

unusual. The incorporation of unsaturated fatty acids into the phospholipids of tumor cells may aid ordinary respiration as opposed to glycolysis, thus inhibiting tumor growth.

The iodine numbers of the phospholipid fatty acids of this tumor are low compared with those of muscle on the same diets. Sinclair⁵ found for muscle: fat-poor diet—101; coconut oil diet—124; and cod liver oil diet—160; with menhaden oil probably in the range of cod liver oil values. There are several plausible explanations for these low iodine numbers. In the first place, the long chain unsaturated fatty acids of the diet may not enter the tumor which, being a carcino-sarcoma, consists of epithelium and connective tissue. Possibly connective tissue does not take up the unsaturated acids of the diet. A study of a pure carcinoma and sarcoma, comparing effects of these diets on similar rat tissues may settle this point.

In the second place, the ratio of saturated to unsaturated fatty acids in the tumor may be different from that in muscle. Determinations of amounts of solid and liquid fatty acids using the lead soap method, will clarify this point.

In the third place, the unsaturated fatty acids of the diet may enter the tumor phospholipids but be immediately reduced or saturated. Boyland¹² and Harris¹³ have shown that tumors contain some as yet unidentified reducing substance in addition to glutathione and ascorbic acid. It is not known whether this substance reduces unsaturated fatty acids.

7839 C

Reaction to Differentiate Vitamin A from Carotene by Means of Antimony Trichloride.*

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The Carr-Price reaction for vitamin A and carotene consists in the development in chloroform solution of a characteristic blue color

¹² Boyland, E., *Biochem. J.*, 1933, **27**, 802.

¹³ Harris, L. J., *Nature*, 1933, **132**, 605.

* The carotene used in our work was obtained from the S.M.A. Corporation of Cleveland, Ohio, and consists largely of β -carotene with small amounts of α -carotene.

with antimony trichloride.¹ Recently Rosenthal and Erdelyi² have described a test in which antimony trichloride in the presence of pyrocatechin serves to distinguish vitamin A from carotene. A blue color is formed at room temperature in a chloroform mixture made up to a total volume of 5 cc., containing a solution of an oil rich in vitamin A, 2 cc. of antimony trichloride reagent, and 1 cc. of 0.5% pyrocatechin in chloroform. Immediately after mixing, the mixture is transferred to a water bath and maintained at a temperature of 60°C. for 1 to 2 minutes. During the treatment with heat the original blue color changes to a violet-red. These investigators report the violet-red color to be more stable than the original blue of the Carr-Price reaction. Under similar treatment a reaction mixture containing carotene yields a blue color which does not change on heating. Recently Rosenthal and Erdelyi³ have reported that in addition to pyrocatechin, other polyphenols or their derivatives, such as hydroquinone, veratrole, and guaiacol, may be employed.

To make the studies of the Rosenthal-Erdelyi reaction, we made use of halibut liver oil assayed to contain 50,000 U.S.P. units of vitamin A per gram. A solution in chloroform was made of such a concentration that 1 cc. was equivalent to 100 units of the vitamin. Carotene solutions in chloroform were made up fresh in concentrations of 0.3 mg. of the pigment per cc. Antimony trichloride reagent was prepared by washing a new sample of antimony trichloride with chloroform and adding 30 gm. of the washed compound to 100 cc. of the chloroform. Two solutions of 0.5% pyrocatechin in chloroform were used. One was a freshly made solution, and the other prepared and aged for 21 days in an ordinary clear glass-stoppered bottle exposed to daylight but not to direct sunlight. The chloroform used for all solutions and employed in all manipulations was the U.S.P. grade containing less than 1% of ethyl alcohol.

Our studies indicate that the use of antimony trichloride and heat is all that is needed to carry out a differential test for vitamin A and carotene. We recommend that 2 cc. of the antimony trichloride reagent be added to about 3 cc. of a chloroform solution of the vitamin A-rich material, or to a solution of carotene. The reaction mixture should be allowed to stand for one minute to permit the blue color to develop to its full intensity before heating at 60°C.

¹ Carr, F., and Price, E. A., *Biochem. J.*, 1926, **20**, 497.

² Rosenthal, E., and Erdelyi, J., *Biochem. Z.*, 1933, **267**, 119; *Biochem. J.*, 1934, **28**, 41.

³ Rosenthal, E., and Erdelyi, J., *Biochem. Z.*, 1934, **271**, 414.

on the water bath for 2 minutes. As a result of heating the blue color at first formed in the reaction between vitamin A and the antimony trichloride changed to pink, rose, red tinted with violet, or a deep wine-red depending upon the quantity of vitamin A present. A rose color developed with 0.03 cc. to 0.1 cc. of a chloroform solution of halibut liver oil (3 to 10 units of vitamin A).

TABLE I.
Antimony Trichloride and Halibut Liver Oil.

Halibut liver oil solution—1 cc. is equivalent to 100 U.S.P. units of Vitamin A	Chloroform	0.5% Pyrocatechin, fresh	0.5% Pyrocatechin, old	Antimony trichloride solution	Color before heating	Color after heating	Color on standing 30 min. after heating
cc.	cc.	cc.	cc.	cc.			
0.00	2.00	1	0	2	none	none	none
0.00	2.00	0	1	2	"	"	"
0.03	1.97	1	0	2	faint blue tint	"	"
0.03	1.97	0	1	2	none	"	"
0.03	2.97	0	0	2	faint blue tint	faint pink tint	faint pink tint
0.10	1.90	1	0	2	blue	pink	pink tint
0.10	1.90	0	1	2	faint blue	none	none
0.10	2.90	0	0	2	blue	rose	rose
1.00	1.00	1	0	2	"	red	"
1.00	1.00	0	1	2	"	rose tint	pink
1.00	2.00	0	0	2	"	wine-red	wine-red

A red-violet color formed with 0.5 cc. of a chloroform solution of halibut liver oil, an amount equivalent to 50 units of vitamin A. A wine-red color appeared with 1 cc. of a solution of the oil (100 units of vitamin A). In the absence of pyrocatechin the color developed contained more red and less violet.

Carotene yields a blue color with antimony trichloride. The blue color persists, however, after heating. A carotene solution containing 0.3 mg. of the pigment gave a greenish blue tint changing immediately to blue and remaining unaltered on the application of heat. The interaction of carotene and antimony trichloride yields the full blue color more rapidly in the absence of pyrocatechin.

Our work indicates that the presence of pyrocatechin, as suggested by Rosenthal and Erdelyi, is unnecessary. In fact, pyrocatechin was found to inhibit the development of the red color with vitamin A during the treatment with heat, and also to inhibit the formation of the blue color with carotene at room temperature. We observed that the intensity of the red color obtained in a heated

reaction mixture containing antimony trichloride, pyrocatechin and halibut liver oil was less than the intensity of the color obtained in a similar mixture with the pyrocatechin omitted. The difference in color intensity was noted over a wide range of vitamin A content, from 10 to 200 units. Rosenthal and Erdelyi³ have also recorded results which indicate that phenols other than pyrocatechin exert an inhibitory effect. They reported a scarcely discernible blue color when antimony trichloride reacted with a solution containing 0.001 mg. of vitamin A per cc., but upon addition of guaiacol and the application of heat the color disappeared. We have been able to obtain a blue color, which changed to rose on heating in the presence of a small quantity of halibut liver oil equivalent to 3 units of vitamin A. A similar quantity of halibut liver oil treated with antimony trichloride and pyrocatechin gave a blue color in the cold, but no pink or rose on heating.

The aged pyrocatechin solution exerted an even greater inhibitory effect than the fresh chloroform solution of the phenol. With 0.03 cc. of the halibut liver oil solution (equivalent to 3 units of vitamin A), 1 cc. of the fresh pyrocatechin solution and 2 cc. of the antimony trichloride solution, a faint blue tint developed at room temperature. When this mixture was heated the blue tint disappeared without further development of color. With a mixture using the same quantity of vitamin and 1 cc. of the old pyrocatechin solution, no color appeared before or after heating. With 0.4 cc. of halibut liver oil solution equivalent to 40 units of vitamin A, a blue color formed in the presence of 1 cc. of old pyrocatechin solution. The color disappeared on heating without the subsequent formation of a red-violet color. In a control experiment in which fresh pyrocatechin was used the blue color appeared before heating, and the red-violet color after heating.

Cod liver oil gave similar responses to haliver oil. Almond oil, olive oil, linseed oil and castor oil gave atypical reactions.

Summary. Antimony trichloride may be used effectively as a reagent to differentiate vitamin A from carotene. In chloroform solution antimony trichloride and carotene interact with the formation of a blue color, which persists after heating on the water-bath at 60°C. Under similar conditions antimony trichloride and vitamin A-bearing oils develop at room temperature a blue color. On the application of heat the blue color changes to rose, violet-red or wine-red, depending upon the concentration of the vitamin.

Pyrocatechin, which Rosenthal and Erdelyi employed along with the antimony trichloride reagent to differentiate vitamin A from

carotene, is not only needless, but it actually inhibits the formation of the blue color with carotene and of the blue color with oils rich in vitamin A at room temperature and of the rose or violet-red after heating. Aged solutions of pyrocatechol exert even greater inhibitory powers than fresh solutions.

7840 P

Cultivation of the Johne's Bacillus in a Synthetic Medium.*

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Twort and Ingram¹ succeeded in isolating the causative organism from the intestine of a cow which had died of Johne's disease, by using a special medium containing the dead cells of tubercle bacilli. They later found that other acid-fast organisms would give the same stimulation, the timothy grass bacillus, *Mycobacterium phlei*, giving especially good results. Since then the cells, various extracts of the cells, or the products of growth of *M. phlei* have been incorporated in the media used to cultivate the Johne's bacillus. The nature of the "essential substance", which may be chemical or physico-chemical has, however, remained obscure.

Johne's disease (paratuberculosis) is a chronic enteritis affecting the cow and the sheep and caused by the multiplication of the Johne's bacillus in the intestinal mucosa, the impairment of the function of which results in the emaciation of the animal. The disease is usually fatal. The disease occurs in isolated herds in the United States but is not yet of serious economic importance. In France, however, the disease has spread within the last 20 years from 2 to 41 departments of the country according to Rinjard.² It would seem, therefore, that the United States would profit by combating the disease before it becomes more widely distributed. In the eradication we are hampered by the lack of a sufficiently good diagnostic agent. A "Johnin" prepared like old tuberculin neces-

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¹ Twort, F. W., and Ingram, G. L. Y., A Monograph on Johne's Disease. Baillière, Tindall and Cox, London, 1913.

² Rinjard, M. P., *La paratuberculose bovine en France*. Office International des Epizooties R 51, 1934.