and the bridge principle of specificity with active-site-directed irreversible inhibitors (14), it should be possible to magnify immensely any small difference between the hydrophobic areas of the tumor tissue and host dihydrofolic reductases; this small difference would be unusable or even undetectable with reversible inhibitors. Since active-site-directed irreversible inhibitors of the dihydrofolic reductase from pigeon liver have been found which utilize the hydrophobic bonding region (15, 16), only a small difference in the hydrophobic region of a tumor tissue dihydrofolic reductase would be sufficient for high specificity—such as a change of a valine for a leucine or a slightly different conformation in the hydrophobic region caused by a single amino acid exchange in another region. Such a small difference could be exploited by attaching a bridging moiety to the hydrophobic bonding moiety of an inhibitor so that the subsequent attack by the bridging group to form a covalent bond to a nucleophilic site on the enzyme is subject to proper juxtapositioning by the hydrophobic area on the enzyme. Such studies are currently being pursued.

REFERENCES

(1) Baker, B. R., Ho, B.-T., and Santi, D. V., *J. Pharm. Sci.*, **54**, 1415(1965).
(2) Baker, B. R., and Ho, B.-T., *J. Heterocyclic Chem.*, **2**, 335(1965).
(3) Baker, B. R., and Lourens, G. J., *ibid.*, **2**, 344(1965).
(4) Baker, B. R., Schwan, T. S., Novotny, J., and Ho, B.-T., *J. Pharm. Sci.*, **55**, 295(1966).
(5) Baker, B. R., Coward, J. K., Ho, B.-T., and Santi, D. V., *ibid.*, **55**, 302(1966).
(6) Baker, B. R., and Shapiro, H. S., *ibid.*, **55**, 308 (1966).
(7) Joshi, J. G., and Handler, P., *J. Biol. Chem.*, **239**, 2741(1964).
(8) Baker, B. R., and Ho, B.-T., *J. Pharm. Sci.*, **53**, 1137 (1964).
(9) Hitchings, G. H., and Burchall, J. H., *Adv. Enzymology*, **27**, 417(1965).
(10) Burchall, J. H., and Hitchings, G. H., *Molecular Pharmacol.*, to be published.
(11) Baker, B. R., Ho, B.-T., and Neilson, T., *J. Heterocyclic Chem.*, **1**, 79(1965).
(12) Baker, B. R., and Ho, B.-T., *ibid.*, **2**, 72(1965).
(13) Modest, E. J., *J. Org. Chem.*, **21**, 1(1956).
(14) Baker, B. R., *J. Pharm. Sci.*, **53**, 347(1964).
(15) Baker, B. R., and Jordaan, J. H., unpublished data.
(16) Baker, B. R., and Shapiro, H. S., unpublished data.

Effect of Certain Drugs on Perfused Human Placenta VI

Serotonin Antagonists

By CHARLES O. WARD and RONALD F. GAUTIERI

The antiserotonin action of several compounds was investigated in the vasculature of the isolated perfused human placenta. Average onset, duration of action, and per cent decrease at maximal antagonism were used to discern the antiserotonin capability of the smallest effective dose of each compound necessary to antagonize the pressor action of serotonin. Their ability to antagonize the vasoconstrictor effect of serotonin, in decreasing order of effectiveness, was: cyproheptadine, LSD, diphenhydramine, chlorpromazine, promethazine, promazine, and dibenamine. Cyproheptadine, which had a relatively short duration of action, caused the greatest decrease to the pressor action of serotonin, while chlorpromazine and diphenhydramine exhibited the longest duration of action. The mechanisms by which these agents antagonized the vasopressor effect of serotonin are attributed to a blockade of α-adrenergic receptors, competition for specific receptor sites, and/or direct negative musculotropic action. It is suggested that the human placenta, rather than tissues of other species, may serve as the organ of choice to evaluate the potential effectiveness of serotonin antagonists useful in therapeutics.

ALTHOUGH the vasoconstrictor property of serotonin has been known since 1869 (1), the interplay between its physiologic and pathologic functions, as well as the elucidation of its pharmacologic mechanisms, awaited the development not only of reliable assays for its estimation in biologic tissues, but also techniques for determining the potency of agents specifically antagonistic to it.

Many compounds have been shown to antagonize the stimulant effect of serotonin on smooth muscle, and among the several organs used to demonstrate this phenomenon are the gastric fundus of the rat (2), estrogen-primed rat uterus (3), isolated rabbit ear (4), sheep artery rings (5), and the guinea pig ileum (6). The effect of serotonin on diuresis (7) and blood pressure (8) in the intact animal also has been used to show the antiserotonin effect of these agents. While these techniques are generally sensitive to minute doses of serotonin, and though the results are usually reproducible in the particular procedure

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TABLE I.—COMPARISON OF SEROTONIN ANTAGONISM BY SEVERAL COMPOUNDS IN PERFUSED HUMAN PLACENTAL VESSELS

Drug	Dose	Expt., No.	Av. Max. Antagonism, min.	Range of Duration of Action, min.	Av. Decrease at Max. Antagonism, %
Chlorpromazine	0.5–1.0 mg.	3	20.0	21–79	67.8
Promazine	0.5–1.0 mg.	5	14.0	9–46	47.4
Promethazine	0.5–2.0 mg.	6	16.2	11–49	52.7
LSD	100.0–200.0 mcg.	6	10.8	11–30	75.6
Cyproheptadine	100.0 mcg.	3	11.0	17–28	86.2
Diphenhydramine	10.0 mg.	3	31.7	24–63	68.9
Dibenamine	2.0 mg.	4	26.3	17–52	46.4

employed, conflicting results occur often enough to make it difficult to predict the effect of these antagonists in humans.

Variation among the several species of animals used is considerable, both in response to serotonin and to its antagonists as well. For example, the intravenous injection of serotonin in dogs increases the heart rate and blood pressure, while the rabbit responds to similar doses with hypotension and bradycardia (8). The gramine derivatives, medmain and methylmedmain, are serotonin antagonists on the rat uterus but have no effect on antidiuresis (7). Morphine is ineffective as a serotonin antagonist on strips of the gastric fundus of the rat (2), the isolated rat uterus, and the isolated rabbit ear, but has potent antiserotonin actions on the guinea pig ileum (6). The ergot alkaloids, however, are poor serotonin antagonists on the guinea pig ileum (9), but give excellent results in other tissues, including sheep artery rings (5), the isolated rat uterus (3), and the blood vessels of the isolated human placenta (10). Hydralazine increases serotonin-induced contractions of the guinea pig gut, with only slight, if any, effect on the rat intestine (11). On the other hand, the vasoconstrictor action of serotonin on the blood vessels of the human placenta is occasionally antagonized by hydralazine (12). Another adrenergic blocking agent, dibenamine, has also been shown to exhibit serotonin-like and antiserotonin actions, in that it inhibits diuresis when given alone to the intact rat, then blocks the antidiuretic effect of subsequent doses of serotonin (13).

The obvious conclusion is that no one animal preparation can be used to assay for all serotonin antagonists, and even if one were available, this would not be an exact indication that the same effects would be noted when the drugs were given to man.

Serotonin has been implicated as a causative factor in the toxemias of pregnancy (14, 15), certain forms of mental disease (16), allergic disorders (17), and collagen diseases (18); in the toxemias of pregnancy the toxic effects of serotonin are thought to be the result of a constriction of the blood vessels of the placenta with resulting ischemia (15).

Thus, it is imperative that a method for evaluating the therapeutic effects of serotonin antagonists in man be available. Therefore, it was the purpose of this investigation to evaluate the antiserotonin activity of several known serotonin antagonists on the vasculature of the human placenta in order to predict more accurately the degree of antagonism of these agents in human tissue.

MATERIALS AND METHODS

Full-term human placentas, obtained from the hospital 15 to 20 min. after normal delivery, were used throughout this investigation. Each was transported to the laboratory in a glass container filled with approximately 1 L. of Tyrode's solution preheated to 38°.

The apparatus employed and the procedures involved in the preparation and perfusion of the placentas, in recording and maintaining the perfusion pressure, and in measuring the inflow and outflow volumes of the perfusate have been described in previous papers (12, 19). In 26 successful placental preparations, each lasting 1–4 hr., a total of 30 experiments were performed. The results obtained from 4 placentas were discarded because the response to a test dose of serotonin was erratic.

Throughout this investigation the agonist, serotonin, was administered before the various antagonists for the purpose of establishing a standard response to its vasoconstrictor effect; the antagonists were administered 5 min. after the pressor action of serotonin subsided. Responses to subsequent doses of serotonin, after the various antagonists, were then compared to the initial response to serotonin at the start of each experiment. The per cent decrease at maximal antagonism of the pressor effect of serotonin was calculated and used to compare the relative antagonistic capability of the compounds employed. The average onset of maximal antagonism, as well as the time interval (duration of action in Table I) before subsequent doses of serotonin would produce pressor responses that approximated half that of the initial standard response, were also used as bases of comparison. The doses of serotonin, administered after the antagonist, were injected at approximately 8–10-min. intervals and were based on those doses used in previous investigations in this laboratory (12,

19), either 25 or 50 mcg. In the later perfusions the dose of 50 mcg. was adhered to because this appeared to give more consistent responses.

One objective of the investigation was to observe the relative antagonism of the compounds employed utilizing the smallest dose which exhibited serotonin antagonism. This dose, for the antagonists, was not always the same in every placenta. Also, because the antagonists varied widely in their inherent ability to antagonize the pressor action of serotonin, they could not be compared on an equidosage basis and still show antiserotonin activity.

The following known serotonin antagonists were injected into the arterial side of the perfusion, in a volume of distilled water not exceeding 2.0 ml., except where noted: 5-hydroxytryptamine creatinine sulfate (serotonin), 0.01%; chlorpromazine hydrochloride,[1] 0.025%; promethazine hydrochloride,[2] 0.05%; promazine hydrochloride,[3] 0.05%; cyproheptadine hydrochloride,[4] 0.01%; D-lysergic acid diethylamide-25[5] (LSD-25), 0.01%; diphenhydramine hydrochloride,[6] 1.0%; dibenamine hydrochloride,[7] 0.4% in 95% U.S.P. alcohol. In addition, 95% U.S.P. alcohol was injected alone to observe the effect of this vehicle on the placental vessels.

RESULTS

The following results, which are summarized in Table I, were obtained on the vasculature of full-term human placentas perfused at pressures between 60 and 108 mm. Hg. This range of perfusion pressures corresponds to inflow rates of 58 to 72 ml. of perfusion fluid [Tyrode's solution modified by the addition of 0.525% polyvinylpyrrolidone (Plasdone C)] per min.

Chlorpromazine.—In doses of 0.5–1.0 mg., chlorpromazine caused a maximum decrease of 52.0 to 85.0% (average 67.8) compared to the standard pressor effect of an initial 25.0-mcg. dose of serotonin. In the 3 experiments performed, the maximum antagonism of serotonin occurred an average of 20 min. (range 10–28) after the administration of chlorpromazine. Approximately 21–79 min. elapsed before further doses of serotonin gave an increase in pressure approximately equal to half the pressure increase produced by the initial dose of serotonin (28.0–119.2 mm. Hg) (Fig. 1). In 2 additional preparations, subsequent doses of serotonin failed to produce pressure increases that even approximated half that caused by initial doses of serotonin.

Promazine.—In a total of 5 experiments, 0.5–1.0 mg. of promazine produced an average maximum antagonism of serotonin of 38.2–72.2% (average 47.4). The antagonistic effect of promazine became maximal at an average of 14 min. (range 9–19) after administration and lasted 9–46 min. before a standard dose of serotonin (25.0–50.0 mcg.) caused an increase in pressure in the placental vessels that was about half that caused by the same dose of serotonin initially (11.0–123.6 mm. Hg) (Fig. 2).

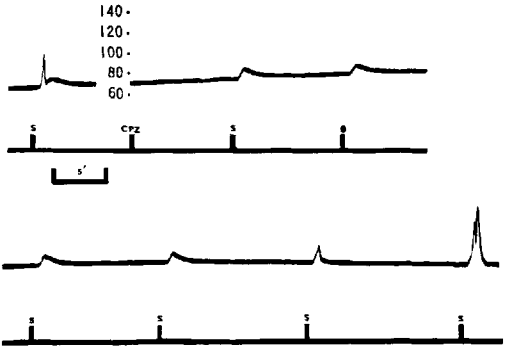

Fig. 1.—Antagonism of serotonin by chlorpromazine (top and bottom). Key: S, serotonin (25 mcg.); CPZ, chlorpromazine (1.0 mg.).

Promethazine.—Promethazine, in doses of 0.5–2.0 mg., produced an average maximum decrease to the pressor effect of 25.0–50.0 mcg. of serotonin of 52.7% (range 16.7–80.1). The maximum antagonism became evident at an average of 16.2 min. (range 11–30) after promethazine was given and lasted from 11–49 min. before the same dose of serotonin gave a response approximately half that of the initial dose (4.8–258.0 mm. Hg). The results presented above were obtained from 6 experiments on 5 separate placental preparations (Fig. 3).

LSD.—Doses of 100.0–200.0 mcg. of LSD reduced the pressor effect of 50.0 mcg. of serotonin by an average of 75.6% (range 28.3–100.0) at maximal antagonism. This effect of the antagonist was observed to reach a maximum at an average of 10.8 min. (range 6–16) after administration and lasted from 11–30 min. before subsequent doses of serotonin produced increases in pressure equal to about half those produced by initial doses (7.8–74.8 mm. Hg). In 4 of the 6 experiments performed, LSD produced an increase in pressure itself equivalent to 1.2–4.4 mm. Hg (Fig. 4).

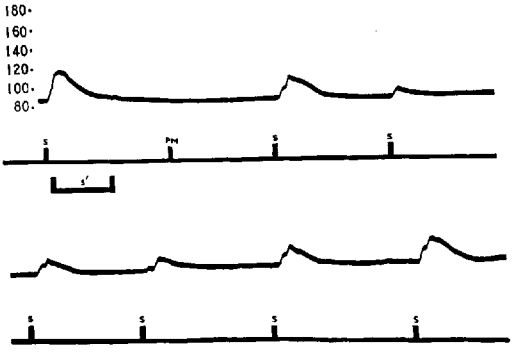

Fig. 2.—Antagonism of serotonin by promazine (top and bottom). Key: S, serotonin (25 mcg.); PM, promazine (0.5 mg.).

[1] Solution used prepared from commercially available ampul. Marketed as Thorazine by Smith Kline & French.
[2] Solution used prepared from commercially available ampul. Marketed as Phenergan by Wyeth.
[3] Solution used prepared from commercially available ampul. Marketed as Sparine by Wyeth.
[4] Supplied through the courtesy of Merck, Sharp and Dohme Laboratories, West Point, Pa. Marketed as Periactin.
[5] Supplied through the courtesy of Sandoz Pharmaceuticals, Hanover, N. J.
[6] Solution prepared from commercially available ampul. Marketed as Benadryl by Parke Davis.
[7] Supplied through the courtesy of Givaudan Corp., Delawanna, N. J.

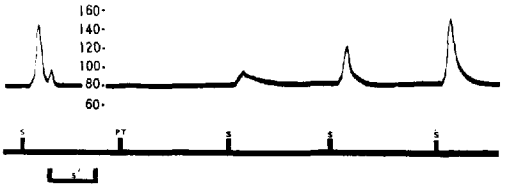

Fig. 3.—Antagonism of serotonin by promethazine. Key: S, serotonin (25 mcg.); PT, promethazine (1.0 mg.).

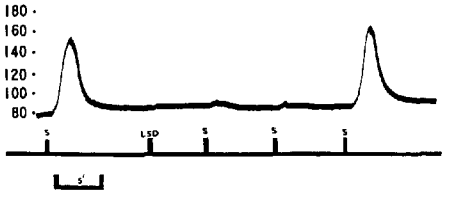

Fig. 4.—Antagonism of serotonin by LSD. Key: S, serotonin (50 mcg.); LSD, lysergic acid diethylamide (200 mcg.).

Fig. 5.—Antagonism of serotonin by cyproheptadine. Key: S, serotonin (50 mcg.); CP, cyproheptadine (100 mcg.).

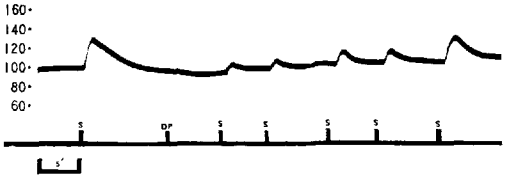

Fig. 6.—Antagonism of serotonin by diphenhydramine. Key: S, serotonin (50 mcg.); DP, diphenhydramine (10 mg.).

Cyproheptadine.—In 3 experiments 100.0 mcg. of cyproheptadine produced an average of 86.2% (range 82.7–92.4) maximum decrease in comparison to the pressor effects of 50 mcg. of serotonin. The maximum serotonin antagonism of cyproheptadine was noted at an average of 11 min. (range 10–12) after administration and lasted 17–28 min. before the administration of another dose of serotonin gave pressure increases equivalent to about half those produced by the control dose (13.6–53.8 mm. Hg) (Fig. 5). In one perfusion, 50.0-mcg. doses of serotonin subsequent to the administration of 100.0 mcg. of cyproheptadine failed to produce an increase in pressure about equal to half that produced by the initial dose of the agonist.

Diphenhydramine.—Diphenhydramine, in a dose of 10.0 mg., produced an average 68.9% decrease (range 63.6–71.4) to the pressor effects of serotonin at maximal antagonism. In the 3 experiments performed, the average maximal antagonism occurred an average 31.7 (range 14–49) min. after administration and lasted 24–63 min. before subsequent doses of serotonin gave responses equivalent to about half the original pressure increase (19.8–46.2 mm. Hg) caused by the initial 50-mcg. dose of serotonin. In 2 of the 3 preparations, diphenhydramine elicited an initial decrease in pressure ranging from 3.1–6.6 mm. Hg (Fig. 6).

Dibenamine.—In a total of 4 experiments, 2.0 mg. of dibenamine caused an average maximum decrease in the response to serotonin of 46.4% (range 14.2–92.8). The average maximum antagonism occurred 26.3 min. (range 7–52) after administration and lasted from 17–52 min. before subsequent doses of serotonin produced about half the pressure increase caused by the control dose of 50 mcg. (7.0–47.0 mm. Hg). In all of the experiments performed, the administration of dibenamine alone caused an increase in pressure ranging from 2.0–5.0 mm. Hg. The administration of a volume of 95% U.S.P. ethyl alcohol (0.5 ml.), equal to that used to dissolve a 2-mg. dose of dibenamine hydrochloride, caused a comparable increase in pressure in 4 separate experiments. In 2 of the 4 experiments utilizing dibenamine, it was noted that the first dose of serotonin subsequent to the antagonist elicited a pressure increase greater than the control response at the beginning of the perfusion. However, all other injections of serotonin resulted in diminished responses.

DISCUSSION

When the antagonists, utilizing their smallest effective dose, were compared on the basis of the per cent that they decreased the pressor response of the placental vessels to serotonin at maximal antagonism, their ability to antagonize the vasoconstrictor effect of serotonin, in decreasing order of effectiveness, was: cyproheptadine, LSD, diphenhydramine, chlorpromazine, promethazine, promazine, and dibenamine. As can be seen from Table I, the onset of maximal antagonism for most of the compounds tested was between 10 and 20 min. after administration, with only diphenhydramine and dibenamine having a more prolonged onset. The range of duration of action, based upon the previously stated criterion, varied greatly, with chlorpromazine being by far the longest acting, as would be expected from the known localization of this compound in body tissues (20). Cyproheptadine, while the most effective antiserotonin agent tested, had the shortest duration of action. Chlorpromazine, in 2 perfusions, and cyproheptadine, in 1, blocked serotonin's vasoconstriction so effectively that subsequent doses never elicited pressor responses that approximated half those produced by the initial standard doses of serotonin during the biological life of the preparation. In all of the perfusions performed the phenomenon of tachyphylaxis to the pressor action of serotonin was never observed. In most instances the effect of the antagonist reached a maximum after 10–31 min.; subsequently the pressor response

to successive doses of serotonin began to approximate half that of the control response.

In a preliminary investigation, leading to the present study, no dose-response relationship to serotonin could be demonstrated in the perfused placental preparation. Consequently, it was not possible to classify the compounds employed as specific or nonspecific antagonists.

Because previous investigations (12, 21) have alluded to the concept that placental vessels contain both α- and β-adrenergic receptors, according to Ahlquist's classification (22), and because it has been postulated that the α receptors are stimulated by serotonin (12), the vasoconstrictor effect of this agonist, as well as the actions of the various antagonists, can be interpreted in the light of these observations, as well as by the well-known stimulant effect of serotonin on most smooth muscle. The phenothiazines used in this investigation, chlorpromazine, promazine, and promethazine, possibly owe their antiserotonin action to a blockade of the α-adrenergic receptors in the placental vessels, since they have been shown to possess adrenergic blocking properties (23). Dibenamine and possibly LSD share this mechanism with the phenothiazines, and a similar interpretation can be applied to the understanding of their antiserotonin action in this preparation. LSD, however, has been shown to have a structural similarity to serotonin, notably the indole configuration, and thus it may compete with serotonin for receptor sites in the smooth muscle (24). In addition, LSD has a stimulant effect on smooth muscle similar to that of serotonin (24) which again may be attributed to its structural similarity to serotonin. This was probably the case in this investigation, as LSD produced pressure increases equivalent to 1.2–4.4 mm. Hg in most of the experiments performed. Cyproheptadine (25) and diphenhydramine (26) both have a negative musculotropic action in other systems, and it is plausible to credit their antiserotonin action in the placental vessels to a similar mechanism. Diphenhydramine and cyproheptadine were both shown to be strong serotonin antagonists in this investigation, being more potent than dibenamine and the phenothiazines which acted predominantly on receptor sites. In fact, diphenhydramine, in 2 of the 3 perfusions, caused a decrease in pressure immediately after it was injected which varied between 3.1 and 6.6 mm. Hg.

In 2 of the 4 perfusions performed using dibenamine, doses of serotonin, subsequent to this antagonist, gave pressor responses greater than the original control response, indicating that the dibenamine was potentiating the vasoconstrictor action of serotonin. However, dibenamine has been shown to possess serotonin-like actions in other preparations, such as its antidiuretic effect in the rat (13); therefore, a similar effect in the placental vessels cannot be disregarded at this time. The administration of dibenamine in a vehicle of 95% ethyl alcohol always caused an initial increase in pressure, ranging from 2.0 to 5.0 mm. Hg; this is due to the vasoconstrictor effect of the alcoholic vehicle since in this, and previous investigations (27), alcohol has been shown to cause vasoconstriction of the placental vessels.

By following the procedures outlined in this investigation, we have evaluated the relative effectiveness of several agents that are antagonistic to serotonin. Consequently, we can hypothesize that those agents which were found to be the most effective serotonin antagonists in this organ may have some therapeutic efficacy in the diseases in which abnormal amounts of serotonin play a significant role in their pathologic manifestations as well as their etiology. The primary example of such a disease is toxemia of pregnancy since the clinical manifestations of this malady are thought to be the result of the vasoconstrictor effect of serotonin on the blood vessels of the placenta (14, 15).

REFERENCES

(1) Janeway, T. C., Richardson, H. B., and Park, E. A., *Arch. Internal Med.*, **21**, 565(1918).
(2) Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344(1957).
(3) Cerletti, A., and Doepfner, W., *J. Pharmacol. Exptl. Therap.*, **122**, 124(1958).
(4) Gyermek, L., *Pharmacol. Rev.*, **13**, 399(1961).
(5) Shaw, E., and Woolley, D. W., *J. Biol. Chem.*, **203**, 979(1953).
(6) Medakovic, M., *Arch. Intern. Pharmacodyn.*, **114**, 201(1958).
(7) Erspamer, V., *Science*, **121**, 369(1955).
(8) Schneider, J. A., and Yonkman, F. F., *J. Pharmacol. Exptl. Therap.*, **111**, 84(1954).
(9) Gaddum, J. H., and Hameed, K. A., *Brit. J. Pharmacol.*, **9**, 240(1954).
(10) Astrom, A., and Samelius, U., *ibid.*, **12**, 410(1957).
(11) Naess, K., and Skramstad, K. H., *Acta Pharmacol. Toxicol.*, **10**, 178(1954).
(12) Mancini, R. T., and Gautieri, R. F., *J. Pharm. Sci.*, **53**, 1476(1964).
(13) Van Arman, C. G., *Arch. Intern. Pharmacodyn.*, **108**, 356(1956).
(14) Krupp, P., and Krupp, I., *Obstet. Gynecol.*, **15**, 237 (1960).
(15) Poulson, E., Botros, M., and Robson, J. M., *Science*, **131**, 1101(1960).
(16) Woolley, D. W., "Biochemical Bases of Psychoses," John Wiley & Sons, Inc., New York, N. Y., 1962, pp. 131, 132.
(17) MacHaffie, R. A., Menebroker, L. R., Mahler, D. J., and Barak, A. J., *J. Allergy*, **31**, 106(1960).
(18) Scherbel, A. L., and Harrison, J. W., *Angiology*, **10**, 29(1959).
(19) Ciuchta, H. P., and Gautieri, R. F., *J. Pharm. Sci.*, **53**, 184(1964).
(20) Emmerson, J. L., and Miya, T. S., *ibid.*, **52**, 411 (1963).
(21) Mancini, R. T., and Gautieri, R. F., *ibid.*, **54**, 1443 (1965).
(22) Ahlquist, R. P., *Am. J. Physiol.*, **153**, 586(1948).
(23) Krantz, J. C., Jr., and Carr, C. J., "Pharmacological Principles of Medical Practice," 6th ed., The Williams and Wilkins Co., Baltimore, Md., 1965, p. 503.
(24) Shaw, E., and Woolley, D. W., *Science*, **124**, 121 (1956).
(25) Ward, C. O., and Gautieri, R. F., unpublished data.
(26) Krantz, J. C., Jr., and Carr, C. J., *op. cit.*, p. 925.
(27) Ciuchta, H. P., and Gautieri, R. F., *J. Pharm. Sci.*, **52**, 974(1963).