of irradiated cholesterol and 12 tubes containing a 0.5% suspension of non-irradiated cholesterol were prepared. The cholesterol suspension was obtained by dissolving the required amount of cholesterol in ether and vigorously stirring the ether suspension into the hot medium. In this manner a finely dispersed suspension was obtained and the medium had a milky opalescent appearance. Each of the 24 tubes was then inoculated with human tubercle bacilli. The tubercle bacilli were obtained from the American Type Culture Collection. Inoculation of the glycerine agar tubes was made evenly by suspending the tubercle bacilli in a small amount of normal saline and inoculating the culture medium with equal amounts by means of a capillary pipette. The tubes were then plugged, corked, incubated and examined at 7-day intervals up to 4 weeks. A luxuriant growth of tubercle bacilli was obtained on all tubes inoculated save one which was overgrown by contaminating yeast. There was no appreciable difference in either rate or amount of growth between those cultures growing on medium containing a suspension of non-irradiated and those growing in medium containing a suspension of irradiated cholesterol.

A culture medium was then employed in which the cholesterol was in solution rather than in suspension. For this purpose Dorset's egg medium was chosen and 24 tubes were prepared as follows: Twelve tubes were filled with ordinary egg medium and 12 with egg medium which had previously been irradiated for one hour at a distance of 50 cm. from the lamp. These 24 tubes were inoculated and incubated in the same manner as the glycerine agar tubes and again no appreciable difference was observed in either the rate or the amount of growth at the end of 4 weeks' incubation.

*Conclusions:* The growth of human tubercle bacilli was not affected by a medium containing a suspension of cholesterol irradiated by ultra violet light or by an irradiated medium containing cholesterol in solution.

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### Development of Vitamin A During Ripening of Tomatoes.

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In the progress of an inquiry into the conditions under which vitamin A is developed in plants a comparison was made of the vitamin

A content of tomatoes ripened under various conditions. The tomatoes were of the variety called San Jose Canner and were supplied us by J. T. Rosa of the University Farm, Davis, California. They were plucked from the vines when green but completely matured, and were divided into 6 lots. One lot, designated lot 1, was ground at once, placed in small air tight glass jars fitted with outlet and inlet glass tubes, the air from which was evacuated and released with carbon dioxide 4 or 5 times. The jars were then stored at 17 degrees below zero centigrade. This process of preparation and storage was used to ensure as much freedom from oxidation as possible during the testing period. The other lots of tomatoes were similarly treated after being ripened as follows: lot 2, ripened in diffused light; lot 3, ripened in the dark; lot 4, ripened in ethylene, 1:2000 parts for 1 week[1]; lot 5, ripened in the dark except for 3 periods of ultra-violet irradiation of 30 minutes each at 12 inches distance from the arc. Temperature, ventilation and humidity were kept constant during ripening for all lots, a precaution indicated by the work of Duggar.[2] In addition lot 6 was later taken from the vines in the ripened condition. An attempt was made to allow all specimens of the fruit to ripen to a uniform color by comparison with Tallquist hemoglobin standards, but the results were not satisfactory since all the tomatoes with the exception of lot 4, showed varied degrees of redness in different parts of the same fruit. The ethylene-ripened specimens, lot 4, were uniformly colored a deep red, the tint of the deepest colored portions of the other fruits. The ripening period occupied 10 days in diffused light, 19 days in the dark, 7 days in ethylene, and 27 days for the ultra-violet treated lot. The oxidase contents of all specimens were determined but these were found to exhibit no significant differences.

Vitamin A deficient young rats were used for testing the value of these preparations by feeding separately carefully weighed portions of the fruit in addition to 0.5 gm. yeast daily and the usual basal diet. All rats used were taken from mothers reared and maintained on identical diet, were placed on this basal diet at 21 days of age and given the curative doses of tomato only after eye disease and loss of weight developed after 30 to 40 days. The basal diet consists of baked and alcohol-extracted casein, 18 parts; irradiated crisco, 5; agar, 2; salt mixture (Osborne and Mendel), 4; dextrin, 71. The feeding period was 56 days, and an attempt made to determine the

[1] Rosa, J. T., *Proc. Am. Soc. Hort. Sci.*, 1926.

[2] Duggar, B. M., "Lycopersicin, the red pigment of tomatoes, and the effect of conditions upon its development." *Wash. Univ. Studies*, I, 1913.

dosage which allowed 6 to 8 gm. increase in weight weekly. This standard was adopted rather than that of 3 gm. increase weekly proposed by Sherman and Munsell,[3] because the rats maintained at the former level are so often rendered abnormal by intercurrent and accidental results of their extremely low vitamin A margin.

The results of the test shown in Table I indicate the relative poverty as to vitamin A of the green tomatoes, and its consistent development in the ripened fruit. This is somewhat unexpected in view of the usual occurrence of this vitamin in green plant products and of the recent work of Dye, Medlock and Crist[4] in which the occurrence and production of chlorophyll and of vitamin A together has been indicated. As the lycopin developed in the plucked tomatoes as well as in those ripening on the vines apparently vitamin A developed also. This would seem to indicate a local production of the vitamin in the fruit itself. Certain findings upon the increase in

TABLE I.

*Vitamin A Content of Tomatoes, Green and Ripened under Various Conditions.*

| Lot No. | Method of preparation | Amount fed daily | No. of animals | Average gain in 8 weeks or ex pt'l period | Remarks. |
|---|---|---|---|---|---|
| | | gm. | | gm. | |
| 1 | Green. | 0.10 | 2 | — | Average survival, 42 days. |
| | pH, 4.86 | 0.20 | 4 | 14 | 2 died, av. survival, 69 days. |
| | | 0.30 | 3 | –5 | All died, av. survival, 54 dys. |
| 2 | Ripened in | 0.00 | 1 | –4 | Died on 35th day. |
| | diffused | 0.10 | 4 | 33 | 1 died on 77th day, 3 killed. |
| | light | 0.20 | 2 | 59 | Very good condition. |
| | pH, 5.21 | | | | |
| 3 | Ripened in | 0.00 | 1 | –2 | Died on 52nd day. |
| | the dark. | 0.10 | 3 | 16 | Average survival, 80 days. |
| | pH, 4.75 | | | | 2 died, 1 killed. |
| | | 0.20 | 2 | 73 | Good condition. |
| | | 0.30 | 1 | 86 | Very good condition. |
| 4 | Ripened in | 0.00 | 3 | –4 | Died in 34 days. |
| | ethylene | 0.10 | 3 | 36 | Fair condition. |
| | 1:2000 for | 0.20 | 2 | 59 | Good condition. |
| | 1 week. | 0.30 | 2 | 90 | Very good condition. |
| | pH 5.04 | | | | |
| 5 | Ripened in | 0.00 | 1 | –4 | Died on 35th day. |
| | dark except | 0.10 | 2 | 4 | Died, av. survival, 63 days. |
| | for 90 min. | | 3 | 47 | Poor condition. |
| | ultraviolet | 0.20 | 2 | 58 | Good condition. |
| | irradiation. | | | | |
| | pH, 4.88 | | | | |
| 6 | Ripened on | 0.00 | 2 | — | Died in 45 days. |
| | the vine. | 0.10 | 5 | 35 | Poor condition. |
| | pH, 4.66 | 0.20 | 2 | 59 | Fair condition. |
| | | 0.30 | 2 | 66 | Very good condition. |

[3] Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, xlvii, 1639.

[4] Dye, M., Medlock, O. C., and Crist, J. W., *J. Biol. Chem.*, 1927, lxxiv, 95.

vitamin A in red bell peppers as compared with the green variety from which they develop after plucking from the vines which were observed in this laboratory several years ago, parallel this experiment.

The vine ripened tomatoes and 4 examples of the fruit ripened off the vines under various light conditions appear to be about equally rich in vitamin A. Some interest attaches to the adequacy of the ethylene ripening process in this connection.

We are now engaged in determining the quantitative relations of the fat-soluble pigments, carotin, xanthophyll, and lycopin with chlorophyll and vitamin A in tomatoes and other plant products when these have been exposed to the action of ultra-violet and other light rays. The curious delay in ripening which was encountered in the ultra-violet treated lot number 5, may have been caused by some injurious action of the ultra-violet light, possibly through stimulated autoxidation, such as Holm, Greenbank and Deysher[5] have shown for unsaturated oils.

*A protecting effect of carotin and lycopin in allowing vitamin A production in plants when these are exposed to sunlight* is suggested as an explanation for the consistent occurrence, and as here shown development, of vitamin A along with one or the other or both of these plant pigments. The work of Coward[6] and of Heller[7] on the production of vitamin A in etiolated and normal wheat shoots under light treatment is not inconsistent with this view, although their choice of seeds and seedlings normally poor in both carotinoid pigments and vitamin A makes interpretation of their data difficult. The effect of changes which accompany germination in the growing plant and in the rate of growth of the plant as influenced by amount and kind of light can not be separated from the changes in vitamin A formation recorded in their experiments. The tomato ripening tests here recorded are independent of the changes involved in plant development although a definite metabolism in the plucked fruit must be postulated.

[5] Holm, G. E., Greenbank, G. R., and Deysher, E. F., *Ind. and Eng. Chem.*, 1927, xix, 156.

[6] Coward, K. H., *J. Biol. Chem.*, 1927, lxxii, 781.

[7] Heller, V. G., *J. Biol. Chem.*, 1928, lxxvi, 499.