previous paper[1] are discussed in relation to the mechanism of replication of viral RNA.

We are indebted to Mr. Morton C. Schneider and Mr. Horace Lozina for help with the large-scale growth of *E. coli*.

* Aided by grants AM-01845-07 and 1-SO1-FR-05099-02 from the National Institutes of Health, USPHS, the Jane Coffin Childs Fund for Medical Research, and E. I. du Pont de Nemours and Co. The results reported in this and in the preceding paper of this series were presented at a Symposium on Protein Synthesis and the Genetic Code at the 146th National Meeting of the American Chemical Society in Denver, Colorado, January 22, 1964. For papers I and II see refs. 3 and 2, respectively. The abbreviations used are the same as in the preceding paper.[1]

† Fellow of the Rockefeller Foundation.

‡ Fellow of the Jane Coffin Childs Fund for Medical Research.

[1] Weissmann, C., P. Borst, R. H. Burdon, M. A. Billeter, and S. Ochoa, these PROCEEDINGS, **51**, 682 (1964).

[2] Weissmann, C., and P. Borst, *Science*, **142**, 1188 (1963).

[3] Weissmann, C., L. Simon, and S. Ochoa, these PROCEEDINGS, **49**, 407 (1963).

[4] Loeb, T., and N. D. Zinder, these PROCEEDINGS, **47**, 282 (1961).

[5] Schmidt, J., in *The Nucleic Acids*, ed. E. Chargaff and J. N. Davidson (New York: Academic Press, 1955), vol. 1, p. 562.

[6] Weissmann, C., unpublished results.

[7] Strauss, J. H., Jr., and R. L. Sinsheimer, *J. Mol. Biol.*, **7**, 43 (1963).

[8] Marmur, J., and P. Doty, *J. Mol. Biol.*, **3**, 585 (1963).

[9] Chamberlin, M., and P. Berg, in *Synthesis and Structure of Macromolecules*, Cold Spring Harbor Symposia on Quantitative Biology, vol. 28 (1963), p. 67.

[10] Hayashi, M., M. N. Hayashi, and S. Spiegelman, these PROCEEDINGS, **50**, 664 (1963).

[11] Marmur, J., and C. M. Greenspan, *Science*, **142**, 387 (1963).

[12] Tocchini-Valentini, G. P., M. Stodolsky, A. Aurisicchio, M. Sarnat, F. Graziosi, S. B. Weiss, and E. P. Geiduschek, these PROCEEDINGS, **50**, 935 (1963).

[13] Geiduschek, E. P., T. Nakamoto, and S. B. Weiss, these PROCEEDINGS, **47**, 1405 (1961).

[14] Chamberlin, M., and P. Berg, these PROCEEDINGS, **48**, 81 (1962).

[15] Baltimore, D., these PROCEEDINGS, **51**, 450 (1964).

[16] Haruna, I., K. Nozu, Y. Ohtaka, and S. Spiegelman, these PROCEEDINGS, **50**, 905 (1963).

[17] Ochoa, S., C. Weissmann, P. Borst, R. H. Burdon, and M. A. Billeter, *Federation Proc.*, in press.

---

*POLYSACCHARIDE AND GLYCOLIPID SYNTHESIS BY CELL-FREE PREPARATIONS FROM TYPE XIV PNEUMOCOCCUS**

BY JACK DISTLER† AND SAUL ROSEMAN

RACKHAM ARTHRITIS RESEARCH UNIT AND DEPARTMENTS OF ZOOLOGY AND BIOLOGICAL CHEMISTRY, UNIVERSITY OF MICHIGAN

Communicated by J. L. Oncley, March 18, 1964

The immediate precursors of oligosaccharide units in glycoproteins and glycolipids are not known, although several possibilities have been considered.[1] As one approach to this problem, we investigated a potential model system, the biosynthesis of Type XIV pneumococcal polysaccharide (S XIV).

Following its isolation by Goebel *et al.*[2] this polysaccharide was studied extensively by both chemical and immunochemical methods.[3] Although the structure is not established, the polymer is branched and apparently composed of N-acetyl-

D-glucosamine, D-glucose, and D-galactose, in ratios of 2:1:3. Di- and trisaccharides isolated after partial acid hydrolysis (Barker *et al.*[4, 5]) included lactose, N-acetylglucosaminido-β1,3-galactose, and glucosyl-β1,4-N-acetylglucosamine. Immunochemical studies by Heidelberger[6] showed that terminal galactopyranosyl end groups were the principal antigenic determinants of the polysaccharide, while the more recent work of Watkins and Morgan[7] and Kabat[8] confirmed and extended these findings. They suggested that the major antigenic determinant disaccharide was galactosyl-β1,4-N-acetylglucosamine. Although this disaccharide has not yet been isolated from S XIV, it has been obtained from blood group substances[9] and is probably responsible for the cross reaction between partially hydrolyzed blood group substances and Type XIV antiserum.[10, 7] The disaccharide is also a constituent of fetuin,[11] orosomucoid,[12] milk oligosaccharides,[13] and certain UDP-trisaccharides[14, 15] and UDP-disaccharides.[16]

Our approach to the biosynthesis of S XIV was analogous to that of Mills and Smith[17] in their studies on other pneumococcal polysaccharides. This report presents evidence for the enzymatic synthesis of polysaccharides similar to, but not identical with, S XIV isolated from whole cells. The enzyme system was a particulate fraction obtained from Type XIV pneumococcus, and the substrates were the UDP derivatives of glucose, galactose, and N-acetylglucosamine. The particulate fraction also catalyzed a rapid synthesis of glycolipids containing glucose and galactose.

Materials and Methods.—The UDP-monosaccharides were prepared by minor modifications of a general chemical method.[18] The sugar-labeled nucleotides were UDP derivatives of: glucose-U-C^{14}, galactose-1-C^{14}, C^{14}-acetyl labeled N-acetylglucosamine, and N-acetylgalactosamine labeled uniformly in the galactosamine moiety.[19] A Packard liquid scintillation spectrometer was used for C^{14} analyses; substances insoluble in the toluene or dioxane solvent systems were counted with the aid of "Hyamine" as recommended by the manufacturer. Paper strips, after electrophoresis or chromatography, were counted in the toluene system.

The organism, *Diplococcus pneumoniae* ATCC 6314, after three mouse passages to increase its virulence, was reisolated and characterized on blood-agar plates and by means of the capsular swelling reaction in the presence of Type XIV antiserum. After culture in Todd-Hewitt liquid broth (Difco), the cells were suspended in sterile skim milk, lyophilized, stored in a vacuum at 5°, and used as primary inocula.

Double diffusion studies were performed in agar by standard methods.[20] "Ionagar" no. 2 (Consolidated Laboratories, Inc., Chicago Heights, Ill.) was made to 0.8% final concentration in a buffer containing the following components per liter: 9.3 gm of Tris adjusted to pH 7.4 with HCl, 7.0 gm of NaCl, and 0.5 gm NaN_3. The polysaccharide samples, placed in outer wells 1 cm from the center well on 3 × 4-inch agar plates, were allowed to diffuse for 1–3 days at 37° before adding antiserum to the center well.

Samples of S XIV were kindly provided by Drs. Elvin A. Kabat, Michael Heidelberger, and Jessie L. Hendry. The three preparations showed the same general pattern on double diffusion, but different turbidity values on a weight basis by the method described below. The sample from Dr. Kabat was used as the "standard" XIV-polysaccharide. Dr. Hendry also provided Type XIV pneumococcal rabbit antiserum; it was used for these studies after 3–4 absorptions[21] with formalin-treated Type III pneumococcal cells. The absorbed antiserum gave no precipitin test with pneumococcal C substance kindly provided by Dr. Emil C. Gotschlich. A sample of "C" absorbed horse XIV antiserum, provided by Dr. Heidelberger, gave results similar to the absorbed rabbit serum.

Quantitative precipitin reactions were studied by modification of a turbidimetric method.[22] The reactions were conducted in cuvettes containing the following components in final volumes of 1.0 ml: 0.40 ml of absorbed rabbit antiserum (equivalent to 0.13 ml of original undiluted antiserum), the Tris-NaCl buffer described above (without azide), and 0.5–2.5 μg standard S XIV. Turbidity

formation was followed at 550 mμ as a function of time using a Gilford recording spectrophotometer. Under these conditions, the antibody was in excess, the final absorbancies (120 min) showed excellent linear correlation with S XIV concentration, and kinetics of turbidity development could be studied. When necessary, the reaction mixture was reduced to final volumes of 0.2 ml.

Preparation and assay of enzyme system: The lyophilized cultures were inoculated into 60-ml portions of Todd-Hewitt broth (Difco), allowed to grow overnight in standing culture at 37°, and 10-ml aliquots transferred to 500-ml portions of fresh broth. When the growth medium reached 0.20 optical absorbancy (Evelyn colorimeter, 2-cm path length, 620 mμ), the culture was immediately chilled (all remaining operations were conducted at 0–4°), and the cells washed with a solution containing 0.15 *M* KCl, 0.05 *M* potassium phosphate buffer, pH 7.2, and 0.01 *M* Na thioglycollate. Cells from 6 l of medium were suspended in 24 ml of fresh buffer, disrupted in a French pressure cell, treated with 6 mg each of crystalline RNAase and DNAase to reduce viscosity (in the absence of added Mg^{++}), and the mixture centrifuged at 10,000 × *g* for 10 min to remove cellular debris. The supernatant fluid was then diluted to 90 ml with the buffer solution, centrifuged at 105,000 × *g* for 30 min, the particulate fraction washed with 90 ml of fresh buffer, and suspended in 6 ml of the buffer (yielding 6.2 mg protein per ml); 100 μmoles of $MgCl_2$ were added to the mixture just before use.

The particulate system contained an active UDP-glucose-4-epimerase,[23] so that incubation with either UDP-glucose-C^{14} or UDP-galactose-C^{14} (in the presence or absence of UDP-N-acetylglucosamine) gave products containing both glucose-C^{14} and galactose-C^{14}. For this reason, incubations were generally conducted with equimolar mixtures of the UDP-hexoses. The particulate fraction also contained UDP-N-acetylglucosamine-4-epimerase,[24, 25] but this enzyme did not interfere in the present studies. Incubation of the system with UDP-N-acetylgalactosamine-C^{14} and UDP-hexoses showed that the C^{14} was incorporated at 25% of the rate observed with the glucosamine derivative. However, hydrolysis of the polysaccharides obtained from UDP-N-acetylgalactosamine-C^{14} yielded glucosamine-C^{14} as the only detectable labeled component. This also indicated that the system did not synthesize pneumococcal C substance, a polysaccharide that contains galactosamine as a major constituent.[26]

Results.—Under the conditions described above, preliminary kinetic experiments showed the following: synthesis of C^{14}-polysaccharide was linear with protein concentration and time over a 2-hr period; the pH optimum was 7.2 in both phosphate and Tris buffers; Mg^{++} was required (other metal ions were not tested), and the optimum concentration was 0.01 *M;* the concentrations of UDP-monosaccharides required for maximum incorporation are indicated in Table 1.

A typical experiment, shown in Table 1, gave the following results: (1) N-acetylglucosamine-C^{14} was not significantly incorporated into the polysaccharide fraction without simultaneous incorporation of the hexoses. (2) In contrast, the hexoses-C^{14} *were* significantly incorporated in the absence of UDP-N-acetylglucosamine; however, the presence of the latter stimulated incorporation of hexoses 4–5-fold. (3) The butanol-soluble material became highly labeled in all experiments involving UDP-hexose-C^{14} but not in those involving UDP-N-acetylglucosamine-C^{14} as the only C^{14}-substrate.

Identification of polysaccharide fractions: The radioactive polysaccharide fractions, isolated as described in Table 1, showed these similarities to standard S XIV: (*a*) they were neither dialyzable nor electrodialyzable; (*b*) they did not migrate upon paper electrophoresis at pH 7.4, phosphate buffer, but did migrate in borate at pH 9.5; (*c*) more than 90 per cent of the C^{14} was precipitated with Type XIV absorbed antiserum; and (*d*) the only radioactive products detected after acid hydrolysis were the expected C^{14}-sugars or C^{14}-acetate, depending upon the C^{14} substrate employed. Glucosamine was characterized by ion-exchange chromatography[27] and borate electrophoresis,[28] and the hexoses by paper electrophoresis in

TABLE 1

INCORPORATION OF C^{14}-SUGARS INTO POLYSACCHARIDES AND GLYCOLIPIDS BY PARTICULATE ENZYME SYSTEM*

Tube no. →	1	2	3	4	5	6
Labeled substrate →	UDP-G, -Gal, -Ag	UDP-Ag	UDP-G, -Gal	UDP-G, -Gal	UDP-Ag	UDP-G, -Gal, -Ag
Nonlabeled substrate →		UDP-G, -Gal	UDP-Ag			
	(cpm)	(cpm)	(cpm)	(cpm)	(cpm)	(cpm)
Total aqueous phase†	115,000	34,200	91,900	20,100	1,920	3,440
Hexosamine	32,200	33,600	0	0		
Hexose	95,000	0	89,900	19,000		
Galactose	63,600	0	59,500	9,690		
Glucose	31,500	0	30,500	9,310		
Total butanol phase	207,000	1,190	275,000	299,000	1,720	25,300

* The complete incubation mixtures contained the following components (in final volumes of 0.5 ml): 0.25 μmole each of uridine diphosphate glucose, UDP-glucose (UDP-G; 1.11×10^8 cpm/μmole) and UDP-galactose (UDP-Gal; 1.06×10^6 cpm/μmole); 0.05 μmole UDP-N-acetylglucosamine (UDP-Ag; 1.14×10^6 cpm/μmole); and 0.3 ml of the enzyme preparation described in the text. Nonlabeled substrates were used in place of C^{14}-substrates where indicated. The nucleotides were omitted from tube 6 until after the reaction was stopped by cooling to 0°. The samples were incubated for 2 hr at 37°, chilled, centrifuged at $35,000 \times g$ for 30 min, and the particulate fraction washed with 1-ml portions of saline until the last wash was essentially free of radioactive substrate (usually 4 washes were used). The combined supernatant fluids contained less than 5% of the C^{14}-polysaccharides present in the particulate matter. To solubilize the latter, the particulate fractions were suspended in 0.05 ml H_2O and mixed with 0.5 ml of *n*-butanol. After vigorous stirring for 15 min, 0.5 ml H_2O was added, the mixture stirred, and centrifuged at $10,000 \times g$. The clear butanol layer was removed from the tube, and the interface and aqueous layers were extracted 4 times with 0.5-ml portions of butanol. The combined butanol extracts were counted and represent the glycolipid fraction described in the text. The remaining aqueous and interface layers were adjusted to 0.5 ml with H_2O and centrifuged; the pellet was washed with 0.5 ml of water and discarded (this fraction contained less than 3% of the C^{14}-polysaccharides). The combined aqueous layers were dialyzed against distilled water for 24 hr, electrodialyzed for 4 hr (at this time the conductivity of the dialysate was the same as that of distilled water), and the resulting, slightly turbid mixture was briefly centrifuged. The precipitate contained essentially all of the ultraviolet-light-absorbing material, but no C^{14}. The C^{14} concentrations of the clarified solutions are given above and are essentially equal to the C^{14} present in the aqueous phase after butanol extraction. Aliquots of the final solutions were used for the turbidimetric and double diffusion studies described in the text. Samples were also treated with excess antiserum, and the precipitates and supernatant fluids counted; the C^{14} content of samples 5 and 6 were too low for accurate assay, but more than 90% of the C^{14} in samples 1–4 was precipitated by the antiserum.

† Aliquots of the aqueous fractions were hydrolyzed with 2 *N* H_2SO_4 for 3 hr at 100°, and the C^{14}-acetate was steam-distilled,[35] collected, and counted; the C^{14}-acetate represents the N-acetylhexosamine incorporated into the polysaccharide. Samples of the polysaccharide fractions were also hydrolyzed with 2 *N* HCl for 3 hr at 100°, followed by drying *in vacuo* to remove the acetate, the residual hexoses separated by paper electrophoresis in 2% Na tetraborate, and their C^{14} content was determined. The ratios shown here, N-acetylglucosamine, glucose, galactose, 1:1:2 are identical to those obtained in a separate experiment using UDP-N-acetyl-galactosamine-U-C^{14} as substrate in place of the C^{14}-acetyl UDP-N-acetylglucosamine. Values were not determined on the samples shown as blank spaces in the table; the value 0 means not detectable (less than 100).

borate buffer,[29] chromatography in two solvent systems,[29] and by reduction with borohydride to the hexitols which were characterized by the same methods.[29]

Despite the similarities, important differences were noted between the C^{14}-polysaccharides and standard S XIV. While the ratio of monosaccharides reported for S XIV is N-acetylglucosamine, 2; glucose, 1; galactose, 3,[30] the ratio of C^{14} sugars incorporated into the polymer fraction was 1, 1, 2. When UDP-N-acetylhexosamine was omitted from the incubation mixture, the resulting polymer contained C^{14}-glucose and C^{14}-galactose in a ratio of 1,1 (Table 1). Immunochemical differences were also detected and are described below.

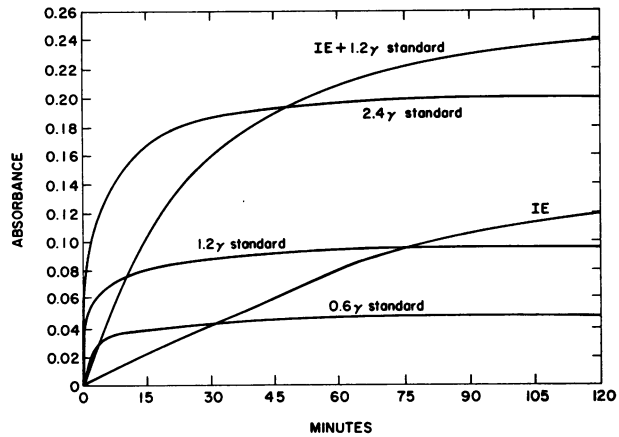

FIG. 1.—Rate of reaction between standard S XIV and rabbit antiserum as measured by turbidity formation. *IE* represents 0.025 ml of the incubated, endogenous polysaccharide preparation isolated from tube 6, Table 1.

Precipitation and diffusion: The preparations discussed below were processed through the electrodialysis step described in the table, their C^{14} contents were determined, and they were further examined by the turbidimetric and double diffusion methods. The polysaccharides were isolated from particulate systems treated as follows: no further treatment (unincubated endogenous, E); after incubation for 2 hr alone or in the presence of UDP-N-acetylglucosamine (tubes 5, 6, Table 1; incubated endogenous, IE); after incubation with the UDP-hexoses (tube 4, Table 1; incomplete incubation mixture, IM); after incubation with the three UDP-monosaccharide derivatives (tubes 1–3, Table 1; complete incubation mixture, CM).

As shown in Figure 1, turbidities varied directly with concentration of standard S XIV. When the latter was added to the incubated endogenous sample, values about 10 per cent higher than theoretical were obtained.

Turbidities given by the polysaccharide preparations are shown in Figures 1 and 2, and indicate: (*a*) The sample from the complete incubation mixture gave about twice the turbidity of the controls. (*b*) The sample from the mixture with UDP-hexoses but no UDP-acetylglucosamine gave a slight but reproducible increase in turbidity above the controls.

Conversion of turbidity values to concentrations of antigenic polysaccharides by use of the standard curve suggested "net synthesis," and agreed within 20 per cent with the values obtained by C^{14}-incorporation. However, this interpretation can be questioned since turbidity is affected by factors[21] such as molecular size and shape, multiplicity of antibodies, antigenic sites on the molecule, etc. Also, the turbidities obtained with standard S XIV rapidly reached constant values, while the enzymatic products gave turbidities that gradually increased with time, a

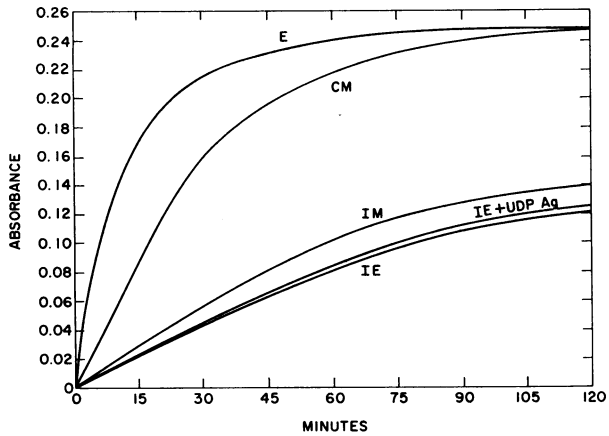

FIG. 2.—Rate of reaction between polysaccharides and rabbit antiserum. The polysaccharides were isolated from the incubation mixtures given in Table 1: *CM*, complete incubation mixture (polysaccharides from tubes 1, 2, or 3 gave the same results); *IM*, incomplete incubation mixture (UDP-N-acetylglucosamine omitted from the complete incubation mixture, tube 4); *IE* + UDP-Ag, polysaccharide isolated from incubation mixture 5; *IE*, incubated endogenous (tube 6). Sample *E* was obtained directly (i.e., without incubation) from the particulate enzyme preparation by the same method. Aliquots, 0.025 ml, were assayed in each case except *E*, where 0.005 ml was used.

phenomenon suggestive of cross reactions. The slow reaction was particularly marked with preparations obtained from the incubated endogenous and incomplete incubation mixtures.

The turbidity data also show another characteristic of the system. The particulate preparation initially contained a large quantity of antigenic polysaccharide that could not be removed by washing with buffer but was solubilized with butanol. Kinetically, in the turbidimetric procedure, it behaved like standard S XIV, but at least 90 per cent disappeared from *the particles* during the 2-hr incubation at 37° alone or in the presence of UDP-N-acetylglucosamine. Preliminary experiments suggest that this behavior was not due to partial hydrolysis of the polysaccharide by a simple hydrolase (like a β-galactosidase) that would alter its antigenic properties, but rather that the S XIV was solubilized and appeared in the supernatant fluid.

The differences between the samples were most convincingly shown by double diffusion (Figs. 3 and 4). All preparations showed two bands differing in their rates of migration; the more slowly migrating substance(s) is called *A* while the rapidly migrating substance(s) is called *B*. The difference between various samples was in the relative concentrations of *A* and *B*. Standard S XIV showed only a trace of *B*. Similarly, the unincubated, particle-bound, endogenous material showed a small quantity of *B*, but consisted mainly of *A* that formed lines of identity with standard S XIV. Upon incubation, the *A*-band of the endogenous samples largely disappeared, resulting in particle-bound polysaccharide in which *A* and *B* appeared almost equal. With the incomplete mixture (i.e., in the presence of UDP-hexoses), a slight but significant increase was noted in band *B*. Finally, a large increase in band *B* was observed in the sample from the complete incubation mixture.

The double diffusion studies therefore substantiated and extended the results obtained with the turbidimetric and isotopic methods. The synthetic C^{14}-polysaccharides differed from the standard S XIV. In contrast, the endogenous, particle-bound (not incubated) material largely resembled the standard, and almost disappeared from the particulate matter during the incubation; other experiments indicate that it was solubilized (degraded?) during the 37° incubation. The presence of sugar-nucleotides during the incubation did not qualitatively affect the solubilization (degradation?).

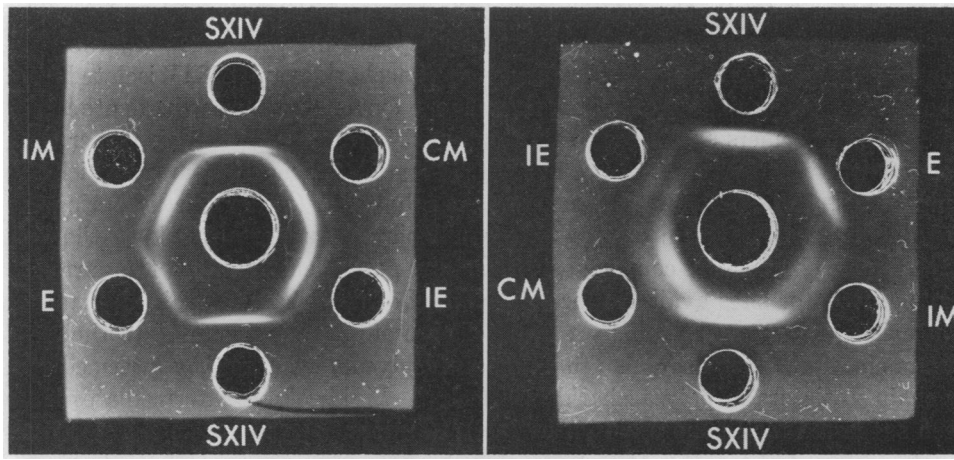

FIG. 3.—Double diffusion with horse antiserum. Conditions are given in the text. S XIV represents standard Type XIV polysaccharide; a solution containing 0.1 mg per ml was added to the indicated wells. The other polysaccharides are those used in Fig. 2.

FIG. 4.—Double diffusion with rabbit antiserum. Conditions as in Fig. 3, except that absorbed rabbit antiserum was added to the center well.

The butanol-soluble fraction: As noted above, when the particulate preparation was incubated with UDP-hexose-C^{14}, but not UDP-N-acetylglucosamine-C^{14}, most of the radioactive product was butanol-soluble (Table 1). The C^{14}-products in this fraction appear to be glycolipids that did not react with antibody, could not be extracted from the butanol phase with water, dilute $NaHCO_3$, or dilute HCl, and were essentially insoluble in water, but soluble in many organic solvents. After acid hydrolysis, the only detectable C^{14}-compounds were glucose and galactose. Fractionation of the butanol-soluble material on silicic acid columns by a method used for glycolipids[31] gave two clearly separable peaks. Fraction I, eluted with chloroform/methanol, 95/5, gave C^{14}-glucose as the only C^{14} product after acid hydrolysis, while Fraction II, eluted with chloroform/methanol, 90/10, gave primarily C^{14}-galactose and a small amount of C^{14}-glucose.

Discussion.—The data show that a particulate fraction from Type XIV pneumococcus catalyzes the incorporation of N-acetylglucosamine, glucose, and galactose into antibody-reactive polysaccharide(s). The product(s) is, however, distinguishable from S XIV isolated from whole cells and from the particulate fraction prior to incubation at 37°.

The complete incubation mixture contained substantially more antibody-reactive polysaccharide than did the controls. These results could be obtained if the enzyme system catalyzed the addition of a few antigenically active di- or oligosaccharide units to the outer branches of endogenous, antigenically inactive polysaccharide; there would be no significant increase in the total quantity of polymer. Alternatively, the antibody-reactive polysaccharide may have been substantially increased by adding many monosaccharide units to endogenous polysaccharide and/or by initiation and building of new chains. Experiments are in progress to resolve this question.

The particulate enzyme system is complex and contains contaminating enzymes like UDP-glucose-4-epimerase that must be removed in order to search for possible

intermediates between the UDP-monosaccharides and the polysaccharides. The results suggest experimental approaches to the following problems: (*a*) the relationship between the enzymatically formed polysaccharides and S XIV isolated from whole cells; (*b*) the possibility that the enzyme system catalyzes the synthesis of a family of polymers analogous to that obtained in glycogen synthesis rather than a monodisperse polymer as in protein synthesis; (*c*) the mechanism by which the endogenous, particle-bound polysaccharide is solubilized (or degraded?) during the 37° incubation (A system of this type could play an important role in the biosynthetic mechanism by continuously removing polysaccharide from the particles, leaving acceptor sites available for the building of new chains. This mechanism, if it exists, suggests a means by which cells secrete large polymers into their environment.); (*d*) the nature and function of the glycolipids (Are they intermediates or chain initiators in polysaccharide synthesis? Lipid-soluble intermediates have been suggested for cellulose biosynthesis.[32]); (*e*) does polysaccharide chain growth involve sequential transfer of monosaccharide units as in the case of the lipopolysaccharide of Salmonella[33] and *E. coli* O-111,[34] or does it involve transfer of larger units from compounds like the UDP-oligosaccharides?[14-16]

The expert technical assistance of Mr. Alan Jacobs in some of these studies is gratefully acknowledged.

* The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies of The University of Michigan. This investigation was supported in part by grants from the National Institute of Arthritis and Metabolic Diseases (AM 00512-10), National Institutes of Health, and the American Cancer Society.

† Predoctoral trainee, Michigan Chapter, Arthritis and Rheumatism Foundation.

[1] Roseman, S., *Federation Proc.*, **21**, 1075 (1962).

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

[3] Stacey, M., and S. A. Barker, *Polysaccharides of Micro-Organisms* (London: Oxford Press, 1960).

[4] Barker, S. A., M. Heidelberger, M. Stacey, and D. J. Tipper, *J. Chem. Soc.*, 3468 (1958).

[5] Barker, S. A., M. C. Keith, and M. Stacey, *Nature*, **189**, 746 (1961).

[6] Heidelberger, M., *J. Am. Chem. Soc.*, **77**, 4308 (1955).

[7] Watkins, W. M., and W. T. J. Morgan, *Nature*, **178**, 1289 (1956).

[8] Kabat, E. A., *Arch. Biochem. Biophys., Supplement 1*, 181–186 (1962).

[9] Yoshizawa, Z., *Tohoku J. Exptl. Med.*, **52**, 103 (1950).

[10] Rege, V. P., T. J. Painter, W. M. Watkins, and W. T. J. Morgan, *Nature*, **200**, 532 (1963).

[11] Spiro, R. G., *J. Biol. Chem.*, **237**, 646 (1962).

[12] Eylar, E. H., and R. W. Jeanloz, *J. Biol. Chem.*, **237**, 622 (1962).

[13] Kuhn, R., A. Gauhe, and H. H. Baer, *Chem. Ber.*, **87**, 289 (1954).

[14] Jourdian, G. W., F. Shimizu, and S. Roseman, *Federation Proc.*, **20**, 161 (1961); *Ann. N. Y. Acad. Sci.*, **106**, 202 (1963).

[15] Kobata, A., *Biochem. Biophys. Research Commun.*, **7**, 346 (1962).

[16] Kobata, A., *J. Biochem. (Japan)*, **53**, 167 (1963).

[17] Mills, G. T., and E. E. B. Smith, *Federation Proc.*, **21**, 1089 (1962).

[18] Roseman, S., J. J. Distler, J. G. Moffatt, and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 659 (1961).

[19] Carlson, D. M., A. L. Swanson, and S. Roseman, *Biochemistry*, **3**, 402 (1964).

[20] Crowle, A. J., *Immunodiffusion* (New York: Academic Press, Inc., 1961).

[21] Kabat, E. A., and M. M. Mayer, *Experimental Immunochemistry* (Springfield, Ill.: Charles C Thomas, 1961), 2nd ed.

[22] Bernheimer, A. W., *J. Exptl. Med.*, **97**, 591 (1953).

[23] Maxwell, E. S., K. Kurahashi, and H. M. Kalckar, in *Methods in Enzymology*, ed. S. P. Colowick and N. O. Kaplan (New York: Academic Press, Inc., 1962), vol. 5, p. 174.

[24] Maley, F., and G. F. Maley, *Biochim. et Biophys. Acta*, **31**, 577 (1959).
[25] Glaser, L., *J. Biol. Chem.*, **234**, 2801 (1959).
[26] Liu, T., and E. C. Gotschlich, *J. Biol. Chem.*, **238**, 1928 (1963).
[27] Gardell, S., *Acta Chem. Scand.*, **7**, 207 (1953).
[28] Jourdian, G. W., and S. Roseman, *J. Biol. Chem.*, **237**, 2442 (1962).
[29] Whistler, R. L., and M. L. Wolfrom, eds., *Methods in Carbohydrate Chemistry* (New York: Academic Press, Inc., 1962), vol. 1.

[30] The ratios reported in the literature are apparently different. Goebel *et al.*[2] found a ratio of glucosamine to hexose, 1:3, by colorimetric analysis of hydrolysates; the hexose was thought to be only galactose. Barker *et al.*[4] methylated and hydrolyzed the polysaccharide, isolated the methylated products, and concluded that the ratio of N-acetylglucosamine, glucose, galactose was 2:1:3. Preliminary studies in this laboratory, involving specific enzymatic assays for glucose and galactose and a colorimetric method for glucosamine, suggest that the ratios in Dr. Kabat's preparation are glucosamine, glucose, galactose, 1, 0.82, 1.7, but are subject to revision since analysis of the hydrolysates was unexpectedly difficult. When comparing the ratios of sugars in the polysaccharides, it is important to note that the material from whole cells is sometimes isolated after lengthy autolysis of the bacteria, a step that may result in significant degradation.

[31] Rapport, M. M., H. Schneider, and L. Graf, *J. Biol. Chem.*, **237**, 1056 (1962).
[32] Colvin, J. R., *Nature*, **183**, 1135 (1959).
[33] Osborne, M. J., S. M. Rosen, L. Rothfield, and B. L. Horecker, these PROCEEDINGS, **48**, 1831 (1962); *Federation Proc.*, **22**, 465 (1963).
[34] Edstrom, R. D., A. D. Elbein, and E. C. Heath, *Bacteriological Proceedings* (1963), p. 82.
[35] Wiesenberger, E., *Microchem. Acta*, **53**, 51 (1948).

ERRATA

In the article entitled "Inferences Concerning the Tertiary American Bird Faunas," by Ernst Mayr, which appeared in the February issue of volume 51 (1964), pp. 280–288, the penultimate sentence in the legend to Figure 1 (p. 284) should read, "On both maps, the first figure gives the number of genera found breeding in the area, and the second figure the number of species," rather than "Map A gives the number of genera now found breeding in the area, and Map B the number of species."

On page 286, seven lines from the bottom of the page, the reference should be to the Fifth Edition of *The A. O. U. Checklist*, rather than to the Fourth Edition.

In the article entitled "Effect of Chemical Control of Stomata on Transpiration and Photosynthesis," by Israel Zelitch and Paul E. Waggoner, which appeared in the July issue of volume 48 (1962), pp. 1101–1108, equation (3) on page 1103 should have been

$$S = [4l/(\pi ab) + 1/\sqrt{ab}] \div n,$$

where a and b are the mean width and length of the stomata.

The correction affects the relations between $1/T$ and $1/P$, the reciprocals of the transpiration and photosynthesis rates, and S, the stomatal diffusion length (Figs. 1 and 2, p. 1107). The S becomes about 3 times as great and the slopes of the regression lines $^1/_3$ of those shown. The fit of the lines to the data is not materially changed (r^2 is increased by only 0.01). The diffusion lengths L and M are estimated