Demonstration of Cell-Bound Antibody by Fluorescent Microscopy in the Intestine of Rabbits Following Live Enteral Cholera Vaccination

While advocating the development of a live oral cholera vaccine, MUKERJEE[1] argued that effective immunity against cholera rests primarily in the cells of the intestinal mucous membrane. There is evidence to indicate that coproantibody arises from the intestinal tissue[2-5] and the work of BURROWS et al.[6], FRETER[7], BHATTACHARYA and MUKERJEE[8], and SANYAL and MUKERJEE[9] suggests that coproantibodies may have a role in protection against cholera. Intestinal epithelial tissue being the primary site exposed to the action of cholera vibrios may be expected to form the first barrier of defence if its constituent cells contain antibody in bound form. Experiments were therefore carried out to investigate the presence of antibodies in the intestinal epithelial cells after live enteral vaccination.

Methods. Adult rabbits (weighing 1.25 kg) were immunized intraintestinally with a single dose of 8×10^9 viable vibrios of the apathogenic vaccine El Tor strain No.W-6[1,10,11]. After a week's interval, intestinal epithelial cells were isolated from groups of immunized and control rabbits according to the method of HARRER et al.[12]. Final suspensions of the isolated epithelial cells were made in Hanks' solution containing 140 units/ml of streptomycin and penicillin. On microscopical examination under phase contrast, the preparation was seen to contain columner epithelial cells essentially free from other cell types. The cell suspensions were sonicated in the MSE ultrasonic disintegrator for 10 min at 20 kc/sec and preserved in the refrigerator at 4 °C.

Sonicated cell suspensions were examined by indirect fluorescence microscopy. On each of three 1 mm wide slides smears of W-6 strain were prepared, the second and third for use as negative and positive controls. The smears were fixed by gentle warming. The test smear was covered with sonicated cell suspension from immunized rabbit and the negative and positive control smears were covered respectively with sonicated cell suspension from normal rabbit and known high titre serum against W-6 strain. The slides were incubated for 40 min in a moist chamber at 37 °C and rinsed twice with phosphate-buffered saline of pH 7.4 for 10 min. The smears were dried and covered with sheep anti-rabbit γ-globulin conjugated with fluorescein isothiocyanate (Nutritional Biochemical Corporation, Cleveland, Ohio) and incubated for another 40 min. The slides were again washed with phosphate-buffered saline for 10 min with 2 changes, dried and mounted in buffered glycerol-saline for examination under the fluorescence microscope.

Results and discussion. The results demonstrate clearly that the intestinal epithelial cells of enterally immunized rabbits contain antibodies combining with the homologous strain (Figure 1). No antibody was present in intestinal epithelial cells of unimmunized rabbits. The relatively higher intensity of fluorescence in the slide prepared with known high titre antivibrio serum (Figure 2) was due to the presence of a higher amount of antibody in the serum than in the sonicated preparation which had been made from a relatively scanty suspension of cells. The epithelial cells had been washed thoroughly, so that the possibility of the antibody being derived directly from the blood may be excluded. It appears that the antibody demonstrated by fluorescence microscopy was originally present in the epithelial cells in bound form. The present data do not permit any conclusion as to whether the cell-bound antibody is derived from the serum and concentrated by the cells from the circulation, or represents antibody formed by lymphoid cells in the intestinal epithelium. It is likely that the antibody occurs bound to one or other of the cell-organelles, most probably to the cell membrane.

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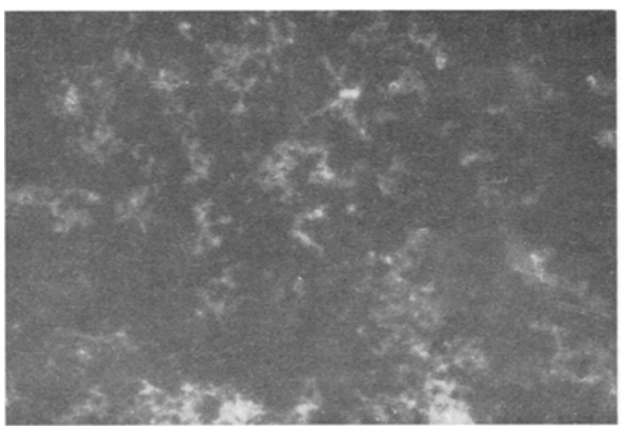

Fig. 1. Slide prepared with sonicated intestinal epithelial cell suspension of immunized rabbit.

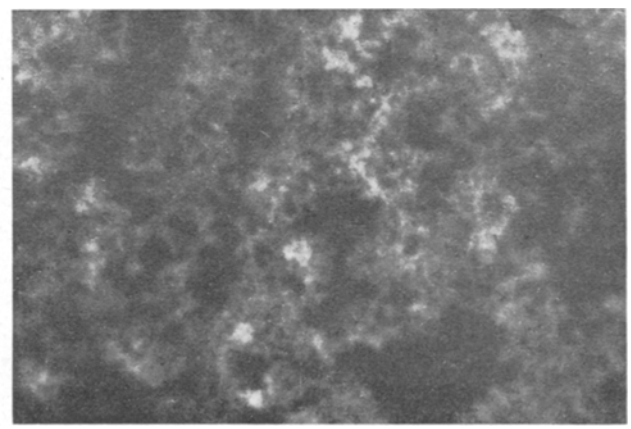

Fig. 2. Slide prepared with known high titre antivibrio serum raised in rabbit.

The appearance of bound antibodies in the intestinal epithelial cells after exposure to live vibrios is of significance because it provides evidence for the importance of local immunity in protection against cholera. Since in cholera the causal vibrios multiply and remain limited in the intestinal lumen, an antibacterial immune mechanism, if it is to be effective, has to operate at the lumenal surface of the mucous membrane lining the intestine. This in fact appears to be the case from the present results.

The cell-bound antibody liberated on cytolysis of the epithelial cells shed into the lumen – a process of known rapidity in the intestine – may form part of the copro-antibody detectable in the lumenal secretions and faeces, and thought to be the main protective factor in cholera[13].

Zusammenfassung. Mit Hilfe der Fluoreszenzmikroskopie lassen sich zellgebundene Immunkörper in isolierten Darmepithelzellen erwachsener Kaninchen nach Darmimpfung mit lebender Kultur des *Vibrio-eltor*-Stammes erkennen. Immunkörper waren in den Epithelzellen nicht immunisierter Kaninchen nicht nachweisbar. Es wird die mögliche Rolle der zellgebundenen Immunkörper beim antibakteriellen Immunmechanismus (Schutz gegen Cholera und Ansteckungsort) diskutiert.

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The Effect of Radiothyroidectomy on Blood Volume, Red Cell Survival and Iron Kinetics in Puppies

The influence of thyroidectomy on erythropoiesis in the dog is well documented. In the studies most carefully done[1,2] ablation of the thyroid by surgical procedures or destruction of the gland by administration of radioiodine (I^{131}) produced anemia and a striking decrease in the red cell renewal rate without change in red cell survival. These studies were done in adult animals and since in general the earlier thyroid insufficiency appears the more far-reaching the effects, at least on some organ systems, the present study was designed to investigate the influence of thyroid destruction on the erythropoietic system in the new-born dog.

Six mongrel 3-week-old dogs of both sexes of the same littermate were used throughout. 3 of them were injected with 1 mc/kg body weight of sterile sodium radioiodine (I^{131}) i.p. while the remaining 3 served as normal controls. The puppies were then left unmolested and determinations of peripheral hemoglobin concentration, blood volume, red cell survival, and plasma and red cell iron turnover rates were performed in each dog 1 year later. The total red cell volume and apparent red cell survival *tl/2* were determined with radiochromium as previously described[3]. Plasma and red cell iron turnover rates were measured by the method of HUFF et al.[4]. Hemoglobin concentration was determined by the cyanmethemoglobin method and hematocrits were done by micromethod. Plasma iron was measured by the method of PETERS et al.[5].

The results obtained are presented in the Table. The mean hemoglobin concentration was 13.8 g/100 ml and the mean hematocrit 44.5% in the normal dogs. Both values showed an approximately 32% decrease in the thyroidectomized ones. The total circulating red cell volume was decreased 27% from the mean control value of 36.1 ml/kg body weight to 26.3 ml/kg in the thyroidecto-

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Hematologic data from normal and thyroidectomized dogs

	Normal	Thyroidectomized	% change
Body weight, kg	15.7 ± 0.1[a]	9.1 ± 1.3	– 42
Hemoglobin concentration, g/100 ml	13.8 ± 0.1	9.4 ± 0.2	– 32
Hematocrit, %	44.5 ± 0.1	30.5 ± 0.5	– 31
Red cell volume, ml/kg body weight	36.1 ± 2.8	26.3 ± 0.2	– 27
Plasma volume, ml/kg body weight	49.8 ± 3.8	66.2 ± 1.3	+ 33
Blood volume, ml/kg body weight	85.9 ± 6.7	92.5 ± 1.1	+ 8
Plasma iron, μg/ml	1.02 ± 0.1	0.97 ± 0.1	– 5
Plasma Fe^{59} half life, min	47.0 ± 3.0	106.0 ± 1.0	+ 126
Plasma iron turnover rate, mg/kg per day	1.08 ± 0.2	0.61 ± 0.1	– 44
Red cell iron utilization, %	75.0 ± 2.1	73.0 ± 2.0	– 3
Red cell iron turnover rate, mg/kg per day	0.81 ± 0.1	0.45 ± 0.1	– 45
Cr^{51} *tl/2*, days	24.0 ± 2.2	24.6 ± 2.9	–

[a] S.E. of the mean.