in 60 ml. of hot water. Cooling produced 1.50 to 1.74 g. (60–68%) of the somewhat impure flavin, m.p. 285–286° dec.

This slightly impure material (4.66 g.) was divided into three lots, each of which was dissolved in hot water and poured into a column packed with a mixture of 150 g. of Magnesol and 150 g. of Hi-Flo Super-Cel. The column was washed with 10% acetone in water and the flavin eluted with 50% acetone in water. The eluate was evaporated to dryness and the residue recrystallized by dissolving in hot water. The yield was 3.09 g. of flavin, m.p. 288–289[18] dec. This material is identical in all respects to that prepared by the previous procedure.[6]

Chromatography.—The flavins were chromatographed by the descending method on Whatman #1 paper using the upper phase of a water (5)–*n*-butyl alcohol (4)–acetic acid (1) system. The flavins were found to have the following R_f values: riboflavin, 0.35; 6-methyl-7-ethylflavin, 0.48; 6-ethylflavin, 0.50; 6-ethyl-7-methylflavin, 0.51, and 6,7-diethylflavin, 0.62.[19]

Ultraviolet Absorption Spectra.—All ultraviolet absorption spectra were determined with a Beckman quartz spectrophotometer, model DU. The compounds were dissolved in water and measurements made on solutions whose concentrations were 5.00 mg./l. for riboflavin and 5.02 mg./l. for the 6-ethyl-7-methylflavin and 6-methyl-7-ethylflavin.

The spectra were almost superimposable. The values for ϵ for the maxima and minima were found to be as follows: riboflavin, maxima: (ϵ) 267 mμ (32,100), 373 mμ (10,400), 447 mμ (12,300); minima: 306 mμ (1050), 402 mμ (6800); 6-ethyl-7-methyl-9-(1′-D-ribityl)-isoalloxazine, maxima: 268 mμ (32,500), 374 mμ (10,300), 447 mμ (12,200); minima: 306 mμ (1,090), 402 mμ (6,800); 6-methyl-7-ethyl-9-(1′-D-ribityl)-isoalloxazine, maxima: 268 mμ (32,100), 375 mμ (10,500), 448 mμ (12,400); minima: 307 mμ (1,090), 402 mμ (6,900).

Acknowledgments.—The author is pleased to acknowledge the assistance given by a number of people: to Drs. J. B. Dickey and E. B. Towne of the Tennessee Eastman Corporation for the use of a battery of high pressure autoclaves; to Dr. F. W. Holly of Merck and Co., Inc., for the D-ribamine used in this study; to Dr. M. Lane of the National Cancer Institute for the information that Magnesol is an excellent adsorbent for the flavins, and to Mr. W. O. Holleman of the Westvaco Chlor-Alkali Division of the Food Machinery and Chemical Corporation for the Magnesol.

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(18) Reference 6 reported m.p. 284–285.

(19) The prevailing atmospheric temperature was between 85 and 90°F. during these runs. On an earlier occasion H. V. Aposhian and J. P. Lambooy, THIS JOURNAL, **77**, 6368 (1955), by means of the ascending method, found the R_f values of three of these flavins to be; riboflavin, 0.30; 6-ethylflavin, 0.43; and 6,7-diethylflavin, 0.54.

[CONTRIBUTION FROM THE INSTITUTE OF MICROBIOLOGY, RUTGERS, THE STATE UNIVERSITY AND THE DEPARTMENT OF MEDICINE, THE COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY AND THE PRESBYTERIAN HOSPITAL]

Immunological Specificities Involving Multiple Units of Galactose. III.[1]

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Further cross reactions of D-galactose-containing polysaccharides with Type XIV antipneumococcal serum are explored. Periodate-oxidized specific polysaccharide of Type XIV pneumococcus precipitates less than one-half of the antibody in the antiserum, confirming the importance of the eliminated non-reducing end groups of D-galactose in the over-all Type XIV pneumococcal specificity. It is also inferred that such groups are present in the specific polysaccharide of the anthrax bacillus, in a cold-soluble fraction of agar, and probably in the mucilage of okra pods. Serological confirmation of the presence of non-reducing end groups of D-galactose also is given for corn fiber hemicellulose.

In the first two papers of this series[4,5] it was shown that the cross-reactivities in Type XIV antipneumococcal sera of a number of galactose-containing polysaccharides of known constitution could be used to obtain advance information on the linkages of the galactose which occur in the capsular, immunologically type-specific polysaccharide (S XIV) characteristic of the virulent, or mucoid, form of pneumococcus Type XIV.[6,7] A study of the hydrolytic products of the methylated derivative of S XIV has already confirmed the prediction of non-reducing end groups of D-galactose and identified as 1,3-linked at least a portion of the galactose predicted as bound in 1,3-, 1,6- or 1,3,6-linkages.[8] Moreover, the uronic acid originally reported in a galactan isolated from beef lung[9] was shown to be D-glucuronic acid by the unexpected reactivity of the galactan in Type II antipneumococcal serum, a finding later confirmed by chromatography.[4] The glucuronic acid could also be shown to be a component of an impurity in, or degradation product of, the principal polysaccharide by the far greater content of uronic acid in the carbohydrate recovered from the specific precipitate in the Type II antiserum than in the polysaccharide derived from the Type XIV precipitate or in the original galactan. An extension of these findings to other galactose-containing polysaccharides seemed indicated by these promising beginnings, and the present paper records some additional results.

Experimental

A preparation of the specific polysaccharide of *B. anthracis*,[10] isolated from cultures grown in the guinea pig,[11] was kindly supplied by Dr. H. Smith, while two other poly-

(1) These studies were carried out mainly under a grant from the National Science Foundation to Rutgers University.

(2) Fellow of the Rockefeller Foundation, from the Department of Chemistry, University of Birmingham, England.

(3) Wallenberg and Swedish Cancer Society Fellow, at Columbia University, from the State Bacteriological Institute, Stockholm, Sweden.

(4) M. Heidelberger, Z. Dische, W. Brock Neely and M. L. Wolfrom, THIS JOURNAL, **77**, 3511 (1955).

(5) M. Heidelberger, *ibid.*, **77**, 4308 (1955).

(6) W. F. Goebel, P. B. Beeson and C. L. Hoagland, *J. Biol. Chem.*, **129**, 455 (1939).

(7) M. Heidelberger, S. A. Barker and M. Stacey, *Science*, **120**, 781 (1954).

(8) Private communication from Dr. S. A. Barker.

(9) M. L. Wolfrom, D. I. Weisblat, J. V. Karabinos and O. Keller, *Arch. Biochem.*, **14**, 1 (1947); M. L. Wolfrom, G. Sutherland and M. Schlamowitz, THIS JOURNAL, **74**, 4883 (1952).

(10) G. Ivánovics, *Z. Immunitätsforsch.*, **97**, 402 (1939); **98**, 373 (1940).

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TABLE I

PRECIPITATION OF POLYSACCHARIDES IN 1.0 ML. OF TYPE XIV ANTIPNEUMOCOCCAL HORSE SERUM 635 AT 0°

Substance	Unabsorbed serum	Nitrogen in μg., precipitated from antiserum absorbed with: Pn C[a]	Oxd. S XIV	Anthrax	Carob	Anthr. + carob	Pn C, red cells	Cold-sol. agar
Pn C[a]	47							
S XIV		1010		867			914	830, 770[c]
IO_4-oxd. S XIV		427		289[b]		257		
Anthrax		219	66		91		209	109
Carob[5]	159			41				52
Guar		204						
Tamarind seed[4]	94		44					
Arabogalactan, Jeffrey pine[5]	245			96				
Cryptococcus A		19						
Cold-sol. agar		257						
Corn fiber hemicellulose		207						
Okra	56	48						

[a] C-, or group-specific, carbohydrate of Pneumococcus. [b] Single determination. [c] Values adjusted for pptn. of 257 μg. N by the agar; *cf.* Table II for actual amounts.

saccharides from *in vitro* cultures of the same microörganism[12] were furnished by Dr. Rydon. Of samples of the specific polysaccharides of Cryptococcus A, B and C[13] sent by Dr. E. E. Evans, only the first precipitated the Type XIV antiserum used. Carob mucilage[14] was kindly supplied by Prof. E. L. Hirst, a sample of the closely related guar mucilage[15] by Prof. Fred Smith, and one of okra mucilage[16] by Dr. R. L. Whistler. Corn fiber (corn hull) hemicellulose[17,18] was furnished by the Corn Products Refining Co. through the courtesy of Mr. Norman Kennedy. A generous sample of the arabogalactan of the Jeffrey pine[19] was supplied by Prof. W. Z. Hassid. The S XIV used was given by E. R. Squibb and Sons, through the courtesy of Mr. T. D. Gerlough, and was further purified by several treatments of its aqueous solution with chloroform and butanol[20] and precipitation with isopropyl alcohol after addition of sodium acetate.[21] Tamarind seed polysaccharide[22] was obtained through the kindness of Drs. E. V. White and F. E. Brauns.

Cold-soluble agar was prepared as follows: 10 g. of Difco "bactoagar" was shaken vigorously for 2 min. with 200 ml. of water and filtered through paper. The filtrate, 110 ml., was treated with 4.5 g. of crystalline sodium acetate, adjusted to *p*H 7.1, and precipitated with 300 ml. of 95% ethanol. After 2 hr. at 0° the mixture was filtered on hardened paper and the precipitate was washed with ethanol and dried; yield 0.19 g. or 2% of the original agar. Subsequent preparations on a larger scale were fractionated and are to be discussed in a separate communication.

S XIV (21.6 mg.) was treated with 76.9 mg. of Na periodate in 50 ml. of water at 25° in the dark for 5 days. Salts were removed by dialysis and the oxidized S XIV (OS) was recovered by freeze-drying. Solutions were prepared by suspension of weighed amounts in 0.9% NaCl solution, addition of a drop of *N* NaOH solution until clear, and neutralization with *N* HCl.

(12) J. E. Cave-Brown-Cave, E. S. J. Fry, H. S. El Khadem and H. N. Rydon, *J. Chem. Soc.*, 3866 (1954).

(13) E. E. Evans, *J. Immunol.*, **64**, 423 (1950); E. E. Evans and J. W. Mehl, *Science*, **114**, 10 (1951); E. E. Evans and J. F. Kessel, *J. Immunol.*, **67**, 109 (1951); E. E. Evans and R. J. Theriault, *J. Bacteriol.*, **65**, 571 (1953).

(14) E. L. Hirst and J. K. N. Jones, *J. Chem. Soc.*, 1278 (1948); F. Smith, THIS JOURNAL, **70**, 3249 (1948).

(15) L. E. Wise and J. W. Appling, *Ind. Eng. Chem., Anal. Ed.*, **16**, 28 (1944); Z. F. Ahmed and R. L. Whistler, THIS JOURNAL, **72**, 2524 (1950); C. M. Rafique and F. Smith, *ibid.*, **72**, 4634 (1950).

(16) R. L. Whistler and H. E. Conrad, *ibid.*, **76**, 1673, 3544 (1954).

(17) R. L. Whistler and W. M. Corbett, *ibid.*, **77**, 6328 (1955); R. L. Whistler and J. N. BeMiller, *ibid.*, **78**, 1163 (1956).

(18) R. Montgomery, F. Smith and H. C. Srivastava, *ibid.*, 2837, 6169; **79**, 698 (1957); R. Montgomery and F. Smith, *ibid.*, **79**, 695 (1957).

(19) W. H. Wadman, A. B. Anderson and W. Z. Hassid, *ibid.*, **76**, 4097 (1954).

(20) M. G. Sevag, *Biochem. Z.*, **273**, 419 (1934).

(21) M. Heidelberger, C. M. MacLeod, H. Markowitz and A. S. Roe, *J. Expil. Med.*, **91**, 341 (1950).

(22) E. V. White and P. S. Rao, THIS JOURNAL, **75**, 2617 (1953).

The Type XIV antipneumococcal horse serum was donated by the division of Laboratories, New York State Department of Health, Dr. G. Dalldorf, Director.

Quantitative determinations of antibody nitrogen precipitated[23] in the cross reactions are given in Tables I and II. As in previous instances the reactions were allowed to stand in a bath at 0° for 6 to 14 days, depending upon the speed of precipitation, before centrifugation at 0° and completion of the analyses. The technical assistance of Mr. Check M. Soo Hoo in this part of the work is gratefully acknowledged.

TABLE II

PRECIPITATION OF TYPE XIV ANTIPNEUMOCOCCAL HORSE SERUM BY VARIOUS POLYSACCHARIDES, PER 1.0 ML.

Wt. of polysaccharide, mg.	Micrograms of antibody nitrogen pptd. at 0° from C-absorbed serum by: Anthrax	Cold-sol. agar	Corn fiber hemicellulose	Guar	Okra
0.05					36
.10	192				48
.2			104		
.3	216	60		204	37
.6	219	73	161		
2		119	204		
4		202,[a] 231[b]	207		
8		257, 364[b]			

[a] The supernatant gave 885 μg. more N with S XIV; total 1087. [b] With a second prepn. of cold-sol. agar; mean value, 298. The combined supernatants gave 729 μg. additional N per ml. original serum with S XIV; total, 1027.

Results and Discussion

1. **Periodate-oxidized S XIV (OS).**—As noted in Table I, OS precipitated 42% of the antibody to S XIV in a Type XIV antipneumococcal horse serum at 0°. Since OS still contains all three sugars known to occur in S XIV[8] it is probable that much of the decrease in precipitating power is due to the oxidative removal of that portion of the D-galactose present as non-reducing end groups. That OS still removes a part of the antibody reactive with such end groups is shown by the precipitation of only 44 μg. of antibody N (Table I) from the OS-absorbed supernatant upon the addition of tamarind seed polysaccharide, in which the multi-

(23) M. Heidelberger and F. E. Kendall, *J. Expil. Med.*, **55**, 555 (1932); **61**, 559 (1935); M. Heidelberger, C. M. MacLeod, S. C. Kaiser and B. Robinson, *ibid.*, **83**, 303 (1946); R. Markham, *Biochem. J.*, **36**, 790 (1942); E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," C. C Thomas, Springfield, Ill., 1948.

ple end groups of D-galactose presumably mediate the precipitation.[4]

2. Specific Polysaccharide of *B. anthracis*.—Of the three samples tested, only that prepared from bacilli grown in guinea pigs[11] precipitated the antiserum. The inverse relationship, namely, agglutination of Type XIV pneumococci by anti-anthrax serum, had been shown many years before.[10] However, the cross reaction of Type XIV antipneumococcal sera with blood group substances[6,24] suggested the possibility that the Smith and Zwartouw polysaccharide might react with the antiserum because of possible contamination with Forssman antigen, which occurs in guinea pigs. In order to test this, Type XIV antiserum was absorbed at 0° with washed sheep erythrocytes, a standard source of Forssman antigen. No agglutination was observed, nor, after removal of the red cells, was the reactivity of the serum toward the Smith and Zwartouw polysaccharide appreciably diminished (columns 3 and 8, Table I), although about 10% of the antibody reactive with S XIV had been absorbed by the red cells.

From column 5 of Table I it is evident that the anthrax polysaccharide precipitates antibody to S XIV, since the sum of the N precipitated by the anthrax substance and that precipitated from the residual serum by S XIV is roughly equal to that given by S XIV with the intact serum. Both the precipitation by the anthrax substance and by carob mucilage are sharply reduced by prior removal of antibody reactive with carob or anthrax, respectively. Since the galactose in carob mucilage is entirely in the form of non-reducing end groups, one may deduce that a part of the reactivity of the anthrax polysaccharide in Type XIV antipneumococcal serum arises from the presence of non-reducing end groups of D-galactose in the anthrax substance. This finding has been communicated to Dr. Smith and it is hoped that its confirmation by the methods of organic chemistry will be attempted. The analyses (Table I) with the arabogalactan of Jeffrey pine, in which only relatively little of the galactose is in the form of non-reducing end groups and most occurs linked 1,6- or 1,3,6-,[19] show that part of the galactose in the anthrax polysaccharide may occur in similar linkages, the more so as absorption of the antiserum with the anthrax substance greatly reduces precipitation with the arabogalactan.

Reciprocal absorption also greatly diminishes the quantities of antibody N precipitated by OS and anthrax substance from the antiserum. Since the D-galactose end groups presumably are missing from OS (note, however, the diminished precipitation of tamarind polysaccharide in OS absorbed serum), the residual reactivity shows that a portion of the precipitation in each instance is due to groupings other than D-galactose end groups. Whether these are galactose residues bound in other linkages or N-acetylglucosamine residues, or both, in the anthrax substance, cannot be stated at present.

The above analysis of the structure of the anthrax substance is only partially in agreement with another based on the behavior of the formazan of the periodate-oxidized substance.[25]

3. Specific Polysaccharide of *Cryptococcus neoformans A*.[18]—Of the specific polysaccharides of cryptococcus A, B and C, only the first gave appreciable precipitation in Type XIV or, indeed, any other available antipneumococcal serum. Although the amount of precipitated N was only 19 μg. per ml. of antiserum, a fractionation of the cryptococcus A polysaccharide was effected by the combination with antibody, much as had been accomplished in the precipitation of gum arabic by Type II antipneumococcal serum.[26] The results will be discussed in greater detail in a forthcoming note.

4. Cold-soluble Agar.—Following the classical observation of Sordelli and Mayer[27] that animals injected with typhoid cultures grown on agar produced precipitating antibodies for agar as well as antibodies to the typhoid bacilli, it was generally assumed[28] that any antibacterial sera which gave a precipitate with agar actually contained antibodies to agar itself. However, it was noted that some supposedly normal sera reacted in this fashion; also that agar extract precipitated 20% of the anticarbohydrate in an antianthrax horse serum.[29] Preliminary qualitative tests were therefore made with a cold-soluble fraction of agar, since this was more reactive and more easily studied through a wide range of concentrations than was agar itself. Many antipneumococcal horse sera were found to precipitate heavily with this reagent, particularly when it was added in milligram concentrations. Since agar cultures are rarely used for the growth of pneumococcus a quantitative study of this finding was instituted. In each antiserum the nitrogen of the precipitate consisted of antibody to the homologous pneumococcal type-specific polysaccharide. In the present communication only the data obtained with the Type XIV antiserum are given (Tables I and II) since the soluble agar fraction contains galactose, as shown on hydrolysis and paper chromatography of the resulting mixture. A cross reaction with the Type XIV antiserum might therefore be explained on this basis. It is evident that the 25 to 30% of antibody precipitated is not random with respect to reactivity with non-reducing end groups of D-galactose, since the agar-precipitated serum gave only one-half as much precipitation with anthrax polysaccharide and one-third as much as the intact serum with carob mucilage. It is therefore probable that the portion of the cold-soluble agar which precipitates the Type XIV antiserum contains non-reducing end groups of D-galactose, and that the reaction is a true cross reaction.

5. Corn Fiber Hemicellulose.—This substance contains end groups of D-galactose linked in at least two different ways and also of L-galactose, to-

(24) W. F. Goebel and P. B. Beeson, *J. Exptl. Med.*, **70**, 239 (1939); S. Leskowitz and E. A. Kabat, THIS JOURNAL, **76**, 4887, 5060 (1954), and earlier papers.

(25) L. Mester and G. Iványovics, *Chemistry & Industry*, 493 (1957).

(26) M. Heidelberger, J. Adams and Z. Dische, THIS JOURNAL, **78**, 2853 (1956).

(27) A. Sordelli and E. Mayer, *Compt. rend. soc. biol.*, **107**, 736 (1931).

(28) For example, H. E. Alexander and M. Heidelberger, *J. Exptl. Med.*, **71**, 1 (1940).

(29) A. M. Staub and P. Grabar, *Ann. inst. Pasteur*, **69**, 268 (1943).

gether with xylose, arabinose and other terminal groupings of glucuronic acid. Since there are only two parts of D- and L-galactose in 33, one might not expect strong cross-reactivity in the Type XIV antiserum. However, about 20% of the antibody is precipitated, so that the few end groups of D-galactose must be favorably placed on the molecule.

6. Guar Mucilage.—Although similar structures have been proposed for the mucilages of carob[14] and guar,[15] D-galactose constitutes about 20% of the former and 33% of the latter. Since at least 90% of the galactose in the guar mucilage, like all of that in the carob polysaccharide is in the form of non-reducing end groups, one might expect this substance to precipitate more antibody from the Type XIV antiserum than does the carob gum. Analyses at a single level of polysaccharide, near the maximum for carob,[5] showed that this is indeed true.

7. Okra Mucilage.—The mucilage of okra pods is made up of D-galactose, L-rhamnose and D-galacturonic acid.[16] Since 4-O-α(?)-D-galactopyranosyl-D-galactopyranose was isolated from the products of partial hydrolysis, only part of the galactose, if any, can be present as non-reducing end groups. Whether or not such a portion could be responsible for the small reactivity observed cannot be stated until the chemistry of this substance is studied in greater detail. However, there is no reason to suppose that α-1 → 4-linked galactose occurs in S XIV, so that if this represents the only linkage between two galactose molecules in okra mucilage, any reactivity of the substance in Type XIV antiserum would appear to be due to the presence of end groups of D-galactose.

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Cross Reactions of Polyglucoses in Antipneumococcal Sera. VI.[1] Precipitation of Type VIII and Type III Antisera by β-Glucans

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The recently elucidated structure of the immunologically specific capsular polysaccharide of Type VIII pneumococcus permitted the prediction, according to a quantitative theory of specific precipitation between antigen and antibody, that all polysaccharides containing cellobiose units would precipitate Type VIII antipneumococcal sera. This was verified for three such substances, barley and oat β-glucans, and Iles glucomannan, the only ones immediately available. It was also predicted that the two glucans, by virtue of their β-1,3-linkages, would precipitate Type III antipneumococcal serum and this also was verified.

The long-known cross-relationship between Type III and Type VIII pneumococci[2-4] was traced to the occurrence of cellobiuronic acid in the specific capsular polysaccharides[5] of both microörganisms. It was shown that S III is a polycellobiuronic acid in which each unit is joined to the next by a 1 → 3-linkage, probably β-,[6] while in S VIII only about 50% of the molecule consists of cellobiuronic acid, extra glucose being present.[4,7] In spite of the urgency of additional knowledge of the structural details of these substances upon which so promising a beginning had been made in the relation of chemical constitution to their immunological specificity, there has been no further elucidation of the stereochemistry of the linkages between the units of S III, and the fine structure of S VIII has only recently been worked out.[8] Galactose was newly discovered to be a constituent of S VIII and all residues were found to be linked 1 → 4:[-O-β-D-glucopyranosyluronic acid-(1 → 4)-O-β-D-glucopyranosyl-(1 → 4)-O-α-D-glucopyranosyl-(1 → 4)-O-α-D-galactopyranosyl-(1 → 4)-]$_n$.

It will be remembered that the absence of extensive branching in the chain of S VIII could be predicted from the quantitative studies of cross-precipitation in Type III and Type VIII antipneumococcal sera carried out many years ago.[4] The newly elucidated structure immediately made additional predictions possible. These, based also on a quantitative theory of specific precipitation in which the formation of precipitates is ascribed to multiple reactive groupings on antigen and antibody,[9] and their verification are described in the present paper.

Experimental

Materials and Methods.—S III and S VIII were supplied by E. R. Squibb and Sons, kindness of Mr. T. D. Gerlough. Type III and Type VIII antipneumococcal horse sera were furnished by the Bureau of Laboratories, New York City Department of Health, through the courtesy of Miss Annabel W. Walter. Samples of barley and oat glucans[10] were kindly supplied by Prof. Ian Preece of Edinburgh and Iles glucomannan, purified by precipitation with Fehling solution,[11] by Prof. Fred Smith of Minneapolis, Minn.

(1) Papers III, IV, V: *J. Immunol.*, **78**, 419, 427, 431 (1957). This study was carried out under a grant from the National Science Foundation to Rutgers University.

(2) J. Y. Sugg, E. L. Gaspari, W. L. Fleming and J. M. Neill, *J. Exptl. Med.*, **47**, 917 (1928); G. Cooper, M. Edwards and C. Rosenstein, *ibid.*, **49**, 461 (1929).

(3) M. Heidelberger, E. A. Kabat and D. L. Srivastava, *ibid.*, **65**, 487 (1937).

(4) M. Heidelberger, E. A. Kabat and M. Mayer, *ibid.*, **75**, 35 (1942).

(5) Herein designated S III and S VIII.

(6) M. H. Adams, R. E. Reeves and W. F. Goebel, *J. Biol. Chem.*, **140**, 653 (1941), and earlier papers.

(7) W. F. Goebel, *ibid.*, **110**, 391 (1935).

(8) J. K. N. Jones and M. B. Perry, THIS JOURNAL, **79**, 2787 (1957).

(9) M. Heidelberger and F. E. Kendall, *J. Exptl. Med.*, **61**, 563 (1935).

(10) I. A. Preece and K. G. Mackenzie, *J. Inst. Brewing*, **58**, 457 (1952); G. O. Aspinall and R. G. J. Telfer, *J. Chem. Soc.*, 3519 (1954); L. Acker, W. Diemair and E. Samhammer, *Z. Lebensm. Untersuch. u. Forsch.*, **102**, 225 (1955).

(11) (a) P. A. Rebers and F. Smith, THIS JOURNAL, **76**, 6097 (1954); (b) F. Smith and H. C. Srivastava, *ibid.*, **78**, 1404 (1956).