CLI. THE PRESENCE OF A SAPOTOXIN IN *XANTHOSOMA ATROVIRENS*, A TROPICAL FOOD-TUBER.

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It has long been known that certain plants containing glucosides of the saponin group cause much disease and high mortality amongst domestic animals in different parts of the world [Ewart, 1931]. It has not however been shown previously that this class of poisons is present in harmful quantities in any plant used in human consumption, excepting perhaps the glyco-alkaloid in the potato (*Solanum tuberosum*) and the sapotoxin of *Agrostemma githago* seeds which are sometimes accidentally present in wheat and ground with it in milling.

The tubers of several plants of the natural order Araceae are largely consumed in tropical countries, and an account of the extraction from the one most extensively eaten in the West Indies of an acid sapotoxin which produces nephritis is of interest, especially in view of the heavy incidence of this disease amongst the poorest class of the population in Trinidad and other tropical countries.

The tuber selected for special examination is the Tannia (*Xanthosoma atrovirens*) as this is eaten all the year round whilst most of the others have their season. The wide adoption of tannia as a food is explained by the fact that it is palatable, abundant and cheap. Further, apart from its unfortunate toxicity, it has a fairly satisfactory composition as a foodstuff. During an investigation of its dietetic value it was found that the addition of cholesterol or of sterol-containing foods to a tannia diet materially lessened its toxic effects. This suggested the presence of a sapotoxin and attempts were made to isolate such a substance.

The peeled tubers were finely minced and mixed with distilled water. After several hours an equal volume of alcohol was added and the precipitated starch removed by filtration. The filtrate was freed from most of the alcohol by evaporation on the water-bath, and the hot solution was treated with 30 % normal lead acetate. A precipitate was formed and collected, but the filtrate retained some of its toxicity. Treatment with basic lead acetate yielded a further smaller precipitate and the filtrate from this, after removal of lead, was found to be free from toxic substance.

The normal lead acetate precipitate, after washing with distilled water, was suspended in water and treated with hydrogen sulphide. The resulting lead sulphide appeared to adsorb much of the toxic material which could not be separated entirely, either by prolonged treatment with hydrogen sulphide or by repeated boiling of the precipitate with water. The filtrate was evaporated under diminished pressure to a syrup from which an excess of cold alcohol precipitated a white hygroscopic solid. This solid had the chemical properties of an acid sapotoxin and showed a high toxicity when its solution in normal

saline was injected intravenously into rats. A large amount of inorganic impurities was present. Dissolution of the solid and repetition of the above processes removed most, but not all, of this impurity.

The alcoholic filtrate, on evaporation, yielded a dark gum. This was dissolved in water and treated with hot baryta solution. The precipitated barium salt was collected and well washed with water, and its suspension in water was treated with 10 % sulphuric acid in slight excess. The excess was neutralised with lead carbonate and the filtered solution evaporated under diminished pressure. The yellow residue was dissolved in alcohol and the solution decolorised with charcoal, filtered and evaporated. The clear gum thus obtained was dissolved in ethyl acetate and the solution filtered from inorganic material and evaporated. Repetition of the treatment with ethyl acetate gave a clear gum which crystallised on standing. Recrystallisation from ethyl acetate gave pure crystals of citric acid, M.P. 152° , either alone or when mixed with an authentic specimen. A solution of this product in normal saline produced little or no discomfort to rats when injected intravenously.

The basic lead acetate precipitate was similarly decomposed by hydrogen sulphide and the filtrate from the lead sulphide evaporated to a syrup. On pouring cold alcohol into this a cream-coloured solid was precipitated. This also proved to be a sapotoxin with an acid reaction and in physiological effect was indistinguishable from that obtained from the normal lead acetate precipitate. It was, however, much smaller in amount than the latter.

The alcoholic mother-liquors of this sapotoxin were evaporated to a gum, which was shown by its chemical and physiological properties to contain a sapotoxin. Various attempts were made to purify this gum, but without complete success. A solution of the material in water was mixed with freshly prepared magnesium hydroxide and evaporated to dryness at 50° , and the resulting mass was powdered and boiled with alcohol to remove any uncombined organic material. The solid was then suspended in water and a rapid stream of carbon dioxide passed through the mixture for several hours. The precipitated magnesium carbonate was removed by filtration and the solution evaporated to small bulk under diminished pressure and poured into a large excess of alcohol. The solid thus obtained was redissolved in a little water and again precipitated with alcohol. This gave a pale brown amorphous powder which still contained magnesium. Its solution in water was treated with basic lead acetate solution, the precipitate collected and well washed. This was decomposed by hydrogen sulphide and the filtrate evaporated to a syrup, which was dissolved in a little alcohol, filtered and diluted with a large volume of ether. This procedure gave a white, amorphous solid which still contained a small quantity of inorganic salts. It was extremely deliquescent, giving a brown gum on exposure to moist air for a few seconds, and possessed the properties of a sapotoxin.

The acid sapotoxins prepared in the above manner were believed to differ only in degree of purity. They were found to contain a little material which reduced Fehling's solution, and this was removed by boiling with alcohol. The product then contained 7 % of ash. Its solution was dextrorotatory and after hydrolysis with dilute sulphuric acid was less strongly dextrorotatory. The hydrolysed solution was neutralised with barium carbonate, evaporated to small volume under diminished pressure and poured into alcohol. A solid, presumably the sapogenin, separated out and was removed by filtration. The alcoholic solution was evaporated to dryness under diminished pressure. The residue reduced Fehling's solution and when heated with strong hydrochloric acid gave a very weak furfuraldehyde reaction to aniline acetate paper. An osazone was

obtained which, microscopically, resembled glucosazone, M.P. 194–196°. Owing to lack of material it was not possible to recrystallise the osazone. When, however, it was mixed with an authentic specimen of glucosazone, the M.P. was raised to 199–200°. The sapotoxin thus appears to contain a hexose which yields glucosazone.

It would be difficult to make even a rough estimate of the toxin content of the tubers owing to the great loss by adsorption during the processes required for its isolation and because the content of the tubers, as indicated by monthly feeding experiments on rats, showed a great variation in the duration of life of animals fed on them. This duration was on an average 4 days in January: 22 days in March, April, May and June: 7 days in the remaining months.

The sapotoxins thus extracted from tannia tubers are highly toxic to rats. Intravenous injection of 0.1 mg. killed a 60 g. rat instantaneously from cardiac and respiratory paralysis: 0.05 mg. killed in 20 minutes. Injection of smaller quantities produced in 50 minutes a rise of blood-sugar ranging from 176 to 415 mg. per 100 ml. At the same time the hepatic glycogen was found to be greatly diminished, usually to the merest trace. The urine, when obtainable, was albuminous. A solution of 1 part of sapotoxin in 20,000 parts of normal saline haemolyses blood *in vitro*, with reduction of the liberated haemoglobin. Spectroscopic examination shows the presence of the bands characteristic of haemato-porphyrin. The cholesterol-saponin compound is neither toxic when injected, nor haemolytic.

The raw tubers have a burning effect upon the tongue which is sometimes severe but usually not unpleasant. Rats eat them readily and their average daily consumption when given no other food is 0.35 g. per g. rat, which is slightly more than the amount consumed of the stock diet. In spite of the adequate food consumption the animals lose weight from the commencement of the experiment. Within 24 hours the faeces become putty-like and clay-coloured. The abdomen becomes greatly distended and the urine albuminous. A gradually increasing lethargy follows which becomes profound as death approaches. Adrenaline injected intravenously rouses the animal rather dramatically, but the stupor returns in a short time and a further injection has little or no effect. The blood-sugar in the terminal phase of the experiment is greatly diminished, amounts as low as 45 mg. per 100 ml. being observed, whilst at the same time it is found that the hepatic glycogen is almost or quite absent.

The *post mortem* signs are remarkably constant. The caecum is enormously distended and its walls much thinned. The average weight of this organ in 27 examinations of rats fed on raw tannia was 14.6 % of body weight (one 20.7 %) compared with a weight of 1.47 % in rats fed on normal diet. The stomach and intestines contain blood in almost every case. The liver, lungs and kidneys are usually very dark, the cortex and medulla in the last-named being hardly distinguishable by their colour. Special interest is attached to the condition of the adrenal glands in view of the disturbed carbohydrate metabolism in tannia poisoning. In the normal rat these are of a pale skin colour and, in the albino rat of 60–70 g. body weight, account for about 0.0275 % of body weight, but in rats of the same strain and weight which have been poisoned by tannia are about 0.0390 % and vary in colour from a dull pink to a purplish tint similar to that of the kidney. Microscopically the kidneys show the appearances characteristic of an acute toxic nephritis. The glomeruli and secretory tubules are much disintegrated and the picture presented closely resembles that produced in poisoning by uranium nitrate. Granules of blood pigment are found abundantly scattered in kidneys, suprarenals and spleen and are especially plentiful in the

reticulo-endothelial cells of the liver. These appearances are found whether the tubers have been ingested or the toxin injected, but in the latter case the effects are more marked in the kidneys and less in the other organs than in the former.

Unstained blood films prepared in the ordinary way from rats poisoned by tannia or injected with its toxin show colourless crystals which are quite new in our experience. These are usually regular, stellate structures and many are six-pointed. They are about twice as large as a red blood corpuscle. We have not been able to find them in the films when freshly spread, but they can be discovered an hour later. They are not very numerous, but are striking and characteristic when found. It is not yet known whether they occur in cases of tannia poisoning in human beings in tropical countries. Ordinary haemoglobin crystals are sometimes found in association with the stellate crystals, being produced no doubt by the saponin haemolysis.

SUMMARY.

A highly poisonous acid sapotoxin, for which the name tanniatotoxin is suggested, has been isolated from the tubers of *Xanthosoma atrovirens*, a food commonly eaten in Trinidad under the name of "tannia."

When injected in doses of 0.1 mg. it kills a 60 g. rat instantaneously from paralysis of heart and respiration.

In smaller doses or when the raw tubers are eaten, a glomerulo-tubular nephritis is produced.

The sapotoxin haemolyses blood *in vitro* and converts the liberated haemoglobin into haematoporphyrin.

Certain stellate crystals which are found in blood films of poisoned rats are described.

It is suggested that certain cases of nephritis occurring in tropical countries may be caused by the consumption of tannia and other aroid tubers.

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REFERENCE.

Ewart (1931). *Council for Sci and Ind. Res., Commonwealth of Australia, Melbourne*, Bull. 50.