Attempt to Stimulate Lymphatic Gland Changes of Fowl Typhoid With Adrenal Cortex Extract.* (23341)

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Young chickens infected with fowl typhoid organisms have hyperactive adrenals which are accompanied by an involution of the thymus and bursa of Fabricius(1). The latter lymphatic gland undergoes a peculiar loss of cells from the follicles, a loss that appears to be closely correlated with the degree of cortical activity. Selye(2) presented evidence indicating that the cortical hormones possibly were responsible for lymphatic involution in animals subjected to stress stimuli.

The present study was conducted to determine if exogenous adrenal cortex extract (ACE) would cause in lymphatic glands the weight losses and histological changes observed for these glands from chickens having fowl typhoid.

Methods. At the beginning of each of 2 trials, 5 groups of chickens were selected. In trial A, each of these groups contained 5 individuals and in trial B, 12 individuals. These groups were comparable with respect to age, sex, and weight. The birds of trial A were 39 days old at the start and those of trial B were 35. The range in initial, average weight for groups of the first trial was 484 to 491 g and 312 to 316 g for the second. In both trials, Group 1 constituted the control while Group 2 was injected intramuscularly with a 1 ml suspension of *Salmonella gallinarum organisms*. Groups 3, 4, and 5 were given ACE daily in single, intramuscular dosages of 0.25, 0.50, and 0.75 ml respectively in trial A. These groups of trial B received respective dosages of 1, 2, and 3 ml daily. Because of the large quantities employed in the latter trial, each of the daily levels was divided into 3 equal doses and given at 8 hour intervals. Insofar as possible all groups were maintained under the same environmental conditions. Whenever ACE was administered to Groups 3, 4, and 5 the controls were given sterile saline injections. The organisms used for infecting Group 2 with fowl typhoid were grown on proteose No. 3 (Difco) slants for 24 hours at 37°C. The colonies on each slant were washed from the agar with sterile saline. This suspension was diluted to 14% light

TABLE I. Effect of Fowl Typhoid and Daily Injections with ACE on Gland and Body Weights of Young Chickens.

	Final wt, g	Pituitary	Adrenal	Bursa	Thymus	Spleen	Liver
		mg/100 g of body wt				g/100 g body wt	
Trial A							
Control	564		10.4	152	560		
Typhoid	377		23.6	67	150		
ACE, ¼ ml	540		11.9	102	360		
½	534		10.4	123	380		
¾	527		9.6	91	350		
Stand. errors	23.7		1.1	18.7	5.8		
Trial B							
Control	412	1.28	13.6	82	690	.28	2.74
Typhoid	311	1.49	19.1	62	350	.60	5.26
ACE, 1 ml*	353	1.36	12.1	62	420	.28	3.22
2 *	341	1.65	12.7	60	440	.25	3.40
3 *	341	1.57	10.9	60	410	.31	3.75
Stand. errors	13.9	.08	.9	4.4	34	.04	.12

* Divided into 3 equal doses and given at 8 hr intervals.

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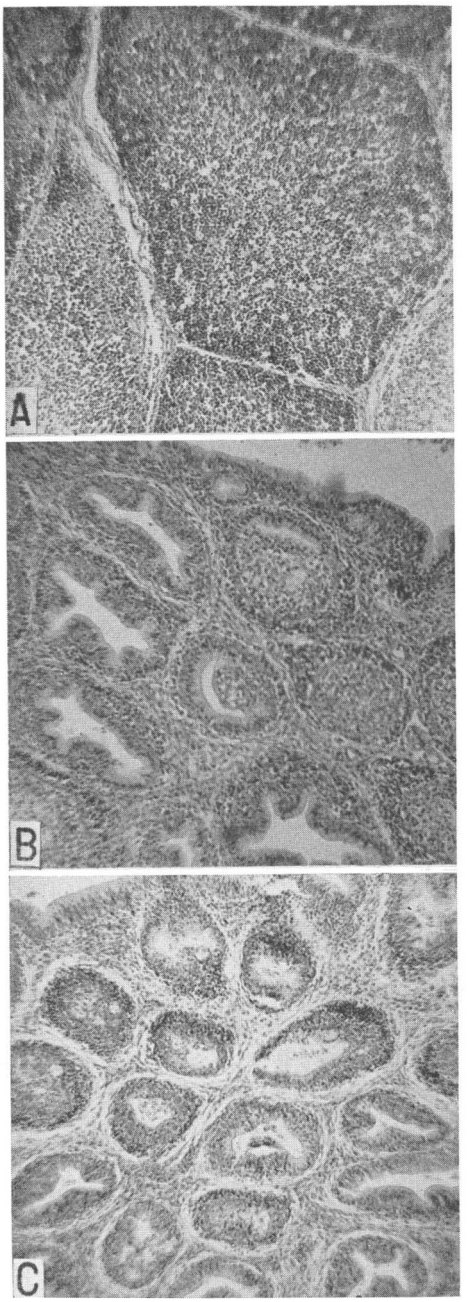

FIG. 1. Photomicrographs of bursa of Fabricius from a control chicken, A; from one infected with fowl typhoid, B; and from one inj. daily for 6 days with 2 ml of adrenal cortex extract, C. (×75).

transmission on a Coleman spectrophotometer model 14, using a PC-4 filter, at wave length of 650 mμ. Each ml of ACE (Upjohn) contained the biological activity equivalent to 100 μg of hydrocortisone acetate. When a chicken in Group 2 died, its control was sacrificed in each of the 4 remaining groups. In addition to noting the lymphatic gland changes, the effect of ACE on body weight and on weight and histology of the pituitary, thyroid, adrenal, gonads, liver, spleen, and thymus was also recorded. Statistical analysis of the gland and body weights included an analysis of variance. Standard errors of the mean in the Table were calculated from the average bird-to-bird variance for each set.

Results. As it developed, the *S. gallinarum* culture used in trial A was very virulent; of the infected group, one chick died on the third day following infection, 2 on the fourth, and 2 on the sixth and final day of the trial. In trial B the culture had apparently become less virulent since none of the infected chickens died during the experimental period of 6 days. However, fowl typhoid was diagnosed in these chickens when they were autopsied.

Gland and body weight data obtained in the 2 trials are given in Table I. In trial B, control chickens were smaller and possessed smaller bursae than control chickens of trial A. This differential in body weight is not unusual in chickens of this age grown at 2 different periods of time. A subsequent study has revealed that frequent handling, as present in trial B, can cause a mild but typical stress response as indicated by reduction in body weight, accompanied by an adrenal hypertrophy and a lymphatic involution. It has also been found that the bursa of Fabricius undergoes a rapid loss in weight when chickens are subjected to relatively short periods of stress(3).

Close comparisons between trials A and B are not possible because of differences in bird size and pathogenicity of the infecting organisms. Then too, the multiple injections employed in trial B would be expected to make these larger levels of ACE more effective. However, general comparisons can be made. In both trials the groups infected with fowl typhoid failed to make gains in body weight and the final weights for these groups were significantly less than the control. A weight loss of 109 g occurred for this group

in trial A. For a given trial, the group having fowl typhoid also displayed larger adrenals and smaller bursae than the control. In terms of mg per 100 g of body wt, the pituitary was larger for the group with fowl typhoid in trial B when this gland was considered. Similar weight changes and histological alterations associated therewith have been reported(1).

In both trials, all levels of ACE administration resulted in weights for thymus and bursa of Fabricius which were significantly less than observed for the control. In trial B, 1 ml daily was sufficient to cause a reduction in weight of the bursa comparable with that noted for the bursae of chickens with fowl typhoid, but was not sufficient to cause comparable histological changes. For the latter purpose, 2 ml daily were necessary. Three ml daily caused histological changes in the bursa which were slightly more severe than generally observed in this gland from chickens of the fowl typhoid-infected group. None of the levels of ACE employed was sufficient to cause a decrease in thymus weight comparable with that observed for groups having fowl typhoid. Actually for a given trial, the largest level of ACE administered failed to cause a significantly greater decrease in thymus weight than did the smallest level administered. The reason for this relationship is not known, particularly in view of the marked involution of the thymus from individuals having fowl typhoid. Possibly the thymus tissue of healthy chickens has a greater regenerative capacity than the same tissue of infected chickens. Then too, other physiological changes besides increased corticoid production may occur with fowl typhoid infection and contribute to the loss of cells from the thymus. And, of course, the highest level of ACE employed in the present study could be lower than necessary to cause a comparable loss of cells from the thymus.

Other results obtained, included (a) ACE injections which caused a significant increase in relative weights of the pituitary gland. This gland was larger than the control on an absolute basis for groups given 2 and 3 ml daily, but not significantly so. No discernible histological changes were noted. (b) Adrenals of the group given 3 ml of ACE daily were significantly smaller than the control. Less cortical tissue was present in groups given 1, 2, and 3 ml of ACE daily than observed in the control group. (c) The spleen weight and gross histology were unaffected by ACE administration. (d) ACE caused an increase in liver weight. Statistical analysis of the data indicated that weight increased in a linear fashion with larger doses of ACE. The yellow appearance of livers from groups injected with ACE suggested that increase in liver weight may have resulted from an increased fat deposition. However, no analysis of fat content was made. Enlarged livers and spleens of groups having fowl typhoid constitute characteristic lesions of the disease (4). (e) ACE administration at all levels caused a retardation in body growth. This reduction was significant for groups given daily levels of ACE in amounts of 1, 2, and 3 ml. This observation suggests the interesting possibility that the high levels of corticoids apparently occurring naturally in chickens with fowl typhoid may contribute to the marked body weight losses associated with the disease. Restriction of feed and water intake to that consumed by chickens with fowl typhoid is not sufficient to cause comparable weight losses noted for chickens with the disease(1). (f) The levels of ACE administered had no significant effect on the weight of the comb, gonads, or thyroid.

Summary. Daily levels of ACE for 6 days in amounts of 0.25 to 3 ml caused a significant reduction in weight of the thymus and the bursa of Fabricius. Two ml daily were adequate to cause both the weight loss and histological changes occurring in the bursa of Fabricius from chickens with fowl typhoid. Other significant changes observed for the groups given 1, 2, and 3 ml daily included: decrease in body weight, increase in relative pituitary weight, reduction in adrenal weight (less cortical tissue), and increase in liver weight which bore a linear relationship with the level of ACE administration.

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Evidence for Adrenergic and Cholinergic Components in Milk Let-Down Reflex in Lactating Rat.* (23342)

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A cholinergic component apparently is involved in activation of the hypothalamo-hypophyseal mechanism for release of antidiuretic and luteinizing hormones and, in addition, an adrenergic link has been proposed for luteinizing hormone and ACTH release(1). However, very little appears to be known about the release of oxytocin from the posterior pituitary gland other than acetylcholine, when injected into the dog, results in increased uterine activity apparently due to liberation of oxytocin(2). Since oxytocin is released as a result of nursing or milking stimuli in lactating animals thereby resulting in milk let-down(3), it seemed of interest to investigate the possibility of cholinergic and adrenergic components in this phenomenon. In the present study, the effects of various blocking agents on let-down in lactating rats have been studied and results compared with normal values previously obtained(4).

Materials and methods. Adult primiparous lactating rats weighing 200-300 g were assayed for milk let-down according to the method of Grosvenor and Turner(4). These were divided into 3 groups: *Ergot Group.* Each mother received a single subcutaneous injection of one of the following ergot drugs‡ 10 minutes prior to nursing: methylergonovine tartrate (Methergine), 1-2 mg/kg; an equi-proportional mixture of methanesulfonate salts of dihydroergocornine, dihydroergokryptine and dihydroergocristine (Hydergine), 2-4 mg/kg; dihydroergotamine methanesulfonate (DHE 45) 2-4 mg/kg. In addition, some mothers received .2 USP/kg oxytocin ("P.O.P." Armour) subcutaneously 8 minutes after Methergine or 10 minutes after Hydergine and DHE 45, *i.e.*, immediately before nursing. *Dibenamine Group.* Each mother received a single subcutaneous injection of Dibenamine HCl§ which was administered to one group in dose of 40-60 mg/kg 15-60 minutes prior to nursing and 60-80 mg/kg 120 minutes prior to nursing to a second group. A third group was injected with 80 mg/kg 120 minutes before nursing followed by .2 USP/kg oxytocin subcutaneously immediately before the animals commenced to nurse. *Atropine Group.* Each animal in this group received a single subcutaneous injection of atropine sulfate in dose of 700 mg/kg either 15-60 minutes or 150-220 minutes prior to nursing. Eighteen of these were anaesthetized with Nembutal (45 mg/kg) and oxytocin administered intravenously while the young were actively sucking. Gross observations were made on effects of each drug on behavior of the animals.

Results. Ergot Group. All ergot drugs, in dosage employed in the present study, sig-

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