prior to or simultaneously with a lethal dose of endotoxin consistently protected the majority of mice from death. There was no protection when cortisone was administered after endotoxin. 2. A transient bacteremia, presumably of intestinal origin, occurred in some animals a few hours after injection of endotoxin, but probably did not contribute significantly to death of the animals. 3. Antibiotics were capable of suppressing bacteremia, but with the exception of streptomycin, which irregularly gave some protection, did not influence mortality rate. 4. Injection of streptomycin or penicillin into animals previously treated with cortisone resulted in significantly higher mortality rates than in animals given cortisone alone. This interference by antibiotics with cortisone protection was demonstrable only when antibiotics were given after cortisone; simultaneous administration of both cortisone and antibiotic gave results which reflected only protective effects of cortisone. 5. In view of the unrelated nature of the antibiotics capable of interfering with cortisone protection against endotoxins, it was suggested that the interference was probably of a nonspecific nature.

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Received July 6, 1954. P.S.E.B.M., 1954, v86.

## Antagonism of Inotropic Responses of Frog Heart to Epinephrine and Acetylcholine.* (21212)

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In a previous study the ratio between the concentrations of epinephrine and acetylcholine present simultaneously was shown to be important in determining the amplitude of contraction of the isolated frog heart(1,2). With a mixture of appropriate concentrations of these drugs a response was obtained whose amplitude was similar to that of the heart before the addition of the mediators. This latter condition was referred to as a balanced response and was characterized by the ratio of epinephrine to acetylcholine necessary to produce it. This balanced response was readily altered by the barbiturates, which decreased the response of the heart to epinephrine.

In this investigation, using the approach

* This investigation was supported in part by a grant from the Smith, Kline and French Foundation.

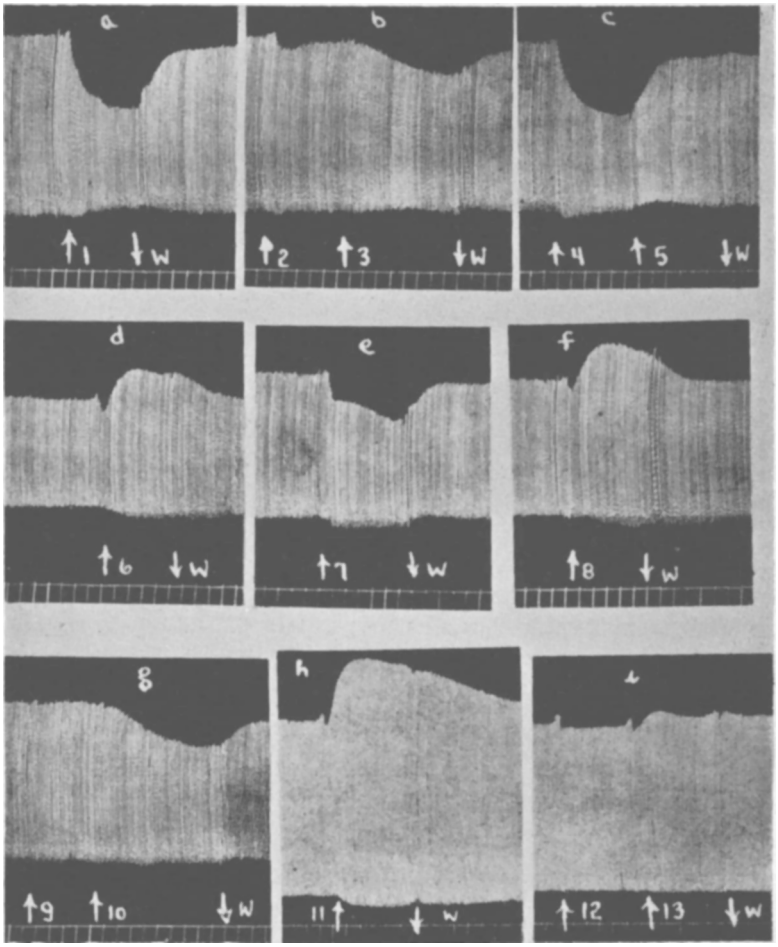


FIG. 1. Effect of atropine on inotropic response of frog heart to epinephrine (Epi) and acetylcholine (Ach).

1. Ach $6 \times 10^{-8}$ M
2. " + atropine $10^{-7}$ M
3. "
4. "
5. " + *idem*
6. Epi $15 \times 10^{-8}$ + Ach $3 \times 10^{-8}$ M
7. " + 6 " (2.5:1)
8. Same as 7 + atropine $10^{-7}$ M
9. Atropine $10^{-7}$
10. Ach $6 \times 10^{-8}$ M
11. Epi $15 \times$ "
12. Atropine $10^{-7}$ M
13. Epi $15 \times 10^{-8}$ M + atropine $10^{-7}$ M

Arrows indicate duration of drug administration. Time in 20 sec. intervals.

described for the barbiturates(1), we studied the effect of some drugs which are known to modify the action of either epinephrine or acetylcholine. It was found that the balanced response was upset by some of these drugs, and that the same agents interfered with the inotropic actions of both epinephrine and acetylcholine under slightly different conditions.

*Method.* The isolated frog heart was perfused with aerated Clark's Frog Ringer solution at room temperature. At the beginning of each experiment the responses to selected doses of epinephrine and acetylcholine were recorded. Only those hearts which showed an adequate response to both agents were used for further investigation. This criterion was used to eliminate preparations which had been either injured during the removal of the heart or were already contracting maximally and could show no further increase in amplitude. The following drugs were prepared in the Clark's solution prior to perfusion: acetyl-

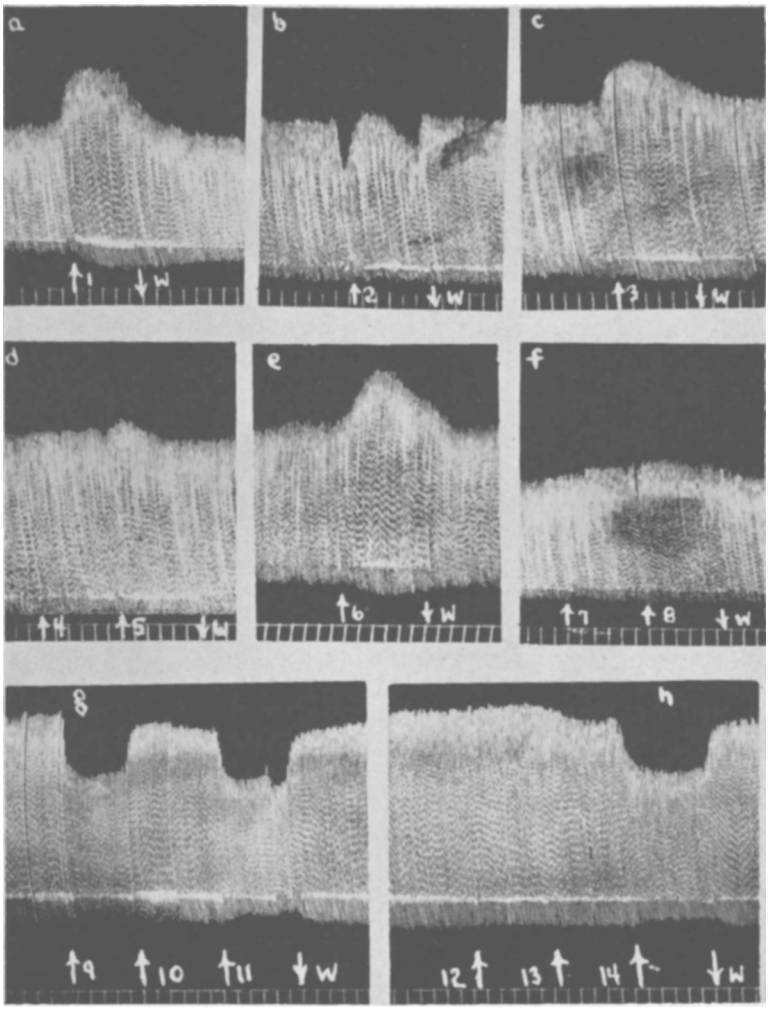


FIG. 2. Action of hexamethonium (Hex) on inotropic response to epinephrine (Epi) and acetylcholine (Ach).

1. Epi 15 × $10^{-8}$M
2. " + Ach 6 × $10^{-8}$M (2.5:1)
3. *Idem* + Hex 5 × $10^{-4}$M
4. Hex 5 × $10^{-4}$M
5. Same as 3.
6. Epi 15 × $10^{-8}$M + Hex 5 × $10^{-4}$M
7. Hex 5 × $10^{-4}$M
8. Epi 15 × $10^{-8}$M + Hex 5 × $10^{-4}$M
9. Ach 6 × $10^{-8}$M
10. " + "
11. "
12. Hex 5 × $10^{-4}$M
13. Ach 6 × $10^{-8}$M + "
14. Ach 6 × $10^{-8}$M

choline chloride, l-epinephrine bitartrate,† atropine sulfate, hexamethonium bromide, tetraethylammonium chloride, and diethylaminoethanol. When a combination of drugs was employed, all were incorporated in the same solution. In some experiments the heart was previously perfused with a drug before testing the response to epinephrine and acetylcholine. This was accomplished by first introducing the drug for a two minute period and then replacing this solution by a mixture containing epinephrine and acetylcholine as well as the drug. All of the drugs were used in concentrations which produced no changes in heart rate. The heart activity was recorded continuously with an end writing lever on a smoked drum.

† Furnished through courtesy of Dr. M. L. Tainter of the Sterling-Winthrop Institute.

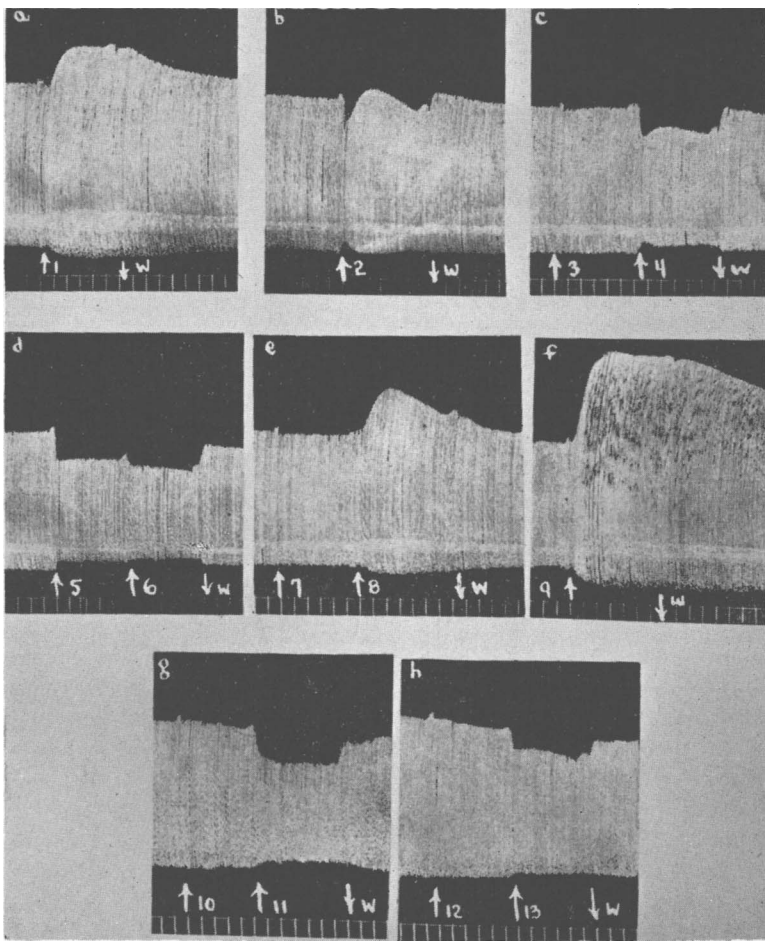


FIG. 3. Influence of diethylaminoethanol (DEA) on response to epinephrine (Epi) and acetylcholine (Ach).

1. Epi 15 × $10^{-8}$M + Ach 3 × $10^{-8}$M (5:1)
2. " + Ach 6 × $10^{-8}$M (2.5:1)
3. DEA $10^{-6}$M
4. Same as 2 + DEA $10^{-6}$M
5. Ach 6 × $10^{-8}$M
6. " + DEA $10^{-6}$M
7. DEA $10^{-6}$M
8. Epi 15 × $10^{-8}$M
9. " + DEA $10^{-4}$M
10. DEA $10^{-6}$M
11. Ach 6 × $10^{-8}$M + DEA $10^{-6}$M
12. DEA $10^{-6}$M
13. Epi 15 × $10^{-8}$M + Ach 6 × $10^{-8}$M (2.5:1) + DEA $10^{-6}$M

*Results. Atropine.* In Fig. 1 atropine given simultaneously with acetylcholine blocked the depressive action of the latter (↑2). Although not illustrated in these records, atropine under similar conditions did not alter the epinephrine positive inotropic response. Atropine also reversed the effects produced by a 2.5:1 mixture of epinephrine and acetylcholine by permitting a more complete response to epinephrine (↑7, ↑8). Prior perfusion with atropine at the same concentration had little effect on the action of acetylcholine (↑9, ↑10) but prevented the response to epinephrine (↑12, ↑13). This antagonism, however, was readily overcome by increasing the concentrations of epinephrine in the mixture. At the concentration employed, $10^{-7}$M, atropine had no inotropic action of its own.

*Hexamethonium and tetraethylammonium.* In Fig. 2 it is seen that the balanced response to a mixture of epinephrine and acetylcholine (2.5:1) was upset by the simultaneous addition of hexamethonium (↑2, ↑3) in such a

manner as to permit the positive inotropic action of the epinephrine component. Prior perfusion with hexamethonium, however, blocked this latter action (↑4, ↑5). Previous perfusion with hexamethonium also interfered with the action of epinephrine when the acetylcholine was omitted (↑7, ↑8). At a higher concentration than that used with atropine, $5 \times 10^{-4}$, hexamethonium simultaneously perfused with acetylcholine reversed the action of the latter (↑10) but under similar conditions did not affect the action of epinephrine (↑6). Initial perfusion with hexamethonium, however, had no effect on the acetylcholine depression (↑14). Results comparable to these were obtained with the same concentration of tetraethylammonium.

*Diethylaminoethanol.* Although not usually classified as an autonomic drug, diethylaminoethanol was included in this group of agents since its effects on the actions of epinephrine and acetylcholine have been studied by others (6). In Fig. 3 we note that the balanced response to a mixture of epinephrine and acetylcholine (2.5:1) was readily decreased by prior perfusion with diethylaminoethanol (↑2, ↑3 and ↑4). Additional studies indicated that previous perfusion with diethylaminoethanol reduced the response to epinephrine (↑7, ↑8) but had no effect on acetylcholine depression. At $10^{-6}$ M as well as at higher doses, diethylaminoethanol when given simultaneously with epinephrine was ineffective against the latter (↑9). Likewise, it failed to block the response to acetylcholine (↑6). At $10^{-6}$ M diethylaminoethanol had no inotropic action of its own.

*Discussion.* In the concentrations used in these experiments atropine, hexamethonium, and tetraethylammonium, when present simultaneously with acetylcholine, blocked the negative inotropic action of the latter. The hypothesis has been adopted by Clark(3) that atropine and tetraethylammonium unite with specific receptors on the heart cell surface and make these sites less accessible to acetylcholine. It would be reasonable to accept a similar explanation for hexamethonium because of its related quaternary ammonium structure(5). This competitive antagonism also explains how the balanced response to a mixture of epinephrine and acetylcholine is upset by these drugs. In blocking acetylcholine depression, they permit an unopposed response to epinephrine. In a previous report it was shown that in the balanced response epinephrine and acetylcholine were acting independently of one another(2). The present results are in accord with this observation. In contrast to their effect on acetylcholine these compounds administered simultaneously with epinephrine do not block its action. Diethylaminoethanol had no effect on the action of either neurohumoral agent when administered simultaneously with them.

Prior perfusion with all of the drugs tested had no effect on acetylcholine depression, but all blocked the positive inotropic action of epinephrine. Riesser and Hergott(6) also observed the inhibitory action of diethylaminoethanol when it was perfused prior to the addition of epinephrine. The reason for the necessity of a short prior perfusion before these drugs will block the action of epinephrine is not clear. It appears unlikely that the time required for penetration of the drugs to the site of action is the sole explanation. Clark(4) found that the frog heart, because of its sponge-like character, permits rapid diffusion of drugs. It appears rather that a certain amount of time is required even after these compounds arrived at their site of action before the changes occur which make the heart less responsive to epinephrine. Increasing the concentration of epinephrine, however, will again give a maximal response. This indicates that during the short preliminary perfusion the threshold for the response to epinephrine is raised but that the heart can still respond to larger doses.

Our results also suggest that the inotropic responses of the isolated heart to the chemical mediators can be altered through at least two mechanisms. The first is specific and directly related to a competitive antagonism to acetylcholine; the second is non-specific and associated with an increased threshold for the epinephrine response. The increased threshold requires a few minutes to be effective, whereas the competitive mechanism occurs instantaneously. Atropine, hexamethonium, and tetraethylammonium influenced both mechan-

isms, whereas, diethylaminoethanol involved only the second.

*Summary.* In the isolated frog heart balanced responses to mixtures of epinephrine and acetylcholine were converted to positive inotropic responses by atropine, hexamethonium and tetraethylammonium. These drugs specifically and competitively antagonized acetylcholine depression when present with the latter. Prior perfusion with them as well as with diethylaminoethanol effectively blocked the positive inotropic action of epinephrine. This antagonism is believed to be non-specific and related to changes which raise the threshold to epinephrine.

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Received July 6, 1954. P.S.E.B.M., 1954, v86.

## Life Maintaining Action of 9 Alpha Chlorohydrocortisone Acetate in Adrenalectomized Rats.* (21213)

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Chlorohydrocortisone acetate is 4 times more active than cortisone acetate in promoting deposition of liver glycogen(1). Furthermore, chloro-F-acetate is more active than hydrocortisone or cortisone acetate in extending survival of adrenalectomized rats given one injection(2). Since single injection experiments appraise duration of action rather than daily hormone requirements, a comparison was made of the daily requirements for chloro-F-acetate and cortisone acetate in adrenalectomized rats.

*Methods.* Immature male rats (Long-Evans strain) were adrenalectomized when 60-65 g. All rats were fed *ad libitum* a diet consisting of 20% casein, 22% dextrose, 25% dextrin, 25% Crisco, 2% corn oil, 2% cellulo flour and 4% Wesson's salt mixture. Each kg of diet was supplemented with 2 g choline chloride and vitamins (Table I). The hormones used were chloro-F-acetate (Squibb)† and cortisone acetate (Merck) in aqueous suspension.

*Results.* Chloro-F-acetate provided 100% survival for 20 days when given to adrenalectomized rats in 15 μg amounts daily whereas 150 μg of cortisone acetate was required to provide similar protection (Table II). Ten μg and 100 μg daily of the 2 steroids proved inadequate for 100% survival of the adrenalectomized rats suggesting a 10:1 ratio of activity. Preliminary studies indicate that 15-20 μg of desoxycorticosterone acetate† (DCA in oil) will provide 100% survival of adrenal-

TABLE I. Vitamin Supplements/100 g of Diet.

| | |
|---|---|
| Alpha tocopherol | 4.0 mg |
| Vit. A | 900 U.S.P. units |
| " D | 180 *idem* |
| | mg |
| 2-methyl-1,4 napth-quinone diacetate | 1.0 |
| Thiamin HCl | .8 |
| Riboflavin | 1.6 |
| Pyridoxine HCl | .8 |
| Niacin | 4.0 |
| Calcium pantothenate | 4.4 |
| Para aminobenzoic acid | 4.0 |
| Inositol | 21.6 |

* Supported by U. S. Public Health Grant A-462.

† Grateful acknowledgement is made to E. R. Squibb & Sons for making the chlorohydrocortisone acetate available and to Ciba Pharmaceutical Products for the desoxycorticosterone acetate (percorten).