EARLY AND DELAYED PHASES OF HIND PAW EDEMA IN RATS

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Abstract—Interaction between early and delayed phases in developmental process of hind paw swelling was investigated in rats using the mixed phlogistics-induced paw edema technique. Swelling induced by a mixture of carrageenin and Kaolin was observed in both the early and delayed phases. Despite of the augmentation of the early phase induced by the mixture, the delayed phase was not altered. The delayed phase was not reduced by treatment with antihistaminics and/or anti-serotonin agents, whereas the early phase was inhibited with those agents. The delayed phase was inhibited with anti-inflammatory agents without altering the early phase. The potentiating effect on the delayed phase was observed in the combined use of flufenamic acid and diphenhydramine. From these results, it is concluded that the delayed phase is independent from the early phase in the developmental process of hind paw edema, but it is related to the early phase in chemotherapy. Furthermore, effects of various agents on the mixed phlogistics-induced hind paw edema, the some of which included 0.1% histamine, 0.01% serotonin, 0.5% carrageenin and 5% Kaolin were investigated by localized and oral application and are discussed herein.

Acute inflammatory reaction induced by various types of stimulus, e.g. thermal injury, UV-injury, bacterial invasion and Arthus reaction, consists of two phases in vascular permeability change: the early and delayed phases (1-4). The early phase is transient and brought about by histamine and serotonin. The delayed phase is long lasting and probably brought about by large molecular substances, such as kininogens, globulin PF, substance P, LPNF etc. (5-7). On the other hand, Vinegar et al. (8, 9) reported that in the rat, hind paw swelling caused by carrageenin or kaolin included both the early and delayed phases, neither of which was inhibited by treatment with antihistaminics, whereas the delayed phase was inhibited with anti-inflammatory agents. However, Di Rosa et al. (10), Crunkhorn et al. (11) and Bhalla et al. (12) reported that serotonin and histamine may play a role in the early phase of carrageenin edema. A part of the delayed phase of carrageenin edema is brought about by the kinin system (13-15).

To investigate the interaction between the early phase and the delayed phase in the inflammatory process, the present experiments were performed on the mixing effects of phlogistics in paw edema and on the effects of various agents on edema induced by the mixed phlogistics in rats.

MATERIALS AND METHODS

Male rats of Wistar strain, weighing 100 to 120g, were maintained in an air-conditioned room at a temp. of 23±1°C, and given free access to water and laboratory food (CE-2, Nippon CLEA, Tokyo). Hind paw volume was determined by immersion of hind paw
down to the lateral malleolus into a glass cylinder containing water (water displacement method).

**Preparation of mixed phlogistics**

The suspension of mixed phlogistics included 0.01% serotonin creatinine sulfate, 0.1% histamine dihydrochloride, 5% kaolin and 0.5% carrageenin in distilled water. The original suspension of mixed phlogistics was prepared by the following procedure; one g of kaolin was suspended in 14 ml of 0.715% carrageenin aqueous solution on a magnetic stirrer, and then 1.0 ml of 2% histamine dihydrochloride solution and 0.2% serotonin creatinine sulfate solution were added to the suspension while stirring. The final suspension of mixed phlogistics was prepared by addition of 0.5 ml of distilled water, drug solution or suspension to 2.0 ml of the original suspension.

**Drug treatments**

Hind paw edema was induced by an s.c. injection of 0.1 ml of the final suspension of mixed phlogistics into the right hind foot pads of rats. For oral administration, drugs were suspended in 0.5% gum tragacanth aqueous solution and administered by stomach tube 30 min before challenge of the mixed phlogistics. For localized treatment, 0.1 ml of the final suspension of mixed phlogistics containing each drug was injected s.c. into the right foot pads of rats. Five to 12 rats were used for one dose of each drug.

**Chemicals**

Histamine dihydrochloride (Nakarai Chemicals, Ltd.), serotonin creatinine sulfate (Nakarai Chemicals, Ltd.), indomethacin (Nippon Merck-Banyu Co., Ltd.), flufenamic acid (Dainippon Pharm. Co., Ltd.), phenylbutazone (Fujisawa Pharm. Co., Ltd.), ibufenac (Kakenyaku Kako Co., Ltd.), aminopyrine (Iwaki Seiyaku Co., Ltd.), Aspirin (Yoshitomi Pharm. Co., Ltd.), benzydamine (Yoshitomi Pharm. Co., Ltd.), prednisolone (Takeda Chemical Ind., Ltd.), diphenhydramine hydrochloride (Dainippon Pharm. Co., Ltd.), cypreheptazine hydrochloride (Banyu Pharm. Co., Ltd.), cinnanserine hydrochloride (Dainippon Pharm. Co., Ltd.), chlorpromazine hydrochloride (Shionogi & Co., Ltd.), haloperidol hydrochloride (Dainippon Pharm. Co., Ltd.) and trasyrol (Bayer) were used.

**RESULTS**

**Interaction between serotonin and histamine on hind paw edema**

Experiments were carried out to determine whether or not there is a potentiating effect between histamine and serotonin, which are mediators in the early phase of acute inflammation.

Hind paw edema was induced by s.c. injection of 0.1 ml of 0.1% histamine dihydrochloride, 0.01% serotonin creatinine sulfate, and the mixture of both solutions into the right hind foot pads, respectively. Time-course of hind paw swelling is shown in Fig. 1. With both histamine and serotonin, the maximum swellings were observed 30 min after challenge of phlogistics, and the subsiding curves during 0.5 to 1 hr were steeper than those after 1 hr. As shown in Fig. 2., the potentiating effect of serotonin on the effect of histamine was not observed, although an additive effect was evident.
FIG. 1. Time-course of hind paw swelling caused by serotonin and histamine. Each point represents the mean ± S.E. of 5 rats. (S); serotonin creatinine sulfate 0.01 mg/paw. (H); histamine 2HCl 0.1 mg/paw. (S+H); (S)+(H). (W); distilled water 0.1 ml/paw.

FIG. 2. Effect of serotonin on histamine-induced hind paw edema. Hind paw volume was measured at 30 min after s.c. injection of 0.1 mg/paw histamine 2HCl (triangle), serotonin creatinine sulfate (white circle) and serotonin added to 0.1 mg/paw of histamine (black circle), respectively. Each point represents the mean ± S.E. of 5 rats.
Interaction between carrageenin and Kaolin on hind paw edema

The swelling induced by s.c. injection of 5 mg/paw of Kaolin into the right hind foot pad lasted longer than that by 0.5 mg/paw of carrageenin, and small shoulders were observed in both curves of the swellings 30 min after challenge of carrageenin and Kaolin (Fig. 3). The potentiating effect of carrageenin on Kaolin-induced hind paw edema was observed only after 7 hr following challenge of carrageenin and Kaolin.

Fig. 3. Time-course of hind paw swelling caused by carrageenin and kaolin. (K); kaolin 5mg/paw. (C); carrageenin 0.5 mg/paw. (K + C); (K) + (C)

Fig. 4. Influence of the early on the delayed phase of hind paw swelling. (A); serotonin creatinine sulfate (0.01 mg) + histamine 2HCl (0.1 mg). (B); carrageenin (0.5mg) + kaolin (5mg). (C); (A) + (B).
Influence of the early phase on the delayed phase

The interaction between the mixture of histamine with serotonin which induces the early phase of acute inflammation and the mixture of Kaolin and carrageenin which induces the delayed phase was investigated. Time-course of hind paw swelling induced by the mixed phlogistics, which included 0.1% histamine dihydrochloride, 0.01% serotonin creatinine sulfate, 5% Kaolin and 0.5% carrageenin in distilled water, is shown in Fig. 4. Swelling induced by the mixed phlogistics was weaker than the sum of the swellings induced by the mixture of histamine with serotonin and the mixture of Kaolin with carrageenin, especially between 2 to 6 hr following challenge of phlogistics. This result demonstrates that the delayed phase of hind paw swelling (4 hr after a challenge with phlogistics) was not potentiated despite augmentation of the early phase (up to 1 hr following challenge of phlogistics).

Effects of antihistaminic and anti-serotonin agents on hind paw edema induced by the mixed phlogistics

As is shown in Fig. 5 and Table 1., diphenhydramine hydrochloride (0.05 to 2.0 mg/
| Dose | n   | 0.5 mean ± s.e. (%) | 3 mean ± s.e. (%) | 5 mean ± s.e. (%) | 7 mean ± s.e. (%) | 9 hr mean ± s.e. (%) |
|------|-----|---------------------|-------------------|------------------|------------------|---------------------|
| Diphenhydramine HCl | vehicle | 6 | 86.9 ± 2.5 | 80.5 ± 3.4 | 78.7 ± 1.6 | 70.7 ± 2.8 | 54.8 ± 3.0 |
|   | 0.05 | 5 | 60.1 ± 3.8 | 70.5 ± 3.7 | 68.3 ± 4.5 | 13.2b | 69.7 ± 5.5 | 1.4 | 52.5 ± 5.7 | 4.2 |
|   | 0.1 | 10 | 42.5 ± 2.8 | 73.4 ± 3.3 | 69.3 ± 3.7 | 61.7 ± 3.6 | 49.3 ± 4.4 |
|   | vehicle | 6 | 74.1 ± 3.8 | 63.9 ± 4.4 | 66.2 ± 5.5 | 4.5 | 62.0 ± 5.1 | - 0.5 | 52.9 ± 5.2 | - 7.3 |
|   | 2.0 | 4 | 16.2 ± 2.5 | 66.7 ± 3.2 | 58.8 ± 3.4 | 21.5c | 61.9 ± 3.5 | - 5.3 |
| Cyproheptazine HCl | vehicle | 6 | 75.3 ± 3.9 | 70.0 ± 4.3 | 65.0 ± 4.4 | 0.05 | 36.7 ± 4.1 | 51.3c | 60.0 ± 4.0 | 14.3 | 63.6 ± 5.0 | 2.2 |
|   | 0.05 | 5 | 76.3 ± 2.7 | 70.7 ± 2.8 | 62.3 ± 3.6 | 47.8 ± 2.8 |
| Cinnanserine HCl | vehicle | 6 | 83.2 ± 3.5 | 73.5 ± 6.4 | 72.0 ± 7.1 | 1.8 | 67.2 ± 6.7 | - 7.9 | 55.4 ± 6.0 | - 15.9 |
|   | 0.05 | 5 | 58.6 ± 3.9 | 73.5 ± 6.4 | 72.0 ± 7.1 | 1.8 | 67.2 ± 6.7 | - 7.9 | 55.4 ± 6.0 | - 15.9 |
|   | vehicle | 6 | 77.8 ± 3.4 | 69.7 ± 6.4 | 61.5 ± 3.0 | 0.1 | 46.3 ± 3.1 | 40.5c | 66.0 ± 3.8 | - 5.6 | 73.0 ± 5.8 | - 18.7 |
| Chlorpromazine HCl | vehicle | 6 | 86.3 ± 2.3 | 70.7 ± 3.9 | 67.9 ± 4.5 | 61.0 ± 3.6 | 50.7 ± 5.5 |
|   | 0.03 | 5 | 70.0 ± 2.8 | 71.8 ± 3.3 | 69.9 ± 5.1 | - 2.9 | 65.1 ± 4.9 | - 6.7 | 49.1 ± 5.3 | 3.2 |
|   | vehicle | 6 | 86.9 ± 2.5 | 80.5 ± 3.4 | 78.7 ± 1.6 | 70.7 ± 2.8 | 54.8 ± 3.0 |
|   | 0.1 | 5 | 40.9 ± 1.8 | 59.6 ± 3.6 | 62.0 ± 5.9 | 21.2 | 59.0 ± 5.1 | 16.5 | 45.3 ± 2.2 | 17.3 |
|   | vehicle | 6 | 82.3 ± 3.5 | 76.0 ± 2.7 | 70.7 ± 2.8 | 62.3 ± 3.6 | 47.8 ± 2.8 |
|   | 1.0 | 5 | 26.2 ± 4.0 | 50.4 ± 7.3 | 33.6c | 51.1 ± 7.4 | 27.7c | 52.4 ± 8.9 | 15.9 | 51.4 ± 9.7 | - 7.5 |
| Haloperidol HCl | vehicle | 6 | 86.3 ± 2.3 | 70.7 ± 3.9 | 69.7 ± 4.5 | 61.0 ± 3.6 | 50.7 ± 5.5 |
|   | 0.1 | 5 | 77.8 ± 2.7 | 77.1 ± 5.1 | 71.8 ± 4.9 | - 5.7 | 66.1 ± 3.6 | - 8.4 | 48.0 ± 3.4 | 5.3 |
| Trasyrol | vehicle | 6 | 86.1 ± 7.6 | 68.4 ± 4.9 | 62.3 ± 4.6 | 60.4 ± 7.0 | 48.4 ± 6.7 |
|   | 350 kiu | 5 | 84.6 ± 2.9 | 58.6 ± 2.4 | 56.5 ± 1.9 | 9.3 | 55.6 ± 2.7 | 7.9 | 45.6 ± 3.8 | 5.8 |

a: (%) inhibition in hind paw swelling compared with each vehicle control. b: 0.01 < P < 0.05 from each vehicle control.

c: P < 0.01 from each vehicle control.
paw), cyproheptazine hydrochloride (0.05 mg/paw), cinnanserine hydrochloride (0.05 to 0.1 mg/paw) and chlorpromazine hydrochloride (0.03 to 1.0 mg/paw) showed potent inhibitory activity on the early phase of swelling, but no significant inhibitory activity on the delayed phase with the exception of chlorpromazine, which inhibited the swelling significantly up to 5 hr after a challenge with phlogistics. Orally, diphenhydramine hydrochloride (40 mg/kg) and cyproheptazine hydrochloride (80 mg/kg) as in the local treatment inhibited the early phase only (Table 2).

Accordingly, the delayed phase was not influenced by modification of the early phase of hind paw swelling.

**TABLE 2. Effect of anti-inflammatory, antihistaminic and anti-serotonin agents on the mixed phlogistics-induced hind paw edema in rats.**

| Drug                  | Dose mg/kg p.o. | n | 1     | 3     | 5 hr |
|-----------------------|-----------------|---|-------|-------|------|
| Flufenamic acid       | 80              | 6 | 5.0   | 4.8   | 14.1 |
| Phenybutazone         | 80              | 6 | 5.1   | 5.0   | 7.7  |
| Indomethacin          | 2.5             | 6 | 20.0  | 13.1  | 4.4  |
| Aspirin               | 160             | 6 | 1.4   | 10.7  | 12.5 |
| Ibuprofen             | 160             | 6 | 1.3   | 0.6   | 8.5  |
| Aminopyrine           | 160             | 6 | 14.6  | 22.4  | 17.9 |
| Benzydamine           | 240             | 6 | 15.2  | 3.0   | 0.6  |
| Diphenhydramine-HCl   | 40              | 6 | 13.8  | 25.6  | 23.5 |
| Cyproheptazine-HCl    | 80              | 6 | 17.0 b| 18.1 b| 21.3 |

Each drug was given per os 30 min before an s.c. injection of 0.1 ml/paw of the mixed phlogistics.

b: $0.01 < P < 0.05$, c: $P < 0.01$ from each vehicle control

**Effect of anti-inflammatory agents on hind paw edema induced by the mixed phlogistics**

Non-steroidal anti-inflammatory agents had a mild inhibitory activity on the swelling of hind paw (Fig. 6. and Table 3). Indomethacin (0.1 to 2.0 mg/paw) and Aspirin (2.0 to 4.0 mg/paw) did not possess inhibitory activity on either the early or delayed phases of swelling, however, flufenamic acid (2.0 mg/paw) and phenylbutazone (4.0 mg/paw) both had significant inhibitory activity on the delayed phase of swelling.

Prednisolone (0.1 to 2.0 mg/paw and 10 mg/kg, p.o.) showed potent inhibitory activity on the delayed phase of swelling, but none on the early phase, as is shown in Fig. 7. and Table 3.

Subsequently, it was found that steroidal and non-steroidal anti-inflammatory agents inhibited the delayed phase without inhibiting the early phase.
FIG. 6. Effect of non-steroidal anti-inflammatory agents on the mixed phlogistics-induced hind paw edema. (e) flufenamic acid 2.0 mg/paw. (f) phenylbutazone 4.0 mg/paw. (g) indomethacin 2.0 mg/paw. (h) Aspirin 4.0 mg/paw.

FIG. 7. Effect of prednisolone on the mixed phlogistics-induced hind paw edema. Prednisolone was given s.c. together with the mixed phlogistics into the hind foot pad of rats (i) and administered per os 30 min before challenge of phlogistics (j), respectively. (i) 2.0 mg, (j) 10 mg/kg.
### Table 3. Effect of anti-inflammatory agents on the mixed phlogistics-induced hind paw edema in rats.

| Dose | n   | 0.5 | 3 (% increase in right hind paw volume) | 5 | 7 | 9 hr |
|------|-----|-----|----------------------------------------|---|---|-----|
|      |     | mean ± s.e. | (%) | mean ± s.e. | (%) | mean ± s.e. | (%) | mean ± s.e. | (%) |
|      |     | (µg/paw) |     | (µg/paw) |     | (µg/paw) |     | (µg/paw) |     | (µg/paw) |     |
| Flufenamic acid |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 12  | 90.3 ± 3.6 | 79.2 ± 3.6 | 77.8 ± 3.7 | 66.7 ± 3.8 | 50.8 ± 3.6 |     |         |     |         |     |
| 0.5      | 10  | 79.4 ± 3.7 | 70.1 ± 4.0 | 66.7 ± 5.0 | 61.1 ± 4.0 | 51.1 ± 4.2 |     |         |     |         |     |
| vehicle  | 6   | 77.2 ± 4.5 | 68.2 ± 3.9 | 69.0 ± 3.9 | 68.3 ± 3.8 | 51.1 ± 4.2 |     |         |     |         |     |
| 2.0      | 5   | 71.4 ± 6.0 | 75.6 ± 8.3 | 45.5 ± 7.0 | 36.0 | 29.0 ± 5.4 |     |         |     |         |     |
| Phenylbutazone |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 6   | 81.6 ± 7.6 | 68.5 ± 4.9 | 62.3 ± 4.6 | 60.4 ± 7.0 | 48.4 ± 6.7 |     |         |     |         |     |
| 2.0      | 3   | 73.6 ± 6.4 | 61.4 ± 5.9 | 65.9 ± 4.9 | 62.1 ± 6.1 | 51.7 ± 5.4 |     |         |     |         |     |
| vehicle  | 6   | 83.7 ± 3.4 | 74.8 ± 4.0 | 74.0 ± 3.8 | 66.8 ± 3.7 | 52.1 ± 2.5 |     |         |     |         |     |
| 4.0      | 5   | 81.6 ± 1.2 | 64.0 ± 1.7 | 63.3 ± 4.4 | 56.3 ± 5.3 | 45.1 ± 6.3 |     |         |     |         |     |
| Indomethacin |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 6   | 75.3 ± 3.9 | 70.0 ± 4.3 | 65.0 ± 4.4 |     |         |     |         |     |         |     |
| 0.1      | 5   | 84.4 ± 2.8 | 66.3 ± 4.6 | 65.7 ± 5.9 | 50.7 ± 3.4 | 43.1 ± 4.0 |     |         |     |         |     |
| vehicle  | 12  | 84.6 ± 2.7 | 75.1 ± 3.5 | 69.0 ± 3.1 | 64.5 ± 4.2 | 50.7 ± 3.4 | 21.1 |     |     |         |     |
| 0.5      | 10  | 84.0 ± 4.8 | 68.1 ± 3.6 | 58.7 ± 3.3 | 53.9 ± 4.7 | 40.0 ± 5.1 |     |         |     |         |     |
| vehicle  | 6   | 85.4 ± 1.8 | 75.3 ± 2.9 | 63.9 ± 2.3 | 62.2 ± 4.7 | 49.3 ± 4.2 |     |         |     |         |     |
| 2.0      | 5   | 93.6 ± 4.9 | 67.6 ± 3.3 | 60.3 ± 3.7 | 56.8 ± 3.6 | 43.1 ± 4.0 |     |         |     |         |     |
| Aspirin  |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 6   | 97.4 ± 3.7 | 82.3 ± 4.4 | 84.8 ± 4.5 | 71.0 ± 4.0 | 53.8 ± 4.7 |     |         |     |         |     |
| 2.0      | 5   | 89.4 ± 3.7 | 74.3 ± 3.7 | 70.2 ± 5.3 | 59.2 ± 5.0 | 46.6 ± 5.0 |     |         |     |         |     |
| vehicle  | 6   | 83.7 ± 3.4 | 74.8 ± 4.0 | 74.0 ± 3.8 | 66.8 ± 3.7 | 52.1 ± 2.5 |     |         |     |         |     |
| 4.0      | 5   | 86.6 ± 1.8 | 65.7 ± 6.7 | 75.6 ± 5.5 | 70.2 ± 4.4 | 50.4 ± 7.5 |     |         |     |         |     |
| Aminopyrine |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 6   | 86.9 ± 2.5 | 80.5 ± 3.4 | 78.7 ± 1.6 | 70.7 ± 2.8 | 54.8 ± 3.0 |     |         |     |         |     |
| 2.0      | 5   | 73.0 ± 3.3 | 71.1 ± 2.6 | 67.0 ± 3.1 | 67.5 ± 1.6 | 55.3 ± 2.4 | 0.9  |     |     |         |     |
| vehicle  | 6   | 85.4 ± 1.8 | 75.3 ± 2.9 | 63.9 ± 2.3 | 62.2 ± 4.7 | 49.3 ± 4.2 |     |         |     |         |     |
| 4.0      | 5   | 72.4 ± 2.5 | 71.0 ± 4.4 | 79.9 ± 3.9 | 78.2 ± 2.3 | 67.8 ± 4.9 | 37.5 |     |     |         |     |
| Prednisolone |     |         |     |            |     |            |     |            |     |            |     |
| vehicle  | 6   | 83.7 ± 3.4 | 74.8 ± 4.0 | 74.0 ± 3.8 | 66.8 ± 3.7 | 52.1 ± 2.5 |     |         |     |         |     |
| 0.1      | 5   | 87.0 ± 6.2 | 64.5 ± 5.0 | 63.1 ± 5.5 | 53.8 ± 4.0 | 43.6 ± 4.0 | 16.3 |     |     |         |     |
| vehicle  | 6   | 77.2 ± 4.5 | 68.2 ± 3.9 | 69.0 ± 3.9 | 68.3 ± 3.9 | 48.2 ± 3.6 |     |         |     |         |     |
| 0.5      | 5   | 90.6 ± 5.2 | 66.5 ± 4.8 | 47.9 ± 4.6 | 43.3 ± 4.4 | 27.5 ± 5.3 | 48.1 |     |     |         |     |
| vehicle  | 6   | 86.1 ± 7.6 | 68.4 ± 4.9 | 62.3 ± 4.6 | 60.4 ± 7.0 | 48.8 ± 6.7 |     |         |     |         |     |
| 2.0      | 5   | 82.4 ± 4.0 | 58.4 ± 3.2 | 41.7 ± 1.8 | 24.5 ± 3.0 | 10.1 ± 1.7 | 79.1 |     |     |         |     |

See Table 1. footnote.
Combined effect of antihistaminic and anti-inflammatory agents

Diphenhydramine inhibited mainly the early phase of hind paw swelling induced by the mixed phlogistics, while flufenamic acid inhibited mainly the delayed phase. The combined effect of diphenhydramine hydrochloride (0.1 mg/paw) with flufenamic acid (0.5 mg/paw) was investigated using a local application.

As is shown in Table 4, both the early and the delayed phases were significantly inhibited by treatment with both agents. The potentiating effect on the delayed phase was particularly evident when both agents were combined.

### TABLE 4. Combined effect of flufenamic acid and diphenhydramine on the mixed phlogistics-induced hind paw edema in rats.

| Dose mg/paw | n | Percent increase in hind paw volume mean ± s.e. |
|-------------|---|-----------------------------------------------|
|             |   | Hind paw volume before (ml) 0.5 1 2 3 4 5 6 7 8 9 hr |
| Control     | 6 | 1.29 ± 0.04 83.2 ± 2.8 78.5 ± 2.9 72.8 ± 2.7 76.0 ± 3.2 70.8 ± 2.8 70.7 ± 3.5 72.5 ± 3.6 62.3 ± 3.5 59.0 ± 47.8 |
| flufenamic acid 0.5 | 5 | 1.16 ± 0.02 71.4 ± 4.0 79.6 ± 3.3 68.4 ± 4.5 70.4 ± 5.4 ± 6.7 ± 6.8 ± 6.4 ± 4.9 ± 6.1 ± 5.8 |
| flufenamic acid 0.5 | 5 | 1.18 ± 0.03 14.9 ± 1.8 23.2 ± 1.5 29.3 ± 2.5 28.6 ± 2.0 32.6 ± 3.1 41.3 ± 5.3 45.9 ± 5.4 41.6 ± 5.5 38.6 ± 5.9 |
| diphenhydramine-HCl 0.1 | 5 | b b b b b b b |

Each drug was injected s.c. together with a mixture of phlogistics into the foot pad of rats. a: 0.01<P<0.05, b: P<0.01 from each control

**DISCUSSION**

The inflammation consists of three stages, that is increase in vascular permeability, leucocyte emigration and proliferation of connective tissue. In the present study, the interaction of the first stage (the increase of vascular permeability) with the second stage (the leucocyte emigration) was investigated using the mixed phlogistics-induced paw edema technique in the rat.

Time-course of each phlogistic-induced edema (Figs. 1. and 3.) was in accord with those of other investigators (16, 17). The slight swelling in histamine-induced edema has been explained by the fact that rats are less sensitive to histamine (18). Histamine and serotonin cause transient swelling of hind paw by increasing vascular permeability, but the swelling caused by carrageenin includes leucocyte emigration (19, 20). Accordingly, it is conceivable that histamine- and serotonin-type swelling is the first stage, and carrageenin- and Kaolin-type swelling is the second stage in the inflammatory process.

Histamine- and serotonin-induced swelling is composed of only the early phase (the first stage), though carrageenin- and Kaolin-induced swelling is composed of both the early and the delayed phases (the second stage). If the early phase plays a role in the development of the delayed phase, the delayed phase would be augmented by the potentiation of the early phase. However, in spite of the potentiation of the early phase by addition of
histamine and serotonin into the mixed suspension of carrageenin and Kaolin, the delayed phase was not altered (Fig. 4). The delayed phase was not reduced by the treatment with antihistaminics or anti-serotonin agents in hind paw edema induced by the mixed phlogistics, whereas the early phase was inhibited by treatment with these agents (Fig. 5), and the delayed phase was inhibited with prednisolone or flufenamic acid with no alteration in the early phase (Figs. 6 and 7). These results demonstrate that the early phase and the delayed phase are independent of each other in the developmental process of hind paw edema. Nakamura et al. (21) reported that granulation tissue formation induced by s.c. implantation of felt-pellets into a rat was accelerated by addition of carrageenin in the pellets, but was not by addition of histamine or serotonin. Accordingly, it may be concluded that the first stage in the inflammatory process is independent from the second and the third stage, that is the first stage is not an indispensable stage to the second and the third.

Shanahan (22) and Riesterer et al. (23) reported that Kaolin and carrageenin induced hind paw edema of rats was inhibited by a local application of non-steroidal anti-inflammatory agents. However, in the study herein, indomethacin, Aspirin and phenylbutazone showed a weak activity on the delayed phase of the mixed phlogistics-induced edema. This discrepancy may be explained by the difference in the severity of the early phase. On the other hand, a potentiating effect on the delayed phase was observed by a combined use of flufenamic acid and diphenhydramine (Table 4). These results imply that the early phase is related to the delayed phase in chemotherapy for hind paw edema.

Chlorpromazine has potent antihistaminic activity and significantly inhibited the early phase in the mixed phlogistics-induced hind paw edema at a s.c. dose of 0.03 mg/paw, however, in both the early and delayed phases inhibition was observed with a dose of 3 mg/paw (Fig. 5 and Table 1). The inhibitory effect of chlorpromazine on the delayed phase may depend on its membrane stabilizing activity (24, 25) rather than its tranquilizing activity, because haloperidol, which possesses about 30 times more potent tranquilizing activity and 3 times more potent anti-edema activity (26, 27) and one third to one thirty times less antihistaminic activity than chlorpromazine (28), did not inhibit the delayed phase as is shown in Table 1. Sigg et al. (29) and Yamasaki et al. (30) reported that antihistaminics, chlorpromazine and non-steroidal anti-inflammatory agents inhibited mast-cell degranulation, while steroidal anti-inflammatory agents did not. Skidmore et al. (31, 32) reported that non-steroidal anti-inflammatory agents inhibited a stabilizing activity on erythrocyte membrane, while diphenhydramine and cyproheptazine did not have this activity. Steroidal and non-steroidal anti-inflammatory agents possess a stabilizing activity on lysosomes (35, 36). From these facts, it appears that agents with antihistaminic and/or anti-serotonin activity inhibit the early phase in the mixed phlogistics-induced hind paw edema of rats, while agents with the membrane and/or lysosome stabilizing activity inhibit the delayed phase. The inhibitory effect of
aminopyrine on the early phase may be depend on its more potent anti-histaminic activity (33) and weaker membrane stabilizing activity than those of non-steroidal anti-inflammatory agents (37).

The delayed phase of carrageenin-induced hind paw edema is inhibited by treatment with trasyrol or SBTI, or pre-treatment of cellulose sulfate (12-15, 38). These results demonstrate that the kinin system is one of the important mediators in carrageenin-induced paw edema of rats. However, in the mixed phlogistics-induced hind paw edema of rats, trasyrol failed to inhibit both the early and delayed phases at a s.c. dose of 350 KIU/paw (Table 1.). Accordingly, the mixed phlogistics-induced hind paw edema technique in the rat is a useful method for investigating the mechanism of action of anti-inflammatory agents.

From the results described above, it may be concluded that in the developmental process of acute inflammation the first stage is independent of the second, that is, the first stage is not an indispensable stage to further development of inflammatory reaction. Therapeutic effects of agents on the second stage are however influenced by the first stage.

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