THE ACTIONS OF DIGITALIS LEAF PREPARATIONS AND OF CARDIAC GLYCOSIDES ON THE ISOLATED RIGHT VENTRICLE OF THE GUINEA PIG

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This communication is concerned with a study of the direct action of digitalis leaf preparations and of cardiac glycosides on heart muscle by employing the isolated right ventricle of the guinea pig. The muscle is housed in a specially constructed glass vessel* which permits the muscle to be oxygenated with pure oxygen without mechanical agitation. The Ringer fluid, maintained at 35°, contains a high concentration of $CaCl_2$ (2·015 g./l.) which produces a large augmentation of the amplitude of contraction. This is recorded isotonically on a kymograph by a lever system. The muscle is stimulated twice each minute from platinum electrodes connected to an electronic stimulator.

When the preparation has settled in the bath a dose of a glycoside is added. This produces an increase in amplitude which passes through a maximum and finally diminishes to zero. Simultaneously the resting length of the muscle may increase or remain unchanged, depending on the dose, during the increase to maximum amplitude and then will invariably decrease as the amplitude diminishes to zero.

Such a tracing* permits the study of the action of the glycoside on heart muscle to be undertaken in detail. The $\log_{10}$ time to/or of: first increase in amplitude, the beginning of the plateau, that is 95 per cent of the maximum amplitude, maximum amplitude, duration of plateau, maximum resting length, that is the relaxation of the muscle relative to initial length, and zero amplitude can be observed, and also the effects produced, namely rate of increase of amplitude, maximum amplitude, and maximum and minimum resting lengths, can be measured.

These metameters can be used to compare the activity of one glycoside preparation with another, as well as yielding information on the several actions which each glycoside possesses.

The study was divided into two parts. Firstly two samples of *Digitalis purpurea* leaf, of different potencies as determined by slow intravenous infusion into guinea pigs, one sample of *D. lanata* leaf, and the 3rd International Standard Preparation of *D. purpurea* were extracted at room temperature with 80 per cent (v/v) ethanol. The samples were assayed in terms of the standard on the right ventricle preparation using a 3 + 3 assay design. Only one dose was allowed to act on each isolated ventricle, and each dose was administered to six preparations. From the metameters dependent on time the results in terms of the standard ranged

* The Figures for this communication appear in the full text which is published in the December number of the Journal on pages 741 to 754.

from 33 to 42 per cent and from 65 to 74 per cent for the two samples of *D. purpurea* leaf respectively, and from 177 to 227 per cent for the sample of *D. lanata*. Within each sample none of the results was significantly different. These results are in close agreement with those obtained for the same extracts by slow intravenous infusion into intact guinea pigs namely 32 per cent and 68 per cent respectively for the two samples of *D. purpurea* and 180 per cent for the sample of *D. lanata*. The potencies from the ventricle from metameters involving changes in amplitude and resting length were higher, namely 57 to 70 per cent and 72 to 111 per cent for the two samples of *D. purpurea* respectively and 243 to 334 per cent for the sample of *D. lanata*. The limits of error are extremely wide, and the results do not therefore differ significantly from those obtained from the time metameters.

The second part of the study was concerned with the actions of digoxin, ouabain, and a sample of digitoxin assaying at 1000 I.U. per g. by the guinea pig infusion method. The experimental technique was similar to that described for the digitalis leaf preparations. The number of doses was increased to cover those from 2·5 μg. to 160 μg. inclusively in a geometric ratio of 2, which permitted a more detailed study of the influence of dose on the relationship between the various effects produced by each glycoside.

Two distinct phases were noted for doses of each glycoside on maximum amplitude. With lower doses the maximum amplitude, corrected for initial amplitude, increased as the dose increased. Beyond a certain dose, different for each glycoside, the maximum amplitude decreased with increase in dose. With lower doses the time to maximum amplitude, beginning and duration of plateau, and zero amplitude was constant while the maximum amplitude itself markedly increased. With larger doses the time to these effects became shorter with increase in dose with a concomitant reduction in maximum amplitude and an increase in the rate of increase of amplitude.

The initial increase in resting length of the muscle varied inversely with the dose for digoxin and digitoxin whereas with ouabain, up to 20 μg. the length increased with the dose. With all three glycosides doses in the middle of the range produced a decrease in the resting length with increase in dose.

The metameters dependent on time gave potency ratios in terms of digoxin of 1·24 to 3·84 for digitoxin and 0·85 to 1·52 for ouabain. Those dependent on the actual changes induced ranged from 1·58 to 2·30 for digitoxin, and for ouabain 1·55 for the decrease in resting length and 1·39 for the phase of increase of maximum amplitude with increase in dose. Other estimates were invalid.

The results for digitoxin were heterogeneous, due to the estimate of 2·24 from the metameter, time to zero amplitude. If this is excluded digitoxin has 1·66 times the activity and ouabain 1·26 times that of digoxin on the right ventricle preparation of the guinea pig.

This implies that the general therapeutic and toxic actions of the three glycosides are similar. It does not mean, however, that each glycoside

possesses only one mechanism of action. The evidence presented suggests that with low doses the glycoside is actively taken up by the muscle and used optimally at a dose which produces the greatest increase of maximum amplitude, and that larger doses may be taken up passively and more rapidly and exert their toxic action on the same mechansim controlling systolic contraction or on another mechanism which is responsive only to higher doses of glycoside. Furthermore, there must be different mechanisms controlling the change in resting length of the muscle.

The comparative results for the three glycosides obtained by slow infusion into guinea pigs were 0·51 for the sample of digitoxin and 2·03 for ouabain in terms of digoxin. These are different from those obtained on the ventricle. Digitoxin is therefore less active in the acute test *in vivo* which may reflect its greater absorption by the non-cardiac tissues such as the liver and plasma. Ouabain is twice as active as digoxin *in vivo* which suggests that it may be less readily absorbed by the extracardiac tissues.

DISCUSSION

The short communication was presented by the AUTHOR.

DR. G. B. WEST (London). Why had the right ventricle been used and not the thicker left ventricle? Had the guinea pig been chosen because it was the animal normally used for testing digitalis, and had the right ventricle in the rat or the rabbit been tried? He also enquired if similar results were obtained with normal Ringer Locke solution containing the usual amount of calcium. The addition of adrenaline might also stimulate the amplitude of contraction.

PROFESSOR J. P. TODD (Glasgow). His colleagues had attempted to record the electrical output of the heart when stimulated with digitalis; electrocardiographs were recorded, but did not demonstrate a difference between cardiotonic and cardiotoxic activities.

DR. T. E. WALLIS (London). Was there a difference between the effect of the leaf and the effect of the glycoside?

The AUTHOR, in reply, said that the right ventricle, being a thinner tissue, was more sensitive to the action of glycoside. The pharmacopoeial method used the guinea pig, and the work was being extended to other parts of that animal. Rat and rabbit hearts had been tried but were both too insensitive. If the ordinary Ringer Locke solution were used, the height of the contraction obtained was too minute for immediate observation. The addition of adrenaline had been found to produce fibrillation. Other glycosides were being screened in the hope of finding some which had the therapeutic properties of the potent glycosides, but which were less toxic. Digoxin was possibly the best of the three glycosides tested, as with an eight-fold change of dose one was still on the phase of increase of maximum amplitude, whereas it was four times for digitoxin and twice for ouabain. Beyond these doses the underlying toxic effects came in; there was a greater width of therapeutic effectiveness with digoxin. He had tried the electrocardiograph technique but there

did not seem to be any correlation between the electrical and the mechanical changes in the muscle. Both leaf and glycoside seemed to give the same pattern of effects on the heart muscle. When the leaf extract was assayed, using the ventricle preparation by the infusion method, agreement was good, but it was not good for the action of the pure glycoside. As Professor Brindle and his colleagues had shown in their infusion experiments in the guinea pig, purpurea glycoside A was more potent than digitoxin, and the difference might be due to the presence of the primary glycoside.