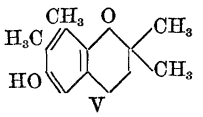
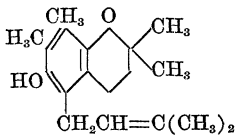
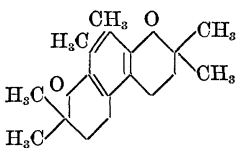
compound from I. The red substance, however, is not a para quinone, but is an ortho quinone, and it has the structure IV. The substance forms a phenazine, m.p. 151–152° with o-phenylene diamine and this phenazine shows a strong greenish yellow fluorescence in ultra-violet light, a property shown by the phenazines of the lapachol group of compounds, similar in structure to IV.[5] That the methyl group in position 5 of I is the group lost in the conversion of I to IV was shown by the fact that the condensation products of o-xylo hydroquinone and isoprene (V, VI and VII), in which the group in position 5 is not methyl, all gave the same red compound IV, m.p. 109–110°, when subjected to the action of nitric acid or silver nitrate.



V



VI



VII

A careful comparison of the absorption curves obtained from IV with absorption curves of known o-quinones also leads unmistakably to the conclusion that the red compounds can not be para quinones, but are ortho quinones.

The red o-quinone from α-tocopherol is an oil. Unfortunately, although it reacts with o-phenylene diamine, the phenazine is also an oil. However, solutions of this phenazine show the same strong greenish fluorescence as is shown by the phenazine of IV, and there can be little doubt but that the tocopherols are also converted into analogs of IV by nitric acid or silver nitrate.

The formation of red o-quinones is not confined to 6-hydroxy chromans, but occurs also with 5-hydroxy coumarans and other related substances. Catechol also produces a red color in the Furter and Meyer reaction, a fact which may be of importance in the examination of natural products by this method, since catechol derivatives are fairly common among natural products.

In these reactions, the mechanism is obscure, although it appears that an alcohol, preferably a primary or secondary alcohol, must be used as the solvent, and the results indicate that the alcohol is probably a reagent as well as a solvent.

Full experimental details of this work will be published elsewhere.

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[5] Hooker, *Jour. Chem. Soc.*, 63: 1376, 1893.

APPEARANCE OF FERMENTABLE POLYSACCHARIDE IN THE BLOOD AND A SIMPLE METHOD FOR ITS DETECTION[1]

ALMOST 40 years ago Besançon and Griffon,[2] Huber,[3] Neufeld[4] and Wadsworth[5] noted that certain bacteria grow more abundantly in the sera obtained from pneumonia patients than in normal sera. When inoculated with pneumococci, a voluminous white precipitate is obtained, while normal sera develop only a slight cloudiness. E. C. Rosenow[6] and Longcope[7] showed that this is due not to bacteria and bacterial debris, as was previously supposed, but to the production of unusually large quantities of acid which precipitate the serum proteins. Longcope noted this phenomenon in sera from patients with pneumococcic and streptococcic infections, from cases of gonococcus endocarditis, acute articular rheumatism, chronic nephritis and uremia. He concluded that "there is . . . some substance which makes its appearance in the blood stream under certain conditions and from which the pneumococcus is capable of forming large quantities of acid." Until the present study was undertaken in 1934, no further work has appeared, and the literature contains scant reference to this very striking phenomenon.

The following simple procedure was adopted early in this work. One cc of the clear sterile serum is transferred to a small sterile test-tube. Serum from strongly hemolyzed blood should not be used. It is inoculated with one drop of an 18-hour culture of a *rapidly growing strain* of pneumococcus. It is then incubated at 37.5° C. The precipitation becomes apparent in about 12 hours; it reaches its maximum in about 36 hours. Normal sera become faintly cloudy. Sera from patients with abnormal states may become opalescent (+), almost opaque (++), opaque with small amount of precipitate (+++) or they may contain a voluminous precipitate (++++). In order to rule out

[1] This study was aided by grants from the Bartlett Memorial Fund and the Douglas Smith Foundation for Medical Research of the University of Chicago.

[2] F. Besançon and V. Griffon, *Ann. Inst. Pasteur*, 14: 449, 1900.

[3] F. O. Huber, *Centralbl. innere Med.*, 23: 417, 1902.

[4] F. Neufeld, *Zeit. Hyg. Infectionskrank.*, 40: 54, 1902.

[5] A. Wadsworth, *Jour. Med. Res.*, 10: 228, 1903.

[6] E. C. Rosenow, *Jour. Inf. Dis.*, 2: 280, 1904.

[7] W. T. Longcope, *Jour. Exp. Med.*, 7: 626, 1905.

any effect due to abnormal quantities of free sugar, it is advisable to determine the reducing sugar in the zinc hydroxide[8] filtrate prepared from 1 to 2 cc of serum. Results from sera which contain more than 125 mg of free sugar per 100 cc should be discarded.

The acids which are produced in normal sera can be accounted for entirely by the free sugar which is consumed. Growth of the organisms appears to stop when the sugar is used up; from 40 to 80 mg of lactic acid are produced. The average yield of lactic acid is 72 per cent.

Unlike normal sera, however, growth continues in positive (+++ or ++++) sera at an apparently undiminished rate after the free sugar is consumed, until a terminal acidity of about pH 5.5 is reached. *The quantity of lactic acid is always greater than can be obtained from the free sugar.* For example, a serum, which contained 104 mg of free sugar, yielded 233 mg of lactic acid. Approximately 75 mg of the acid were derived (by calculation) from the free sugar; 158 mg of lactic acid were therefore derived from some other source.

As has long been known, normal sera contain considerable quantities of protein-polysaccharides.[9-12] By using a standard technique[13] throughout (increase of reducing sugar after 6 hours of hydrolysis with acid), we have invariably obtained higher results in +++ and ++++ sera than in normal ones. None of the sera contain measurable quantities of glycogen. The polysaccharide content is not perceptibly decreased by the growth of the organisms in the normal serum, but large quantities may be rapidly removed in the serum in which precipitation is noted. The production of acids parallels the consumption of the hydrolyzable sugar. Our results therefore indicate that *the phenomenon is due to the presence of abnormal quantities of a polysaccharide which supports rapid growth and which is readily fermented by the pneumococcus.* It thus differs from the normally present polysaccharide, which is not apparently metabolized by the micro-organism, since growth stops in normal sera when the free sugar is consumed.

Besides the conditions mentioned by Rosenow and by Longcope, the phenomenon can be noted in sera from many other diseases. We have most often observed it in acute bacterial infections which are associated with a sustained high temperature. Negative results were obtained from the majority of sera from chronic osteomyelitis. Of sera which were obtained from 9 patients with tuberculous infections, 5 were negative. The polysaccharide appears also in the serum in some conditions in which there is no direct evidence of an infection. It is present in the majority of sera in acute nephritis, in about one half of the sera from hypertension in pregnancy and in about one half of the sera taken during parturition in human subjects.

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[8] M. Somogyi, *Jour. Biol. Chem.*, 86: 655, 1930.
[9] B. Glassmann, *Zeit. physiol. Chem.*, 158: 113, 1926.
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[11] H. Bierry, F. Rathery and Mlle. Levina, *Paris Medical*, 83: 137, 1932.
[12] L. F. Hewitt, *Biochem. Jour.*, 32: 1554, 1938.
[13] T. E. Friedemann, *Jour. Bact.*, 35: 527, 1938.

LISTERELLA FROM A PREMATURE BOVINE FETUS

PREMATURE birth of calves in Bang's disease free herds in Illinois has repeatedly caused concern on the part of cattle owners and veterinarians. Recently a seven months-old bovine fetus from a Bang's disease free herd was brought to the Laboratory of Animal Pathology and Hygiene for examination. Cultural examination and animal inoculation proved negative to *Brucella,* pyogenic organisms and mycoses. The results of microscopic examination for *Tritrichomonas foetus* were negative. However, a gram positive organism in apparently pure culture was isolated on liver agar plates from the stomach of the fetus. The cultural, pathogenic, tinctorial, serologic and biochemic characters of the organism are quite indistinguishable from those of the genus *Listerella.* There was no definite history of encephalitis in the herd in which the fetus originated. Unfortunately, no study could be made of the aborting cow since she was sold for slaughter immediately after aborting.

The organism is a hemolytic gram-positive rod. It is slightly motile in semisolid agar stabs and in hanging drop preparations. Acid, but no gas is produced in dextrose, maltose, levulose, rhamnose, and salicin. Slight acid is produced in lactose and very slight acid in inosite after ten days, while sucrose is not fermented. A rabbit inoculated subcutaneously with the stomach contents of the calf fetus died ten days later, and the organism was recovered from its heart blood. A rabbit and two chickens were inoculated intravenously and a guinea pig was inoculated intraperitoneally with a saline suspension of the organism. The rabbit died two days later, and the organism was recovered from the heart blood and brain. The guinea pig died six days after inoculation, and the micro-organism was recovered from both heart blood and brain. It has been recognized that ocular instillation of Listerella cultures may cause a transitory conjunctivitis in different species of animals, and Julianelle and Pons[1] suggest that the production of conjunctivitis in rabbits

[1] L. A. Julianelle and C. A. Pons, *Proc. Soc. Exp. Biol. and Med.*, 40: 362, 1939.