ACTH produced less pronounced changes in the serum lipids (Table II). A small drop in total serum cholesterol was noted in 6 of 9 patients during the first few days of treatment as originally observed by Conn(6), but this was usually followed by a subsequent moderate rise, and the overall average for the group showed an increase of 4%. There was an average increase of 9% in the phospholipids with 9 of the 12 exhibiting a rise, and an average decrease of 16% in the neutral fat, with 6 of 8 showing this effect. None of the changes which occurred during ACTH therapy were statistically significant.

Of special interest was the fact that the fasting sera of 7 of the 15 cortisone-treated patients and 3 of the 12 ACTH patients became turbid ("lipemic") despite the fact that the level of neutral fat had decreased. This may indicate that the rise in phospholipids and cholesterol was due to the production of a lipid which was less soluble than normal, or that there was a change in the conjugated lipids (possibly lipoproteins).

It is evident, then, that there was a difference between the effects of cortisone and ACTH on the serum lipids. This difference of effects may be explained by the different nature and points of attack of these substances. Cortisone is a cortical hormone of a well-defined structure, while ACTH is an organ extract of the anterior lobe of the pituitary which stimulates the production of several cortical hormones. Evidence is available(1) that the adrenal cortex of man when stimulated by ACTH secretes Compound F rather than cortisone (Compound E). It is also possible that serum cholesterol represents the material for the synthesis of cortical hormones under ACTH stimulation(6), and that prolonged stimulation results in functional exhaustion and atrophy of the adrenal cortex, as well as in the production of antihormones(7).

Summary. This investigation indicates that cortisone produced well-characterized changes of serum lipids in man. The effects of ACTH on the serum lipids were less pronounced.

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Positive Inotropic Action of Ouabain on Rat Ventricle Strips.* (18076)

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Previous work in this laboratory on the metabolism and functional activity of the rat heart(1,2) led us to an investigation of the effect of cardiac glycosides on the force of contraction of rat ventricle muscle. The present paper reports a positive inotropic action of ouabain on the isolated rat ventricle strip, and the effects of various factors upon this response. A literature survey reveals that this is the first conclusive demonstration of a cardiotonic effect of ouabain on rat myocardium.

Method. Adult male rats (225-275 g) were killed by decapitation and a strip prepared from the right ventricle, attached to a semi-isometric optical lever, and mounted according to the method developed in this

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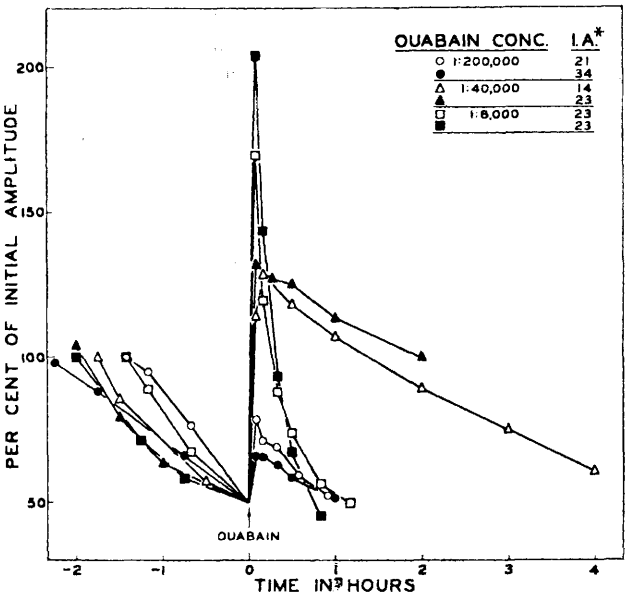

FIG. 1.
Effect of ouabain concentration. * I.A. Initial amplitude of contraction in mm ×65 (after equilibrating 1 hr).

laboratory by Feigen *et al.*(3). The tissue was placed in a medium containing 0.9% NaCl, 0.042% KCl, 0.036% $CaCl_2$, 0.1% glucose, and 0.00105 M phosphate buffer (pH 7.2) at 37.5°C, and gassed with oxygen through a sintered glass plate in the bottom of the vessel. The muscle, with a diastolic tension of 0.75 g, was stimulated at a constant rate of 100 per minute by an Electrodyne stimulator. After an equilibration period of one hour, the solution in the vessel was renewed. The contractions, magnified 65-fold, were recorded photographically every 15 or 30 minutes during the "fatigue" period in a manner similar to that of Cattell and Gold(4,5), who first developed the technic for demonstrating the positive inotropic effect of cardiotonic glycosides on the cat papillary muscle. When the force of contraction had decreased to approximately 50% of the initial value, ouabain was added and a record was taken every 5 to 15 minutes. Only one addition of ouabain was made for each ventricle strip tested.

Results. Effect of ouabain concentration. When ouabain was added to the hypodynamic ventricle strip, there was a very rapid increase in the force of contraction, usually reaching a maximum in 3 to 5 minutes. Typical results of 3 different concentrations are presented in Fig. 1. A fairly uniform response was obtained with each concentration. At 1:40,000 ouabain there was a pronounced positive inotropic effect which remained above the 0 time value (Fig. 1) for more than 4 hours, whereas the force of contraction following 1:8,000 returned to the 0 time value in approximately one hour even though the maximum developed tension was greater. Inasmuch as a concentration of 1:40 million to 1:75 million produces a cardiotonic effect on the cat papillary muscle(4), an effective concentration of 1:40,000 is not unexpected for the rat ventricle preparation, as the toxic dose for a rat is 100 to 1000 times greater (depending on the rate of injection) than for a cat(6-8).

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Investigation of time-response to ouabain. As already mentioned, the maximum effect on the rat ventricle strip occurred within 5 minutes. Yet, when the papillary muscle of the cat's heart was exposed to 1:5 million ouabain, the maximum tension was not reached until 45 and 90 minutes in 2 experiments, thus confirming the observations of Cattell and Gold(4). This time difference could be due (a) to a species variation or (b) to a difference in the part of the heart used. Rat papillary muscles from the left ventricles of 2 rat hearts were tested in the same manner as the right ventricle strip. Although the initial force of contraction of the rat papillary muscle was considerably less than the ventricle strip, the maximum increase in force of contraction following the addition of ouabain (1:40,000) also occurred in approximately 5 minutes. It is probable, therefore, that the difference in time-response to ouabain between the cat papillary muscle preparation and the rat ventricle strip is due to a species difference.

Effect of calcium concentration on ouabain response. The relation between the ouabain response and calcium concentration was investigated, and Fig. 2 shows the response at 3 calcium concentrations. Feigen *et al.*(3) observed that the rat ventricle strip exhibited maximal contraction at a concentration of 0.062% $CaCl_2$ and above or below this amount there was a decrease in activity. With an increase in calcium from 0.012% to 0.036%, we found an increase in the ouabain response, but at a concentration of 0.062% $CaCl_2$ ouabain produced a smaller effect than at the two lower concentrations.

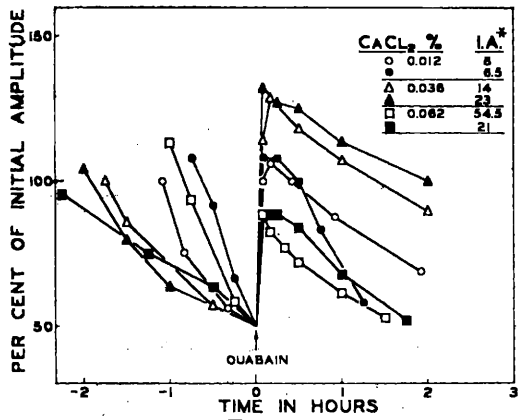

FIG. 2.

Effect of calcium concentration on ouabain response. 1:40,000 ouabain was added at 0 time. * I.A. See footnote under Fig. 1.

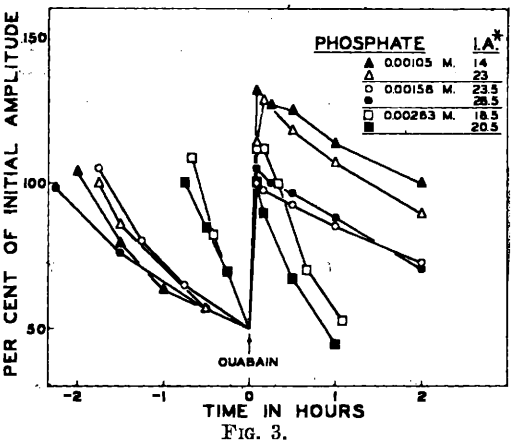

FIG. 3.

Effect of phosphate concentration on ouabain response. 1:40,000 ouabain was added at 0 time. * I.A. See footnote under Fig. 1.

Effect of phosphate concentration on ouabain response. White and Salter(9) found that the cat papillary muscle preparation would contract in bicarbonate buffer without much decrease in force over long periods of time, but in phosphate buffer a rapid decrease in mechanical activity occurred. We have, therefore, investigated the effect of various phosphate concentrations on the ouabain response. Fig. 3 shows that ouabain produced the greatest effect in the presence of a low concentration of phosphate. The highest concentration of phosphate produced the most rapid "fatigue," and even after ouabain this depressant effect of phosphate was apparent.

Effect of initial tension. The amount of diastolic tension on the muscle was varied to determine its effect on the ouabain response. The maximum response to ouabain (1:40,000) at diastolic tensions of 0.25 g and 1.5 g was approximately two-thirds that obtained at a tension of 0.75 g.

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Summary. A positive inotropic effect of ouabain has been demonstrated on the rat ventricle strip. At a given ouabain concentration the magnitude of the response was quite uniform. The effects of variation in the concentration of ouabain, calcium, and phosphate, and in initial tension on the positive inotropic response have been investigated.

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Band-Pass Filter Action of the Cochlea During Nembutal Anesthesia.* (18077)

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In the course of studies of the potentials evoked in the cerebral cortex by auditory stimulation, we have noted changes in the threshold of the electrical response of the cochlea which merit further description.

Experimental. The observations were made in cats anesthetized with nembutal. The auditory stimuli, provided by a variable frequency sine-wave generator (Hewlett-Packard No. 202B) were led into the ear from a headphone via a plastic tube fixed in the external meatus. Recording of the cochlear potential was accomplished through a wick electrode placed at the round window and led through a suitable amplifier to a cathode-ray oscilloscope. The cochlear audiograms were recorded by determining the threshold intensity of stimulus at various frequencies from 100 to 5000 cycles/sec.

Results and discussion. Typical audiograms in 3 cats, A, B, and C, are charted in Fig. 1. The duration of anesthesia, the body temperatures, and the respiratory rates are indicated at the right of the chart.

During nembutal anesthesia the change in the cochlear response consists of a rise in threshold, initially for the higher frequencies, and later for the lower frequencies. Characteristically, there is a relative sparing of the frequency band around 1700 cycles, as recorded in the audiograms of cats B and C. This differential sparing may result in a relatively narrow frequency band with a threshold 6 or more db below any other.

At low intensities of stimulation, therefore, the cochlea may respond only to a narrow frequency band and its functioning may be likened to that of a band-pass filter.

Riesco-MacClure *et al.*(1) have reported antemortem failure of the cochlear response, especially to higher frequencies, in guinea pigs under Dial anesthesia. They considered this failure as possibly due to "cumulative irreversible or slowly reversible effects of prolonged partial anoxia." Our observations with nembutal anesthesia confirm the finding of these authors that the level of anesthesia, *per se*, and the usual fall in body temperature are not necessarily implicated in the depression of the cochlear response. This depression may occur when the level of anesthesia is light as judged by the electrical activity of the cerebral cortex and by the presence of reflex withdrawal responses. An audiogram with normal frequency distribution may be obtained when body temperature has fallen as in cat A, and a depressed cochlear response may be obtained with a similar fall in body temperature, as in the audiogram B_2.

Although the depression of the cochlear

* Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

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