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## Condensation Products of Lipoids and Chemo Immunity. I. Synthesis of Azo Derivatives of Cholesterol Aryl Esters.

A. O. TISCHER AND E. GEBAUER-FÜLNEGG. (Introduced by C. J. Farmer.)

*From the Departments of Biochemistry and Research Bacteriology, Northwestern University Medical School.*

It has been proposed by Forssman,[1] Meyer,[2] Wernicke and Sordelli,[3] Taniguchi,[4] Landsteiner,[5] and Landsteiner and Simms[6] that some heterogenetic antigens may be composed of 2 chemically different substances, one being represented by a lipoidal substance containing the specific group but possessing no antigenic properties. The other component is of the nature of a protein. Since Landsteiner and Simms[6] reported investigations only with simple mixtures of these 2 systems, and Klopstock and Selter[7] those with mixtures of lecithin and diazotised atoxyl, it was of interest to study the possibility of chemically combining lipoidal substances with proteins and their degradation products to obtain a product of a definite entity which then could be used for immunological experiments. From a different aspect combination of lipoidal substances with proteins or their degradation products is interesting because of the colloidal nature of such condensations in an aqueous system. Such a physical state of the antigen is claimed to be important in immunity reactions.

The present report deals with experiments in which we prepared condensation products of cholesterol derivatives with certain amines, amino acids, and peptides. There are also indications of successful condensations with proteins.

Cholesterol, especially purified over the dibromide, was esterified with 4-nitrobenzoylchloride. The ester obtained was reduced with a Pt-catalyst as described by Shriner and Ko[8] to form the corresponding cholesteryl-4-aminobenzoate. For condensation with the above mentioned substances (amines, amino acids, peptides, etc.),

[1] Forssman, *Biochem. Z.*, 1911, **37**, 78.

[2] Meyer, *Z. f. Immunitatsf.*, 1911, **11**, 211; 1912, **15**, 245; 1913, **20**, 367; *Biochem. Z.*, 1921, **122**, 225.

[3] Wernicke and Sordelli, *Rev. Inst. bact. Buenos Aires*, 1919, **2**, 281.

[4] Taniguchi, *J. Path. Bact.*, 1921, **24**, 217.

[5] Landsteiner, K., *Biochem. Z.*, 1921, **119**, 294.

[6] Landsteiner and Simms, *J. Exp. Med.*, 1923, **38**, 127.

[7] Klopstock and Selter, *Z. f. Immunitatsf.*, 1928, **57**, 174.

[8] Shriner and Ko, *J. Biol. Chem.*, 1928, **80**, 1.

diazotisation of the amino benzoyl ester was studied. A number of difficulties were encountered. It was found, for example, that while the hydrochloride of the cholesteryl-4-aminobenzoate can also be prepared by carefully grinding it with an excess of conc. HCl, the resulting salt is not soluble in water.* The action of alkali nitrite therefore proved not to be effective, resulting in an incomplete diazotisation.

Finally, diazotisation with amylnitrite in chloroform, ether, butyl alcohol, etc., was investigated and chloroform found best suitable for this purpose. This procedure necessitated a condensation in the 2 layer system, chloroform-water (containing an alkali, preferably sodium carbonate). Good stirring or shaking is important during the reaction. The following combinations were studied: diazotised cholesteryl-4-aminobenzoate with histidine, carnosine, and histamine. The reaction products are soluble in chloroform, the color red to orange-red. Under analogous conditions tyrosine and tyramine gave a yellow condensation product. Experiments with egg white, edestine, and serum are in progress. Because of the relative ease with which diazotised cholesteryl amino-benzoate was found to condense with beta-naphthol this reaction was used in determining the best conditions for diazotisation. The resulting dye can be obtained in crystalline form when butyl alcohol is used as a solvent.

In analogy with experiments by Golodetz,[9] who prepared cholesteryl salicylate, the 3-nitro-salicylate was synthesized and reduced with a Pt-catalyst to the corresponding 3-amino-derivative. Difficulties were encountered in diazotising this substance, possibly due to the vicinal position of the substituents in the aryl radical.

This study is continued with the intention of extending it to other lipoidal substances.

* Shriner and Ko prepared the hydrochloride by passing HCl through ether solution of cholesteryl-4-aminobenzoate and also reported insolubility in water.

[9] Golodetz, *Chem. Ztg.*, 1906, **81**, 1215.