IMMUNODEPRESSIVE ACTIVITY OF FRACTIONS ISOLATED FROM A CELL-FREE EXTRACT OF *Escherichia coli* BY GEL FILTRATION

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A cell-free extract from *Escherichia coli* 020: K4 and M-17 was fractionated on columns with Sephadex G-150 or G-200, yielding two fractions (peaks). The first fraction contained most of the carbohydrates of the original extract, together with protein and possessed immunodepressive activity relative to antibody production in mice against xenogeneic erythrocytes. The second fraction contained protein and more nucleic acids than the first fraction; it did not possess immunodepressive activity. It was concluded from these experiments that the immunodepressive substance localized in the cytoplasm of *E. coli* is a macromolecular complex composed of protein and carbohydrates.

Previous investigations [1-3] have shown that a cell-free extract (cytoplasmic fraction) from *Escherichia coli* 04: K12 inhibits humoral antibody production against typhoid VI-antigen and against sheep's erythrocytes and also immunity against allogeneic skin grafts in mice. This paper describes the isolation of an immunodepressive substance from bacterial extract and its chemical characteristics.

EXPERIMENTAL

The bacterial extract, an original unpurified preparation, was obtained from nonpathogenic strains of *E. coli* 020: K4 or M-17 by the methods described previously [1]. Strain 020:K4 was grown on meat-peptone agar (pH 7.2-7.4) in flasks, while strain M-17 was grown in nutrient broth with aeration in boilers. Extracts were thus prepared from both agar and broth cultures. In the latter case large quantities of extract could be made without the need for a large series of experiments.

The liquid extracts were sterilized by filtration through Chamberland candles and the filtrate was fractionated on a column with Sephadex G-200 or G-150. Columns measuring 90 × 3 cm were used and elution was carried out with distilled water (pH 7.0). Samples of 3-4 ml were collected and tested spectrophotometrically at 230, 260, and 280 nm. Glucose and rhamnose were determined in the original preparation and in the fractions by Dische's method [4] and protein was estimated by Lowry's method. Experiments were carried out on BALB/C mice or on (CBA × C57BL/6) F_1 hybrids immunized by intraperitoneal injection of sheep's erythrocytes (0.5 ml of a 20% suspension of erythrocytes to each animal). The original preparation and the fractions were injected intraperitoneally into the animals in a dose of 2 mg dry weight per mouse 24 h before immunization.

The state of immunological reactivity of the experimental animals was estimated from the blood hemolysin level.

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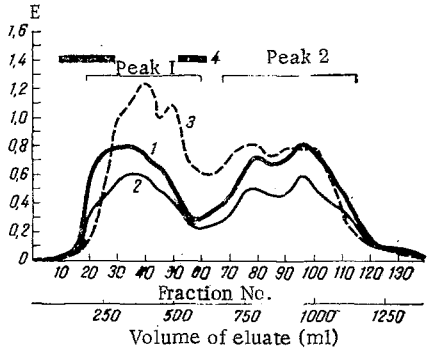

Fig. 1

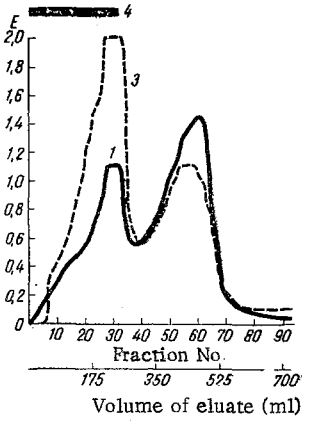

Fig. 2

Fig. 1. Results of fractionation of bacterial extract on column with Sephadex G-200. 1) Extinction at 260 nm; 2) extinction at 280 nm; 3) extinction at 230 nm; 4) fractions of eluate with immunodepressive activity. Rate of elution 20-36 ml/h.

Fig. 2. Results of fractionation of bacterial extract on column with Sephadex G-150. Rate of elution 50 ml/h. Legend as in Fig. 1.

TABLE 1. Immunodepressive Effect of Fractions Obtained by Filtration of Extract from E. coli on Column with Sephadex G-200

Fraction No.	No. of mice in expt.[1]	No. of mice dying	Content (%)		Hemolysin titer after immunization ($\log_2$ + tm)		
			glucose	rhamnose	5-th day	15-th day	30-th day
Original preparation . .	6	0	1,8	2,7	2,0±0,0	3,0±0,0	0,5±0,6
17-51	10	4	10,8	16,2	3,0±0,0	1,0±0,0	0,5±0,5
52-82	10	0	not determ.		0,0±0,0	2,7±2,6	0,0±0,0
83-102	10	0	0,8	0,9	6,0±0,5	4,3±3,3	1,0±0,2
103-128	10	0	1,3	1,9	5,0±0,8	3,7±1,3	0,6±0,2
Control[2]	10	0	—	—	5,7±1,3	2,5±0,5	0,6±1,0

[1]BALB/C mice.
[2]Mice not treated with extract.

EXPERIMENTAL RESULTS

During gel-filtration on a column with Sephadex G-200 the extract was eluted as two peaks (Table 1). It was concluded from the results of spectrophotometry at 230 nm and of estimation of glucose and rhamnose that most of the carbohydrates were concentrated in the fractions of the first peak. The spectrophotometric tests at 260 and 280 nm showed that a protein was present in the fractions of both peaks, while the content of nucleic acids was greater in the fractions of the second than of the first peak. The presence of protein also was confirmed by Lowry's method.

Tests of the immunodepressive activity of the various samples obtained by gel-filtration showed that such activity was clearly present in the fractions of the first peak. The fractions of the second peak either had no immunodepressive activity or (in some experiments) they stimulated hemolysin production slightly.

Similar results were obtained in the study of extracts obtained from the agar and broth cultures.

The results of gel-filtration of extract from E. coli on a column with Sephadex G-200 and the study of the immunodepressive activity of the fractions are given in Fig. 1; titers of hemolysins in mice treated with the fractions are given in Table 1.

In the next series of experiments gel filtration of the extracts was carried out on a column with Sephadex G-150. Under these conditions clearer separation of the extract into two peaks was obtained than on the column with Sephadex G-200. The results of gel-filtration of the extract of E. coli on a column with

Sephadex G-150 are given in Fig. 2. In this case also the immunodepressive activity was mainly found in the fractions of the first peak, which contained more carbohydrates than the fractions of the second peak.

The experiments thus showed that the immunodepressive substance localized in the cytoplasm of E. coli is a macromolecular complex consisting of protein and carbohydrates.

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