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CARDIOTONIC ACTION OF TWO TANNINS

BY

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(WITH AN APPENDIX BY K. BOWDEN)

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A tannin isolated from *Paullinia pinnata* Linn., and tannic acid, have cardiotonic actions on the isolated perfused frog heart. Paullinia tannin is more firmly "fixed" than tannic acid. Tannin solutions contain peroxide, but the cardiotonic action is not dependent on this, since drugs believed to prevent peroxide formation, and sodium pyruvate which destroys peroxides, do not prevent the cardiotonic action. Maximal stimulation by tannin greatly reduces subsequent stimulation by ouabain. If calcium is omitted from the Ringer solution tannins cannot stimulate the heart. In this respect they differ from ouabain. However, the ouabain stimulation can be prevented by prior perfusion with tannin. It is suggested that the antagonism between tannin and ouabain is due to the former preventing ouabain from reaching its receptor sites, and that tannin stimulation is dependent on the formation of a calcium-tannin complex at the heart surface. In the isolated perfused mammalian heart preparation tannins increase diastolic tonus and coronary flow.

Paullinia pinnata Linn. is a member of the *Sapindaceae* family. It is a liana found in tropical West Africa and South America. Its alleged toxicity to fish, and its medicinal uses, are described by Hutchinson & Dalziel (1955). Extracts of the leaves exert a cardiotonic action on the isolated perfused frog heart. The active substance was isolated and is a tannin of the flavotannin type. The method of isolation and the identification of the tannin are described by Dr Bowden in an Appendix to this paper. This tannin is for convenience termed "paullinia tannin," but should not be confused with the tannin from the well-known *Paullinia cupana* Kunth described by Nierenstein (1922). *P. cupana* is of medical interest since "Guarana," a paste formed from its seeds, contains caffeine and theobromine.

METHODS

Perfusion of the isolated frog heart. Frogs were stored at 3° C. The dissection of the frog heart was similar to Bülbring's method as described by Burn (1952). Cannulae were tied into the inferior vena cava and into the left aorta. The right aorta was tied off. The perfusion method is shown diagrammatically in Fig. 1. The venous cannula can be fed with either Ringer solution or Ringer solution containing the drug to be tested. The solutions are contained in Marriotte bottles raised above the level of the heart. A constant head of pressure is maintained in the venous cannula by continuous suction through a tube connected to a filter pump. In most experiments the venous pressure was maintained at 2.5 cm. The aortic cannula is bent at right angles to itself, the height above the heart being adjustable. Fluid leaving the aortic cannula is collected by a small funnel leading to a drop-recording assembly. The cardiac output is recorded, in drops in a given time (usually each 10 sec or 30 sec), by a Thorp impulse counter. Heart movements are recorded on a smoked drum by a

lever situated below the heart. A perspex chamber protects the heart above and laterally from air movements and so reduces drying of the preparation.

The Ringer solution had the following composition: NaCl 6.5 g (112.0 mM), $CaCl_2$ 0.12 g (1.08 mM), KCl 0.14 g (1.88 mM), $NaHCO_3$ 0.5 g (5.95 mM), distilled water to 1 litre. In

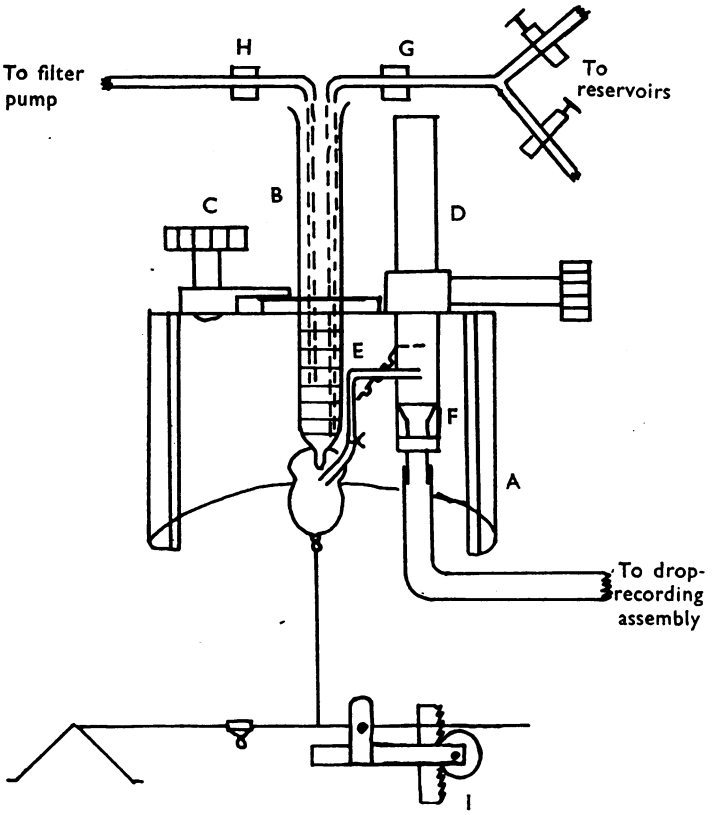

Fig. 1. Diagram of the frog heart perfusion apparatus. Frog Ringer solution is fed to the venous cannula (B) by means of tube (G). The cannula is graduated in cm, and the venous level is kept constant by aspiration via tube (H) leading to a filter pump. The aortic cannula (E) is supported by wire attached to the funnel support, and outflow from the cannula is collected by the funnel (F) leading to a drop recording assembly. Screw (C) holds the venous cannula in position. Screw (D) adjusts the height of the bar supporting the funnel and the aortic cannula. The heart is shielded from draughts above and laterally by a cylinder (A), the front part of which is removable and not shown in the diagram. The aortic cannula and the tubing are of polythene, and the rest of the construction is perspex. The level of the lever recording ventricular movements can be adjusted at "I."

low-calcium Ringer the calcium chloride content was reduced to 0.03 g (0.27 mM). In experiments where all calcium chloride was omitted, the apparatus was first washed through several times with distilled water to remove as far as possible traces of calcium. In attempts to suppress peroxide effects, the suppressant drug, either cysteine 1×10^{-4}, glutathione 2.5×10^{-4}, or sodium pyruvate 1×10^{-3} was added to the low-calcium Ringer solutions before the beginning of the experiment. The heart was perfused first with low-calcium Ringer containing suppressant drug, and then with the same solution with added tannin.

Estimation of peroxide. Peroxide was estimated by a modified Lommel's reagent consisting of 3-aminophthalhydrazide 0.1 g, 5 mg of mesoporphyrin, 2 drops of 5% ferric chloride and sodium carbonate 1 g made up in 100 ml. of distilled water. The solution was freshly prepared. Five ml. of the reagent was mixed with 10 ml. of the solution to be tested. Observations were made in the dark-room after thorough adaptation of the eyes. The luminescence produced by test solutions was compared with that of freshly prepared dilutions of hydrogen peroxide (Krayer, Linstead & Todd, 1943).

Isolated perfused mammalian heart (Langendorff's preparation). The hearts of rabbits and cats were perfused with Ringer–Locke solution aerated with oxygen. The heart was enclosed in a perspex chamber so that drying was reduced. Injections of drug were made close to the orifices of the coronary arteries via a polythene cannula. The heart rate was recorded by a Thorp impulse counter, and the coronary flow by a float recorder.

RESULTS

Isolated perfused frog hearts. When perfused continuously with tannin dissolved in low-calcium Ringer both the force of the ventricular contraction and the cardiac output were increased (Fig. 2). With paullinia tannin this effect lasted for about 20 min, after which force of contraction and cardiac output were reduced. Systolic arrest of the ventricles sometimes occurred. The effect on the heart rate was inconstant, but usually there was a slight increase at the height of stimulation, followed by slowing as toxic effects supervened. Tannic acid washed out more readily than paullinia tannin. Maximal stimulation with tannin largely prevented subsequent stimulation with ouabain. Paullinia tannin was active in a concentration of 1×10^{-5}, though in most experiments with tannins concentrations of 1×10^{-4} were used. When freshly prepared no precipitation appeared in the Ringer solution. In full-calcium Ringer the stimulant action of the tannin was less evident. Hearts perfused with calcium-free Ringer became very feeble and sometimes failed to move the heart lever. Ouabain 2×10^{-6} regularly stimulated in the absence of calcium, though there was sometimes a long latent period before the stimulation began. Tannins did not stimulate in calcium-free Ringer (Fig. 3). But in other experiments using calcium-free Ringer it was found that paullinia tannin prevented subsequent stimulation by ouabain even though the tannin itself caused no stimulation. Tannin caused slight stimulation of isolated frog hearts depressed by acetylcholine 1×10^{-8} dissolved in the Ringer. D-catechin 1×10^{-4} showed no cardiotonic action. Gallic acid monohydrate 1×10^{-4} was tested on four hearts. On two preparations it produced depression ; on the other two there was a cardiotonic action which was more prolonged than that produced by the tannins.

Presence of peroxide in tannin solutions. Since peroxides have cardiotonic actions (Mendez, 1944), low-calcium Ringer solutions of tannins were tested for the presence of peroxides by means of Lommel's reagent. Concentrations of 1×10^{-4} of paullinia tannin contained, in various experiments, from 1 part in 500,000 to 1 part in 2 million w/v peroxide when compared with dilutions of hydrogen peroxide. In some experiments perfusate from the aortic cannulae of stimulated hearts was also tested and found to contain similar quantities of peroxide. Tannic acid *B.P.* and gallic acid monohydrate, both in concentrations of 1×10^{-4}, contained about 1 part in 1 million of peroxide. No peroxide could be detected in solutions of paullinia tannin

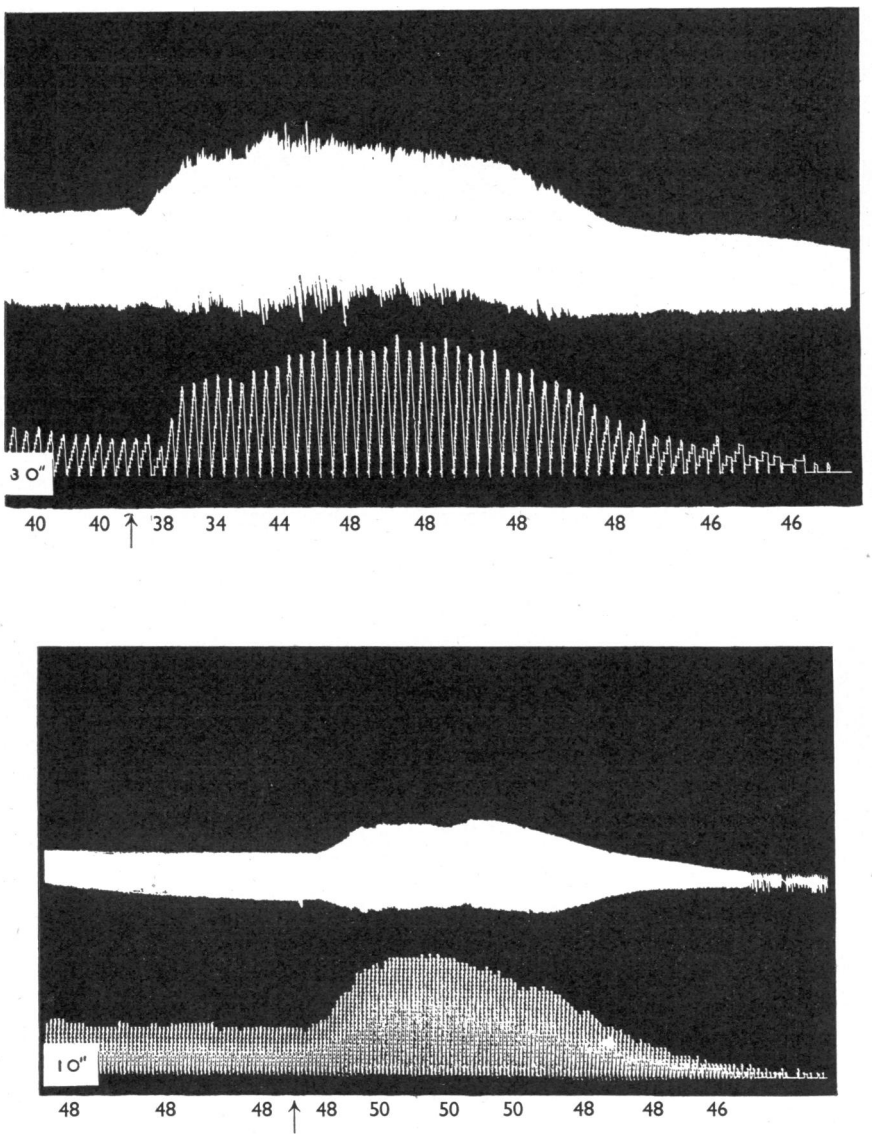

Fig. 2. *Upper recording:* An isolated frog heart perfused with low-calcium Ringer. From above downwards are recorded ventricular movements (systole being shown by an upwards movement), cardiac output each 30 sec, and the heart rate in beats per min. At the arrow the perfusion fluid was changed to low-calcium Ringer containing paullinia tannin 0.1 mg/ml. *Lower recording:* Preparation as before. At the arrow the perfusion fluid was changed from low-calcium Ringer to low-calcium Ringer containing tannic acid 0.1 mg/ml. The cardiac output is recorded each 10 sec. Paullinia tannin and tannic acid both increase the force of ventricular contraction and the cardiac output.

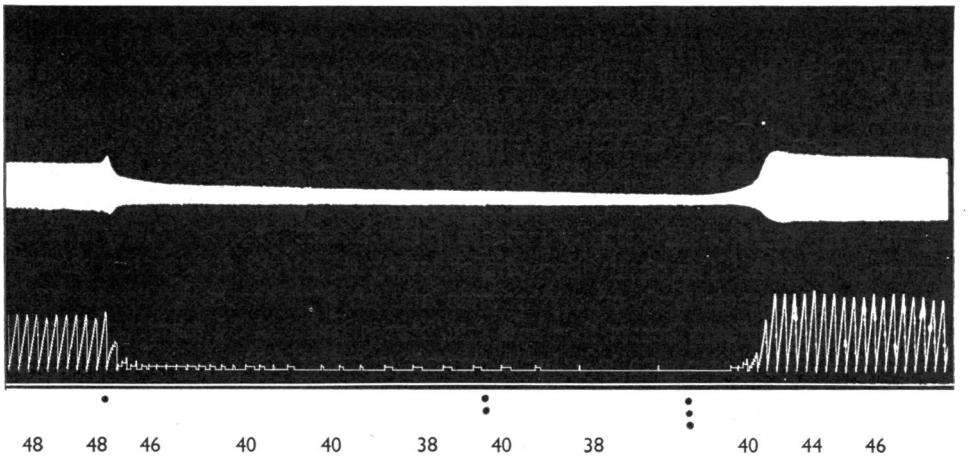

Fig. 3. Isolated frog heart perfused first with low-calcium Ringer. This was changed to calcium-free Ringer at the dot, then to calcium-free Ringer containing paullinia tannin 0.1 mg/ml. (2 dots), and finally (3 dots) back to low-calcium Ringer. Cardiac output is recorded each 30 sec. The heart rate is in beats/min. Paullinia tannin does not stimulate the heart when there is no calcium in the perfusion fluid.

1×10^{-5}, D-catechin 1×10^{-4} or ouabain 2×10^{-6}. The lower limit of sensitivity of the peroxide test, in these experiments, varied between 1 part in 2.5 millions and 1 part in 5 millions of peroxide.

Drugs believed to suppress the formation of peroxide, such as cysteine and glutathione, did not prevent the action of the tannins. Nor did sodium pyruvate, which destroys peroxide. Addition of pyruvate, in a concentration of 1×10^{-3}, to low-calcium Ringer reduced the peroxide content from 1 part in 1 million to less than 1 part in 2.5 millions—the limit of sensitivity of the test—yet a cardiotonic action was still seen.

Isolated perfused mammalian heart (Langendorff's preparation). On cat and rabbit hearts small doses of paullinia tannin (1 to 2 mg) produced a slowly developing increase in diastolic tonus. This was sometimes preceded by a brief increase in the force of the systolic movements. The coronary flow was increased.

DISCUSSION

Possible actions of tannins on the heart do not seem to have aroused much interest, though Handovsky & Masaki (1923) noticed that " Japanese " tannin caused systolic arrest of the frog heart and, in smaller amounts, prevented the action of saponin on the heart. Our experiments indicate that two tannins, at least, must be added to the long list of drugs that stimulate the isolated perfused hypodynamic frog heart. Several cardiotonic drugs, ascorbic acid (Krayer, Linstead & Todd, 1943), and some unsaturated lactones (Mendez, 1944), are believed to act by forming

peroxides in their solutions. Solutions of the two tannins contain small quantities of peroxide; but the cardiotonic action is apparently not due solely to peroxide, since 1×10^{-5} dilutions of paullinia tannin were active when no peroxide could be detected. Also it was possible to destroy peroxide by adding sodium pyruvate 1×10^{-3} and still obtain a cardiotonic action. Glutathione and cysteine, believed to prevent peroxide formation, did not abolish cardiac activity. No attempt was made to estimate peroxide in glutathione and cysteine solutions, as Mendez (1944) showed that these could actually increase luminescence in the strongly alkaline Lommel's reagent. The observation of Handovsky & Masaki, that tannin prevents the action of saponin both on erythrocytes and on frog hearts, led us to try to stimulate with ouabain hearts that had first been stimulated by tannin. Maximal stimulation with tannin greatly reduces ouabain stimulation.

The stimulant action of Ca^{++} on the frog heart is well known and has been the subject of recent studies (Moulin & Wilbrandt, 1955; Niedergerke & Lüttgau, 1957; Niedergerke & Harris, 1957). Several cardiotonic drugs have in common the properties of being fixed by heart cells and forming sparingly soluble calcium salts. They may act by forming a calcium-drug complex at the heart surface. Such drugs are lipoids, oleates and fluorides (Loewi, 1955). Tannins form insoluble calcium salts and the stimulant action seems to be dependent in some way on the presence of Ca^{++} in the perfusion fluid. This was demonstrated by perfusing hearts with Ringer from which calcium was omitted. The tannins did not then stimulate. In this respect they differ from ouabain, which can regularly stimulate though sometimes after a long latent period. This is not conclusive evidence that Ca^{++} is not required for the stimulant actions of ouabain, since traces of the metal may be present in the perfusion fluid or there may be mobilization of calcium already present in the heart (see Hadju & Leonard, 1959). However, it is clear that, whereas stimulation by tannin is dependent on greater than trace quantities of Ca^{++} in the perfusion fluid, stimulation by ouabain is not. Though tannins cannot stimulate in the absence of Ca^{++} it seems that tannin is still being fixed by the heart since ouabain stimulation is prevented. This may be compared with the action of tannic acid on erythrocytes, where tannin protects against the lytic action of soaps and saponins by attaching itself to protein of the cellular membrane. The permeability of the erythrocyte membrane to anions is also reduced (Edelberg, 1952; 1953).

Experiments with isolated mammalian hearts confirm that a brief application of paullinia tannin produces prolonged effects. The main action is an increase in the diastolic tonus of the heart. The coronary dilatation is worthy of note.

Finally, the presence of tannin in the leaves of *Paullinia pinnata* Linn. offers a rational explanation for the folk uses of the plant as a local haemostatic and in the treatment of dysentery.

It is a pleasure to acknowledge the technical assistance of Mr T. A. Stanbridge, and to thank him and Mr D. Groves for the construction of the isolated frog heart assembly. Mr D. Opara, of the University College of Ibadan, Nigeria, supplied the *Paullinia pinnata* leaves. Dr W. A. Bain has kindly read the manuscript and made helpful suggestions.

APPENDIX

ISOLATION FROM *PAULLINIA PINNATA* LINN. OF MATERIAL WITH ACTION ON THE FROG ISOLATED HEART

BY

K. BOWDEN

Dried, powdered leaves of the plant (90 g) were extracted for 8 hr with boiling methanol in a Soxhlet apparatus. Ether was carefully added to the hot methanolic extract until no further precipitation occurred. The mixture was cooled in a refrigerator overnight, the precipitate centrifuged down, washed with ether and dried in vacuum. The solid so obtained was dissolved in water (300 ml.), filtered to remove the small amount of insoluble material, and 20% aqueous lead acetate (50 ml.) was added to precipitate the active material as a lead salt. This was centrifuged, washed with water and then dissolved in 2N-acetic acid (50 ml.). Lead was removed by adding 20% aqueous disodium hydrogen phosphate (50 ml.) and the precipitated lead phosphate centrifuged down and washed with water. The combined aqueous solutions were freeze-dried and the active material extracted from the resulting solid by boiling with methanol (300 ml.), filtering hot and adding ether to the methanolic filtrates. The precipitate was finally washed with ether and dried in vacuum. By this method 2 g of a fawn-coloured amorphous powder was obtained. It contained no nitrogen, but ignition indicated the presence of some inorganic material which could be reduced, but not completely removed, by taking up in a small amount of hot methanol, filtering hot and reprecipitating with ether. The material did not melt below 200° C, but above this temperature it slowly charred.

Chemical nature of the cardio-active material. An aqueous solution of the material showed strong absorption at 200, 278 and 327 mμ. Its lead salt, examined as a disc mixed with potassium bromide, absorbed strongly at 1,612, 1,491 and 800 cm^{-1}, indicating the presence of benzenoid rings.

The material reduced ammoniacal silver nitrate slowly and became reddish-brown on standing in dilute alkali. It gave a bluish-black precipitate with aqueous ferrous sulphate, indicating the presence of polyhydric phenolic groups. Aqueous solutions of gelatine or quinine sulphate gave immediate precipitates with the substance, pointing to a tannin-like structure. An immediate precipitate with bromine water indicated a catechol, rather than pyrogallol, tannin. A negative reaction for phloroglucinol compounds was obtained in the pine splint/hydrochloric acid test. A positive reaction with formaldehyde/hydrochloric acid tended to confirm the presence of a catechol tannin. Attempts to hydrolyse the material and detect gallic acid and a carbohydrate gave no trace of either, again tending to rule out a gallic acid tannin. Further evidence in support of this view was the absence in the infra-red

of absorption which might be attributed to ester groups. Preliminary indications are, therefore, that the cardio-active material present in *Paullinia pinnata* Linn. is a condensed tannin (flavotannin).

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