Reactions of Dextrans with Antiserums of Rabbits Immunized with *S. typhi.** (19132)

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The use of partially hydrolyzed dextrans as plasma volume extenders makes it important to accumulate more complete information on the serological properties of dextrans in general. Previous reports have established that types 2, 20, and 12 pneumococcus antiserums cross react with both the native (unhydrolyzed)(1-9) and the clinical (partially hydrolyzed) products(10). The purpose of this paper is to extend the information on the cross-reacting capacities of these compounds by presenting data on the reactions obtained with antiserums of rabbits immunized with *S. typhi*. Zozaya(11) in 1932 reported cross reactions between a dextran and antiserums of various bacteria, including *S. typhi*. However, Zozaya's evidence is weakened by the facts that the reactions were obtained only with low dilutions of dextran, that only single samples of antiserum were used to represent the cross-reacting bacterial species, and that no controls were included on the possible dextran-reactivity of pre-immunization samples of serum. The validity of the *S. typhi* cross-reaction is established on a better basis by the data in this paper.

Materials. The samples of dextran were supplied by Dr. Edward J. Hehre. The 2 native dextrans (designated *A* and *B*) were prepared by the methods and from the strains of *Leuconostoc mesenteroides* described by Sugg and Hehre(1). The 2 clinical dextrans (designated *C* and *D*) had been prepared for use as plasma volume extenders by controlled acid treatment of native dextrans, and represent partially hydrolyzed products of lower molecular size than native dextrans; they were from different manufacturers and presumably different strains of leuconostoc bacteria had been used in the production process. All the serums were from rabbits. The pre-immunization samples were taken a few days before the first injection and the immune bleedings 6 days after the last injection of vaccine. The immunization consisted of 7 injections at 1 or 2 day intervals over a period of 10 or 11 days. The vaccines used will be indicated in the presentation of the data in Table I.

Experimental. Precipitation tests were made with the 4 dextrans in a series of dilutions ranging from 1:20,000 to 1:2 million against a series of dilutions of all the serums; the test mixtures (0.2 ml of dextran plus 0.2 ml of serum) were incubated 3 hours at 37°C and then stored in the icebox for 5 days. Complement fixation tests were made only with the clinical dextrans; the test mixtures comprised 0.2 ml each of dextran, serum, complement (2 units), and sensitized sheep r.b.c.; 2 hours at 37°C was allowed for the fixation and 1 hour at 37°C for the hemolysis test.

Results. As shown in Table I, the antiserums from all the rabbits immunized with

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TABLE I. Precipitation Reactions of *S. typhi* Antiserums in Tests Against Dextran Solutions.

Immunizing material	Rabbit No.	Highest dilution* of serum that gave precipitation with a series of dilutions of the dextran sol.											
		Native dextran A			Native dextran B			Clinical dextran C			Clinical dextran D		
		20000	200000	2000000	20000	200000	2000000	20000	200000	2000000	20000	200000	2000000
					Vaccines for human prophylaxis†								
S. typhi, S. paratyphi, A, B	1	40	40	10	40	20	10	10	10	—	U	U	U
	2	5	5	U	5	5	U	5	5	—	0	0	0
S. typhi	3, 4	10	10	5	10	10	5	5	5	—	U	U	0
	5-8	5-10	5	U-5	5-10	U-5	U	5	U-5	—	0	0	0
					Experimental vaccine‡								
S. typhi	9, 10	40-80	20-40	10	80	20	10	10	5-10	U	U	U	U
	11-14	10-20	10-20	5	10	5	5	5	U-5	0	U	0-U	0-U
					Normal serums								
Pre-immunization	2, 9-11	U	U	U-0	U	U	U-0	0	0	0	0	0	0
	Others	0	0	0	0	0	0	0	0	—	0	0	—

* U, undiluted serum; —, no test made; 0, negative reaction with undiluted and diluted serum.

† Bacterial cells from agar cultures, heated at 53°C for 1 hr; both vaccines contained 1 billion *S. typhi* per 1 ml plus 500 million each of *S. paratyphi A* and *B* in case of the polyvalent vaccine; Rabbit No. 1 received total of 6 ml; No. 2 and 3, 10 ml; No. 4, 16 ml; No. 5-8, 18-23 ml. (These vaccines were made by the Laboratories of the N. Y. City Department of Public Health and were kindly supplied to us by Dr. G. I. Steffen).

‡ Bacterial cells from broth cultures of the same strain (779) as in the human prophylactic vaccines, heated at 63°C 30 min.; 1 ml contained approximately 1 billion *S. typhi* per ml; all rabbits in this series received 16 ml, except No. 13, which received 3.5 ml.

S. typhi gave precipitation with the solutions of native dextrans *A* and *B*. There was considerable variation in potency among the antiserums from the different rabbits, which is to be expected in the case of any cross-reaction. However, all the antiserums gave precipitation in 1:5 dilution, and the most potent ones (No. 1,9,10) gave reactions in 1:20 or higher dilution against the 1:20,000 and 1:200,000 dilutions of dextran and in 1:10 dilution against 1:2,000,000 dextran. The fact that undiluted pre-immunization serums of 4 of the rabbits (No. 2, 9, 10, 11) precipitated the native dextrans illustrates the importance of including controls with normal serums, but does not invalidate the significance of the reactions obtained with the immune serums. That the latter reactions were due to antibodies evoked by immunization was shown by absorption experiments in which the capacity to react with the dextrans was removed by treatment of the antiserum with *S. typhi* bacterial cells.†

Both the clinical dextrans also gave precipitation with the typhoid antiserums.‡ But, as shown in Table I, dextran *C* had a higher degree of reactivity than did *D*. That is, *C* gave precipitation with 1:5 dilutions of all the antiserums, whereas *D* reacted only with the more potent antiserums and with them only when the serums were tested undiluted.

Additional evidence of difference between the 2 products was obtained from complement fixation experiments, in which 1:5 to 1:160 dilutions of all the serums were tested against the dilutions of the dextrans given in

† Although the immunological validity of the cross-reaction seems definitely established by the data, one can not entirely exclude the possibility that the reactions may be due to some accompanying substance rather than to dextran itself. This possibility is being investigated in studies we are making on the serological properties of non-dextran components of leuconostoc bacteria.

‡ The reactions of the clinical dextrans with the antityphoid serums differed from the reactions of the same products with type 2 antipneumococcus serums (Hehre and Sugg) (10) in that the antigen prozone which was prominent with the pneumococcus antiserum was not evident in the case of the antityphoid serums.

TABLE II. Number of Antiserums from 14 Typhoid-Immunized Rabbits* that gave Complement Fixation in Tests with 2 Clinical Dextrans.

Dilutions of		Dilutions of serum 5	10	20	40	80	160
Dextran C	20000	14	14	13	6	1	0
	200000	14	14	11	6	4	1
Dextran D	†	0	0	0	0	0	0

* The pre-immunization serums from all the rabbits gave negative reactions with both *C* and *D*.

† Dextran D was tested in 5 dilutions ranging in ten-fold increments from 1:2000 to 1:20000000.

Table II. It is apparent from the data that all of the 14 antiserums gave fixation with dextran *C* but not with dextran *D*; moreover, it should be noted that 6 of the antiserums gave positive reactions with both 1:20,000 and 1:200,000 dilutions of *C* throughout the serum dilution range of 1:5 to 1:40. The results with these 6 more potent antiserums are especially convincing because the difference between the 2 products is manifested over a broad zone of serum and antigen dilution. The question of whether the difference in capacity to react with antityphoid serums was due to a difference in molecular size or to differences in the strains of leuconostoc bacteria used to produce the 2 dextrans was not determined.

However, it was of particular interest to test samples which had shown differences in tendency to give untoward reactions in studies made upon presumably similar groups of people. Five samples which met those requirements were kindly supplied to us by Col. Hinton J. Baker. Samples No. 1 to 4 (from the manufacturer of *C*) had given fairly frequent untoward reactions; Sample No. 5 (from the manufacturer of *D*) had given no untoward reactions up to the time the samples were sent to us.§ The 5 samples were tested for fixation against our 6 most potent *S. typhi* antiserums, employing the same serum and dextran dilutions as used in the experiment reported in Table II. Samples No 1 to 4 proved essentially the same as *C* in capacity to fix complement, whereas Sample No. 5, was like *D* in giving entirely negative reactions over the entire range of serum and antigen dilutions tested. Thus, in the case of these particular samples, there was some correlation between the tendency to give untoward reactions and the capacity to fix complement with *S. typhi* antiserums.

Other Salmonella. Antiserums produced with *S. oranienburg* and with *S. typhi* strain 42-A-58 (often called Panama No. 58 and commonly used in making human prophylactic vaccines) were also studied. Their reactions with the dextrans were comparable to those reported in Tables I and II for the antiserums of *S. typhi* strain 779, with the exception that the *S. oranienburg* antiserums did not show as sharp a distinction between the 2 clinical dextrans. A number of other Salmonella antiserums,‖ produced at the Lederle Laboratories for diagnostic use, were tested for capacity to precipitate the native dextrans. Positive reactions were obtained with antiserums produced by immunization with the following species or varieties: *S. paratyphi A var. durazzo, S. oranienburg, S. berta, S. cholerae suis,* and a combined vaccine of *S. thompson* and *S. newport*. The dextran-reactivity was weaker than that of the antiserums in Table I, and in the majority of instances precipitation occurred only with undiluted serum. However, the results were sufficient to indicate that the capacity to evoke dextran-reactive antibodies is possessed by many other *Salmonella* as well as by *S. typhi* and *S. oranienburg*.

Comment. That dextrans† produced by *Leuconostoc mesenteroides* react with antibodies evoked by *S. typhi* is of some academic interest simply because of the phylogenetic distance between the 2 species of microorganisms. However, the reactions with the clinical dextrans, which had been prepared for intravenous administration, may be of practical

§ These samples (different lot numbers than *C* and *D*) had been used in the studies carried out at the Brooke Army Hospital at Fort Sam Houston, reported by Col. Baker and Col. Pulaski at a conference held by the National Research Council in Washington Jan. 30, 1951. We wish to express our appreciation to Col. Baker for sending the samples and for permission to designate them in this paper as ones that had been used in studies by their group.

‖ The serums were obtained through the courtesy of Dr. H. D. Piersma and Mr. W. S. Hammond.

importance. It is noteworthy that, among 5 samples which had been used in studies on humans, the four which had given frequent untoward reactions showed a much greater complement-fixing capacity with *S. typhi* antiserums than did the fifth sample which had given no untoward reactions. This apparent correlation was of course not evidence that the difference in the tendencies to give untoward reactions was due to the difference in the capacities of the samples to react with *S. typhi* antibodies. The data are insufficient to permit definite conclusions. Nevertheless, until evidence to the contrary is obtained, the capacity of clinical dextrans to react with *S. typhi* antiserums is one of the factors to take into account in the use of those products. Theoretically, the importance of this particular factor would vary in different geographical and occupational groups, depending upon the frequency of prophylactic typhoid vaccination and upon the incidence of typhoid infection. With some people, the capacity of dextrans (or of accompanying substances) to cross-react with other antibacterial antibodies might be more important.

Summary. Solutions of dextran, both in the unhydrolyzed or native form and in the partially hydrolyzed or clinical form, gave precipitation reactions with antiserums of rabbits immunized with *S. typhi,* including vaccines prepared for human typhoid prophylaxis. Reactions also occurred with antiserums of some other *Salmonella,* particularly *S. oranienburg.* Among 5 samples of clinical dextran that had previously been used in studies on humans, 4 that had given fairly frequent untoward reactions had greater capacities to fix complement with *S. typhi* antiserums than did the one that had given no untoward reactions.

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Proliferation and Vascularization of Adult Human Epithelium in Subcutaneous Tissues of X-Irradiated Heterologous Hosts.* (19133)

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Recently a preliminary report was made on the growth and transplantation of human neoplasms in the subcutaneous tissues of x-irradiated rats and mice(1). The present paper describes the proliferation ad vascularization of normal adult human epithelium in similarly treated rats and hamsters. The findings are of interest in that successful transfer of tissues between heterologous species has been considered as possible only when embryonic or malignant tissues are used(2).

Material and methods. Young female albino rats 30-50 days of age (Carworth Farms) were used as hosts in all 6 experiments and 50-70 g female hamsters (*Mesocricetus aurats*) were employed in 3. These animals were of "weanling" age since it had been learned that human tumors could be supported successfully in such x-irradiated hosts for approximately 15 days, in contrast to the 8-10 days reported for older animals (1). Half of the experimental animals were x-irradiated, both the rats and the hamsters receiving a total of 2 doses of 150 r each on alternate days from two 180 kv tubes (one above, one below the animal) with the last irradiation treatment usually on the day prior to implantation. The other half of the ex-

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