not damaging and the toxic effects of Lavollay's "Catèchin actif" were less noticeable than the sample of epimerized *d*-catechin obtained from Hoffmann-LaRoche. Catechin red was especially damaging in doses greater than 0.01 γ. It is probable that the damaging effect of the partially decomposed catechin preparations was due to the presence of catechin tannins formed during the decomposition. This is also supported by the observation by Parrot and Cotereau[18] that these colored substances, which they term "antivitamin P," antagonize the action of active catechin ("vitamin P"), and increase capillary fragility.‖

It is probable that some of the biological and clinical effects ascribed to "vitamin P" can be explained on the basis of a vasoconstrictor effect on the capillaries rather than to an indirect effect on these vessels through an epinephrine-sparing action[4-9] or by a direct action on capillary walls.[16]

Summary. 1. A mammalian capillary bed preparation was modified from the rat mesoappendix mesenteric preparation of Zweifach and Chambers, and used to estimate the relative activities of various topically applied "vitamin P"-like compounds on microscopically visible vasomotor responses of the vessels.

2. Rutin, rutin sodium acid succinate, hesperidin, hesperidin sodium acid succinate, methylated hesperidin chalcone, esculin and adrenochrome were inert in the concentrations used.

3. The sodium acid phthalates of rutin and hesperidin were slightly active vasoconstrictors.

4. The catechins were highly active vasoconstrictors, including *d*-catechins, epimerized *d*-catechins, and especially *l*-epicatechin, in doses as low as 0.0001 γ or less.

5. Catechin red (catechin tannin or anhydride) was highly toxic, inducing irreversible vasodilatation and stasis.

‖ Preliminary investigations in these laboratories indicate that catechin tannins, like tannic acid, are powerful endothelium poisons, producing marked hemorrhagic symptoms when administered intravenously, which supports the observations of Parrot and Cotereau.[18]

15911

Relative Gastro-Intestinal Stability of Carotene and Vitamin A and Protective Effect of Xanthophyll.*

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Previous work[1,2] from this laboratory has demonstrated that carotene administered to vitamin A-deficient rats is destroyed in the gastro-intestinal tract by the simultaneous feeding of unsaturated fat acid esters. The gastro-intestinal destruction of carotene was prevented by feeding α-tocopherol. Similar results have been reported by Quackenbush, Cox and Steenbock,[3] and by Hickman, Harris and Woodside.[4] Hove[5] has shown that the mucosa of the rat stomach contains an enzyme, carotene oxidase, which greatly accelerates the destruction of carotene *in vitro* in the presence of methyl linolate.

Under conditions used in biological assay procedures for vitamin A, the potency of vita-

* Published with the approval of the Director of the Alabama Agricultural Experiment Station.

1 Sherman, W. C., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 199.

2 Sherman, W. C., *Proc. Fed. Am. Soc. Exp. Biol.*, 1942, **1**, 134.

3 Quackenbush, F. W., Cox, R. P., and Steenbock, H., *J. Biol. Chem.*, 1942, **145**, 169.

4 Hickman, K. C. D., Harris, P. L., and Woodside, M. R., *Nature*, 1942, **150**, 91.

5 Hove, E. L., *Science*, 1943, **98**, 433.

min A is reported to be about twice that of β-carotene on a weight basis. Values ranging from 0.3 μg[6] to 0.25 μg[7,8] of vitamin A have been reported to be equivalent to 0.6 μg of β-carotene (1 IU). However, no information is available on the relative potencies of vitamin A and carotene under conditions that would favor gastro-intestinal destruction of these factors.

The possibility of xanthophyll exerting a sparing effect upon the gastro-intestinal destruction of vitamin A and carotene appeared worthy of investigation in view of the similarity in the molecular structure of xanthophyll and carotene and the susceptibility of both classes of compounds to oxidative destruction.

In the present investigation the response of vitamin A-deficient rats was used to determine the effect of xanthophyll upon the effectiveness of vitamin A alcohol, vitamin A acetate, and β-carotene under conditions that would favor gastro-intestinal destruction of these factors.

During the vitamin A depletion period, weanling rats were fed a diet of the following composition: alcohol-extracted casein 18, salt mixture No. 5[9] 4, sucrose 77, and cottonseed oil 1. The following amounts of vitamins were added in mg per kg of diet: calciferol 0.125, α-tocopherol 50, *i*-inositol 200, thiamin 2, pyridoxine 2, riboflavin 4, calcium pantothenate 10, niacin 20, and choline chloride 2000. Cottonseed oil and α-tocopherol were fed during the depletion period to minimize possible complications from the development of deficiencies of vitamin E and unsaturated fat acids. The rats were maintained upon the depletion diet until growth had ceased and ophthalmia had developed, at which time the daily feeding of supplements was started and the animals were changed to a test diet. The test diet differed from the depletion diet as follows: α-tocopherol and the cottonseed oil were omitted from the test diet and sucrose was increased to 78%. Fresh lard, which is known to contain only a small amount of antioxidants, was fed during the test period as a source of unsaturated fat acids.

The xanthophyll used in these studies was prepared from fresh Kentucky blue grass according to the method of Strain.[10] Strain's procedure was modified slightly in that the material, after being purified by adsorption from carbon disulphide on a chromatograph column of magnesium oxide, was crystallized twice from hot methanol without the addition of petroleum ether. The recrystallized xanthophyll was dried in vacuum over potassium hydroxide and calcium chloride. The adsorption spectrum of the xanthophyll in ethanol solution was determined by means of a Beckman spectrophotometer. The absorption curve had sharp maxima at 447 and 476 mμ and was qualitatively identical to the curve for lutein reported by Zscheile and associates.[11] The specific absorption coefficients of the xanthophyll at 447 and 476 mμ were 231 and 204, respectively. These and all other specific absorption coefficients throughout the spectrum were approximately 90% of the values found by Zscheile, indicating that the crystals were 90% pure lutein. It is probable that the crystalline material contained solvent, since it was not heated in drying. According to Zscheile,[11] lutein crystallized from carbon disulphide-ethanol retained approximately 10% of solvent, and heating at 84°C for 4 hours under high vacuum was necessary to remove the solvent.

Tests of the material by di-phasic fractionation between 90% methanol and petroleum ether showed that no carotene was present. When a *n*-hexane solution of the crystals was chromatographed through a column of alumina, there was no indication of carotene or

[6] Mead, T. H., Underhill, S. W. F., and Coward, K. H., *Biochem. J.*, 1939, **33**, 589.

[7] Baxter, J. G., and Robeson, C. D., *J. Am. Chem. Soc.*, 1942, **64**, 2407.

[8] Baxter, J. G., and Robeson, C. D., *J. Am. Chem. Soc.*, 1942, **64**, 2411.

[9] Salmon, W. D., *J. Nutrition*, 1947, **33**, 155.

[10] Strain, H. H., Monograph, Carnegie Inst. of Wash., 1938.

[11] Zscheile, F. P., White, J. W., Jr., Beadle, B. W., and Roach, J. R., *Plant Physiol.*, 1942, **17**, 331.

TABLE I.
Responses of Vitamin A-Deficient Rats.

Daily supplements*	No. of rats	Avg wt gain at end of 3rd week g	Avg wt gain at end of 7th week g	Mortality %	Avg time to death,† days
2 μg carotene	14	22	33	14.3	74
2 " " + 5 μg xanthophyll	6	25	57	0	—
2 " " + 1 mg α-tocopherol	5	46	93	0	—
2 " " + 5 μg xanthophyll + 1 mg α-tocopherol	5	40	90	0	—
1 μg vit. A alcohol	19	—22	—50‡	100.0	25
1 " " " " + 5 μg xanthophyll	12	—17	—12	75.0	31
1 " " " " + 1 mg α-tocopherol	5	26	48	0	—
1.15 μg vit. A acetate	7	3	1	43.0	35
1.15 " " " " + 5 μg xanthophyll	7	5	29	14.3	37

* All rats received 0.2 ml of fresh lard with the above supplements.
† Only animals dying were included in the averages.
‡ This value represents the average weight gain at the end of the 5th week, beyond which no animal survived.

cryptoxanthin impurities. Nearly all of the pigment was contained in a single distinct band (lutein). A very small quantity of the pigment was strongly adsorbed to the upper surface of the alumina. This was probably a trace of other xanthophylls or oxidation products of lutein. The xanthophyll was dissolved in acetone for feeding in sufficient concentration so that the daily dose of 5 μg was contained in 0.2 ml of solution.

Crystalline vitamin A alcohol and vitamin A acetate (Distillation Products), pure β-carotene (prepared in this laboratory)[12] and α-tocopherol (Merck) were dissolved in a sufficient volume of *n*-hexane, so that the daily supplement of 1.0 μg of vitamin A alcohol, 1.15 μg of vitamin A acetate or 2.0 μg of carotene was continued in about 0.2 ml.

The supplements were fed each morning by pipetting the requisite amounts on a small amount of basal diet. After the solvents had completely evaporated, the fresh lard was added by pipette and the supplement jars were placed in the cages. The main portion of the basal diet was fed in the afternoon. Supplements were stored at 1°C. The carotene and vitamin A solutions were analyzed at weekly intervals in order to make any necessary changes in aliquots for feeding.

Results. The results of the feeding tests with rats are given in Table I. A marked superiority of 2 μg carotene over 1 μg vitamin A alcohol or 1.15 μg vitamin A acetate is evident under the conditions of this experiment. Although optimum growth was not obtained with 2 μg of carotene alone, the animals grew slowly and only 2 rats died during the 7-week test period. With 1 μg vitamin A alcohol all animals lost weight rapidly and died in an average of 25 days. Vitamin A acetate gave results intermediate between carotene and vitamin A alcohol; the mortality rate was 43% and the average time to death was 35 days.

Xanthophyll definitely improved the growth of rats receiving carotene or vitamin A acetate and reduced the mortality rate to 0. Xanthophyll added to vitamin A alcohol reduced the mortality rate and increased the average time to death. Rats receiving xanthophyll and vitamin A alcohol did not grow, but the loss in weight was considerably less and slower than was obtained with vitamin A alone. Although the growth stimulation obtained with xanthophyll was not as great as was obtained with α-tocopherol, only 5 μg of xanthophyll was fed as compared with 1 mg of α-tocopherol.

Discussion. The most logical explanation of the gastro-intestinal destruction of carotene and vitamin A in the absence of intestinal antioxidants is that the destruction is enzymatic in nature as is indicated by the results of Hove.[5] Nothing is known of the

[12] Sherman, W. C., and Koehn, C. J., *Ind. and Eng. Chem.*, in press.

mechanism of the reaction or of the specificity of the enzyme, except that the presence of unsaturated fat acids is apparently required. The results reported in the present paper show that vitamin A alcohol is destroyed to a considerably greater extent than vitamin A acetate. The work of Hickman,[13] showing that vitamin A esters are more stable than free vitamin A when fish oils are aerated, is of interest in connection with these results. The effect of xanthophyll can probably be explained on the basis of the nonspecificity of the enzyme that results in the gastro-intestinal destruction of considerable quantities of the ingested xanthophyll, thereby sparing corresponding amounts of vitamin A or carotene. It is possible that, with certain human dietaries and animal feeds supplying vitamins A and E in suboptimum quantity, the protective effect of xanthophyll may be of practical significance.

Summary. Vitamin A-deficient rats receiving a daily supplement of 1 μg of vitamin A alcohol with fresh lard in the absence of added α-tocopherol lost weight rapidly and 100% of the animals died. Carotene fed under the same conditions at a level of 2 μg/rat/day produced fair growth and a mortality rate of only 14.3%. Intermediate results were obtained with vitamin A acetate. The addition of xanthophyll apparently decreased the gastro-intestinal destruction of carotene, vitamin A alcohol, and vitamin A acetate and, thus, enhanced the response to the 3 vitamin A sources at the levels fed.

[13] Hickman, K. C. D., *Ind. and Eng. Chem.*, 1937, **29**, 1107.

15912

Use of Dubos Medium for Culture of *M. tuberculosis* from Sputum.

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The liquid culture medium described by Dubos[1] can initiate rapid growth of *M. tuberculosis* from small inocula and maintain this growth in diffuse form. These media may offer, therefore, a great advantage for detection of *M. tuberculosis* in pathological material. Dubos tried his culture method on sputum (liquefied by sodium hydroxide), but concluded that "there is no indication that in its present form, the medium can be utilized with advantage for diagnostic work." At about the same time, Foley[2] reported successful culture of *M. tuberculosis* on Dubos medium from 165 pathological specimens (among them 31 sputa); thus demonstrating the suitability of the new medium for routine diagnosis. However, Foley emphasized the handicaps of some routine culture methods as applied in the use of Dubos media; "the digestion even for as short a period as 15 minutes with hydrochloric acid is liable to sterilize a specimen which contains only a few bacilli." "Little advantage results from the use of sulfadiazine to eliminate contaminating organisms in the specimens inoculated without digestion." It should be remembered that other authors, for instance Blacklock,[3] have studied the sterilizing effect of the alkaline digestion of sputa. Thus, the "digestion" (concentration) and the considerable elimination of contaminating bacteria without damage for acid-fast organisms, seem to be the essential condition for successful use of Dubos media in routine diagnosis.

Experimental. We have found in our pre-

[1] Dubos, R. J., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 361; Dubos, R. J., *J. Exp. Med.*, 1946, **83**, 409.

[2] Foley, G. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 298.

[3] Blacklock, J. W. C., *Med. Res. Council Rep.*, 1932, p. 5.