rivative. After adsorption on silicic acid, the product was eluted with mixtures of ether and ethyl acetate and mixtures of ethyl acetate and acetone. Crystallization from a mixture of acetone, ether and pentane gave 25 mg. (68%); m.p. 152–153°, $[\alpha]^{27}D$ +90 ± 2° (in chloroform, *c* 0.90). *Anal.* Calcd. for $C_{20}H_{27}O_{10}NS$: C, 50.73; H, 5.75. Found: C, 50.60; H, 5.84.

Methyl 2-Acetamido-2-deoxy-4,6-di-*O*-methyl-3-*O*-*p*-tolylsulfonyl-α-D-galactopyranoside (IV).—Four hundred and eighty mg. of III was methylated with methyl iodide and silver oxide as previously described.[3] Purification was carried out by chromatography on silicic acid. Mixtures of ethyl acetate and acetone eluted 490 mg. (95%) of sirup (IV); $[\alpha]^{27}D$ +89 ± 2° (in chloroform, *c* 1.17). *Anal.* Calcd. for $C_{18}H_{27}O_8NS$: C, 51.78; H, 6.52; OCH_3, 22.30. Found: C, 51.76; H, 6.58; OCH_3, 22.49.

Methyl 2-Acetamido-2-deoxy-4,6-di-*O*-methyl-α-D-galactopyranoside (VII).—To a solution of 610 mg. of sirupy IV in 30 ml. of 90% methanol was added 9 g. of 2.5% sodium amalgam. After shaking overnight, the mixture was diluted with 20 ml. of water, neutralized with CO_2, filtered and evaporated to dryness *in vacuo*. The residue was extracted with chloroform and filtered. After concentration to dryness, crystallization from a mixture of methanol and ether afforded 341 mg. (89%) of fine needles; m.p. 227–229°, $[\alpha]^{29}D$ +141 ± 2° (in methanol, *c* 0.79). *Anal.* Calcd. for $C_{11}H_{21}O_6N$: C, 50.18; H, 8.04; OCH_3, 35.36. Found: C, 49.84; H, 8.65; OCH_3, 35.13.

Thirty-six mg. of VII was methylated with methyl iodide and silver oxide as previously described. After crystallization from a mixture of acetone and pentane, the theoretical yield of methyl 2-acetamido-2-deoxy-3,4,6-tri-*O*-methyl-α-D-galactopyranoside (VIII) was obtained. The product melted at 191–192° and did not depress the m.p. in admixture with authentic material[4]; $[\alpha]^{28}D$ +147 ± 2° (in methanol, *c* 1.00).

Acetylation of 38 mg. of VII with acetic anhydride and pyridine in the usual way gave the **3-*O*-acetyl derivative.** Crystallization from a mixture of acetone, ether and pentane gave 26 mg. (60%); m.p. 111–112°, $[\alpha]^{28}D$ +106 ± 2° (in chloroform, *c* 0.94). *Anal.* Calcd. for $C_{13}H_{23}O_7N$: C, 51.14; H, 7.59. Found: C, 51.32; H, 7.69.

4,6-Di-*O*-methyl-α-D-galactosamine Hydrochloride (2-Amino-2-deoxy-4,6-di-*O*-methyl-α-D-galactose Hydrochloride) (VI).—A solution of 145 mg. of VII in 3 *N* hydrochloric acid was treated as previously described.[3] The residual sirup was crystallized from a mixture of acetone and methanol, affording 112 mg. (84%) of small square platelets, decomposing at 190°. The compound showed mutarotation from $[\alpha]^{26}D$ +107° (after 5 minutes) to $[\alpha]^{26}D$ +91 ± 2° (after 48 hours in water, *c* 1.02). *Anal.* Calcd. for $C_8H_{18}O_5NCl$: C, 39.43; H, 7.44; OCH_3, 25.47; Cl, 14.55. Found: C, 39.56; H, 7.57; OCH_3, 25.51; Cl, 14.65.

2-Deoxy-2-(2′-hydroxynaphthylideneamino)-4,6-di-*O*-methyl-D-galactose (V).—The preparation was carried out on 59 mg. of VI as previously described.[3] Purification was obtained by chromatography. Elution with mixtures of ethyl acetate and acetone gave 32 mg. (39%) of yellow microcrystals, m.p. 183–186°, $[\alpha]^{26}_{5461}$ +223 ± 3° (at the equilibrium in methanol, *c* 0.62). *Anal.* Calcd. for $C_{19}H_{23}O_6N$: C, 63.14; H, 6.41. Found: C, 63.01; H, 6.37.

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[CONTRIBUTION FROM THE DEPARTMENTS OF NEUROLOGY, MICROBIOLOGY AND BIOCHEMISTRY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, THE NEUROLOGICAL INSTITUTE, PRESBYTERIAN HOSPITAL AND THE BIOPHYSICS SECTION, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

Precipitation of C^{14}-Labeled Dextran by Human Anti-dextran[1a]

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A large proportion of C^{14}-labeled clinical dextran was specifically precipitated by an excess of anti-dextran produced by the injection of 1 mg. of dextran into human beings. An unrelated specific precipitate of blood group A substance with human anti-A formed in the presence of C^{14}-labeled dextran did not carry down significant quantities of radioisotope.

Recent studies from this Laboratory[1b] and confirmed by Maurer,[1c] have demonstrated that purified dextrans are antigenic in man; injection of small quantities of dextran stimulates the production of precipitins and of skin sensitivity. In addition to other lines of evidence indicating that the precipitins formed were indeed antibodies to dextran and not to some contaminating substance, it was considered desirable to show that dextran itself was contained in the precipitate formed by the antidextran. The recent preparation[2] of highly purified dextran randomly labeled with C^{14} by biosynthesis from C^{14}-sucrose made it possible to establish that the antibody formed in humans injected with various dextrans[1b] specifically precipitated a substantial portion of the C^{14}-labeled dextran.

Materials and Methods

Human Antidextran.—Serum samples 20_{D-2} and 30_{D-2} from individuals who had been injected with 1 mg. of clinical Swedish dextrans OP155 and OP163, respectively, were available. The quantitative precipitin data on the reaction of these antisera with various native and clinical dextrans as well as with the C^{14} clinical dextran and C^{14}-dextran fractions have already been published.[1b]

C^{14}-Labeled Dextrans.—The C^{14}-labeled clinical dextran[2] Lot 21-2-Ci-L-D was obtained through the National Research Council. Fractions 1 and 9 of this C^{14}-dextran obtained by fractional precipitation with methanol were prepared at the National Bureau of Standards[3] and provided through the kindness of Drs. S. G. Weissberg and H. S. Isbell; fraction 1 had a number average molecular weight of 60,800, while fraction 9 had a number average molecular weight of 13,230.

Control Materials.—A sample of anti-A prepared by injection of hog blood group A substance into humans of blood group B[4] and a sample of purified hog blood group A substance (Hog 14)[5] were employed.

Procedure.—From the quantitative precipitin curves for the reactions of the C^{14}-dextran and dextran fractions with

(1) (a) This investigation was carried out in part under a grant (RG 34) from the National Institutes of Health, U. S. Public Health Service and under the William J. Matheson Commission.

(1) (b) E. A. Kabat and D. Berg, *Ann. N. Y. Acad. Sci.*, **55**, 471 (1952); *J. Immunol.* **70**, 514(1953); (c) P. H. Maurer, *Proc. Soc. Exp. Biol. and Med.*, **83**, 879 (1953).

(2) N. J. Scully, H. E. Stavely, J. Skok, A. R. Stanley, J. K. Dale, J. T. Craig, E. B. Hodge, W. Chorney, R. Watanabe and R. Baldwin, *Science*, **116**, 87 (1952).

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antiserum,[1b] suitable multiples of antiserum and antigen were selected to give an amount of specific precipitate in the region of excess antibody sufficiently small to be satisfactorily handled by the micro-quantitative precipitin technique and yet sufficiently large to make possible estimations of radioactivity; with serum 20_{D-2} this involved the use of six times and with serum 30_{D-2} ten times the quantities used for the quantitative precipitin curves. Amounts of anti-A and of A substance to give a comparable quantity of specific precipitate were used. Thirty-six or 40 μg. of dextran was added in quadruplicate to measured volumes of antiserum in 12-ml. conical centrifuge tubes, the contents of the tubes mixed, incubated at 37° for one hour and kept in the refrigerator for one week. The precipitates were centrifuged off in a refrigerated centrifuge and washed twice in the cold with 3.0-ml. portions of chilled saline. To avoid losing traces of precipitate, all supernatants were decanted and recentrifuged and given one additional washing[6] with chilled saline. For the control determinations 40 μg. of C^{14} clinical dextran was added to 5.0 ml. of anti-A serum. After the procedure described above a very small quantity of precipitate was isolated. To the supernatant 200 μg. of Hog 14A substance was added and the A anti-A precipitate obtained as above.

The specific precipitates and solutions containing similar quantities of C^{14}-dextran diluted with a suitable carrier containing comparable amounts of carbon were analyzed for C^{14} activity independently in two laboratories. At the College of Physicians and Surgeons, the combustion mixture was that described for general use by Van Slyke and Folch.[7] With the small samples being analyzed the blank due to the reagents was found to be appreciable and was eliminated by mixing the solid and liquid reagents in proper proportions just before use and heating to 150°. For the combustion the apparatus was suitably set up so that the CO_2 liberated was washed through two acid permanganate traps and collected in saturated barium hydroxide in a 15-ml. centrifuge tube. The sample of specific precipitate or of C^{14}-dextran plus a known weight of sucrose as carrier was placed in the apparatus and CO_2-free nitrogen was passed through the system for 15 minutes. With the barium hydroxide receiver in the apparatus, 5 ml. of the combustion mixture was then added without opening the system and the reaction vessel heated for 2–3 minutes. N_2 was passed through for another 15 minutes, the $BaCO_3$ was centrifuged, washed three times with water, suspended in a few ml. of ethanol and filtered directly on to a weighed 1 or 2 sq. cm. stainless steel filter plate, dried, the amount of $BaCO_3$ determined, and counted in an end window Geiger–Müller counter. All samples were counted for a sufficient time to give a statistical error of less than 10%.

At the Sloan-Kettering Institute, the specific precipitates and similar quantities of C^{14}-dextran diluted with unlabeled dextran as carrier were combusted by the Van Slyke method[7] collecting the CO_2 formed in sodium hydroxide. To avoid losses of specific precipitates the combustion apparatus was modified so that both tubes containing the washed specific precipitates were entirely immersed in the combustion mixture. The alkali containing the Na_2CO_3 was transferred to the carbonate conversion train and the liberated carbon dioxide was counted by internal gas counting using the method described by Eidinoff.[8] The results are expressed in disintegrations per minute per sample; calibration of the apparatus has been described.[9]

Results and Discussion

The data in Table I clearly show that most of the radioactivity of C^{14} clinical dextran and of the two dextran fractions was specifically precipitable by the samples of human anti-dextran. That this reaction is specific is established by the failure of C^{14}-dextran to react appreciably with human antibody to blood group A substance and by the failure of any appreciable quantity of radioactivity to be carried down non-specifically when A substance was used to precipitate the anti-A in the presence of quantities of C^{14}-dextran comparable to those used in the experiments with anti-dextran. The low intrinsic radioactivity[2] of the C^{14}-dextran available 5.68 μc./g. was not optimal for immunochemical experimentation. Despite this the assays in the two laboratories were generally in good agreement, the variation in the experiment with anti-dextran 30_{D-2} might be at about the limit of experimental error; under these circumstances it is

TABLE I

PRECIPITATION OF C¹⁴-DEXTRAN BY HUMAN ANTI-DEXTRAN

C^{14}-Dextran added, μg.	Total activity of C^{14}-dextran added	Antibody N pptd.,[h] μg.	Antibody carbon in ppt.,[a] μg.	C^{14}-Activity in ppt.	C^{14}-Dextran in ppt., %
6.0 ml. of anti-dextran serum 20_{D-2}					
C^{14}-dextran					
36	21,500[b]	225	774	12,600[b]	59
36	11,200[c]	225	774	6,900[c]	62
36	572[d]	225	774	399[d]	70
36	572[d]	225	774	389[d]	68
				Av.	65
Fraction 1					
36	16,640[b]	209	719	14,800[b]	89
36	10,650[c]	209	719	8,440[c]	79
36	528[d]	209	719	436[d,e]	83
				Av.	84
Fraction 9					
36	14,050[b]	212	730	11,500[b]	82
36	9,700[c]	212	730	5,770[c]	60
36	518[d]	212	730	287[f]	56
				Av.	66
5.0 ml. of anti-dextran serum 30_{D-2}					
C^{14}-dextran					
40	20,700[b]	332	1142	18,600[b]	90
40	11,600[c]	332	1142	12,100[c]	104
40	577[d]	332	1142	376[d]	65
40	577[d]	332	1142	374[d]	65
				Av.	86
5.0 ml. of anti-A + 40 μg. of C^{14}-dextran					
40	20,700[b]	2.2[g]	7.6	990[b]	4
40	577[d]	2.2	7.6	11[d]	2
				Av.	3
5.0 ml. of anti-A + 40 μg. of C^{14}-dextran + 200 μg. Hog 14 A substance					
40	20,700[b]	208	716	2430[b]	12
40	10,650[c]	208	716	0[c]	0
40	577[c]	208	716	*ca.* 40	7
40	577[d]	208	716	*ca.* 10	2
				Av.	5

[a] Carbon and nitrogen determinations on two samples of human γ-globulin, fractions II-1, 2 and II-3 by Dr. Adelbert Elek gave C/N ratios of 3.38 and 3.50, respectively. The average 3.44 was used to convert N to C. [b] Counts per minute per 2 cm.² at infinite thickness. [c] Counts per minute per 1 cm.² at infinite thickness. [d] Disintegrations per minute per sample. [e] An additional determination discarded. [f] One determination lost. [g] Diluted with sucrose as carrier for analysis. [h] Calculated from data in reference IIa.

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not possible to establish any immunological differences between the two dextran fractions 1 and 9 which differed in molecular weight. Despite these limitations, however, and in view of the high purity of the C^{14}-dextran used,[2] the data clearly establish that the precipitin produced by human beings in response to the injection of small amounts of dextran is indeed antibody to dextran.

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Streptothricin. I. Preparation, Properties and Hydrolysis Products[1]

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Streptothricin is a basic antibiotic with an empirical formula of about $C_{20}H_{34}N_8O_9$. On hydrolysis, it yields ammonia, carbon dioxide and three ninhydrin-positive components, none of which give significant ninhydrin–carbon dioxide values. One of these hydrolysis products has been identified as β,ϵ-diaminocaproic acid. A large-scale carbon chromatogram is described which conveniently affords pure sulfates or hydrochlorides of streptothricin or streptomycin.

Streptothricin is a basic antibiotic produced by *Streptomyces lavendulae*, and first isolated by Waksman and Woodruff[3] in 1942. Although of little clinical importance because of its nephrotoxicity,[4] streptothricin is of considerable interest due to its high antibacterial activity. A study of its structure and properties has been undertaken, therefore, and the present paper records initial data on the properties and degradation products of streptothricin. Fried and Wintersteiner[5] reported preliminary data on streptothricin, and later Peck, *et al.*,[6] prepared the crystalline helianthate and reineckate, reporting analytical data and properties for these salts.

The streptothricin used in these studies was prepared in the research laboratories of The Upjohn Company. Essentially pure sulfates of either streptothricin or streptomycin were obtained by chromatography of neutral aqueous solutions on columns of active carbon. Very dilute acetone (1%, v./v.) in water was found to be a suitably selective eluting agent for obtaining the pure antibiotic sulfates.

The crystalline helianthate and the amorphous hydrochloride were prepared and analyzed. The data obtained agree reasonably well with those for salts of a tribasic substance of empirical formula $C_{20}H_{34}N_8O_9$. The helianthate analyses approximate those of Peck, *et al.*, as do the rotation and the analyses of the hydrochloride. The tribasic nature of the antibiotic is also demonstrated by the titration data which indicate the presence of three groups with pK_a' values of 7.1, 8.2 and 10.1.[7] These data are not consistent with the presence of a free carboxyl group, and the pK_a' of 10.1 suggests the possible presence of a guanido group. Analyses disclosed the absence of O-methyl C-methyl and N-methyl groups.

The Van Slyke nitrous acid determination liberated 25% of the total nitrogen, whereas the ninhydrin–carbon dioxide value was essentially zero. Streptothricin showed only end-absorption in the ultraviolet and took up no hydrogen in the presence of platinum catalyst.

A study was made of the behavior of streptothricin toward a variety of color reagents and the results (Table I) are in agreement with the more limited data of Fried and Wintersteiner.

TABLE I

RESULTS OF COLOR TESTS WITH STREPTOTHRICIN

Test	Result	Test	Result
Benedict	+	Ninhydrin	+
Fehling	+	Sakaguchi	−
Tollens	+	Molisch	−
Neutral permanganate	+	Anthrone	+(weak)
Schiff	−	Elson–Morgan	+
Ferric chloride	−	Hopkins-Cole	−
Biuret	+	Millon	−
Pauly	+		

The negative Sakaguchi test is not consistent with the presence of a monosubstituted or asymmetrically disubstituted guanidine group. Thus, if the basic group of pK_a' 10.1 is a guanido group, it is not of the type present in streptomycin. The Pauly test did not give the typical color of an imidazole, and may be due to an active methylene group.

Because streptothricin gave positive Pauly, biuret and ninhydrin tests, and amino groups were liberated during hydrolysis, a peptide structure for this antibiotic seemed probable. The products of acid and alkaline hydrolysis were, therefore, investigated.

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