Immediately after application of the current to the prosthesis ceased, a decrease in the tissue blood flow to its initial level was thus observed compared with its value during application of the electrical potentials.

The results of these experiments showed that during application of a positive potential to an electrically conducting prosthesis, i.e., during electrolysis of the silver framework of the prosthesis implanted into the abdominal aorta, the muscle tissue blood flow in the hind limbs of the dog increases. After application of the current to the prosthesis has ended and no more silver ions enter the blood stream, a decrease in the tissue blood flow to its initial values is observed.

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ALBINO MOUSE PAW EDEMA – A TEST FOR ESTIMATING *Escherichia coli* ENTEROTOXIN ACTIVITY

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UDC 576.851.48.097.29.087.5

The mouse paw edema test was evaluated as a means of detecting activity of *Escherichia coli* (strain P-99) enterotoxins. The paw edema test was shown to be simple, sensitive, and reproducible, and to permit determination of activity of the thermostable and thermolabile enterotoxins and endotoxin. This test is particularly useful for the evaluation of endotoxin preparations in the course of their isolation and purification.

KEY WORDS: *Escherichia coli*; enterotoxin; edema.

To determine the activity of enterotoxins of *Escherichia coli* the method of the ligated segment of rabbit small intestine [6] is often used. This test is somewhat laborious and its reproducibility is low, and if conducted in parallel with other tests (the skin test or cell culture) it gives contradictory results. False positive reactions also are often obtained by its use [9, 13, 15]. The skin test [5] is a sensitive test for the detection of the vascular permeability factor of enterotoxin, but this is not necessarily identical with the diarrheogenic factor tested by the ligated intestinal segment method [1].

To assess the activity of the cholerogen, several workers have used the mouse, rat, and golden hamster paw edema test [2-4, 7, 10], and in their opinion it is a highly reproducible and sensitive laboratory test.

The object of this investigation was to study the possibility of using the mouse paw edema test to determine activity of *E. coli* enterotoxins.

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(Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 150-152, February, 1978. Original article submitted May 16, 1977.

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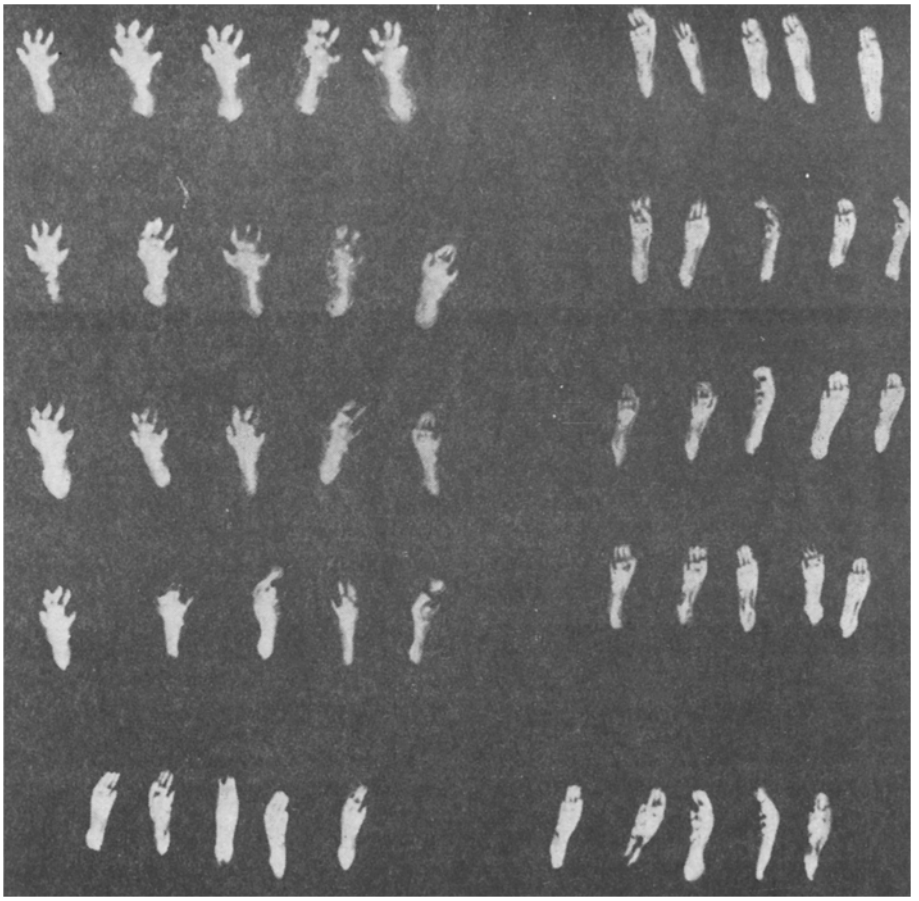

Fig. 1. Edema of albino mouse paws after injection of concentrate of E. coli enterotoxin. Paws into which concentrate was injected shown on left; row 1) whole concentrate, row 2) diluted 1:4; row 3) diluted 1:16; row 4) diluted 1:64; row 5) physiological saline. Control paws shown on right.

EXPERIMENTAL METHOD

Enterotoxigenic strain E. coli P-99 (O141; K85ab; K88ab; H 4), described by Mitchell [12] and generously provided by him, was used. The culture was grown on Hottinger's broth (pH 7.2-7.4) for 18 h at 37°C on a shaker. The bacterial cells were sedimented by centrifugation (15,000g, 30 min, 4°C) and the supernatant was used as the original preparation of enterotoxin; the original preparation was concentrated by precipitation with ammonium sulfate at 60% saturation. The residue was dissolved in one tenth of the initial volume and dialyzed against tap and distilled water until a negative reaction was obtained with barium chloride. This preparation was described as the concentrate.

Protein [11] and hexoses [14] were determined in the test preparations.

Enterotoxin activity was determined from the degree of dilatation of the ligated segment of rabbit intestine and by edema of the mouse paw. For the paw edema test noninbred albino mice weighing 12-14 g were used. The test preparations were injected into the pad of the right paw in 0.05 ml isotonic sodium chloride solution, and the left paw served as the control. Paw edema was read after 48 h. The hind limbs were amputated at the level of the ankle and weighed on torsion scales (Fig. 1). The degree of edema was determined from the difference in weight of the right and left paw of each mouse, after which the arithmetic mean value of the edema and its confidence interval at $P = 0.05$ were calculated. The following preparations were tested as the controls: isotonic sodium chloride solution; lipopolysaccharide isolated from the bacterial cells by the aqueous phenol method [16]; Hottinger's broth; bovine serum albumin.

EXPERIMENTAL RESULTS

To determine the completeness of precipitation of active enterotoxin by ammonium sulfate the concentrate and supernatant obtained after removal of the concentrate from the original enterotoxin were tested. Contradic-

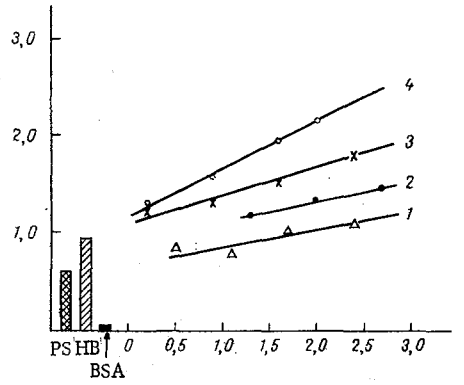

Fig. 2. Biological activity of freeze-dried preparations of E. coli enterotoxin: 1) supernatant; 2) original preparation; 3) concentrate after heating; 4) concentrate before heating. HB) Hottinger's broth; BSA) bovine serum albumin; PS) physiological saline. Abscissa, dose of preparation (log mg); ordinate, edema of paws (log mg).

TABLE 1. Activity of Enterotoxin Concentrate and Supernatant after Ammonium Sulfate Precipitation

Preparation	Protein content, μg/ml		Dose, μg protein	Edema of paws, mg ($\bar{x}$ and confidence interval)
	before dialysis	after dialysis		
Concentrate	5600	3000	150	110,8 (184,8—36,8)
			37,5	39,4 (45,9—32,9)
			9,4	24,0 (39,2—8,8)
			2,4	5,6 (8,8—2,4)
Supernatant	1500	170	8,5	7,4 (8,8—6,0)
			2,1	4,8 (11,5—1,9)
			0,5	3,8 (8,2—0,6)

tory results were obtained by the ligated intestinal segment test: In about 50% of cases the concentrate caused dilatation of the segment of rabbit intestine whereas in 50% of cases negative results were obtained. The supernatant caused no appreciable dilatation of the segment of intestine. In the mouse paw edema test, in which liquid preparations were injected after dialysis, clear and reproducible results were obtained (Table 1): A sharp increase was observed in the activity of the preparation in the course of concentration with ammonium sulfate. Only a very small part of the activity remained in the supernatant.

Compared with the ligated segment of rabbit intestine test, the mouse paw edema test was much more sensitive. In the subsequent experiments the ligated segment of intestine method was therefore not used.

The above-mentioned preparations were freeze-dried and tested in different doses (as dry weight) in the paw edema test. In this case also the supernatant was found to produce much less severe edema than the concentrate of enterotoxin (Fig. 2).

The concentrate was most interesting for further study: Its activity was an order of magnitude higher than that of the original preparation (Fig. 2). The activity of the concentrate decreased on heating (56°C, 1 h). The residual activity was probably connected with the presence of thermostable toxin in the concentrate. However, during concentration of the enterotoxin with ammonium sulfate the possibility of accompanying precipitation of endotoxin [8], some of which is secreted into the medium during culture, cannot be ruled out. It was accordingly postulated that the activity of the heated enterotoxin concentrate may be due both to thermostable enterotoxin and to thermostable endotoxin, i.e., lipopolysaccharide (LPS). Evaluation of the LPS by the paw

edema test showed that it causes not only edema, but also death of the animals. After injection of LPS in a dose of 4 μg the animals did not die, and the extent of edema of the paws was similar to its extent after injection of an equal dose of heated enterotoxin concentrate. LPS in a dose of 0.04 μg produced no appreciable paw edema. Considering that the content of LPS in the enterotoxin concentrate does not exceed 1% and that this amount does not cause edema of the paws, it can be concluded that the activity of the heated enterotoxin concentrate is in fact attributable entirely to the presence of thermostable toxin.

The mouse paw edema method can therefore detect activity of both the thermolabile and the thermostable enterotoxins of E. coli.

To this it must be added that the mouse paw edema test is simple, reproducible, and sensitive.

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STUDY OF COMPENSATION AFTER UNILATERAL LOSS OF VESTIBULAR FUNCTION

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UDC 616.283-008.66-031.4

The dynamics of compensation of the after-effects of unilateral destruction of the labyrinth was studied in rabbits. Destruction of the labyrinth was followed by nystagmus, an increase in the external respiration and heart rates, and EEG activation. The investigations revealed differences in the rate of extinction of these reactions with time. In the late stages after labyrinthectomy marked asymmetry of the nystagmic response of the eyes to angular accelerations of equal intensity but opposite direction was observed. Stimulation of the intact otolith apparatus was accompanied by the appearance of positional nystagmus. The results point to imperfection of the mechanisms of compensation after total unilateral loss of vestibular function.

KEY WORDS: vestibular system; deprivational and positional nystagmus; compensation of disturbed functions.

Clinical observations and experimental investigations on animals of different species have shown that a whole series of sensory, motor, and autonomic disorders develops after trauma to or surgical removal of one

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 2, pp. 152-155, February, 1978. Original article submitted July 4, 1977.