secretion as early as after 1 week. Toward the end of the investigation their titer reached 20% of that in the blood serum. Meanwhile, 1 week after infection specific antibodies against *Sh. flexneri* were found in the intestinal secretion, and by the 21st day their titer exceeded 1:4096. Antibodies appeared in the serum at the 6th week, in low titers (1:16, 1:32; Table 1). Consequently, synthesis of specific antibodies found in the intestinal secretion of germfree rats infected with *Sh. flexneri* began sooner in the intestinal wall, i.e., the local immune response was more marked than the generalized response. However, persistence of *Sh. flexneri* in the intestine of germfree rats did not come to an end, but a clincally healthy bacterial carrier state developed in the immune animal.

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SYNTHESIS OF A HIGH-CAPACITY IMMUNOSORBENT BASED ON A CELLULOSE SUSPENSION

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Antibodies and antigens fixed to an insoluble base (immunosorbents — IS) are now widely used in immunology and molecular biology [2, 5, 7]. To increase the total surface on which interaction takes place between antigen and antibody molecules, the writers previously used a very fine suspension of cellulose ester, obtained by reprecipitating cellulose from cuprammonium solution, in order to prepare IS [3, 4].

In this paper a simple method of obtained IS with higher capacity and with a better ratio between the quantities of fixed antigen and antibodies bound to it, is suggested. For this purpose the protein was bound to the cellulose suspension through aldehyde groups [6, 9].

Cellulose powder* was used as the insoluble base for IS. The cellulose was converted into a finely dispersed suspension by reprecipitation from cuprammonium solution by the method described previously [3], the difference being that cellulose and not its ester was taken. The cellulose was oxidized with sodium periodate ($NaIO_4$, from Reanal, Hungary) [6]. The content of reducing aldehyde groups was determined by Szabolks' method [10]. Rabbit γ-globulin (RGG) (from Calbiochem, USA) was used as antigen, and donkey antiserum against RGG, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, as antiserum. To

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TABLE 1. Dependence of Properties of IS on Quantity of Protein Added during Synthesis

Expt.	$NaIO_4$ concentration, M	Duration of oxidation, min	Content of CO groups, mg/g	Quantity of added RGG, mg/g	Quantity of RGG fixed to carrier		Quantity of attached antibodies, mg/g	Antibody–antigen ratio
					mg/g	%		
1	0,1	30	12,0	25	24,0	96,0	192	8,0
				50	34,3	68,5	202	5,9
				100	64,4	64,4	416	6,5
				200	63,6	31,8	531	8,3
				400	68,8	17,2	616	8,1
2	0,2	30	21,2	25	24,2	96,8	152	6,3
				100	72,9	72,9	434	6,0
•				400	175,5	43,7	936	5,3

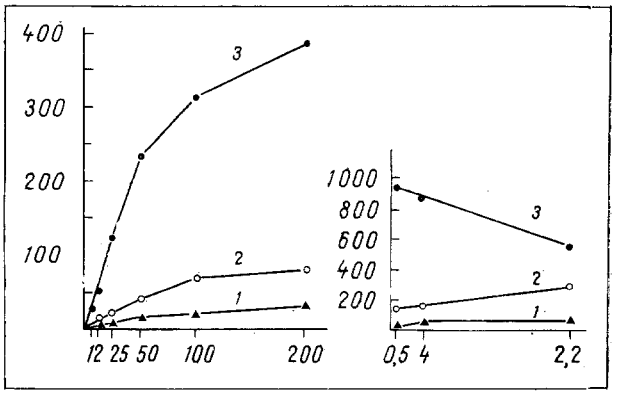

Fig. 1 Fig. 2

Fig. 1. Dependence of properties of IS on $NaIO_4$ concentration. Abscissa, $NaIO_4$ concentration (in mM); ordinate, concentration of CO groups (or protein) (mg/g IS). 1) Concentration of CO groups; 2) quantity of fixed antigen; 3) quantity of attached antibodies. For synthesis of each version of IS 100 mg of suspension and 10 mg of RGG were used.

Fig. 2. Effect of duration of oxidation on properties of IS. Abscissa, duration of oxidation (in h); ordinate, content of CO groups or protein (in mg/g sorbent). Suspension oxidized with 100 mM $NaIO_4$; 100 mg suspension and 40 mg RGG taken to synthesize each IS. Remainder of legend as to Fig. 1.

stabilize bonds between protein and cellulose and to reduce unreacted aldehyde groups, the material was treated with an equimolar (relative to sodium periodate) quantity of sodium borhydride ($NaBH_4$, from Koch-Light, England). The quantity of RGG attached to cellulose was determined by binding bromphenol blue [1], which was eluted with 0.1 N NaOH. Antibodies were eluted from the sorbent with 0.1 N NaOH and determined quantitatively by Lowry's method [8] after neutralization of the eluate with 0.1 N HCl.

The first task was to select the conditions for oxidation of the cellulose. It has been shown that during a change in the $NaIO_4$ concentration from 6 to 200 mM the concentration of aldehyde groups in the suspension (after oxidation for 30 min) increases from 3 to 30 mg/g suspension. The duration of oxidation was no less important: With an increase in time from 30 min to 22 h the content of CO-groups was increased about tenfold to 75 mg/g cellulose. Consequently, under the conditions selected, of every 100 glucose residues from 1 to 24 were oxidized. According to these results and data in the literature [10], the content of aldehyde groups does not decrease at least during the first month of storage of the cellulose.

Dependence of the properties of IS on the degree of oxidation was studied next. The results of an experiment showing that an increase in the concentration of oxidizing agent led to an equivalent rise in the number of aldehyde groups are illustrated in Fig. 1. The ability of the product to bind antigen increased at the same time. Depending on the degree of oxidation of the cellulose suspension, from 7 to 76% of added protein was fixed to it.

With an increase in the quantity of fixed antigen in the synthesized product, the quantity of attached antibodies also increased: For every antigen molecule incorporated into the sorbent, 5-7 antibody molecules were attached.

Data showing the effect of duration of oxidation of cellulose on the properties of the IS are given in Fig. 2. Too high a degree of oxidation, despite an increase in the quantity of attached antigen, did not lead to any further increase in the quantity of extractable antibodies. The IS obtained after oxidation of the cellulose suspension for 22 h had high capacity but less than that of the IS obtained after oxidation for 30 min. The ratio between the fixed antigen and attached antibodies also changed for the worse, from 7.0 to 1.9.

The properties of IS were largely determined by the quantity of protein added during conjugation with the carrier. This will be clear from the data in Table 1. With an increase in the concentration of added RGG the quantity of fixed protein increased, although the attached fraction steadily diminished. This was particularly clearly seen when cellulose with a lower degree of oxidation was used. So far as capacity is concerned, this increased with an increase in the number of antigen molecules fixed to the matrix.

On the basis of the results the following method of preparing IS can be recommended. To 100 mg of the washed suspension 10 ml of 0.2 M $NaIO_4$ is added. The reaction mixture is stirred for 30 min in the dark at room temperature. The oxidized cellulose is washed twice with water and twice with 0.1 M carbonate—bicarbonate buffer, pH 9.0, by centrifugation (3000 rpm). To the residue suspended in 4 ml of 0.1 M carbonate—bicarbonate buffer, 10 mg antigen and 2 ml of the same buffer are added and the sample is stirred for 16-18 h at 4°C. The suspension is centrifuged and the residue treated with 10 ml of 0.2 M $NaBH_4$ in 0.85% NaCl. After mixing for 1 h at 4°C the IS is washed successively with 0.85% NaCl, 0.1 M carbonate—bicarbonate buffer, pH 9.0, 0.85% NaCl, 0.1 M acetate buffer, pH 4.0, and 0.85% NaCl. The washed IS is kept at 4°C in 0.85% NaCl.

The simple method suggested can be used to synthesize an IS which can bind almost its own weight of antibodies; moreover, for every antigen molecule incorporated into the sorbent 5-8 molecules of antibodies are bound.

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