# Acta Medica Okayama

Volume 12, Issue 2

1958 July 1958

Article 1

Effect of histamine releasers and of anti-inflammatory drugs on the egg-white edema of rat's hind paws in relation to skin histamine

Shozo Irino\*

\*Okayama University,

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# Effect of histamine releasers and of anti-inflammatory drugs on the egg-white edema of rat's hind paws in relation to skin histamine\*

Shozo Irino

### Abstract

1. A method was described for a fairly accurate judgement of the effect of drugs inhibiting the edema in hind paws of a rat caused by local injection of egg white. 2. The degree of inhibition of egg-white edema by single doses of sinomenine, compound 48/80, or dextran was in parallel with histamine reduction in skin and other tissues of the paws (and the skin of abdomen), although prevention of the edema by prolonged treatment with sinomenine was incomplete even when the releasable histamine of the skin was practically exhausted. 3. Sodium salicylate, aminopyrine, butazolidine sodium, cortisone, and guaiazulene were capable of inhibiting egg-white edema without modifying the content of skin histamine. These drugs and a small dose of phenergan potentiated the inhibition by dextran of egg-white edema and inhibited the release of histamine by dextran. These actions lasted for over 24 hours with the exception of guaiazulene. 4. Irgapyrin and a large dose of phenergan, which possess actions of histamine release and of histamine release inhibition and also antihistaminic action, caused a slight reduction of skin histamine and a comparatively marked inhibition of the edema. 5. In adrenalectomized or hypophysectomized rats, the edemainhibiting effect of salicylate and aminopyrine decreased but that of cortisone increased. The effect of guaiazulene remained unchanged. 6. The observations that inhibition of egg-white edema is caused by (a) histamine releasers, (b) histamine-release inhibitor, and (c) drugs exerting both histamine release and inhibition of the release were discussed with the consideration to a relationship between egg-white edema and skin histamine.

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Acta Med. Okayama 12, 93-111 (1958)

## EFFECT OF HISTAMINE RELEASERS AND OF ANTI-INFLAMMATORY DRUGS ON THE EGG-WHITE EDEMA OF RAT'S HIND PAWS IN RELATION TO SKIN HISTAMINE<sup>1,2</sup>

#### Shozo IRINO

### Department of Pharmacology, Okayama University Medical School, Okayama, JAPAN (Director : Prof. H. Yamasaki)

Received for publication June 17, 1958

In spite of the wide variety of stimuli, the acute inflammatory tissue responses caused by them show a series of common changes chiefly in the peripheral blood vessels, such as vasodilatation, increased capillary permeability, local changes in the blood flow, and infiltration of definite cells, sometimes leading to necrosis. This fact has led us to the idea that the inflammatory reaction may be mediated by the release or formation of a chemical substance or substances, capable of causing similar vascular reactions. Of the possible mediators possessing such actions, histamine is an important substance because of wide distribution in the mammalian tissues and of the fact that it is easily liberated by various physicochemical and biological stimuli, including antigen-antibody reaction. Other mediators proposed as responsible for inflammatory reaction are leukotaxine and 5-hydroxytryptamine (5-HT), whose close relationships with histamine have recently been pointed out  $^{1-4}$ . What should be found out at present is not whether histamine plays any rôle in inflammatory reaction, but how much or how great is its rôle.

The present paper is concerned with studies designed to give some answer to the afore-mentioned question. In this work, quantitative examinations were made on the effect of histamine releasers and some antiinflammatory drugs on the egg-white edema in the hind paws of rats, a typical sample of acute inflammation, in relation to changes in local histamine content as reflected by changes in histamine of the abdominal skin. Some considerations were given in this experiment on the relationship of the pituitary-adrenal system to the effect of these drugs. The

<sup>1)</sup> Aided by a grant for Fundamental Scientific Research from the Ministry of Education.

<sup>2)</sup> Preliminary notes in Folia pharmacol. japon. 52, 197 § (1956).

anti-inflammatory action of the drugs tested seemed to be related to one or more of (a) action causing histamine depletion, (b) action of inhibiting histamine release, and (c) counteraction against histamine action. Such findings obtained in the present experiment may give some suggestions for elucidation of this problem.

#### METHODS

Throughout the present series of experiments, male albino rats weighing 100-150 g. were used because it is known that the skin histamine content is approximately definite in this weight range<sup>5</sup> and male rats are more sensitive to egg white than the females<sup>6</sup>. They were fed on a mixture of Oriental compressed diet MC5 and wheat, fresh vegetables, and water. Adrenalectomized or hypophysectomized rats were given 1 per cent saline solution in place of drinking water and placed at a room temperature of  $22 \pm 3^{\circ}$ C. A group of six rats was used for each dosage level of each drug and the determined values were represented as the mean value with S. E.

Measurement of the intensity of egg-white edema. The egg-white edema was caused by the injection of 0.1 c.c. of fresh egg white diluted to 1:10 with 0.85 per cent saline solution, under the skin of dorsa of hind paws of the rat. As was reported by GROSS<sup>7</sup> and by WILHELMI<sup>8</sup>, the egg-white edema is reliably produced in all the rats by this method and, as shown in Fig. 1, the edema was produced in approximately equipotent reaction on the left and right paws in one rat. The rats in one group were injected with egg white prepared from one egg preserved in an ice chamber.

The edema was measured every 15 minutes for the first hour after injection of egg white, every 30 minutes for subsequent 1 hour, and once 1 hour later. The intensity of edema was measured with a micrometer gauge with specially weakened spring and was represented as the difference of the dorso-planter thickness in mm. of the hind paw, before and after injection of egg white. Error of measured values was within 0.2 mm.

Measurement of the effect of edema inhibition. All the drugs to be tested were administered the day after the intensity of control edema was measured. A definite time after injection of a drug, the edema produced on the other hind paw by injection of the same egg white was compared with the control edema. In the control edema, the maximum intensity appeared 30 to 120 minutes after injection of egg white (Fig. 1), but inhibition of edema by drug administration reached approximately the maxi-

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mum degree around 2 hours (Figs. 4, 5, and 6). The percentage inhibition was calculated from the following equation and used in jndging the anti-inflammatory effect of a drug.

Rate of edema inhibition by a drug  $(\%) = \frac{E_n - E_t}{E_n} \times 100$ , where  $E_n$  is intensity of control edema (mm.) and  $E_t$  is intensity of edema (mm.) after drug administration.

Measurement of release of skin histamine and its inhibition. A small piece (100-200 mg.) of the skin on one side of the abdomen was excised aseptically under light ether anesthesia. This was performed three days before administration of test drug with consideration of the period for recovery of change in skin histamine value due to remote injury<sup>9</sup>. Similar excision of the skin specimen was made on the corresponding side of the abdomen between 24 and 30 hours after drug administration in order to examine the release of skin histamine.

For examination of the effect of drugs in inhibiting histamine release, in one group of rats 3600 mg./kg. of dextran was injected intraperitoneally, and in another group a drug to be tested was administered at a definite interval before dextran injection. The degree of percentage reduction of the skin histamine in these two groups was compared.

Assay of histamine. Extraction and assay of histamine were made by the methods described by SANUKI<sup>10</sup> of our laboratory. The histamine content was expressed by the weight of the base.

Adrenalectomy and hypophysectomy. Adrenalectomy was performed by lumbar approach through median incision. The absence of accessory adrenals was ascertained by postmortem autopsy. The surgery was performed within 5 minutes under light ether anesthesia. The test drugs were administered three days after adrenalectomy in order to avoid the effect of the operation<sup>11,12</sup>. In the control experiment, the same surgical invasion was made, only without adrenalectomy.

Hypophysectomy was performed by TANAKA's modification<sup>13</sup> of KOYAMA's external auditory canal method<sup>14</sup>. The hypophysectomized animals were used for the experiment one week after the operation.

*Histological examination.* The hind legs were cut off 1, 2, 4, or 6 hours after injection of egg white, fixed in 20 per cent formalin solution, and paraffin sections were stained with hematoxylin-eosine. For observation of changes in the mast cells, the hind legs 2 hours after egg-white injection were fixed for over one week in 4 per cent basic lead acetate solution in 50 per cent ethanol. The fixed tissue pieces were immersed for 5-6 hours in 70 per cent ethanol containing 1 per cent of

hydrochloric acid, washed in running water, and lyophilized section was stained with 0.1 per cent toluidine blue solution.

Drugs. Sinomenine hydrochloride(Shionogi), compound 48/80(through the courtesy of Dr. Edwin de Beer, Wellcome Research Laboratories, New York), dextran (mol. wt., 75, 000  $\pm$  2, 500) (Nagoya Seito), and Irgapyrin (Fujisawa) were used as a histamine releaser. Anti-inflammatory drugs tested were phenergan (Shionogi), butazolidine sodium (Fujisawa), aminopyrine, sodium salicylate, cortisone acetate, and guaiazulene (1, 4-dimethyl-7-isopropylazulene). Guaiazulene was used as an 1 per cent suspension with tragacanth. Other drugs were all dissolved in 0.85 per cent saline solution in concentrations so that the required doses were contained in about 1 c.c.

#### RESULTS

1. Gross and microscopic characters of the egg-white edema. The edema of the hind paws of rats began to swell a few minutes after injection of egg white, attained the maximum swelling in 30 minutes, which continued for 120 minutes, and began to decrease gradually thereafter, disappearing almost completely after 24 hours (Fig. 1). Reddening was observed a few minutes after egg-white injection, the color became most



FIG. 1. Equipotent development of the edema induced by subcutaneous 0.1 c. c. of 10% egg-white saline solution in the bilateral hind-paws of the rat. ● (○: mean): edema before intraperitoneal injection of 1 c.c. of 0.85% saline. ▲ (△: mean): edema in the opposite paw 1 hour after the saline injection. Six male rats.

intense after 15-30 minutes, and decreased gradually thereafter. The intensity and course of these edema and reddening were not affected by intraperitoneal injection of 1.0 c.c. of physiological saline solution (Fig. 1).

Microscopic observations revealed typical pattern of acute inflammation such as hyperemia, exudation and vascular accumulation of polymorphonuclear leucocytes (within 60 minutes), followed in addition by cell infiltration (120 minutes), swelling and softening of vessel wall (4 hours), and hemorrhage (6 hours) (Fig. 2).



FIG. 2. Histologic pictures stained with hematoxylin-eosine (upper) and mast cells with toluidine blue (lower) of the skin of rat's hind-paw, (1) 2 hours after injection of 0.1 c.c. of 10% egg-white saline solution beneath of dorsa of the paws. Note the striking edema, and hyperemia and cellular infiltration. (2) 6 hours after. Note the severe haemorrhage with cellular infiltration and slight edema. (3) Normal mast cells. (4) Disruption with swelling of the mast cells 2 hours after the same injection. Upper : × 110, lower : × 350.

Two hours after egg-white injection, mast cells of the skin and subcutaneous tissue of the paw were slightly swollen, with dissociation of intergranular spaces, and with irregular and indistinct cell border. Ex-

trusion of granules was also observed, extending over a wide region (Fig. 2). Such disruption of mast cells was similarly produced after subcutaneous injection of the same volume of croton oil (1 per cent in corn oil), xylene, chloroform, and formalin solution (10 per cent in saline) in the hind paws The intensity of edema and alteration of mast cells seemed to be approximately in parallel. Disruption of mast cells by croton oil which revealed scattering of all granules into the surrounding area was marked-ly of stronger intensity than in the case of other substances.

2. Reduction of skin histamine by sinomenine and other histamine releasers. Single intraperitoneal injection of 50 mg./kg. of sinomenine, 1 mg./kg. of compound 48/80, and 3,600 mg./kg. of dextran caused reduction of abdominal skin histamine, as indicated in Table 1. Vascular dilatation or reddening was observed around the ears, around the mouth and snout, sole of all paws, and the genital area, a few minutes after the injection of the first two, and 5-10 minutes after the latter. The animals scratched the face with forelegs. Cyanosis was accompanied by severe dyspnea and prostration. These symptoms weakened 15-30 minutes after the injection, with gradual appearance of edema in portions with reddening and this lasted for about 2 hours.

TABLE 1. Inhibition of egg-white edema of the hind-paw and reduction of hista-<br/>mine of the abdominal skin of the rat by histamine releasers. Egg-white<br/>edema for test was induced 24 hours after the end of injections for sino-<br/>menine, 48/80 and dextran; and 1 hour after for Irgapyrin and phenergan.

Drugs	mg./kg. No. of (i.p.) rats		Edema inhibition (%)	Histamine reduction (%)
Sinomenine	50	6	$45.6 \pm 1.6$	32.5 + 3.7
Sinomenine	500*	6	$62.9~\pm~2.6$	39.8 + 2.7
Sinomenine	2100†	6	$68.4 \pm 1.3$	$88.2 \pm 2.0$
48/80	1	6	$24.3 \pm 2.0$	18.4 + 0.78
Dextran	3600	6	<b>43</b> .0 ± 2.9	$30.4 \pm 3.5$
Irgapyrin	200	6	<b>30.9</b> ± <b>2.9</b>	$13.6 \pm 4.5$
Phenergan	20‡	6	$50.7~\pm~1.5$	$12.3~\pm~0.87$

\* 50 mg./kg.  $\times$  2, for 5 days.

† 50 to 300 mg./kg. imes 2, daily increasing for 6 days.

‡ Subcutaneously.

The reduction of skin histamine after 10 repeated injections of 50 mg. /kg. of sinomenine during 5 days (a total of 500 mg. /kg.) was larger only slightly than that caused by a single injection of 50 mg. /kg. How-

ever, when an increasing amount of sinomenine was injected for 6 days, starting with 50 mg./kg. twice a day and increasing 100 mg./kg. in daily doses (a total of 2, 100 mg./kg.), an average of 88.2 per cent reduction of histamine in the abdominal skin was reached (Table 1). The course and degree of skin histamine depletion by the daily increasing doses of sinomenine were the same either by intraperitoneal or intravenous injection (Fig. 3.). In these cases, appearance of symptoms decreased gradually after subsequent injections in spite of increasing doses. This fact indicates that inherent toxicity of sinomenine, other than that through histamine liberation, is very low.



FIG. 3. Effect of sinomenine on histamine content of rat's skin from abdomen. Six days' treatment with increasing doses of sinomenine. ×… ×: Intraperitoneal injections, the 1st day 50 mg./kg., thereafter daily increasing by 50 mg./kg.; ○—○: Intravenous injections, the same doses as described above.

Since it has been indicated that the degree of histamine depletion by these substances was approximately the same in the abdominal skin, skin and other tissues (excluding the bone) of the paws (Table 2), rate of change in abdominal skin histamine was used as a reflection of that in histamine of the paw tissues for the present experiment.

Intraperitoneal injection of 200 mg./kg. of Irgapyrin and 20 mg./kg. of phenergan caused a slight decrease of the skin histamine (Table 1) but in these cases, above-mentioned symptoms were not observed. This is probably due to the antihistamine action of these substances<sup>15,16</sup>.

3. Inhibition of egg-white edema by sinomenine and other histamine releasers. The time course of inhibition of egg-white edema by the aforementioned drugs is shown in Fig. 4, and in Table 1 the per cent inhibition of edema two hours after the injection of egg white is compared with

Drugs	mg./kg. (i. p.)	No. of rats	Tissues	Histamine reduction (%)
Sinomenine	50	5	Abdominal skin Skin of paw Musc. & tend. of paw	$\begin{array}{c} 32.1 \pm 4.2 \\ 47.3 \pm 6.5 \\ 46.8 \pm 7.1 \end{array}$
Dextran	.3600	5	Abdominal skin Skin of paw Musc. & tend. of paw	$\begin{array}{c} 30.0 \pm 3.4 \\ 49.7 \pm 6.6 \\ 34.1 \pm 3.9 \end{array}$
48/80	1	5	Abdominal skin Skin of paw Musc. & tend. of paw	$18.3 \pm 3.4 \\ 17.0 \pm 5.6 \\ 13.4 \pm 3.3$

TABLE 2. Comparison of histamine reductions of the abdominal skin and of the tissues of the hind-paw by histamine releasers.

Note. Of 15 rats in this table, histamine contents before the treatments of the abdominal skin, the paw skin, and the muscle with tendon of the paw were  $43.9 \pm 1.6$ ,  $86.4 \pm 3.2$  and  $36.7 \pm 2.1 \ \mu$ g./g. respectively.



FIG. 4. Inhibitory effects of histamine releaser substances on the egg-white edema of rat's hind-paw (cf. Table 1).

the rate of reduction of skin histamine of the same rat.

With single doses of sinomenine, compound 48/80, and dextran, edema inhibition was effected approximately in parallel with the degree of depletion of skin histamine. This suggests that the edema inhibition by these drugs is related to the depletion of local histamine. However, edema inhibition by prolonged treatment of sinomenine, until about 90 per cent of the skin histamine had been depleted, was not more than 70 per cent, so that the cause of egg-white edema cannot be entirely due to histamine release alone.

Injection of Irgapyrin (200 mg. /kg.) and a large dose of phenergan (20 mg. /kg.) gave a different result from that of the foregoing histamine releasers; inhibition of edema was rather marked in spite of the slight reduction of histamine. This fact indicates that the inhibition of edema by these drugs is due to some causes other than histamine reduction.

4. Edema inhibition with prevention of histamine release by some anti-inflammatory drugs. Table 3 is the summarized result of the effects of sodium salicylate, aminopyrine, butazolidine sodium, cortisone, and guaiazulene on the egg-white edema (Fig. 5) and skin histamine content. These drugs inhibited edema to a greater or lesser degree in the doses used, but none of these drugs gave any change in the skin histamine content, so that the inhibition of egg-white edema by these drugs is not due to histamine release. Recent reports<sup>17-20</sup> on the inhibitory effect of salicylate and some antipyretics against *in vitro* anaphylactic histamine release suggest the possibility that these drugs might also have similar action against *in vivo* histamine release by egg white in the rat, because there are some indications that cortisone and a small dose of antihistamines inhibit the action of other releasers *in vivo*.<sup>10,21,22</sup>

Drugs	mg./kg. (i. p.)	No. of rats	Edema inhibition (%)	Change of histamine content (%)	
Sod. salicylate	250	6	$26.2 \pm 1.2$	$0 \pm 1.1$	
Aminopyrine	100	6	$28.1 \pm 2.4$	$0.6 \pm 0.97$ (increase)	
Butazolidine sod.	100	6	$26.0~\pm~1.7$	$0.4 \pm 0.72$ (increase)	
Cortisone	100*	6	$18.2 \pm 1.4$	$6.6 \pm 1.4$ (increase)	
Guaiazulene	200	6	$51.6 \pm 1.6$	$4.0 \pm 2.3$ (increase)	
Guaiazulene	100	6	$24.9~\pm~2.3$		
Guaiazulene	50	6	$18.1 \pm 2.3$	-	

TABLE 3. Inhibition of egg-white edema of the hind-paw and change in hista-mine content of the abdominal skin by anti-inflammatory drugs. Drugswere injected 1 hour before edema production.

\* 50 mg./kg. (i.m.) 24 hours and 1 hour before edema.



FIG. 5. Inhibitory effects of some anti-inflammatory drugs on the egg-white edema of rat's hind-paw (cf. Table 3).

Therefore, examination was made on the effect of the foregoing doses of sodium salicylate, aminopyrine, cortisone, guaiazulene, and a small amount (5 mg. /kg.) of phenergan on the rate of release of skin histamine by dextran. These drugs were administered 0.5-1 hour or 24 hours prior to dextran injection. The results are shown in Table 4 from which it is evident that these drugs, in the doses used, suppress the *in vivo* histamine release by dextran. This suggests that the edema inhibition of these drugs is probably due to the inhibition of histamine release by egg white.

Drngs (mg./kg.)*		Edema inhibition (%)	Histamine reduction (%)	No. of rats‡	Histamine reduction (%)
Dextran (3600) alone	6	$43.0~\pm~3.0$	$30.4 \pm 4.2$	6	$30.4 \pm 4.2$
Sod. salicylate(250) $+$ Dextran(3600	) 6	$57.2 \pm 2.8$	$4.3 \pm 1.8$	6	$10.2 \pm 1.6$
Aminopyrine (100) + "	5	54.9 ± 3.3	$18.5 \pm 3.5$	6	$20.8 \pm 1.2$
Cortisone (100) + "	6	$59.6 \pm 2.2$	$13.9 \pm 2.5$	6	$15.9 \pm 1.2$
Guaiazulene (100) + "	5	$51.5 \pm 3.7^{\circ}$	0.27± 2.7	6	$17.3 \pm 2.1$
Phenergan (5) + "	6	$46.1 \pm 1.9^{\circ}$	$15.9 \pm 1.9$	6	$21.7\pm2.5^{\text{\$}}$

TABLE 4. Effects of anti-inflammatory drugs on the edema inhibition (hindpaws) and the histamine reduction (abdominal skin) induced by dextran.

\* Dextran was injected intraperitoneally.

<sup>+</sup> In these series of experiments sodium salicylate, aminopyrine and guaiazulene were given intraperitoneally 1 hour before, phenergan subcutaneously 30 minutes before dextran; cortisone intramuscularly 50 mg./kg., 24 hours and 1 hour each before dextran.

 $\ddagger$  In these series cortisone was given 50 mg/kg.  $\times$  2, 48 and 24 hours, and the other drugs 24 hours before dextran by similar routes in the  $\dagger$  series.

 $\S$  No significant difference from the effect of dextran alone at the value of P as 0.05.

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FIG. 6. Potentiation by some histamine-release inhibitors of the edema-inhibiting effect of dextran (cf. Table 4).

5. Inhibition of egg-white edema by dextran potentiated by antiinflammatory drugs. The effects of sodium salicylate, aminopyrine, cortisone, guaiazulene, and phenergan (5 mg./kg.) on the egg-white edema inhibition by dextran are indicated in Table 4 and in Fig. 6. It is interesting to see that the edema-inhibiting effect of a histamine releaser, dextran was potentiated by the concurrent use of salicylate and other drugs which inhibited histamine release, because the histamine depletion of paw tissues should be apparently smaller by the concurrent use of these drugs than by dextran alone. Probable explanation of this phenomenon is the possibility that utilization of local histamine for development of egg-white edema becomes more difficult by the concurrent use of the histamine release inhibitors with dextran than by the use of dextran alone. There are evidences<sup>23,24</sup> suggesting that histamine is bound in the tissue in different levels of stability. Inhibitory action of these drugs on further release of available histamine which remains after dextran, may have given these results. In order that such assumption be realized, an evidence must be demonstrated that the action in inhibiting histamine release of these anti-inflammatory drugs extended to 24 hours or more, because the injection of egg white for edema production was made 24 hours after. The figures in the right-hand column of Table 4 indicate that these drugs were still inhibiting histamine release even after 24 hours.

Relatively greater rate of declination during this period of the action of guaiazulene probably explains the reason why this drug failed to show any significant potentiation of edema inhibition of dextran.

6. Effect of anti-inflammatory drugs on the skin histamine and egg-white edema in the adrenalectomized or hypophysectomized rat. The 34 rats in which the adrenalectomy was proved to have been executed perfectly lived for an average of eight days (5-11 days). The hypophysectomized rats seemed to be otherwise normal except for failure of gaining body weight.

 

 TABLE 5.
 Effect of adrenalectomy and hypophysectomy on the histamine content of the abdominal skin and the development of egg-white edema in the rat. Percentages are comparisons with the values before the operations.

Rats	No. of rats	Degrees of edema (%)	Histamine content (%)
Adrenalectomized	6	$8.3 \pm 1.9$ (increase)	$23.1 \pm 3.2$ (decrease)
Sham-operated	6	$1.2 \pm 1.1$ (increase)*	$2.7 \pm 2.8$ (increase)*
Hypophysectomized	6	$21.8 \pm 1.5$ (decrease)	$0.92\pm$ 1.4 (increase)*

\* Not significant at the value of P as 0.05.

Table 5 shows the histamine content in the abdominal skin and intensity of egg-white edema three days after adrenalectomy or seven days after hypophysectomy as percentage increases or decreases in the same animals before the operations. In hypophysectomized rats, no change occurred in the content of skin histamine but in adrenalectomized rats, 23.1 per cent decrease was indicated. The latter result does not agree with those of ROSE and BROWN<sup>25</sup> and of MARSHALL<sup>26</sup> who reported an increase in the tissue histamine content in rats after adrenalectomy. Eggwhite edema was intensified by an average of 8.3 per cent after adrenalectomy while it became weaker by an average of 21.8 per cent in the case of hypophysectomy. The changes of edema and of skin histamine in the adrenalectomized rats were statistically significant in comparison with the control of sham-operated group.

The rats three days after adrenalectomy died in an average of 45 minutes after intraperitoneal injection of 50 mg. /kg. of sinomenine, and in an average of 10 hours after similar injection of 250 mg. /kg. of sodium salicylate or 100 mg. /kg. of guaiazulene. On the contrary, two intramuscular doses (on the 2nd and 3rd day after operation) of 50 mg. /kg. of cortisone extended the life of adrenalectomized rats for an average of 4 days. Intraperitoneal injection of 20 mg./kg. of phenergan or 100 mg./kg.

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TABLE 6. Inhibition of egg-white edema by anti-inflammatory drugs in adrenalectomized and hypophysectomized rats. Edema was induced 24 hours after sinomenine and 1 hour after the other drugs. Figures in parentheses refer to percentages as supplemented with  $8.3 \pm 1.9$  per cent, an increase of edema by adrenalectomy alone (cf. text).

Drugs	mg./kg. (i. p. )	No. of rats	Edema inhibition (%)		
			Adrenalectomized rats	Hypophysecto- mized rats	
Sinomenine	50	6	·	$43.2 \pm 1.6^{+}$	
Sod. salicylate	250	6	$13.8 \pm 2.1 \ (20.4 \pm 3.2)$	$20.7 \pm 1.2$	
Aminopyrine	100	6	$10.2 \pm 2.4 \ (17.1 \pm 3.4)$	$16.5 \pm 1.8$	
Cortisone	100*	6	$24.7 \pm 3.7 (30.5 \pm 5.0)$	$33.3 \pm 2.4$	
Guaiazulene	100	6	$17.0 \pm 2.3 (23.4 \pm 3.4)^{\dagger}$	$23.9 \pm 2.0 +$	
Phenergan	20	6	$34.2 \pm 2.2 (39.3 \pm 3.7)$	$50.4 \pm 1.7$ †	

\* Intramuscularly 50 mg./kg., 24 hours and 1 hour each before the edema.

+ Differences are not significant at P value as 0.05 as compared with the effect in normal intact rats shown in Tables 1 and 3.

of aminopyrine did not change the life span of adrenalectomized rats.

The edema inhibition by these drugs in adrenalectomized or hypophysectomized rats is presented in Table 6 in order to compare with the effect of these drugs in normal intact rats which is shown in Tables 1 and 3. In adrenalectomized rats, the effect of these drugs at first sight appear to decrease with the exception of cortisone, the effect of which potentiated. These figures are compared with the intensity of edema before adrenalectomy whereas the comparison should theoretically be made with the values added with  $8.3 \pm 1.9$  per cent increase of egg-white edema after adrenalectomy. The percentages of edema inhibition calculated by an addition of this figure are parenthetized in the table and indicate that the effects of aminopyrine and phenergan are decreased by adrenalectomy. In hypophysectomized rats the edema inhibition, based on the control obtained under the same condition, was intensified in the case of cortisone and decreased in aminopyrine and sodium salicylate, but no significant difference was indicated in guaiazulene and sinomenine as compared with that in normal intact rats.

#### DISCUSSION

Various methods are available for measuring the degree of edema produced in hind paws of rats, for the purpose of evaluating the efficacy of drugs inhibiting edema.<sup>7,8,27–31</sup> However, the majority of these methods does not give much considerations for the difference in the degree of edema

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in individual animals. In the present experiments, the edema caused in the same animal before and after administration of drugs was compared by the direct measurement of the paw diameter, based on the confirmation that the degree of edema produced is invariably the same in the left and right paws of the same animal, and this is believed to have given a fairly accurate reading of the drug effect. The effect of histamine releasers in inhibiting egg-white edema was observed with single doses of sinomenine, dextran, and compound 48/80 and the effect was in parallel with the degree of reduction of histamine in the skin and other tissues of the paw. Prolonged treatment with sinomenine caused depletion of skin histamine to only 10 per cent of the control level. This level is the maximum reduction that can be reached by known histamine releasers<sup>23,24</sup> and indicates that releasable histamine is in the state of practical exhaustion. Even in this case, however, inhibition of edema was not perfect. This fact indicates that histamine release alone is not responsible for the production of egg-white edema. Recent reports claim that the anaphylactoid reaction caused by egg white or dextran in the rat is chiefly mediated by the release of 5-HT<sup>28,31-35</sup>. It is not known yet whether sinomenine also depletes 5 HT the same as compound 48/80 although there is such a possibility. If the edema inhibition by sinomenine is due to the reduction of both 5-HT and histamine, then the intensity of edema inhibition by prolonged treatment with this substance should be much stronger. Therefore, insufficient inhibition of edema in this case is more likely to be due to some other reasons.

The present experiments indicated that sodium salicylate, aminopyrine, cortisone, and guaiazulene do not themselves change the content of skin histamine but do suppress the depletion of histamine produced by dextran. TRETHEWIE<sup>17</sup>, UNGAR and DAMGAARD<sup>18</sup>, HAINING<sup>19</sup>, and MON-GAR and SCHILD<sup>20</sup> have indicated the inhibiting effect of salicylate on the in vitro anaphylactic histamine release, and UNGAR and DAMGAARD<sup>18</sup> have shown the similar action of salicylate against histamine release from lung slice of a guinea pig by Tween 20 or octadecylamine. MONGAR and SCHILD<sup>20</sup> observed that butazolidine and aminopyrine also showed inhibition of the foregoing histamine release. The inhibitory action of guaiazulene on histamine release was suggested by STERN and MILIN<sup>36</sup> and recent studies of YAMASAKI et al. 37 in our laboratory have clearly demonstrated, by quantitative determination, the inhibition by this substance of histamine release in vitro as well as in vivo in rats and guinea pigs by different releasers. GOTH et al. 38,39, HALPERN et al. 40, and SCHAYER et al.<sup>41,42</sup> have given evidences that cortisone inhibits resynthesis of de-

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pleted histamine in the tissue. On the other hand, SANUKI<sup>10</sup> reported that cortisone inhibited histamine depletion from the mouse skin caused by prolonged treatment with sinomenine, and KONDO43 observed in cortisone-treated rats that the increase in the urinary excretion of histamine induced by injection of various histamine releasers, including egg white, is inhibited. These evidences indicate that cortisone is capable of exhibiting inhibitory action on histamine release under different conditions. Analogy may be applicable to the inhibitory effect of the anti-inflammatory drugs against histamine release by dextran, observed in the present experiments, that it will be also extended over the release of histamine by a fairly wide range of releasers includings egg white. In fact, KONDO<sup>43</sup> has recently proved this is true with aminopyrine and guaiazulene. Thus the inhibition of egg-white edema by these drugs is to the advantage of assumption that histamine is responsible for this edema. KAMIMURA<sup>44</sup> and TASAKA<sup>45</sup> of our laboratory have reported that Irgapyrin, but not aminopyrine and butazolidine, release histamine from the chopped tissues of guinea-pig lungs and dog skin, and this observation agrees with the findings in vivo in the present series of experiments. The present observations that a large dose of phenergan liberates histamine while its small dose inhibits the liberation also agree well with recent reports of YAMA-SAKI and TASAKA<sup>21</sup> with other antihistamines, but the effect of edema inhibition of Irgapyrin and a large dose of phenergan is much greater than that of other histamine releasers, in comparison with the degree of the histamine releasing activity. Therefore, their effect of edema inhibition must partly be explained by other actions. It is possible that Irgapyrin, which is a combination of aminopyrine and butazolidine, possesses inherent ability of inhibiting histamine release, besides the action of releasing histamine, the same as phenergan. Based on the fact that the edema-inhibiting effect of dextran is potentiated by the concurrent use of histamine-release inhibitors, the comparatively strong edema-inhibiting effect of the above two drugs suggests that the assumption that egg-white edema is largely mediated by histamine is reasonable.

Evidences are not available to the question of whether or not the anti-inflammatory drugs tested here also inhibits liberation of 5-HT. However, mediation of edema caused by egg white through 5-HT is probably not large enough to deny the significance of histamine since the injection of reserpine, a specific releaser of 5-HT, does not produce edema<sup>32</sup> and because the depletion of 5-HT is not sufficient to prevent eggwhite edema<sup>31</sup>. I have observed in a separate experiment<sup>46</sup> that the weight increase and histologic edema of the wall of granuloma pouch caused by

croton oil is suppressed after repeated injections of histamine (histamine desensitization). It is difficult to find any reason for denying that the edema caused by egg white, which produces disruption of mast cells like croton oil, is mediated by histamine. Suppression of increased permeability of capillaries may be partly responsible for the anti-inflammatory mechanism of cortisone and a certain derivatives of pyrazolone, but there are no available evidences that such an action exists in the anti-inflammatory drugs without regard to histamine-release inhibition.

From results of the present experiments inhibition of egg-white edema by the drugs tested may be explained by (a) depletion of histamine to be utilized for release by egg white (sinomenine, compound 48/80, and dextran), (b) prevention of histamine release by egg white (sodium salicylate, aminopyrine, butazolidine, guaiazulene, and small amount of phenergan and cortisone), and (c) a combined effect of release and suppression of release of histamine (Irgapyrin and a large dose of phenergan). In addition, the effect of some of these drugs, i. e. pyrazolone derivatives and antihistamine agents, may partly be attributable to counteraction against the liberated histamine.

Decrease in inhibition of edema by salicylate and aminopyrine in adrenalectomized or hypophysetomized rats suggests that a part of the effect of these drugs is also mediated by the cortisone-like hormones from the adrenal cortex. On the other hand, edema-inhibiting effect of cortisone was fortified in these operated animals. According to UDA<sup>47</sup>, in normal rats, the inhibition by cortisone of dextran edema appears rather stronger in smaller doses than that used in the present experiments. In adrenalectomized animals, in which cortisone-like hormone in the body has been depleted, the tissue level of cortisone administered is lower than that in the case of normal rat and this may be the reason for striking appearance of the edema inhibition.

#### SUMMARY

1. A method was described for a fairly accurate judgement of the effect of drugs inhibiting the edema in hind paws of a rat caused by local injection of egg white.

2. The degree of inhibition of egg-white edema by single doses of sinomenine, compound 48/80, or dextran was in parallel with histamine reduction in skin and other tissues of the paws (and the skin of abdomen), although prevention of the edema by prolonged treatment with sinomenine was incomplete even when the releasable histamine of the skin was practi-

cally exhausted.

3. Sodium salicylate, aminopyrine, butazolidine sodium, cortisone, and guaiazulene were capable of inhibiting egg-white edema without modifying the content of skin histamine. These drugs and a small dose of phenergan potentiated the inhibition by dextran of egg-white edema and inhibited the release of histamine by dextran. These actions lasted for over 24 hours with the exception of guaiazulene.

4. Irgapyrin and a large dose of phenergan, which possess actions of histamine release and of histamine release inhibition and also antihistaminic action, caused a slight reduction of skin histamine and a comparatively marked inhibition of the edema.

5. In adrenalectomized or hypophysectomized rats, the edema-inhibiting effect of salicylate and aminopyrine decreased but that of cortisone increased. The effect of guaiazulene remained unchanged.

6. The observations that inhibition of egg-white edema is caused by (a) histamine releasers, (b) histamine-release inhibitor, and (c) drugs exerting both histamine release and inhibition of the release were discussed with the consideration to a relationship between egg-white edema and skin histamine.

#### REFERENCES

- 1. ROCHA E SILVA, M. and DRAGSTEDT, C. A.: Observations on the trypan blue capillary permeability test in rabbits. J. Pharmacol. 73, 405, 1941.
- MILES, A. A. and MILES, E. M. : Vascular reactions to histamine, histamineliberator and leukotaxine in the skin of guinea-pigs. J. Physiol. 118, 228, 1952.
- ELDRIDGE, E. and PATON, W.D.M.: Unpublished observations, quoted by PATON, W.D.M.: Histamine release by compounds of simple chemical structure. *Pharmacol. Rev.* 9, 269, 1957.
- 4. FELDBERG, W. and SMITH, A.N. : Release of histamine by tryptamine and 5hydroxytryptamine. *Brit. J. Pharmacol.* 8, 406, 1953.
- 5. HARDWICK, D.C.: Age changes in the histamine content of rat skin. J. Physiol. 124, 157, 1954.
- 6. LÉGER, J., MASSON, G. and PRADO, J.L. : Hypersensitivity to egg white in the rat. Proc. Soc. exp. Biol., N.Y. 64, 366, 1947.
- 7. GROSS, G. : Zur Auslösung und Beeinflussung der Oedembildung nach Injektion von Hühnereiweiss an der Ratte. Arch. exp. Path. Pharmak. 211, 421, 1950.
- WILHELMI, G. and DOMENJOZ, R. : Die Beeinflussung des Hühnereiweiss-Oedems an der Rattenpfote durch Pyrazole sowie Cortison und ACTH. Arzneim.-Forsch. 1, 151, 1951.
- 9. GEIRINGER, E. and HARDWICK, D.C. : Changes in skin histamine after remote injury. J. Physiol. 119, 410, 1953.
- 10. SANUKI, K. : The analgesic effect induced by repeated administration of histamine and histamine liberators. Jap. J. Pharmacol. 6. 69, 1957.

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#### S. Irino

- 11. GROLLMAN, A. : The Adrenal, Williams and Wilkins Co., Baltimore, 1936.
- 12. NAKAO, T. and MATSUBA, M. : On the methods of biological assay of adrenocortical hormones. *Med. Sci.* 1, 73, 1949. (in Japanese)
- TANAKA, A. : A simple method of hypophysectomy on rats. Modification of Koyama's external auditory canal method. Ann. Rep. Shionogi Res. Lab. 5, 678, 1955. (in Japanese)
- KOYAMA, R. : Experimentelle Untersuchungen über die Hypophysenextirpation an Ratten und die Wirkung des Vorderlappenextraktes. Jap. J. med. Sci. IV Pharmacol. 5, 41, 1931.
- 15. WILHELMI, G. : Ueber die pharmakologischen Eigenschaften von Irgapyrin, einem neuen Präparat aus der Pyrazolreihe. Schweiz. med. Wschr. 79, 577, 1949.
- 16. NISHIYAMA, R. : The effect of an antihistamine agent on the gastric secretion induced by sinomenine and irgapyrin. Acta med. Okayama 10, 164, 1956.
- 17. TRETHEWIE, E. R. : The influence of sodium salicylate and acetyl salicylic acid on the release of histamine in anaphylaxis. Aust. J. exp. Biol. med. Sci. 29, 443, 1951.
- UNGAR, G. and DAMGAARD, E. : Tissue reactions to anaphylactic and anaphylactoid stimuli : Proteolysis and release of histamine and heparin. J. exp. Med. 101, 1, 1955.
- 19. HAINING, C.G. : Inhibition of histamine release by sodium salicylate and other compounds. Brit. J. Pharmacol. 11, 357, 1956.
- MONGAR, J.L. and SCHILD, H.O. : Inhibition of the anaphylactic reaction. J. Physiol. 135, 301, 1957.
- YAMASAKI, H. and TASAKA, K. : Dual action of antihistamines on histamine release. Acta med. Okayama 11, 290, 1957.
- 22. SANUKI, K., IRINO, S. and KAWAMOTO, S. : Synergism of antihistamines with histamine and sinomenine. Foila pharmacol. japon. 52, 157, 1956. (in Japanese)
- FELDBERG, W. and TALESNIK, J. : Reduction of tissue histamine by compound 48/80. J. Physiol. 120, 550, 1953.
- BROCKLEHURST, W. E., HUMPHREY, J. H. and PERRY, W. L. M. : The rôle of histamine in cutaneous antigen-antibody reactions in the rat. J. Physiol. 129, 205. 1955.
- 25. ROSE, B. and BROWN, J.S.L.: The effect of adrenalectomy on histamine content of the tissues of the rat. *Amer. J. Physiol.* 131, 589, 1941.
- MARSHALL, P. B. : The influence of adrenal cortical deficiency on the histamine content of rat tissues. J. Physiol. 102, 180, 1943.
- HALPERN, B. N. and BRIOT, M. : Étude pathogénique et thérapeutique du syndrome ödémateux provoqué chez le rat par l'ovalbumine. Arch. int. Pharmacodyn. 82, 247, 1950.
- ROWLEY, D.A. and BENDITT, E.P. : 5-hydroxytryptamine and histamine as mediators of the vascular injury produced by agents which damage mast cells in rats. J. exp. Med. 103, 399, 1956.
- 29. CERLETTI, A. and ROTHLIN, E. : The pharmacological basis of calcium-antihistamine combination. Int. Arch. Allergy 6, 230, 1955.
- COURVOISIER, S. and DUCROT, R. : Action de la chlorpromazine (4,560 R.P.) sur la syndrome ödémateux provoqué par le dextrane chez le rat. Arch. int. Pharmacodyn. 102, 33, 1955.
- 31. PARRATT, J.R. and WEST, G.B.: 5-hydroxytryptamine and the anaphylactoid reaction in the rat. J. Physiol. 139, 27, 1957.
- 32. PARRATT, J. R. and WEST, G. B. : The location and possible function of tissue 5-hydroxytryptamine in the rat. J. Physiol. 134, 11 P, 1956.

- 33. PARRATT, J. R. and WEST, G. B. : Oedema-producing substances in the rat. J. Physiol. 135, 10 P. 1957.
- PARRATT, J. R. and WEST, G. B. : Inhibition of oedema production in the rat. J. Physiol. 135, 24P, 1957.
- 35. SPARROW, E. M. and WILHELM, D. L. : Species differences in susceptibility to capillary permeability factors : histamine, 5-hydroxytryptamine and compound 48/80. J. Physiol. 137, 51, 1957.
- 36. STERN, P. and MILIN, R. : Die antiallergische und antiphlogistische Wirkung der Azulene. Arzneim. Forsch. 6, 445, 1956.
- 37. YAMASAKI, H., IRINO, S., UDA, T., UCHIDA, K., OHNO, H., SAITO, N., KONDO, K., JINZENJI, K. and YAMAMOTO, T. : Pharmacology of guaiazulene, with special reference to anti-inflammatory effect due to histamine-release inhibition. Folia pharmacol. japon. 54, 362, 1958. (in Japanese)
- 38. GOTH, A., ALLMAN, R. M., MERRIT, B.C. and HOLMAN, J. : Effect of cortisone on histamine liberation induced by Tween in the dog. Proc. Soc. exp. Biol., N. Y. 78, 848, 1951.
- 39. GOTH, A., HOLMAN, J. and COPENHAVER, J. H. : Mechanism of action of cortisone in experimental hypersensitivity. *Fed. Proc.* 11, 349, 1952.
- 40. HALPERN, B. N., BIOZZI, G., BRIOT, M. and BENACERRAF, B. : Modalités de la recharge des tissus en histamine après sa déplétion par une substance libératrice d'histamine et action empéchante de la cortisone sur cette recharge. C. R. Soc. Biol., Paris 147, 1180, 1953.
- SCHAYER, R. W., SMILEY, R. L. and DAVIS, K. J.: Inhibition by cortisone of the binding of new histamine in rat tissues. *Proc. Soc. exp. Biol.*, N. Y. 87, 590, 1954.
- 42. SCHAYER, R. W., DAVIS, K. J. and SMILEY, R. L. : Binding of histamine in vitro and its inhibition by cortisone. *Amer. J. Physiol.* 182, 54, 1955.
- 43. KONDO, K. : Yet unpublished.
- 44. KAMIMURA, Y. : A quantitative study of histamine release by chemical substances, using minced tissues of guinea-pig lung. Folia pharmacol. japon. 53, 836, 1957. (in Japanese)
- 45. TASAKA, K. : A quantitative study of histamine release by chemical substances using chopped skin tissue of the dog. Okayama Igakkai-Zasshi 69, 2853, 1957. (in Japanese)
- 46. IRINO, S. : Granuloma pouch and skin histamine of the rat. Acta Med. Okayama 12, 112, 1958.
- 47. UDA, T. : Yet unpublished.