In one rabbit 0.75 c.c. of a 2 per cent. solution of the acid sulphate of berberine was also tried and there was no indication of any inflammation. The drug, therefore, can safely be given in 1 or 2 per cent. solutions hypodermically or intramuscularly. The greater solubility of the acid sulphate makes it possible to give smaller amounts of fluid and minimises the discomfort produced by larger amounts. The actions of the two salts are identical.

Berberine sulphate was also tried clinically in cases of oriental sore and gave very satisfactory results.

Technique.—1 to 2 c.c. of a 1 per cent. solution of the sulphate or 0.5 to 1 c.c. of a 2 per cent. solution of the acid sulphate, according to the size of the sore, is injected as follows. The needle is inserted just above the margin of the sore and the solution injected near its edge. Four or more punctures are made so as to infiltrate the sore from all sides. The injections are repeated a week after and as a rule not more than three injections are required to bring about a complete cure. Two injections are often quite sufficient.

The solutions are very stable and can be preserved in sterile tubes with rubber caps, requisite amounts being withdrawn by the syringe whenever required. The treatment is not very painful and does not involve any risk as the injections are given locally. No expert skill is required to give the injections and the treatment can be given in any outlying dispensary by any medical man, the only apparatus required being a simple hypodermic syringe. The drug is very cheap; the approximate cost of the drug required for treating one sore being somewhat less than 3 pice.

Following is a summary of reports of cases treated with berberine sulphate:—

Case No. 1.—B. C. Hindu male, age 13 years.
An ulcer about the size of an eight-anna piece on the forearm. Duration 4½ years. Smear from the edge showed a large number of *L. tropica.*
Six injections of stiburea commencing from 0.05 grm. and rising up to 0.15 grm. were given twice a week, without any improvement in the lesion.
Two injections of berberine sulphate were then given by the infiltration method using 1.5 c.c. of a 1 per cent. solution. The scab fell off leaving a raw surface and the sore was then dressed with ung. zinci for 3 or 4 days. The sore healed up perfectly.
Case No. 2.—Mrs. P. Hindu female, age 32 years.
Lesions.—Two ulcers on fore-head, 1 on cheek, 2 on the back of wrist.
Duration.—The oldest one appeared 8 months back.
Lab. findings.—Parasites present in all lesions.
Each sore was treated with 3 weekly injections of 1.5 c.c. of 1 per cent. solution of berberine sulphate. All healed up within a month.
Case No. 3.—Mr. B. Hindu male, age 50 years.
Lesions.—Three sores on fore-arm. All appeared about the same time.
Lab. findings.—Parasites present in all.
He had 2 injections of berberine sulphate 1.5 c.c. of 1 per cent. solution. Discharged cured.
Case No. 4.— Mr. S. Hindu male, age 45 years.
Lesions.—Two sores on the leg, both chronic, one of 8 years duration, the other about 1 year.
Lab. findings.—Old lesion was negative to leishmania, but scanty bottle bacilli were found.
Culture from the 2nd gave a good growth of flagellates.
The patient had received 22 injections of antimony (no information about the salt and the dose) and the sore was cauterized twice.
Four injections of berberine sulphate were given at weekly intervals. The patient had a good recovery.
Case No 5.—Mrs. S. Hindu female.
Lesion.—A sore on the nose; was treated with mercuric ointment.
Lab. finding.—Leishmania were present. Berberine sulphate injected as before; 2 days after the 1st injection, smear negative; culture after 5 days, negative. One more injection was given and the sore was completely healed.

In case No. 2, material from the lesion on the cheek which had not yet ulcerated was cultured on N.N.N. medium and a rich growth of flagellates was obtained. 0.2 c.c. of water of condensation from 2 tubes of N.N.N. culture medium was injected into the shaved skin of an English mouse. A small nodule was produced showing scanty leishmania. The nodule was then treated with berberine sulphate solution (10 times weaker than that used for human beings). The nodule disappeared within 7 days. The mouse died later of some intercurrent disease.

Conclusions.

(1) Berberine sulphate inhibits the growth of *L. tropica* even in high dilutions like 1 in 80,000. Stibosan has no local action on the parasite. This may be the reason why antimony ointments are useless in the treatment of oriental sore.

(2) Berberine sulphate given by infiltrating the tissues around the edge of the sore, brings about complete cure in 2 or 3 weeks. The drug promises to be a specific cure for oriental sore.

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TERMINALIA ARJUNA: ITS CHEMISTRY, PHARMACOLOGY AND THERAPEUTIC ACTION.

By R. N. CHOPRA, M.A., M.D. (Cantab.),
LIEUTENANT-COLONEL, I.M.S.,
In-charge, Indigenous Drugs Inquiry, Indian Research Fund Association,
and
SUDHAMOY GHOSH, D.SC., F.R.S.E.
(From the Department of Pharmacology and Chemistry, Calcutta School of Tropical Medicine and Hygiene.)

HISTORY, BOTANY, USES, ETC.

Terminalia arjuna is a large deciduous tree attaining a height of 60 to 80 feet which is common throughout the sub-Himalayan tracts of the United Provinces, and in the Deccan, Southern Bihar, Chota Nagpur, Burma and Ceylon. It is

called "Arjun" in Hindi and Bengali and "Arjuna" in Sanskrit. The bark is $\frac{1}{3}$ inch thick and is considered by the Sanskrit writers to be a cardiac tonic. Chakradatta, the great Hindu physician, described it as tonic, astringent and cooling, and prescribed it in heart disease and for those purposes for which astringents are generally applied. In heart disease he recommended it to be given as a decoction with milk and treacle and water or as a "ghrita" (preparation with "ghee" or melted butter) made with the decoction and powder of the bark.

The bark and preparations made from it are reputed to have a marked action on the heart even to the present day in this country. The "Kabirajes" use them for all sorts of conditions of cardiac failure and dropsy. Some of the practitioners of western medicine believe in its stimulant effect on the heart and use it as cardiac tonic. A liquid extract prepared from the bark is on the market in Calcutta. It was for this reason that we took up investigation of this drug.

CHEMICAL COMPOSITION.

A reference to the literature shows that this drug has interested many previous investigators. Hooper (1891) mentioned that the bark yields 34 per cent. of ash consisting of almost pure $CaCO_3$; the watery extract contains as much as 23 per cent. of calcium salts and 16 per cent. of tannins. Ghoshal (1909) made a detailed chemical and pharmacological study of the bark. He found it to contain the following substances:—(1) sugar, (2) tannins, (3) a colouring matter, (4) a body of glucosidal nature and (5) carbonates of Ca and Na and traces of chlorides of alkali metals. He also found that the total tannin content amounted to 12 per cent. and the content of ash to 30 per cent.

The present investigation was started in order to determine the active principle which might be responsible for the alleged stimulant action of the drug on the heart. The bark was obtained from a reliable source and was dried in air. As the drug was said to contain glucosides, an enormous amount of careful and laborious work extending over a year was done to prove or disprove their presence. A portion of this work was done by one of us (S. G.) in the Pharmaceutical Institute of Berlin. A brief summary of the work and the procedure adopted is given in the following paragraphs:—

For a systematic examination, about 200 grms. of the powdered bark was carefully weighed out and extracted in a large glass soxhlet for several days with petroleum ether (B. P. 35°—50° C.). The yield of the petroleum ether extract, freed from the solvent was only 0.13 per cent. It contained no oily matter and when fractionally crystallised from dilute alcohol gave a minute quantity of colourless crystals which gave the reactions of a phytosterol. Nothing else could be isolated from the other fractions.

The residual powder, after extraction with petroleum ether, was extracted with ether for some days until the extraction was complete. The ethereal extract, freed from the solvent, amounted to 0.37 per cent. The substance was recrystallised from ether and some colourless crystals were isolated. The dried crystals softened at 225° and melted with decomposition at 230°C. On testing they were found to be those of an organic acid but the yield was very small. Nothing else was found in the ethereal fraction.

The residual powder, after extraction with ether, was then extracted for several days with absolute alcohol until the extraction was complete. The yield of the absolute alcoholic extract, freed from solvent, amounted to 18.12 per cent. The residue was dark red, brittle and amorphous and gave strong colour reactions for tannins.

To test for alkaloids, a portion of its solution in alcohol was treated with a few drops of acetic acid, the alcohol was removed and the residue taken up in water. The filtered aqueous solution was made slightly alkaline with Na_2CO_3 and shaken with ether and $CHCl_3$. No alkaloid could be thus traced in the alcoholic extract.

The alcoholic extract was soluble in acetone, hardly so in acetic ester and chloroform and partially soluble in amyl alcohol. A separation of the constituent tannins and colouring matters was attempted by fractional precipitation with different organic solvents but no satisfactory separation could be effected.

To determine the nature of the tannins, an aqueous solution was tested with various reagents. With $FeCl_3$ it gave a bluish-black precipitate. When poured into a solution of KOH, it gave a deep red colour. With concentrated H_2SO_4, a few drops of the solution gave a red colour. With a solution of iron alum it gave an olive colour and on adding some solid sodium acetate there was a bluish black colour. 50 c.c. of the original solution and 25 c.c. of a mixture of HCl and formalin (100 c.c. of concentrated HCl, 100 c.c. water and 150 c.c. of 40 per cent. formaldehyde) were boiled under reflux for $\frac{1}{2}$ hour. A precipitate was obtained showing the presence of pyrocatechol tannins. The filtrate gave with iron alum and solid sodium acetate a bluish black colour. With bromine water the original solution gave a precipitate. A portion of the solid alcoholic extract was fused with KOH and the melt dissolved in water, filtered, acidified with dilute H_2SO_4, filtered and extracted with ether. The residue from ether was decolourised in alcoholic solution with animal charcoal and the filtrate evaporated to dryness. The residue dissolved in water gave a green colour with $FeCl_3$ which was turned red with Na_2CO_3 and violet with Na-acetate; with Br_2 water it gave no precipitate. With ammonium molybdate, it gave a reddish brown colour.

All these reactions show that the tannin in the arjun bark is mainly a *pyrocatechol tannin*.

An attempt was also made to determine if any substance of glucosidic nature could be separated. The alcoholic extract was dissolved in hot water and treated with lead acetate and basic lead acetate. The filtrate was freed from lead by H_2S and concentrated *in vacuo* to a small bulk. The concentrated solution was filtered and evaporated to dryness in a vacuum desiccator over H_2SO_4. Nothing separated out during the evaporation over H_2SO_4. The residue was taken up in absolute alcohol and filtered. The filtrate was concentrated to a small bulk, treated with excess of chloroform and filtered. The filtrate was evaporated to dryness and the residue taken up in water. It did not reduce Fehling's solution. On hydrolysis with HCl, a white precipitate was obtained but the hydrolysed product did not reduce Fehling's solution.

As the amount of the above substance was too small for further work, a large quantity of the bark was extracted with alcohol. The alcohol was recovered and the residual powder dissolved in hot water. To avoid the presence of acetates for pharmacological trials, the aqueous solution was treated with excess of lead hydroxide (freshly precipitated and washed free from soluble salts). The filtrate was freed from Pb by H_2S and the filtered solution concentrated *in vacuo* to a small bulk. The filtered liquid was finally evaporated to dryness *in vacuo* over H_2SO_4 and treated in the above way. The residue was optically inactive. It was easily hydrolysed by HCl but the hydrolysed solution was also inactive. It did not reduce Fehling's solution either before or after hydrolysis. It was also found to be pharmacologically inactive. It was, therefore, not a glucoside but probably an ester-like compound.

The original bark, extracted with petroleum ether, ether and absolute alcohol, was next extracted 5 times with large bulks of boiling water. The aqueous filtrates were concentrated *in vacuo* to a very small bulk and precipitated by a large bulk of alcohol.

The precipitate was dried in a desiccator. When burned it gave a large amount of ash. It probably consisted of inorganic salts and salts of organic acids and was not deemed of pharmacological interest. The ash showed the presence of Al, Mg and large quantities of Ca.

The filtrate, freed from alcohol, was precipitated by lead acetate and then by basic lead acetate, the filtrate being freed from Pb and concentrated *in vacuo*. It showed the presence of only sugars. The lead acetate and basic lead acetate precipitates, which were small, were also tested separately but nothing of any interest could be detected.

100 grams of the arjun bark were then tested for glucosides by Bourquelot's process by the use of pure emulsin. No change of rotation could be observed showing the absence of glucosides (excluding tannins and colouring matters).

Estimation of tannins.—

(1) Hyde powder method: Schroder's process was used and gave 11.21 per cent. of tannins.

(2) Blood method: The hæmolytic process of Brandt was followed using fresh ox blood collected from a slaughter house. The first series of experiments gave the limit between 10 and 20 per cent. and the second series, with higher dilution, gave the final value at 12 per cent. (The method is described as approximate but comparable.)

An estimation by the Pb method was also carried out as a control to see the upper limit. The value obtained was 15 per cent. (It is generally high, since lead acetate precipitates not only the tannins but also the colouring matters and some other substances.)

The result of the chemical analysis may be summarised as follows:—

(1) About 12 per cent. of tannins, consisting mainly of pyrocatechol tannins.

(2) Some colouring matters.

(3) An organic acid with a high melting point and a phytosterol.

(4) An organic ester easily hydrolysed by mineral acids.

(5) Large amounts of calcium salts with smaller amounts of Al and Mg salts.

(6) Sugar, etc.

Pharmacological Action.—It will be seen that analysis of the bark of *terminalia arjuna* does not reveal the presence of active principles which could account for its cardiac tonic effects, which are so widely believed in in this country We carefully tested the different fractions obtained from petroleum ether, alcoholic and aqueous extracts during analysis, but found that with the exception of calcium compounds, no other constituent produced any effect on the heart or on any of the other tissues. The colouring matter was separated and tested with the same result. It will be seen, therefore, that we could not detect any pharmacologically active bodies in the bark of *Terminalia arjuna* either by chemical or biological methods.

Clinical Trials.—An alcoholic extract was prepared from the bark in our laboratory. It was carefully tested in a number of patients suffering from failure of cardiac compensation with or without dropsy. In none of the patients the drug did produce any marked effects such as are produced by drugs of the digitalis or caffein groups. The rate, frequency, force of the heart beat and the blood pressure remained appreciably unaltered. The secretion of urine was not markedly affected in these cases. Any therapeutic effects attributed to the drug may be accounted for by the high calcium content to which reference has already been made.

Summary and conclusions.

(1) An analysis of the bark of *Terminalia arjuna* does not reveal the presence of any active principles of the nature of alkaloid, glucoside or essential oil. With the exception of large amounts of calcium salts, tannins, organic acids, an organic ester and sugars no other substances could be detected.

(2) Different fractions obtained during the course of analysis from the bark including the petroleum ether, alcoholic and aqueous extracts and colouring matter were found not to show any marked physiological activity.

(3) An alcoholic extract prepared from the bark was tried in a number of patients suffering from cardiac decompensation but it did not show any appreciable effects such as are produced with the cardiac tonic drugs of the British Pharmacopœia.

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A PRELIMINARY NOTE ON THE ACTION OF *VASOPRESSIN* AND *OXYTOCIN*.*

By J. CHRISTODOSS DAVID, M.B., B.S.,
Professor of Pharmacology,
and
C. VAREED, M.B., B.S.,
Demonstrator in Pharmacology.
(*From the Department of Pharmacology, Madras Medical College.*)

THE physiological properties of extracts of the posterior lobe of the pituitary body are well known. Briefly, they cause marked vaso-constriction and raise the blood pressure; they stimulate uterine contractions and are also diuretics. As opposed to this last observation, they markedly reduce the secretion of urine in the disease called diabetes insipidus. But chemical information regarding the physiologically active constituents of pituitary extracts is very meagre. Much controversy has raged round the question, whether these various physiological activities are due to one hormone or to more than one active principle. The general conclusion that can be drawn, from the work that has been done, is that the physiological activity of extracts of the posterior lobe of the pituitary body is due chiefly to two active principles, one tending to cause a rise of blood pressure and the other having a specific oxytocic action. According to Schafer, the principle which acts upon intestinal muscle differs from the above two and there is little doubt that it is histamine. It is probably histamine that is also responsible for the primary fall of blood pressure observed in some samples. Such samples of the extract as do not exhibit this phenomenon have little action on the intestinal muscle.

Recently O. Kamm and his associates (1928) working in the research laboratory of Parke Davis & Co. have succeeded not only in demonstrating the presence of the two important active principles but they have also been abled to separate these two and concentrate them in the form of potent solid preparations. They have shown that solutions of these separated active principles can be recombined to form a pituitary extract identical with the original from which they were prepared, thus proving that no decomposition has taken place. Both active principles are said to be basic bodies, presumably amines; practical manufacturing methods have been developed for the separation of these two hormones and these have been made available for experimental and clinical study. The oxytocic principle named *a-hypophamine* is put up in ampoules and designated *oxytocin*. The pressor principle or *b-hypophamine* has been labelled *vasopressin*. There is at present only one official standard for extracts of the posterior lobe of the pituitary gland and this is based upon the oxytocic test as described in the U. S. P. The amount of activity contained in 1 c.cm. of the official extract has been designated "10 international units." When extracts are prepared from a good grade of gland there is a fairly constant relation between the amount of oxytocic and pressor activities. So the original workers have designated the amount of pressor activity in 1 c.cm. of the official extract as "10 pressor units." Ordinary pituitrin as marketed for obstetrical purposes, therefore, contains 10 oxytocic units and 10 pressor units per c.cm. Pituitrin as marketed for surgical purposes contains 20 oxytocic units and 20 pressor units. The oxytocin put up for experimental work is said to assay 10 oxytocic activity per c.cm. but its pressor activity is only ½ a unit per c.cm. It is claimed that vasopressin contains 20 pressor units per c.cm. Its oxytocic activity is less than one unit per c.cm. From the results shown above, these authors claim that the purified active principles are bound to have a wider range of clinical use.

These active principles have been subjected to a more complete physiological study by J. H. Gaddum (1928). According to him, oxytocin has, in addition to its action on the uterus, a depressor (blood pressure) action on the fowl and, in certain circumstances, on the cat. Vasopressin, in addition to its effects on the blood pressure and on the kidney (diuresis), has a specific stimulant action on the bowel of the rabbit and a dilator action on the melanophores of the frog; the latter effect is apparently due to a different principle, so that vasopressin is not yet a physiologically pure preparation. A. W. Bourne and J. H. Burn (1928) examined the action of the separated constituents of pituitary extract on the human uterus in labour. They found that oxytocin possesses the typical stimulant action whereas vasopressin has no effect even in large doses. A given dose of oxytocin (2 units) was found to produce a large response in one patient and a small one in another. Discussing the rare cases of pituitary shock which have led to a disinclination on the part of some obstetricians to use pituitary extract, they recommend that oxytocin, having none of the vasomotor effects which are responsible for the collapse, may be confidently used. F. R. Curtis and J. W. Pickering (1928) studied the effect of these active principles on the blood. According to them, vasopressin increases the coagulability of blood both in fasting

* Messrs. Parke Davis & Co., have now changed these names to Petressin and Pitocin respectively.