In addition, echo-6 was recovered from 3 cases of aseptic meningitis in Rhode Island during the summer of 1954. Together with evidence being obtained in other laboratories, these data strongly support the view that echo virus type 6 is one of the etiological agents of the aseptic meningitis syndrome.

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Epinephrine-Chlortetracycline Induced Arrhythmia in the Isolated Rat Heart. (22631)

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(Introduced by C. H. Hine.)

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The effect of antibiotics on the host has received considerable attention. Their possible effect on cardiac action has been emphasized. One of the authors, with another worker(2) found that epinephrine-chlortetracycline induced arrhythmia can be demonstrated in isolated frog and turtle hearts. This led to the experiments reported here on the effect of epinephrine-chlortetracycline on the isolated mammalian heart.*

Methods. A white rat was killed by a blow at the base of the skull. The heart was excised and the coronary arteries were perfused through a modified Straub cannula in the aorta. The heart was allowed a 10-15 minute stabilization period and its contractions were recorded kymographically. After an additional 10 minutes, the drug was administered by injection into the perfusate and in approximately 20-25 minutes following this a challenge dose of epinephrine (1 μg) was added to the perfusate.

The temperature for each experiment was kept to within $\pm$ 0.5°C and ranged between 34-37°C in different experiments. The perfusate was well-oxygenated Lockes' solution; a glucose concentration of 0.2% strength rather than the lower limit of 0.1% aided in reducing irritability and in stabilizing the heart (Fig. 1). The elapsed time per experiment averaged 1.9 hours although a normal record without irregularity of beat for up to 3.4 hours was made.

Results. An isolated rat heart treated with 100 mcg of chlortetracycline-HCl† followed by 1 μg of epinephrine developed both inotropic and chronotropic irregularities (Fig. 2) in which the normal beat was interrupted for from 5-30 minutes, usually 15-20 minutes, by one or more of several different kinds of arrhythmia as follows: 1. Cyclic variation in amplitude; 2. Periods of increased amplitude followed by 2-20 dropped beats or by contractions of smaller amplitude; 3. Series (2-5) of beats irregular in rate and amplitude; 4. Complete cessation of beat (Fig. 5).

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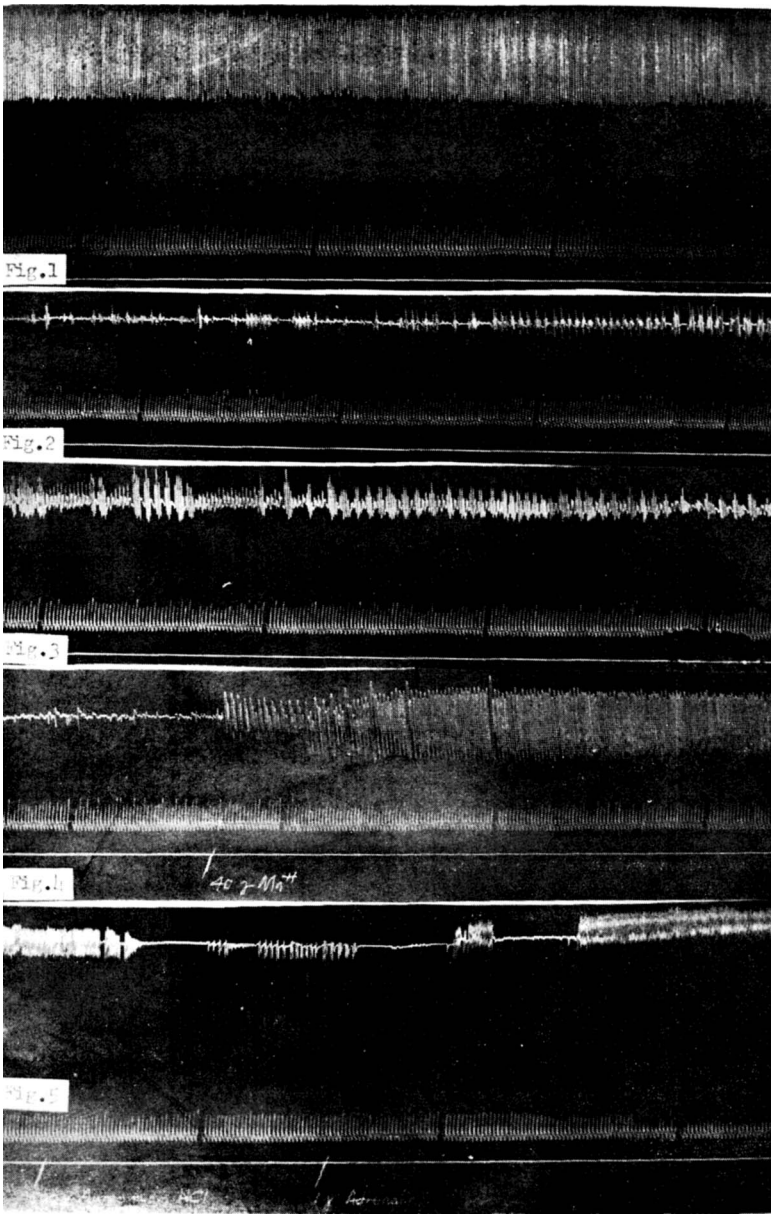

FIG. 1. Segment of normal kymographic tracing of beat of isolated rat heart 0.9 hr following theoretical drug addition. The entire tracing resembled this segment.

FIG. 2. Effect of 100 μg of chlortetracycline-HCl on beat of isolated rat heart 1.3 hr following addition of drug.

FIG. 3. Effect of 20 μg of tetrasodium versenate on beat of isolated rat heart 1.0 hr following addition of drug.

FIG. 4. Alleviation of arrhythmia in isolated rat heart by 40 μg of Mn^{++} (as $MnCl_2, 4H_2O$) 1.2 hr following addition of 100 μg of chlortetracycline-HCl.

FIG. 5. Cessation of beat of isolated rat heart due to 500 μg of chlortetracycline-HCl.

(Timer markings in seconds in all tracings.)

Twenty μg of a known chelating agent, tetrasodium versenate, were added to the perfusate in place of chlortetracycline-HCl in 4 experiments. Cardiac irregularities developed which were almost identical with irregularities induced by chlortetracycline-HCl and which appeared in approximately the same time intervals (Fig. 3). In 3 experiments, the addition of 40 μg Mn^{++} as manganous chloride to the perfusate following onset of arrhythmia that had been induced by chlortetracycline alleviated the irregularities temporarily with resumption of a normal beat (Fig. 4).

Discussion. To account for the effect of chlortetracycline-HCl on the isolated frog heart, Yang and Harvey(5) proposed alteration of acetylcholine regulation of cardiac action as a possible explanation. If alteration of acetylcholine control resulted in accumulation of acetylcholine, the inhibitory effect of this mediator on heart rate would occur and inotropic and chronotropic irregularities would appear. Alteration of acetylcholine regulation of cardiac action might result from chelation of the co-factor of cardiac cholinesterase by chlortetracycline. Thus, cholinesterase would be unable to destroy acetylcholine which would accumulate and cause cardiac irregularity. Weidenheimer, Reed and Upham(4) demonstrated the ability of chlortetracycline to form complexes with 11 different metallic ions. Mn^{++} was not included in this list. However, Albert(1) showed that chlortetracycline will chelate Mn^{++} and Saz and Slie(3) found that Mn^{++} will reverse the inhibition of nitro reductase by chlortetracycline.

Summary. The effect of chlortetracycline-HCl on the isolated rat heart is practically identical with the effect of a known chelating agent, tetrasodium versenate. This effect can be reversed temporarily by Mn^{++}. Thus, chlortetracycline seems to cause irregularities in the isolated rat heart by chelation of the co-factor of cholinesterase which inactivates this enzyme and permits acetylcholine to accumulate and to exert its inhibitory effect on the heart. The co-factor of cardiac cholinesterase may be Mn^{++}.

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Regulation of Heat Production in Cold-Adapted Rats.* (22632)

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Recent studies(1,2) of adaptive changes induced in small mammals by prolonged exposure to cold have indicated that there is a reduction of muscular activity after adaptation. This demonstration along with the observation that heat production in the cold is increased(3,4) suggests that a regulatory mechanism not dependent on excitation of skeletal muscle is effective after adaptation. Such control of metabolic heat production was postulated by Rubner(5) to account for increased heat production not accompanied by increased muscular activity.

Regulation not due to muscular activity has been postulated to be mediated through release of epinephrine from adrenal medulla (6,7). The increase in such thermogenesis on cold adaptation may be due either to greater release of epinephrine or to greater sensitivity of adapted animals to the hormone.

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