quence is alcohols<phenylacetates<diphenylacetates<benzilates.

Summary and conclusions. Generally, it has been found that *cis* and *trans* isomers of 1,2-tertiary aminocyclanols and their esters, studied largely in the cyclohexane series, are capable of exhibiting convulsant activity in the mouse and the cat. From graded activities in the mouse it is observed that with few exceptions each *cis* isomer of a given *cis-trans* pair is more potent than its corresponding *trans* isomer. Within the tertiary amino compounds as a class, it appears that convulsant activity in the mouse, which is roughly paralleled in the cat, runs in the increasing sequence alcohols<phenylacetate esters<diphenylacetate esters≤benzilate esters. The *cis* quaternary alcohols 3 and 7 are active in cats and mice, whereas the *trans* isomers 4 and 8 are strikingly inactive in cats. Further, the probability that active and inactive members of a quaternary *cis-trans* pair (*e.g.* 7 and 8, and 4 and 3) reach the same receptor centers in the cat is indicated by the observations that 8 protects against the action of 7, and 4 against 3. Finally, the quaternary *cis* and *trans* benzilates 18 and 19 appear to show strong species specificity, with high activity in mice (*cis* better than *trans*) and low activity in cats.

1. Friess, S. L., *J. Am. Chem. Soc.*, 1957, v79, 3269.
2. Baldridge, H. D., McCarville, W. J., Friess, S. L., *J. Am. Chem. Soc.*, 1955, v77, 739.
3. Friess, S. L., Baldridge, H. D., *ibid.*, 1956, v78, 2482.

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Effect of Homologous Spinal Cord in Freund's Adjuvant on Cockerel Comb, Testicular and Body Growth.* (24322)

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During investigations concerning production of experimental allergic encephalomyelitis, Lipton and Steigman found (unpublished) that 4-week-old White Rock chickens were suitable hosts for production of the disease, both clinically and histologically, when injected with homologous spinal cord in Freund's adjuvant. Subsequent experiments were designed to determine the influence of age of chicken on its susceptibility. Casual observation revealed that injected groups had paler and smaller combs as well as smaller body weights than uninjected controls. The differences were most striking in birds injected at 3 to 7 days of age, and less so in birds injected at 14 days.

Methods. For Freund water-in-oil emulsions, *M. butyricum* was suspended in an Aracel-light mineral oil mixture to a concentration of 4 mg/ml. A local poultry provided adult chicken heads and necks from which spinal cord tissue was selected. Liver tissue was obtained from normal chickens killed in this laboratory. The tissue was suspended in 0.5% phenol water at 30% concentration and permitted to incubate 4-6 hours at room temperature. Emulsions were made by adding the aqueous phase to butyricum-oil suspension in a 1:1 proportion and emulsifying by a hypodermic syringe. In the group receiving *M. butyricum* alone, organisms were suspended in phenol solution in concentration of 2 mg/ml. White leghorn cockerels from a single source were used. All were received within 24 hours after hatching and living, attenuated Newcastle disease vaccine was instilled into the conjunctiva upon receipt. Diet was "starter mash" (Aubrey Feed Mills, Louisville) and water *ad lib.* Each bird received 0.7 ml of material subcutaneously divided into 7 sites. Seven-day-old chicks were injected,

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TABLE I. Average Comb, Body and Testes Weights of White Leghorn Cockerels Injected at Day 7.

Material inj.	No. birds	Avg comb wt, g ± S.E.‡	P§	Avg body wt, g ± S.E.‡	P	Avg testes wt, g ± S.E.‡	P	Comb wt/Body wt × 10⁻³ ± S.E.‡	P	Test. wt/Body wt × 10⁻³ ± S.E.‡	P
Exp. 1—Terminated at Day 54											
Chick cord and adj.*	8	2.40 .46		696 40				3.32 .76			
Adjuvant alone*	10	5.37 .48	<.001	823 24	<.05			6.61 .62	<.01		
Arlacel and oil†	9	5.30 1.19	<.05	862 35	<.02			6.03 1.16	<.1		
Chick liver and adj.*	10	4.79 .75	"	737 35	<.5			6.49 1.01	<.05		
M. butyricum (aq)	5	4.95 .33	<.01	800 27	<.1			6.17 .34	<.01		
Uninjected	20	4.28 .4	<.01	824 12	<.02			5.17 .44	<.02		
Exp. 2—Terminated at Day 53											
Chick cord and adj.*	9	2.96 .70		523 37		.19 .027		5.32 1.17		.36 .057	
Adjuvant alone*	10	7.15 .70	<.001	727 23	<.001	.38 .032	<.001	9.09 .84	<.01	.52 .040	<.02
Uninjected	20	6.98 .59	"	757 16	"	.42 .045	"	9.72 .70	"	.50 .036	"

* Emulsion made with Arlacel, light mineral oil, 0.5% phenol water and killed *M. butyricum*.
† Emulsion as above, but without *M. butyricum*.
‡ Stand. error of mean.
§ P values comparing cord inj. groups with control groups.

Liver *vs* cord $<p = .5$
" " uninjected $<p = .05$
" " adj. alone $<p = .10$
" " Arlacel & oil $<p = .05$
" " *M. butyricum* $<p = .20$

observed for 46-47 days and sacrificed at age 53-54 days. Serial body weights and comb areas were recorded. Periodically determined comb areas reflected trends in comb growth and correlated well with comb weights determined at sacrifice. The data (Table) presented here, however, are confined to body and comb weights at sacrifice. Two experiments were done. In the first, the following control groups were included: Adjuvants with killed Mycobacteria, adjuvants sine Mycobacteria, complete emulsion containing chicken liver instead of CNS tissue, aqueous mycobacterial suspension and uninjected normals. In the second experiment control groups consisted of adjuvants with Mycobacteria and uninjected normals. The number of animals are given in Table. Due to development of fatal allergic encephalitis or occasional accidental inclusion of a pullet, the number of birds in each group is not the same. At termination of experiment, the animals were sacrificed and exsanguinated. The combs were removed and weighed. In the second experiment, testes were also weighed. Ratios of comb weight to body weight were calculated in both groups to determine whether depressions in comb weights reflected depressions in body weight or whether there was more specific depression of comb weight. Each group was subjected to statistical analysis using the t test, standard errors and P values.

The results including P values comparing experimental with control groups are included in the Table, which reveals that in both experiments comb weights were significantly lower in groups injected with spinal cord and adjuvants than in any control group. The results in animals injected with control materials did not differ significantly from the uninjected ones.

Body weights in Exp. 1 also showed differences, although less marked than comb weight differences. The same trends were apparent with the exception of the liver-in-adjuvant group. This group did not differ significantly from the group receiving chicken cord emulsion. Like that group, it did differ significantly from the other control groups.

In Exp. 2, body weights of cord injected

group were significantly lower than in both control groups.

In both experiments the ratios of comb weight to body weight were significantly lower in experimental than in control groups. Such findings are interpreted as evidence that retardation of comb growth in animals receiving cord emulsion exceeded retardation in body growth. This ratio is of particular interest in comparing the group receiving liver-in-adjuvant and the spinal cord group. Although body weights of these groups did not differ significantly from each other, this ratio did reveal statistically significant differences.

In Exp. 2, testicular weights and ratios of testicular to body weight were significantly lower in the experimental than in control groups. There were no statistically significant differences in control groups.

Discussion and summary. Injection of homologous spinal cord in Freund's adjuvant into 7-day-old cockerels retards body growth with disproportionate retardation of comb and testicular growth. Preliminary microscopic examination of combs revealed decreased mucoid matrix of the intermediate layer of the dermis in experimental animals, suggesting a hormonal mechanism(1). Freund and co-workers(2) reported production of aspermatogenesis in guinea pigs by injection of homologous testicular tissue and adjuvant. In studying the effects of other tissues, these investigators found mild testicular damage and aspermatogenesis after injecting homologous *CNS* tissue and adjuvant into guinea pigs. The effects of these injections on secondary sex characteristics were not studied. They believed that, although CNS and testes shared a common antigen, the testicular injury was secondary to physio-pathologic changes associated with meningo-encephalitis (loss of body weight and other symptoms). In our chickens the incidence of encephalomyelitis was very low. Although body weights were retarded in these animals and in animals receiving liver-in-adjuvant, ratio of comb weight to body weight was lower only in the experimental group, indicating a disproportionate retardation of comb growth. It appears that some factor more specific than decreased body weight and changes associated with encephalomyelitis is operative. Whether it is a common antigen shared by CNS tissue and testes or whether it is inhibition of hypothalamic-hypophyseal function is purely speculative.

1. Hardesty, Mary, *Am. J. Anat.*, 1931, v47, 277.
2. Freund, J., Lipton, M. M., Thompson, G. E., *J. Exp. Med.*, 1953, v97, 711.

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Effects of 11-Oxy Corticoids on Renal Excretion of Water and Electrolytes in Force-Fed Intact Rats.* (24323)

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Hydrocortisone, prednisolone, or prednisone were considered equally effective in augmenting urine volume and renal excretion of Na and K in water-loaded adrenalectomized rats (1). Recently, Houlihan and Eversole (unpublished) found that these and other 11-oxy steroids augmented urine volume and potassium excretion in force-fed adrenalectomized rats. Our experiments were conducted to determine (1) whether presence of adrenals would modify renal responses to 11-oxy corticoids and (2) relative effectiveness of corticoids in inducing adrenal atrophy.

Methods. Eleven groups of Long-Evans male rats (approx. 230 g) were used. The control group received no hormone while the

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