In an effort to throw more light on the question under consideration, an allergic state was induced in rabbits by 5 or 10 successive daily intravenous injections of 2 cc. of 2% casein in 0.1% of sodium carbonate. It was found that after 5 to 10 casein injections 10 out of 15 animals showed a definite, 5 a less definite, *hyperergic skin reaction* to injection of casein. No morphologically detectable effects of the casein treatment, especially on the endocardium, were found in controls with the usual histological methods. On intravenous injection of 1 to 2 cc. of an 18-hour *Staphylococcus aureus* culture 5 out of 7 animals developed a definite endocarditis.

We feel justified to draw the inference that bacterial localization on the endocardium—in our experience—is due to an allergic state of the endocardium, *i. e.*, an altered capacity to react.

It is also to be inferred that a relationship between that allergic state and the subsequent infection in the sense of species-specificity is not a requirement for bacterial localization on the endocardium, and that furthermore, that allergic state may be induced by a non-bacterial foreign protein-matter. The immun-biological definition of that allergic state remains a matter of further study.

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Adaptation of the Bloor Oxidation Procedure to the Determination of Small Quantities of Cholesterol as Digitonid.

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Micro methods for the estimation of cholesterol have hitherto been based almost entirely upon the color reaction with acetic anhydrid and concentrated sulphuric acid. The presence of interfering color producing substances in blood and tissue extracts frequently makes it highly problematical whether or not this reaction gives a true picture of the cholesterol concentration. The gravimetric estimation as the digitonid, proposed by Windaus[1] is impracticable where the amounts to be weighed are as small as those available in the average blood analysis.

We have, therefore, attempted to adapt the digitonin precipitation

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[1] Windaus, *Z. f. Physiol. Chem.*, 1910, lxv, 110.

to micro determination by oxidation of the digitonid according to the method used by Bloor[2] for fatty acids and phospholipins; *i. e.*, with silver-chromate sulfuric acid (Nicloux reagent) and normal $K_2Cr_2O_7$. Since this oxidation is not specific, the largest problem involved has been the separation of the digitonid from the excess digitonin used in precipitation, and from contaminating substances of a fatty nature. Digitonin is a saponin. Consequently, the surface tension of its water solutions is such as to make separation of the digitonid by centrifugalization impossible. Filtration of the digitonid from water solutions of digitonin has proved quantitative. However, the physical nature of the digitonid precipitate is such as to make quantitative removal of very small amounts from the filter and sides of the flask impracticable. We have, therefore, devised, with the assistance of Mr. D. J. Kooyman, a small Gooch filter tube made from a piece of Pyrex glass tubing 45 mm. long and 18 mm. in diameter, constricted somewhat at one end and fitted with a perforated porcelain disk (cut from the bottom of an ordinary Gooch crucible). This, after the filtration and washing is completed, may be placed bodily inside the flask used for the oxidation in such a way that the oxidizing solution may be made to come in contact with every part of the tube and the asbestos mat. The asbestos used for the filter must be prepared by long continued (72 to 120 hrs.) heating at 120-130° C. with bichromate-sulphuric acid which is frequently renewed. The asbestos is washed free of bichromate and suspended in distilled water in the usual way. This filter differs from that used by A. von Szent Györgyi[3] in that it makes possible complete oxidation of the digitonid by allowing control of time and temperature.

The procedure consists, briefly, of (a) precipitation of the digitonid by adding alcoholic digitonin solution to a measured amount of the cholesterol containing extract in the oxidation flask, (b) evaporation just to dryness, (c) removal of fatty substances by washing with anhydrous alcohol-free ethyl ether, pouring off the ether through the prepared filter tube described above, (d) removal of excess digitonin and water soluble impurities by washing repeatedly with hot and cold distilled water, filtering through the same tube into a fresh flask (to avoid back pressure of ether vapor), (e) transfer of the tube back to the original stoppered Pyrex Erlenmeyer, washing the mouth of the flask and the stirring rod with 1 cc. of concentrated H_2SO_4, (f) addition of the measured Nicloux reagent and

[2] Bloor, W. R., *J. Biol. Chem.*, 1928, lxxvii, 53.

[3] Györgyi, A. von Szent, *Biochem. Z.*, 1923, cxxxvi, 1123.

normal $K_2Cr_2O_7$, as described by Bloor. A control containing a filter tube, asbestos mat, the sulfuric acid and the oxidizing agents is prepared at this time, (g) heating to 90° C. for 1½ hours to 120° to 125° C. for 15 minutes, (h) dilution, addition of KI, and titration of the excess $K_2Cr_2O_7$ with thiosulfate.

The accuracy to be expected from the method may be judged from the following table. Theoretically, if the digitonid has the formula $C_{82}H_{140}O_{29}$, 1 mg. of cholesterol should require 10.62 cc. of N/10 $K_2Cr_2O_7$ for oxidation. If, however, the cholesterol is crystallized from alcohol and contains alcohol of crystallization, the value is reduced to 9.51 cc. N/10 $K_2Cr_2O_7$.

TABLE I.

Date	Cholesterol* taken	Cc. N/10 $K_2Cr_2O_7$ used per mg	Cholesterol Found		Deviation from theory	Variation from average	
			mg.	%	%	%	
Feb.							
12	1 mg.	10.30	0.969	96.9	—3.1	—0.3	water bath
		10.10	0.951	95.1	—4.9	+2.5	1 hour
13	1 mg.	10.36	0.976	97.6	—2.4	—1.0	
		10.32	0.972	97.2	—2.8	—0.6	
		10.22	0.962	96.2	—3.8	+0.4	
		10.32	0.972	97.2	—2.8	—0.6	
		10.27	0.967	96.7	—3.3	—0.1	
14	1 mg.	10.57	0.958	95.8	—4.2	+0.8	
		10.22	0.962	96.2	—3.8	+0.4	
15	1 mg.	10.22	0.962	96.2	—3.8	+0.4	
		10.27	0.967	96.7	—3.3	—0.1	1 hour
		10.13	0.954	95.4	—4.6	+1.2	20 min.
		10.04	0.946	94.6	—5.4	+2.0	
18	0.5 mg.	10.38	0.484	96.8	—3.2	—0.2	
		10.38	0.484	96.8	—3.2	—0.2	1 hour
		10.56	0.497	99.4	—0.6	—2.8	20 min.
		10.38	0.484	96.8	—3.2	—0.2	
				Averages	—3.4	0.76%	

* Cholesterol crystallized from petroleum ether.

It is our purpose to adapt the method to estimation of free cholesterol, "total cholesterol" and total unsaponifiable matter in blood and tissue.