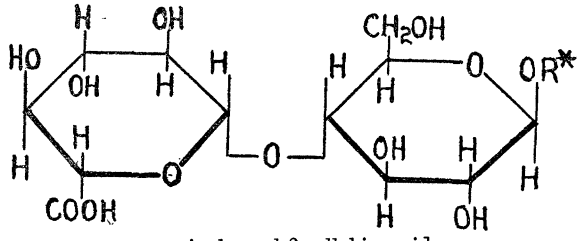
SPECIAL ARTICLES

IMMUNITY TO EXPERIMENTAL PNEUMOCOCCAL INFECTION WITH AN ARTIFICIAL ANTIGEN CONTAINING A SACCHARIDE OF SYNTHETIC ORIGIN

EXPERIMENTAL investigations on the chemical and immunological properties of bacterial polysaccharides and artificial antigens containing simple saccharides of known chemical constitution have revealed many of the factors which govern the immunological specificity of encapsulated microorganisms. With the gradual accumulation of this knowledge we have come to the belief that it should be possible to incite antibacterial immunity in experimental animals with artificially compounded antigens containing immuno-specific groups of synthetic origin rather than with antigenically complex bacterial cells as they naturally occur.

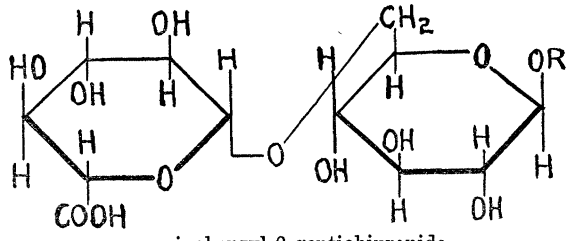
Recently we have shown that an artificial antigen containing the azo benzyl glycoside of cellobiuronic acid evokes in rabbits antibodies which agglutinate Type III pneumococci and confer passive immunity on mice against infection with virulent pneumococci Types II, III and VIII.[1] Cellobiuronic acid, obtained from the acid hydrolysis products of the specific polysaccharide of Type III pneumococcus[2] is constituted from molecules of glucose and glucuronic acid linked in β-glucuronosidic union on the fourth carbon atom of the hexose. Several years ago we described the synthesis of an isomeric aldobionic acid, gentiobiuronic acid or 6-β-glucuronosido-glucose.[3] We have now succeeded in preparing the acetohalogen derivative from heptaacetyl gentiobiuronic acid methyl ester, and from this the corresponding amino benzyl aldobionide has been obtained.[4] An artificial antigen has in turn been prepared by combining the diazonium salt of the aldobionide with horse serum globulin. We have at hand, therefore, two antigens, one containing the azo benzyl glycoside of the naturally occurring aldobionic acid 4-β-glucuronosido-glucose, the other, the corresponding glycoside of the synthetic aldobionic acid, 6-β-glucuronosido glucose. The structural relationship of these two aldobionides is represented by the following graphic formulae.



p-aminobenzyl β-cellobiuronide

* R = $NH_2C_6H_4CH_2$–

Fig. I



p-aminobenzyl β-gentiobiuronide

Fig. II

Since the two saccharides differ only in the position but not the configuration of the glucuronosidic linkage, any differences in their immunological properties can be directly correlated with these differences in chemical constitution. When rabbits are injected with the antigens containing the azo benzyl glycosides of these two aldobionic acids the antibodies evoked are in each instance specifically directed toward the saccharides in question. The specificity of the antibodies is best demonstrated by the fact that the homologous precipitation reactions are in each instance inhibited only by the homologous and not by the heterologous amino benzyl aldobionide.

As previously pointed out, the antiserum to the cellobiuronic acid antigen agglutinates Type III pneumococci and confers passive protection on mice against infection with virulent pneumococci of Types III and VIII. The serum of rabbits injected with the gentiobiuronic acid antigen, on the other hand, exhibits none of these phenomena. It is apparent, therefore, that the position of the glucuronosidic linkage determines the immunological specificity of antigens containing these acids in so far as their capacity to evoke antibacterial immunity to Types III, VIII pneumococci is concerned.

In addition to conferring protection on mice against Types III and VIII pneumococcal infection, the antiserum to the cellobiuronic acid antigen also confers immunity against infection with Type II pneumococci. The antiserum evoked by the antigen containing the synthetic gentiobiuronic acid, although failing to protect mice against infection with either Types III or VIII pneumococci, does afford passive immunity against infection with as much as 100,000 minimal lethal doses of Type II organisms. That this is a property peculiar to the two uronic acid antigens is evidenced by the fact that antigens containing the disaccharides cellobiose and gentiobiose evoked antibodies which show no protective action against Type II pneumococcal infection in mice. In view of these

[1] W. F. Goebel, *Jour. Exp. Med.*, 69: 353, 1939.

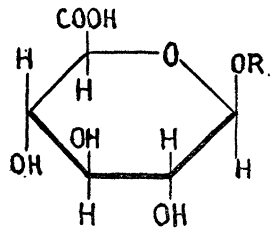
[2] M. Heidelberger and W. F. Goebel, *Jour. Biol. Chem.*, 74: 613, 1927; R. D. Hotchkiss and W. F. Goebel, *Jour. Biol. Chem.*, 121: 195, 1937.

[3] R. D. Hotchkiss and W. F. Goebel, *Jour. Biol. Chem.*, 115: 285, 1936.

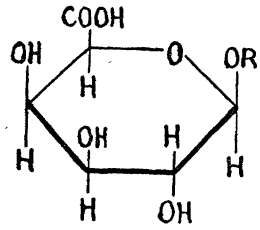
[4] The synthesis of these derivatives will be described in a later communication.

experiments one is faced with the necessity of explaining a new and peculiar fact, namely, the reason underlying the protective action exhibited by the antisera elicited by antigens containing isomeric aldobionic acids against infection with the same microorganism. It is possible to demonstrate experimentally that the protective action of cellobiuronic and gentiobiuronic acid antisera can be attributed to the antibody evoked by the glucuronic acid component common to the two aldobionic acid antigens.

Two artificial antigens, one containing the azo benzyl glycoside of glucuronic acid, the other that of galacturonic acid, have been prepared. The amino benzyl glycosides of these two uronic acids differ only in the configuration of the fourth carbon atom where the position of the H and OH groups is interchanged, as can be seen in Figs. III and IV.



p-aminobenzyl glucoronide
R = $NH_2C_6H_4CH_2$–
Fig. III



p-aminobenzyl galacturonide
Fig. IV

This alteration in constitution has a profound influence in determining the serological specificity of these antigens, as was shown in a study from this laboratory several years ago.[5] That this alteration in configuration of but one carbon atom will determine the capacity of the hexuronic acid antigens to evoke antibacterial immunity is likewise evident, for it has now been found that the antisera of rabbits injected with the glucuronic acid antigen protect mice against infection with 100,000 minimal lethal doses of Type II pneumococci (though not to Types III and VIII), whereas the antiserum to the galacturonic acid antigen is devoid of any protective action whatsoever.

In view of this experimental evidence it is justifiable to conclude, first, that passive immunity to Types III and VIII pneumococcal infection can be conferred on mice by the sera of rabbits immunized with an artificial antigen containing an aldobionic acid having an exact and appropriate configuration; second, that it is possible to confer passive immunity to Type II pneumococcal infection on mice with the sera of rabbits injected with artificial antigens containing saccharides of synthetic origin. The immunity conferred appears to be attributable to an antibody directed toward glucuronic acid. If the molecular configuration of but one of the carbon atoms of the hexuronic acid constituent of the antigen is altered, its capacity to evoke immunity to Type II pneumococcal infection is lost.

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[5] W. F. Goebel and R. D. Hotchkiss, *Jour. Exp. Med.*, 66: 191, 1937.

THE DETERMINATION OF THE TOTAL D-AMINO ACID CONTENT OF HUMAN TUMORS AND NORMAL TISSUES BY MEANS OF D-AMINO ACID OXIDASE

THE reports in recent months by Kögl and co-workers[1] of the characteristic occurrence of amino acids of unnatural configuration in malignant tumors in man and rabbit have been followed by several papers[2] tending both to confirm and to discredit this claim of obviously profound and immediate interest for the biochemistry of tumors. Most of the reports concerned make evident certain difficulties involved in establishing or rejecting such a conclusion on the basis of isolation procedures. We have, therefore, utilized the specific *d*-amino acid oxidase preparation of Krebs[3] for the determination of the *total d*-amino acid content of hydrolysates of a fairly representative variety of benign and malignant human tumors, normal human tissues and some proteins of especial interest: the urinary Bence-Jones protein, because of its close correlation with multiple myeloma in man; insulin as a hormone; and gliadin because of its very high (45 per cent.) glutamic acid content. The hydrolyses were conducted essentially as described by Kögl, in initially 25 to 35 per cent. HCl for 7 to 15 hours, usually with 10 gm acetone-dried tissue, or occasionally directly with fresh tissue. After elimination of the excess HCl, and appropriate neutralization and dilution, the *d*-amino and total nitrogen contents of aliquot portions were determined as reported in Table 1, which gives all data of this nature obtained by us to date.

All biologically significant *d*-α-amino acids are oxidatively deaminated by the Krebs enzyme, but at widely varying rates, so that to obtain reasonably complete oxidation of the slowly reacting *d*-amino acids the experiments were run for 20 hours with concentrated enzyme preparations stabilized by gum ghatti, and with thymol crystals added as antiseptic. As indicated in the table, 70 to 80 per cent. of the *d*-glutamic acid, the slowest reacting amino acid (*cf.* Krebs, 3,

[1] F. Kögl and H. Erxleben, *Verh. Kon. Ned. Akad. Wet.*, II, 38: 1, 1939; *Z. physiol. Chem.*, 258: 57, 1939, 261: 154, 1939; *Naturwissensch.*, 27: 486, 1939; *Nature*, 144: 111, 1939; F. Kögl, *Klin. Wochensch.*, 18: 801, 1939; *Z. f. Krebsforsch.*, 49: 291, 1939; F. Kögl, H. Erxleben and A. M. Akkerman, *Z. physiol. Chem.*, 261: 141, 1939.

[2] (Confirmatory) L. E. Arnow and J. C. Opsahl, SCIENCE, 90: 257, 1939; J. White and F. R. White, *Jour. Biol. Chem.*, 130: 435, 1939. (Non-confirmatory) A. C. Chibnall, M. W. Rees, G. R. Tristam, E. F. Williams and E. Boyland, *Nature*, 144: 71, 1939; S. Graff, *Jour. Biol. Chem.*, 130: 13, 1939; E. Chargaff, *Jour. Biol. Chem.*, 130: 29, 1939; C. Dittmar, *Z. f. Krebsforsch.*, 49: 397, 444, 1939.

[3] H. A. Krebs, *Biochem. Jour.*, 29: 1620, 1935.