quickly: the cGMP level reached its maximum 3 min after the beginning of addition of ACh, and after 8 min the cGMP level had fallen below its initial value (Fig. 2). The effect of ACh was abolished by atropine, but depended only a little on extracellular calcium: The rise of the cGMP level during the action of ACh on B lymphocytes was still present in medium with EDTA. The effect of ACh depended on dose: ACh in a concentration of 10^{-8} M was enough to cause the cGMP level to rise close to its peak value (Fig. 3).

Elevation of both intracellular cGMP and calcium levels is thus accompanied by stimulation of B lymphocyte mobility; ultimately elevation of the cGMP level stimulates B lymphocyte mobility through calcium mechanisms. Since the effect of cGMP and of substances raising its level in the cell depends only a little on extracellular calcium, it seems very probable that cGMP can influence the redistribution of calcium in the cell.

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SYNTHESIS OF A HIGH-CAPACITY IMMUNOSOLVENT WITH ORIENTED IMMOBILIZATION OF Fab'-FRAGMENTS FOR ANTIGEN ISOLATION

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Immunosorbents containing immobilized antibodies are being used on an ever-increasing scale for the isolation and determination of antigens [1, 2, 5, 7, 9, 10]. To obtain such immunosorbents various carriers are used, mainly sepharose, to which a fraction of immunoglobulins or pure antibodies is covalently bound.

However, when these methods are used the antigen molecules, on binding with the matrix, are oriented randomly, as a result of which only some (1 of 4) of the active centers react with antigen.

The writers have attempted to overcome this shortcoming by binding Fab'-fragments of antibodies in an oriented manner to the carrier through thio groups, which are located at the opposite end from the active center. This possibility arose in connection with the development of methods of specific binding of thiol-containing molecules to the matrix by thiol-disulfide exchange [3, 8].

Donkey antibodies against rabbit IgG were isolated by means of a cellulose immunosorbent [1]. $F(ab')_2$-fragments were isolated by the method described previously [6] and reduced with 0.02 M dithiothreitol (from Koch-Light, England) in 0.1 M Tris-HCl buffer, pH 8.0, with 0.5 M NaCl and 0.001 M EDTA. The Fab'-fragments thus obtained were separated from dithiothreitol on a column with P6 Bio-Gel (from Bio-Rad, USA), equilibrated with the same buffer, and were

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TABLE 1. Characteristics of Immunosorbent

Expt. No.	Quantity of sorbent, mg	Quantity of Fab' added to sorbent, μg	Quantity of bound Fab', μg	Quantity of normal rabbit serum (in ml) or of IgG (in mg) added	Quantity of antigen eluted (0.01 M HCl, 0.5 M NaCl, μg)	Molar ratio Fab'/IgG†
1	12	3000	1208	1,0 ml	2240	0,69
2	12	1500	780	0,7 ml	1890	0,89
3	12	1050	575	0,55 ml	1498	0,96
4	12			1,0 ml	84	
5	12	750	438	3,0 ml	1080	0,91
6	12	1120	630	0,5 ml	1330	0,68
				0,5 ml*	1162	0,78
7	12	1000	545	1,5 ml	1281	0,87
8	12	1000	571	4,0 ml	1189	0,77

Legend. *) Reapplied to column after 1st elution of antigen, †) molecular weight of IgG 150,000 daltons, of Fab' 55,000 daltons.

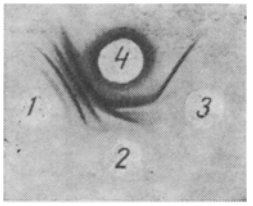

Fig. 1. Identification of eluted material by immunodiffusion in gel with oriented immobilization of Fab'-fragments: 1) normal rabbit serum; 2) rabbit IgG (from Calbiochem, USA); 3) eluted material; 4) donkey immune serum against rabbit serum.

used at once for binding to the matrix. Porous cellulose balls (PCB), oxidized with sodium metaperiodate [1], were used as matrix.

The intermediate ligand was introduced by treating 300 mg PCB for 1 h at room temperature with a 0.02 M solution of 1,5-diaminopentane (from LobaChemie, Austria) in 0.1 M carbonate–bicarbonate buffer, pH 9.5. To reduce the unreacted aldehyde groups and also the Schiff bases the matrix was washed on a glass filter with 100 ml of 2% $NaBH_4$ (from Koch-Light). Synthesis continued with some modifications to the method in [8]. To incorporate thio groups the matrix was transferred into 0.1 M carbonate–bicarbonate buffer, pH 10.6, with 0.001 M EDTA, 0.015 M N-acetyl-homocysteine-thiolactone was added, and the mixture was incubated for 2.5 h at room temperature. After the matrix had been washed with 0.1 M Tris-HCl buffer, pH 8.0 with 0.001 M EDTA, 10 ml of 0.001 M 5,5-dithio-bis-(2-nitrobenzoic acid) in the same buffer was added and the reaction was allowed to proceed for 30 min at room temperature. The product was washed with the same buffer, with 0.5 M NaCl, and finally with buffer, and kept at 4°C. The content of thio groups was determined by the method described in [4]. The synthesized product contained 630 μM of thio groups per gram dry weight. The protein content was determined from the SF-16 spectrophotometer. For IgG, $F(ab')_2$, and Fab', it was taken that $E^{1\%}_{280} = 14.0$.

To bind the Fab'-fragments, PCB modified by the method described above were placed in a column (0.6 × 14 cm, 10-15 mg of dry substance), equilibrated in 0.1 M Tris-HCl buffer, pH 8.0, with 0.5 M NaCl and 0.001 M EDTA. The Fab' fragments were added to the column in dif-

ferent quantities and the emerging fractions were passed through the column again 4 or 5 times. Unbound protein was washed off with buffer and estimated quantitatively. The quantity of Fab' bound to the matrix was judged from the difference between added protein and unbound protein. In some cases the quantity of bound Fab' was determined after removal with 0.05 M cysteine. The results obtained by the two methods virtually coincided: 545 and 571 mg and 560 and 578 mg respectively. The later results are given in in Table 1. Fab'-fragments bound to the matrix in an amount up to 100 mg/g dry weight (40-50% of the amount added).

To determine the specificity and capacity of the resulting immunosorbent, different quantities of normal rabbit serum, diluted 1:2 with 1 M NaCl or a purified preparation of rabbit IgG were added to the columns. The serum was treated beforehand with 0.01 M iodoacetamide (from BDH, England) to prevent addition of its components, containing free thio groups. After washing the sorbent to remove unbound proteins, the bound material was eluted with 0.01 M HCl with 0.5 M NaCl or with 0.14 M NaCl. It will be clear from Fig. 1 that the preparation isolated on the resulting immunosorbent was identical with rabbit IgG. The capacity of the synthesized sorbent varied from 100 to 200 mg antigen/g. The molar ratio between the quantity of immobilized Fab'-fragments and the quantity of eluted antigen depended on the quantity of fixed Fab'. With smaller quantities of Fab' (500-600 μg) 0.8-0.9 mole of antigen was bound per mole Fab'. With large quantities of fixed Fab'-fragments, this ratio was reduced somewhat. This was evidently due to steric hindrances for addition of the macromolecular antigen. These hindrances will perhaps not be present if the sorbent is used to isolate antigens with lower molecular weight. Nonspecific adsorption on the original product did not exceed 4%.

The suggested method of oriented immobilization of Fab'-fragments of antibodies can thus be used to obtain an immunosorbent in which ability of the active centers of the antibodies to bind with antigen is utilized practically completely.

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