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

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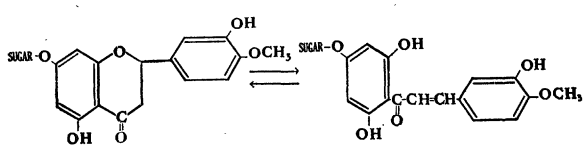
THE ISOLATION OF A NEW OXIDATION-REDUCTION ENZYME FROM LEMON PEEL (VITAMIN P)[1]

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.

[2] L. Armentano, A. Bentsath, T. Beres, St. Rusznyak and A. Szent-Györgyi, *Dtsch. med. Wschr.*, 33: 1325, 1936.

[3] A. Szent-Györgyi, *Zeit. f. physiol. Chem.*, 255: 126, 1938.

[4] J. Shinoda and S. Sato, *Jour. Pharm. Soc. Japan*, 48: 791, 1928.

[5] A. Russell and S. Clark, *Jour. Am. Chem. Soc.*, 61: 2651, 1939.

kept in solution by such a mechanism. The chalcone-protein complex was found to be easily reduced by sodium hyposulfite and to be reoxidized by oxygen.

The chalcone-protein and its prosthetic group, hesperidin chalcone, can serve as hydrogen transporters in mammalian tissue. This was demonstrated in the l(+)-glutamic acid dehydrogenase system obtained from liver by the method of v. Euler, Adler, Günther and Das.[6] This system involves l(+)-glutamic acid, apodehydrogenase, codehydrogenase I or II and diaphorase; whether the chalcone is reduced by diaphorase or directly by the codehydrogenase we do not know at the present time. As previously mentioned, the chalcone is autoxidizable and, hence, under aerobic conditions can increase the oxygen consumption of such a respiratory system. Work is now in progress to determine the various respiratory systems in which the chalcone-protein may play a role and the exact location of this substance in the hydrogen transport chain. We believe that this material and perhaps similar substances play a part in tissue respiration of both plant and animal cells.

Finally, preliminary experiments have shown that the chalcone exerts a beneficial effect upon the state of the capillaries, decreasing the fragility and preventing localized hemorrhages.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

AN APPARATUS FOR CONTINUOUS FILTRATION IN BLOOD AND PLASMA TRANSFUSIONS

DESPITE the use of adequate amounts of anticoagulant, fibrin clots will form in stored blood or plasma. The amount of fibrin will increase with the time of storage, but may be present in blood shortly after it is drawn. The blood and plasma, therefore, must be filtered before administration to prevent embolism.

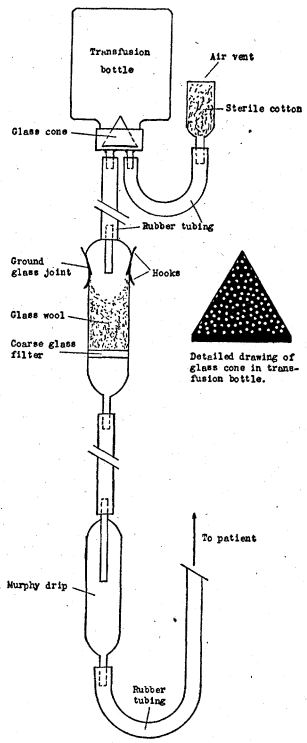

FIG. 1. Diagrammatic sketch of transfusion system, showing apparatus for continuous filtration.

In the methods employed at present, the most widely used materials are cotton gauze and metal screening. In many cases filtration through cotton gauze is carried out in the open air, permitting air contamination with bacteria. Furthermore, cotton gauze offers no uniformity for standardization, and no evaluation as to its pore size is possible. Metal screens, aside from the usual objection to metals for intravenous fluids, have been too coarse to retain all fibrin particles.

We have successfully used a filtering system which is fine enough to remove all fibrin particles. It consists first of a glass cone with coarse openings which holds back the large clots and prevents plugging of the rubber tubing. The blood is then filtered through glass wool (or glass cloth), and a fused glass filter which removes the remaining particles. "Pyrex" glass wool is adequate for this purpose, and, together with the fused glass filter, may be cleaned before use with cleaning fluid. The fused glass filter may be as fine as desired. It may retain the red cells or it may permit their easy passage. We have found that a filter made of fused glass particles, size 80 to 100 mesh, allows the rapid passage of the red cells and is still fine enough to hold back the fibrin particles.

The filtering apparatus permits administration in a closed system from the bottle into which the blood or plasma was drawn originally. This type of filter may be used to filter plasma in the field, either with the transfusion set as illustrated or by any other method. It satisfies the need for an adequate filter in the emergency treatment of shock with plasma.

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[6] H. v. Euler, E. Adler, G. Günther and N. Das, *Zeit. f. physiol. Chem.*, 254: 61, 1938.