is used as a standard is briefly discussed.

Addendum. During the progress of this work we received a copy of a paper by H. W. Long and O. L. Kline(17) in which, from studies similar to ours, it was postulated that both vit. B_{12} and vit. B_{12b} following heating in the basal medium are converted to a form of vit. B_{12} more readily utilizable by *L. leichmannii.*

The assistance of Mrs. Lucy Smith is gratefully acknowledged.

17. Long, H. W., and Kline, O. L., *J. Assn. Official Ag. Chemists,* in press.

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Estrogen-Androgen Antagonism: Histology of Mammary Glands and Vaginal Grafts of Male Mice Receiving Estrogens.* (18640)

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The concept of an essential antagonism between estrogens and androgens has been the subject of much endocrinological investigation and considerable debate. In the early endocrine literature, Steinach and Kun(1), Lipschutz and others(2,3) refer to what they considered to be antagonistic actions between the gonads of the opposite sexes and later applied this concept to the sex hormones as well. However, extensive work culminating in the classic publication of Moore and Price (4), refuted this hypothesis, contending that gonad hormones stimulate homologous reproductive accessories but are without direct effect upon heterologous accessories and have no direct effect on the gonads of either the same or opposite sex, gonadal activity being governed by the function of the pituitary gland. Although most instances of "hormonal antagonism" observed prior to 1932 probably were effected through the suppression of pituitary function, several more recent reports seem clearly to demonstrate the existence of both cooperative and antagonistic interactions between estrogenic, androgenic and progestational hormones(5-16). Although there are certain differences in the usage of the terms hormone "antagonism" and "cooperation", it would seem best to restrict the term "antagonism" to those situations in which one hormone reduces the magnitude of the response of a target tissue or end organ to another hormone. To demonstrate such an-

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† Clinical Fellow in Surgery, Am. Cancer Soc.

1. Steinach, E., and Kun, H., *Biol. Gen.,* 1926, v2, 815.

2. Lipschutz, A., *Endocrinology,* 1925, v9, 109.

3. Lipschutz, A., and Vinals, E., *C. R. Soc. Biol.,* 1927, v97, 1400.

4. Moore, C., and Price, D., *Am. J. Anat.,* 1932, v50, 13.

5. Korenchevsky, V., *Nature,* 1936, v137, 494.

6. Korenchevsky, V., and Hall, K., *J. Path. and Bact.,* 1937, v45, 681.

7. Deansly, R., and Parkes, A., *Quart. J. Exp. Physiol.,* 1936-37, v26, 394.

8. Mühlbock, O., *Proc. Internat. Cong. Obstet. and Gynec.,* 1938, v2, 491.

9. Mühlbock, O., *Acta Brevia Neerlandica,* 1938, v8, 50.

10. Gardner, W., and Pfeiffer, C., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 599.

11. Shorr, E., Papanicolaou, G., and Stimmel, F., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 759.

12. Huggins, C., and Clark, P., *J. Exp. Med.,* 1940, v72, 747.

13. Mühlbock, O., *Acta Brevia Neerlandica,* 1942, v12, 85.

14. Albert, S., *Endocrinology,* 1942, v30, 454.

15. Smith, O., and Smith, G., PROC. SOC. EXP. BIOL. AND MED., 1944, v57, 198.

16. Mühlbock, O., *Acta Brevia Neerlandica,* 1948, v16, 1.

tagonism it would seem desirable, if not essential, to arrange the hormone doses in such a way that the stimulating effects of one hormone are completely inhibited or abolished by the antagonizing hormone. If, however, the response of a target tissue is altered in a qualitative manner but not inhibited by the addition of a second hormone, "cooperative actions" may be said to exist.

The present study was prompted by the demonstration in mice that testis function will inhibit the stimulating effects of estrone for both the glandular epithelium of the mammae (16.17) and the epithelial lining of vaginal grafts(17). Evidence that this represents an estrogen-androgen antagonism is suggested by the work of both Mühlbock(16) and Gardner(18), who noted that the administration of large doses of testosterone propionate counteracted the proliferative influence exercised by injections of estrone and estradiol benzoate on the development of the mammary glands of male mice. The purpose of this investigation is to extend the observations concerning the action of testicular function in inhibiting mammary gland and vaginal mucosal proliferation induced by steroid estrogens, and to ascertain if a similar antagonism exists between testes function and the actions of a non-steroid estrogen such as diethylstilbestrol.

Materials and methods. Closely graded doses of two steroid estrogens (estrone and estradiol‡) and one stilbene estrogen (diethylstilbestrol) were administered to groups of castrate and non-castrate male mice bearing subcutaneous vaginal grafts. It has been shown in a previous investigation(17) that the epithelial lining of such vaginal grafts present essentially the same histology as normal vaginal mucosa and will undergo the same histological changes when subjected to hormonal stimulation. Intact testes were chosen as the source of androgen because of the extensive and contradictory literature dealing with the possible "estrogenic actions" of injected androgens on the mammary gland and vaginal mucosa of female mice(7,19-21). Shortly after weaning, male mice of the F_1 hybrid cross between the A and Z stocks§ received implants of vaginal tissue in the deep subcutaneous tissues of the groin, the donors being female mice of the same age and strain. Castrations were carried out after the grafts had been allowed to vascularize for 4 weeks and hormone injections were begun the following day. The estrogenic substances were administered in 10% alcohol-aqueous solutions in 2 daily injections of 0.2 cc each. Injections were continued for 21 days with the exception of one series that received estrone .04 γ per day 4 days of the week for 3 weeks. All animals were sacrificed within 6 hours of the last injection. A total of 196 mice divided into 27 series were studied. The mammary glands were studied by the whole mount technic. Multiple sections of the vaginal grafts were examined microscopically and the accessory sex organs were examined grossly and in certain groups microscopically in order to estimate testicular function (to rule out evidence of pituitary suppression).

Results. The mammary glands of the uninjected control mice revealed no evidence of stimulation and consisted of only a few narrow branching ducts without the formation of end buds. The mucosa of the vaginal grafts in these animals was atrophic and consisted of a double layer of low, cuboidal cells, similar to the vaginal mucosa of recently ovariectomized female mice. It would therefore appear that testicular function alone does not alter the histology of this mucosa.

In order to be certain that the estrogenic substances injected did not cause significant pituitary suppression, resulting in reduction of testicular function, the accessory sex organs

17. Huseby, R., and Bittner, J., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 321.

18. Gardner, W., *Recent Progress in Hormone Research*, 1947. p. 234 (Academic Press, Inc., New York, N. Y.).

‡ The estrone and estradiol were kindly supplied by Schering Corporation.

19. Leonard, S., and Reece, R., *Endocrinology*, 1942, v30, 32.

20. McEven, C., Selye, H., and Collip, J., PROC. SOC. EXP. BIOL. AND MED., 1936, v35, 56.

21. Von Heuverswyn, J., Folley, S., and Gardner, W., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 389.

§ Supplied through the courtesy of Dr. J. J. Bittner.

TABLE I. Male Mice Bearing Vaginal Grafts Injected with Estrone.*

γ per day		Vaginal mucosa		Mammary gland development	
.04 γ 4 days/wk	Castrate	Cornification and mucification	(4)	0	(8)
				Slight	(1)
	Non-castrate	Atrophic	(5)	0	(10)
	Adrenalectomized castrate	Cornification	(4)	Good	(4)
	Adrenalectomized non-castrate	Atrophic	(5)	0	(5)
.05	Castrate	Cornification and mucification	(5)	Good	(5)
	Non-castrate	Development—almost complete mucification	(5)	0	(4)
.10	Castrate			Good	(6)
	Non-castrate			0	(5)
.15	Castrate	Complete cornification	(7)	Good	(9)
	Non-castrate	Almost complete cornification	(4)	0	(3)
				Slight	(1)
Uninj. controls	Non-castrate	Atrophic	(18)	0	(18)

* The number in parentheses indicates number of mice in each group.

of the animals were carefully studied, especially in the groups receiving the higher estrogen doses. The accessory sex organs of the animals were found to be normally developed in all of the non-castrate animals. This suggests that relatively little or no pituitary suppression occurred under the conditions of these experiments. It is also of some interest to record that in the castrate males no evidence of squamous metaplasia of the seminal vesicular or prostatic epithelia occurred with the relatively small doses of estrogens employed in these studies.

Estrone series (Table I). Evidence for androgenic antagonism to the proliferation of the mammary gland induced by a steroid estrogen was noted at the .05 γ dose of estrone¶ and was exhibited through the .15 γ dose level. At these dose levels there was good development present in all of the castrate animals, (all mammary glands contained numerous club-shaped swellings at the end of lactiferous ducts with numerous mitotic figures), while the glands of all the non-castrate animals receiving the .05 γ and the .10 γ doses and 3 of the 4 glands of the non-castrate animals given the .15 γ dose were without development. (Fig. 1 and 2). Similar antagonism for the vaginal mucosa was observed at the .04 γ dose of estrone when administered 4 times per week. In the castrate group at this dose level there was definite development in the form of cornification and mucification while there was no histological evidence of stimulation in the non-castrate group. (Fig. 3 and 4).

It is apparent from the study of the vaginal epithelium that antagonism, when it occurs, is exhibited within a rather narrow range of dosage. As the dose of estrone is increased above this critical range, the antagonism appears to be overcome and then as, at the .05 γ level, the cooperative effects of the hormones, as evidenced by epithelial mucification, become apparent, while at still higher doses, for example .15 γ, neither antagonism nor cooperation is evident and the epithelium is completely cornified reflecting only the estrogenic stimulation. In many strains of mice following gonadectomy the adrenal cortex has been shown to produce rather large amounts of estrogenic hormones. Therefore, an experiment was carried out to ascertain if the differences noted between the non-castrate and castrate groups of animals

¶ In an earlier study(17) the mammae of castrate male mice of this hybrid cross receiving .05 γ estrone per day were reported as not being developed because at that time we considered both end bud formation and definite duct extension as being necessary criteria of development. A review of these earlier slides reveals that the glands of all 4 of the animals contained several end buds and should have been recorded as showing development.

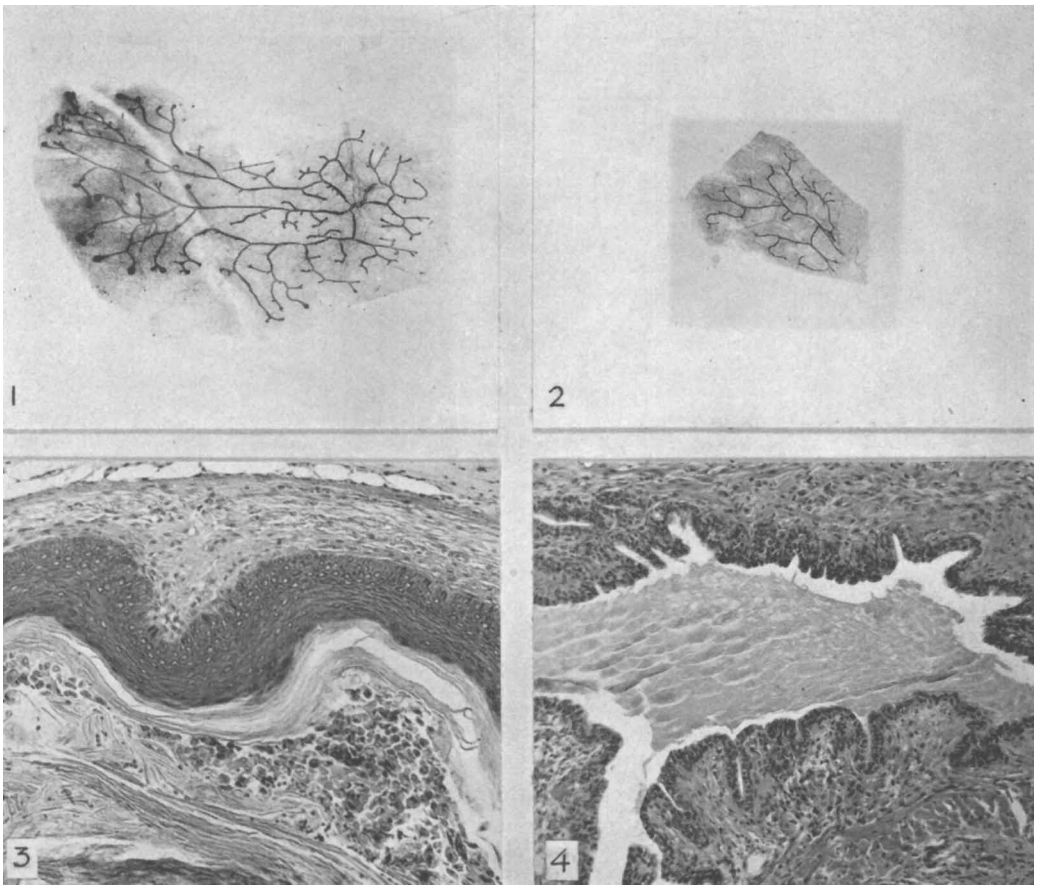

FIG. 1. Mammary gland of a castrate male mouse receiving .10 γ estrone per day. Note the presence of numerous end buds, and the definite increase in the size of the gland that has occurred.

FIG. 2. Mammary gland of a non-castrate male mouse receiving .10 γ estrone per day. No end buds are present and the gland is similar to those seen in uninjected control male mice of this stock.

FIG. 3. Vaginal graft of a castrate male mouse receiving .04 γ estrone 4 days of the week. The epithelium is almost completely cornified.

FIG. 4. Vaginal graft of a non-castrate male mouse receiving .04 γ estrone 4 days of the week. No evidence of epithelial stimulation is seen and the graft is similar to those seen in uninjected control male mice.

could be due to differences in adrenal function.‖ The result of this study has been included in Table I. It can be seen that the differences between the castrate and non-castrate mice are still present after adrenalectomy. In fact, the castrate adrenalectomized mice appear somewhat more sensitive to estrone than do the castrate non-adrenalectomized animals.

Estradiol series (Table II). Similar evidence for androgenic antagonism to the proliferation of the mammary gland occurred at the .01 γ per day and .02 γ per day doses of estradiol. At the .01 γ per day dose level, there was development present in the glands of 8 of the 9 castrate animals, while the glands

‖ Month-old mice were castrated and adrenalectomized and then were maintained on sodium chloride and glucose drinking water and a semi-synthetic diet in which sodium salts had been substituted for the potassium salts in the salt mix (graciously supplied by Dr. J. T. King). Studies have indicated that on this regimen young adrenalectomized mice grow well and show essentially normal development of their secondary sex characteristics.

TABLE II. Male Mice Bearing Vaginal Grafts Injected with Estradiol.*

γ per day		Vaginal mucosa		Mammary gland development	
.0025	Castrate	Atrophic	(4)	Slight	(1)
				0	(4)
	Non-castrate	"	(3)	0	(5)
.01	Castrate	Cornification	(2)	Good	(7)
		Cornification and mucification	(1)	Slight	(1)
				0	(1)
	Non-castrate	Atrophic	(3)	0	(8)
		Slight mucification	(1)	Slight	(1)
.02	Castrate			Good	(8)
	Non-castrate			0	(9)

* The number in parentheses indicates number of mice in each group.

TABLE III. Male Mice Bearing Vaginal Grafts Injected with Diethylstilbestrol.

γ per day		Vaginal mucosa		Mammary gland development	
.004	Castrate	Atrophic	(5)	0	(6)
				Slight	(1)
	Non-castrate	"	(4)	0	(3)
				Slight	(2)
.008	Castrate	Slight development	(9)	0	(12)
		Some mucification		Slight	(2)
	Non-castrate	Slight development	(9)	0	(8)
		Some mucification		Slight	(1)
.016	Castrate	Mucification with beginning cornification	(9)	Good	(9)
	Non-castrate	Mucification	(9)	"	(9)
.033	Castrate	Almost complete cornification	(5)	"	(5)
	Non-castrate	Almost complete cornification	(5)	"	(5)
.066	Castrate	Complete cornification	(4)	"	(4)
	Non-castrate	" "	(4)	"	(4)

* The number in parentheses indicates number of mice in each group.

of 8 out of 9 of the non-castrate animals were without development (one gland of one non-castrate animal contained 2 end buds). The glands of all of the castrate animals receiving .02 γ per day were well-developed in contrast to a complete absence of development in all of the non-castrate animals receiving the .02 γ per day dose. Evidence for androgenic antagonism to the development and cornification of the vaginal mucosal grafts was present also at the .01 γ per day dose level of estradiol. All of the grafts in castrate animals were cornified (a small amount of mucification accompanied the cornification in two cases) whereas all but one of the grafts in non-castrate animals remained completely atrophic. A slight amount of mucification was present in this one graft.

Diethylstilbestrol series (Table III). Evidence of estrogen-androgen antagonism seemed to be lacking when the non-steroid diethylstilbestrol was the estrogenic substance administered. In contrast with the estrone and estradiol series there was a striking similarity when the mammary gland and vaginal graft histology of the castrate animals was compared with that of the non-castrated animals receiving the same doses of diethylstilbestrol (Fig. 5, 6, 7, 8). This varied from no development in the .004 γ per day dose to complete development of both epithelia at the .066 γ per day dose. The only appreciable differences demonstrable between the castrate and non-castrate animals occurred in the vaginal mucosa at the .016 γ per day dose. The grafts of these castrate animals revealed mucification with beginning cornification, while only mucification was present in the

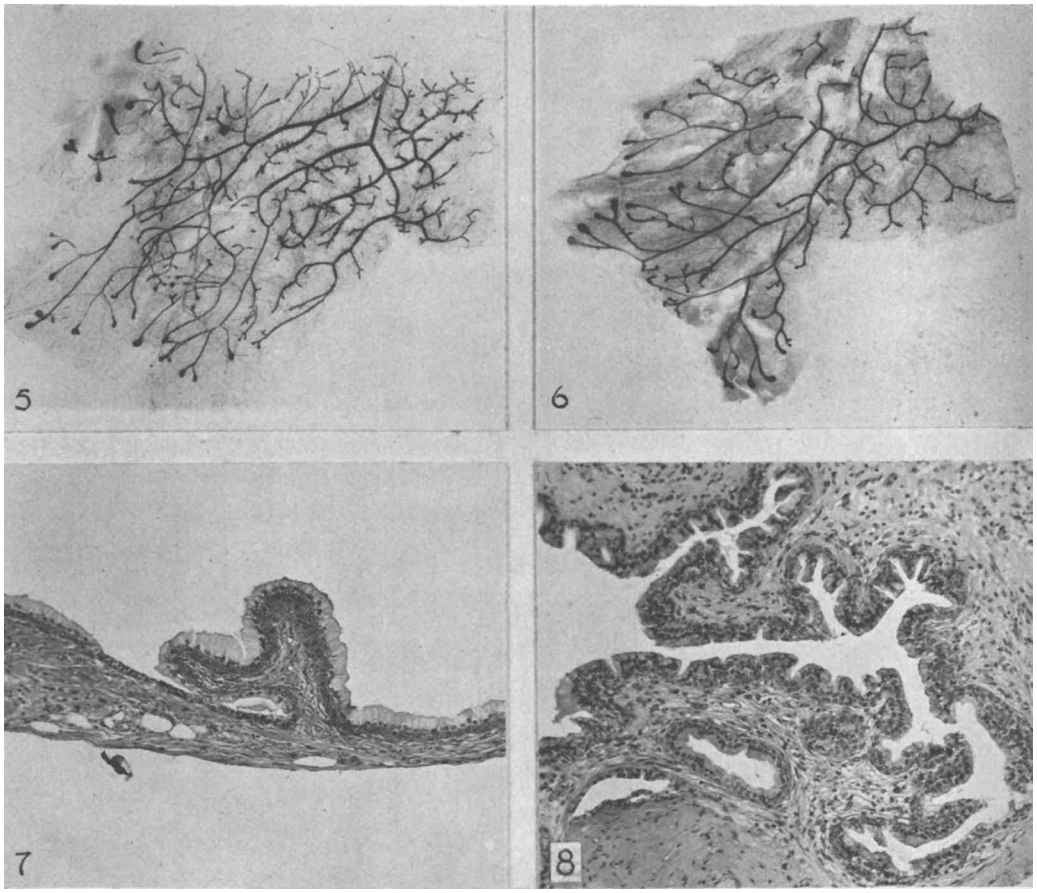

FIG. 5. Mammary gland of a castrate male mouse receiving .033 γ diethylstilbestrol per day. Several end buds are to be seen and the duct system has extended.

FIG. 6. Mammary gland of a non-castrate male mouse receiving .033 γ diethylstilbestrol per day. This gland is similar to those seen in the castrate male mice receiving the same dose of diethylstilbestrol.

FIG. 7. Vaginal graft of a castrate male mouse receiving .008 γ diethylstilbestrol per day. There is evidence of a minimal stimulation consisting only of mucification of the luminal cells.

FIG. 8. Vaginal graft of a non-castrate male mouse receiving .008 γ diethylstilbestrol per day. The degree of stimulation is similar to that seen in the grafts of the castrate males receiving this dose of diethylstilbestrol.

non-castrate animals. This is interpreted as indicating that with functioning testes cooperative effects were evident over a wider range of injected estrogen.

Discussion. The presence of an estrogen-androgen antagonism seems fairly well established by the results of investigations with estrone and estradiol. It is evident that testicular function is capable of completely inhibiting the stimulating effects of certain doses of both of these steroid estrogens on the mammary and vaginal epithelia. That this antagonism is due to testicular androgen is supported by the studies of Mühlbock and Gardner(16,18) in which the injection of relatively large doses of testosterone propionate inhibited the mammary stimulating effects of estrone and estradiol.

The apparent lack of pituitary suppression as evidenced by the normal testicular stimulation of the accessory sex organs of the non-castrate animals (in all series regardless of estrogen dosage) strongly supports the concept of estrogen-androgen antagonism as a direct or essential phenomenon for certain target tissues. It seems probable that in many

experiments in the past, such as those reported by Moore and Price(4), antagonistic or neutralizing effects were not observed due to the use of relatively large doses of the stimulating hormones. In our study of the vaginal epithelium, if only the 0.15 γ per day or larger doses of estrone had been employed no inhibitory or cooperative effects of testicular function would have been observed. If instead of graded doses, a single low level such as 0.05 γ had been employed, only the cooperative effects would have been observed in the target tissue.

Of particular interest is the apparent lack of estrogen-androgen antagonism in the studies with diethylstilbestrol. Although an explanation of the mechanism for hormone antagonism must await further experimental data, the interactions of the naturally occurring estrogens and androgens may possibly represent another instance of biological competition occurring between structurally related compounds similar to the competitive inhibition exhibited between the metabolite thiamine and its structural analogue pyrithiamine(22). One might hypothecate further that the lack of such competitive inhibition between diethylstilbestrol and testicular androgens might suggest that the androgens are not able to successfully compete with diethylstilbestrol for some particular intermediary substance, possibly an enzyme, whereas they can successfully compete for this substance with estrone or estradiol. This difference in action would also result if the metabolic pathways for the utilization of, or the stimulation by, the two types of estrogens were somewhat different so that the step at which androgens inhibit or block the steroid estrogens is not involved in diethylstilbestrol stimulation of these target organs.

Summary. 1. Studies of the effect of injections of steroid and non-steroid estrogenic substances on the mammary gland and vaginal mucosa of castrate and non-castrate male mice bearing subcutaneous vaginal grafts are reported. 2. It is suggested that antagonistic actions exist between the naturally-occurring estrogens (estrone, estradiol) and testicular androgens in regard to the mammary gland and vaginal mucosa. 3. Such antagonism did not seem to occur when the synthetic estrogenic substance diethylstilbestrol was employed.

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22. Woolley, D., *Physiol. Rev.*, 1947, v27, 308.

Propagation in Chick Embryos of Dengue Virus, Hawaiian Strain. II. Findings in Infected Eggs.* (18641)

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It has been reported(1) that propagation of the Hawaiian strain of dengue virus was facilitated by progressive adaptation of the virus to mice by means of intracerebral passages. After 101 such passages, the virus was transferred to chick embryos, and it has to date been carried through more than 90 passages in eggs without demonstrable changes in its pathogenicity for mice and without change in its limited host range. The sera of human beings and of monkeys convalescent from infection with the original, human Hawaiian strain react specifically with the egg-adapted virus. Of two human volunteers inoculated with it, only one had a mild febrile reaction, but both responded with production of neutralizing antibody against the mouse-adapted virus(1).

This paper will describe certain characteristics of the infection in eggs. Of main interest is the finding that the pronounced neurotropism, acquired by the virus as a result of passage through mice, has persisted in the

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1. Schlesinger, R. W., *Am. J. Hyg.*, 1950, v51, 248.