

## Studies on Anaphylactic Manifestation in Guinea Pig's Atria by Means of Albumin Labeled with Fluorescein\*<sup>1</sup>

Tsutomu FUJITA\*<sup>2</sup>

*Department of Pharmacology,  
Nagasaki University  
School of Medicine, Nagasaki*

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It has been widely accepted that the anaphylactic reaction might be caused principally by chemical substances released from the tissues as a result of antigen-antibody reaction. Atria isolated from guinea pigs sensitized by egg-albumin were mainly used for this experiment. When the sensitized atrium and the non-sensitized guinea-pig's ileum were suspended together in the same bath, there appeared strong contraction of the ileum along with the provocation of anaphylaxis in the sensitized atrium. Although such contraction of ileum was abolished almost completely by diphenhydramine or diphenylpyraline, the atrial anaphylaxis was not affected. The anaphylactic reaction was also evoked by perfusing solution containing the antigen. However, the atrial anaphylaxis showed little influence on the contractile tension of the non-sensitized atrium suspended together in the same bath. The above facts suggest that the anaphylactic contraction would not be caused solely by mediators, including hitherto unknown chemical substances. So the further attempt was carried out to examine in vitro the site of antigen-antibody interaction in the atrium by using the antigen labeled with fluorescein isothiocyanate. The specific fluorescence about the muscle cells was observed along their boundary of the highly sensitized atrium. Through the results in these experiments, together with the reports from our laboratory, it could be assumed that in the atrial anaphylaxis the antigen-antibody reaction itself induces some changes on the cell membrane leading to muscular contraction.

H.H. DALE et al<sup>2)</sup> found that histamine action in animal is very similar to anaphylactic shock, and postulated<sup>3)</sup> that histamine released from the tissue as a result of the antigen-antibody reaction is a substance principally responsible for the anaphylactically induced contraction of

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\*<sup>2</sup> 藤田 勉

smooth muscle. C. A. DRAGSTED also reported that histamine is found in the blood of dog during anaphylactic shock as a substance stimulating smooth muscle. On the other hand, C. H. KELLAWAY<sup>8)</sup> reported that rat uterus relaxed by histamine also evoked contractile enhancement as an anaphylactic reaction. According to H. O. SCHILD<sup>14)15)</sup>, the amounts of histamine actually released from the tissue is considerably less than the amounts which must be added exogenously to produce a similar response.

Y. NAKAZAWA<sup>11)</sup> reported that the anaphylactic reaction in the isolated atria of the sensitized guinea pigs occurred as enhancement of cardiac movements, and pointed out that the heart was a suitable organ for analyzing tissue anaphylaxis pharmacodynamically. And then extensive studies were performed in his laboratory: significance of considerable substances in anaphylactic reaction, prophylactic survey of the reaction, or electrophysiological characteristic of the reaction and so on. And their results<sup>6)7)11)12)16)17)</sup> indicate that the anaphylactic reaction in the isolated atrium of the sensitized guinea pigs differs from effects caused by exogenously given histamine, acetylcholine and serotonin, and suggest that dynamic responses of cardiac muscle during anaphylaxis may not solely be caused by chemical substances.

In the present paper, the attempt was made to search what significance a substance mediated during anaphylaxis had in the dynamic responses of anaphylaxis and where antigen-antibody union was taken place.

## I. PHARMACODYNAMIC EXPERIMENTS

### MATERIALS AND METHODS

The anti-egg-albumin serum was obtained from rabbits following repeated intravenous injections of alum-precipitated egg-albumin, up to serum shows above  $1 \times 256$  antibody titer.

The method of sensitization is described in detail elsewhere.<sup>9)</sup> Male and female guinea pigs weighing 280 to 300 g were used throughout the experiment, and anaphylactic sensitization of the guinea-pig was carried out passively by intravenous injection of anti-egg-albumin rabbit serum. The injection of the antiserum (0.3 ml per 100 g body weight) was made 24 hours prior to the experiment. The atrium and the ileal segment (a terminal portion of ileum was used) were removed immediately after exsanguination. Then, the sensitized atrium was suspended together with the non-sensitized atrium or the non-sensitized ileum in a bath containing 10 ml of Lock's solution composed of NaCl 0.9%, KCl 0.042%, CaCl<sub>2</sub> 0.024%, Glucose 0.1%, NaHCO<sub>3</sub> 0.05% under constant aeration with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at 30°C. And isometric records of their contractions were made by connection with the unbonded strain

gage transducer (Shinkoh UL-10-120). In the first place, preparations showing almost same sensitivity to histamine ( $0.01 \mu\text{g/ml}$ ) were used in this experiment. Solution of egg-albumin as the antigen was added to the bath at  $2 \text{ mg}\%$  every time.

## RESULTS

### 1. Evidence of presence of active principle released during anaphylaxis.

The contractile tension of a sensitized atrium was markedly increased 10 to 30 sec after applying egg-albumin to the bath, and simultaneously contractile tension of non-sensitized ileum suspended together in the same bath were found to increase along with enhancement of the atrial movement (Fig. 1).

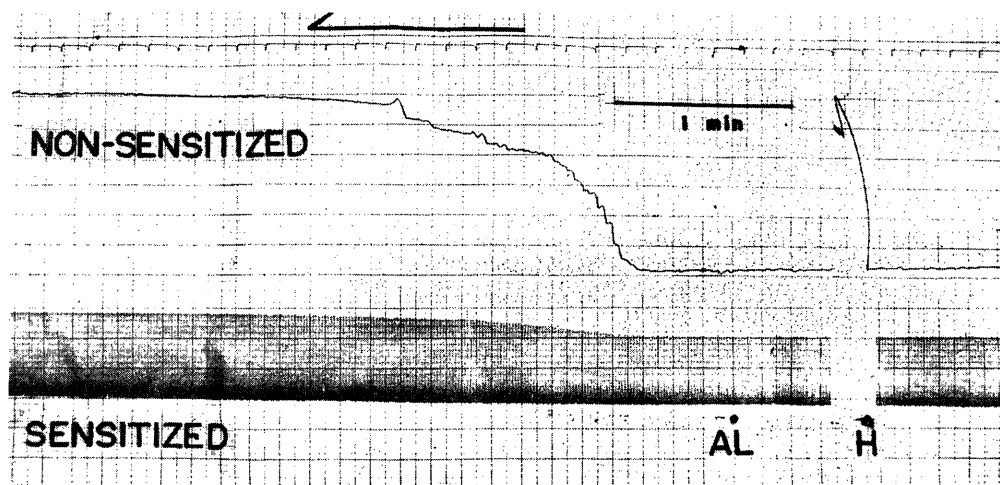


Fig. 1. Anaphylactic reaction appeared in the sensitized atrium and response of the non-sensitized ileum suspended together in the same bath.  
 NON-SENSITIZED: Ileal segment isolated from non-sensitized guinea pigs  
 SENSITIZED: Atrium isolated from guinea pigs sensitized by egg-albumin  
 H : Histamine  $0.01 \mu\text{g/ml}$   
 AL : Antigen (egg-albumin  $0.2 \text{ mg/ml}$ )

### 2. Elimination of the active principle by perfusion of nutrient solution.

When both non-sensitized ileal segment and the sensitized atrium were set together in the same bath were perfused continuously by antigen-containing Locke's solution, the atrium showed a marked anaphylactic response, but no significant contraction of the ileum was observed (Fig. 2.).

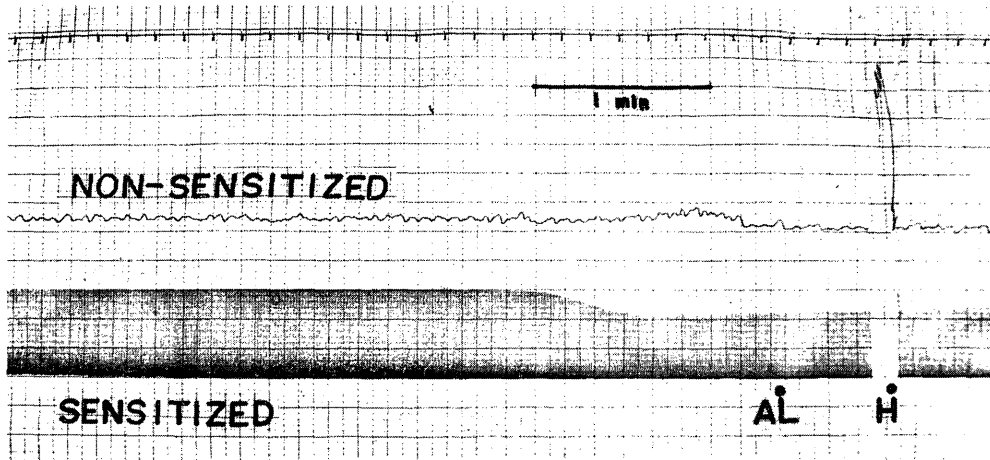


Fig. 2. Anaphylactic reaction appeared in the sensitized atrium and response of the non-sensitized ileum suspended together in the same bath perfused with antigen-containing solution.

NON-SENSITIZED : Ileal segment isolated from non-sensitized guinea pigs

SENSITIZED : Atrium isolated from guinea pigs sensitized by egg-albumin

H : Histamine 0.01  $\mu$ g/ml

AL : Antigen (egg-albumin 0.2 mg/ml)

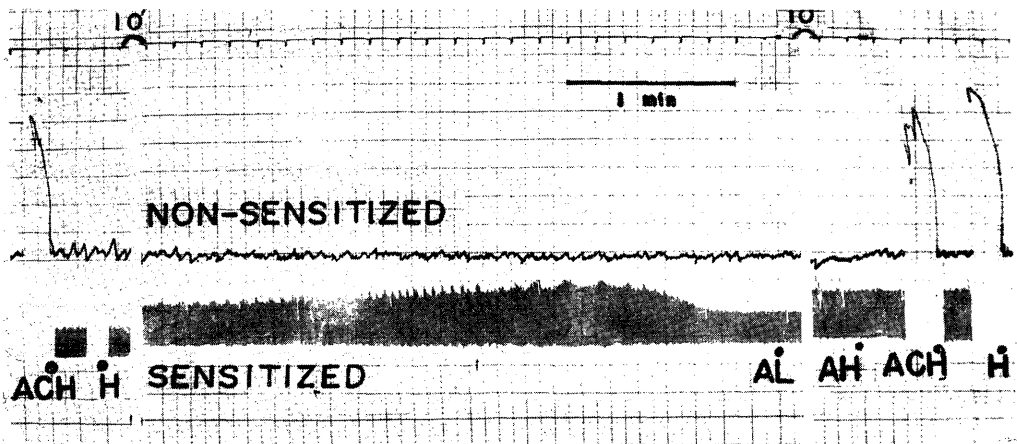


Fig. 3. Anaphylactic reaction appeared in the sensitized atrium and response of the non-sensitized ileum in the presence of diphenhydramine (each preparation is as in Fig. 1.). And the effects of histamine and acetylcholine on the ileum before and after administration of diphenhydramine.

NON-SENSITIZED : Ileal segment isolated from non-sensitized guinea pigs

SENSITIZED : Atrium isolated from guinea pigs sensitized by egg-albumin

H : Histamine 0.01  $\mu$ g/ml

ACH : Acetylcholine 0.005  $\mu$ g/ml

AH : Diphenhydramine hydrochloride 0.03  $\mu$ g/ml

AL : Antigen (egg-albumin 0.2 mg/ml)

Above facts clearly indicate that contractile principle against ileum is demonstrated as diffusible during atrial anaphylaxis.

### 3. Effects of antihistaminic agents

After 10 minutes applying diphenhydramine hydrochloride ( $0.03 \mu\text{g/ml}$ ), antigen was added to the bath in which a non-sensitized ileal segment was suspended together with the sensitized atrium. The dynamic response of the atrium anaphylaxis was observed similarly without antihistaminics, while the contractile response expected to be produced in the ileum was almost completely abolished. On the other hand histamine did not react on the ileal segment after treatment with antihistaminics ( $0.03 \mu\text{g/ml}$ ), but contractile action of acetylcholine on the segment was not clearly inhibited (Fig. 3).

Similar response in the sensitized atrium and the non-sensitized ileum against the specific antigen was also observed by application of diphenylpyraline hydrochloride ( $0.02 \mu\text{g/ml}$ ).

It is very probable that the active principle released during atrial anaphylaxis to contract ileal segment may be histamine.

### 4. Significance of the active principle on dynamic response of the atrium.

Although marked increase in the contraction and beat rate was evoked in the sensitized atrium by adding of antigen, it was not

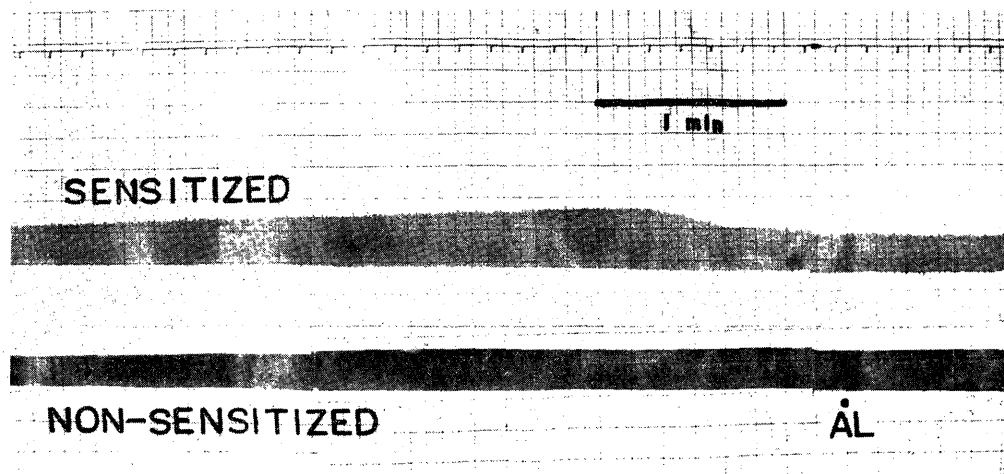


Fig. 4. Anaphylactic reaction appeared in the sensitized atrium and response of non-sensitized atrium suspended together in the same bath.

SENSITIZED : Atrium isolated from guinea pigs sensitized by egg-albumin

NON-SENSITIZED : Atrium isolated from non-sensitized guinea pigs

AL : Antigen (egg-albumin  $0.2 \text{ mg/ml}$ )

significant in a non-sensitized atrium suspended together with a sensitized one (Fig. 4). That is to say, the active principle released during anaphylaxis is effective to contract ileal segment, but not enough to cause dynamic reaction in the atrium.

## II. HISTOLOGICAL EXPERIMENTS INCLUDING USE OF FLUOROANTIGEN

### MATERIALS AND METHODS

Fluorescent antigen was made by conjugation of egg-albumin with fluorescein isothiocyanate in a ratio of 0.05 mg of dye per mg of protein according to advanced method of RIGGS et al<sup>13)</sup> from method of CCONS<sup>1)</sup>. After removing excess fluorescein isothiocyanate by dialysis against 0.15 M NaCl for 5 days, the conjugated albumin were stored at 4–5°C. Immediately before the experiment, unspecific fluoroprotein was eliminated by adsorption with liver powder<sup>1)</sup> of guinea pig. Sensitization of guinea pig carried out by intravenous injection of profuse amounts of anti-egg-albumin rabbit serum (over 1.2ml/100g body weight).

The spontaneously beating atrium was suspended in a 30°C bath containing Locke's Solution whose pH was adjusted in 7.1 by sodium phosphate and aerated with oxygen. After atrium was repeatedly washed with the nutrient solution, the fluoroprotein was added and allowed to contact for 1 minute. During this time apparent anaphylactic reaction was observed by dynamic recording. Then atrium was repeatedly washed with Locke's solution, and was frozen indirectly by ethanol dryice mixture. And tissue sections 4–6  $\mu$  in thickness were made in a Cryostat<sup>1)</sup>. After mounting on a dry slide, the sections were fixed in acetic acid-ethanol<sup>5)</sup>. A drop of glycerin of pH 7.1 was placed on the tissue and then the coverslip was put on. And the tissue was examined under Fluoromicroscope (CARL ZEISS).

On the other hand, some sections were stained with hematoxylin-eosin, Van Gieson's solution and toluidine blue for original microscopic examination.

Fluoromicroscopic studies included following control studies: (a) use of a non-sensitized atrium; and (b) blocking of fluorostaining by preceding application of non-fluorescent antigen.

### RESULTS

For the microscopic examinations of the distributions of fluoro-antigen, adequate filter was used to activate emission of fluorescence, and specific fluorescence originated from fluorescein isothiocyanate was confirmed by the filter.

Fluorescent findings from the sections were always compared with those of the sections stained with hematoxylin-eosin and Van Gieson's

solution. Specific fluorescence was found as fine-granular fluorescence distributed along the boundary of each cardiac muscle fiber, as shown in Fig. 5. The intracellular fluorescence could not be observed in these muscle cells. In vessels the strong specific fluorescence could be seen,

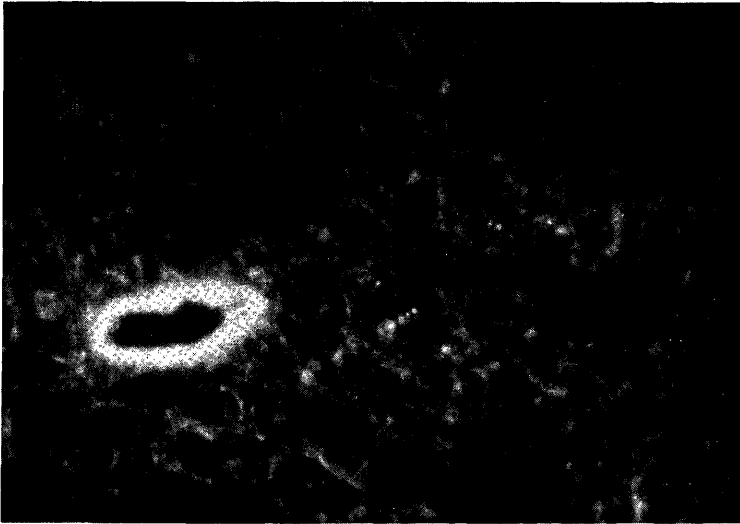


Fig. 5. Fluorescence photomicrograph of  $4 \mu$  section of sensitized guinea-pig's atrium. White area corresponds to specific fluorescence of labeled proteins (cross section : approx.  $\times 1000$ )

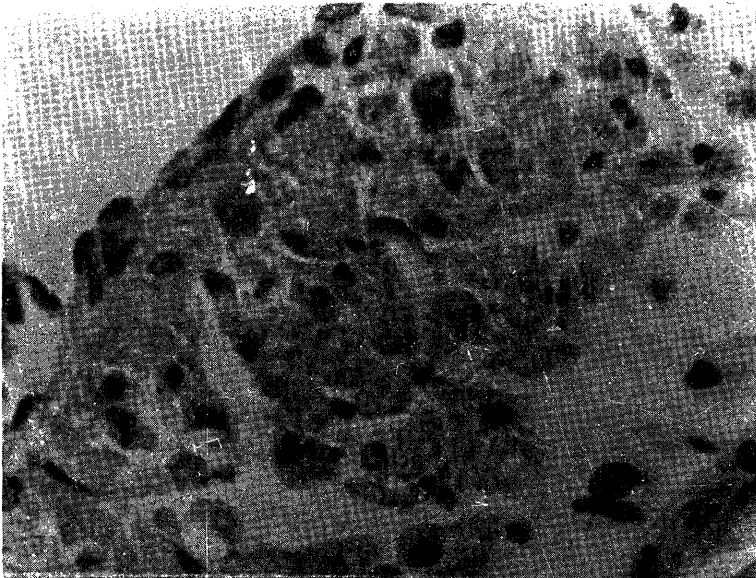


Fig. 6. The tissue similar to that seen in Fig. 5., but stained with hematoxylin-eosin (cross section : approx.  $\times 1000$ )

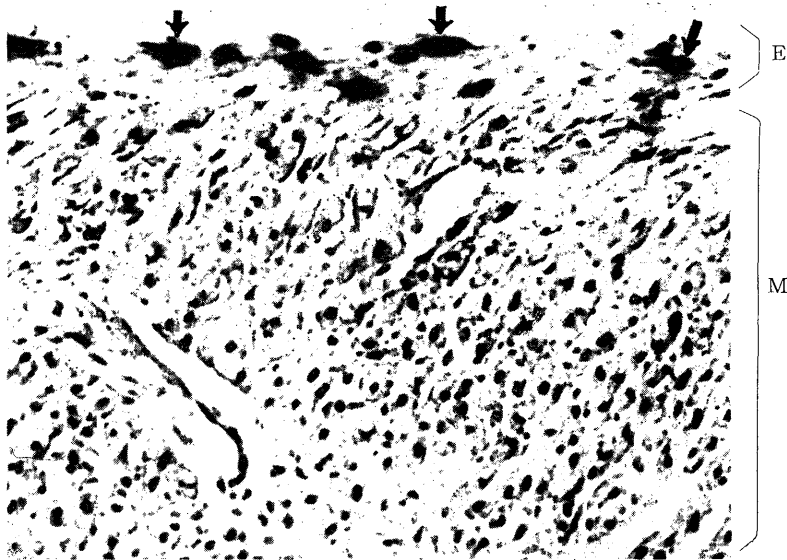


Fig. 7. The tissue similar to that seen in Fig. 5., but stained with toluidine blue in  $20\ \mu$  section. Black areas correspond to tissue mast cells, and they are distributed numerously in epicardium (cross section : approx.  $\times 300$ )  
 E : Epicardium  
 M : Myocardium

but no specific fluorescence could be found in the control sections.

On the other hand, it has been generally believed that histamine is released mainly from mast cells during anaphylaxis. So, distribution of mast cells was also examined in the tissue stained with toluidine blue according to the method described by MOTA<sup>10)</sup>. The granules of these cells turned to purple, nuclei to shade of blue, cardiac muscle cells to yellow. And these cells could be discerned apparently from the others in these tissues. At a thickness of  $20\ \mu$ , a number of mast cells were observed in epicardium, but only few cells were found in myocardium (Fig. 7) and the cells disseminated in this area were round, oval or spindle form having much metachromatic granules. At a thickness of  $4\ \mu$ , these cells were hardly observed in myocardium. So, it is considered that the specific fluorescence observed along muscle fibers may not be related to the mast cells.

## DISCUSSION

The theories in anaphylaxis, in any cases, have been studied about the substances released from the tissues as a result of the antigen-antibody union. Pharmacodynamically there appeared the strong contraction of the non-sensitized ileum along with the provocation of anaphy-



laxis in the sensitized atria suspended together in the same bath. This fact indicates that in the anaphylaxis some substance diffused out rapidly from the isolated atria of sensitized guinea pigs into the surrounding fluid, by which guinea pig's ileum should be stimulated. Such effects of the substances on the non-sensitized ileum were inhibited almost completely in the presence of diphenhydramine ( $0.03 \mu\text{g/ml}$ ) or diphenylpyraline ( $0.02 \mu\text{g/ml}$ ). The concentrations of these agents used in this experiment have little effect on the ileum without antagonizing action to histamine. Therefore, the active substances released from the atria during anaphylaxis are almost entirely histamine, including hitherto unknown active substances.

The anaphylactic reaction in the sensitized atrium was not significantly affected by antihistaminic agents, which has been also reported in very detail by F. KIHARA<sup>7)</sup>. A marked anaphylactic reaction in the atrium was also provoked normally in perfusing bath in which perfusate contained the antigen, while active substances released from the tissue should be removed with outlet fluid. On the other hand, no significant change was observed in a non-sensitized atrium suspended together with a sensitized one by adding antigen, whereas marked reaction occurs in the sensitized one.

These findings mentioned above indicate that the anaphylactically induced dynamic reaction in the atrium does not concern solely with released chemical substances. Therefore, direct effect of antigen-antibody reaction should be considered.

Histamine release from the muscle tissue has been generally accepted. It comes mostly from tissue mast cells. Although numbers of mast cells in the guinea pig's myocardium are incomparably fewer than those in the epicardium, histamine released from the former amounts to half of the latter as M. YASUDA<sup>19)</sup> has described in this Acta. This result may indicate that in atrial anaphylaxis histamine also comes from the cardiac muscle fibers, not only from mast cells. This also suggests that the antigen-antibody reaction would take place in muscle cells themselves.

The localization of antigen-antibody interaction in the highly sensitized atrium searched by using the antigen labeled with fluorescein isothiocyanate demonstrates the binding site in the sensitized tissues. Specific fluorescence is found as fine-granular fluorescence distributed along the boundary of each cardiac muscle cells. It is concerned with the cell membrane. It has been already supposed<sup>16)</sup> that egg-albumin as antigen may be conjugate with antibody series at the surface of the cell membrane. Y. NAKAZAWA and A. UENO<sup>17)</sup> have reported through the finding in the contour of the transmembrane action potentials that a change on the cell membrane of the cardiac muscle occurs during atrial anaphylaxis, and that potassium contracture<sup>18)</sup> in the sensitized atria is not modified at all by addition of the specific antigen. Thus,

it is considered that concerning the mechanism of the anaphylactic contraction in the isolated atria of sensitized guinea pigs, the antigen-antibody reaction itself induces some changes in the cell membrane leading to the muscular contraction.

On the other hand, active substances are surely released during anaphylaxis and these are mainly histamine responsible to various functions. Therefore it is considered that antigen-antibody reaction itself plays a role in the dynamic response in the first, but histamine and other possible substances also have a concern with the reaction.

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