duced at skin sites was generally found to disappear in 10 to 14 hours. No difference in disappearance from the skin was observed between precipitating antitoxin, non-precipitating antitoxin and skin sensitizing antitoxin.

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Effect of Topical Application of Hydrocortisone on Dermic Lesions Produced in Rabbits by Vaccinia Virus. (26846)

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Cortisone and its derivatives are commonly employed as topical medication in several skin and mucosal diseases. Inflammatory reactions are thereby diminished and symptoms such as rash and pruritus are alleviated in many cases. However, in other cases local application of cortisone involves hazards which it is important to consider. These hazards occur in some viral diseases, among which those produced by herpes simplex virus are good examples. In fact, in man, conjunctival lesions of such an origin grow and deepen as a result of the local action of corticoids, as has been observed in experimental infection of the rabbit(1,2). Trachoma is also aggravated during the acute phase or may be reactivated during later stages when treated locally with those substances(3). Nevertheless, the literature does not mention similar observations in relation to other viruses that produce epidermal lesions.

In this work the local effect of hydrocortisone on dermal lesions produced in the rabbit by vaccinia virus is studied.

Material and methods. The strain employed belonged to the Instituto Nacional de Higiene virus seed whose lots 32 and 38 of vaccinia virus were prepared in calves. In the lymph potency test, determined by Force and Leake's technic(4), confluent lesions were observed at 1:10,000 dilution of lymph and isolated lesions at 1:30,000.

Once the inoculum potency had been determined, 24 healthy albino rabbits were selected at an average weight of 4,500 g. Two areas on the back of each rabbit, measuring 4 cm $\times$ 4 cm and situated 5 cm apart, were depilated. One percent hydrocortisone ointment was applied on one of the areas and neutral vaseline on the other. Both applications were performed daily. Six days after the treatment had begun the rabbits were inoculated intracutaneously through scarification with 0.3 ml of a viral suspension diluted 1:30,000 in saline. The inoculum was carefully spread throughout the area. Two hours later, when the fluid had thoroughly soaked the skin, ointments were applied and the treatment was continued for 6 days more.

To detect the appearance of vesicles the rabbits were observed daily until maximal development of lesions. As soon as the vesicles appeared they were measured every 24 hours by applying a glass slide over the affected

area in such a way that the outline of the lesions observed through the glass could be traced with India ink. The area of the lesions was determined by setting the slides on millimetric paper. Another group of 12 inoculated rabbits, treated in the described way, was assigned to provide virus for titration from the lesions found in the 2 areas when maximal development was reached. To harvest the virus for titration the areas were first washed with soap and water and the lesions were thoroughly scraped with a scalpel. The material obtained from each area was separately ground in a mortar adding sterile saline until a volume of 10 ml was reached. From this suspension, which was regarded as undiluted, serial dilutions were made with a factor of ½ log, and inoculated at doses of 0.2 ml onto the chorioallantoic membrane of chick embryos (6 per dilution). The eggs were incubated for 72 hr, then kept in the freezer 4 hours more and the chorioallantoic membrane harvested. Titers were estimated by the pock counting method(5). Using 6 tubes per dilution of rhesus monkey kidney cell cultures maintained with 60% Hanks' solution, 20% lactalbumin hydrolysate and 20% normal horse serum, a parallel titration was performed with 0.2 ml of viral suspension. The inoculated tubes were incubated for 30 minutes before adding the culture medium. During the next 10 days the tubes were observed for CPE typical of vaccinia virus infection in this type of cells, as described elsewhere(6,7). Titers were estimated by the Reed and Muench method(8).

Results. On the appearance of lesions, usually 72 hr after inoculation, results were evaluated by comparing the total extent of injured areas in corresponding sites on the 24 rabbits. From the fourth day it became apparent that the size of the injured surface in the areas treated with cortisone was larger than control areas (70% greater). This difference increased in the following days. Measurements taken when the lesions reached maximal development are shown in Table I. There is a significant difference between the extent of affected surface in treated and control areas.

It should be pointed out that in control areas the lesions reached maximal development on the seventh day, whereas in areas treated with cortisone the vesicles continued to enlarge until the ninth day. Moreover the involutive phase of cortisone-treated lesions involved 17 days, in comparison to 10 days in the controls. The total process lasted 26 days in areas treated with cortisone and 17 days in control areas, a difference of 9 days. These data can be seen in Fig. 1. After healing, treated and control areas presented different aspects. The site of lesions on the cortisone-treated skin was marked by a small depressed area, while complete restoration to normal appearance of the skin occurred at control sites.

Comparative titrations obtained from virus contents of the lesions found in treated and control areas were performed on the chick embryo chorioallantoic membrane and in

TABLE I. Action of Cortisone on Number and Total Area of Lesions Produced by Vaccinia Virus in 2 Cutaneous Scarifications in 24 Rabbits.*

Rabbits	No. of lesions in area 4 cm × 4 cm		Total injured area, cm²		Diff., cm²
	+ Cort.	− Cort.	+ Cort.	− Cort.	Δ
1	3	4	3.5	2.6	.9
2	4	4	3.8	2.0	1.8
3	5	4	3.6	2.4	1.2
4	3	4	3.4	2.4	1.0
5	4	3	3.4	1.8	1.6
6	4	5	3.8	2.8	1.0
7	3	4	3.2	2.4	.8
8	3	3	3.6	1.5	2.1
9	4	4	3.4	2.2	1.2
10	4	3	3.6	1.6	2.0
11	3	4	3.5	2.0	1.5
12	4	5	3.4	2.5	.9
13	4	4	3.6	1.8	1.8
14	3	4	3.6	1.8	1.8
15	4	4	3.2	2.2	1.0
16	4	3	3.4	1.4	2.0
17	5	4	3.8	2.0	1.8
18	5	4	4.2	2.4	1.8
19	4	3	3.6	1.6	2.0
20	4	4	3.6	1.8	1.8
21	4	4	3.4	1.8	1.6
22	4	5	3.2	1.8	1.4
23	4	4	3.2	2.4	.8
24	5	4	3.8	2.8	1.0
Avg	3.9	3.9	3.5	2.08	1.45
			Total:		34.8

$\bar{\Delta}$ Differs significantly from zero ($p < 0.01$).

* Calculations were made from measurements taken at time the lesions reached maximal development; on the 7th day for control areas and on the 9th for cortisone-treated areas.

TABLE II. Titers of Material Tested in Embryonated Eggs and in Tissue Cultures.

Type of material	Titration in chorioallantoic membrane	Titration in *M. rhesus* kidney cell cultures
With cortisone	8.2×10^8 infectious particles/ml	$10^{8.3}ID_{50}$ per ml
Without cortisone	8.0×10^8 " " "	$10^{8.1}ID_{50}$ "

rhesus monkey kidney cell cultures. By either method, the results disclosed no significant differences in the virus titer of the two materials tested (Table II).

Discussion. In view of the results obtained in similar experiments with other viruses(2,9), our findings were to be expected, *i.e.*: that topically applied cortisone on rabbit skin enhanced the manifestations of the infection produced by the vaccinia virus. In our experiments, cortisone caused an increase in size of the lesions and an extension of total evolution time, mainly by an increase of the involutive phase. These two effects of cortisone may be explained by its inhibitory properties upon inflammatory and reparative processes. In fact, no differences were observed in either area during the first 4 days, *i.e.*, at the time when important inflammatory changes were not expected to occur. From the fourth day on, cortisone caused progressive increase in the size of the lesions.

As no differences were encountered in the total number of lesions appearing in cortisone-treated areas as compared with those in control areas, it seems that the susceptibility of the cells was not essentially modified. This idea finds further support in the results of the virus titrations on material from treated and control areas. However, differences in amount of virus from the 2 sites might be expected to parallel the differences in size of lesions, and such differences (less than 2-fold) would probably not have been detected by the methods used.

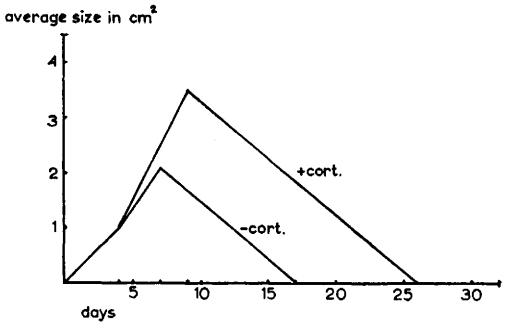

FIG. 1. Evolution time of the lesions and size in areas treated with cortisone and in control areas.

Summary. The effect of topically applied cortisone upon vaccinia virus infection in the rabbit skin was studied. No difference was observed in the number of lesions that appeared in treated as compared with untreated areas, but lesions in cortisone-treated sites attained a greater size than did those in control sites (70% greater area). Also the pathologic process was prolonged by cortisone, from 17 days in control to 27 days in treated areas, namely, by increasing the reparative phase. Comparative titrations, performed in chick embryos and in rhesus monkey kidney cell culture, of the viral contents of lesions from the 2 sites, disclosed no significant difference. These findings may be explained by the inhibitory effect which cortisone exerts upon inflammatory and reparative processes. It seems that cortisone did not essentially modify cell susceptibility to the vaccinia virus.

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Hexosamine and Hydroxyproline Content of Fracture Callus in Normal and Aminoacetonitrile Treated Rats.* (26847)

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Aminoacetonitrile (AAN) administered to adult rats with experimental fractures induces formation of a voluminous fibrocartilaginous callus which ossifies well during the second week of fracture and becomes very osteoporotic during the third week. Connective tissue cells and immature cartilaginous cells are extremely abundant in the callus. Histological and histochemical studies have indicated that the ground substance and collagen fibers are defective(1). We are reporting here studies on the hexosamine and hydroxyproline content of fracture callus of normal and AAN-treated animals obtained 5, 10, 15 and 21 days following the experimental fracture.

Methods. Forty Sprague Dawley male rats weighing from 150 to 155 g were used. Half of the animals received finely ground, laboratory Purina chow and the other half received the same diet containing 0.075% AAN. A fracture in the middle third of both tibiae was produced manually in every rat under anesthesia by bending the leg over the dull edge of a large knife held in a vise. The fractures were not immobilized.

Five experimental and 5 control animals were sacrificed with ether 5, 10, 15 and 21 days following the fracture. Both hind legs were immediately dissected and the fracture callus was collected and placed in dry ice. Also both femurs were obtained, but only the shaft was used, and placed in dry ice. The callus tissue and femurs were pooled and homogenized in acetone in a Virtis blendor, dried and the acetonic powder was used as a starting material for the analysis.

Chemical analysis: The acetonic powder was dried in an oven at 104°C at constant weight and was hydrolyzed in HCl 4N during 15 hours and refluxed at 100°C. After hydrolysis, the solution was dried in a flash evaporator. The residue was dissolved, filtered, and the volume made up with water to 25 ml. This solution was used for the analysis.

The hexosamine was determined with a modification(2) of Boas' method(3). The hydroxyproline was determined according to the method of Neumann and Logan(4) modified by Leach(5).

The hexosamine content in the femoral shaft (including marrow) varied from 5.8 to 7 γ/mg of dried weight and the hydroxyproline content in the femoral shaft varied from 14 to 18 γ/mg in both experimental and control animals. These values did not change markedly throughout the experiment.

Discussion. In the fracture callus of the control animals fed normal laboratory diet an increase of hexosamine was noted which reached its maximum 15 days following fracture. If it is assumed that hexosamine is an index of mucopolysaccharide content of the callus our results agree with the work of Aoike *et al.*(6) who found large increase of chondroitin sulfate in the 2-week-old fracture callus using S^{35} as index of the chondroitin sulfate synthesis. The increase of hexosamine in the callus of the AAN-treated animals is small as compared with the controls. In the 15-day-old callus, the hexosamine of the AAN-treated rats was half that found in the controls and hexosamine decreased very

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