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A Quantitative Study of Histamine H_2-receptor Blockade by Burimamide in Isolated Atria[1] (38490)

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Histamine receptors can be distinguished on the basis of antagonist specificity, analogous to adrenergic receptor behavior. Sites which can be blocked by the classic antihistaminics are now being designated H_1-receptors, while those sites which are resistant to blockade by these compounds are considered H_2-receptors.

We have been studying the effects of histamine on isolated atria and have shown that promethazine is an irreversible inhibitor of the rabbit atrial receptor (1). Therefore, we were interested in the results reported by Black *et al.* (2), using a new compound, burimamide, which blocked H_2-receptors. They studied several tissues, including guinea pig atria, and using the method of Schild, concluded that burimamide is a competitive inhibitor. There are two other reports (3, 4) concerning the inhibitory effect of burimamide on guinea pig atrial H_2-receptors.

We have been able to show that an inverse plot of the increase in atrial rate versus the concentration of histamine is a straight line (5). We thought this relationship might prove to be useful in elucidating atrial activity, not only in response to histamine, but after H_2-receptor blockade. Most of our research has been done with rabbit atria, therefore, we used this tissue as our primary test object. Guinea pig atria were also studied to confirm the validity of our mathematical approach and to compare our results with those obtained by the other groups (2–4).

Material and Methods. Forty-two white rabbits (average weight 1.5 kg) and twelve guinea pigs (average weight 0.6 kg), unselected as to sex, were killed by a cervical fracture. The chest was opened along the midline and the whole heart was removed and immersed in Tyrode's solution which was gassed with 95 % O_2–5 % CO_2. The atrial pairs were dissected free of all extraneous tissue and a thread passed through the tip of each auricular apex.

The atria were mounted in a tissue bath (50 ml) with one atrium tied to a glass hook and the other to a Statham strain gauge. The average control tension was 0.6 g. The tissues were allowed to equilibrate at 36° for 60–90 min before experimental procedures were begun. Signals from the transducers were recorded on Beckman Biomedical Dynograph recorders.

Drugs were injected into the tissue bath through a fine calibre, polyethylene tube which extended to the base of the tissue bath. The gas mixture was bubbled into the tissue chamber through a sintered glass base and served as a source of oxygenation, regulation of pH, and rapid mixing of the injected drug solutions with the bathing medium. Drugs were injected in a total vol of 0.5 ml or less, followed immediately by the injection of 1.0 ml of air to clear the drug from the tubing.

Tissue baths were immersed in a large volume thermostated water bath, which maintained the temperature at 36° ± 0.2°. The bathing solution in the tissue chambers could be changed rapidly by a continuous flow from a 6 liter reservoir. The bathing medium was gravity fed through glass coils (also in the thermostat) into the base of the tissue baths and the chamber volume kept constant by suction applied to the side arm at the 50 ml level. At the end of a drug response period the baths were completely emptied three times by suction and refilled. Flow was stopped during the drug response period by clamping the reservoir tubing.

Four types of experimental procedures were performed which will be delineated in conjunction with the results obtained.

The Tyrode's solution had the following

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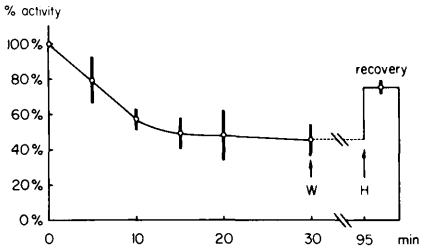

FIG. 1. Time course of burimamide inhibition of the atrial response to histamine. Histamine (1.6×10^{-6} *M*) was administered at zero time, 10 min before the addition of burimamide (9.4×10^{-6} *M*) The increase in rate caused by histamine was normalized to 100% so that three rabbit atrial pairs could be compared. (W) wash and (H) histamine repeated, showing incomplete recovery. t 0.50 = 6.4 min and t 0.93 = 15 min. Ordinate: % of rate in response to histamine before the inhibitor. Abscissa: time in minutes. Vertical bars: S.D. (Standard Deviation of the mean in this and succeeding figures).

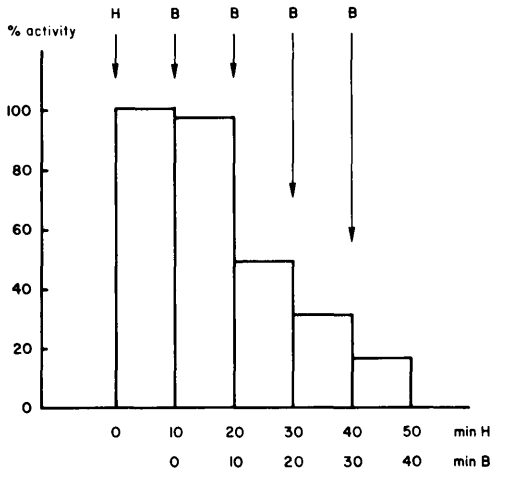

FIG. 2. Inhibition of the atrial response to histamine by various concentrations of burimamide. (H) Histamine (3.2×10^{-6} *M*) given at zero time, increments of (B) Burimamide (3.8×10^{-6} *M*, 1.1, 2.3, and 4.2×10^{-5} *M*) given at 10 min intervals (2 rabbit atria). Ordinate: % of histamine control. Abscissa: time in minutes.

composition in m*M*/L: Na^+ 145, K^+ 5.9, Ca^{2+} 1.6, Mg^{2+} 1.7, Cl^- 132, HCO_3^- 25 and glucose 9. The pH was 7.40 ± 0.05. Drugs were dissolved in or diluted with deionized water. Molar concentrations specified are those attained after injection into the tissue bath and are indicated in the section on results. The drugs used and their sources are: histamine diphosphate (Eli Lilly), isoproterenol hydrochloride (Winthop) and burimamide (a generous gift of Smith, Kline, and French). Statistical calculations were done on the Monroe Scientific Calculator (Model 1860).

Results. Our first approach was to determine the time necessary to reach maximum inhibition by a single dose of burimamide. This was done by giving a test dose of histamine (1.6×10^{-6} *M*) to rabbit atria; when the atrial rate had reached a steady state (10 min), the increase in rate (beats/min) above the pre-drug spontaneous rate was normalized to 100% for each atrium. Burimamide (9.4×10^{-6} *M*) was then added to the bath and the rate declined, the percentage of the histamine induced activity was then calculated at various intervals of time. A new steady state was approached in approximately 15 min (Fig. 1), with approximately a 50% inhibition of histamine activity.

A second type of experiment was done to determine an inhibitory dose range for burimamide that would be effective with rabbit atria. Histamine (3.2×10^{-6} *M*) was administered, followed in 10 min by the first dose of burimamide (3.8×10^{-6} *M*). Additional increments of burimamide were added to the bath at 10 minute intervals, to a final concentration of 4.2×10^{-5} *M* (Fig. 2). In this type of experiment, the highest concentration of burimamide inhibited the response to histamine approximately 84% in 10 min.

A third type of experiment was then done to check the reversibility of the burimamide blockade. The response to a control dose of histamine was established, the atria washed and time was allowed for the rate to return to the predrug control level. Burimamide was then introduced into the bath and after 15 min, the control dose of histamine was repeated. The tissues were again washed, which removed both the burimamide and the histamine. The histamine response was monitored two additional times, after appropriate wash and recovery periods (Fig. 3). All of the free burimamide was removed during the wash. However, low concentrations of histamine (1.6×10^{-6} *M*) did not completely reverse the burimamide blockade

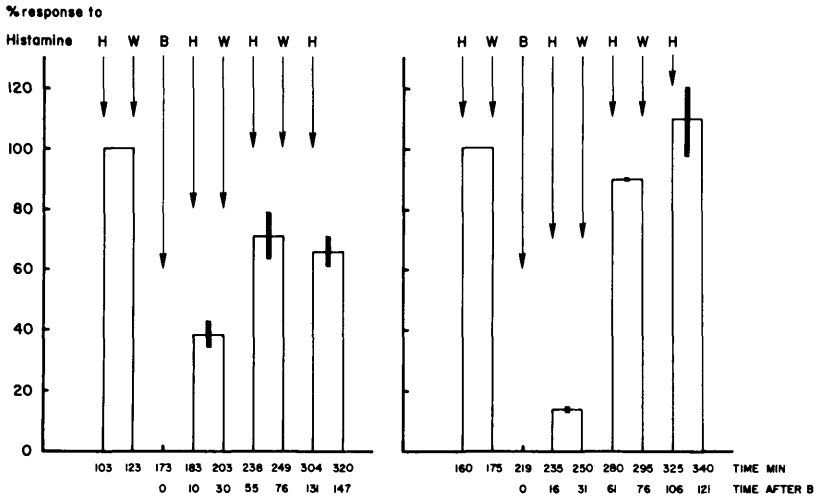

FIG. 3. Reversibility of burimamide inhibition at two concentrations of histamine and of burimamide. Left (H) Histamine (1.6×10^{-6} *M*) and (B) Burimamide (1.9×10^{-5} *M*), (W) wash (2 rabbit atria). Right (H) (4.5×10^{-6} *M*) and (B) (9.4×10^{-5} *M*) (2 rabbit atria). Ordinate: control response to histamine considered 100% and response to histamine after burimamide as percentage of the control response ($Rate_B/Rate_C$). Abscissa: time in minutes—upper set of numbers—time after suspension of atria in bath; lower set—time after burimamide was added to bath (total 4 rabbit atrial pairs). Vertical bars: S.D.

even after several washes, while a higher concentration of histamine (4.5×10^{-6} *M*) did. This suggests that burimamide dissociation from the H_2-receptor is slower than the initial association.

The fourth type of procedure involved the use of dose-response curves. The time involved in completely washing out burimamide made series of inhibitory curves done with the same atria impractical. Therefore, a control dose response curve was paired with a single burimamide inhibitory curve.

The concentration of histamine used with rabbit atria to produce the dose-response curves ranged from 0.25 to 2.5 μg histamine base/ml of the bathing medium (2.2×10^{-6} *M* to 2.2×10^{-5} *M*). A series of burimamide concentrations (0.8–80 μg/ml of bathing medium) were tested for their modification of the histamine response. A minimum of two rabbits were used at each concentration of burimamide tested and a total of 13 different levels of the inhibitor were used.

Control curves were done, histamine was washed out, and time allowed for the atrial rate to decline to the predrug level. Burimamide was then administered, and (after 15 min) a second dose-response curve was done in the presence of the inhibitor. The tissues were again washed and time allowed for them to return to their initial rate. To determine the reversibility of H_2-receptor inhibition following the inhibitory curve and wash out, a single dose of histamine (4.5×10^{-6} *M*) (the concentration of histamine which will cause one-half-maximum increase in rabbit atrial rate) was administered. The post-inhibitory response to histamine was compared to the response obtained for that same dose during the control and burimamide curves. In all cases some inhibition remained even when low concentrations of burimamide (3.8×10^{-6} *M*) were used.

Four representative blocking experiments are illustrated in Figure 4. Doses of burimamide of 4.7×10^{-5} *M* or less suggest competitive inhibition, while at concentrations of 9.4×10^{-5} *M* or greater inhibition no longer appears to be strictly competitive.

Black (2) had checked the specificity of the burimamide blockade and we also tested the atrial response to isoproterenol before and after the addition of burimamide. There was no attenuation of the response to isoproterenol even in the presence of 1.88×10^{-4} *M* burimamide.

It was noted that high concentrations of burimamide ($>4.2 \times 10^{-5}$ *M*) tended to increase the atrial rate by a few beats per minute even before histamine was added.

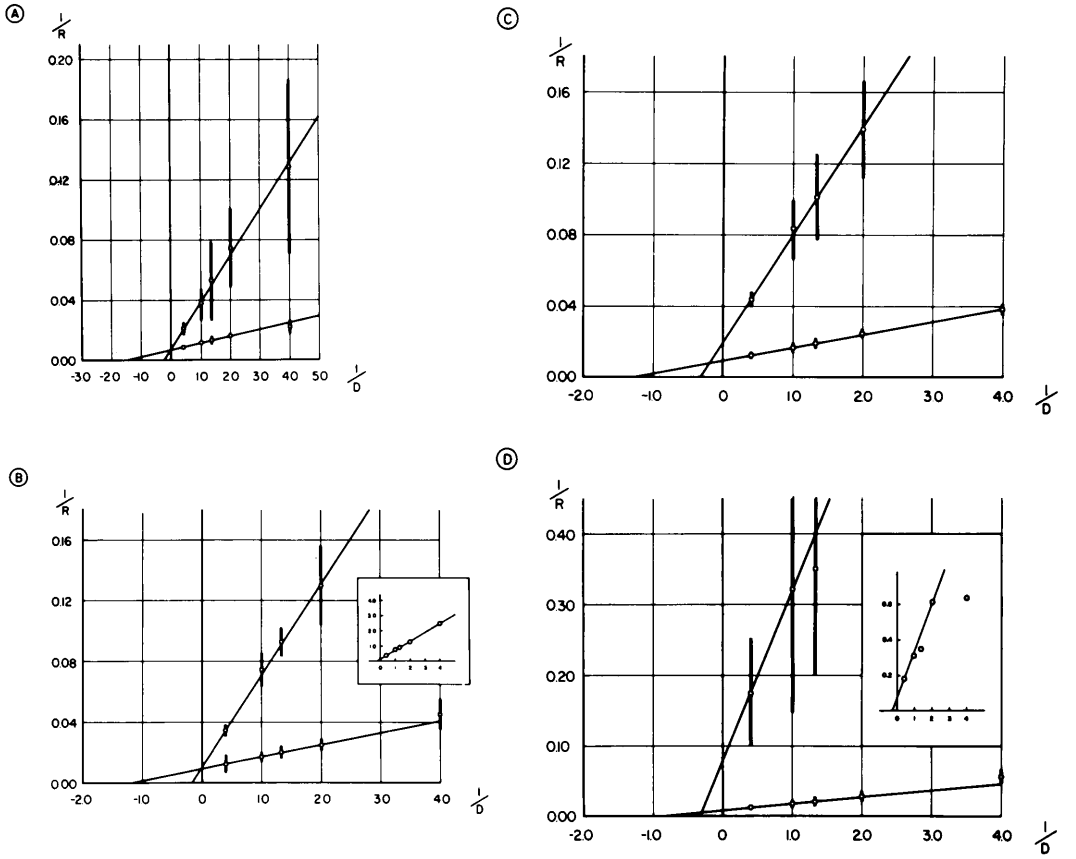

FIG. 4. Reciprocal plots of rabbit atrial rate responses with histamine and burimamide. A. Control (lower line) and burimamide (2.4×10^{-5} *M*) inhibition curves (upper line). (Each point represents average of three atrial pairs). B. Control (lower line) and burimamide (4.7×10^{-5} *M*) curves (upper line) (3 atrial pairs). Coincidence of $1/R$ intercepts for control and burimamide curves consistent with competitive inhibition for both A & B. C. Control (lower line) and burimamide (9.4×10^{-5} *M*) curves (upper line) (two atrial pairs). Ratio of $1/R$ intercepts for the two curves shows that the theoretical maximum increase in rate for the inhibition curve has been reduced by 47% from the control. D. Control (lower line) and burimamide (3.8×10^{-4} *M*) curves (upper line) (2 atrial pairs). Non-coincidence of $1/R$ intercepts of control and inhibition curves suggest inhibition is no longer strictly competitive in both C & D. (Inserts show complete inhibitory plot on a reduced scale). R (Beats/min): increase in atrial rate above control. D (μg histamine base/ml bathing solution): Vertical bars: S.D. These plots are fitted by eye.

It is possible that this increase in rate is due to a small agonistic effect prior to blockade or to release of endogenous catecholamines or other cardiotonic substances.

Guinea pig atria were also tested; cardiac muscle from these animals is much more sensitive to histamine than similar tissue from rabbits. The dose response curves covered a range of 0.01–5.0 μg histamine base/ml of the bathing medium. Four concentrations of burimamide were studied with guinea pig atria and Fig. 5A and B is representative of the results obtained at a low and higher concentration of burimamide.

It would appear that in the lower concentrations tested burimamide behaved as a competitive inhibitor in the rabbit (Fig. 4) and guinea pig (Fig. 5). Even the highest concentration of burimamide (2.6×10^{-4} *M*) used did not cause a complete blockade ($\pm 93\%$) and no longer appears to be competitive (Figs. 4 and 5).

Discussion The increase in atrial rate which is initiated by increasing concentra-

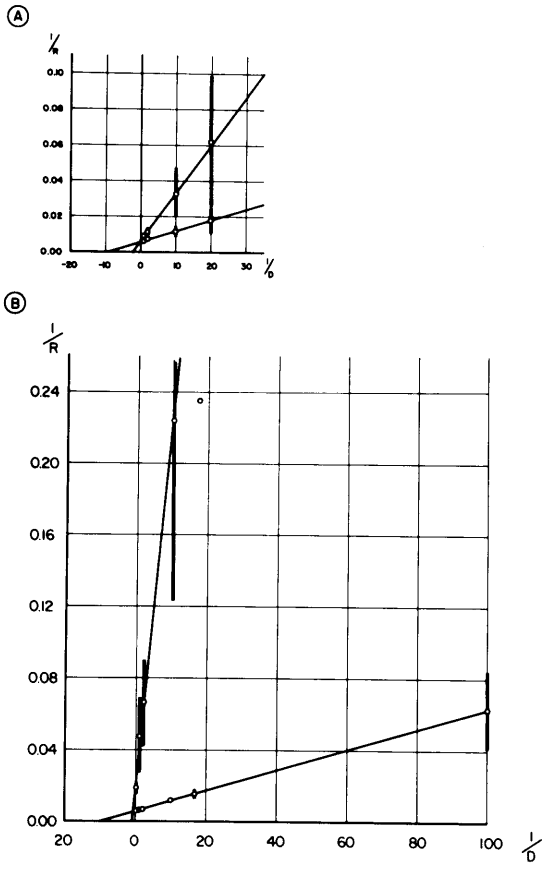

FIG. 5. Reciprocal plots of guinea pig atrial rate responses to histamine with and without burimamide. A. Control (lower line) and burimamide (2.4×10^{-5} *M*) inhibition curves (upper line) (3 atrial pairs). Coincidence of $1/R$ intercepts for control and burimamide curves consistent with competitive inhibition. B. Control (lower line) and burimamide (1.9×10^{-4} *M*) curves (upper line) (3 atrial pairs). Non-coincidence of $1/R$ intercepts for control and inhibition curves indicate that inhibition is no longer strictly competitive. R (beats/min): increase in atrial rate above control. D (μg histamine base/ml bathing solution): Vertical bars.

tions of histamine, when plotted, produces a hyperbolic curve which can be linearly transformed (5).

Not all dose response curves can be treated in this manner and most tissue responses to increasing drug concentrations *cannot* be linearly transformed. Therefore, log plots are typically used as Black (2) did in reporting his results with burimamide and H_2-receptors.

There are several advantages of using linear transformations whenever possible (5). Analogous to enzyme kinetics, the theoretical maximum increase in rate at infinitely large drug concentrations (the point at which the line intercepts the 1/R axis), can be estimated directly from the plot. In Fig. 5A the lines cross 1/R at 0.006 giving a theoretically maximum increase in rate in response to histamine of 167 beats/min.

The apparent association constant (again analogous to enzyme kinetics), or the drug dose which will cause a half-maximum increase in rate can be read directly from the point at which the line crosses I/D. In Fig. 5A, the line for the histamine responses cross I/D at -9.5 or a dose of approximately 0.1 μg histamine/ml of the bathing medium (the dose necessary to cause a half-maximum response). The same atria, after treatment with burimamide (2.4×10^{-5} *M*) show no change in the theoretical maximum increase in rate (1/R intercept), however, the I/D intercept now occurs at -2.2, which indicates that approximately 0.46 μg histamine is necessary to get a half-maximal response, more than a fourfold change. This type of plot immediately suggests competitive inhibition and does not necessitate the more laborious calculations used by Black (2). It is possible to calculate the apparent association constant K_I (using a low concentration of the reversible inhibitor burimamide) characterizing the presumed interaction between inhibitor and receptor. From the calculations of "classical" receptor theory (6) and the analogous calculations of enzyme kinetics (7), we define an apparent equilibrium constant, *K*(app), characterizing the activity of the *system* under the influence of a reversible, competitive inhibitor:

$$(1) \qquad K(app) = K_D\left[1 + \frac{(I)}{K_I}\right]$$

where K_D is an apparent equilibrium constant for the interaction of drug with receptor (or of substrate with enzyme) and (I) is the concentration of inhibitor. If Eq 1) is applicable to the relation between control [for which (I) = 0] and inhibited curves, it implies that every dose of drug must be multiplied

by the factor, $(1 + (I)/K_I)$, to produce the same effect in the presence of the inhibitor as in its absence. (This is the case whatever complexity may be incorporated in the term K_D; that is, irrespective of its relation to the "true" equilibrium constant. If we define the factor F as the "dose-ratio" (6), we have:

$$(2) \quad F = 1 + \frac{(I)}{K_I} \quad \text{or} \quad K_I = \frac{(I)}{F - 1}$$

F can be read from our curves, using the numbers from the 1/D intercept in Figure 5A F = 9.4/2.2 = 4.3. The concentration of burimamide (I) is known, therefore, we may estimate K_I. From Figs. 4 and 5 we measure the following: ^{F}(rabbit) = 7.5, (I) = 2.4×10^{-5} M; ^{F}(guinea pig) = 4.6, (I) = 2.4×10^{-5} M. Substituting these values into Eq 2), we find (in Tyrode's solution at 36°): K_I (rabbit) 3.7×10^{-6} M; K_I (guinea pig) 6.7×10^{-6} M. These values are in agreement with the value of 7.8 (6.4–9.6) $\times 10^{-6}$ M recently obtained by Black *et al* (2) using a different method with data obtained from 11 guinea pig atria bathed in McEwen's solution at 35°.

Our graphical method does not distinguish partial from completely competitive inhibition; nor does the method of Schild (8), which was used by Black *et al.* (2). The structural similarity of histamine and burimamide lends strong support to the supposition that they do occupy the same site on the receptor and therefore at lower concentrations of burimamide equation 1) may apply. However, this does not appear to be true when higher concentrations of the burimamide are used; a similar situation was reported (9) for epinephrine and an antagonist, (SY28) *N*-(2 bromoethyl)-*N*-ethyl-1-naphthalenemethylamine·HBr where low doses of the inhibitor resulted in plots that appeared to be competitive in character while higher concentration suggested noncompetitive inhibition.

It is not possible to state from our evidence using isolated tissue whether this inhibition affects some specific enzyme. It is probable that some biochemical change induced by the activation of the receptor, is ultimately responsible for the increase in atrial rate. There have been several reports (10–12) that histamine stimulates cardiac adenyl cyclase and a recent abstract (3) reports that burimamide inhibits histamine's activation of this enzyme in guinea pig atria.

Summary. The onset of burimamide inhibition of histamine stimulation of rabbit atria is rapid, and a near steady-state blockade occurs at approximately 15 min (≧ 90% complete). The blockade is reversible but requires several washings suggesting the disassociation is slow. The administration of histamine may accelerate the decay of the burimamide effect. Reciprocal plots (rate response versus histamine concentration) of dose-response curves are linear for both rabbit and guinea pig atria. In the presence of low concentrations of burimamide, (2.4×10^{-5} M), the displacement of curves suggests a competitive type of inhibition both for rabbit and guinea pig atria. The apparent association constants calculated from these curves are: K_I (rabbit) 3.7×10^{-6} M and K_I (guinea pig) 6.7×10^{-6} M. These results for guinea pig atria are in satisfactory agreement with the value obtained in another laboratory (2). At higher concentrations of burimamide, inhibition curves showed distinct evidence of departure from competitive character for both guinea pig and rabbit atria.

1. Hughes, M. J., and Coret, I. A., Amer. J. Physiol. **223,** 1257 (1972).
2. Black, J. B., Duncan, W. A. M., Durant, C. J., Gannellin, C. R., Parsons, E. M., Nature (London) **236,** 385 (1972).
3. McNeill, J. H., Verma, S. C., Lyster, D. M., Fed. Proc. **32,** 808 (1973).
4. Levi, R., and Capurro, N., Fed. Proc. **32,** 808 (1973).
5. Coret, I. A., and Hughes, M. J., Arch. Int. Pharmacodyn. **208,** 117 (1974).
6. Gaddum, J. H., Hameed, K. A., Hathway, D. E., Stephens, F. F., Quart. J. Exp. Physiol. **40,** 49 (1955).
7. Webb, J. L., Enzyme and Metabolic Inhibitors. Vol. 1, Academic Press. New York. (1963).

8. Arunlakshana, O., and Schild, H. O., Brit. J. Pharmacol. Chemother. **14,** 48 (1959).
9. Chen. G., and Russell, D., J. Pharmacol. Exp. Therap. **99,** 401 (1950).
10. McNeill, J. H., and Muschek, L. D., J. Mol. Cell Card. **4,** 611 (1972).
11. Klein, I., and Levey, G. S., J. Clin. Invest. **50,** 1012 (1971).
12. Wollenberger, A., Schulze, W., and Krouse, E. G., J. Mol. Cell Card. **5,** 427 (1973).

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