vascular ruptures and petechial hemorrhages to occur earlier and more prominently.

Summary. 1. Hyaluronidase modifies the inflammatory response of tissue due to certain oil and water-soluble irritants. 2. Hyaluronidase affects the vascular response. The spread of trypan blue in the tissues representing increased vascular permeability, appears earlier and is greater when the irritants are administered with hyaluronidase; and where the nature of the irritant is such as to produce hemorrhages, the areas of these hemorrhages are also greater and occur earlier. 3. Hyaluronidase does not alter the solubility or absorption of an oil-soluble irritant. 4. Use of hyaluronidase with oil or water-soluble irritants eliminates edema from the classical picture of inflammation. This may have practical applicability where prevention of edema resulting from contact with irritants is important. 5. These studies also indicate that hyaluronidase is a useful tool for investigating the edema aspect of the inflammatory reaction.

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Use of "Granuloma Pouch" Technic in the Study of Antiphlogistic Corticoids.* (20108)

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The "granuloma pouch" technic has been devised as a procedure for the quantitative analysis of factors regulating inflammation and wound healing. Its principle is that by injecting a given amount of air into the loose subcutaneous tissue of the rat, an almost perfectly symmetrical, spherical or ellipsoid air space, of any desired size, can be created. This acts as a mold for the subsequent formation of a granulomatous membrane, into which the internal surface of this cavity can readily be transformed, by injection of some irritant (*e.g.,* croton oil, formalin, kaolin) into the air space. The thickness and structure of the granuloma pouch, as well as the quantity and constitution (cellular and chemical constituents) of the fluid, which gradually replaces the air, can be largely determined at will by the selection of appropriate irritants. The salient features of this technic and its principal applications have been outlined elsewhere(1,2).

In the present communication, we would like to report upon experiments demonstrating the striking changes which occur, in the granuloma pouch, upon topical administration of hydrocortisone acetate.

Experimental observations. In the *1st experiment,* 16 female Sprague-Dawley rats, weighing 128 to 151 g, were subdivided into 2 equal groups. In all the animals of both groups, the dorsal region was depilated with barium sulfide. Immediately afterwards, the 8 rats of one group were treated with hydrocortisone micro-crystals. The suspension was made up by diluting the original preparation of Hydrocortone Merck (which contains 25 mg per ml) with the original suspension fluid (kindly supplied by Merck and Co., Montreal), in the amount required to obtain a concentration of 10 mg per ml. Of this suspension, 0.1 ml (*i.e.,* 1 mg) was then injected in the interscapular region. In order to obtain

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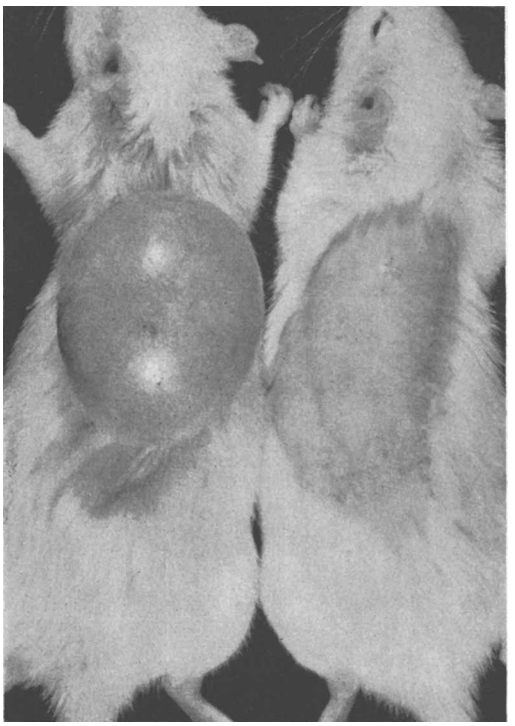

FIG. 1. On left, control rat received no hormone treatment. Note the large "granuloma pouch" filled, under pressure, with hemorrhagic exudate. On right, similarly treated rat in which whole inflammatory response was suppressed by a single topical inj. of 1 mg of hydrocortisone (much of which is visible, near cranial margin of depilated area, in the form of a whitish crystal deposit).

good distribution over a large area, this was done by inserting the entire length of a 3/4 inch, No. 27 hypodermic injection needle just underneath the skin, parallel with the vertebral column, through the loose subcutaneous tissue of the back. By injecting while the needle was then withdrawn, a long streak of hormone deposit was created. The 8 control animals remained untreated. *Twenty-four hours later,* in all animals of both groups, granuloma pouches were made by the following procedure: 25 ml of air were injected deep into the loose connective tissue between the shoulder blades through a No. 27 hypodermic needle. This was immediately followed by the injection of one ml of a 1% croton oil solution (in mazola oil) into the resulting air space, through the same needle. During the *first 2 days,* the air-filled pouches were essentially similar in the 2 groups. However, beginning at about the fourth to sixth day, the wall of the air bubble began to thicken, in the untreated control group, and transillumination revealed that a hemorrhagic brownish fluid was beginning to fill the cavity. In the hydrocortisone treated animals, the walls of the pouch remained so thin, that they could not be palpated and transillumination revealed only the transparent oily solution, which we had originally injected. Throughout the experiment the hydrocortisone deposit remained visible, by naked-eye inspection, just under the skin in the peripheral wall of the pouches. During the following days, the accumulation of exudate and the thickening of the granuloma-pouch wall proceeded constantly in the untreated controls. In the hydrocortisone treated animals, some of the air was absorbed and no fluid was formed to replace it, so that the pouch gradually collapsed.

The experiment was terminated on the 14th day after the croton oil injection, and Fig. 1 illustrates the typical appearance of one rat from each group. In the control animals, the

FIG. 2. Another pair of a control (left) and a hydrocortisone treated (right) rat, which underwent same procedure for production of granuloma pouches as in Fig. 1. The dorsal skin was removed, but the pouch remained attached to it, hence, entire region can be viewed from the inside. Again note the thick, non-transparent, granulomatous membrane, which forms a very regular ellipsoid. It is filled, under pressure, with hemorrhagic fluid. The empty pouch itself weighed 3.16 g and contained 40 ml fluid. The pouch of the hydrocortisone treated rat is not delimited from the surrounding tissue. It is so transparent that one can see the millimeter-scale, in its lumen, almost as clearly through the membrane as in those places where it is directly exposed through accidental holes. Perfect dissection was impossible, but even with much adjacent tissue still adherent the dissected mass weighed only 0.40 g.

TABLE I. Effect of Hydrocortisone on Granuloma Pouch (1st Experiment).

Treatment	Controls									Hydrocortisone treated								
No. of rats	1	2	3	4	5	6	7	8	Avg	1	2	3	4	5	6	7	8	Avg
Initial body wt, g	150	133	128	142	146	147	150	145	143	142	138	129	133	151	128	146	134	138
Final body wt, g	165	150	151	152	160	158	161	157	157	161	150	148	149	162	145	160	142	152
Fluid in pouch, ml	12	32	35	28	26	24	25	40	28	*	*	*	*	2.0	*	*	*	—
Air in pouch, ml	3.0	3.1	3.5	3.0	1.0	1.0	3.0	3.2	2.6	3.1	2.1	2.5	2.8	3.0	3.3	3.4	3.2	2.9
Wt of pouch wall, g	3.6	3.8	4.1	4.2	3.1	4.6	3.5	3.1	3.7	0.4	0.3	†	†	2.0	†	†	†	—

* Only about 1 ml of the original oil present; no macroscopically detectable exudate.
† Pouch too thin to be dissected and weighed.

pouch was filled by a large amount of hemorrhagic exudate, which accumulated under considerable pressure and hastened the absorption of air. Consequently, the pouch became extremely hard. On the other hand, in the hydrocortisone treated animals, only a slight elevation of the skin revealed some remnant of the air injected. Here, the skin was quite flabby and the air was obviously not under pressure.

In the control animals, not hormone treated, the pouch could easily be dissected; indeed, after making a skin-incision, mere pressure sufficed for the blunt enucleation of the ellipsoid granuloma pouch. Conversely, in the hormone treated animals, there was no definite membrane to dissect. Upon carefully splitting the skin, some remnant of air and the apparently unchanged oil solution were visible in virtually unchanged connective tissue. In the untreated controls, the pouch contained 1.0 to 3.5 ml of air and between 12 and 40 ml of hemorrhagic exudate. The carefully washed empty granuloma pouch itself weighed between 3.1 and 4.6 g (Fig. 2 and Table I).

Apparently, very small amounts of hydrocortisone suffice to inhibit the inflammatory response under these conditions. It must be kept in mind that with this technic we produced an inflammatory focus whose total weight (granuloma tissue and exudate) amounted to approximately ¼ of the total body weight, yet, as judged by macroscopic estimation, the size of the hydrocortisone deposit under the skin has hardly diminished during the 14 days of this experiment. Obviously, only a very small fraction of the hormone was actually involved in this intense antiphlogistic effect. It is rather important to emphasize, however, that in order to be so effective, the hormone must be injected into the connective tissue, which will subsequently become the granulomatous pouch wall. Numerous control experiments showed that if the same amount of hydrocortisone is injected at a distance of even only 1 mm from the pouch, it is virtually inert.

In a *2nd experiment,* conducted in exactly the same way, we used 3 groups of rats. Group I acted as not hormone treated controls; Group II received 1 mg of hydrocortisone acetate on the first day of the experiment in the same manner as the rats of the first experimental series; Group III was injected with the same amount of hormone directly into the air space. In Group I the granuloma pouch developed as in the untreated controls of the first series; it was completely inhibited in all the animals of Group II, which received hydrocortisone by injections into the adjacent subcutaneous tissue (which subsequently came to form the pouch wall): in the rats of Group III, which were given the same amount of hormone by injection into the pouch cavity, no significant inhibition was noted.

The question arose whether the croton oil solution (which remained in a seemingly unchanged condition within the connective tissue of the air space in the hydrocortisone protected rats) was still irritating to unprotected normal tissues at the end of the 14-day experiment. To examine this, we collected the oil from all the pouches in the hydrocortisone protected group (Group II of the second series) and injected 0.2 ml, just underneath the plantar aponeurosis of the right hind paw, in an additional group of 6 female Sprague-Dawley rats weighing 135 to 146 g. 0.2 ml of mazola oil was injected, as a blank control, into the left hind paw in these same animals.

TABLE II. Comparison between Effect of Hydrocortisone when Injected into Wall or Cavity of Granuloma Pouch (2nd Experiment).

Treatment No. of rats	Controls									Hydrocortisone in wall									Hydrocortisone in air-space								
	1	2	3	4	5	6	7	8	Avg	1	2	3	4	5	6	7	8	Avg	1	2	3	4	5	6	7	8	Avg
Initial body wt, g	120	112	115	130	150	134	150	151	*133*	137	135	125	116	135	140	144	133	*133*	141	131	144	120	134	142	150	140	*138*
Final body wt, g	148	123	145	149	168	142	150	172	*150*	160	142	143	148	150	152	180	153	*153*	160	190	175	145	145	152	160	160	*161*
Fluid in pouch, ml	17	12	19	10	20	27	28	19	*19*	2.6	*	*	*	2.0	*	*	*	—	11.5	32	22	13	12	11	28	17	*18*
Air in pouch, ml	1.0	2.0	1.5	2.0	1.0	0.6	2.0	1.5	*1.4*	1.0	1.5	1.0	2.0	1.5	1.0	1.7	2.0	*1.7*	1.0	0.6	1.2	1.0	2.0	1.5	0	1.5	*1.1*
Wt of pouch wall, g	3.5	3.2	3.6	4.1	4.5	3.5	3.0	3.1	*3.6*	†	†	0.5	0.3	†	†	2.6	†	—	3.0	2.5	1.6	2.5	3.6	2.1	2.0	1.6	*2.4*

* Only about 1 ml of the original oil present; no macroscopically detectable exudate. † Pouch too thin to be dissected and weighed.

TABLE III. Comparison between Effect of DOA and Δ^5-Pregnenolone upon Granuloma Pouch (3rd Experiment).

Treatment No. of rats	Controls									DOA									Δ^5-pregnenolone								
	1	2	3	4	5	6	7	8	Avg	1	2	3	4	5	6	7	8	Avg	1	2	3	4	5	6	7	8	Avg
Initial body wt, g	158	160	141	146	160	159	155	157	*154*	145	140	160	153	160	160	159	161	*155*	158	160	156	140	155	160	146	150	*153*
Final body wt, g	201	195	182	183	201	179	182	198	*190*	182	210	194	200	200	195	198	201	*198*	200	204	204	200	220	191	192	198	*201*
Fluid in pouch, ml	20	12	17	19	22	14	17	13	*17*	14	22	14	16	16	20	15	16	*17*	26	10	19	19	15	9	12	*	*16*
Air in pouch, ml	3	4	7	3	5	6	6	5	*5*	5	5	6	5	6	7	5	6	*6*	6	8	7	7	6	5	6	7	*6*
Wt of pouch wall, g	3.6	2.1	3.1	3.6	4.1	2.6	3.0	2.6	*3.0*	3.6	3.4	2.8	3.7	3.4	2.9	3.1	3.4	*3.3*	4.5	2.1	3.8	2.4	2.3	2.6	3.5	3.1	*3.0*

* This specimen was accidentally lost.

FIG. 3. Results of topical-irritation-arthritis tests. On left (top) paw of rat which received 0.2 ml of the oil, recovered from the hydrocortisone-protected "granuloma-pouch" region of the rat illustrated in Fig. 2. Note necrosis, near heel, and some hemorrhagic imflammatory response around ankle region. The other foot (bottom) was injected with same amount of mazola oil for control purposes and shows no reaction. On the right, another animal whose one paw (top) was inj. with 0.2 ml of a fresh 1% croton oil solution and shows a very intense hemorrhagic response throughout. Here again the other paw (bottom) was inj. with ordinary mazola oil and shows no reaction.

For comparative purposes, another group of 6 female Sprague-Dawley rats weighing 134 to 141 g was given 0.2 ml of a fresh 1% croton oil solution in mazalo oil into the right hind paw and again ordinary mazola oil into the left hind paw. Fig. 3 shows the result of this treatment after 24 hours. The mazola oil in itself caused no visible irritation, while the fresh 1% croton oil produced a very marked, hemorrhagic inflammatory response. The oil removed from the pouches of hydrocortisone protected rats was still very irritating, but evidently less so than the croton oil solution which had been originally introduced into the pouch.

It may be concluded that, even after 14 days of sojourn in the pouch, which showed no sign of irritation, the croton oil solution still retained much of its phlogistic effect. It is not possible to judge by our observations whether the decrease in phlogistic potency was due to partial absorption of the croton oil from the mazola oil solvent, to inactivation, or to the admixture of hydrocortisone which may have penetrated into the oil. In any case, however, it is obvious that, under these conditions, the cells of the pouch wall were pro-

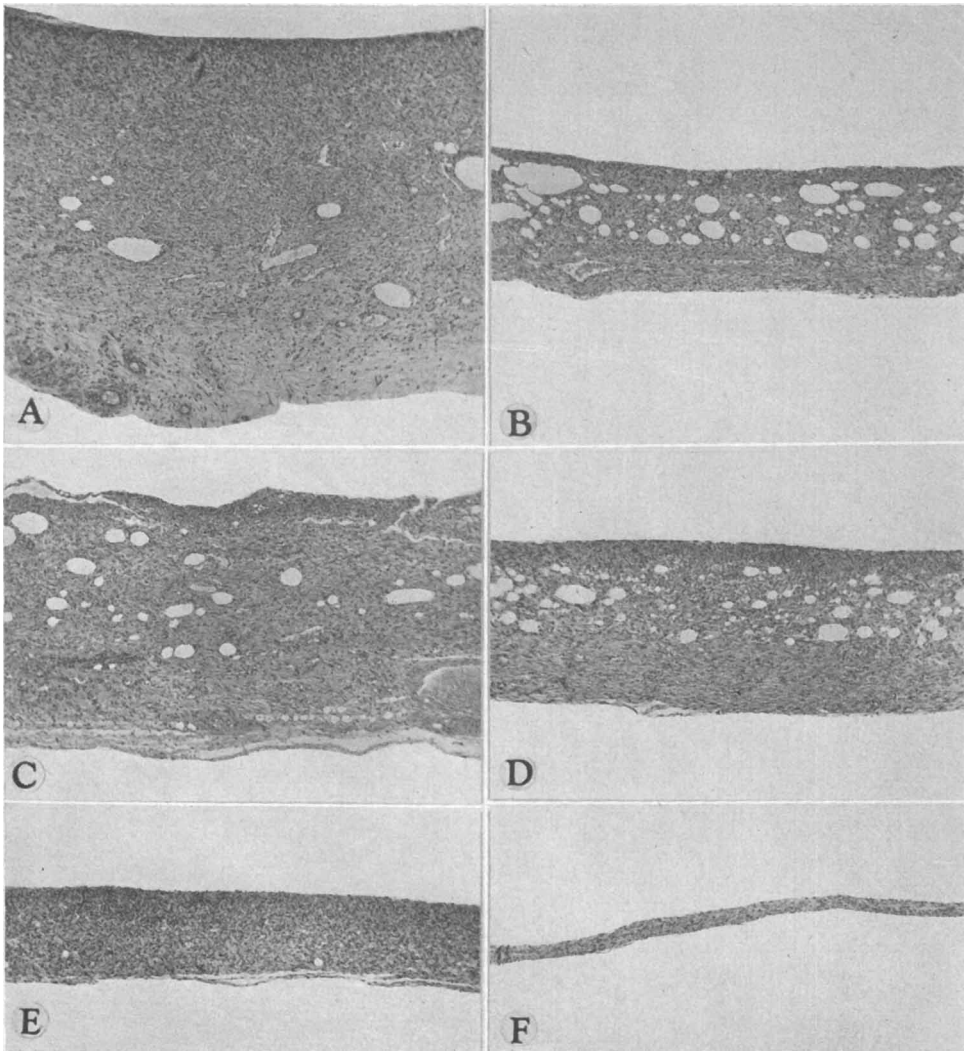

FIG. 4. Histologic appearance of "granuloma pouch" wall as influenced by various steroids. A and B: Rat No. 1 of Δ^5-pregnenolone series. C and B: Rat No. 1 of DCA series. E and F: Rat No. 1 in hydrocortisone treated group of first experiment. In all instances proximal wall (left) of pouches, that which rests on spinal column, is thicker than the peripheral portion (right), situated under the skin. Everywhere the walls consist of a rather fibrous granuloma tissue. In rats treated with DCA or pregnenolone (as in untreated controls, not shown here), fibroblasts and fibers of connective tissue are well developed, the capillaries are dilated and the granulomatous tissue contains numerous sudanophilic, presumably oil-containing, vacuoles, which appear here as empty holes. On the other hand, the hydrocortisone treated animal's pouch is thin; it consists of rather atrophic connective tissue and small fibroblasts with narrow capillaries and contains no fat vacuoles.

tected by hydrocortisone against the irritating action of the croton oil solution which was in contact with them for as long as 2 weeks.

A *3rd experiment* was then conducted essentially in the same manner as the first and second, except that here 1 mg of desoxycorticosterone acetate (DCA) micro-crystals and 1 mg of Δ^5—pregnenolone, respectively, were injected into the pouch wall in the experimental animals of Groups II and III, respectively. Here again Group I acted as untreated controls. Unlike in the first 2 experiments, all the animals of this third series were killed on the 9th day, by which time it had

become obvious, from mere macroscopic inspection, that these steroids fail to inhibit the inflammatory reaction when administered at the same dose level as the hydrocortisone acetate (Fig. 4 and Table III).

Table III indicates that, at this dose level, neither the formation of exudate, nor the development of the granuloma-pouch wall were significantly altered by these steroids. In this 9-day experiment, irrespective of treatment, the volume of the residual air is higher in all groups than in the first two 14-day experiments.

These findings, concerning the predominantly exudative, hemorrhagic response to croton oil, essentially confirm earlier animal experiments on the effect of gluco-corticoids upon inflammatory reactions in general(3-6). However, the use of a new technic permitted the experimental production of an inflammatory focus whose initial size and shape are always identical. It also made possible the objective, quantitative, separate determination of exudate and granuloma tissue formation, the recovery of the eliciting irritant and the accurate introduction of hormone preparations into the granuloma wall proper.

Summary. 1. Using the "granuloma pouch" technic, it was possible to produce very large amounts of inflammatory tissue and fluid (corresponding, in some cases, to as much as 25% of total body weight) in the rat, without interfering with the general well-being of the animal. 2. The introduction of small amounts of hydrocortisone, directly into the wall of the pouch, completely prevents the development of these inflammatory structures. 3. Introduction of the same amount of hydrocortisone into the lumen of the pouch, or into connective tissue at a distance of only about one mm from the pouch, is virtually ineffective. 4. The croton oil used to produce these granuloma pouches retained part of its irritating effects, even after a sojourn of 14 days, in hydrocortisone protected connective tissue. Although here it failed to cause inflammation, it still produced considerable damage, with hemorrhagic necrosis, when it was introduced into the unprotected connective tissue of other recipient animals.

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Effect of X-Irradiation on Weight and Contents of Rat Stomach, Small and Large Intestine.* (20109)

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The vulnerability of the gastrointestinal tract to ionizing radiation has long been recognized. Quastler *et al.*(1), Cronkite(2), Bond (3), and others, have demonstrated the importance of the relationship of gastrointestinal damage to early deaths in animals seen after large doses of total body irradiation. Even with relatively low doses, the extreme sensitivity of the system to radiation has been demonstrated by certain functional changes, such as increased tone and amplitude of contractions of the rat intestine which occurred during irradiation or immediately thereafter, followed by a period of atonicity and lack of motility lasting for several days(4). Pharma-

* The opinions or assertions contained herein are the private ones of the author and are not necessarily to be construed as official or reflecting the views of the Navy Department or the naval service at large.