(1), which reduces intramammary pressure, may play important roles in the mammogenic and galactopoietic responses to nursing. On the other hand, there may not be a proportional relationship between prolactin secretion rate and pituitary prolactin content.

Pituitaries contained .014, .020, .015, and .021 IU of prolactin per mg at 5 a.m., 11 a.m., 5 p.m. and 11 p.m., respectively, ($P > 0.05$). Thus, contrary to results in the virgin rats of Clark and Baker(10) prolactin concentration in lactating rats did not fluctuate according to diurnal rhythms. Nursing stimuli may have masked any diurnal changes in pituitary prolactin potency. Nursing frequency did not influence pituitary weight (Table II).

Contrary to prolactin, ACTH, although always present in the pituitary, did not appear ($P > 0.05$) to vary with nursing frequency (Table II). This confirms our previous results based on nursing intensities applied by varying litter size(5). Although nursing frequency did not alter pituitary ACTH, the present study does not rule out the possibility that basal levels of ACTH may synergize with other pituitary mammogens to maintain cell structure and metabolic activity. ACTH content averaged 258.5, 310.1, 405.7 and 255.0 milliunits/mg of pituitary at 5 a.m., 11 a.m., 5 p.m. and 11 p.m., respectively, ($P > 0.05$). Although diurnal variation in ACTH content was not statistically significant, trends of data may suggest a peak ACTH content at 5 p.m. which agrees well with the data of Critchlow *et al*(11).

Summary. Increasing the nursing frequency in rats from non-nursed levels (controls) to 1×, 2×, 4× and *ad libitum* frequencies progressively increased mammary DNA, RNA and pituitary prolactin content 4.3-, 21.1- and 2.1-fold, respectively. Pituitary ACTH content, although always detectable, was unaffected by nursing frequency.

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Studies on the Mechanism of Action of Polymyxin B On *Cholera vibrios.* (32377)

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Gan and Tjia(1) and Powell and Finkelstein(2) found that *Vibrio cholerae* are highly sensitive to polymyxin B and El Tor vibrios relatively resistant. Roy, Mrihda and Mukerjee(3) reported *V. cholerae* to be sensitive to 15 μg/ml concentration of polymyxin B in nutrient agar media whereas El Tor vibrios were resistant to this concentration.

Few and Schulman(4) working with 7 different sensitive and resistant bacteria and Newton(5) with *Pseudomonas aeruginosa* found that when polymyxin is added to washed suspensions of sensitive organisms, it causes the release of soluble constituents from the cells. These workers suggested that the bactericidal activity of polymyxin is due to its combining with and disorganizing the structure of the bacterial cell wall, which is

responsible for the maintenance of the osmotic equilibrium of the cell. Studies by Newton(6) with protoplasts of *Bacillus megaterium* and *Pseudomonas aeruginosa* which were treated with a fluorescent derivative of polymyxin B indicated that the antibiotic acts on the cell membrane. Roy and Mukerjee(7) studied the action of polymyxin B on El Tor vibrios. Their results suggested that the antibiotic is adsorbed on the cell surface of the vibrios.

The present work was undertaken to investigate sequentially the mechanism of action of polymyxin B on the vibrio cell, using protoplasts of vibrios to clarify the role of the cell wall in determining the degree of resistance to polymyxin B.

Materials and methods. Vibrio strains and medium. Strain no. Bom 46/64 and Bg 76/65 were used as representative strains of *V. cholerae* and *V. eltor*, respectively.

Polymyxin B: 'Aerosporin' brand polymyxin B sulphate manufactured by Burroughs Wellcome & Co. (India) Private Ltd. was used, one μg being equivalent to 6 International Units.

Development of polymyxin-resistant strain of V. cholerae. Nutrient agar plates containing polymyxin B in a concentration lower than the minimal inhibitory strength for *V. cholerae* was used. On the plate a loopful of 4-hr culture of *V. cholerae* in nutrient broth was spotted. After incubation overnight at 37°C, the surviving colonies were picked up and allowed to grow in nutrient broth for 4 hours. They were subcultured repeatedly on nutrient agar containing progressively higher concentrations of the antibiotic. By 20 passages a strain resistant to polymyxin B at 60 μg/ml concentration could be obtained.

Preparation of protoplast. A 2-hour nutrient broth culture of vibrios was added in broth containing 6.35 M sucrose and 3% glycine(8) and incubated with slow shaking at 37°C to obtain protoplasts. On microscopic examination it was seen that protoplasts began to form in 2-4 hours by depolymerization of the cell wall and after 18-24 hours a dense growth of protoplasts could be obtained.

Action of polymyxin B on normal cells and protoplasts. Suspensions consisting of about 10^4-10^6 cells/ml of normal vibrio cells and vibrio protoplasts from a 24-hour culture were taken in conical flasks having a side arm. Papain broth and 3% plycine with 0.35 M sucrose in papain broth were respectively used for normal cells and protoplasts with and without polymyxin at different concentrations. The suspensions were incubated at 37°C for 24 hours with slow shaking. The growths of the cultures were measured photometrically every hour by placing the side arm of the flask directly in the Leitz-photometer, using a 550 mμ filter. Every hour a 0.1 ml portion from each sample was taken in normal saline for determination of viable count by plating after suitable dilution.

Results. Growth pattern of protoplasts. On examination under the microscope vibrio protoplasts could be seen within 2 hours treatment in the glycine-sucrose broth. Initially there was little or no growth of the protoplasts of vibrios in broth containing glycine and sucrose, and growth started only after 6 hours, whereas the normal cells in nutrient broth reached the exponential growth phase within 2 hours. The rate of rise in viable count of the culture of protoplasts appeared to be considerably lower than that of the normal cells of homologous strains of vibrios (Fig. 1).

Action of polymyxin on normal cells and protoplasts of vibrios. For this study protoplasts as well as normal cells of representative strains of (a) El Tor vibrios, (b) polymyxin-sensitive *V. cholerae* and (c) polymyxin-resistant (60 μg/ml) *V. cholerae* were used.

I. *Estimation of growth rates by turbidimetric method.* The results are given in Table I. With 5 μg/ml of polymyxin B in papain broth normal cells of *V. cholerae* showed an initial fall in optical density and growth started only at 4-6 hours. But when polymyxin B was added in the concentration of 15 μg/ml to a suspension of normal *V. cholerae* the optical density fell sharply and tended to reach zero within half an hour. After this the optical density of the suspen-

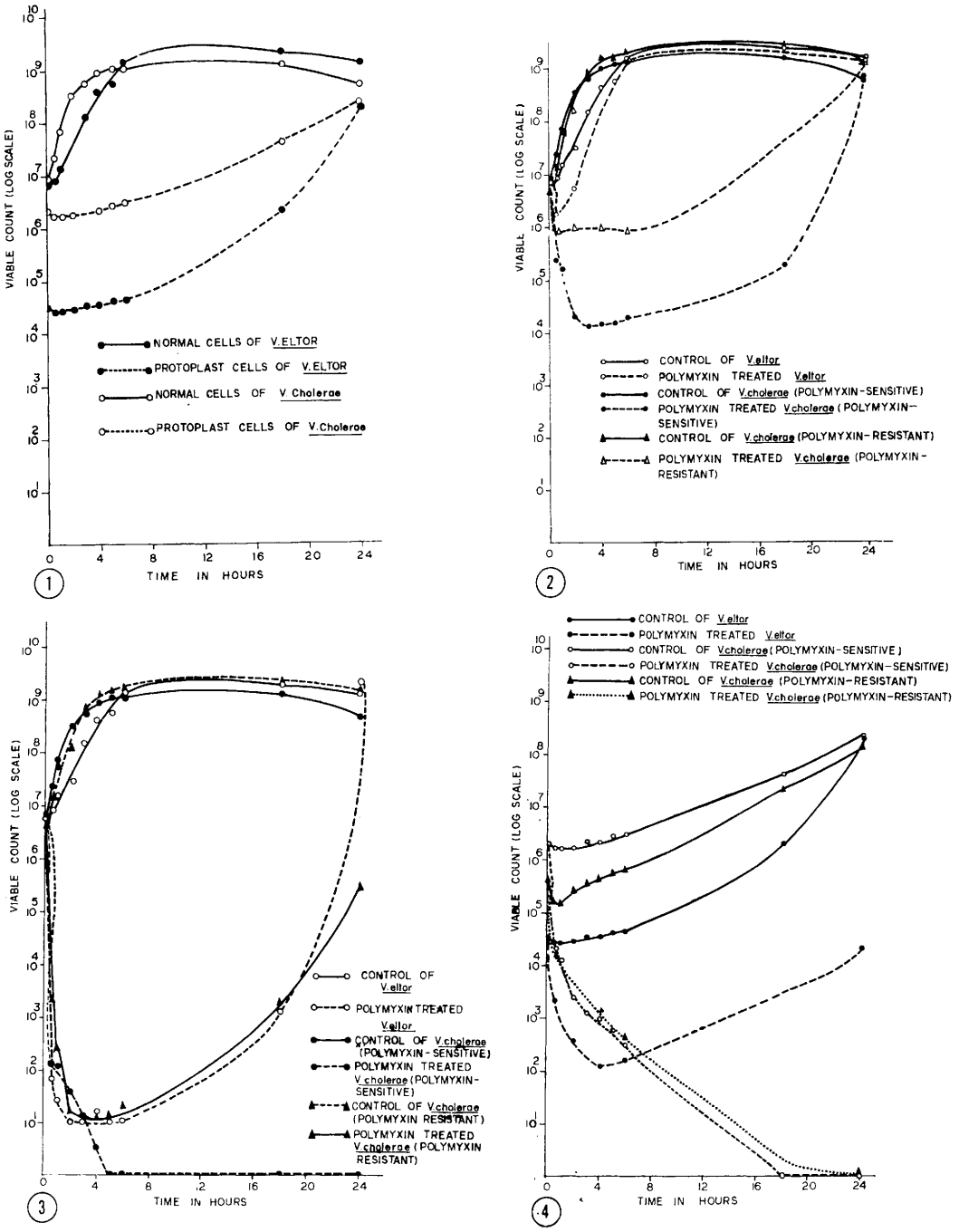

FIG. 1. Growth curve of normal and protoplast cells of vibrios.

FIG. 2. Effect of 5 μg/ml. Concentration of polymyxin B on the growth curve of normal cells of vibrios.

FIG. 3. Effect of 15 μg/ml. Concentration of polymyxin B on the growth curve of normal cells of vibrios.

FIG. 4. Effect of 5 μg/ml. Concentration of polymyxin B on the growth curve of protoplast cells of vibrios.

TABLE I. Effect of Polymyxin B on Growth of Vibrios.

Sample No.		Optical density at different hours							
		0 hr	½ hr	1 hr	2 hr	4 hr	6 hr	18 hr	24 hr
Polymyxin-sensitive *V. cholerae*	A	0.004	.004	.022	.061	.229	.328	.538	.602
	B_1	"	.002	.002	.002	.002	.018	.229	.319
	B_2	"	.000	.000	.000	.000	.000	.000	.000
	C	0.013	.013	.013	.022	.032	.036	.215	.432
	D_1	"	.006	.006	.009	.009	.009	.000	.000
	D_2	"	.004	.004	.004	.000	.000	.000	.000
Polymyxin-resistant *V. cholerae*	A	0.006	.006	.018	.051	.174	.328	.523	.620
	B_1	"	.002	.002	.002	.002	.018	.367	.482
	B_2	"	.002	.002	.002	.002	.004	.194	.398
	C	0.018	.018	.018	.027	.032	.036	.201	.284
	D_1	"	.002	.002	.002	.002	.000	.000	.000
	D_2	"	.002	.002	.002	.002	.000	.000	.000
V. eltor	A	0.006	.006	.027	.071	.244	.398	.921	1.000
	B_1	"	.009	.006	.027	.168	.237	.620	.824
	B_2	"	.004	.004	.002	.002	.002	.699	.824
	C	0.018	.018	.018	.027	.032	.041	.229	.310
	D_1	"	.013	.013	.013	.013	.004	.009	.006
	D_2	"	.009	.009	.009	.009	.004	.004	.004

A = Control (normal cells). B_1 = Normal cells treated with 5 μg/ml of polymyxin. B_2 = Normal cells treated with 15 μg/ml of polymyxin. C = Control (protoplasts). D_1 = Protoplasts treated with 5 μg/ml of polymyxin. D_2 = Protoplasts treated with 15 μg/ml of polymyxin.

sion did not rise even after 24 hours and viable count on plating indicated a vibriocidal effect. The polymyxin-resistant strain of *V. cholerae* differed from the sensitive strain only in its ability to resume growth at 6-18 hours even on treatment with 15 μg/ml of the antibiotic. Otherwise the effect of polymyxin at 5 and 15 μg/ml was similar to that of the sensitive strain. The effect of polymyxin on *V. eltor* cells was qualitatively similar to that on resistant *V. cholerae* cells, at both concentrations examined. Protoplasts of these strains were affected by polymyxin at both 5 μg/ml and 15 μg/ml concentrations. Protoplasts of all 3 strains behaved in the same way in turbidimetric estimation, irrespective of the degree of polymyxin sensitivity of the corresponding normal cells. The protoplasts showed an immediate fall in optical density and only in the case of El Tor protoplasts was there slight resumption of growth which could be estimated by viable count only at 24 hours.

II. *Estimation of growth rates by viable count.* Treatment of El Tor cultures in papain broth with 5 μg/ml of polymyxin B had no effect on growth rates of the normal vibrios (Fig. 2). But when an El Tor culture was treated with 15 μg/ml of polymyxin, there was an initial sharp fall in the viable count within an hour. After 6 hours exponential growth started and reached a maximum at 24 hours as in the control without the antibiotic (Fig. 3). On treatment of protoplast of the same El Tor strain with 5 μg/ml and 15 μg/ml concentrations, there was a steady fall of viable count up to 4-6 hours and most of the cells were lysed. After 6 hours the growth was resumed and the viable count again showed a slow rise until 24 hours (Fig. 4 and 5).

A polymyxin-sensitive *V. cholerae* strain when treated with 5 μg/ml of polymyxin, showed an initial lysis followed by slow growth which ultimately was equal to the control in 24 hours (Fig. 2). In the same polymyxin-sensitive strain of *V. cholerae* on treatment with 15 μg/ml of polymyxin, a sharp fall of viable count within half an hour was found and the cells underwent complete lysis within 5 hours (Fig. 3). Behaviour of protoplast of the same strain to polymyxin was interesting. On treatment with 5 μg/ml and 15 μg/ml of polymyxin, protoplast underwent complete lysis within 18 hours and 5 hours, respectively (Fig. 4 and 5). When polymyxin-resistant *V. cholerae* was treated with 5 μg/ml of the antibiotic, initially there

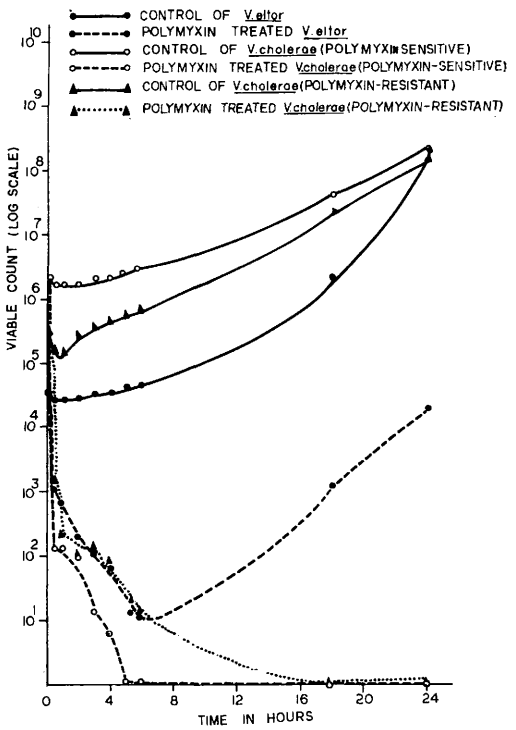

FIG. 5. Effect of 15 μg/ml. Concentration of polymyxin B on the growth curve of protoplast cells of vibrios.

was a little inhibition of growth which equalled the growth of control at 24 hours (Fig. 2). The same strain when treated with 155 μg/ml of the antibiotic, reached the logarithmic phase after an initial fall up to 3 hours and showed considerable growth within 24 hours (Fig. 3). Protoplasts of the same strain showed the same result as the sensitive strain when treated with 5 and 15 μg/ml of the antibiotic (Fig. 4 and 5). The cells were completely lysed within 24 hours.

Discussion. The 3 strains of vibrios examined in this study, namely, polymyxin-sensitive *V. cholerae,* polymyxin-resistant *V. cholerae* and *V. eltor* exhibited in that order an increasing degree of resistance to polymyxin. On exposure to 15 μg//ml of polymyxin the latter 2 strains were able to resume growth after an interval during which viable count fell and growth remained inhibited. In contrast to this, the protoplasts of the former 2 strains exhibited a similar degree of sensitivity to polymyxin; even at 5 μg/ml concentration of the antibiotic, protoplasts of these 2 strains were gradually lysed, and there was no recovery of growth even after 24 hours. Protoplasts of *V. eltor* on treatment with 5 μg/ml and 15 μg/ml concentration of polymyxin showed almost complete lysis, like the polymyxin-sensitive and polymyxin-resistant *V. cholerae* strains. After this the growth phase was resumed and at 24 hours there was again an increase in viable count although that was much less than in the control flask without polymyxin. This increase in viable count indicated that some other factors than cell wall structure, such as the difference in the cell-membrane of *V. eltor* from that of *V. cholerae,* contribute to the differences in polymyxin sensitivity of *V. cholerae* and *V. eltor* strains. This finding throws interesting light on the factors determining sensitivity to polymyxin. When the cell membrane is directly exposed to the antibiotic, it lyses the cell even at lower concentrations. From the results it appears that the cell wall of *V. eltor* and of polymyxin-resistant *V. cholerae* acts as the main barrier against polymyxin reaching the cell membrane. It appears also that the degree of resistance of a vibrio strain to polymyxin is determined chiefly by the properties of its cell wall, since no differences in degree of resistance could be found in protoplasts of polymyxin resistant and sensitive *V. cholerae* and only a slight difference was found to be present in the protoplasts of *V. cholerae* and *V. eltor* strains. The difference in reactivity of these two types of vibrios in soda-serum agglutination, soda-sublimate-precipitation tests(9) and chemical flocculation test with copper sulphate solution(10) also suggests the same kind of difference in the physio-chemical characteristics of their cell walls.

As this difference in the cell wall characteristics of the two types of vibrios may be related to the nature of the lipid component, the present results provide support for the views put forward by Newton(6). According to him the chemical nature of the polymyxin-binding component of bacterial cells may be a phospholipid. He found that cell walls of resistant organisms absorb only about one-fifth the amount of polymyxin absorbed by

the walls of sensitive organisms and in the case of resistant organisms there was relatively less penetration of polymyxin through the cell wall to the underlying membrane or osmotic barrier.

Summary. While normal cells of polymyxin-sensitive *V. cholerae,* polymyxin-resistant *V. cholerae* and *V. eltor* were found to exhibit a degree of resistance to polymyxin, increasing in that order, protoplasts of all 3 strains were sensitive to a polymyxin concentration insufficient to cause irreversible inhibition of growth of the normal vibrio cells. From the results it appears that the cell wall of El Tor vibrios differs in character from that of *V. cholerae* and this difference may be an important factor responsible for the resistance of *V. eltor* to polymyxin. Further, some other factor(s) in addition to the cell wall structure, such as a difference in the cell membrane of *V. eltor* from that of *V. cholerae,* may contribute to the difference in the sensitivity of the two types of vibrios to polymyxin.

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Cholesterol-Lowering Effects of Certain Grains and of Oat Fractions in the Chick.* (32378)

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An anti-hypercholesterolemic effect has recently been demonstrated with chicks for complex carbohydrates, including pectin, various gums, and scleroglucan(1-3). In a continuing effort to explore the relationship between certain complex carbohydrates and cholesterol metabolism, we have examined a number of common cereals in relation to their possible cholesterol-lowering activity, and now wish to report on the efficacy of oats in this regard. An earlier report by De Groot *et al*(4) suggested a cholesterol-lowering effect from rolled oats with rats and humans that the authors believed to be, at least in part, due to the polyunsaturated fat contained in this cereal.

Materials and methods. With one exception, one-week-old male chicks, in duplicate groups of from 6 to 10 chicks per group, were fed *ad lib* a hypercholesterolemic diet after one week on a standard chick ration. In one experiment they were placed on the experimental diet when one day old. The experimental diet consisted of 25% whole egg powder, 25% soybean meal, 3% cellulose, 2% dicalcium phosphate, 1% trace mineral concentrate,† 0.5% NaCl, 0.2% vitamin mixture,‡ 0.2

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† Mico concentrate, Limestone Products Corp. of America, Newton, N. J. Composition, in %: calcium, 32.0; magnesium, 2.6; manganese, 1.0; iodine, 0.225; iron, 0.175; copper, 0.125; fluorine, 0.01; cobalt, 0.01; zinc, 0.009.

‡ Provides, in mg/kg diet: thiamine HCl, 25; riboflavin, 16; Ca-pantothenate, 20; pyridoxine HCl, 6; biotin, 0.6; folic acid, 4; menadione sodium bisulfite complex, 5; cyanocobalamine, 0.02; nicotinic acid, 150; ascorbic acid, 250, In I.U./kg diet: vitamin A, 10,000; vitamin D_3, 600; vitamin E, 5.