1. Fekete, E., Woolley, G. W., Little, C. C., *J. Exp. Med.,* 1941, v74, 1.
2. Woolley, G. W., Little, C. C., *Cancer Research,* 1945, v5, 193.
3. Dickie, M. M., Woolley, G. W., *ibid.,* 1949, v9, 372.
4. Smith, F. W., *ibid.,* 1948, v8, 641.
5. Christy, N. P., Dickie, M. M., Atkinson, W. B., Woolley, G. W., *ibid.,* 1951, v11, 413.
6. Frantz, M., Kirschbaum, A., Casas, C., PROC. SOC. EXP. BIOL. AND MED., 1947, v66, 645.
7. Dickie, M. M., Atkinson, W. B., Fekete, E., *Anat. Rec.,* 1957, v127, 187.
8. Atkinson, W. B., Dickie, M. M., Fekete, E., *Endocrinol.,* 1954, v55, 316.

Received July 23, 1958. P.S.E.B.M., 1958, v99.

Effect of Behavior on Development of Resistance in Trichinosis.* (24319)

DAVID E. DAVIS AND CLARK P. READ (Introduced by J. J. Christian)
School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.

In recent years the effect of external conditions on resistance to a pathogen has received increasing attention. This paper describes the effect of behavior upon resistance to *Trichinella spiralis* infections in mice. A series of papers(1,2,3,6) has demonstrated that crowding in mice produces hypertrophy of the adrenal glands and a chain of physiological consequences, especially with respect to reproductive functions. In addition it seemed probable that these adrenal changes would affect resistance. Resistance here is defined as response of a host to past or present experience with a parasitic organism or a chemically related entity, whereas the term insusceptibility is restricted to the properties of a host which render it unsuitable as a habitat for a parasite but which do not represent a response of the host to the parasite(11). This paper presents the first of a series of studies designed to determine alterations of development of resistance in respect to density of the population. The parasite *Trichinella spiralis* was selected because considerable information was available on the mechanisms of resistance and because it was possible to set up an experiment in which transmission from mouse to mouse did not occur(7). It has been shown that during the initial phase of a *Trichinella* infection there is an inflammatory response in the wall of the gut. This response is thought to be involved in determining length of life of the adult worms(9). It has been known for several years that resistance to *Trichinella* is altered by cortisone treatment(10,12). Coker(4,5) showed that when mice are given injections of cortisone the intestinal inflammation in trichinosis is reduced. As a consequence, the life of the adult worms is prolonged and more larvae are presumably deposited in the host's tissues than is the case in hosts not receiving cortisone treatment. Resistance to certain other animal parasites is altered by administration of cortisone to the host(13).

Methods. The general method consisted of infecting mice with *Trichinella,* then arranging part of the mice as a group to contrast the effects of interaction of individuals with the condition of isolation. The mice are called wild strain because they are raised in large cages from mice that are regularly replaced by mice caught in the wild. These mice have been used for a variety of studies of the adrenals(1,6). The young mice are weaned at 20 days and placed in jars separated from each other by partitions so that one mouse cannot see another mouse. Food and water are provided and the mice live in these jars until they are sexually mature at an age of about 4 months. Only male mice are used in these studies.

The grouping procedure consists of placing 6 mice together in a large can for about 4 hours a day. At the end of this time each mouse is put back in his own jar which is kept isolated from other mice. The large can does not have food or water. The mice begin

* This research was supported in part by a grant from the U. S. Public Health Service.

TABLE I. Number of Trichinella Found in Isolated and in Grouped Mice.

Exp. I				Exp. II			
Isolated		Grouped		Isolated		Grouped	
Wt of mouse	No. of worms	Wt of mouse	No. of worms	Wt of mouse	No. of larvae	Wt of mouse	No. of larvae
				per g			
28.3	6	32.4	26	28.8	880	30.0	1433
31.5	18	30.2	38	27.7	1043	19.2	1443
27.8	0	28.8	24	25.5	1026	29.2	1543
27.1	0	32.7	29	26.9	1006	26.9	1733
30.1	0	28.5	51	27.5	1093	21.8	1626
27.2	0	24.1	28	27.0	1273		
29.0	3	30.0	40				
28.7	0	27.7	16				
27.2	0	26.5	27				
28.3	0	25.5	50				
25.6	0	24.7	20				

to fight as soon as they are placed in the can and fight vigorously for most of the first 4 hours. During this time the social rank is pretty well settled but some further definition may occur on subsequent days. This grouping is done for 10 days. Generally speaking, while in the can the top-ranking mouse will attack and defeat other mice who in turn may attack lower-ranking individuals. The fighting is severe at first but, by the second or third day, involves only an occasional encounter. The mice spend most of their time sitting in different parts of the can licking themselves or simply resting. Such grouped mice will be referred to as interaction groups.

Control and experimental mice were chosen as follows: The 24 mice were listed according to weight beginning with the heaviest (32.5 g) and ending with the lowest (25.7) and alternately assigned to each group. Thus, the 12 control mice averaged 27.1 g while the mice in the 2 interaction groups averaged 26.6.

Trichinella spiralis was maintained in Princeton Swiss mice and rats of the Sprague-Dawley strain. Worms for experimental infections were obtained by 45 minute peptic digestion of blendorized muscle from the stock animals. The larvae were washed with Ringer's solution by centrifugation and suspended in Ringer's solution containing 0.2% agar at a concentration of 625 larvae per ml. The hosts in all experiments were given a 0.2 ml aliquot of the suspension by stomach tube. The suspension was thoroughly agitated before removal of each aliquot. Mice of the groups were infected in alternative order. Determinations of adult worm burden were made by the methods of Larsh and Kent(8) and larval worm burden by peptic digestion of the weighed muscles and direct counting of aliquot samples. Determinations of worm burden were made by the junior author who did not know the history of the animal in the experiment.

Results. In Exp. I, 24 mice were infected with the standard dose of *T. spiralis* larvae on Day 1. Twelve animals were divided into 2 lots of 6. These were interaction groups during Days 2 through 11, and the remainder were isolated controls. In this experiment, the mice in the interaction groups behaved in the usual manner. Severe fighting persisted to such an extent that the mice were kept together for only 3 hours a day and carefully watched to avoid deaths. During the 10-day period, one experimental and one control mouse died. A social rank in the interaction groups was obvious by the third day; in both groups the second heaviest mouse was dominant. The mice were killed on the fifteenth day after infection, and a determination made of the number of adult *T. spiralis* harbored by each animal. The data obtained are presented in Table I. The adrenals of the 11 control animals averaged 3.92 mg (S.D. = 0.38) while those of the 11 experimentals averaged 4.19 (S.D. = 0.55). This difference is not significant.

In Exp. II the procedures were identical except that the mice were held for a longer

period of time after infection. The animals were infected with *T. spiralis* in Day 1, the interaction group was brought together on Days 2 through 11, and the animals were killed on Day 30. Counts were made of the larvae in the muscles of these animals. The data obtained are presented in Table I. Adrenals of the controls averaged 4.24 mg (S.D. = 0.70) while those of the experimentals averaged 5.08 mg (S.D. = 0.65). This difference is significant (P = 0.01).

Discussion. The results of Exp. I support an hypothesis that the initial resistance response to the adult *Trichinella* has been markedly suppressed. Although histological studies were not made to prove the point, it seems that this is a manifestation of the suppression of intestinal inflammation. The results obtained in Exp. II show that there is an increased burden of larval worms in the fighting animals which is attributed to an alteration of the resistance response. This might well have a dual basis. Since the female worms remain in the intestine for a longer period of time in these animals, it is presumed that more larvae are deposited by the adults. However, we have not ruled out the possibility that suppression of concomitant and subsequent humoral immune responses to the larval worms may also be involved.

Although the mice arranged themselves in a rank the adrenal weights did not increase regularly with decrease in rank. However, as expected the adrenals of the 3 dominant mice were on the average, smaller (4.24 mg) than those of the lower-ranking mice. The number of adult *Trichinella* was not related to rank in any group.

The present experiments have shown that degree of development of resistance to an infectious agent may be in part dependent on socio-psychological factors. These experiments were arranged so that transmission among the mice in the interaction groups was impossible. Food supply was not a limiting factor during the experiments. In some situations the stress of nutritional insufficiency might enhance the effects of increasing social contact. It has been pointed out(11) that dissociation of variables in the field or laboratory analyses of resistance and insusceptibility is difficult and that some of the experimental demonstrations of alterations of immunity in malnutrition may involve adrenal responses rather than direct chemical deficiencies of antibody-synthesizing systems.

It may be noted that these results were obtained with a "wild" strain of mice that have larger adrenals than do domesticated strains. The domesticated strains show the same responses in principle(1) but to a considerably lesser degree. Thus experiments with domesticated mice might not show a detectable response although we have not examined this point.

The possible significance of the present findings to host populations is manifest. Under conditions of increased social contact, the members of a population may be less capable of developing resistance to an infectious agent. In many cases this effect would be compounded by enhanced contagion. The data suggest, though they do not establish, that those individuals that are highest in the social hierarchy would be least affected by the infectious agent. Probable effects in the evolution of vertebrate populations are obvious. The alteration of host resistance would also have implications for parasite populations. Suppressed host resistance responses might allow the exploitation of new host species by some parasitic species, with subsequent selection of individuals adapted for life in the new host.

Summary. The demonstration that behavioral factors cause hypertrophy of adrenals, and that injection of corticoids decreased the resistance of mice to *Trichinella spiralis* suggested that behavior might affect infections under relatively natural conditions. "Wild" strain house mice were infected with embryonated larvae. Twelve mice were isolated in jars and 12 were placed in 2 groups in a large can for 3 hours a day for 10 days. When killed on the fifteenth day the grouped mice had from 16-51 worms in the intestine while only 3 of the isolated mice had worms. To determine the effect on subsequent stages, 12 mice were infected. Six were isolated and 6 were grouped for 10 days. All were killed on the thirtieth day. The isolated mice averaged 1054 larvae per gram of muscle while the

grouped mice averaged 1556. It is concluded that behavior may affect resistance and it is suggested that this phenomenon may be an important factor in epidemics and in development of host-parasite relations.

1. Christian, J. J., *Ecol.*, 1956, v37, 258.
2. ———, 1959, in press.
3. Christian, J. J., Davis, D. E., *J. Mamm.*, 1956, v37, 475.
4. Coker, C. M., *J. Inf. Dis.*, 1956, v98, 187.
5. ———, *J. Parasitol.*, 1956, v42, 479.
6. Davis, D. E., Christian, J. J., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 728.
7. Gould, S. E., *Trichinosis*, Chas. C. Thomas, Springfield, 1945, p356.
8. Larsh, J. E., Jr., Kent, D. E., *J. Parasitol.*, 1949, v35, 45.
9. Larsh, J. E., Jr., Race, G. J., *J. Inf. Dis.*, 1954, v94, 262.
10. Markell, Edward K., *ibid.*, 1958, v102, 158.
11. Read, C. P., *Rice Institute Pamphl.*, 1958, v45, 36.
12. Stoner, R. D., Godwin, J. T., *Am. J. Path.*, 1954, v30, 913.
13. Weinstein, P. P., *Am. J. Trop. Med. Hyg.*, 1955, v4, 61.

Received July 25, 1958. P.S.E.B.M., 1958, v99.

Enzyme Studies in Muscular Dystrophy. III. In Hereditary Muscular Dystrophy in Mice.*† (24320)

I. M. WEINSTOCK,‡ SANDRA EPSTEIN AND A. T. MILHORAT
Depart. of Medicine, Cornell University Medical College, Russell Sage Institute of Pathology, and N. Y. Hospital, N. Y. City

Michelson, Russell and Harman(1) reported on spontaneous occurrence of a genetic mutation resulting in muscular dystrophy in a highly inbred strain of mice. The muscular areas involved, the nature of the lesions in these muscles, and lack of involvement of the nervous system, are similar to those seen in muscular dystrophy in man. By these criteria, this form of muscular dystrophy in the mouse would appear to be related more closely to muscular dystrophy in patients than are the experimentally induced types of muscle wasting. To characterize further this myopathy in mice, and if possible, to find some relationship to other forms of muscle wasting, we have investigated a number of enzyme systems in the muscles. Our results will be discussed in relation to those of comparable studies in muscular wasting of Vit. E-deficiency and of neurotomy, and that occurring in clinical forms of muscular dystrophy in man.

Methods and materials. Mice of strain 129 (dystrophic animals and normal litter mates) obtained from the Roscoe B. Jackson Memorial Laboratory were given a commercial mouse pellet diet. (Purina Laboratory Chow). The animals were allowed free access to food and water. According to the original investigators(1), symptoms of muscular dystrophy are evident by the third week of life, and dystrophic mice survive from one to 6 months, or occasionally longer. In these investigations, age of the dystrophic mice averaged 68 days (46-110), and weight averaged 14.1 g (9.7-19.0), when they were sacrificed. The control group consisting of heterozygous litter mates of the dystrophic mice, averaged 63 days (47-116) of age, and 21.4 g (16.4-25.4) in weight when they were studied. Dystrophic animals were smaller in size and lighter in weight than controls, and exhibited various degrees of ataxia, atrophy, and paralysis. However, there was no close correlation between age or weight of the animals, within the ranges observed, and gross symptoms of muscular dystrophy.

* Aided by grants from Muscular Dystrophy Associations.

† A preliminary report was presented before Fed. Am. Soc. Exp. Biol., March, 1957.

‡ Present address: Dept. of Physical Medicine and Rehabilitation, N. Y. Medical College, and Bird S. Coler Hospital, N. Y. City.