ANTIRICKETIC SUBSTANCES.

III. THE CATALYTIC FORMATION OF AN ANTIRICKETIC CHOLESTEROL DERIVATIVE.

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It has been demonstrated by Hess, Weinstock, and Helman (1), Rosenheim and Webster (2), and Steenbock and Black (3) that under the influence of ultra-violet rays cholesterol is transformed into an antiricketic modification. In a previous communication (4) I have shown by a reaction with *n*-butyl nitrite that in one respect the activated cholesterol behaves chemically the same as the antiricketic vitamin.

Now the researches of Windaus (5), Dorée and Gardner (6), Schrötter and Weitzenböck (7), and Mauthner (8) would indicate that cholesterol is an olefinic terpene. If such is the case it is not strange that the molecular structure of cholesterol should be altered by physical agents, light among others. To illustrate: The olefinic structure in cholesterol appears to be a vinyl radical, and Klatte and Rollett (9) observed that vinyl compounds polymerize under ultra-violet rays. Moreover, the terpene structure appears to be related to pinene, which according to Urbain and Scal (10) is extensively altered by a metallic arc.

The light-sensitive groups in cholesterol—the olefine and terpene groups—are subject also to polymerization by the catalytic action of floridin.[1] Gurvich[2] (11), Venable (12), and Lebedev and Filonenko (13) studied the action of floridin on pinene and other ter-

[1] A fuller's earth from northern Florida possessing marked catalytic properties.

[2] I am indebted to Dr. L. J. Pessin of the Johns Hopkins University for translating the work of Gurvich from the Russian.

penes, and on amylene and other olefines. In brief, they found the effect to consist of polymerization, followed by degradation of the polymers. Of particular significance is the observation of Kawai and Kobayashi (14) that cholesterol when fused with "Japanese acid clay" yields fluorescent, petroleum-like hydrocarbons.

Following the analogy suggested by the above work, I investigated the action of floridin on cholesterol. The catalyst was obtained as a yellowish white, "200 mesh" powder from the Jamieson, Florida, mine of the Floridin Company. It contained 19.1 per cent of water expellable at 880°.

Some floridin was activated by heating until the moisture content was reduced to 8.9 per cent. When cholesterol was fused with this product the ensuing reaction was uncontrollable, and I therefore proceeded to experiment with cholesterol dissolved in various solvents. 2.50 gm. of the activated floridin were added to 0.50 gm. of cholesterol dissolved in 15 ml. of xylene. The mixture was refluxed for 2 hours, a Kjeldahl flask being used to minimize the effects of violent bumping.

Almost immediately the suspension darkened in color. In 5 minutes it was deep purple; in 15 minutes, blue; and after 30 minutes it remained a dark greenish blue. The suspension after 2 hours was filtered. The filtrate was yellow with a blue fluorescence. The floridin residue was washed repeatedly with xylene and with benzene, but no pigment passed into solution except a little of the yellow substance which appeared to have been firmly adsorbed on the floridin. However, it was found that ethyl ether or acetone would release the pigment and restore the floridin to its normal color. The completely extracted pigment was not greenish blue, but a deep orange color! Evidently the greenish blue appearance was a physical effect, associated with the adsorption of the pigment in the pores of the floridin.

The combined xylene and benzene filtrates and ether extracts were evaporated to dryness by gentle warming. There remained an odorous, orange resin. That the resin certainly is a product of the action of floridin on cholesterol was demonstrated by refluxing xylene with floridin but without cholesterol, and with cholesterol without floridin; there was no reaction in either case.

Inasmuch as the boiling point of xylene is about 139°, I decided

to investigate the action in solvents which can be refluxed at lower temperatures. 2.50 gm. of activated floridin, 0.50 gm. of cholesterol, and 15 ml. of toluene (b.p. 110°) were refluxed 2 hours as before. The color changes in the floridin suspension occurred a little more slowly than with xylene, and at the end of 2 hours the color was a deep blue. The toluene filtrate was yellow with a blue fluorescence. The ether extract upon evaporation left an odorous, orange resin.

A similar preparation was then made with benzene (b.p. 80°). The color changes in the floridin suspension were decidedly slower than with toluene, so that after 2 hours only the purple stage had been reached. The benzene filtrate was pale yellow and slightly fluorescent. The extracted resin possessed little odor, and was lighter in color than the product from toluene.

A similar preparation was made with carbon tetrachloride (b.p. 76°). The color changes in the floridin suspension were even slower than with benzene, although in 2 hours the purple stage was reached. However, the carbon tetrachloride filtrate and the ether extracts were somewhat more colored than those with benzene. The fluorescence was nil, and the resin after evaporation was odorless.

From the foregoing experiments one might assume that the rate of catalytic alteration of cholesterol dissolved in various solvents depends upon the temperature at which the solvent is refluxed. However, this is not true in all cases. There is a remarkable specificity in the behavior of solvents, so that in certain solvents there is no catalysis, even though the boiling point be not particularly low. 0.50 gm. of cholesterol were dissolved in 15 ml. of each of the following solvents: ethyl acetate (b.p. 77°), absolute ethyl alcohol (b.p. 78°), *n*-propyl alcohol (b.p. 97°), and isobutyl alcohol (b.p. 107°). To each solution 2.50 gm. of activated floridin were added, and the suspensions refluxed for 2 hours as before. In no case was there any color change, and from the filtrates crystals having the melting point of unaltered cholesterol were obtained.

An investigation of the chemical changes given by floridin and other catalysts with cholesterol will be reported later, but for the present it will suffice to describe some biological tests with one of the reaction mixtures. To this end a cholesterol-carbon tetrachloride-floridin preparation was made under carefully regulated

conditions. A second portion of floridin was activated by heating for 2 hours at 280° ± 5°. The moisture content was reduced to 4.7 per cent, as compared with 8.9 per cent in the first activation. Incidentally, the second sample was much the more active, for it gave the series of color changes almost three times as rapidly. The cholesterol was a specially purified product, the preparation of which I have described elsewhere (4).

1.25 gm. of cholesterol dissolved in 37.5 ml. of carbon tetrachloride were refluxed for 5 hours with 6.25 gm. of activated floridin. The color changes in the floridin suspension (indicative of the rate and extent of reaction) were: 5 minutes, noticeable darkening; 15 minutes, old rose; 45 minutes, reddish purple; 2 hours, bluish purple; 3 hours, purplish black; 5 hours, black. The suspension was filtered, and the floridin residue extracted with ether and acetone until no more coloring passed into solution. The combined filtrate and extracts exhibited a deep orange color with a slight fluorescence.

In testing antiricketic concentrates or irradiated cholesterol, I have lately been adding to these preparations small amounts of Newfoundland seal oil which serves the purpose of dissolving and protecting the antiricketic material and assisting its trituration with the diet. 5 gm. of seal oil were added to the combined filtrate and extracts, and the mixture was gently evaporated onto a sufficient quantity of McCollum's Diet 3143 to make 250 gm. of a modified ration containing 0.5 per cent of catalyzed cholesterol and 2 per cent of seal oil. The diet was administered to five rickety rats for 5 days. The Shipley line test was performed on the tibias and it showed advanced healing of the ricketic lesions (Table I).

Two control preparations were made. In the first, 1.25 gm. of cholesterol were refluxed 5 hours with 37.5 ml. of carbon tetrachloride without any floridin. There was no color change. 5 gm. of seal oil were added and the mixture evaporated onto Diet 3143 as before. No healing was induced, which shows that the antiricketic substance is not produced in the absence of floridin. In the second control 6.25 gm. of activated floridin were refluxed for 5 hours with 37.5 ml. of carbon tetrachloride without any cholesterol. There was no color change. The floridin was filtered and extracted, the seal oil was added, and the modified diet ad-

ministered as before. No healing was induced, which shows that the antiricketic substance is not produced in the absence of cholesterol.

TABLE I.

[For convenience in tabulation, the quality of healing observed by the line test is here expressed on a scale of 4 degrees. One plus sign (+) indicates a just perceptible healing; two plus signs (++), a distinct healing; three plus signs (+++), an advanced healing; and four plus signs (++++), a practically complete healing. The reader should realize that these values bear no numerical relation to each other.

Rat No.	Sex.	Preparation administered.	Grade of test.	Weight.	Average daily consumption.
				gm.	*gm.*
1677	M.	Diet 3143 control.	—	94–98	8.4
1678	F.	" 3143 "	—	72–75	6.8
1679	"	" 3143 "	—	70–72	6.8
1680	M.	" 3143 "	—(?)	92–93	7.4
1681	F.	" 3143 "	—	69–71	7.6
1682	M.	Catalyzed cholesterol ½ per cent plus seal oil 2 per cent.	+++	81–87	8.2
1683	"	" " "	++	73–74	6.4
1684	F.	" " "	+++	77–83	7.2
1685	M.	" " "	+++	72–74	6.6
1686	"	" " "	+++	78–81	8.0
1628	F.	Cholesterol ½ per cent refluxed without floridin, plus seal oil 2 per cent.	—	68–75	7.8
1612	M.	" " "	—	83–83	7.0
1611	F.	" " "	—	70–72	5.8
1610	"	" " "	—	78–82	7.6
1609	"	" " "	—	75–77	6.6
1613	F.	Floridin refluxed without cholesterol, extracted, plus seal oil 2 per cent.	—	70–72	7.2
1614	"	" " "	—	84–84	8.6
1615	"	" " "	—	69–69	7.0
1617	M.	" " "	—	72–72	7.0
1651	F.	" " "	—	77–77	8.6

The foregoing experiments should not be misinterpreted. They do not prove that the antiricketic substance present among the

products of the catalytic alteration of cholesterol is identical with the antiricketic vitamin, or even with the antiricketic cholesterol modification produced by irradiation. Either of these possibilities may be true, but it is equally possible that a new antiricketic derivative was formed, or that the healing resulted from a systemic disturbance following mild poisoning by one of the evidently numerous reaction products.

SUMMARY.

1. When a solution of cholesterol in xylene, toluene, benzene, or carbon tetrachloride is refluxed with activated floridin a catalytic action takes place, resulting in the formation of fluorescent and resinous products.

2. There is a specificity among solvents in accordance with which no catalysis occurs in ethyl acetate, ethyl alcohol, *n*-propyl alcohol, or isobutyl alcohol.

3. An antiricketic substance of unknown nature was demonstrated among the products of the action of floridin on cholesterol in carbon tetrachloride.

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