Therapeutical and Pharmacological Section.

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Professor W. E. DIXON, F.R.S., President of the Section, in the Chair.

The Influence of Ions upon the Action of Digitalis.

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IN the experiments described below I have studied the action of the digitalis glucosides upon the isolated frog's heart, and have endeavoured to ascertain to what extent this action is modified when the composition of the perfusion fluid is altered.

(I) METHOD.

The method was one described by Hartung [2]; I have modified it slightly (fig. 1). As shown in the figure, a cannula connected with a small reservoir is ligatured into the sinus venosus of the frog, a second cannula, connected with a short piece of bent tubing, is inserted into one aorta, the other aorta is ligatured, the superior venæ cavæ are ligatured, the frog's heart is cut free from the body, and the apparatus is fixed on to a piece of cork with pins, being so arranged that the fluid circulates freely. By varying the size of the reservoir any quantity of fluid from ½ c.c. to 10 c.c. can be circulated through the heart for any length of time desired, the pressure remains constant, and the circulation of the fluid provides aeration, rendering oxygen unnecessary. The movements of the heart are recorded by a light lever on a smoked drum. By means of the two T-tubes and clips, shown in the figure,

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the cannula can be washed out without altering the pressure. In the experiments described below the diastolic pressure was always about 4 cm., and the systolic pressure 5 cm. of water; the temperature varied between 12° C. and 16° C.; Ringer's fluid of the following composition was used—NaCl 0·65 per cent., KCl 0·018 per cent., $CaCl_2$ 0·024 per cent., $NaHCO_3$ 0·02 per cent. In this medium a frog's heart maintained a strong, steady beat for twenty-four hours, without the addition of glucose or the passage of oxygen. Most of the experiments were performed with crystalline digitoxin, kindly supplied by Messrs. Merck

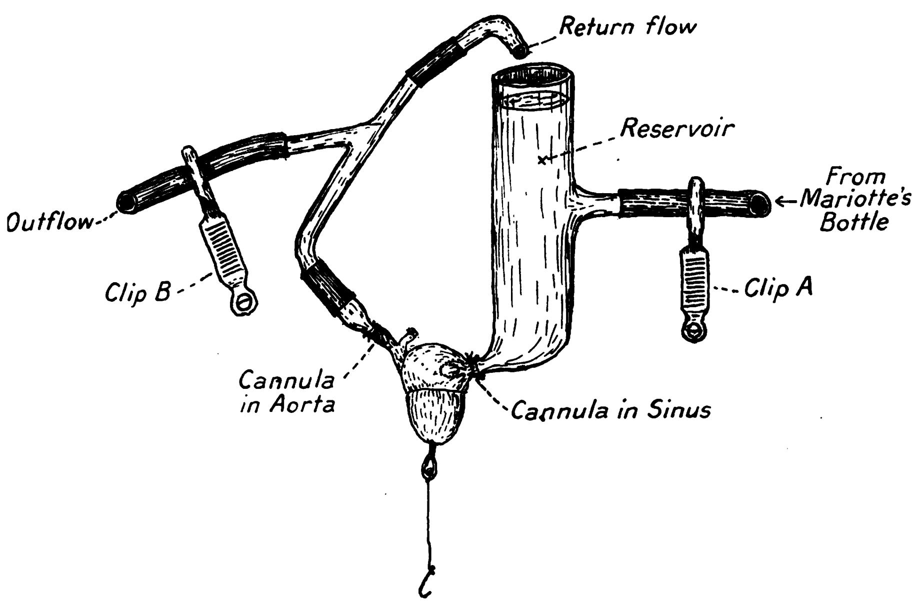

FIG. 1.

Apparatus for perfusion of isolated frog's heart. (Natural size.)

and Co. A few experiments were performed with strophanthin (commercial preparation from *Strophanthus hispidus*—Burroughs Wellcome and Co.). The drug was dissolved in alcohol (1 mgrm. per cubic centimetre), and stock solutions kept in this form. It was proved by experiment that the solutions in alcohol suffered no loss of strength on keeping, but dilute solutions of digitoxin in Ringer's fluid showed a marked loss of activity after twenty-four hours, therefore freshly made solutions were always employed.

(II) The Effect of Concentration upon the Action of Digitoxin and Strophanthin.

Schmiedeberg [6], Krailsheimer [5], Holste [4], and Werschinin [9] investigated the action of these drugs, using Williams's apparatus, in which 50 c.c. of fluid are perfused through the heart, which works under a diastolic pressure of over 10 cm. of water. Trendelenburg [8], Straub [7], and Heffter and Sachs [3] did similar experiments, using Straub's apparatus, in which 1 c.c. or 2 c.c. of fluid are used and the diastolic pressure is only 2 to 3 cm. of water. Werschinin and Straub obtained the following results :—

Table I.

Observer	Drug used	Minimal concentration of drug in milligrammes per cubic centimetre producing systolic arrest
Werschinin ...	Gratus strophanthin (Thoms) ...	0·01
	Amorphous strophanthin (Böhringer) ...	0·001
	Digitoxin cryst. (Merck) ...	0·01
Straub	Gratus strophanthin cryst. (Merck) ...	0·0025 to 0·00125

From these results it appears that gratus strophanthin and digitoxin are of nearly the same strength, but that amorphous strophanthin is many times more toxic. It will also be seen that with Straub's apparatus much lower concentrations of gratus strophanthin produce systolic arrest than produced systolic arrest with Williams's apparatus. Straub found that if ½ c.c. of fluid were used instead of 1 c.c. the same concentration of strophanthin still produced exactly the same effect; he therefore concluded that the action of strophanthin depended entirely upon the concentration of the drug, and was not influenced by the total quantity of the drug present.

I investigated this question with the apparatus already described, using quantities of fluid varying from ½ c.c. to 100 c.c. With the varying quantities of fluid the minimal concentration of digitoxin was ascertained which, within one hour, would produce a systolic effect from which the heart did not recover (fig. 2). From these results it will be seen that if quantities of fluid varying from 2 c.c. to 100 c.c. are used, the minimum lethal concentration of the drug remains constant, but when smaller quantities of fluid are used slightly higher concentrations are required to produce systole. This rise in concentration, although slight, is nevertheless quite definite, and is greater than can be accounted for by the fact that when small quantities of perfusion fluid are used the volume of the heart muscle may cause some error in calculating the dilution. The weight of the heart of a 25-grm. frog was found to be only 0·06 gr.

A few experiments were performed with strophanthin; the minimal lethal concentration was found to be between 0·0005 and 0·00025 mgrm. per cubic centimetre.

I find the minimal concentrations of digitoxin and strophanthin, which produce systole, to be much lower than the figures obtained by Werschinin with these drugs.

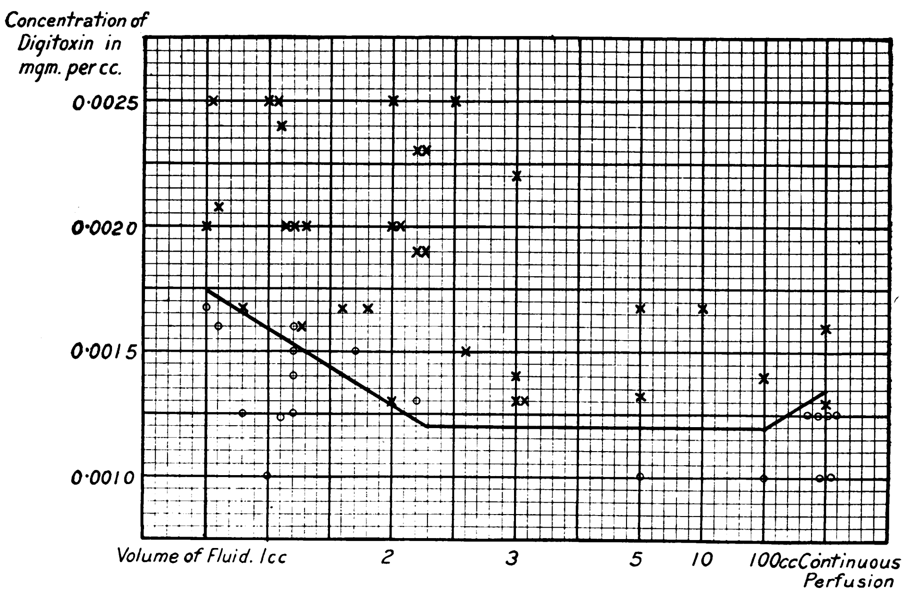

FIG. 2.

This figure shows the minimal concentration of digitoxin which will produce systolic arrest in an isolated frog's heart; it also shows how this minimal concentration varies when the quantity of perfusion fluid is altered. **X**, systolic effect produced upon frog's heart within sixty minutes, and subsequent death of the heart in systole; **O**, no systolic effect produced, or slight systolic effect followed by recovery.

(III) DIASTOLIC EFFECT OF DIGITALIS GLUCOSIDES.

Werschinin [10] follows Schmiedeberg in describing a diastolic effect produced by the digitalis glucosides in low concentrations, and in some of his work he takes considerable pains to ascertain the minimal concentration of strophanthin that will produce a systolic, as opposed to a diastolic, arrest. Straub [7] denies that the digitalis glucosides have any diastolic action, and ascribes the diastolic effects observed by Werschinin to instrumental error, for he points out that Williams's apparatus, which was used by Werschinin, produces an excessive diastolic pressure. I agree with Straub's conclusion; the lowest concentrations of digitoxin and strophanthin that have any action produce at first a systolic effect, but the heart may partially recover from systole and continue beating slowly and regularly for some hours, finally dying in semi-systole: this occurs most frequently when large quantities of fluid are passed through the heart (fig. 3). If such a heart were

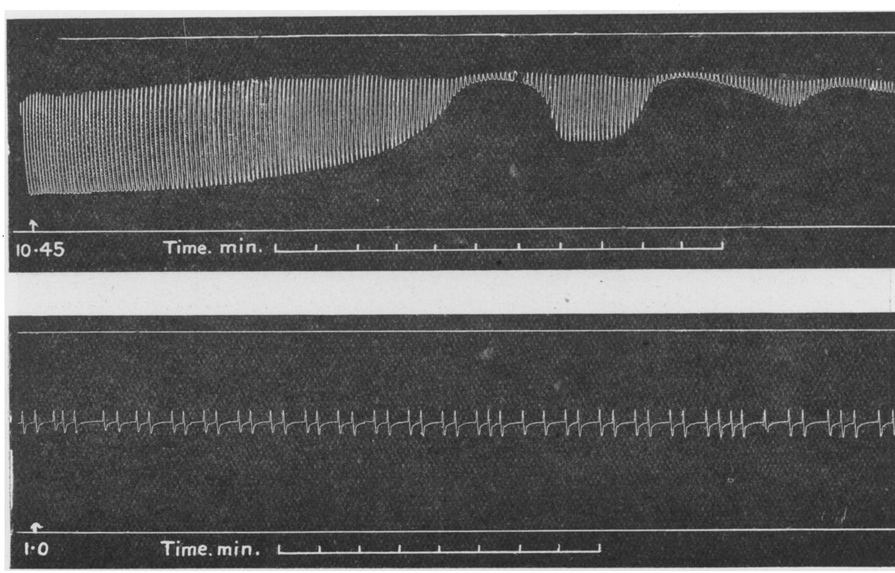

FIG. 3.

Isolated frog's heart perfused with 150 c.c. Ringer's fluid, containing 0·00125 per mille digitoxin; an initial systolic effect is produced, followed by partial recovery. There is an interval of two hours between the two tracings. (Tracing reads from left to right.)

subjected to an excessive diastolic pressure it would probably die in complete diastole. In some instances where very minute quantities of drug were used the heart, after showing an initial systolic effect, recovered completely and maintained a strong regular beat for many hours (fig. 4). Hearts which, before any drug was added, were beating feebly or irregularly, often were rapidly arrested in diastole after the addition of digitoxin, but this never occurred with healthy hearts.

I consider, therefore, that the diastolic effect, described by Werschinin, is due to instrumental error, and that the distinction he draws between the systolic and diastolic effect of strophanthin is not of any importance.

(IV) The Absorption of Digitalis Glucosides by the Frog's Heart.

Straub [7] investigated this question by transferring 1 c.c. of fluid containing 0·0025 mgrm. of strophanthin through a series of hearts. After passing the fluid through six hearts a marked diminution in its

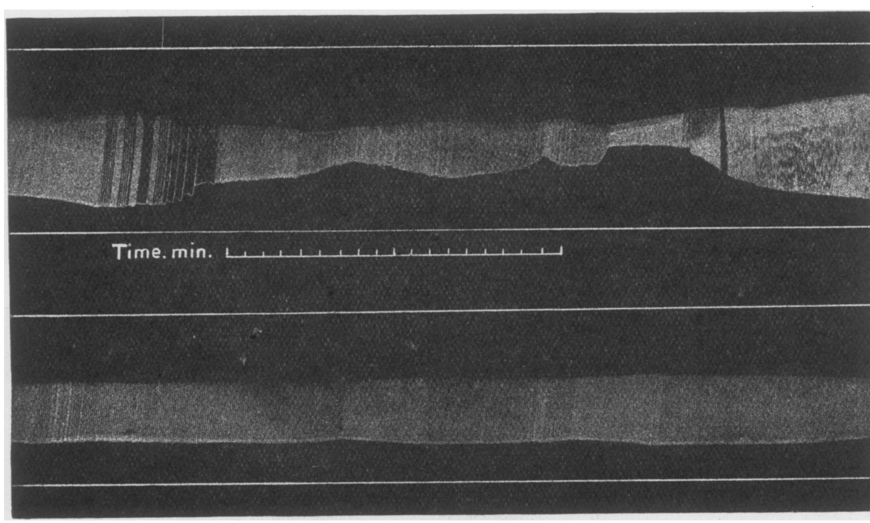

Fig. 4.

Isolated frog's heart perfused with 0·5 c.c. of Ringer's fluid containing 0·00083 mgrm. digitoxin; initial systolic effect produced, which is followed by a complete recovery. (Tracing reads from right to left.)

action was observed, and Straub calculated that each heart absorbed 0·0002 mgrm. of the drug. Using digitoxin I obtained the following results: 2 c.c. of Ringer's fluid, containing 0·005 mgrm. digitoxin, were passed successively through three frog's hearts perfused by Straub's method. The first heart died in systole in thirty-seven minutes; the second heart died in systole in thirty-six minutes, but no systolic effect was produced upon the third heart.

Now a concentration of 0·0012 mgrm. per cubic centimetre of digitoxin produces a systolic effect, therefore the fluid at the end of the experiment must contain less than 2 × 0·0012 = 0·0024 digitoxin. Therefore the two hearts must have absorbed 0·0026 mgrm.; therefore each heart must have absorbed 0·0013 mgrm. of digitoxin.

Using strophanthin I obtained the following results: 1 c.c. of Ringer's fluid containing 0·00066 mgrm. strophanthin was passed successively through four frog's hearts which were perfused by Hartung's method. The first heart died in systole in twenty minutes; the second heart died in diastole in ten minutes; the third heart died in systole in sixty minutes, but no systolic effect was produced upon the fourth heart.

Only 0·7 c.c. was recovered at the end of the experiment, some fluid being lost between Nos. 2 and 3.

Now strophanthin in a concentration of 0·0002 mgrm. per cubic centimetre just fails to produce a systolic effect, therefore not more than 0·0002 mgrm. was present at the end of the experiment; 0·3 c.c. fluid was lost, which corresponds to 0·00020 mgrm.; therefore three hearts absorbed 0·00066 − 0·0004 = 0·00026 mgrm., and therefore each heart absorbed about 0·00008 mgrm. of strophanthin. Since the strophanthin I used was about four times as toxic as the gratus strophanthin used by Straub, this figure agrees with his result.

Using digitoxin, a much higher absorption figure was obtained, but the experiment took four hours to carry out, and it was found that dilute solutions of digitoxin in Ringer's solution suffered a very marked loss of strength on standing many hours; therefore the absorption figure for digitoxin is very possibly inaccurate.

(V) Other Factors influencing the Action of Digitalis Glucosides.

Trendelenburg [8] investigated the length of time taken by strophanthin to kill the isolated frog's heart; he found that all concentrations between 0·25 and 0·005 mgrm. per cubic centimetre took the same time to kill the heart—namely, ten minutes, but weaker dilutions took a steadily increasing time. Schmiedeberg [6] found with gratus strophanthin that 0·003 mgrm. per cubic centimetre took thirteen minutes, 0·002 mgrm. per cubic centimetre took twenty-five minutes, and that 0·001 mgrm. per cubic centimetre took thirty-two minutes to kill a frog's heart, using Williams's apparatus. I found that the time at which a heart died—that is to say, the time at which the last spontaneous beat occurred—varied very greatly, but that the time taken to produce a maximal systolic effect was roughly proportional to the degree of dilution (fig. 5).

The effect of concentrations above 0·004 mgrm. per cubic centimetre was not investigated.

The effect on the heart of digitalis glucosides in high concentrations is a permanent one, and the heart cannot be restored to activity by washing out, but Straub showed that with weak concentrations the heart after it had been arrested in systole could be restored to activity by washing out with Ringer's fluid. I tried the effect of washing out the heart with Ringer's fluid at various stages of digitoxin poisoning. If 0·003 mgrm. per cubic centimetre of digitoxin is allowed to act on

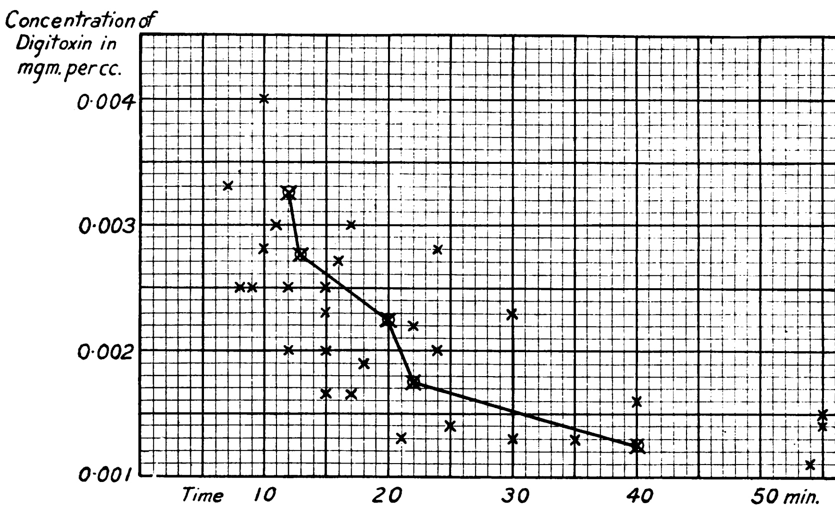

FIG. 5.

Curve showing the times at which different concentrations of digitoxin produce their maximal systolic effects upon isolated frogs' hearts.

X = single observation. ⊠ = average of a group of observations.

the heart for less than five minutes, and then the heart is washed out, no systolic effect follows; but if the digitoxin is allowed to act for eight minutes, and is then washed out, a systolic effect occurs (fig. 6). This systolic effect occurring after the digitoxin has been removed suggests that either digitoxin enters into some form of combination with the muscle from which it can only be slowly removed by washing out the heart, or else that the digitoxin initiates some slowly developing change in the heart muscle, which change can be continued in the absence of the drug. In fig. 4 it will be seen that when a very small amount of digitoxin is used the heart may develop a partial systole, and then recover without any of the drug being removed; this can only be explained by supposing that the heart either acquires a tolerance for, or else destroys, the drug.

(VI) The Mode of Action of Digitalis Glucosides.

Straub found that only minute traces of strophanthin were absorbed by hearts upon which it acted, and that the action depended entirely

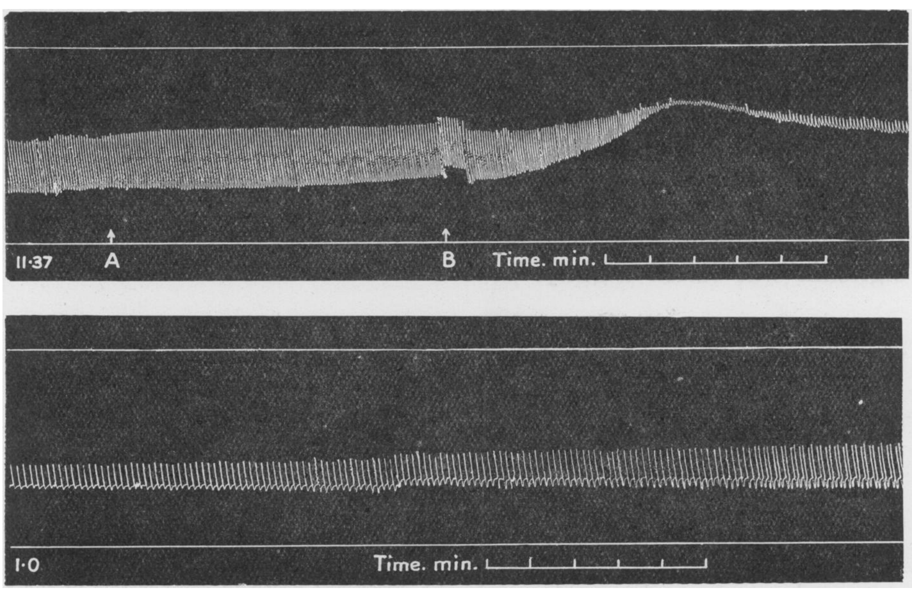

Fig. 6.

Isolated frog's heart perfused with 3 c.c. Ringer's fluid. **A**, 0·01 mgrm. digitoxin introduced; **B**, heart washed out with Ringer's fluid. Interval of one hour between the two tracings. (Tracing reads from left to right.)

upon the concentration of the drug; from these facts he concluded that strophanthin could not act by entering into combination with the heart muscle, and he suggested that its action was of a physical nature, possibly connected with an alteration of the surface tension. I agree with Straub's facts, except that I find that with minute quantities of fluid the action is slightly modified by the total quantity of drug present. I do not consider that these facts exclude the possibility of a chemical action. The amount of the drug taken up by the heart was very small, but in the case of strophanthin I found it was at least one-quarter of the amount which would kill a heart if introduced in 1 c.c. of fluid. Straub also found that 0·001 mgrm. of strophanthin in ½ c.c. of fluid killed a heart in systole, and that 0·0002 mgrm. was taken up by each heart. On the other hand, a chemical union between the heart muscle and the drug is certainly suggested by the fact that systolic effects, due to the action of digitoxin, may first appear when the digitoxin is no longer in contact with the heart, as is shown in fig 6.

(VII) The Effect of Alterations in the Composition of the Perfusion Fluid.

(*A*) The effect of reducing the osmotic pressure of the perfusion fluid was first investigated.

(*a*) Ringer's fluid diluted with an equal volume of water. The minimal lethal concentration of digitoxin was raised slightly.

(*b*) Ringer's fluid (NaCl half normal concentration, other constituents of Ringer natural). The minimal lethal concentration of digitoxin was raised slightly.

Table II.—Action of Digitoxin when the Ringer's Fluid is Diluted.

Number of experiment	Fluid used	Volume of cannula	Concentration of digitoxin	Effect
2.xv.8	Ringer diluted one-half	c.c. 0·75	0·004	Systolic effect in seven minutes; partial recovery; death in semi-systole in four hours
2.xv.9	Ditto	0·65	0·003	Systolic effect in fifteen minutes; partial recovery; death in semi-systole in three hours
2.xiv.3	Ditto	0·9	0·002	Slight systolic effect in twenty minutes; complete recovery
2.xv.7	Ditto	0·5	0·0016	No effect
2.xxi.1	Ringer, NaCl 0·3 per cent.	1·0	0·0025	Systolic effect in thirty minutes; recovery

(*B*) Effect of altering the concentrations of ions, the osmotic pressure being kept constant.

(*a*) Diminution in the concentration of NaCl. The osmotic pressure was kept equal to that of normal Ringer by the addition of cane-sugar; 6 per cent. of cane-sugar exercises an osmotic pressure equal to 0·65 per cent. of sodium chloride. In all these experiments large quantities of fluid were perfused through the heart.

TABLE III.—ACTION OF DIGITOXIN IN RINGER'S FLUID, NACL DIMINISHED, CANE-SUGAR ADDED IN SUFFICIENT QUANTITY TO MAINTAIN NORMAL OSMOTIC PRESSURE.

Number of experiment	Composition of fluid	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
2.xxv.7	Ringer, NaCl 0·26 per cent.	0·001	Systolic effect in ten minutes; complete recovery
2.xxv.3	Ditto	0·00125	Systolic effect in twenty minutes; complete recovery
2.xxvi.2	Ditto	0·00125	Systolic effect in forty minutes; death in semi-systole in a hundred and thirty minutes
2.xxv.1	Ringer, NaCl 0·13 per cent.	0·001	No systolic effect
3.xxvi.4	Ringer, NaCl 0·1 per cent.	0·0016	Ditto
4.o.1	Ditto	0·002	Systolic arrest in sixty minutes

If the NaCl was reduced below 0·1 per cent. the heart no longer maintained a steady beat. From the above table it will be seen that reduction of the NaCl content to 0·1 per cent. has no marked effect upon the action of digitoxin; the diminution in the toxicity of the digitoxin observed when the Ringer was diluted and no cane-sugar added must have been due to alteration of the osmotic pressure, and not to diminution in the concentration of the sodium ions.

(*b*) *Diminution of the Concentration of the Calcium Ions.*—If calcium-free Ringer is perfused through the heart the beat becomes steadily feebler and finally the heart is arrested in diastole; but if calcium-free Ringer is perfused through the heart until the beat is very feeble, and then circulation, with a small quantity of fluid, is established, the heart

TABLE IV.—ACTION OF DIGITOXIN IN CALCIUM-FREE RINGER.

Number of experiment	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
2.xxii.4.	0·004	Systolic arrest in twenty minutes
2.xxviii.3.	0·004	No systolic effect in thirty minutes
3.gg.1.	0·003	No systolic effect in sixty minutes
2.xxiii.3.	0·003	No systolic effect in one hundred and eighty minutes
2.xxii.2.	0·0023	No systolic effect in two hundred and forty minutes
2.xxii.2.	0·0023	No systolic effect in sixty minutes

will maintain this feeble beat for many hours; if digitoxin is now added it causes a stronger beat but no systolic effect is produced unless the concentration is more than twice the concentration that is sufficient to kill a heart in ordinary Ringer's fluid.

But if a little calcium is added to one of these hearts upon which the digitoxin has acted for some time, immediate systolic arrest occurs. Moreover, if the digitoxin is allowed to act upon the heart for some time and then the heart is washed out for ten minutes with calcium-free Ringer containing no digitoxin and then a little calcium is added,

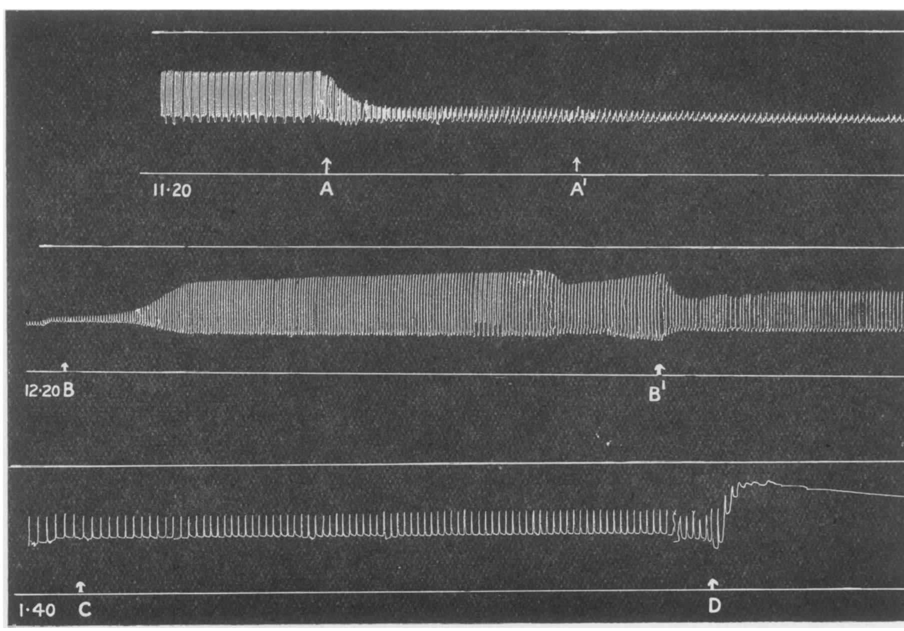

FIG. 7.

Tracing of isolated frog's heart. At **A**, perfused with Ringer's fluid containing no calcium; at **A'**, perfusion stopped and circulation of 3 c.c. of fluid established; at **B**, a trace of calcium chloride added; at **B'**, 0·01 mgrm. digitoxin added; at **C**, washed out with Ringer containing no calcium; at **D**, calcium chloride added to make 0·02 per cent. (Tracing reads from left to right.)

immediate systolic arrest occurs (fig. 7). From the figure it will be seen that the systolic arrest is not in the least like the systolic arrest produced by digitalis, but closely resembles the systolic arrest produced by barium chloride. A similar systolic effect is produced by adding calcium to a heart beating in 0·65 per cent. NaCl., but no similar effect can be produced by adding calcium even in excess to a heart beating in calcium-free Ringer unless the heart has been acted upon by one of the digitalis glucosides. It is, of course, impossible to free the heart entirely of calcium, but it would appear that digitalis acts upon the heart in such a way that when calcium is present the muscle contracts into systole, but that when calcium is absent systolic contraction cannot occur.

(*c*) *Calcium in Excess.*—Werschinin found that excess of calcium increased the systolic action of strophanthin, with normal Ringer 0·01 mgrm. per cubic centimetre of strophanthin was required to produce systolic arrest, but if the calcium content were increased to 0·09 per cent. then 0·002 mgrm. per cubic centimetre of strophanthin would produce systolic arrest. I found that increasing the calcium content above normal did not greatly affect the toxic action of digitoxin.

TABLE V.—ACTION OF DIGITOXIN IN PRESENCE OF EXCESS OF CALCIUM.

Number of experiment	Volume of cannula	Concentration of calcium	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
3.M.3.	2·0	0·1 per cent.	0·002	Death in systole in twenty minutes
2.xxxvii.2.	3·2	0·1 ,,	0·0013	No systolic effect
3.J.1.	2·5	0·12 ,,	0·0008	Death in semi-systole in one hundred and twenty minutes

Excess of calcium does not increase the toxic action of digitoxin. The percentage of calcium in Ringer's solution is about the optimum for an effective digitalis action.

(*d*) *Effect of diminishing the Concentration of the Potassium Ions.*—The heart usually would not maintain a regular beat in Ringer's solution without potassium, even when only a small volume of fluid was used, but when a regular beat did occur the action of digitoxin was slightly increased by the absence of potassium.

(*e*) *Effect of increasing the Concentration of the Potassium Ions.*—Increase in the amount of potassium present slightly diminished the action of digitoxin.

Alterations in the concentration of potassium do not appear markedly to influence the action of digitoxin.

(*f*) *Effect of altering the Alkalinity of Ringer's Fluid.*—Excess of alkali slightly increased the activity of digitoxin. If no alkali was present and the fluid was made faintly acid with lactic acid there was

TABLE VI.—SHOWING EFFECT OF VARYING THE CONCENTRATION OF POTASSIUM.

Number of experiment.	Concentration of potassium chloride	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
2.XXVII.3	*Nil*	0·002	Death in diastole in twenty-two minutes
2.XXVII.5	,,	0·001	Death in systole in twenty minutes
2.XXX.2	,,	0·0008	Death in systole in thirty-eight minutes
4.II.2	0·2 per cent.	0·004	Systolic arrest in ten minutes
2.36.1	0·04 ,,	0·0027	No effect in three hundred minutes
3.M.6	0·2 ,,	0·0025	Slight systolic effect followed by recovery
3.FF.3	0·2 ,,	0·0025	Systolic arrest in twenty minutes
4. *S*. 1	0·2 ,,	0·002	Systolic arrest in forty minutes
4. *S*. 2	0·1 ,,	0·002	Diastolic arrest in forty minutes

immediately a great diminution in the strength of the beat, and the heart often died in diastole, some hearts survived, and to these digitoxin was added. The presence of acid sometimes prevented systole occurring, but did not prevent the digitoxin killing the hearts, which died in diastole.

TABLE VII.—EFFECT OF EXCESS OF ALKALI OR PRESENCE OF LACTIC ACID UPON THE ACTION OF DIGITOXIN.

Number of experiment	Perfusion fluid	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
3.J.1.	Na_2CO_3 0·1 per cent.	0·001	Systolic arrest in thirty minutes
3.O.2.	,, 0·15 ,,	0·001	Systolic arrest in one hundred and twenty minutes
3.D.1.	Lactic acid 0·09 per cent.	0·0018	Systolic arrest in twenty-five minutes
3.D.5.	,, 0·02 ,,	0·0016	Diastolic arrest in thirty minutes
3.E.1.	,, 0·045 ,,	0·0016	Diastolic arrest in sixty-four minutes
3.D.7.	,, 0·045 ,,	0·001	No effect

The presence of acid does not therefore increase the action of digitoxin, nor does it protect the heart, but it may cause the heart to die in diastole rather than in systole.

(VIII) THE ACTION OF THE DIGITALIS GLUCOSIDES IN THE PRESENCE OF BLOOD SERUM.

Werschinin [9] investigated the action of strophanthin upon the heart, both in the presence of blood serum and also in the presence of certain isolated constituents of the serum. He determined the minimal concentration of the drug which would produce systolic arrest in the various fluids employed. He found that 0·01 to 0·008 mgrm. of strophanthin per cubic centimetre was required to produce systolic arrest in Ringer's solution, but that one-tenth this concentration (0·001 mgrm. per cubic centimetre) would produce systolic arrest if blood serum was added to the Ringer. He also found that the minimal lethal concentration was reduced, in a less marked manner, after the addition of washed rabbits' red blood corpuscles, of lecithin, of alcholic solution of the lipoid substances of the blood serum, or, finally, of excess of calcium. Using the serums of various animals I have obtained the following results:—

TABLE VIII.—ACTION OF DIGITOXIN AND STROPHANTHIN IN PRESENCE OF SERUM.

Hartung's apparatus—cannula 1 to 3 c.c.

Number of experiment	Fluid used	Drug	Concentration of digitoxin in milligrammes per cubic centimetre	Effect
3.8.5	Frog's serum ...	Digitoxin	0·0026	Systolic arrest in one hundred and twenty minutes
4.N.7	Dog's serum, two-thirds; aqua dest., one-third	,,	0·001	Slight systolic effect followed by complete recovery
3.L.3	Frog's serum ...	Strophanthin	0·001	Systolic arrest in one hundred minutes
3.P.3	Ditto	,,	0·001	Systolic arrest in sixty minutes
3.EE.2	Ditto	,,	0·00025	No systolic effect
3.F.2	Rabbit's serum, two-thirds; aqua dest., one-third	,,	0·0023	Systolic arrest in ninety minutes
3.F.3	Ditto	,,	0·0023	Systolic arrest in one hundred and twenty minutes
3.F.1	Ditto	,,	0·0015	Systolic arrest in one hundred and twenty minutes
3.G.3	Ditto	,,	0·001	Systolic arrest in one hundred and fifty minutes
4.r.2	Cat's serum, two-thirds; aqua dest., one-third	,,	0·00025	No systolic effect

The serum in these experiments was obtained by centrifugalizing fresh defibrinated blood. As will be seen, the action of both digitoxin and strophanthin was almost unaltered by the addition of serum, the minimal concentration of the drug causing systolic arrest was the same when dissolved in serum as when dissolved in Ringer's fluid, the only difference being that the arrest took rather longer to develop.

This result is very different from that obtained by Werschinin, but I believe this writer's results were due to instrumental error caused by the excessive diastolic pressure of the Williams's apparatus, for this tends to force any feebly beating heart into a condition of diastolic standstill; but a frog's heart to which serum has been added is in a much more vigorous condition than one perfused by saline alone, hence in a Williams's apparatus a dose of strophanthin given with serum will cause a heart to contract into systole, whilst the same dose given with saline produces an apparent diastolic arrest. With the apparatus that I used the diastolic pressure was low, and hence there was no difference between the action of strophanthin in serum or in saline.

SUMMARY.

(1) The action of digitoxin depends entirely upon the concentration of the drug, except when very small quantities of fluid are used.

(2) Digitoxin and strophanthin have no diastolic action upon a frog's heart perfused in the manner described in this paper.

(3) When digitoxin and strophanthin act on the frog's heart only minute quantities are absorbed, but the quantity is not so minute as to exclude the possibility of chemical combination between the drug and the heart muscle.

(4) The systolic action of digitoxin upon the frog's heart is dependent upon the presence of calcium.

(5) Diminution in the quantity of calcium in the Ringer's fluid diminishes the systolic action of digitoxin.

(6) The presence of excess of alkali or the absence of potassium slightly increases the systolic action of digitoxin.

(7) The presence of excess of calcium, or of blood serum, does not influence the systolic action of digitoxin.

Addendum.—Since the above article was written Grünwald [1] has published a paper dealing with the absorption of digitalin pur. (Merck) by the frog's heart perfused in Williams's apparatus. He found that the minimal concentration of digitalin which produced systolic arrest was 0·026 per mille; Werschinin [9] found the corresponding concentration of digitoxin was 0·01 per mille; digitalin is therefore about one-third as toxic as digitoxin. But Grünwald found that after perfusing 50 c.c. of a solution containing 1·3 mgrm. of digitalin through five hearts in succession a loss of toxicity occurred which corresponded to 0·07 mgrm. of digitalin per heart, and this would correspond to over twenty times the amount of digitoxin that I found was destroyed by a frog's heart. The figure obtained by Grünwald may possibly be due to dilute solutions of digitalin in Ringer losing strength on standing, in the same way that I found to occur with digitoxin. Grünwald also found that if hearts were perfused continuously, systolic arrest was produced by about one-half of the concentration of digitalin that was required to produce arrest when the fluid was circulated. This result appears very extraordinary, for I found that the concentration of digitoxin which was required to produce arrest was slightly higher when large quantities of fluid were perfused than when small quantities (e.g., 3 c.c.) were circulated.

I desire to take this opportunity to express my thanks to Professor Dixon for his valuable help, and advice, given during the course of these experiments.

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REFERENCES.

[1] GRÜNWALD. *Arch. f. exper. Path. u. Pharm.*, Leipz., 1912, lxviii, p. 231.
[2] HARTUNG. *Ibid.*, 1911, lxvi, p. 1.
[3] HEFFTER und SACHS. *Biochem. Zeitschr.*, Berl., 1912, xl, p. 83.
[4] HOLSTE. *Arch. f. exper. Path. u. Pharm.*, 1911, lxvi, p. 131.
[5] KRAILSHEIMER. *Ibid.*, 1910, lxii, p. 296.
[6] SCHMIEDEBERG. *Ibid.*, 1910, lxii, p. 305.
[7] STRAUB. *Biochem. Zeitschr.*, 1910, xxviii, p. 392.
[8] TRENDELENBURG. *Arch. f. exper. Path. u. Pharm.*, 1909, lxi, p. 256.
[9] WERSCHININ. *Ibid.*, 1909, lx, p. 328.
[10] *Idem.* *Ibid.*, 1910, lxiii, p. 386.

DISCUSSION.

The PRESIDENT (Professor W. E. Dixon, F.R.S.) said that the contribution contained many points new to him. First, Dr. Clark had thrown light on the question of how digitalis produced its effect. Straub contended that it was a matter of concentration, that digitalis, in other words, produced its action by some physical action, and that this action was directly proportional to the degree of concentration of the digitalis. But according to Dr. Clark this was not strictly true, so that the law previously enunciated held good only to a limited extent. It was clear that the total quantity of digitalis in the fluid was also an important factor to remember. The other point which he regarded with considerable interest was that if the frog's heart was perfused with digitalis or strophanthus until it was killed in systole, that heart had not taken up enough digitalis or strophanthus from the perfusing fluid to produce any decided action on another heart. It did not look to him, in view of this fact, as if the systolic standstill could be entirely a chemical phenomenon. If it were, one would surely expect the heart to take up enough digitalis from the solution to produce a decided physiological effect on another heart. As a matter of fact, the frog's heart specifically absorbed such a small quantity of digitoxin that it was within the range of experimental error. The last point he desired to refer to was that concerning calcium. Dr. Clark's experimental results in regard to that were most important, namely, that digitalis in three or four times a lethal dose produced little or no effect on the heart unless calcium was present, and that if digitalis in a calcium-free saline were perfused through a heart, it would go on beating for hours until a small quantity of calcium was given. Then the moment calcium was added the heart went into tonic contraction. He hoped that Dr. Clark might be able to tell them something as to the significance of this phenomenon.

Dr. H. H. DALE said he was impressed with this departure from Straub's rule as to the independence of the effect on volume, and its dependence solely on the degree of concentration when very small volumes were used. When one put the small volume into the frog's heart that organ was wet, it had not been dried, and so the interstices were filled with blood and serum. And if it were washed out with small quantities of Ringer's solution there must be an effect of dilution. That might be inappreciable when employing a considerable volume, but when using a small quantity it would be appreciable, and the smaller the quantity the greater would be the effect of that dilution. He wondered whether this was a valid criticism of Dr. Clark's contention that, with a very small volume one found a departure from Straub's rule.

Professor CUSHNY, F.R.S., said that he believed there was a curious diastolic effect in digitalis which Dr. Clark had not observed. If one gave a frog a minimal dose of any one of the digitalis series and opened the chest afterwards, when the circulation had ceased one would often find the heart in diastole, and it was constant if the dose was the smallest quantity which would stop the heart. But if one touched the heart, with the scissors or with a pin, one caused it to go into systole; not the whole heart, but if one drew a line with the pin along the diastole one would find a white line following upon the pin. There was a diastolic effect apart from the systolic, which was not an inhibitory phenomenon, because it occurred under atropine. Of course Straub did not get this, and he supposed that Dr. Clark did not get it either, because they had employed the perfused heart, and the cannula had the same effect as the pin. If one had an intact frog it would be found that the heart stopped in diastole. This was an old observation, which was not seen nowadays, because everyone used the heart attached to something which irritated it. But the early observers all saw this diastole effect. It was described forty years ago by Boehm. And Straub's idea that digitalis did not enter into combination with the heart was robbed of much of its significance, because digitalis or strophanthus acted in such inordinately small quantities—the amount which acted on the human heart was extremely small; ¼ mgrm. was distributed through the human body and yet it was sufficient to change the heart in an extraordinary way. The quantity was so minute that it could not be detected chemically.

Dr. CLARK, in reply, said he had not known about the heart dying in diastole as mentioned by Professor Cushny. With regard to the question of absorption of strophanthin, this was rather one of figures. The amount of strophanthin absorbed was considerably smaller than the amount of the drug required to kill a heart, even if only ½ c.c. or ¼ c.c. of fluid was used. This was the line Straub took up, that if one used such a small amount of fluid one could just kill the heart with a certain dose of strophanthin, but the amount which the heart took up was smaller than this dose of strophanthin. With regard to the question of the volume of the heart, the way in which he estimated the quantity of fluid was at the end of the experiment to weigh the heart and cannulæ with their contained fluid, then to empty and dry the heart and cannulæ, and weigh again, thus obtaining the weight of the fluid; in this way he was able to ascertain exactly the amount of fluid. The weight of the moist frog's heart was 60 mgrm., but the smallest quantity of fluid he used was 500 mgrm., so that the volume of heart was only 12 per cent. of the volume of the fluid. The minimal concentration of digitoxin which would kill a heart with only ½ c.c. of fluid was 0·0018 per cent., whereas with 3 c.c. the minimal concentration was 0·0012 per cent., so there was a difference of 33 per cent. between the lethal doses. These doses were so very small that one did not like to be too sure; it was so easy to have a slight error in that way. He was convinced that there was a definite increase in the concentration of digitoxin required to kill the heart when quantities of fluid below 1 c.c. were used.