for 30′ with fresh rabbit serum does not destroy the active agent. Preservatives such as formaldehyde (0.1%), phenol (0.5%) and merthiolate (1:10,000) do not affect it. It can be freeze-dried with little loss of its activity.

*Summary.* Fluid from the coagulating gland of the guinea pig injected intravenously or intramuscularly produced toxic effects (miosis, urination, defecation, salivation) and death in rabbits, recalling the parasympatomimetic action of certain drugs. When it was injected into guinea pigs intravenously, it caused urination, defecation, prostration and death in about one hour. In guinea pigs, the intramuscular injection of this material caused regional clonus. Fluid from the coagulating glands of rats and mice injected into rabbits did not produce a similar effect.

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## Comparative Effects of Ouabain Upon Contractile Force of Guinea Pig Diaphragm and Heart.* (22942)

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The positive inotropic action of cardiac glycosides upon heart muscle has been well established in studies with isolated papillary muscles(1) and ventricle strips(2) and with intact heart preparations(3-5). The effect of these drugs upon the force of contraction of skeletal muscle is less clear, however. For example, no significant positive inotropic action was observed in a variety of skeletal muscle preparations(6-10). On the other hand, a number of investigations using different preparations or experimental conditions have revealed an increased force of contraction(6,7,9-16). Similarly, negative inotropic actions have been reported (7,9,15-17) as well as failure to observe such an effect with other preparations or conditions(6,8,9). The conflicting results obtained above are in part inexplicable. It might be noted, however, that in much of the work (with *in situ* as well as isolated preparations) the experimental conditions were not defined precisely, nor were concentration-action relationships explored fully. In addition, direct comparisons of the action on skeletal and cardiac muscle of the same animal have not been made.

The effects of these drugs assumes an especial importance in view of recent work on their mechanism of action, in which skeletal muscle and contractile protein preparations from skeletal muscle have been used(18-21). The significance of such investigations is in large dependent upon demonstration that cardiac glycosides produce a positive inotropic action upon skeletal muscle. We have therefore investigated the problem in simple skeletal and cardiac muscle preparations of guinea pig *in vitro,* using well-defined conditions and a wide range of concentrations.

*Methods.* The isolated left phrenic nerve-diaphragm preparation of the guinea pig (150-200 g males) was used. Preparation of the strip from the diaphragm was carried out as described originally in the rat by Bülbring (22), and the muscle stimulated with a Grass stimulator (Model S-4-B) with square wave shocks. The muscle was stimulated either directly (the base of the muscle wedge rested upon a platinum electrode of the same length as the muscle base) with a stimulus of 1 millisecond duration and 40 volts, or indirectly

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through the phrenic nerve with a stimulus of 0.1 millisecond duration and 20 volts. Stimuli of these intensities gave contractions of equivalent force through nerve and muscle. At the above voltages, the force of contraction on stimulation of the muscle was essentially constant on direct stimulation at durations of approximately 0.5-1.5 milliseconds and on indirect stimulation at 0.01-0.2 millisecond. Stimuli of longer duration resulted in an increased force of contraction, due presumably to multiple, "fused" contractions as described previously(23,24), while stimulation of the muscle directly at durations of about 0.5 millisecond or less usually produced contractions of less force, due presumably to submaximal stimulation. In some experiments, stimulation of the muscle directly was established by the addition of *d*-tubocurarine chloride (1-2 $\gamma$/ml), which abolished the response to stimulation through the nerve without affecting the response to direct stimulation. All experiments were performed with the muscle stimulated directly, unless otherwise noted. Krebs-Ringer bicarbonate medium, gassed with 5% $CO_2$-95% $O_2$, was used in most of the work(25). A few experiments were performed in a phosphate-buffered medium similar in composition to that used commonly in studies on the action of cardiac glycosides upon cat papillary muscles(1). The phosphate medium contained 0.9% NaCl, 0.042% KCl, 0.036% $CaCl_2$, and 1 *mM* sodium phosphate buffer. The medium had a final pH of 7.4, and was gassed with 100% $O_2$. Both media contained 0.1% glucose. All experiments were performed at 37°C, with the muscle preparation suspended in 150 ml of medium. The ouabain used was U.S.P. grade (Penick), and was dissolved in about 1.0 ml of medium and added to the bath. The contractions were recorded optically, using a semi-isometric torsion lever. The shortening of the muscle was magnified 59 x by the optical system, and a deflection of the beam of 4 mm corresponded to a developed tension of 1.0 g. The resting tension on the muscle was maintained at 2.0 g by means of a micrometer arrangement described previously(26), in order that any change in length of the muscle at this tension could be measured. The muscle was stimulated at a rate of 6 per minute. The activity was recorded for a control period of 2 hours prior to the addition of the drug, during which time the force of contraction declined only slightly. The average force exerted by the muscle at the time of addition of the drug was 6 g. Some experiments were performed on isolated, electrically-stimulated strips prepared from the right ventricular wall of the guinea pig heart, in a manner similar to that described for the rat heart (26). The animal was killed by crushing the skull, and all manipulations involved in the preparation of the strip were done at room temperature. The strip was stimulated at a frequency of 100 per minute at a maintained resting tension of 0.75 g, and the contractions recorded optically with a semi-isometric optical lever system with which a force of 100 mg gave a deflection of 30 mm (amplification of shortening of muscle 62 x). The average force exerted by the muscle at the time of addition of the drug was approximately 100 mg. The experiments were performed in Krebs-Ringer bicarbonate medium at 37°C.

*Results. Actions of ouabain upon diaphragm in bicarbonate medium.* Fig. 1 shows typical experiments over a range of concentrations of ouabain. A positive inotropic action occurred at least briefly over a wide range of concentrations, with a maximal effect at 0.5 $\gamma$/ml. The higher concentrations depressed the force of contraction markedly after a brief period of stimulation, as can be seen from a comparison with the control preparations to which no drug was added. The magnitudes of both the positive and negative inotropic responses were the same, whether the muscle was stimulated directly or through the phrenic nerve. The relation between force of contraction and duration of stimulus at constant voltage was the same during the phases of positive and of negative inotropic action as prior to the addition of the drug, *i.e.*, the force was approximately constant at durations of 0.5-1.5 or 0.01-0.2 milliseconds when stimulated directly or indirectly, respectively. The changes noted in developed tension cannot, therefore, be explained on the basis of alterations in refractory period or threshold of the muscle. Changes in length

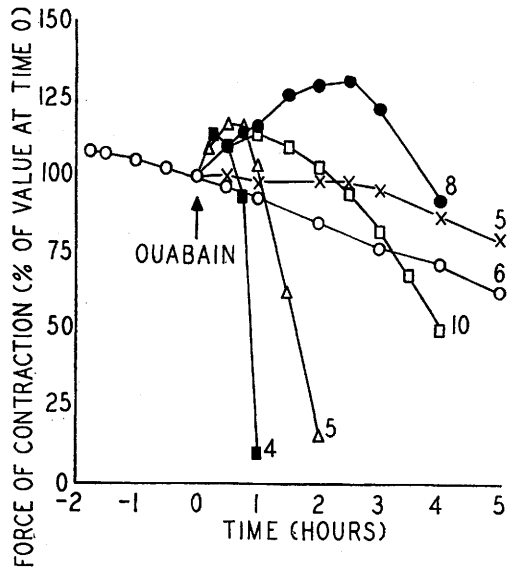


FIG. 1. Effect of ouabain upon force of contraction of diaphragm in Krebs-Ringer bicarbonate medium (muscle stimulated directly). Ouabain added at zero time above. Curves indicate average values for each concentration of ouabain (no. of experiments indicated on each curve). -○—○- controls (no drug), -×—×- 0.1 γ/ml, -□—□- 0.3 γ/ml, -●—●- 0.5 γ/ml, -△—△- 1.0 γ/ml, -■—■- 3.0 γ/ml.

of the muscle at constant resting tension during the periods of increased or decreased force were slight and unrelated in time to the changes in developed tension.

*Actions of ouabain upon diaphragm in phosphate medium.* In view of the fact that most investigations on the positive inotropic

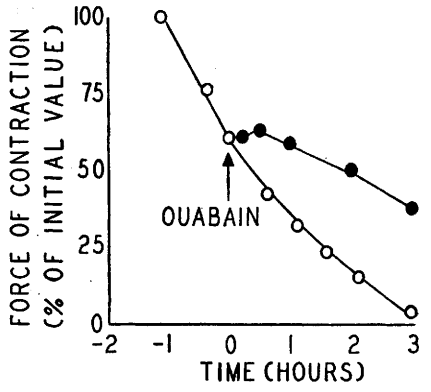


FIG. 2. Effect of ouabain upon force of contraction of diaphragm in phosphate medium (muscle stimulated directly). Ouabain (0.5 γ/ml) was added at zero time above. Curves indicate average values for 8 experiments with ouabain and 4 controls. -○—○- control (no drug), -●—●- ouabain.

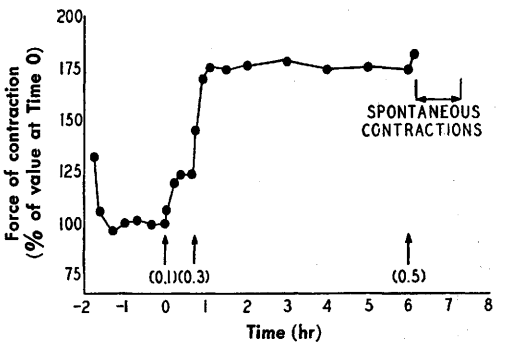


FIG. 3. Effect of ouabain upon force of contraction of ventricle strips. Ouabain added at arrows. *Total* concentration of ouabain in bath in γ/ml indicated in parentheses beneath arrows.

action of the cardiac glycosides on such preparations as the cat papillary muscle have been done in phosphate-buffered media(1,27), we determined the response of the diaphragm to ouabain in such a medium (see *"Methods"*). As seen in Fig. 2, the force of contraction decreased rapidly by comparison with the bicarbonate medium used above. This difference in the stability of isolated muscle preparations is well-known for the papillary muscle(27) and the rat diaphragm(28). Fig. 2 shows that some increase in force is produced by ouabain by comparison with the control, but it is of small magnitude compared with the response in bicarbonate medium (Fig. 1) or with the response of cardiac muscle in phosphate medium(1,2).

*Actions of ouabain upon isolated ventricle strips in bicarbonate medium.* It was of interest to compare the actions of ouabain upon the force of contraction of cardiac muscle in bicarbonate medium with those described above, and for this purpose the isolated guinea pig ventricular strip was used (see *"Methods"*). A positive inotropic effect was produced at a concentration of ouabain of 0.1 γ/ml, and a considerably greater response occurred when the concentration was increased to 0.3 γ/ml, as shown in the typical experiment in Fig. 3. Concentrations of 0.5 γ/ml resulted frequently in the appearance of automaticity, with the muscle contracting spontaneously for brief periods or for hours at a rate of 150-200/minute and independently of the frequency of the stimulator.

Such automaticity represents a toxic response to the digitalis glycosides(29). The muscle usually went into a contracture-like state following such automaticity, with a great increase in resting tension.

Thus, both a positive inotropic action and toxic effects were produced in heart and diaphragm with similar concentrations, although the magnitude of the former was greater in heart. The experimental conditions under which the drug was tested were the same with both tissues, except for the frequency of stimulation. It was not desirable to use the same frequency, as the force of contraction of diaphragm declined rapidly at high rates and on the other hand the initial developed tension of cardiac strips was very low at slow rates of stimulation. The possibility that the quantitative differences in magnitude of the positive inotropic action in diaphragm and heart might be related to activity of the muscle cannot, therefore, be determined from our results.

*Actions of ouabain upon intact guinea pigs.* In view of the fact that the effective concentrations of ouabain upon the diaphragm and heart were of approximately the same order of magnitude, we investigated the action of the drug in the intact animal in order to determine if skeletal muscle function was affected at doses which did not cause cardiac arrest. Doses of ouabain of approximately 0.25 mg/kg were administered intramuscularly to unanesthetized guinea pigs. Within a period of one-half hour the animals began to display signs of skeletal muscle paralysis, which reached maximum intensity in about one hour. Paralysis of the hind legs and neck muscles was marked, with the hind quarters and head resting upon the floor. The animals showed great difficulty in righting themselves when placed on their backs. At this point, electrocardiographic tracings (Lead II) were taken and compared with tracings taken prior to the administration of the drug. Prolongation of PR interval was sometimes noted, but indications of cardiac toxicity (*e.g.*, arrhythmias) were usually not seen when the animals were tested shortly after the development of muscular paralysis. The animals were observed again after completion of the electrocardiographic tracings and the paralysis was found to persist for several hours after administration of the drug. Some animals died within an hour after the appearance of the muscular paralysis, but many recovered completely.

*Discussion.* The present study has demonstrated that ouabain possesses a positive inotropic action upon isolated diaphragm of guinea pig. Previous studies with diaphragm of rat and mouse did not reveal such an action (6-8). Our results represent the first demonstration in an isolated mammalian skeletal muscle preparation of a positive inotropic action of a cardiac glycoside. Lendle and Oldenberg(10) observed an increased force of contraction in the masseter muscle preparation of the rat stimulated *in situ* with needle electrodes with submaximal but not with maximal stimuli; their results are in contrast with ours, which were obtained with supramaximal stimuli. They attributed the effect to an increased excitability of the muscle under the influence of the drug, but we found no change in the intensity of stimulus required to produce a maximal contraction. In view of the fact that a positive inotropic response occurred at concentrations lower than those producing paralysis, and further that the latter can occur at least in limb and neck musculature with non-fatal doses of the drug, it is possible that an increase in skeletal muscle force might occur with therapeutic doses of the drug.

No negative inotropic action of cardiac glycosides occurred in the phrenic nerve-diaphragm preparation of the rat or mouse(6-8) except in the presence of high extracellular concentrations of potassium(7), although such an action has been reported in a variety of other skeletal muscle preparations(7,9,15-17). We found that a marked skeletal muscular paralysis occurred on administration of ouabain to unanesthetized guinea pigs, prior to or in the absence of significant cardiac toxicity. Skeletal muscular paralysis has been reported to occur in the neck and leg muscles of rats(7), and respiratory paralysis occurred frequently prior to cardiac arrest on adminis-

tration of cardiac glycosides to rats(30) and guinea pigs(31-34). It is of interest that LaDue(35) reported marked skeletal muscular weakness in a patient with digitalis poisoning and a decreased work ability of dogs on a treadmill following digitalis administration. The incidence and possible clinical significance of muscular paralysis in digitalis-poisoned patients might well be explored further.

Our results imply that whatever system(s) in cardiac muscle are affected by the cardiac glycosides with resultant increased force of contraction are probably also present in skeletal muscle, and hence the latter could reasonably be used in studies on the mechanism of action of the drugs. In view of the clinical use of the drug and the smaller effect upon the diaphragm, however, it would appear that such studies should be done with cardiac muscle, unless special considerations preclude its use. If skeletal muscle were to be used for such studies, however, as in investigations on potassium exchange and content(18,19), the effective concentrations of the drug for the production of the positive inotropic action should be first established on a contracting muscle preparation. The results presented here show that concentrations greater than those required to produce an increased force of contraction are depressant to the diaphragm, and any changes in ionic content of the cells or rate of ionic exchange, or in electrical, metabolic, or other types of activity at these higher toxic concentrations would not contribute to an understanding of the mechanism of the positive inotropic action of the drugs.

*Summary.* Ouabain increased the force of contraction of isolated guinea pig diaphragm, stimulated directly or indirectly with supramaximal shocks. At higher concentrations of the drugs, the positive inotropic action was followed by a depressant effect. The concentrations at which these effects occurred were similar to those which produced an increase in force of contraction or toxic effects upon isolated guinea pig ventricular strips. Skeletal muscular paralysis in intact guinea pigs following the administration of ouabain was frequently not accompanied by evidence of cardiac toxicity. The significance of these findings in relation to studies on the mechanism of action of the cardiac glycosides which employ skeletal muscle preparations was discussed.

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## Differential Concentration of Porphyrin in Various Divisions of the Central Nervous System.* (22943)

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The observation that white matter of the central nervous system exhibited a band fluorescence spectrum centered at 625 m led to the conclusion that the nervous system contained coproporphyrin(1). From further evidence based on solubility characteristics, Klüver(2) identified protoporphyrin and coproporphyrin type III isomer. Chu and Watson(3), using the fluorescence quenching technic, also identified the coproporphyrin isomer as type III and reported the average amount of this porphyrin in 10 determinations of normal rabbit brain to be 3.56 γ/100 g brain. The isolation of crystalline coproporphyrin III from beef brain was carried out by Blanshard(4). His method of extraction yielded 2 γ/100 g brain.

Earlier observation,‡ on the fluorescence of various parts of the central nervous system of mice and rats, gave the impression that red fluorescent material was more abundant in the medulla oblongata and brainstem than in other areas. The quantitative distribution of coproporphyrin III in gross anatomical subdivisions of the central nervous system was studied to ascertain whether porphyrins exhibit a gradient distribution in the nervous system and to obtain a clue as to the possible functional significance of the nervous system porphyrins.

*Methods.* Pig brains were collected at the abattoir, stripped of membranes, and washed to remove blood. Equal amounts (250 g) of cerebrum, midgrain, medulla oblongata, cerebellum, and spinal cord were homogenized with 4:1 ethyle acetate-glacial acetic acid, (4) and cooled to −15°C. The supernate was decanted and filtered under suction. Extractions were repeated until no color was observed in the supernate. The material remaining was mixed with Solka-Floc BW 40, a purified wood cellulose filter aid, and placed in a large Büchner funnel, and covered with 200 ml 3N HCl which filtered off slowly. This solution was neutralized with sodium acetate and extracted with ethyl acetate-glacial acetic acid(4:1). The extract was added to the filtered supernate decanted previously. Extractions were carried out 5 times on subdivisions of the central nervous system with exception of the medulla oblongata which was repeated 6 times. The extracted porphyrins were measured fluorimetrically.

*Results.* Highest concentration of copro-

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† Fellow, Nat. F. For Infantile Paralysis.

‡ Figge, unpublished.