the inherited susceptibility of the H rats to hypertension may be associated with an inherited anomaly in sodium transport.

Résumé. Nous avons étudié le flux du sodium (Na^{22}) dans des hématies provenant de 2 souches de rats obtenues par croisement consanguin et possédant une susceptibilité différente à l'hypertension artérielle. La tension artérielle chez les rats hypertendus était 158 ± 11 mm Hg vs 123 ± 7 mm Hg chez les rats normotensifs ($p < 0.01$). L'efflux du sodium, par heure, était plus rapide chez les animaux hypertendus (1.38 ± 0.26) que chez les normotensifs (1.03 ± 0.08, $p < 0.01$).

[13] This work was supported in part by a grant from the joint research fund of the Hebrew University-Hadassah Medical School, Jerusalem and by a grant in aid from Merck, Sharpe and Dohme.

[14] Acknowledgment. The advice of Mrs CHANNA WALD and the technical assistance of Mrs YAEL KEREN-TSOUR are acknowledged.

D. BEN-ISHAY[13], A. AVIRAM and R. VISKOPER[14]

Department of Medicine A and the Nephrological Service, The Hebrew University – Hadassah Medical School, P.O. Box 1172, Jerusalem (Israel), 4 February 1975.

Inhibitors of the Adhesiveness of Enteropathogenic *E. coli*

Many species of enterobacteriacae have nonflagellar appendages called fimbriae. The roles of fimbriae are at the present not well-defined. Strong evidence has been provided by DUGUID[1] that they confer adhesive properties on bacilli and that only the fimbriate bacteria are able to adhere to different types of cells, including the epithelial cells of intestinal mucosa. Mutations can affect the synthesis of fimbriae[1]. DAREKAR et al.[2,3] have shown in mice challenged with the fimbriate strain of *S. typhimurium* or the non-fimbriate mutant derived from it, that the fimbriate strain produced greater number of infections and had greater opportunities for dissemination and spread to susceptible hosts. FUBARA and FRETER[4] have given indirect evidence that the adhesive properties play a

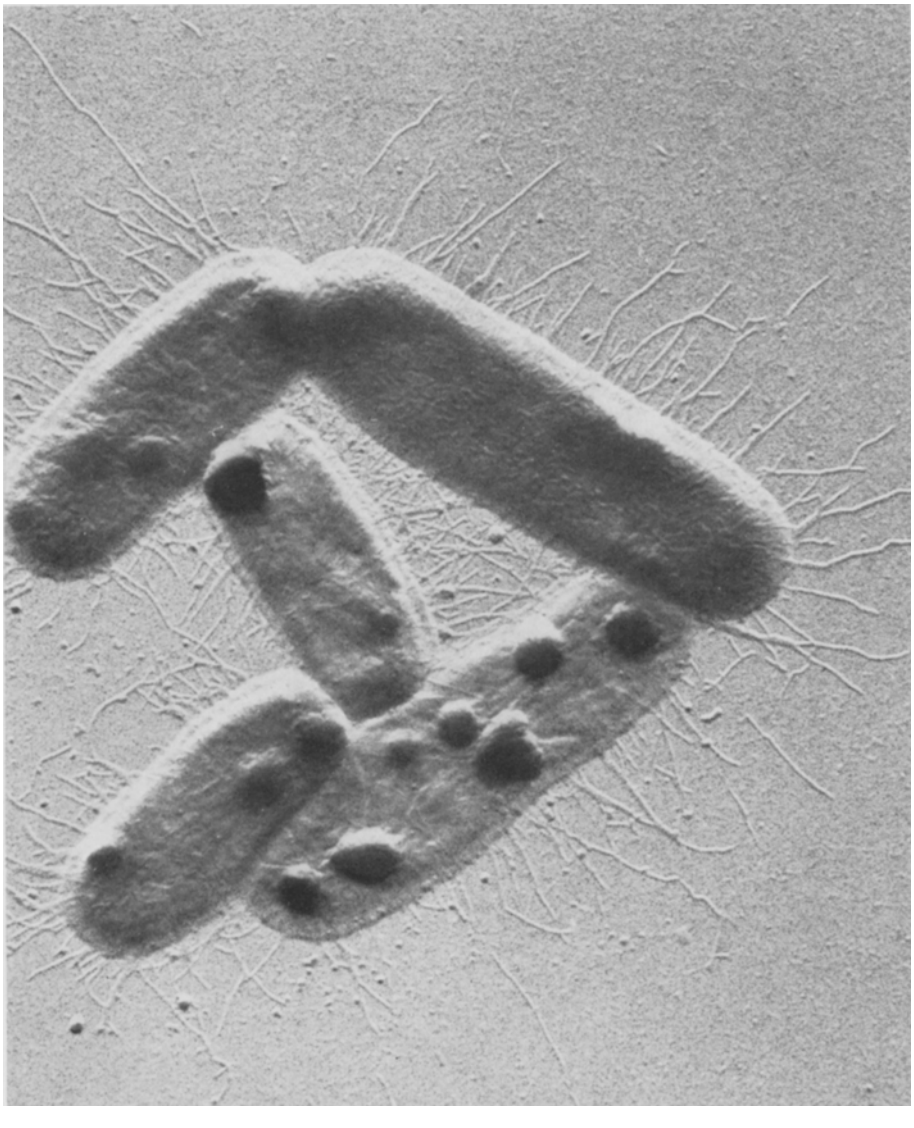

Fig. 1. Electron-microphotograph of fimbriate-*Escherichia coli* 0125: K70. Shadow-cast, ×27,000.

role in the pathogenesis of cholera, allowing the vibrios to adhere to intestinal mucosa, grow and produce enterotoxin. By way of analogy, it can be supposed that the same adhesive properties enhance the pathogenicity of some enteropathogenic *E. coli* strains or explain the chronicity of urinary infections caused by *E. coli*.

The aim of this work was to study inhibitors of the adhesiveness of enteropathogenic *E. coli*.

Material and methods. E. coli strain. The strain *E. coli* 0125: K 70 isolated from the stools of a diarrhoeic child has been selected because of its strong hemagglutinating activity and its richly fimbriate appearance under electron microscope (Figure 1). This strain was given to us by Dr H. HILPERT from Nestlé SA (Vevey, Switzerland). The bacteria are grown for 48 h at 37 °C in Nutrient Broth (Oxoid, London, England). Formaldehyde was added to the culture at a final concentration of 0.25%. The bacteria were washed 3 times and resuspended in phosphate buffer saline (PBS).

Preparation of isolated fimbriae. The bacteria were grown for 48 h at 37 °C in Nutrient Broth. After mechanical treatment of the bacterial culture, the fimbriae were isolated, according to BRINTON [5], by precipitation at the pH (3.9) followed by paracristallization with Mg Cl_2 0.1 *M*. 1 l of culture produced about 2 mg of protein as determined by KJELDAHL or by microbiuret method. The electronmicroscopic examination displayed the typical morphology of fimbriae (Figure 2).

Hemagglutination test. Guinea-pig erythrocytes were washed 3 times in PBS and used as a 3% suspension in PBS. The test was performed on plastic trays by mixing 50 µl of the formalized bacterial suspension or of the

[1] J.-P. DUGUID, Revue latinoam. Microbiol. *7*, suppls. 13 ,1 (1964).
[2] M. R. DAREKAR and J.-P. DUGUID, Proc. Indian Acad. Sci. *75*, 283 (1972).
[3] M. R. DAREKAR and H. EYER, Zentbl. Bakt. (I. Abt. Orig. A) *225*, 130 (1973).
[4] E. S. FUBARA and R. FRETER, J. Immun. *111*, 395 (1973).
[5] C. C. BRINTON, Trans. N. Y. Acad. Sci. *27*, 1003 (1965).

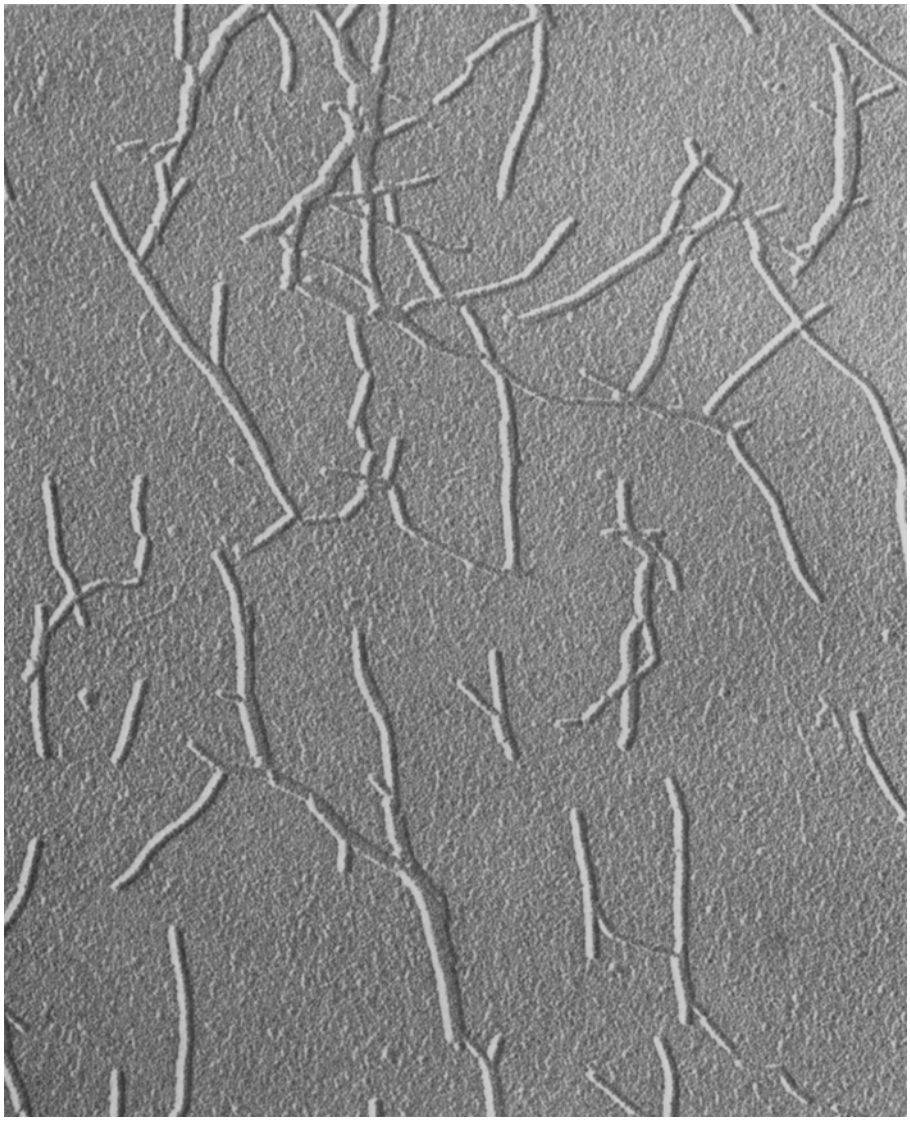

Fig. 2. Electron micro-photograph of isolated fimbriae from *E. coli* 0125: K 70 Shadow-cast, ×78,000.

fimbrial suspension with 50 µl of the erythrocyte suspension. In the experiments of inhibition, a minimal concentration of bacteria or of fimbriae was previously mixed with the inhibitors, and then added to the erythrocytes.

Carbohydrates. D-mannose, D-glucose and D-lactose have been purchased from Merck, Darmstadt, Germany. Mannane from Sigma, St-Louis, Missouri. α-methyl-mannoside, L-mannose, N-acetylglucosamine, N-acetyl-galactosamine, D-fucose and N-acetylneuraminic acid, from Fluka AG, Buchs, Switzerland.

Electron microscope preparations[6]. The culture of bacteria (48 h at 37 °C in Nutrient Broth) was fixed by addition of formaldehyde to a final concentration of 0.25%. The bacteria were washed in distilled water and prepared in films shadow-cast with Platinium (Figure 1). The suspension of isolated fimbriae was diluted to approximately 0.2 µg protein per ml and prepared in the same way as the whole bacteria (Figure 2).

Results. Under the conditions used the minimal concentration of *E. coli* 0125: K 70 to give visible hemagglutination was of the order of 10^8 per ml (ca. 1 bacteria per erythrocyte). When using fimbriae instead of whole bacteria, hemagglutination varied from one preparation to another and could be observed generally at protein concentrations of 10 to 100 µg and as low as 1 µg fimbrial protein per ml.

The results reported in the Table showed that, among the carbohydrates tested, only D-mannose and its derivatives (α-methyl-mannoside, mannane) were able to inhibit the hemagglutination caused either by the whole bacteria or their isolated fimbriae. L-mannose, D-glucose, D-lactose, N-acetylglucosamine, N-acetylgalactosamine, D-fucose and N-acetylneuraminic acid did not exert any inhibitory effect.

Discussion. DUGUID and GILLIES[6] found that D-mannose inhibited the agglutination of human and guinea-pig erythrocytes and of the cells of *Candida albicans* by fimbriate *Shigella flexneri* or *E. coli* strains. Other carbohydrates such as D-glucose, D-galactose, D-xylose, D-raffinose, L-arabinose, maltose, lactose, sucrose, mannitol, sorbitol and inositol did not inhibit the adhesive properties of these fimbriate bacteria. In our work, we have confirmed the effect of D-mannose, using a pathogenic strain of *E. coli*. We have shown the activity of D-mannose, to be stereospecific, since L-mannose is non inhibitory.

D-mannose is an essential constituent of the red blood cell membrane. Other carbohydrates found in such membranes, such as N-acetylglucosamine, N-acetyl-galactosamine, D-fucose and N-acetylneuraminic acid are unable to suppress the adhesive properties of *E. coli*.

The experiments done with the isolated fimbriae have given analogous results. It must be pointed out that bacterial contamination was difficult to avoid in BRINTON's procedure which was used throughout this work to prepare isolated fimbriae. However, it was possible to rule out the possibility of hemagglutination caused by bacteria contaminating the fimbrial preparation: 1. The concentration of bacteria in the preparation of isolated fimbriae was of the order of 10^4 bacteria per ml which was 10^4 times lower than the minimal concentration of bacteria causing hemagglutination. 2. After removing the bacteria by further centrifugation on a gradient of sucrose (10–50%) for 1 h at 100,000 *g*, the isolated fimbriae have been shown to cause hemagglutination.

The D-mannose exerts its specific effect also on the hemagglutination caused by isolated fimbriae suggesting that these proteins bind to cell membranes at specific sites containing a D-mannose residue.

The possibility of using some of the carbohydrate compounds outlined above as therapeutic or prophylactic agents in human *E. coli* infections will depend upon their experimental evaluation in a suitable laboratory animal.

Effect of various carbohydrates on the hemagglutination caused by *E. coli* 0125:K 70 or its isolated fimbriae

	Minimal concentration (mg/ml) of carbohydrate inhibiting the hemagglutination caused by	
	Whole bacteria	Fimbriae
D-mannose	0.012	0.003
α-méthylmannoside	0.006	0.003
mannane	0.100	0.025
L-mannose	>100	100
D-lactose	>100	>100
D-glucose	>100	100
N-acetyl-D-glucosamine	>100	>100
D-galactose	>100	>100
N-acetyl-D-galactosamine	>100	>100
D-fucose	>100	>100
N-acetylneuraminic acid	> 9	> 9

Each experiment has been repeated 2 to 5 times and the difference between inhibitory and non inhibitory carbohydrates has been found highly significant.

Summary. The entero-pathogenic strain of *E. coli* 0125: K 70 was able to adhere to washed guinea-pig erythrocytes and to cause their agglutination. Electron microscopy revealed this strain to be rich in fimbriae. They were able to cause hemagglutination by themselves, generally at protein concentration of 10 to 100 µg per ml. D-mannose and α-methylmannoside were able to inhibit hemagglutination by the whole bacteria or their isolated fimbriae at concentrations of 0.003 to 0.012 mg/ml. L-mannose, D-lactose, D-glucose, N-acetylglucosamine, D-galactose, N-acetyl-D-galactosamine, D-fucose, did not exert any inhibiting effect at concentrations as high as 50 mg/ml. N-acetyl-neuraminic acid was also uneffective at concentration as high as 9 mg/ml.

D. A. RIVIER and M. R. DAREKAR[7]

Institute of Biochemistry, University of Lausanne, 21, rue du Bugnon, CH-1011 Lausanne (Switzerland), 12 March 1975.

[6] J.-P. DUGUID and R. R. GILLIES, J. Path. Bact. *74*, 397 (1957).

[7] Acknowledgments. We should like to thank Prof. H. ISLIKER who made possible the completion of this work and helped us with advice and criticism, and Miss M. DYSLI for her technical assistance. We should like to thank also Dr D. KARAMATA, Dr A. GAUTIER and Mr J.-P. BARBLAND who gave their advice for the electron microscopy. The study was supported by a grant from Nestlé, SA, Vevey, Switzerland.