TABLE III. Assays of Chloro F Acetate for Sodium-Retaining Activity, 4 Experiments.

No. of animals	Potency (DCA = 1)
40	9.5
50	12.3
36	16.9
36	10.2

pooled data listed in Table III, is 10.8 times that of DCA with 95% confidence limits of 6.8 to 17.1. The analysis of variance showed that the departure from parallelism and curvature are not significant. This indicates that the requirements for a valid assay have been met.

The mineralo-corticoid-like activity of Chloro F acetate is of considerable interest in view of the previously reported unusually high glucocorticoid-like action of this compound(1,6). Thus, in the rat this compound is unique in that it manifests both gluco- and mineralo-corticoid-like properties to such a high degree.

It is also of interest to note that growth and survival can be markedly enhanced by a substance, such as Bromo F acetate, which has little sodium-retaining activity. Conversely, a compound with sodium-retaining activity, such as Fluoro F acetate, is not always effective in stimulating growth and survival.

Summary. 1. Growth and survival studies on adrenalectomized immature rats following single injections of aqueous suspensions of steroids indicate that Chloro F acetate is 10 to 20 times and Bromo F acetate 2 to 5 times as active as DCA. E acetate, F acetate, and Fluoro F acetate are less effective than DCA. 2. Sodium retention tests with Na^{22} indicate that Chloro F acetate is 10.8 times as potent as DCA, and that Fluoro F acetate is somewhat more effective and Bromo F acetate considerably less effective than DCA. The dose-response curves suggest that the steroids cause sodium retention by more than one mechanism. 3. The data indicate that, with the steroids studied, there is not always a direct correlation between sodium-retaining activity and growth-survival activity.

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Effects of an Antihyaluronidase Substance and of Hyaluronidase on Growth of Virus-Induced Fibromas. (21169)

JOHN M. PEARCE AND ANTONIO F. LASORTE.

From the Departments of Surgery and Pathology, the New York Hospital-Cornell University Medical Center.

The spreading effect of hyaluronidase has been amply demonstrated by Duran-Reynals (1) and Sprunt(2,3) for India ink, vaccinia virus, fibroma virus, and virus III. We have obtained results similar to theirs with the fibroma virus but have not published these previously since augmentation of the lesion in the rabbit skin brought about by the simultaneous injection of hyaluronidase and virus seemed such an obvious and predictable phenomenon. However, the recent *in vitro* development of an "antihyaluronidase substance" by Hadidian and Pirie(4) stimulated the experiments here described. These indicate that an antihyaluronidase prepared outside of the animal body has an effect exactly the reverse of that of hyaluronidase on the formation of virus-induced fibromas in that it can prevent the formation of the fibroma or markedly diminish its size.

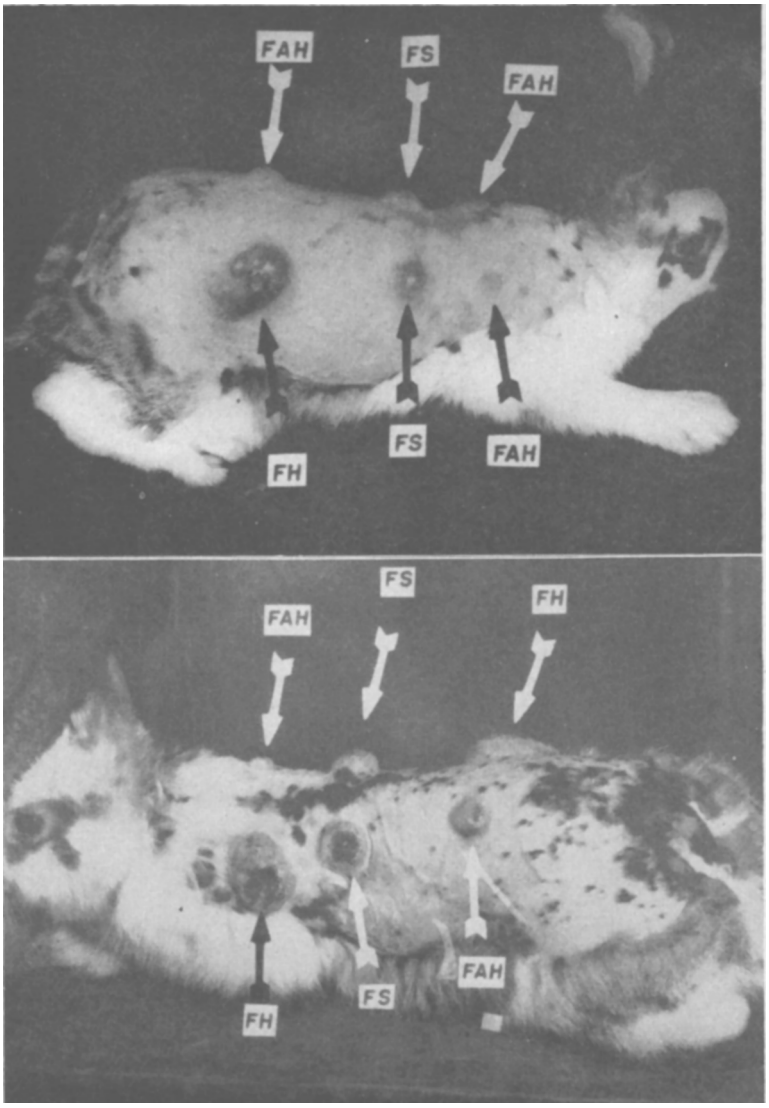

FIG. 1. Effect of hyaluronidase and antihyaluronidase on development of fibroma-induced tumors in the rabbit skin. FH indicates fibroma virus suspension and hyaluronidase injection sites. FS—fibroma virus suspension and saline solution. FAH—fibroma virus suspension and antihyaluronidase. In the rabbit in upper photograph lesions are seen in profile on left side and in lower photograph lesions are seen in profile on right side.

Method and material. The neoplastic or proliferative lesions upon which the hyaluronidase and antihyaluronidase substances acted were produced by the Shope fibroma virus.*

Twelve male rabbits, mixed breeds, weighing approximately 3000 g were used. Six sites of injection were chosen in symmetrical positions on the back of each. One-half cc of a 50% mixture of fibroma virus and antihyaluronidase was injected intradermally into one of the 3 sites on each side. Into another on each side was injected 0.5 cc of a 50% mixture of fibroma virus and hyaluronidase and into the third 0.5 cc of a 50% mixture of fibroma virus and physiological saline. These 3 combinations of material were so placed on the back that the fibroma and antihyaluronidase was at the same level as the fibroma and hyaluronidase. By making this double set of injections there were not only 12 experimental

TABLE I. Effect of Antihyaluronidase on Growth of Virus-Induced Fibromas Compared with That of Hyaluronidase and a Saline Control.

No. of lesions	Fibroma virus + saline 24	Fibroma virus + antihyaluronidase 24	Fibroma virus + hyaluronidase 24
	cm		
Avg greatest diam.	2.3	1.3	3.5
" least "	2.1	1.2	2.9
" thickness	.5	.2	.5
" vol.	2.4	.3	5.1

animals but each animal was his own control, the similarity of the lesions on the 2 sides of the same animal confirming the validity of the results. The preparations of hyaluronidase and antihyaluronidase were provided by G. D. Searle and Co. of Chicago. The former was their commercial preparation Alidase. The latter was their solution SN752, as described by Hadidian and Pirie(4). Each injection of Alidase consisted of 0.25 cc of the solution or 37.5 turbidity reducing units. Each injection of the antihyaluronidase consisted of 0.25 cc of SN752 or 2.5 mg.

The fibroma virus suspension used for injection was made by grinding portions of the skin tumor produced by a recent serial passage in a mortar and pestle with sterile sand and saline. The same suspension was used in each rabbit.

Results. In every instance the fibroma induced by the virus and a dilution of saline was uniform in size, time of appearance and duration. In every instance the fibroma induced by the virus was inhibited by antihyaluronidase, appeared later, if it appeared at all, and never reached as large a size. In every instance the fibroma induced by the virus is augmented by hyaluronidase, appeared earlier and grew larger (Fig. 1). Since the reactions in the fibroma-saline control sites seemed to reach their height at approximately the 7th day, this time was chosen to record the dimensions of the individual lesions. The tumors were measured in their greatest diameter and in their least diameter, paralleling the skin surface, and in their greatest elevation above it or thickness. The cubic volume was then arrived at by multiplying these 3 figures. These dimensions are listed in Table I. There are other variations in the appearance of the lesions which do not appear in this numerical tabulation. The fibroma-saline lesions were invariable symmetrical, flattened hemispheres. The fibroma-hyaluronidase tumors were not only larger but tended to be asymmetrical, the dependant portions extending downward toward the ventral surface of the animal. There were in addition pseudopodial projections and not uncommonly satellite nodules separated from the main mass by intervening grossly uninvolved skin. These latter never measured more than 2-4 mm in diameter. In the fibroma-antihyaluronidase tumors there was also frequently a marked asymmetry but unlike the hyaluronidase affected lesions this did not seem to be the result of gravity but was quite irregular. Again there were pseudopods and occasionally small satellite masses, but always all of these manifestations were on a minimal scale as compared to the fibroma-hyaluronidase tumors. These differences in the lesion produced by the 3 combinations of injected material were always striking and were constant. There was little deviation among the individual experimental animals from the averages listed in the table.

Discussion. These results as illustrated and as tabulated are so definite that only the mechanism of their accomplishment remains to be considered. Theoretically, there are 2 obvious ways in which the growth of the tumor could be inhibited by the antihyaluronidase. One of these is that the agent that incites growth is no longer active. If the virus is killed by the antihyaluronidase, either in the mixture as prepared before its injection or in the skin site itself, it of course could not pro-

* The fibroma virus used in these experiments was the strain A virus that was originally isolated by Shope(5) in 1932. It was obtained from Doctor Shope in 1938 in the laboratories of the Department of Animal Pathology of the Rockefeller Institute and has been maintained by serial skin passages through the rabbit in the laboratory of the senior author since then. During that time the reaction caused by it has changed in that there is a greater degree of interstitial mucin production and a smaller amount of cellular proliferation. The clinical disease in the rabbit, however, remains unchanged.

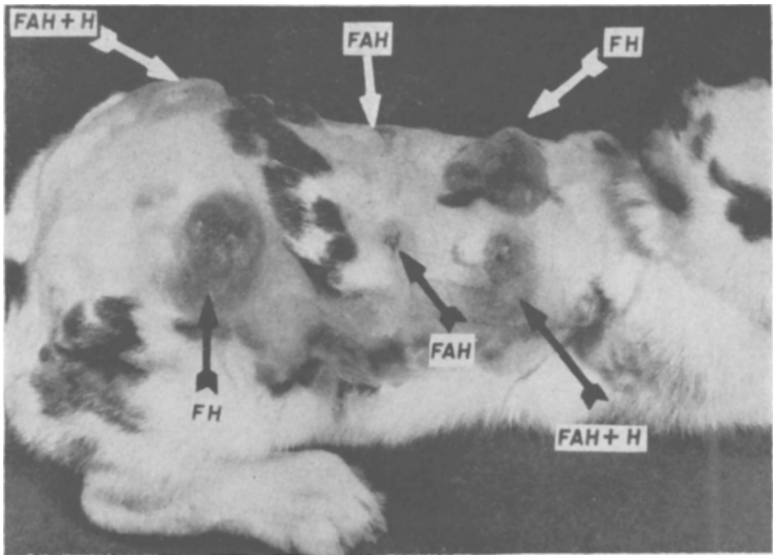

FIG. 2. Effect of a neutralizing amount of hyaluronidase added to fibroma suspension-antihyaluronidase mixture as compared to fibroma virus and antihyaluronidase and fibroma virus and saline. FAH + H indicates the 1st of these combinations, FH the 2nd and FS the 3rd. FAH + H lesions if not entirely equal in size are at least comparable with FH lesions and much larger than FS lesions.

duce tumors. The other possibility is that the power of growth in the reactive cells is inhibited in one way or another.

In order to eliminate the first of these factors, the death or inactivation of the tumor-causing virus, an experiment was set up in duplicate as described above except that at one of the sites of injection on each side a mixture of fibroma virus and antihyaluronidase to which a neutralizing amount of hyaluronidase had been added, was introduced. If the virus was destroyed by the antihyaluronidase no tumor would appear. If the hyaluronidase would counteract or neutralize the antihyaluronidase the tumor would appear and it would be proven that the virus was not killed and the anti-tumor factor was in the action of the antihyaluronidase on the tissues of the host rather than on the inciting virus. The latter proved to be the case. At the sites of injection of the fibroma virus and antihyaluronidase a minimal lesion occurred (Fig. 2). At the sites at which this same mixture with an added equal amount of hyaluronidase was injected, large lesions appeared comparable in size to those at the sites at which the mixture of the fibroma virus and hyaluronidase was injected. This indicates that there is no *in vitro* inactivation of the virus but that the effect of the antihyaluronidase on the lesions produced by the virus is the result of its action on the tissues of the host and not on the inciting virus.

Since in some animals the lesion produced by the virus-antihyaluronidase mixture was minimal or entirely absent it would seem likely that this is not a simple prevention of spread of introduced virus but that it is a prevention of the proliferation of the fibroblast and production by that cell of the large amount of interstitial substance by which this tumor is characterized.

Summary. The action of an antihyaluronidase substance that strikingly inhibits the growth of the tumor produced by the Shope fibroma virus is described. This inhibition is brought about by the action of this material on the cells or ground substance of the host and not by its action on the virus. As previously reported, hyaluronidase increases the size of the tumor-like lesions produced by the Shope fibroma virus in the skin.

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Effect of ACTH upon Artificially Induced Trichinosis in Rats with Special Reference to Eosinophilia. (21170)

M. POLLAY, B. WEIN, AND H. A. HARTMANN. (Introduced by D. M. Angevine.)
From the Department of Pathology, University of Wisconsin Medical School, Madison, Wisconsin.

A few reports have recently been published dealing with the therapeutic possibilities of ACTH and Cortisone in the treatment of trichinosis. To study the problem further under controlled experimental conditions, it was deemed advisable to determine the effects of ACTH upon the course of an artificially induced Trichina infection in rats. Such an experiment afforded an opportunity to study the tissue reactions to the parasite in different sites in both treated and untreated animals. The magnitude of the effect of ACTH upon an artificially induced eosinophilia could also be determined(1).

As early as 1904, Opie(2), had described the peripheral blood leucocytosis with both relative and absolute increases of eosinophils and neutrophils in trichinosis in guinea pigs. He also noted myelocytic hyperplasia of the bone marrow and neutrophilic, eosinophilic, and lymphocytic tissue infiltration due to the encysted larvae in muscle(3,4). Luongo(5), *et al.*, reported the use of ACTH for cases of trichinosis in humans. In patients so treated there was observed a diminution in fever, loss of muscle pain, and a striking reduction of other subjective symptoms. Also reported in the same paper were observations on guinea pigs experimentally infected with *Trichina spiralis* and treated with ACTH. A drop in circulating eosinophils and a reduction in fever were reported, but the authors were unable to demonstrate any modification of the inflammatory response.

During our study a paper by Stoner and Godwin(6) appeared in which the effects of ACTH and Cortisone upon the susceptibility to trichinosis in mice was described. They found that these drugs increased the susceptibility of mice to trichinosis, but did not alter the course of the disease or the mortality to a lethal dose of Trichina. In these mice there was no significant change in the degree or type of inflammatory response in treated or untreated animals.

Experimental. Twenty young adult white male rats (Sprague-Dawley strain) weighing on the average from 60 to 80 g were used because of their susceptibility to trichinosis as well as the availability of blood for study. The animals were separated into 4 groups

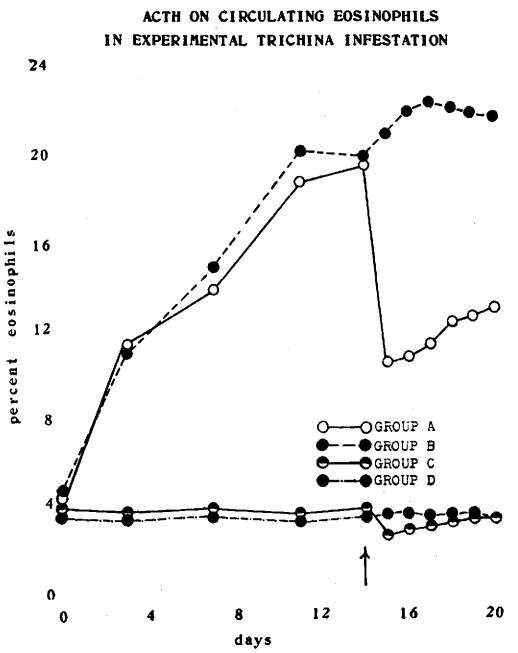

FIG. 1.