order to avoid forming thrombin from the prothrombin present in the oxalated plasma. Under these conditions (*Am. Jour. Physiol.*—in press), thrombin is one third less efficient; the thrombin titers were therefore multiplied by the factor, 1.5, and the results were then plotted in terms of standard units.

In light of these findings, it is of great interest that plasma alone, without a supplement of heparin, also caused the thrombin titer to fall to the 1,500-unit level (tube B). The fall occurred quite slowly, however—almost an hour was required. It is known that plasma contains small amounts of heparin, and this heparin may be an essential catalyst, without which the reaction can not occur in finite time. It will be necessary to isolate the co-factor in order to determine whether, in the absence of heparin, it possesses any detectable antithrombic activity. It may well be that it does not.

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THE ULTRAVIOLET SPECTROGRAPHIC EXAMINATION OF THE FAT FRACTION OF MOUSE MILK AND MAMMARY GLANDS[1]

THE presence of a mammary tumor-producing substance in the milk of high tumor strain mice has been adequately demonstrated by Bittner,[2] Andervont,[3] DeOme.[4] It is known to be present also in the blood and certain tissues of high tumor strain females.[5,6] If this factor is similar to the estrogens or known synthetic carcinogenic hydrocarbons, it should be demonstrable by a comparison of the ultraviolet absorption spectra of the fat fractions of the milk and the mammary glands of high and low tumor strain mice. Milk and non-tumorous mammary glands from lactating high tumor strains, A, C3H and dba and from the low tumor strain C57Black were studied.

Milk was obtained by means of a miniature milking machine. Only mammary glands engorged with milk were used. Freshly excised mammary glands were plunged into liquid air and macerated with CO_2 as described by Strait and Aird.[7] The macerate was extracted by a solvent-separation procedure involving the use of acetone, ether, alcohol and isooctane. Alcohol soluble and insoluble fractions were studied independently. Preliminary control experiments indicated that extreme care is necessary in the use and purification of solvents if spurious results are to be avoided. The extraction procedure permitted study of the ultraviolet region down to 2300 Å, a range sufficient to include compounds of the type expected. Five samples of milk from A and C57Black mice averaging 3.26 g and eleven samples of mammary glands averaging 3.62 g were used. The absorption spectra of the extracts in spectroscopically pure isooctane were photographed in the ultraviolet with a Hilger medium quartz spectrograph. The extraction procedure is known to recover 1, 2, 5, 6-dibenzanthracene and the estrogens triphenylethylene and oestradiol.

A comparison of the ultraviolet absorption spectra of milk and mammary glands of high and low tumor strain mice showed no significant differences. These results would indicate that (1) the milk-borne factor either is not carried in the fat fraction or, if it is (2) it is not spectrographically similar to the carcinogenic hydrocarbons or estrogens, or (3) it is present in quantities too small to be detected spectrographically. Triphenylethylene may be detected in concentrations of 0.025 milligrams per gram, the naturally occurring estrogens in somewhat higher concentrations, and the carcinogenic hydrocarbons in lower concentrations.

Since the initiation of these experiments, recently published ultracentrifugation data[8] have indicated also that the active agent is primarily in the non-fat fraction. Ultrafiltration experiments by one of us on

[1] This work was in part supported by a grant in aid from the Blanche and Frank Wolf Foundation. The authors wish to express their appreciation to Dr. I. H. Perry for her valuable counsel.

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the non-fat portions of milk and mammary tissues are now in progress.

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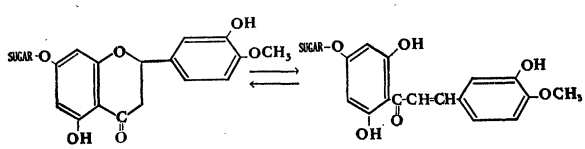
THE ISOLATION OF A NEW OXIDATION-REDUCTION ENZYME FROM LEMON PEEL (VITAMIN P)[1]

SZENT-GYÖRGYI and collaborators in 1936[2] postulated the existence in lemon peel of a new vitamin, designated the permeability vitamin or P, concerned with the regulation of capillary permeability and fragility. Since then much experimental and clinical work has been carried out with crude preparations indicating the presence of factors influencing the capillary state, the blood pressure and related phenomena. It was the purpose of the present work to isolate in pure form the capillary or vitamin factor.

Vitamin P was believed by Szent-Györgyi[3] to be citrin, a mixture of the flavanone glycosides, hesperidin and eriodictin. We initially prepared citrin by a method similar to that of Szent-Györgyi, which consisted in extraction of the ground lemon peel with acetone, precipitation with $Ba(OH)_2$, and liberation of the pigment with H_2SO_4 in a small volume of methanol. On standing for several days a white precipitate of hesperidin slowly separated out, accompanied by the bleaching of the solution. This observation, along with others in the course of the work, led us to regard the yellow eriodictin of Szent-Györgyi as the chalcone of hesperidin. This explains the difficulty encountered by Szent-Györgyi in separating hesperidin from his yellow material, inasmuch as there is an equilibrium between the flavanone and its open chalcone isomer:



hesperidin (flavanone glycoside) — hesperidin chalcone (3,2′,4′,6′-tetrahydroxy, 4-methoxy-chalcone glycoside)

This equilibrium is shifted to the right in alkaline medium and to the left in acid medium. It has been previously observed[4, 5] that the 2′-hydroxy-chalcones exhibit a marked tendency to isomerize to the corresponding flavanones.

The pure chalcone was prepared by dissolving several-times recrystallized hesperidin in cold alkaline solution with subsequent neutralization, upon which the chalcone rapidly crystallized and was filtered, washed with acetone and ether and dried. This crystallization is immediate, whereas the ring-closure proceeds at a much slower rate. The chalcone was obtained in the form of bright yellow microscopic crystals of melting point 251–252° C. (uncorr.), soluble in pyridine, very slightly soluble in methanol and insoluble in water. The chalcone could easily be reverted to hesperidin when suspended in absolute methanol (traces of anhydrous HCl increased the rate of this reaction), and the resulting product gave no depression of the melting point of the original hesperidin (261–262° C.).

An active group such as occurs in the chalcone (—CO—CH=CH—) should be easily susceptible to reduction. Upon solution of the chalcone in pyridine and treatment with small amounts of zinc and glacial acetic acid in an oxygen-free atmosphere, reduction took place rapidly as evidenced by the loss of color. Shaking the solution in air restored the yellow color. This chalcone is, therefore, a member of that class of naturally occurring substances capable of being reversibly oxidized and reduced.

Since most of these substances are associated with proteins as enzymes within the tissues a new method of isolation was developed in order to obtain the intact protein complex. An aqueous extract of lemon peel was saturated with $(NH_4)_2SO_4$ and the resulting precipitate filtered, redissolved, reprecipitated and dried *in vacuo*. This tan powder was exhaustively extracted in a Soxhlet with ether yielding a bright yellow solution and a pale tan residue. The ether-soluble pigment was shown by standard tests to be neither a carotenoid nor a benzopyrone type of pigment and will be the subject of further investigation in this laboratory. The protein residue was extracted with pyridine which served to split the pigment from the protein. This pigment was shown both by physical and chemical tests to be the chalcone of hesperidin. The denatured protein was repeatedly washed and found to give positive reactions to the standard protein tests.

When hesperidin opens to form the chalcone there is established an extensive system of conjugated double bonds, which greatly increases the absorption in the blue and near ultra-violet. Combination of the chalcone with the protein shifted the ultra-violet absorption maxima from 3320 Å. to 3270Å and from 3080 Å to 3020 Å without altering the shape of the curve. The chalcone forms complexes with other proteins, and it is probable that within the tissues it is

[1] One of us is indebted for a fund from the California Fruit Growers Association.

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