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Differential Reactions Between Carotene and Oils Rich in Vitamin A.

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It has recently been demonstrated that carotene* gives characteristic color reactions with trichloracetic acid,[1] with chloral hydrate[1] and with a reagent containing a mixture of sulphuric acid and formaldehyde.[2] Tested on the rat, carotene in a biologic sense behaves in a manner similar to vitamin A. From a chemical standpoint, carotene shows a striking resemblance to vitamin A. One molecule of vitamin A is equivalent to half a molecule of carotene. Carotene and vitamin A both yield a characteristic blue color with antimony trichloride. The blue chloroform reaction mixture obtained with carotene in the presence of antimony trichloride yields, however, an absorption band at 590 mu, while the reaction mixture with vitamin A yields a wider absorption band at 610-630 mu.

Recently Rosenthal and Erdelyi[3] have demonstrated the possibility of distinguishing between carotene and vitamin A by applying the antimony trichloride test in the presence of pyrocatechol. They observed that when oil rich in vitamin A or vitamin A concentrate is dissolved in absolute chloroform and heated with antimony trichloride reagent and 0.5% pyrocatechol in chloroform solution, the blue changes to a purple or violet-red color comparable with the one characteristic of an aqueous solution of potassium permanganate. More recently Rosenthal and Erdelyi[4] have demonstrated that in addition to pyrocatechol, hydroquinone, quaiacol and veratrole may be employed. Andersen and Levine[5] have studied their procedure and report positive tests with antimony trichloride with vitamin A-

* The carotene used was obtained from the S.M.A. Corporation, Cleveland, and consisted of a mixture containing a very large quantity of β-carotene and a small quantity of α-carotene.

[1] Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 335.

[2] Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 335.

[3] Rosenthal, E., and Erdelyi, *J. Biochem. Z.*, 1933, **267**, 191.

[4] Rosenthal, E., and Erdelyi, *J. Biochem. Z.*, 1934, **271**, 414.

[5] Andersen, A. C., and Levine, V. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 737.

rich oils in the absence of pyrocatechol or any other phenol. They observed that pyrocatechol is even detrimental, since it inhibits the reaction and diminishes its sensitivity.

Using carotene, and haliver oil and cod liver oil as sources of vitamin A, we have compared the action of formaldehyde-sulphuric acid reagent, the trichloracetic acid reagent and the chloral hydrate reagent. Since the above reagents also react with sterols, we have included in Table I the results obtained with ergosterol and with cholesterol.

TABLE I.

Reagent	Carotene	Haliver Oil	Ergosterol	Cholesterol
Formaldehyde Sulphuric Acid	purple ring	bright red in acid layer, blue to purple in chloroform layer	red layer above, with acid layer below showing green fluorescence	cherry pink above, with green fluorescence in acid layer below
Trichloracetic Acid	blue	brilliant blue, changing to purple on heating	red changing to blue, the blue unaffected by heat	no color, pink on heating
Chloral Hydrate	blue	brilliant blue, changing to purple	red to greenish blue to final blue, the blue unaffected by heat	slight pink changing to red

The trichloracetic acid reagent contains 9 parts by weight of crystallized acid and 1 part of water. Three drops of this reagent were mixed with 1 cc. of a chloroform solution. The chloral hydrate (0.5 gm.) was liquefied on the water bath and 1 to 2 drops of a chloroform solution of carotene or oil added, together with a drop of concentrated hydrochloric acid. The formaldehyde-sulphuric acid was made fresh by the addition of one volume of 37 to 40% pure formaldehyde to 50 volumes of concentrated sulphuric acid. Equal volumes of chloroform solution of carotene or oil were used in order to carry out the test.

It can readily be seen that trichloracetic as well as chloral hydrate serve to differentiate carotene, vitamin A-rich haliver oil, ergosterol and cholesterol. The more or less permanent blue color, characteristic of carotene treated with chloral hydrate or trichloracetic acid reagent is changed to an evanescent blue and a more stable purple when heat is applied to a mixture containing vitamin A.

The characteristic tests are obtained with 0.001 mg. of haliver oil representing 0.05 units of vitamin A. Ergosterol may be differen-

tiated from carotene and vitamin A because of the fact that an initial red appears in the reaction mixture and a final blue. Cholesterol reacts but slowly with trichloracetic acid, forming no color at room temperature, but a slight pink on heating. With chloral hydrate heat is applied in order to liquefy the reagent, and the reaction in the presence of cholesterol leads to the development of a slight pink to red color. The formaldehyde-sulphuric acid reagent differentiates carotene from vitamin A, and carotene and vitamin A from cholesterol and ergosterol, but not ergosterol from cholesterol.

The trichloracetic acid reagent and the chloral hydrate reagent have been tested out with chloroform solutions of cod liver oil, butter, wheat germ oil, olive oil, cotton seed oil, linseed oil and sesame oil. The results are shown in Table II.

TABLE II.

Oil Tested	Reagent		
	Trichloracetic Acid		Chloral
	In the cold	On heating	
Cod liver oil	purple	purple	purple
Butter	light purple	light purple	light purple
Wheat germ oil	light gray	gray with definite purple tinge	brown
Olive oil	no coloration	no coloration	muddy orange tinge
Cottonseed oil	light orange	muddy green	brown
Almond oil	blue on standing	blue changing to pronounced green	light brown
Linseed oil	muddy brown	bluish black to black with slight purplish tinge	brown
Sesame oil	salmon pink	reddish brown	brown

Cod liver oil and butter yield an immediate purple when treated with trichloracetic acid or with chloral hydrate. The reaction is much stronger for cod liver oil than for butter. Wheat germ and linseed oil give evidence of a purple tinge on heating with trichloracetic acid, but no indication of the presence of any vitamin A with the chloral hydrate reagent. Wheat germ is known to contain traces of vitamin A.

Summary. Carotene (provitamin A) can be differentiated from vitamin A-containing oils by means of several chemical reagents. Antimony trichloride, trichloracetic acid and chloral hydrate each yield with carotene and with halibut liver oil a characteristic blue color. The blue color persists when the reaction mixture containing carotene is heated on the water bath. Heat, however, transforms the color of the reaction mixture containing halibut liver oil from blue to purple. With cod liver oil and with butter fat the trichlor-

acetic acid reagent or the chloral hydrate reagent yields without the aid of heat an immediate purple.

A reagent containing sulphuric acid and formaldehyde also serves to differentiate carotene from vitamin A-bearing oils. With carotene a purple zone is formed; with halibut liver oil a bright red is developed in the acid layer and a blue to purple in the chloroform layer.

Trichloracetic acid and chloral hydrate also serve as reagents to distinguish between carotene, vitamin A-rich oils, ergosterol and cholesterol. The formaldehyde-sulphuric acid reagent differentiates from one another carotene, vitamin A and the sterols, cholesterol or ergosterol, but does not distinguish ergosterol from cholesterol.

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Intestinal Motor Inhibition by Parasympathetic Drugs.

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It was noted by Bernheim[1] that relaxation usually occurred when pilocarpine was added to a strip of guinea pig intestine contracted by histamine. This was confirmed[2] and several instances were noted in which abrupt relaxation occurred when pilocarpine was added to a strip of guinea pig intestine contracted by physostigmine. We found also that in most instances when the intestine was tonically contracted by pilocarpine, it was relaxed by physostigmine. This result was obtained in 4 of 7 trials using the duodenum and jejunum. The investigation has been extended to other parasympathetic drugs using the same method, *viz.*, suspension of the strip in Ringer's solution.

On adding pilocarpine to the bath containing an intestinal strip contracted by acetyl choline, we obtained relaxation of the duodenum, jejunum, ileum, proximal, mesial, and distal colon. In only 3 of the 66 trials was a motor effect produced by the subsequent addition of the second parasympathetic drug (pilocarpine). This effect is only occasionally reversible, that is, acetyl choline relaxes the intestine contracted by pilocarpine only in a minority of the trials. In each intestinal strip tested acetyl choline caused a con-

[1] Bernheim, Frederick, *J. Pharm. and Exp. Ther.*, 1931, **43**, 509.

[2] Craven, Jean D., and McCrea, F. D., *J. Pharm. and Exp. Ther.*, 1934, **51**, 421.