accord with the work of Keating and Albert (7) who state that following radioactive triiodothyronine treatment, there is a greater accumulation of I^{131} in the thyroid than after radioactive thyroxine treatment.

Summary. Antigoitrogenic tests in rats showed L-triiodothyronine to be about 3.5 times as active as L-thyroxine on a molar basis. In addition, in thyroidectomized rats, the L-triiodothyronine had a more potent calorigenic effect than L-thyroxine; in this case, the molar potency ratio was not less than 3.5:1. When the response was in the hyperthyroid range, the ratio was even greater than 3.5:1. Calorigenic tests in normal, intact rats showed a somewhat different effect in that both the triiodothyronine at a 1:1 molar dosage ratio and the triiodothyronine at 1:3.5 molar dosage ratio (triiodothyronine:thyroxine) produced changes not too far different from that obtained with the thyroxine.

The 3:5:3′-L-Triiodothyronine used in this study was prepared by Glaxo Laboratories Ltd., England.

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Dextran, an Antigen in Man.* (20521)

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Kabat and Berg have recently reported(1)† that various dextran preparations are antigenic in man. Quantitative immunochemical studies were presented on the reaction of various native and partially hydrolized dextrans with homologous human antidextran sera. As the implications of these original findings to the use of dextran as a blood substitute are manifold, it was agreed to have the results checked independently in another laboratory. Dextrans have been shown to cross react with types II, XII, and XX horse and rabbit antipneumococcal sera(2-4). Therefore, it is conceivable that the individuals who had high concentrations of precipitins and showed skin sensitivity to dextran may have produced antibodies to a cross reacting pneumococcal polysaccharide. A complete analysis for antibodies to these polysaccharides was performed in both the pre- and post-immunization sera.

The persistence of antibodies to many pneumococcal polysaccharides has been shown by Heidelberger, *et al.*(5-8). However, the pneumococcal polysaccharides(9), in contrast to the dextrans(10,11), are not metabolizible and may persist for some time. It was of great interest, therefore, to see what the concentration of antibodies against a metabolizible polysaccharide would be a year after the antigen was injected.

Experimental dextrans. Five samples of native dextran were used. Three of these, B 1254, B 742, and B 512, were obtained from the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill.

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TABLE I. Some Chemical and Physical Properties of Dextran Samples.

Dextran sample	Source	N, %	Branching ratio, 1:6 linkages / non 1:6	Molecular wt
		Native		
B 1254	NRRL*	<.01	11	
B 742	NRRL	<.01	1.9	
B 512	NRRL	.01	21	
3079 (Swedish)	NRC†	.05	7.1	95000000
N 279	CSC‡	.018	18	1940000
		Clinical		
B 742	NRRL	<.01	1.9	
OP 163 (Swedish)	NBS§		7.1	28300
R 229	CSC	.005	32-49	53000
258 A 7‖	CSC	.009	32-49	83000

Analytical data obtained from source:
* Northern Regional Research Laboratory.
† National Research Council.
‡ Commercial Solvents Corp.
§ Nat. Bureau of Standards.
‖ Not a routine clinical dextran preparation. Prepared for special test purposes for the NRC.

through the courtesy of Mr. C. E. Rist and Dr. A. Jeanes. These are dextrans with different degrees of branching, the B 1254 from *Streptococcus dextranicum* and the other two from strains of *Leuconostoc mesenteroides*. Dextran sample number 3079 was a native preparation obtained from Pharmacia Co. through the National Research Council. Sample 279 was a native dextran supplied by Drs. F. Schulz and M. E. Bachman of Commercial Solvents Corp. This was prepared from the NRRL B 512 strain and similar to sample B 512. Four samples of clinical dextran were available. Swedish sample OP 163 was obtained from Dr. E. A. Kabat. Samples R 229 and 258 A 7 were supplied by Commercial Solvents Corp. B 742 was obtained from the Northern Regional Research Laboratory. A summary of chemical and physical properties of some of the dextrans is given in Table 1 of reference 1. The properties of dextrans used in this study are given in Table I. Samples of C polysaccharide (S 146) from a type I pneumococcus, and the type specific pneumococcal polysaccharides from types II (Squibb) and type XII (Squibb 115 A) were kindly supplied by Dr. M. Heidelberger. Type XX pneumococcal polysaccharide was obtained through the courtesy of Dr. Rachel Brown of the New York State Department of Health.

Analytical solutions of dextran were prepared by dissolving samples dried to constant weight over P_2O_5 *in vacuo*. Solutions were kept with a drop of chloroform. For injections, suitable dilutions were made in 0.85% saline containing 0.25% phenol and tested for sterility.

Immunization procedure. Healthy medical student volunteers were used. The procedures followed for pre-immunization bleedings, intracutaneous skin testing, injections, post-immunization bleedings, and skin testing were those outlined in reference 1. All serum samples were handled with sterile precautions and in addition 'Merthiolate' (0.01%) and phenol (0.25%) were added.

Quantitative precipitin studies. The pre- and post-immunization serum samples were assayed for antibody nitrogen by the quantitative precipitin method of Heidelberger and MacPherson(12). A complete description of the procedure is in reference 1. Results are given in μg antibody nitrogen precipitated per ml of serum although 3 ml samples were used for analysis (Table II). Quantitative precipitin curves were obtained with sera obtained from individuals who had shown the best antibody response. Their sera, both from pre- and post-immunization bleedings were analyzed for antibody N against C polysaccharide from type I pneumococcal poly-

TABLE II. Response of Humans to Injection of Dextran (1 mg).

Dextran sample used	Subject	Nitrogen precipitable by dextran from serum: Pre-immunization, μg N/ml	Nitrogen precipitable by dextran from serum: Post-immunization, μg N/ml	Cutaneous reaction: Pre-immunization	Cutaneous reaction: Post-immunization
CSC clin. R 229	1	—	.5	—	—
	2	—	0	—	—
	3	—	.1	—	—
	4	—	0	—	—
	5	—	0	—	—
	6	—	.1	—	—
	7	—	.5	—	—
	8	—	1.6	—	—
	9	—	.1	—	—
	10	—	.7	—	—
	11	—	0	—	—
	12	—	.1	—	—
	13	—	0	—	—
CSC clin. 258 A 7	14	.3	10.7	—	++++
	15	.1	12.1	—	++++
	16	.4	9.6	—	+++
	17	.6	.4	—	—
	18	.0	.3	—	—
	19	.1	.8	—	—
CSC native N 279	20	1.2	3.5	—	—
	21	1.8	7.1	—	+++
	22	0	0	—	—
	23	0	.3	±	—
	24	0	2.2	—	—
Swedish clin. 163	25	.4	9.9	—	++
	26	.2	24.0	—	++++
	27	0	.8	—	—
	28	.1	1.1	—	—
	29	.2	6.6	±	+
	30	.2	3.1	—	—
Swedish 3079	31	0	1.4	—	+
	32	0	1.5	±	+++
	33	0	0	+	—
	34	1.5	17.3	+	++++
	35	1.9	2.4	—	—
	36	1.6	13.1	+±	++++
NRRL B 742 (clin.)	37	0	0	—	—
	38	.3	4.8	—	—
	39	.2	.9	—	—
	40	.4	.7	—	—
	41	.2	.3	—	—
NRRL B 742 (native)	42	.4	.4	—	—
	43	3.0	3.9	+++	++
	44	5.1	8.3	+++	++++
	45	2.2	2.8	+	++
NRRL B 1254 (native)	46	1.4	.8	—	++
	47	.5	.9	+	+++
	48	0	.6	—	—
	49	.5	4.4	+++	++++
	50	.8	.9	—	++
NRRL B 512 F (native)	51	3.1	21.8	+++	++++
	52	.5	3.7	—	+++
	53	1.6	9.2	+	++++
	54	.3	.7	+	+
	55	.6	1.1	—	—

saccharide and pneumococcal polysaccharides, types II, XII, and XX. The methods for analysis were described by Heidelberger *et al.*(5).

Results. Table II summarizes the results obtained in 55 individuals immunized with the various dextrans. They agree well with those reported by Kabat and Berg. A substantial number of volunteers showed rises in nitrogen precipitable by dextran in the post-immunization serum as compared with that in the pre-immunization sample. There was no significant reaction in any of the 13 volunteers to the CSC clinical dextran R 229. Although this would seem to indicate that this preparation is non-antigenic, it is quite possible that these individuals are not "reactors." Kabat and Berg encountered a similar observation and found their group of nonreactors to CSC clinical dextran to be nonreactors to a second course of CSC clinical dextran and also to CSC native dextran. The reaction noted in 3 of 6 volunteers given the CSC clinical dextran 258 A 7 does not mean that CSC clinical dextran is highly antigenic. This preparation was not a regular clinical preparation but was prepared for special test purposes for the National Research Council. As can be seen from Table I, it has a higher average molecular weight than that of the CSC R 229. Three out of 5 students injected with CSC native 279 showed a significant increase in antibody N in the post-immunization sera. As discussed by Kabat and Berg, an increase of 2 μg or more in precipitable nitrogen per ml from the pre- to post-immunization sample is considered significant. From the rest of Table II it is evident that there are good reactors to all the remaining dextran preparations. It is also of interest that 3 of the 4 individuals injected with the most highly branched native dextran, B 742, had relatively high levels of precipitins in their serum before immunization and not much higher levels after immunization. The presence of significant precipitin levels to NRRL B 742 native dextran in most human sera (about 3-5 μg antibody N per ml) has been confirmed in about 250 sera received from Army volunteers(13).

Comparison of the post-immunization precipitin levels with the skin tests showed that 19 out of 21 individuals with 2 μg antibody N per ml or more had positive skin tests and of the 36 individuals, with antibody levels less than 2 μg N per ml, 31 were negative or $\pm$. This difference is highly significant as pointed out in(1). Furthermore, it is evident from Table II that a definite antibody response on immunization was accompanied by the appearance of cutaneous erythema and wheal reactions in individuals who had negative or $\pm$ skin tests before immunization. In those cases where initial sensitivity existed, an increase in antibody level was usually accompanied by an increased skin reaction.

As discussed by Kabat and Berg, the number of individuals injected with each dextran is not sufficient to permit inferences to be drawn as to the relative antigenicity of each dextran sample.

Results obtained on the complete quantitative precipitin curves from the best anti-dextran sera will not be reported. Suffice to say that a summary of the observations reported here are similar to those reported in (1). 1—Native dextrans precipitated more antibody that the clinical dextran fractions. 2—Inhibition of precipitation by excess antigen required smaller quantities of the clinical dextran than of the native dextrans. 3—Among the native dextrans, B 742, which was the most highly branched, required much larger quantities to precipitate equivalent amounts of antibody nitrogen than used for other native dextrans.

The values presented in the last 2 columns in Table III indicate that there is not much difference in the anti-dextran antibody nitrogen level before or after absorption with the various cross reacting pneumococcal polysaccharides. It is also significant to note that immunization with the dextran did not cause much of a change in the antibody levels to the specific pneumococcal polysaccharides. Table IV presents the data showing that significant amounts of antibodies to the various dextrans in some of the sera persist one year after immunization.

Discussion. The data presented clearly confirm the original findings of Kabat and Berg that the injection of small amounts (1 mg) of native or clinical dextran into hu-

TABLE III. Micrograms Antibody N Precipitated per ml of Serum from Medical Volunteers Receiving Injections of Various Dextrans.

Subject No.	Inj. with dextran	Bleeding	Anti- CI	SII	SXII	SXX	Anti-dextran after absorption with pneumococcal polysaccharides	Anti-dextran before absorption with pneumococcal polysaccharides
15	CSC clin. 258	Pre-immunization	2.0	1.4	2.6	.0	.4	.1
	A 7	Post "	2.2	2.9	1.3	.1	10.7	11.5
21	CSC native N 279	Pre "	.3	1.1	.9	1.0	.2	1.8
		Post "	1.2	1.4	.7	1.0	5.9	6.0
26	Swedish clin. OP	Pre "	1.6	1.4	.8	.0	.5	.2
	163	Post "	1.0	.4	.4	.0	19.8	19.8
34	Swedish native	Pre "	.9	.5	.9	.5	1.1	1.5
	3079	Post "	1.0	1.3	.4	.6	14.9	16.2
38	NRRL B 742	Pre "	1.3	1.7	4.0	1.7	1.8	.3
	clin.	Post "	1.2	3.0	3.7	3.5	3.8	4.4
44	NRRL B 742	Pre "	1.5	.0	.6	.6	3.0	5.1
	native	Post "	1.3	.0	1.6	1.2	6.5	6.3
49	NRRL B 1254	Pre "	.7	2.8	.0	.0	.8	.5
		Post "	.5	2.9	.4	.7	4.1	4.1
51	NRRL B 512 F	Pre "	1.6	.0	1.2	.3	2.7	3.1
		Post "	1.2	.0	.0	.5	19.4	17.1

mans leads to the production of specific antibodies. It would appear then that the allergic reactions reported previously(14-16) are due to the antibodies to dextran. However, it should be borne in mind that the response of an individual or animal to a small dose of antigen is not necessarily the same as that elicited upon exposure to a massive infusion or infusions. The phenomenon of "immunological paralysis" which has been reported by other workers with polysaccharide antigens (17,18) cannot be overlooked. Experiments are now underway in our laboratory which would indicate that it is possible to fatigue the immune response in rabbits to bovine serum albumin and human serum albumin (19) by repeated massive injections of the protein solution. It is realized that inferences should not be drawn from studies on experimental animals to the human.

The possible explanation advanced by Kabat and Berg that individuals who showed precipitins and skin sensitivity to dextran may conceivably have produced antibodies to some cross reacting substance such as type II, XII, or XX pneumococcal polysaccharides has to be ruled out at least as far as the pneumococcal polysaccharides are concerned. This would be more evidence in favor of the conclusions advanced that the antibodies produced after injection of dextran are actually specific antibodies against the dextrans.

The persistence of antibodies to dextran (Table IV) parallels closely the persistence of antibodies to the pneumococcal polysaccharides. It has been shown and discussed (20) that "contrary to the behavior of antitoxin in the human subject which drops off to one-half or less during the second week period and continues to decrease at a slower rate, the type specific anti-carbohydrate in human sera remains level at or near the top for 5-8 months."

Explanations advanced for the difference in behavior are that toxins and toxoids being proteins are attacked by tissue enzymes whereas the pneumococcal polysaccharides are extremely resistant to attack. The persistence of antigen (polysaccharide) explains the production of antibody according to the Breinl-Haurowitz-Mudd-Alexander-Pauling group. According to Burnet, the presence of antigen is necessary only for the initial modification of the antibody producing mechanism. Antigen need not persist for antibody production to occur. A compromise between the 2 theories has been advanced by Heidelberger to explain both the anamnestic reaction observed with toxoid and not with polysaccharide antigen, and the persistence of antibody levels to

TABLE IV. Persistence of Antibodies in Human Sera after Injection of Various Dextrans.

Subject	Inj. with dextran	Date of bleeding	μg antibody nitrogen per ml serum
15	CSC clinical 258 A 7	5/28/52	12.1
		6/26/52	11.5
		2/ 9/53	9.0
21	CSC native	2/ 5/52	7.1
		2/25/52	6.0
		3/ 5/53	6.0
26	Swedish clinical	2/25/52	24.0
		3/18/52	19.8
		3/ 5/53	12.3
34	Swedish native	2/ 6/52	17.3
		2/25/52	16.2
		3/ 5/53	15.6
38	NRRL B 742 clinical	2/25/52	4.8
		3/19/52	4.4
		3/ 5/53	4.2
44	NRRL B 742 native	2/14/52	8.3
		3/20/52	6.3
		3/ 5/53	5.8
51	NRRL B 512 F	2/25/52	21.8
		3/19/52	17.1
		3/ 5/53	18.0

the polysaccharide antigen(20).

The persistence of antibodies to dextran after a year or so does not aid in the elucidation of this problem. It would appear that here, a metabolizible polysaccharide antigen injected in so small an amount (1 mg) should certainly be gone in the course of a few weeks and therefore lend support to the Burnet theory. However, Neill, Hehre, and coworkers(2) have shown that dextran is a contaminant of sugar samples and also that the human gastrointestinal tract contains dextran-producing organisms(21). In addition, several investigators have reported the presence of dextran in various tissues(22,23). It is quite possible that these factors may be providing a sufficient antigenic stimulus to keep the antibody level up.

Summary. It has been confirmed that native and clinical dextrans are antigenic in man. Three weeks after injection of 1 mg of dextran precipitins and cutaneous wheal and erythema sensitivity developed. The presence in human sera of antibodies to the pneumococcal polysaccharides which cross react with dextrans in other species (rabbit and horse) appears to have little to do with the reaction of humans to dextran. Antibodies produced after immunization with dextran have been shown to be specific for dextran. The persistence of antibodies to dextran has been shown.

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