COEXISTENCE OF ENERGY DEPENDENT AND NON-DEPENDENT PROCESSES IN HISTAMINE RELEASE MECHANISM OF COMPOUND 48/80 AND SINOMENINE: EXPERIMENTS ON CHOPPED SKIN FROM VARIOUS ANIMAL SPECIES

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Compound 48/80 releases histamine from mast cells with accompanying morphological changes characterized by degranulation. These actions are considered to be dependent on energy harnessing processes in mast cells, because the histamine release or degranulation of mast cells was inhibited by anoxia or inhibitors of oxidative phosphorylation when the medium was devoid of glucose, and glucose blocked these inhibitions (1-6). Since, however, evidences for the energy dependent processes involved in the histamine release action of 48/80 have been obtained from the experiments on only limited animal species, such as rat (2-9), cat (1) or hamster (10), despite the fact that not all the species are equally susceptible to the action of 48/80, further investigations on this point are to be extended over other animal species. Mongar and Schild (11) reported that the *in vitro* histamine release by 48/80 from guinea-pig lung tissue was not inhibited by anoxia and metabolic inhibitors while the histamine release induced by antigen was clearly inhibited.

Sinomenine releases histamine from rat mast cells in a very similar fashion to 48/80 (12). *In vitro* histamine release from guinea-pig lung by this compound was also not inhibited by anoxia like the release by 48/80 (13).

In the present study, *in vitro* histamine release by compound 48/80 as well as sinomenine from chopped skin of different animal species including guinea pig was compared under aerobic and anaerobic conditions, and it was found that the release of histamine in all these species consisted of two different types of release: the one which is dependent on energy-yielding processes and the other which does not depend on the processes. Concerning the mechanism of the latter type of histamine release further studies were made.

MATERIALS AND METHODS

Preparation of chopped skin tissue

The skin used was obtained from dog, cat, rabbit, guinea pig, rat, mouse and man. The human skin was procured from the sides of midventral line at surgical operation. Dog and cat were anesthetized with nembutal, rabbit, guinea pig, rat and mouse struck on the

head and bled to death. Shaved skin was cut out at the midventral region. After removing the subcutaneous fat tissue the skin was chopped into pieces of about $0.5\text{ mm} \times 3\text{ mm}$ with razor blade in a cold room. These pieces were rinsed in Krebs-Ringer or in Tyrode solution for a few minutes, then gross moisture was removed with a filter paper and these were divided into 200 mg mass each.

With the lung from guinea pig and rat, small pieces of about $1\text{ mm} \times 1\text{ mm} \times 3\text{ mm}$ were prepared and treated in a similar fashion as with skin.

In vitro histamine release from the chopped skin

For this experiment Warburg's flasks were employed. In the main chamber of the flask 1.8 ml Krebs-Ringer solution [NaCl 128 mM, KCl 5.1 mM, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.8 mM, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.3 mM, and phosphate buffer pH 7.4 ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ 100 mM and HCl 20 mM) 10% v/v] or Tyrode solution not containing glucose (NaCl 137 mM, KCl 2.7 mM, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 1.8 mM, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ 1.1 mM, $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ 0.4 mM and NaHCO_3 11.9 mM, pH 8.0—hereafter simply referred as Tyrode solution unless otherwise mentioned) were placed, and in these solutions 200 mg of the chopped tissues were suspended. If necessary, metabolic inhibitors were contained in the medium of the main chamber. The side arm contained 0.2 ml of histamine releaser, compound 48/80, sinomenine hydrochloride or decylamine hydrochloride, dissolved in the same medium at 10-fold to its final concentration. For the determination of spontaneous release, the medium in the side arm was free of histamine releaser.

Air inside the flasks was replaced by oxygen or nitrogen by passing about two liters of respective gas. After preliminary shaking of the flask for 10 minutes in a bath at 37°C , the histamine releaser in the side arm was transferred to the main chamber. The shaking was further continued for 30 minutes, and the flask with manometer was taken out of the bath and while cooling the flask with ice, it was disconnected from the manometer. The content of the flask was filtered quickly through a small funnel plunged with cotton ball. The rate of histamine release was computed from the histamine contents in the filtrate and remaining tissue fragments.

Experiments on the effects of pH and temperature

By adjusting the concentrations of NaHCO_3 of Tyrode solution in the flask and of CO_2 in the gas phase, it was possible to obtain the desired pH (14). For the aerobic conditions oxygen was maintained at 68% and the remaining 32% was supplemented with the mixture of CO_2 and N_2 , adjusting CO_2 to the required concentration to achieve the desired pH. The pH adjusted in this manner was found to rise slightly after shaking flask for 40 minutes. This elevation of pH was slightly greater under anaerobic than under aerobic conditions. This seems to be due to the absence of CO_2 from the tissue under anaerobic conditions. The final pH's are given in the data. For determining the effects of temperature several flasks were shaken separately in baths of different temperatures.

Histamine assay

The tissue filtrate was added with two drops of N HCl and immersed in the boiling

water bath for two minutes, and after cooling, neutralized with N NaOH. To the tissue fragments 2 ml of 0.3 N HCl was added and placed in the boiling water bath for about 5 minutes, and by cooling fat was solidified on the surface of liquid. The filtrate of this solution was neutralized with 0.3 N NaOH. The histamine in these solutions was assayed on guinea-pig ileum in the Tyrode bath containing 10^{-7} g/ml of atropine sulfate. In the case where the drug contained in the test solution affected the histamine assay, an equal amount of the drug was added simultaneously with the addition of standard histamine.

Compound 48/80 was kindly supplied by Dr. J.J. Burns of Burrough Wellcome & Co., sinomenine hydrochloride by Shionogi Research Laboratory and decylamine hydrochloride by Nihon Yushi & Co. Other chemicals were obtained from standard commercial sources.

RESULTS

1. Histamine release from the chopped skin by compound 48/80 and sinomenine under aerobic and anaerobic conditions

FIG. 1. Histamine release from chopped skin of various animal species under oxygen and nitrogen gas phases. In the experiment with shaded column Krebs-Ringer medium contained 5.6 mM glucose. Sinomenine hydrochloride 2 mg/ml, compound 48/80 1 mg/ml. Histamine release for 30 minutes at 37°C. Spontaneous release is deducted in this and subsequent Figures except Fig. 7.

Histamine was released from chopped skin of mouse, rat, guinea pig, rabbit, cat, dog and man by 1 mg/ml of compound 48/80 and 2 mg/ml of sinomenine hydrochloride in Krebs-Ringer solution under oxygen and nitrogen gas phases. Under oxygen with either one of these releasers the rate of histamine release was between 30 and 50% from the skin of all the species, except the dog skin which was less than 20%, as shown in Fig. 1. The histamine contents in these skin tissues were 47.5 in mouse, 31 in rat, 4.1 in guinea pig, 5.4 in rabbit, 12.3 in cat, 10.6 in dog, and 7.3 $\mu\text{g/g}$ tissue in man (each is the average value from 2–3 individuals). Therefore, there is no correlation between the rate of histamine release and the histamine content of the skin. Under nitrogen (in anoxia) the release of histamine was markedly inhibited in all the species, and as indicated with guinea-pig and human skins in Fig. 1, in the presence of 5.6 mM glucose in the medium this inhibitory effect was considerably or largely overcome, showing that the histamine release by 48/80 or by sinomenine from the chopped skin of all the animal species is largely dependent upon the energy production by the respiratory metabolism or glycolysis. However, we should take note of the fact that such a histamine release was not completely abolished by oxygen-lack even in the glucose-free medium.

2. Effect of the aging of skin tissue on the histamine release

In our early observations we experienced that when the chopped skin samples of mouse had been prepared in a relatively long time a marked decrease occurred in the aerobic histamine release from the skin by 48/80 while there was no change in the anoxic histamine

FIG. 2. Effect of preincubation on aerobic and anaerobic histamine releases from mouse and rat skin induced by compound 48/80 (1 mg/ml). Skin tissues were preincubated at 37°C under air for different periods of time before addition of 48/80. Histamine release for 30 minutes in Krebs-Ringer solution at 37°C.

release. Therefore, the effect of aging of the tissue on the histamine release from mouse and rat skins was studied both under aerobic and anaerobic conditions. Chopped skin samples from these animals were prepared as rapidly as possible and these samples were preincubated under air at 37°C in Krebs-Ringer solution for various lengths of time. Then, these tissues were placed in Warburg's flasks under oxygen or nitrogen and incubated at 37°C in the presence of 48/80.

As shown in Fig. 2, under oxygen there occurred a marked decrease in the histamine release from the skin in proportion to the prolongation of such a preincubation but under nitrogen the rate of histamine release remained unchanged. The decrease in the rate of aerobic histamine release due to the aging of skin tissue was rather marked in mouse skin than in rat skin. This decrease of aerobic histamine release suggests that the energy producing capacity of the mast cells upon which the histamine release by 48/80 is dependent is impaired by the aging of tissue.

3. Comparison of histamine releases from skin and lung tissues

It is reported (11, 13) that oxygen-lack does not inhibit histamine release from chopped lung tissue of guinea pig by 48/80 and sinomenine, but in our experiments the histamine release from the skin tissue of this animal was clearly inhibited by the oxygen-lack. This discrepancy may be due to the experimental conditions under which previous workers used the Tyrode whereas we used Krebs-Ringer medium. In order to clarify this point, the histamine releases from the chopped tissues of skin and lung from the same guinea pig induced by 48/80 and sinomenine were compared both in Krebs-Ringer and Tyrode media.

As illustrated in Fig. 3, in Krebs-Ringer medium the histamine release from the lung tissue of guinea pig by either liberator was negligible both under oxygen and nitrogen, but in Tyrode solution it was greater under either gas phase and also the amount of the release was the same both under aerobic and anaerobic conditions. In other words, no anoxic inhibition was observed. In contrast to this, the histamine release from skin tissue occurred to a considerable extent in

FIG. 3. Different effects of oxygen-lack on histamine releases from the skin and lung of guinea pig. Histamine release by sinomenine hydrochloride 2 mg/ml and compound 48/80 1 mg/ml in Krebs-Ringer and Tyrode solutions for 30 minutes at 37°C.

FIG. 4. Effects of oxygen-lack on histamine releases in the skin and lung of rat induced by compound 48/80 (1 mg/ml). For others as in Fig. 3.

FIG. 5. Influence of pH on histamine release from rat skin induced by compound 48/80 (1 mg/ml) under aerobic and anaerobic conditions. pH of Tyrode solution was modified as 6.2, 7.2 and 8.0 by varying quantities of NaHCO₃ in the medium and of CO₂ in gas phase. Final pH's are indicated. Histamine release for 30 minutes at 37°C.

either medium and anoxic inhibition was clearly recognized. These results indicate that the non-dependency of histamine release by these compounds on oxygen as pointed out by previous workers (11, 13) holds true with the lung tissue of this animal but it is not so with other tissues, at least with the skin tissue. There are two aspects that require a consideration in this experiment. One is the fact that anoxic release both from skin and lung is greater in the Tyrode solution than in the Krebs-Ringer. Another is that in the skin tissue the histamine release only dependent on oxygen, that is the part of release in terms of aerobic release subtracted by anoxic release is smaller in Tyrode solution. The results of experiment conducted in a similar manner with skin and lung of rat are shown in Fig. 4. In this species there is no difference in the patterns of histamine release between the lung and skin tissues, showing similar findings as those of the skin, not of the lung, of guinea pig.

The pH's of Krebs-Ringer and Tyrode solution in the flasks were initially 7.4 and 8.0 respectively. The pH of the former at the termination of shaking did not show any change but the latter was 8.2 under oxygen and it rose up to 8.4 under nitrogen. There is a possibility that the difference in pH of these two media is associated with the difference in histamine release. The following experiment was conducted to clarify this possibility.

4. Effect of pH on the histamine release from skin tissue

By adjusting the concentrations of

both NaHCO_3 contained in Tyrode solution and CO_2 in the gas phase of Warburg's flasks, the initial pH was set to 6.2, 7.2, and 8.0 respectively. In these media histamine release from rat skin tissue by 1 mg/ml of 48/80 was assessed. The results are shown in Fig. 5. The final pH of each medium in the flask is seen shifted from its initial pH slightly to the alkaline side as illustrated in the Figure. The histamine release in the anoxic media is increased along with the rise in pH, whereas the oxygen-dependent histamine release as shown by the difference in the releases under aerobic and anaerobic conditions is clearly smaller at the pH between 8.2 and 8.4 than that at other lower pH's. These results indicate that the difference in the histamine releases as observed in the Krebs-Ringer medium and in the Tyrode is due to the difference in pH rather than to that in the composition of the medium itself.

5. Concentration of compound 48/80 and histamine release under aerobic and anaerobic conditions

The histamine release by 48/80 in increasing concentrations was estimated in Krebs-Ringer medium. The results, shown in Fig. 6, revealed that the aerobic release increases rapidly until the concentration of 48/80 rises to 10 $\mu\text{g/ml}$ but when rises beyond it, the rate of increase of release rather declines. On the contrary, the anoxic release increases rather rapidly in higher concentration of the releaser.

6. Effects of metabolic inhibitors on anoxic histamine release

In order to determine whether or not the histamine release under anaerobic condition is due to the utilization of glucose contained in the tissue, effects of several inhibitors of glycolysis on the anoxic histamine release by 48/80 were studied. As shown in Table 1, 2-deoxyglucose and phlorizin that blocked the glucose-dependent anoxic histamine release in the mast cells isolated or in tissue (6, 12, 15) had no effect on the histamine release by 48/80 from mouse skin (in Krebs-Ringer solution) and guinea-pig lung (in Tyrode solution) under nitrogen. *N*-Ethylmaleimide, iodoacetate and arsenite that inhibited glucose-dependent, anaerobic mast-cell degranulation (6, 12) also did not inhibit anoxic histamine release and the first two of these compounds rather enhanced it. Ouabain that clearly suppressed glucose-dependent, anaphylactic histamine release from guinea-pig lung (15) likewise did not show any effect on the histamine release.

FIG. 6. Dose-response curves for compound 48/80-induced histamine releases from rat skin under oxygen and nitrogen. Histamine release for 30 minutes at 37°C in Krebs-Ringer solution.

TABLE 1. Effect of metabolic inhibitors on anoxic histamine release from mouse skin and guinea-pig lung induced by compound 48/80 1 mg/ml.

Inhibitor	Per cent histamine release by 48/80*	
	Mouse skin (Krebs-Ringer)	Guinea-pig lung (Glucose-free Tyrode)
None	15.9	17.7
2-Deoxyglucose (5.6 mM)	12.6	14.8
Phlorizin (5.6 mM)	13.9	18.3
None	14.2	
Ouabain (0.1 mM)	15.3	
Ouabain (0.01 mM)	16.1	
None	17.1	17.7
N-Ethylmaleimide (0.5 mM)	17.5	22.4
Iodoacetate (1 mM)	15.4	23.0
Arsenite (1 mM)	15.3	16.9

* Means of 2-4 experiments.

FIG. 7. Influence of temperature on histamine release from rat skin induced by compound 48/80 (1 mg/ml). Histamine releases for 30 minutes in Krebs-Ringer solution under oxygen and nitrogen.

FIG. 8. Influence of temperature on histamine release from rat skin induced by decylamine (100 µg/ml). For others as in Fig. 7.

7. Effect of temperature on aerobic and anaerobic histamine release

As shown in Fig. 7, the aerobic histamine release from rat skin by 48/80 had its peak at 37–40°C while the anaerobic release increased along with the rise in temperature. The

latter type of release could be detected by decylamine (100 $\mu\text{g/ml}$) which is known to act by non-enzymatic mechanism (8,13), not only under anaerobic but also under aerobic conditions (Fig. 8). The *in vitro* histamine release by decylamine was greater under nitrogen than under oxygen.

DISCUSSION

A considerable species variation is known in the susceptibility to the histamine releasing action of compound 48/80 and sinomenine. Rat (16–18), hamster (10, 19), cat (20–23), dog (16, 20, 23, 24) and man (20, 23, 25, 26) are sensitive to the histamine releasing action of these compounds but rabbit (25, 27–29), mouse (19, 30, 31) and guinea pig (32–35) are not. Repeated administration of these releasers hardly liberated histamine from tissues of guinea pig, and they did not bring about morphologic alterations of mesentery mast cells *in vitro* (10, 34). In spite of these reports, in the present experiment histamine release from skin under the action of these releasers was observed *in vitro* not only in rabbit and mouse but also in guinea pig nearly in the same rate as observable in other species of animals. This is not necessarily due only to a relatively high concentration of the releasers used, because the histamine release from the lung of guinea pig was hardly detected even with the same concentration in the same medium (Kerbs-Ringer solution).

The histamine release by 48/80 and sinomenine was markedly inhibited by oxygen-lack in every skin of seven species including guinea pig. Further it was demonstrated in the skin of guinea pig and man that this anoxic inhibition is largely overcome by the presence of glucose. These facts indicate that a feature common to the histamine release in every species is that a considerable portion of the histamine release is dependent on the energy production by the respiratory or glycolytic system. However, in every species the inhibition by oxygen-lack of the histamine release from the skin was incomplete even in a glucose-free medium. Since this still observable portion of the histamine release was not affected by inhibitors of glycolysis, it is not associated with energy production by the utilization of endogenous glucose. It is reasonable to assume that under oxygen there is included a portion of release which is independent of energy harnessing processes by respiratory system or by glycolysis.

The aging of skin tissue, i.e. the prolonged preincubation of the skin tissue at 37°C in the same medium prior to the exposure to releasers, did not at all affect the extent of anoxic histamine release. In contrast, under oxygen, however, this procedure markedly reduced the release and ultimately lowered to the same level as that of anoxic release. This suggests that the activities of the enzymes involved in the energy metabolism of tissues or mast cells are rapidly impaired by the aging. From these observations it may be deduced that a portion equivalent to the aerobic release subtracted by anoxic release is “oxygen-dependent release”, i.e. the release that is dependent upon the energy production by the cellular respiratory metabolism.

In the histamine release from guinea-pig lung by 48/80 and sinomenine there was no difference between the aerobic release and anaerobic one. This means that in this tissue

the histamine release does not have the portion that is dependent upon the energy metabolism. These data substantiate the findings of Mongar and Schild (11) that the aerobic histamine release by 48/80 was not inhibited by metabolic inhibitors. However, our observations clearly demonstrated an oxygen-dependent histamine release by these releasers in rat lung tissue. Consequently, as far as the histamine release *in vitro* from the lung tissue, aside from the skin tissue, is concerned, the difference in these two species of animals should be admitted.

The pH optimum was around 7.0 in the degranulation or histamine release in rat mesentery or peritoneal mast cells by 48/80 or sinomenine (5, 6, 12). The oxygen-dependent portion of the release from skin tissue was decreased at pH 8.2 when compared with that around 7.0. On the other hand, the anoxic release increased markedly as the pH shifted to alkaline side from 6.6, 7.2 to 8.4. This increase of the histamine release in the alkaline medium may be explained by the increase in the proportion of undissociated molecules of these basic compounds, that are more lipophilic and with a greater cell permeability.

The aerobic histamine release from skin tissue by these compounds increased most rapidly at the concentrations up to 10 $\mu\text{g/ml}$ but above this range of the concentration the rate of the increase gradually declined. In contrast, the anoxic release started to increase at the concentration below 10 $\mu\text{g/ml}$ of 48/80, and beyond that concentration its increase was gradually accelerated. While the aerobic release reached its maximum around 37–40°C and beyond that temperature it declined, the anoxic release exhibited a rapid increase after passing 37°C. These two types of different rate of release indicate clearly the difference in the release mechanism. It seems that the anoxic release is of a non-enzymatic nature and is brought about by physico-chemical processes that permit these releasers access to mast cells and further to granules, and by binding to acidic residues of intragranular matters, they free histamine. This may be supported by the fact that the curves related to temperature and pH in the histamine release by sinomenine from mast-cell granules of dog liver, as reported by Jinzenji (36), resemble intimately respective curves of the anoxic release from skin in the present experiment. The histamine release by decylamine from skin, which is considered to be due to non-enzymatic, probably lytic properties, increased in parallel with the rise in temperature even under oxygen.

These observations indicate that in the histamine release mechanism of compound 48/80 and sinomenine, and probably of some other basic histamine liberators, there coexist two types of processes: one that depends on cellular energy metabolism and another that does not, namely, purely physico-chemical processes. Since the latter type of processes plays a greater role at higher concentration of releaser, alkaline pH and higher temperature, it requires a serious consideration in the *in vitro* experiment.

In our recent experiments (37) it has been demonstrated that histamine release by 48/80 from mast cells isolated from rat peritoneal fluid become difficult to be blocked by any metabolic inhibitors if the releaser is made to act at a high concentration and in an

alkaline medium. Therefore, the results in the present experiment seem to be quite relevant to the mechanism of histamine release from mast cells or mast-cell granules.

SUMMARY

Compound 48/80 and sinomenine release histamine from chopped skin tissues of mouse, rat, guinea pig, rabbit, cat, dog and man without notable species differences. All these histamine releases are incompletely inhibited by anoxia and this inhibition is largely overcome by the presence of glucose. Inhibitors of glycolysis does not decrease the anoxic histamine release in glucose free medium.

The anoxic release of histamine is increased in alkaline medium and at higher temperature than 40°C, whereas oxygen-dependent release is decreased. On increasing concentration of the releasers the anoxic release increases rather rapidly, while the rate of increase in oxygen-dependent release becomes smaller. The prolonged preincubation of the tissues diminishes and ultimately abolishes the oxygen-dependent release, while the anoxic release remains unchanged.

In the guinea-pig lung there is no difference between aerobic and anaerobic histamine releases by 48/80 or sinomenine, indicating absence of oxygen-dependent histamine release in this tissue, while the oxygen-dependent release is distinct in the rat lung tissue.

It is concluded that there are two different types of processes coexisting in the histamine release by the basic histamine releasers: the one depending on energy-yielding cellular processes and the other entirely of physico-chemical nature.

REFERENCES

- 1) WESTERHOLM, B.: *Acta physiol. scand.* **50**, 300 (1960)
- 2) DIAMANT, B. AND UVNÄS, B.: *Ibid.* **53**, 315 (1961)
- 3) ROTHSCCHILD, A.M., VUGMAN, I. AND ROCHA E SILVA, M.: *Biochem. Pharmac.* **7**, 248 (1961)
- 4) DIAMANT, B.: *Acta physiol. scand.* **55**, 11 (1962)
- 5) UVNÄS, B. AND INGA-LISA THON: *Expl Cell Res.* **23**, 45 (1961)
- 6) SAEKI, K.: *THIS JOURNAL* **14**, 375 (1964)
- 7) JUNQUEIRA, L.C.U. AND BEIGUELMAN, B.: *Tex. Rep. Biol. Med.* **13**, 69 (1955)
- 8) HÖGBERG, B. AND UVNÄS, B.: *Acta physiol. scand.* **48**, 133 (1960)
- 9) MOTA, I. AND ISHII, T.: *Br. J. Pharmac. Chemother.* **15**, 82 (1960)
- 10) BORÉUS, L.O.: *Acta physiol. scand.* **49**, 251 (1960)
- 11) MONGAR, J.L. AND SCHILD, H.O.: *J. Physiol.* **135**, 301 (1957)
- 12) YAMASAKI, H. AND SAEKI, K.: *Proc. Japan Acad.* **41**, 958 (1965)
- 13) YAMAMOTO, T.: *Folia pharmacol. japon.* **56**, 1141 (1960)
- 14) UMBRETT, W.W., BURRIS, R.H. AND STAUFFER, J.F.: *Manometric Techniques*, 3rd Ed., p. 18, University of Wisconsin (1957)
- 15) YAMASAKI, H. AND ENDO, K.: *THIS JOURNAL* **15**, 48 (1965)
- 16) FELDBERG, W. AND TALESNIK, J.: *J. Physiol.* **120**, 550 (1953)
- 17) MOTA, I., BERALDO, W.T., FERRI, A.G. AND JUNQUEIRA, L.C.U.: *Ciba Found. Symposium on Histamine*,

p. 47, Churchill, London (1956)

- 18) IRINO, S.: *Acta Med. Okayama* **12**, 93 (1958)
- 19) WEST, G.B.: *Int. Archs Allergy* **13**, 336 (1958)
- 20) PATON, W.D.M.: *Br. J. Pharmac. Chemother.* **6**, 499 (1951)
- 21) FELDBERG, W. AND PATON, W.D.M.: *J. Physiol.* **114**, 490 (1951)
- 22) SMITH, A.N.: *Ibid.* **121**, 517 (1953)
- 23) MAYEDA, H.: *THIS JOURNAL* **3**, 62 (1953)
- 24) KUMÉ, M.: *Okayama-Igakkai-Zasshi* **71**, 3929 (1959)
- 25) FEINBERG, S.M. AND STERNBERGER, L.A.: *J. Allergy* **26**, 170 (1955)
- 26) SICUTERI, F., MICHELACCI, S. AND FRANCHI, G.: *Int. Archs Allergy* **22**, 408 (1963)
- 27) SCHACHTER, M.: *Br. J. Pharmac. Chemother.* **8**, 412 (1953)
- 28) LECOMTE, J.: *Int. Archs Allergy* **8**, 343 (1956)
- 29) KOBAYASHI, K.: *Folia pharmacol. japon.* **35**, 119 (1942)
- 30) RILEY, J.F. AND WEST, G.B.: *Archs int. Pharmacodyn. Thér.* **102**, 304 (1955)
- 31) SANUKI, K.: *THIS JOURNAL* **6**, 69 (1957)
- 32) FELDBERG, W. AND MONGAR, J.L.: *Br. J. Pharmac. Chemother.* **9**, 197 (1954)
- 33) SMITH, A.N.: *Br. J. Surg.* **46**, 157 (1958)
- 34) MOTA, I. AND VUGMAN, I.: *Br. J. Pharmac. Chemother.* **11**, 304 (1956)
- 35) KAMIMURA, Y. AND NISHIYAMA, R.: *Okayama-Igakkai-Zasshi* **67**, 873 (1955)
- 36) JINZENJI, K.: *Ibid.* **71**, 6173 (1959)
- 37) YAMASAKI, H. AND ENDO, K.: To be published.