# Effect of Crowding on Experimental Granuloma Formation in Mice.* (24358)

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Mice, rats and voles respond to increased population density with adrenocortical hypertrophy and diminished growth and reproductive function(1-4). Although adrenocortical function was not measured directly, it was assumed that cortical hyperplasia and hypertrophy indicated increased activity. Involution of the thymus and depressed eosinophile counts associated with adrenal hypertrophy supported this assumption(2-5). Cortisone, hydrocortisone, and corticosterone suppress inflammation(6,7) and increase the pathogenicity of various organisms(6,9-12). It is known that animals from natural populations of high density become less resistant to infectious diseases and their effects than animals from populations of lower density (13). In view of these facts it is logical to ask whether the decreased resistance in animals at high densities is a result of suppression of normal defensive responses to pathogens, particularly inflammation, by increased adrenocortical activity. The present experiments were designed to explore the effect of increased population density, or crowding, on inflammation in albino male mice.

*Methods.* A. *Procedure for inducing granuloma formation.* Several methods of assaying anti-inflammatory actions of steroids have employed the ability of these compounds to suppress formation of experimentally induced granulomas(14-16). A modification of the technic utilizing subcutaneous cotton pellets to induce granuloma formation was used in the present experiments(15,16). Using aseptic procedures, a sterile 5 mg cotton pellet wet with turpentine was inserted beneath the skin of an ether-anesthetized mouse through a mid-dorsal longitudinal incision. The incision was closed with a clip after insuring that the pellet was sufficiently far from the incision so that it would be neither sloughed nor involved in the reaction to the incision. The modification in the original technic was wetting the pellets with turpentine and then blotting off the excess prior to insertion rather than using cotton alone. We had found that granulomas induced in rats by cotton wet with turpentine weighed 3 times as much as those induced by cotton alone and with proportionally less variability in their weights (Table I). A 5 mg pellet of cotton with turpentine was inserted subcutaneously on one side and a 5 mg pellet of cotton only on the other side of the back in each of 9 male Sprague-Dawley rats weighing between 130-150 g. Six days later the rats were sacrificed and granulomas weighed. B. *Effects of crowding on granuloma formation.* Two similar experiments were performed to determine the effect of crowding on weights of granulomas induced by subcutaneous pellets of cotton with turpentine in male albino mice. In both experiments male mice weighing 9-10 g were segregated at weaning, each mouse in its own cage. Three weeks later 5 mg pellets of cotton plus turpentine were inserted subcutaneously and each mouse was either returned to its original cage to serve as a segregated control or it became one of a group of 5 in a single 7″ x 10″ cage, constituting experimental crowding. All mice were sacrificed 6 days later and the granulomas weighed. There were 14 segregated mice and 5 groups of 5 each in the first experiment, and

TABLE I. Mean Weights of Granulomas Induced in Male Albino Rats by Inserting Cotton Pellets Alone and Cotton Pellets Wet with Turpentine Subcutaneously. Cotton alone was placed on one side and cotton plus turpentine on the other side of the mid-dorsum of each rat.

| Treatment | No. of granulomas | Mean wt of granulomas ± S.E., mg |
|---|---|---|
| 5 mg cotton | 9 | 148.8 ± 39.7 (26.7% of mean)* |
| *Idem* + turpentine | 9 | 443.4 ± 74.8 (16.9% of mean)* |

* P difference of means $<0.01$.

* The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or naval service at large.

TABLE II. Mean Weights of Granulomas Induced by Subcutaneous Insertion of 5-mg Pellets of Cotton Wet with Turpentine in Male Mice, and Effect of Grouping and Segregation on Weights of Granulomas. Mean value from each group of mice was the unit of measurement used for statistical treatment except as noted.

| | Isolated | | Grouped | | Isolated & grouped combined | | |
|---|---|---|---|---|---|---|---|
| Exp. | No. of mice | Mean wt of granulomas, mg | No. of groups of 5 mice each | Mean wt of granulomas, mg | No. | | P for diff. of means* |
| 1 | 14 | 148.2 ± 11.1† | 5 | 118.9 ± 8.6† | 19 | 140.5 ± 8.9 | <.0001 |
| 2 | 21 | 272.9 ± 12.2 | 6 | 232.8 ± 9.0 | 27 | 264.0 ±11.6 | |
| Combined | 35 | 223.0 ± 13.4 | 11 | 181.0 ±23.5 | | | <.05 ‡ |

* P values determined by analysis of variance using mean values for each cage as unit of measurement.

† ± stand. error.

‡ If individual values for weights of the granulomas for each mouse are used rather than the means of the groups, P value for the difference of the means of granuloma weights for crowded *vs* segregated mice becomes <.005.

21 segregated and 6 groups of 5 each in the second experiment (Table II). Unless otherwise noted, only the mean value for each group was used for statistical evaluation.

*Results.* The results of experiments on the effect of crowding on weights of experimentally induced granulomas in mice are summarized in Table II. There were significant differences between crowded and segregated mice and between experiments in mean weights of granulomas (Table II). However, in each experiment the weight of granulomas from the crowded mice was significantly less ($P<0.05$ and $P<0.01$ respectively) than from the segregated mice. For the combined experiments mean weight of granulomas from crowded mice was 18.8% ($P<0.05$) less than that from segregated mice.

In addition to differences in weights of granulomas between crowded and segregated mice, it was noted that the inflammatory reaction in segregated mice was usually more discrete and clearly circumscribed than in crowded mice.

Mean body weight of segregated mice (27.5 g) did not differ significantly from that of crowded mice (27.6 g) at the end of the experiment.

*Discussion.* Crowding clearly had a suppressive effect on granuloma formation in mice in these experiments. This, together with the known anti-inflammatory action of adrenal glucocorticoids(6,7) and the positive correlation between adrenal weight and population density in mice(1-4), supports the hypothesis that there is a sufficient increase in adrenocortical activity as a result of crowding to depress inflammation. It is distinctly possible that increased adrenocortical activity may be responsible, at least in part, for decreased resistance to disease commonly found in populations of high density(13).

In a parallel series of experiments Davis and Read(17) have shown that crowding increased the ability of *Trichinella* to invade the intestinal wall of mice. These experiments are of interest because ability of this parasite to penetrate the intestinal wall depends on degree of the hosts inflammatory response to it (17). Increased penetration following crowding presumably resulted from depressed inflammatory response; so that these results are in accord with those reported here on granuloma formation.

Depression of inflammation in both series of experiments is believed to be due to increased glucocorticoid activity, although conclusive proof remains to be obtained. In any event, the fact that increased population density decreases resistance to infection by depressing normal defenses, such as inflammation, has obvious importance for epidemiological studies.

*Summary.* 1) Granulomas were induced subcutaneously by 5 mg pellets of cotton wet with turpentine in 90 male albino mice. Thirty-five of these mice were segregated, 55 were in groups of 5 each. Granulomas of grouped mice weighed 19% less than those of segregated mice. These results were interpreted to mean that crowding increased adrenocortical activity sufficiently to have a suppressive effect on inflammation. 2) Granulomas were induced in rats with cotton and cotton plus turpentine. Cotton plus turpen-

tine approximately tripled the weights of the induced granulomas over those resulting from cotton alone.

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## Some Distinctive Biological Characteristics of Echo-10 Virus.* (24359)

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It has been reported(1,2) that kidney cells from various monkey species had different degrees of susceptibility to enteric viruses, and host cell range was suggested as a useful screening procedure for grouping certain viruses and facilitating their identification. Recently this study has been extended to include the susceptibility of kidney cells from non-primates to a variety of enteric viruses isolated from man and animals. The present report is concerned with ECHO-10 virus which behaves differently from all other enteroviruses that were tested in its ability to grow in non-primate as well as primate tissue cultures. Similar results have been found only with certain enteroviruses isolated from animal species other than man.

*Material and methods.* A. *Tissue culture.* Kidneys were obtained from the following: 1) Primates: rhesus (*Macaca mulatta*), patas (*Erythrocebus patas*) and capuchin (*Cebus capuchinus*) monkeys. 2) Domestic animals: swine, cat, dog, and calf. 3) Laboratory animals: rabbit and guinea pig. All kidneys were decapsulated aseptically and trypsinized by the method of Rappaport(3). The trypsinization fluids were centrifugated and the packed cells resuspended in growth media containing Hanks' salt solution, 0.5% lactalbumin hydrolysate and various amounts of calf serum, depending upon the animal species used. Details of the preparation of the different monolayer cultures will be published separately. B. *Virus strains*: The strains used were rhesus monkey kidney culture passage materials, and included the following: Poliovirus types 1, 2, 3; Coxsackie virus A-9, B 1-5; ECHO virus types 1-14,** ECMO (enteric cytopathogenic monkey orphan) virus (2) groups A 7853, $SV_{23}$†; Group B $SV_{16}$†; Group C $SV_6$†; ECBO (enteric cytopathogenic bovine orphan) virus‡ isolated by Moll and Finlayson(4). All ECHO-10 virus titra-

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‡ ECBO virus was supplied by Dr. T. Moll, State College of Washington, Dept. of Veterinary Microbiol., Pullman.

** All strains used were the prototype suggested by the ECHO viruses Committee (*Science*, 122 (3181): 1187-1188, 1955) except ECHO virus types 3 and 9. In these instances the Berardi and Bourn strains were respectively used.