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The Detection of Strychnine in Carcasses and Corpses.

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I. INTRODUCTION.

In forensic medicine it is of the utmost importance to know whether or not strychnine is present in corpses and carcasses in cases of suspected strychnine poisoning. The vital importance of this point is easily realised in cases of suspected malicious poisoning in human beings.

This investigation, the preliminary results of which are recorded in this article, was prompted by the fact that a difference of opinion exists among those concerned with forensic medicine as to whether or not, the biological test is essential in the detection of strychnine in corpses and carcasses. Some maintain that the taste and chemical (colour) tests yield sufficient evidence as to the presence of strychnine in extracts prepared from corpses and carcasses, whilst others are of opinion that it is essential that the results of the chemical examination be confirmed by biological tests.

II. TESTS EMPLOYED IN THE DETECTION OF STRYCHNINE.

A. Taste.

Strychnine has an intensely bitter taste, which is still detectable in very dilute solutions. In the literature there is a striking discrepancy in the dilutions of strychnine in which this bitter taste is detectable: (*a*) Cloetta (1866) states that if 1 c.c. of a 1:250,000 solution of strychnine in distilled water be placed on the tongue it still has a bitter taste. On further dilution the bitter taste disappears. Some investigators state that a solution of 1:600,000 is still bitter, Cloetta however disagrees; (*b*) according to Gadamer (1924) the bitter taste of strychnine is still detectable in dilutions of 1:40,000-67,000; (*c*) Autenrieth (1928) and van Itallie and Bylsma (1928) state that the bitter taste of strychnine is still detectable in aequeous solutions of 1:670,000; (*d*) Glaister (1931) referring to strychnine writes " 1 grain will impart to a gallon of water (1 in 70,000) a perceptible bitterness ", and (*e*) according to Klein (1933) a delution of 1:700,000 still has a bitter taste.

The above discrepancies are due probably, firstly to a difference in the susceptibility of the taste nerves of the different individuals, and secondly, to the fact that the different individuals took different quantities of the solutions to be tasted into their mouths. It is obvious that a bitter taste may not be perceptible when a drop of a very dilute solution of strychnine is placed on the tongue, whilst when 1 or 2 c.c. of the same solution is taken it will be bitter. When expressing a view as to the perceptibility of a bitter taste of strychnine in certain dilutions both the dilution and the quantity of the solution tasted should be mentioned. It is obvious that different dilutions of strychnine should not be tasted immediately after each other as the taste-nerves become exhausted very soon and hence are unable to record the bitter taste of a solution even if it is more bitter than the one tasted previously. An hour or more should be allowed to elapse between the tests.

The author was unable to detect a bitter taste in dilutions of strychnine beyond 1 in 200,000 in distilled water. A standard quantity of 1 c.c. of each dilution was placed on the tongue. The author failed to detect a bitter taste when only a drop of 1:200,000 strychnine was placed on the tongue.

B. Chemical Tests.

Only *well purified extracts* should be used for chemical reactions (precipitation and colour tests) as impurities may interfere with the reactions.

(*a*) *Precipitation Tests*

It was decided to ascertain the sensitivity of certain commonly used alkaloidal reagents for strychnine. The dilutions were prepared by dissolving strychnine sulphate in distilled water slightly acidified with sulphuric acid. The precipitating agents for alkaloids mentioned in the table given below were prepared according to prescriptions given by Fulton (1932):—

Table I.

Sensitivity of alkaloidal reagents for strychnine.

Precipitating Agents.	Dilutions.							
	1: 1,000.	1: 2,000.	1: 4,000.	1: 8,000.	1: 16,000.	1: 20,000.	1: 30,000.	1: 50,000
"Platinum Chloride"...	+	±	−	−	−	−	−	−
Potassium chromate.....	−	−	−	−	−	−	−	−
Potassium cyanide.....	−	−	−	−	−	−	−	−
Phosphotungstic acid.....	−	−	−	−	−	−	−	−
Phosphomolybdic acid....	+	+	−	−	−	−	−	−
Wagner's reagent No. 1...	+	+	+	+	+	−	±	−
"Gold Chloride".....	+	+	+	−	−	−	−	−
Mayer's reagent.....	+	+	+	+	±	±	−	−
Mercuric sodium nitrite...	−	−	−	−	−	−	−	−
Picric acid.....	+	+	±	−	−	−	−	−
Tannic acid.....	+	+	−	−	−	−	−	−

+++ = Very strongly positive (heavy precipitate).
++ = Strongly positive.
+ = Positive.
± = Faintly positive.
− = Negative (no precipitate).

The above tests were made by placing one drop of the different dilutions of strychnine sulphate on a watch-glass and then adding a drop of the precipitating agent. From the above table it is evident that Wagner's reagent No. 1 still gives a macroscopic recognisable precipitate with strychnine in dilutions of 1 in 20,000. The approximate amount of strychnine sulphate in a drop of a dilution of 1 in 20,000 is 0·0031 mgm. In the course of this article (see biological tests) it will be seen that the author adopted the following method of testing the extracts prepared from carcasses: The purified residue of the chloroform extract of the organs is dissolved in 1·5 c.c. of physiological saline, slightly acidulated with sulphuric acid. If one drop of this dissolved extract were macroscopically to yield a positive precipitation test with Wagner's reagent No. 1 there should be at least 0·074 mgm. strychnine sulphate contained in the 1·5 c.c. That is, the amount of strychnine contained in 1·5 c.c. extract must be

approximately nine times the minimum amount of strychnine that is detectable by the biological test (see biological test). It is therefore clear that fairly large quantities of strychnine must be present in order to render these precipitation tests of any value in the identification of strychnine. If a sufficient quantity of strychnine be present in the extract all the above precipitating agents could be used as the results of this test will give an indication as to the amount of strychnine present. Wagner's reagent No. 1 and Mayer's reagent are the most sensitive.

If large amounts of strychnine (above 5·0 mgm.) are present in the extracts to be tested the method of identifying alkaloids by precipitation described by Fulton (1930) may be found useful in confirming the evidence obtained by other chemical reactions and the biological test.

According to Seka (Klein, 1933) strychnine, weakly acidified with nitric acid, is still precipitated in the following dilutions by the undermentioned precipitants: 1:400,000 by potassium bismuth iodide, 1:100,000 by potassium mercury iodide, 1:300,000 by silicotungstic acid, 1:600,000 by phosphotungstic acid in the presence of 1 per cent. hydrochloric acid, 1:10,000-11,000 with trinitrothymol and hexanitro-diphenylamine, and 1:9,000-10,000 with picric acid. Precipitates are also formed with chlorine and bromine water. [Seka, (Klein, 1933) and Gadamer, 1924.]

(b) Colour reactions for strychnine.

(1) In 1827 Orfila (Ranke, 1879) found that an evaporated alcoholic extract of intestines to which strychnine had been added gave a red colour with nitric acid. On the other hand Gadamer (1924) and Seka (Klein, 1933) state that in concentrated nitric acid strychnine is dissolved with a yellow colour and brucine with a red colour.

(2) Cloetta (1866) states that (*a*) when strychnine is dissolved in concentrated sulphuric acid a violet colour appears when strong oxidising agents (e.g. potassium bichromate) are added; and (*b*) strychnine and chromic acid form a combination, which is almost insoluble in water. According to von Dragendorff (1879), however, strychnine chromate is *not* very insoluble in water and its precipitation can be retarded or prevented by certain foreign substances in the solution.

The sulphuric acid-potassium bichromate test, which was first proposed by Otto (Poe and Bailey, 1933) in 1846, is furthermore referred to by Ranke (1879), Witthaus (1911), Heiduschka and Meisner (1923 and 1927), Gadamer (1924), Autenrieth (1928), van Itallie en Bylsma (1928), Glaister (1931), Seka (Klein, 1933), and Poe and Bailey (1933).

If to a purified extract, as described under IV (A) a few drops of sulphuric acid be added and a small crystal of potassium bichromate be then pushed about in it with a glass rod a deep blue colour, which rapidly changes into purple, crimson and red, and then slowly fades away, results. Very similar colour reactions are obtained, if instead

of potassium bichromate (Otto's test), the following oxidising agents are used: potassium permanganate (Wenzell's test), manganese peroxide, lead peroxide, potassium chlorate and potassium iodide, potassium ferricyanide, cerium oxide (Sonnenschein's reagent) and ammonium vanadate (dissolved in concentrated H_2SO_4 (Mandelin's reagent). These colour reactions are not specified for strychnine but are also seen in all ethyl derivative of aniline and tetrahydrochinolin, provided that the position *para* to the nitrogen atom is unsubstituted [Seka (Klein, 1933) and Gadamer, 1924].

When sulphuric acid and manganese carbonate are added to strychnine a blue colour, which changes into violet and then into pink, appears.

Witthaus (1911, p. 1061) states that (1) the alkaloid *geissospermin*, contained in *Pareira brava* "behaves like strychnine with sulphuric acid and potassium dichromate; " (2) *hypaphorin*, an alkaloid obtained by Gresshof from the seeds and bark of *Hypaphorus subumbrans*, cultivated in Java as a shade-tree, and investigated by Plugge, is said to " cause a beautiful violet color, like that with strychnin " which " changes more rapidly, and soon disappears altogether with this test " and (3) " *anilin* also gives a blue-violet colour with potassium dichromate and dilute sulphuric acid, but this colour does not change to red and yellow, but to black, while a peculiar odor, somewhat resembling that of bitter almonds, is given off, which is not observed with strychnine."

According to Wormley (Poe and Bailey, 1933) *curarine* and cod liver oil give colour reactions with Otto's test similar to those seen in strychnine. He also states that a number of substances (amongst others morphine, quinine, sugar, brucine, and tartar emetic) will interfere with this test.

Fuller (Poe and Bailey, 1933) found " that the petroleum ether residues from *gelsemium* and *yohimbe* give the strychnine test "; presumably the Otto test for strychnine.

Poe and Bailey (1933) state that " Mameli made a study of the interference of certain substances employed in therapeutics on the Otto colour reaction. He found a number of drugs which more or less interferred with the test." It is unfortunate that the drugs concerned are not mentioned by Poe and Bailey as the publication of Mameli is not obtainable in South Africa.

Poe and Bailey (1933) tested a large number of organic compounds with the Otto reaction for strychnine and found a number (e.g. cryptopine, papaverine, piperine, arbutin, benzanilide, etc.), which yielded results similar to those seen in strychnine. They also found that certain organic compounds (e.g. aesculine, meta-aminophenol, azoxybenzene, benzilic acid, benzohydrol, beta-naphthol, etc.) completely covered up the Otto test for strychnine when present in equal amounts.

According to de Vry and van der Burg (Cloetta, 1866) $\frac{1}{60,000}$ grain (= 0·0011 mgm.) strychnine is detectable by the Otto test. Cloetta (1866) however disagrees and states that the smallest amount of strychnine detectable by means of this test is $\frac{1}{7,000}$ grain (= 0·0095 mgm.).

Gadamer (1924) states that the sulphuric acid-potassium bichromate test is still positive with 0·001 mgm. strychnine. Heiduschka and Meisner (1927) were able to demonstrate 0·000125 mgm. strychnine by means of this test, whilst Glaister (1931) refering to this test, states that "this play of colours is characteristic of strychnine, and can be perceived with the $\frac{1}{10,000}$ grain of the poison" ($\frac{1}{10,000}$ grain = 0·0066 mgm.). A glance at these figures shows an enormous discrepancy.

Repeated tests conducted by the author showed only the faintest violet colour with 0·007 mgm. strychnine sulphate. With quantities smaller than this amount of strychnine no characteristic and reliable play of colours was obtained. The tests were conducted as follows: 1 gm. of strychnine sulphate was dissolved in 1 liter of distilled water. From this stock solution weaker solutions were prepared. Of each dilution (ranging from 1:1,000 to 1:800,000) 1 c.c. was taken and evaporated on a waterbath and the residue submitted to the sulphuric acid-potassium bichromate test.

(3) Heiduschka and Meisner (1923) describe a sublimation test for strychnine. The strychnine is sublimated *in vacuo* and the following tests are then applied: (i) Precipitation with potassium bichromate by Behrens method. Strychnine bichromate is formed. (ii) Precipitation with sulphuric acid as the acid strychnine sulphate. If the sublimate is dissolved in dilute sulphuric acid, acid strychnine sulphate crystalises out in long needles. (iii) Furthermore, colour tests (Wenzel's, Mandelin's and Otto's tests) are applied.

Kempf and Eder (Gadamer, 1924, pp. 378-382) also refers to the microsublimation of strychnine.

(4) To 4 c.c. of a strychnine solution add an equal amount of concentrated hydrochloric acid and 2-3 gm. of pure granulated zinc, heat to the boiling point and leave standing for 3-4 minutes. If a drop of a 1/10 per cent. sodium nitrite solution be added to 2 c.c. of the above cooled solution a red colour immediately appears. 0·003 mgm. strychnine in 1 c.c. of the solution tested still yields a positive result (Malaquin-Denigès), (Gadamer, 1924).

In regard to this test Seka (Klein, 1933) adds: If to the remaining portion of the solution one to two drops of bromine water be added, a purplish-red colour appears. If more bromine water be added a precipitate which dissolves with a red-violet colour in alcohol, is formed.

(5) Erdmann's and Froehde's reagents give no colour reactions with strychnine. [Erdmann's reagent—" sulphuric acid containing nitric acid, prepared by adding to 20 c.c. of pure concentrated sulphuric acid 10 drops of a mixture of 10 drops of concentrated nitric acid and 100 c.c. of water " (Autenrieth, 1928). Froehde's reagent—7. " A solution of molybdic acid in sulphuric acid, prepared by heating gently and dissolving 5 mgm. of molybdic acid or sodium molybdate in 1 c.c. of pure concentrated sulphuric acid. The solution which should be colourless does not keep long." (Autenrieth, 1928).]

(6) According to Aloy, Valdiguié and Aloy (1926) " strychnine in H_2SO_4 is unaffected by the addition of small amounts of UO_3 or acetate. But on exposure to the sunlight the solution becomes violet, as a result of oxidation. This method may be used for the *detection of* 1×10^{-5} *parts of* strychnine."

(7) Wharton's test- " Dissolve the substance to be tested in a dry condition in chloroform. Put this solution in a small test-tube and evaporate the chloroform by setting the tube in a larger one containing boiling hot water. When the substance is dry or nearly so, add a few drops of mixture of equal parts of strong sulphuric acid and water and dissolve by shaking. Now introduce bromine vapour carefully and move the tube to and fro so that the solution takes up bromine. Replace the tube in boiling water to expel excess of bromine vapour. If strychnine is present, a carmine-red colour will appear in a few minutes, increasing in intensity as the bromine evaporates. This colour fades after a time. Instead of bromine vapour, a solution of a drop of bromine in 2 c.c. of chloroform may be used. If the quantity of strychnine present is small, only a little bromine should be added to the solution " (Autenrieth, 1928).

According to Fujiwara (1933) a reagent of sulphuric acid and sodium molybdate is specific for strychnine. No details of the tests are described in the abstract and unfortunately the Tokyo Journal of Biochemistry is not obtainable in South Africa.

(c) *Physical and Chemical properties of Strychnine.*

The crystallography of strychnine and its salts is useful in the identification of this poison. The crystals of strychnine when combined with picric acid, picrolonic acid, p-nitro- and trinitro-benzoic acid, hydroferrocyanic acid, perchloric acid and iodic acid, are characteristic [Seka (Klein, 1933)].

According to Klobusitzky (1934) the following strychnine salts yield characteristic crystals with a 4 per cent. sodium glycerophosphate solution sulphate, chloride, nitrate, phosphate, and the glycerophosphate.

(C) Detection of Strychnine by the Dialysis Method.

Nunn (1932) describes a method of detecting strychnine with the use of a Graham dialyser. The specimen (organ or stomach contents) is cut into small pieces, placed in a glass jar, and then mixed with two or three ounces of water containing 2 per cent. hydrochloric

acid. The glass jar is then immersed in hot water and its contents allowed to digest for two or three hours. The contents of the glass jar are then poured into the dialyser, which is immersed in distilled water and allowed to stand for twenty-four hours. This process is repeated a second and third time, if considered necessary. The distilled water is tested with Mayer's reagent for the presence of alkaloids. If the result is negative there is no need to proceed, and if positive, the distilled water is evaporated on a water-bath to one ounce and filtered if necessary. The liquid is now made alkaline and shaken with chloroform. The chloroform is evaporated and the residue tested with sulphuric acid and potassium bichromate.

(D) Biological Tests.

(*a*) *The Solvent.*

Before discussing the biological tests we have to consider the solvent necessary for dissolving the residue of the purified chloroform extract, which is to be injected, into white mice or frogs.

The experiment shown in Table II which was repeated twice with the same results, was conducted with physiological saline solution and distilled water, both of which were slightly acidified with sulphuric acid.

From Table II is is clear that physiological saline solution should be used as a solvent in preference to distilled water, which when injected intraperitoneally in excessive quantities may cause severe and continuous clonic spasms of the hindlegs and death. These spasms resemble, to a certain extent, those seen in strychnine poisoning in white mice.

It is not advisable to inject more than 1·5 c.c. of physiological saline solution intraperitoneally into three weeks old white mice weighing approximately 10 gm., and not mroe than 1 c.c. in the two weeks old white mice weighing approximately 5-6 gm.

(*b*) *The Animal.*

Hall (Ranke, 1879) was the first to recognise the importance of the "frog" in the detection of minute amounts of strychnine, especially in forensic medicine. He suggested that "frogs" be immersed in the solutions to be tested for strychnine. Harley (Glaister, 1931) modified Hall's method by injecting some of the solution to be tested into the thoracic or abdominal cavity of the frog. Harley was able to detect 0·004 mgm. ($= \frac{1}{16{,}000}$ grain) strychnine and Hall 0·013 mgm. ($= \frac{1}{5{,}000}$ grain) by their respective methods. Pickford (Ranke, 1879) produced severe tetanic spasms in "frogs" with 0·006 mgm. strychnine injected subcutaneously. Unfortunately the specific names of the frogs used are not given. According to von Rautenfeld (Weiss and Hatcher, 1922) *Rana temporaria* is unsuited to the quantitative estimation of strychnine. He found that *Rana esculenta* is 25 times more susceptible to strychnine than *R. temporaria* (Kobert, 1906). Lovett (Weiss and Hatcher, 1922) refers to the difference in susceptibility of "frogs" to strychnine.

TABLE II.

The effects of physiological saline solution and distilled water on white mice.

Mouse No.	Age.	Weight in grams.	Fluid injected intraperitoneially.	Quantity of fluid injected.	Result.
1	3 weeks.....	11	Physiological saline solution	0·5 c.c.	Negative.
2	3 weeks.....	11	ditto	1·0 c.c.	Negative.
3	3 weeks.....	10	ditto	1·5 c.c.	Twenty minutes after injection the animal was apathetic, breathing fairly heavily, and had a staring coat. One and a half hours after injections it appeared normal.
4	3 weeks.....	13	ditto	2·5 c.c.	Result as in No. 3.
5	3 weeks.....	10	ditto	3·5 c.c.	Ten minutes after injection the animal was very apathetic, breathing heavily, and had a staring coat. Two and a half hours after injection these symptoms had disappeared.
6	3 weeks.....	10	Distilled water	0·5 c.c.	Negative.
7	3 weeks.....	14	ditto	1·0 c.c.	Slight transient apathy.
8	3 weeks.....	11	ditto	1·5 c.c.	Thirty minutes after injection the animal appeared very apathetic, had a staring coat and breathed heavily. Recovered overnight.
9	3 weeks.....	11	ditto	2·5 c.c.	Twenty minutes after injection—condition as described in No. 10. Animal was still on its side (apparently paralysed) six hours after injection. Recovered overnight.
10	3 weeks.....	10	ditto	3·5 c.c.	Ten minutes after injection the animal was breathing heavily, and had a staring coat. Whole body shivering markedly. Severe and continuous clonic spasms of hindlegs. Difficulty in walking. There was a tendency to extend the hindlegs as in strychnine poisoning. Deep and slow respiration. Not falling in convulsions when cage is knocked. Walking with hindlegs extended. Ultimately lying on left side, unable to move and showing clonic spasms of hindlegs, which are still slightly extended. Respiration became progressively slower until death occurred 40 minutes after injection.

He attributes this phenomenon to the " well-known difference in vitality of summer and winter frogs." No mention is made of the specific names of the frogs used in his experiment.

Ipsen (Weiss and Hatcher, 1922) suggests that the mouse be used in quantitative determinations of strychnine in preference to the " frog " as the latter is subject to seasonal variations in its susceptibility to strychnine.

Hatcher (Weiss and Hatcher, 1922) found that the fatal dose of strychnine sulphate for the " frog " is 0·45 mgm. per Kg. body weight, whilst according to Sollman the fatal dose is 5·5 mgm. per Kg. body weight. Kobert (1906) states that the lethal dose of strychnine injected subcutaneously into the " frog " is 2·0 mgm. per Kg. body weight. Unfortunately none of these authors mention the species of frog concerned.

It is evident from the literature consulted that frogs of the same species vary in their susceptibility to strychnine during the course of single investigations conducted over short periods of time. This is a serious disadvantage as far as the quantitative determination of strychnine by the frog-method is concerned.

The following is a summary of experiments conducted by Weiss and Hatcher (1922): (i) The common grass frog, or leopard frog (*Rana pipiens* Shreder) can be used in the quantitative estimation of strychnine after a period of fasting until the metabolism is reduced to a minimum. The period of fasting lasted about three to four weeks. Unfasted frogs are less susceptible than fasted frogs to strychnine. (ii) 0·15 mgm. strychnine sulphate per Kg. body weight induces perceptible increased reflex excitability in fasted frogs. (iii) The susceptibility of frogs to strychnine can be reduced by suitable feeding. (iv) " The removal of the liver of the frog during a period of minimal metabolism (after long fasting) has little influence on the size of the dose of strychnine required to induce increased reflex excitability. The removal of the liver during active metabolism causes an increase in its susceptibility toward small doses of strychnine so that it then behaves like a frog which had fasted for a long period, or until its metabolism was minimal." (v) " Variations in the weights of the animals within wide limits are without influence of the amount of strychnine per gram of weight required to cause increased reflex excitability."

Führer (Autenrieth, 1928) states that " between 0·02-0·05 mgm. of strychnine nitrate is the smallest quantity capable of producing tetanic convulsions in a medium-sized frog." The exact weight of the frog and the species should have been mentioned. As the sentence stands it conveys nothing to the reader in regard to the susceptibility of the frog to strychnine.

Priestley (1930) states that either " *Rana pipiens* " or " *Rana palustris* " can be used in the quantitative determination of strychnine and that the average percentage error is 10 and rarely exceeds 15. He states that various factors (diet, temperature, etc.), have to be considered in connection with the susceptibility of frogs to strychnine.

Koll (Klein, 1933) states that tadpoles in the stage of changing from larva to frog, are very susceptible to strychnine. They show typical tetanic convulsions after subcutaneous injection of 0·0003-0·0004 mgm. strychnine. Neither species nor weights of frogs are mentioned.

According to Falck [Kofler (Klein, 1933)] young mice weighing 4-5 gm. are very susceptible to strychnine. Not more than 0·5 c.c. of the solution to be tested is injected into the skin of the back just above the tail. Quantities of strychnine as small as 0·0002 mgm. are detectable in this way. The tail is stiff and shows tremors, which are characteristic of strychnine and which can be registered on a kymograph.

As the author intended conducting experiments in order to ascertain for what period after death strychnine is detectable in carcasses, is was thought advisable to determine which animal (frog or mouse) is most suited to the biological test. Consequently a number of experiments were conducted with white mice and frogs (*Rana aqualensis* Bocagd and *Xenopis laevis* Daudin). The experiments with the white mice were repeated twelve times, and those with the frogs twice. In each case the same result was obtained. All the white mice used in these and subsequent experiments were obtained from the same breeder. This allowed of obtaining mice of a definite age and strain and all of which received the same diet. Table III embodies the result of the effects of different quantities of strychnine on three weeks old white mice.

From Table III it is evident that it is possible to detect 0·008 mgm. strychnine sulphate when it is injected intraperitoneally into three weeks old white mice weighing from 10-12 gm. It was found that fourteen day old white mice weighing about 6 gm. possess the same degree of susceptibility per unit body weight. As the latter animals weigh much less than three weeks old mice it is advisable to use the younger animals as smaller quantities of strychnine are detectable in this way.

*Rana aqualensis** was found to possess the same degree of susseptibility as three weeks old white mice, namely, recognisable strychnine spasms were still produced by 0·008 mgm. strychnine sulphate (injected into the dorsal lymph sac) per 10 gm. body weight of frog. The frogs varied in weight from 10-25 gm. They were very young but unfortunately their age could not be determined owing to lack of knowledge of the species, which is now being studied by Mr. Fitzsimons.

Nine times the amount of strychnine sulphate that is detectable with *Rana aqualensis* had no effect on *Xenopis laevis* The weights of the two species of frogs used were approximately the same. All frogs were starved for sixteen days and kept under identical conditions before being used.

* The frogs were kindly provided by Mr. White and identified by Mr. Fitzsimons, both of the staff of the Transvaal Museum, Pretoria.

Table III.

The Susceptibility of White Mice to Strychnine.

Mouse No.	Age.	Weight in grams.	Amount of strychnine sulphate injected intraperitoneally per Kg. body weight.	Result.
A.....	3 weeks.....	10	0·3 mgm.	Negative.
A'.....	3 weeks.....	10	0·3 mgm.	Negative.
B.....	3 weeks.....	12	0·5 mgm.	Negative.
B'.....	3 weeks.....	10	0·5 mgm.	Negative.
C.....	3 weeks.....	10	0·7 mgm.	Negative.
C'.....	3 weeks.....	10	0·7 mgm.	Negative.
D.....	3 weeks.....	12	0·8 mgm.	Slight strychnine-like spasms of hind-legs, 10 minutes after injection. These were more severe when animal was excited (e.g., cage knocked). These symptoms disappeared 1 hour after injection.
D'.....	3 weeks.....	10	0·8 mgm.	ditto ditto
E.....	3 weeks.....	13	1·0 mgm.	Fairly severe strychnine-like spasms 15 minutes after injection. Recovered 1 hour after injection.
E'.....	3 weeks.....	10	1·0 mgm.	Severe convulsions 6 minutes after injection. Died 20 minutes after injection.
F.....	3 weeks.....	10	1·5 mgm.	Died with convulsions 12 minutes after injection.
F'.....	3 weeks.....	11	1·5 mgm.	Died with convulsions 15 minutes after injection.
G.....	3 weeks.....	10	2·0 mgm.	Died with convulsions 11 minutes after injection.
G'.....	3 weeks.....	10	2·0 mgm.	Died with convulsions 10 minutes after injection.
H.....	3 weeks.....	13	2·5 mgm.	Died with convulsions 11 minutes after injection.
H'.....	3 weeks.....	11	2·5 mgm.	Died with convulsions 8 minutes after injection.
I.....	3 weeks.....	12	3·0 mgm.	Died with convulsions 4 minutes after injection.
I'.....	3 weeks.....	10	3·0 mgm.	Died with convulsions 5 minutes after injection.
J.....	3 weeks.....	12	3·5 mgm.	Died with convulsions 4 minutes after injection.
J'.....	3 weeks.....	11	3·5 mgm.	Died with convulsions 3 minutes after injection.

In view of the above facts and also because (1) *Rana aqualensis* is not obtainable during autumn and winter and (2) mice react much more promptly than frogs to small amounts of strychnine (see symptoms of poisoning) it was decided to use white mice in all biological tests to be conducted. Three weeks old white mice were employed as in the determination of unknown quantities of strychnine it was at times necessary to inject 1·5 c.c. of the liquid to be tested. This volume of fluid (injected intra-abdominally) is excessive for a fourteen day old mouse weighing 5-6 gm.

Symptoms of Strychnine Poisoning in White Mice.

If amounts of strychnine slightly less than the M.L.D. be injected intra-abdominally into mice restlessness and increased excitability set in about 5-10 minutes after injection. The animals run about continuously and soon show an occasional clonic spasm of the hindlegs. The spasms increase in severity and the spasm-free interval becomes progressively shorter. Symptoms of weakness (paresis, ataxia) set in about 10-15 minutes after injection. These are soon followed by complete paralysis, the animal lying on its abdomen or side unable to move, and showing severe attacks of clonic spasms of the whole body and legs with the hindlegs rigidly extended backwards. Some affected mice " shivered " (continuous clonic spasms) continuously for more than an hour. The tail is extended upwards or in line with the body and if the animal lies on its side the front legs are extended at right angles to the body or backwards along the abdomen. The interval between the attacks depends on the severity of the case. It may vary from a fraction of a second to a few minutes. Convulsions may be brought on in spasm-free periods by lightly tapping the cage. After an attack of convulsions the respiration may stop for quite a while.

In acute cases of strychnine poisoning clonic spasms set in 2-4 minutes after injection. The symptoms are more severe than those described above and death may ensue from 5-15 minutes after injection. The head may be thrown backwards and the tail rigidly extended upwards.

In peracute cases the mouse suddenly gives a few short jumps with stiff legs and falls into convulsions with the hindlegs rigidly extended backwards and without any prodromal symptoms, a few seconds to a minute after injection. Death may occur almost instantaneously or after one or more attacks of convulsions, which follow at an interval of a second or less. In some cases the head is thrown backwards and the tail rigidly extended upwards at right angles to the body.

The rigid backward extension (in the same line as the body) of the hindlegs during attacks of convulsions is characteristic of strychnine poisoing.

Symptoms of Strychnine Poisoning in Rana aqualensis.

Young frogs weighing 18 gm., which had received 0·0144 mgm. strychnine sulphate (i.e. 0·8 mgm. per Kg. body weight) in the dorsal lymph sac, developed fairly severe tetanic convulsions fifty-five

to sixty-five minutes after injection. If left undisturbed the intervals between the convulsions varied from a few seconds to a few minutes. During these intervals the animals sat up again and appeared quite normal. If the slightest sound was made, or if the beaker containing the injected frogs was gently tapped, the animals immediately fell into convulsions again. During severe convulsions the frogs lay on their backs, the head was held up, the hindlegs were rigidly extended backwards showing continuous tetanic spasms, and the front legs were crossed. The animals recovered overnight.

Young frogs weighing 25 gm. which had received 0·05 mgm. strychnine sulphate (i.e. 2·0 mgm. per Kg. body weight) developed severe convulsions ten to twelve minutes after injection. The legs which were continuously extended backwards for an hour and a half before death, showed uninterrupted clonic spasms. Death occurred about four hours after the injection.

The symptoms of strychnine poisoning in " frogs " (species not mentioned) described by Ranke (1879) and Autenrieth (1928) are similar to those described above.

(E) Quantitative Estimation of Strychnine.

(*a*) *By Weighing.*—This method is applicable only in those cases where a ponderable amount of strychnine was isolated.

(*b*1) *By titration of the free base with* $\frac{1}{100}$ *N acid or with mercuric iodide: potassium iodide.*—Kobert (1906) refers to this method, which obviously cannot be used in the estimation of minute quantities of strychnine. Seka (Klein, 1933) states that when strychnine is dissolved in 50 per cent alcohol, it should be titrated with bromine phenol blue as an indicator. Amrhein (1934) uses methyl red as indicator and titrates with 0·02 N acid to a faint pink colour.

(*b*2) *By the volumetric method.*—Jonesco-Matui (1926) describes a volumetric method of determining strychnine by means of titration with 0·1 N. NaCl. According to his determinations 1·0 c.c. 0·1 N. NaCl is equivalent to 0·014 gm. strychnine. It is impossible to determine fractions of a milligram of strychnine by this method

(*c*) *Biological assay.*—See (D) Biological tests.

(*d*) *By colour reactions.*—The intensity of the colour reactions, for example, Otto's test, is an indication of the approximate amount of strychnine present. Fairly reliable results are obtainable if the colour of the material tested is compared with a series of colours of known amounts of strychnine.

III. FOR WHAT PERIOD AFTER DEATH IS STRYCHNINE DETECTABLE IN CORPSES AND CARCASSES.

In 1866 it was still impossible with the methods known at that time to demonstrate the presence of strychnine in the blood, organs and urine of animals and individuals that had died from strychnine poisoning (Cloetta, 1866).

It seems unnecessary to refer to all the experiments conducted in connection with the fate of strychnine in corpses and carcasses and only a few outstanding cases will be mentioned.

Thompson (Cloetta, 1866) found strychnine in the stomach of a dog four months after it had died from this poison. Taylor (Cloetta, 1866) was unable to detect strychnine in the stomach of a person, who had taken and died from the poison ten years before.

Cloetta (1866) added 1 grain (=0·066 gm.) strychnine nitrate to the stomachs of human beings, and placed these in glass vessels, which were closed up and buried three feet deep. After eleven and a half months he still detected strychnine in the stomach.

In 1856 Riekker (Ranke, 1879) placed the heart, lungs and liver of a bull in glass vessels and after having stirred 5 grains (=0·33 gm.) strychnine nitrate in solution into their contents the vessels were closed up with paper stoppers. They were then packed into a case of sawdust and stored. Eleven years and five weeks later Riekker still detected strychnine in the contents of the vessels. Ranke also refers to experiments conducted by other investigators.

Ranke (1879) killed seventeen dogs with 0·1 gm. strychnine nitrate. None of the animals vomited. Nine dogs were buried 1·5 metres deep in loose sandy soil and the remaining eight in loam soil. Carcasses were exhumed 100, 135, 260 and 330 days after burial respectively, and the specimens (stomach, intestine, liver and spleen) sent to different chemists for analysis. On the 330th day the organs were not recognisable and specimens consisting of decomposed muscle and intestines were collected for analysis. None of the chemists were able to demonstrate with any amount of certainty the presence of strychnine by means of chemical tests (colour tests) and crystallography. Biological tests conducted upon frogs however revealed the presence of strychnine even in the carcasses exhumed 330 days after burial.

Cram and Meserve (1910-1911) report on an interesting case. The body of a man, who had died from poisoning, was exhumed four months after death. "The body had been frozen most of the time but the grave when opened was full of water which was allowed to drain off. An embalming fluid of acid reaction had been used when the body was first buried, which made it appear likely that any strychnine would be dissolved out." No strychnine was obtainable from 454 gm. of the lung, 133 gm. of kidney, 446 gm .of muscle, 850 gm. of small intestine and 560 gm. of brain. 0·0015 gm. of strychnine was obtained from 803 gm. of liver and 0·0033 gm. from the spinal cord, which weighed 25 gm.

Gadamer (1924) and Autenrieth (1928) state that Kratter detected strychnine in corpses six years after death.

Ipsen (van Itallie and Bylsma, 1928) concluded from his experiments that strychnine was detectable in corpses for years after death provided there was no loss of the poison from the corpse.

TABLE IV.

The following dogs were drenched per stomach tube with strychnine sulphate dissolved in distilled water on the 7th September, 1933.

No. of Dog.	Approximate Age in years.	Weight in Kg.	Amount of strychnine sulphate (given per Kg. body weight).	Period that elapsed from time of drenching to death.	Period that elapsed from death to complete rigor mortis.
1247	4	8	5·0 mgm. (+ approximately 5 M.L.D.)	3 hours	35 minutes.
1253	5	14	ditto	10 minutes	15 minutes.
1309	5	8·5	ditto	15 minutes	20 minutes.
1310	6	8·5	ditto	12 minutes	20 minutes.
1311	6	15	ditto	10 minutes	20 minutes.
1312	5	16	ditto	10 minutes	20 minutes.
1313	5	15	ditto	11 minutes	15 minutes.
1314	5	15	ditto	15 minutes	30 minutes.
1317	6	14	ditto	20 minutes	20 minutes.
1318	4	11·5	ditto	15 minutes	30 minutes.
1319	5	12	ditto	20 minutes	20 minutes.
1320	6	12	ditto	12 minutes	10 minutes.
1321	5	9	ditto	8 minutes	12 minutes.
1322	5	13	ditto	Developed fairly severe spasms ½ hour after drenching. After nine attacks improvement set in, the animal appearing normal 3 hours after drenching. It was shot 4 hours after drenching	1 hour.
1323	5	13	ditto	20 minutes	30 minutes.
1324	6	15	ditto	25 minutes	30 minutes.
1325	10	31·5	ditto	13 minutes	20 minutes.
1328	6	23	ditto	15 minutes	20 minutes.
1329	5	20	ditto	10 minutes	15 minutes.
1330	8	23·5	ditto	12 minutes	20 minutes.
1331	6	18	ditto	30 minutes	45 minutes.
1332	4	7·5	ditto	35 minutes	40 minutes.
1333	4	10	ditto	10 minutes	15 minutes.
1334	5	7	ditto	12 minutes	20 minutes.

Onderstepoort Experiments.

The object of these experiments was (*a*) to determine for what period after death strychnine was detectable in carcasses of poisoned dogs and (*b*) to compare ptomaines, which were isolated from control dogs, chemically (colour reactions) and biologically with the purified extracts obtained from dogs killed with strychnine.

Twenty-four dogs were killed with strychnine sulphate on the 7th September, 1933.

From Table IV it would appear that the shorter the period that elapsed between drenching with strychnine and death the sooner *rigor mortis* set in. It is of interest to note that dog 1322 survived the effects of about 5 M.L.D. of strychnine.

On the same date five control dogs (1251, 1315, 1316, 1326 and 1327) were shot.

All the dogs were placed in strong wooden boxes with lids. The boxes were placed on stones in holes five feet deep in black clay soil, and then covered with sheets of corrugated iron, supported by iron standards placed across the boxes. The layer of clay soil over the iron sheets was about three feet deep. The holes were five feet apart. At various intervals after burial dogs killed with strychnine and control dogs were exhumed and specimens collected for analysis. The dogs were then re-buried.

The method employed in the extraction of strychnine from organs and stomach contents is that described by Glaister (1931). It was, however, found that if the alcohol extract was filtered through filter paper instead of cloth (muslin) (as suggested by Glaister) the process of shaking out the alkaline aequeous extract with chloroform was much less laborious in the case of decomposed organs. In this way the possibility of the formation of an emulsion is reduced. Should such an emulsion be formed, separation of the fluids may in many cases be achieved if to the "alkaline aequeous extract-chloroform emulsion" a fair quantity of ether be added and the mixture shaken vigorously and placed alternately in an incubator at about 38° C. and in a refrigerator. If in spite of this procedure no separation of the fluids occur the only alternative is to centrifuge. In the case of decomposed carcasses (organs) the evaporated chloroform extract, which almost invariably was dirty brown in colour and had an unpleasant odour, was purified, as it was realised that only thoroughly purified extracts could be used in the colour and biological tests.

In a preliminary experiment various amounts of strychnine sulphate were dissolved in distilled water and then thoroughly mixed with minced livers. After twenty-four hours the author was able to recover 95 per cent. of the amount of strychnine added to the different specimens of liver.

From previous discussions it is clear that the least amount of strychnine required in order to apply both the potassium bichromate-sulphuric acid (Otto) test and the biological test on three weeks old

white mice is approximately 0·015 mgm., as at least 0·007 mgm. strychnine is required for the former test and 0·008 mgm. for the biological test. If 14-day old mice are used it would be possible to detect 0·012 mgm. strychnine.

As the biological assay of strychnine is much more accurate and less time consuming than a quantitative estimation by means of comparing the intensity of the colour reaction of the tested material with a series of colour reactions of known amounts of strychnine, it was decided first to apply the biological test (see next paragraph) and if sufficient material were left it was made alkaline and shaken with chloroform and the bichromate colour (Otto) test applied to the chloroform residue.

The purified extract was accordingly dissolved in 1·5 c.c. warm physiological saline solution slightly acidified with sulphuric acid. Three weeks old white mice weighing 9-12 gm. were then injected intraperitoneally with this solution commencing with 0·1 c.c. and either increasing or decreasing the dose according to whether the result was positive or negative.

From the above it is obvious that if an extract contains less than 0·015 mgm. strychnine only the biological test can be applied.

Table V embodies the results of tests conducted with the carcasses of the dogs killed with strychnine.

From Table V the following points are evident:

(*a*) *Degree of decomposition of carcasses:*

(1) Six days after death advanced state of decomposition; skin almost hairless; organs still recognisable.

(2) Twelve days after death—organs still recognisable.

(3) Six weeks after death—organs still slightly recognisable.

(4) Ten weeks after death—organs not recognisable.

(5) Eighteen weeks after death—boxes containing the carcasses completely immersed in (and filled with) water. The boxes were continuously immersed in water for about five weeks on account of very heavy rains.

(6) Eleven months after death: bones almost dry but still covered with a small amount of fatty substance.

(*b*) *Presence or absence of strychnine in carcasses.*

(1) Three hours after death (dog 1325) strychnine was found in the lung, stomach, kidney, brain and spinal cord, spleen, muscles on posterior aspect of left femur, and the heart. Unfortunately owing to an accident the amount of strychnine in the liver could not be determined. Strychnine was found most concentrated in the stomach, lung, spleen and kidney. No strychnine was detectable in the left tibia and fibula.

TABLE V.

Carcasses of Dogs killed with Strychnine on 7th September, 1933.

Dog No.	Degree of decomposition of carcass.	Organs examined and amount of strychnine sulphate isolated per 100 gm. organ.	
1325	*Three hours after death.*—No signs of decomposition. 12 *days after death :* In advanced state of decomposition ; organs still recognisable	*Organs examined 3 hours after death.* Right lung (150 gm.) : 0·1 mgm. Extract bitter. Half of liver (450 gm.) : Flask broken, hence amount of strychnine not determined. Posterior half of stomach (wall + contents = 200 gm.) : 7·5 mgm. Extract bitter. Left kidney (40 gm.) : 0·08 mgm. Extract bitter. Half of brain and spinal cord (cut longitudinally) (60 gm.) : 0·02 mgm. Extract bitter. Half of spleen (cut longitudinally) (30 gm.) : 0·1 mgm. Extract bitter. Left tibia and fibula (200 gm.) : No strychnine found. Extract not bitter. Muscles of posterior aspect of left femur (200 gm.) : 0·012 mgm. Extract bitter. Half of heart (cut longitudinally through both ventricles = 100 gm.) : 0·016 mgm. Extract bitter.	*Organs examined 12 days after death.* Left lung (150 gm.) : 0·08 mgm. Extract bitter. Remaining half of liver (200 gm.) : 0·3 mgm. Extract bitter. Anterior half of stomach (wall + contents = 80 gm.) : 5·0 mgm. Extract bitter. Right kidney (60 gm.) : 0·26 mgm. Extract bitter. Hardly anything left of brain and spinal cord. Remaining half of spleen (30 gm.) : 0·5 mgm. Extract bitter. Right tibia and fibula (200 gm.) : 0·03 mgm. Extract bitter. Muscles on posterior aspect of right femur (200 gm.) : 0·08 mgm. Extract bitter. Remaining half of heart (30 gm.) : 0·2 mgm. Extract bitter.
1330	*Six days after death :* In advanced state of decomposition ; skin almost hairless and very moist ; organs still recognisable. *Eleven months after death :* Bones almost dry but still covered by a certain amount of a fatty substance.	*Organs examined 6 days after death.* Stomach wall—no contents present (100 gm.) : 2·4 mgm. Extract bitter. Muscles from posterior aspect of femur (300 gm.) : 0·013 mgm. Extract bitter. Lungs (200 gm.) : 0·06 mgm. Extract bitter. Left tibia and fibula (100 gm.) : No strychnine found. Extract bitter. Liver (200 gm.) : 0·6 mgm. Extract bitter. Heart (70 gm.) : 0·43 mgm. Extract bitter. Kidneys (75 gm.) : 0·023 mgm. Extract bitter. Spleen (30 gm.) : 0·08 mgm. Extract bitter.	*Organs examined 11 months after death.* Bones (almost dry) (400 gm.) : 0·004 mgm. Extract bitter.
1312	*Six weeks after death :* Carcass well preserved ; abdominal and thoracic organs still slightly recognisable. *Eleven months after death :* Same as dog 1247.	*Organs examined 6 weeks after death.* Abdominal contents (400 gm.) : 0·3 mgm. Extract bitter. Bones (both femurs and skull) (200 gm.) : 0·04 mgm. Extract bitter.	*Organs examined 11 months after death.* Bones (almost dry) (400 gm.) : No strychnine detectable. Animal showed slight transient apathy after injection. Extract not bitter.
1328	*Six weeks after death :* Carcass very decomposed ; bones already exposed..... *Eleven months after death :* Bones almost dry but still covered by a certain amount of a fatty substance.	*Organs examined 6 weeks after death.* Abdominal contents (300 gm.) ; 0·04 mgm. Extract bitter. Bones (both femurs and skul) (300 gm.) : 0·02 mgm. Extract bitter.	*Organs examined 11 months after death.* Bones (almost dry) (350 gm.) : 0·005 mgm. Extract has a slight bitter taste.

Dog No.	Degree of decomposition of carcass.	Organs examined and amount of strychnine sulphate isolated per 100 gm. organ.	
1313	*Ten weeks after death :* Only skeleton, which fairly dry, and hair left. *Eleven months after death :* Same as dog 1247.	*Organs examined* 10 *weeks after death.* Bones (skull and left tibia and fibula) (270 gm.) : 0·09 mgm. Extract bitter.	*Organs examined* 11 *months after death.* Bones (almost dry) (300 gm.) : 0·005 mgm. Extract bitter.
1314	*Ten weeks after death :* Only skeleton, which is fairly dry, and hair left.	*Organs examined* 10 *weeks after death.* Bones (skull and left tibia and fibula) (300 gm.) : No Strychnine detectable.	*Organs examined* 11 *months after death.* Bones (almost dry) (500 gm.) : 0·003 mgm. Extract bitter.
1311	*Eleven months after death :* Same as dog 1247. *Ten weeks after death :* Only skeleton, which is fairly dry and hair left. *Eleven months after death :* Same as dog 1247.	*Organs examined* 10 *weeks after death.* Bones (skull and left tibia and fibula) (300 gm.) : 0·04 mgm. Extract bitter.	*Organs examined* 11 *months after death.* Bones (almost dry (200 gm.) : No strychnine detectable. Animal showed slight transient apathy after injection. Extract and a slight bitter taste.
1310	*Ten weeks after death :* Only skeleton, which is fairly dry, and hair left. *Eleven months after death :* Same as dog 1247.	*Organs examined* 10 *weeks after death.* Bones (skull and left tibia and fibula) (180 gm.) : 0·13 mgm. Extract bitter.	*Organs examined* 11 *mobths after death.* Bones (almost dry) (260 gm.) : No strychnine detectable. The animal developed no symptoms of poisoning after injection. Extract slightly bitter.
1247	*Eighteen weeks after death :* Box full of water. Decomposed matter floating on water in box ; bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry. *Eleven months after death :* Bones almost dry but still covered by a certain amount of a fatty substance.	*Organs examined* 18 *weeks after death.* Bones (skull and left tibia and fibula) (120 gm.) : No strychnine detectable. The injected mouse ran about in an excited manner for about 3 minutes and showed ataxy, but no spasms. Recovered 30 minutes after injection. Extract not bitter.	*Organs examined* 11 *months after death.* Bones (almost dry) (150 gm.) : No strychnine detectable. The animal developed laboured respiration, symptoms of paresis, and clonic spasms of the hind-legs, which were not extended backwards. There was continuous nodding of the head. Two and a half hours after injection the animal appeared normal again. Extract intensely bitter.
1309	*Eighteen weeks after death :* State as described in dog 1247.	*Organs examined* 18 *weeks after death.* Bones (skull and left tibia and fibula (400 gm.) : 0·005 mgm. Extract not bitter.	
1253	*Eighteen weeks after death :* State as described in dog 1247.	*Organs examined* 18 *weeks after death.* Bones (skull and left tibia and fibula (200 gm.) : The symptoms exhibited by the mouse injected with the total quantity of extract (1·5 c.c.) exhibited symptoms which were not very typical of strychnine. Hence a toxic ptomaine may be the cause. Extract is tasteless.	
1317	*Eighteen weeks after death :* State as described in dog 1247.	*Organs examined* 18 *weeks after death.* Bones (skull and left tibia and fibula (172 gm.) : Symptoms exhibited were not very typical of strychnine. Extract not bitter.	

Twelve days after death strychnine was found less concentrated in the stomach and more concentrated in other organs. This was due to the fact that strychnine diffused through the decomposed stomach wall thus permeating the whole carcass. This explains the phenomenon that no strychine was detectable in the left tibia and femur collected three hours after death, whilst twelve days later the poison was detectable in the right tibia and femur.

It is of interest to note that no strychnine was detectable in the left tibia and fibula of dog 1330 six days after death, whilst the other organs contained large quantities of strychnine.

(2) Six weeks after death strychnine was still detectable in the "abdominal contents" and bones of dogs 1312 and 1328.

(3) Ten weeks after death strychnine was present in the bones of dogs 1313, 1311 and 1310, whilst it could not be detected in the bones of dog 1314.

(4) Eighteen weeks after death strychnine was present in the bones of dog 1309, whilst it was not detectable in the bones of dogs 1247, 1253, and 1317. It should be mentioned that the carcasses were found immersed in water at the time when the specimens were collected. It is quite conceivable that the strychnine present in the bones was dissolved out by the water. The less "fatty" the bones are the greater the likelihood of this happening.

(5) Eleven months after death the bones of dogs 1330, 1328, 1313, and 1314 still contained strychnine whilst the poison was not detectable in the bones of dogs 1312, 1311, 1310 and 1247. It is of interest to mention that a number of the extracts prepared from the bones of dogs killed with strychnine had a bitter taste in spite of the fact that no strychnine was detectable in them.

IV. DISCUSSION AND RECOMMENDATIONS AS TO THE MOST RELIABLE METHOD OF DIAGNOSING STRYCHNINE POISONING.

A. Method of Extracting Strychnine.

The method described by Glaister (1931) yields most satisfactory results. A high degree of purification of the extracts prepared from organs in an advanced state of decomposition can be achieved by taking up the evaporated chloroform extract in a few c.c. of distilled water acidified with sulphuric acid and shaking this out with a small quantity of ether. The aequeous solution is then rendered alkaline with potassium hydroxide and again shaken out with chloroform. This process should be repeated if necessary. In this way the salts of ptomaines, which are soluble in ether will be removed. This is an advantage as some ptomaines (see IV. D. Ptomaines) are chemically (colour reactions) and biologically almost indistinguishable from strychnine.

Weiss and Hatcher (1922) found that "the Stas-Otto method for the extraction of poisons from animal tissues does not permit of the recovery of strychnine quantitatively when only very small amounts (but such as may be present exceptionally at the time of death) are present, but widely diffused in the organs." They proposed the liquefication of tissues with 20 per cent. sodium hydroxide and heat and then shaking out with chloroform.

As stated before the author obtained most satisfactory results with the method used by himself.

Priestley (1930) described a method of isolating strychnine from blood and organs, which according to him is less time-consuming and as accurate as methods used by other investigators.

B. The Detection of Strychnine *intra vitam* and in Corpses and Carcasses

(*a*) *Intra vitam.*

In cases of acute strychnine poisoning the vomit (if vomition occurs) and the urine should be collected and tested for the presence of strychnine. The method of extraction described by Weiss and Hatcher (1922) could be used.

Weiss and Hatcher state that "the kidneys excrete amounts equal to 20 per cent. of that administered at one time, and a much lower percentage of larger doses taken by the mouth over periods of twelve and twenty-eight hours, respectively. The percentage of the strychnine excreted by the kidneys is a measure of the eliminative efficiency of the liver, rather than that of the kidney itself, for the kidney excretes only that which the liver *fails* to excrete.

Diuresis hastens the elimination of strychnine by the kidney, but it does not necessarily increase the total amount eliminated in the urine after a single dose injected intramuscularly, and it may, in fact, be attended with the renal elimination of a smaller total than would occur in a similar experiment without diuresis."

According to Gadamer (1924) 50-75 per cent. of the strychnine, administered in large but non-lethal doses, is excreted by the kidneys.

From the literature it appears that no strychnine is detectable in the urine a few days after administration, the greatest proportion of the amount eliminated in the urine being excreted within the first few hours.

Strychnine is not detectable in the faeces (Autenrieth, 1928).

(*b*) *A Short Period after Death.*

The amount of strychnine recoverable from the corpse or carcass depends on the following circumstances.

(1) *The dose administered and the method in which it was administered.*

Large doses of strychnine cause death within a few minutes or hours, especially when administered subcutaneously or intravenously,

as there is little time for elimination in the urine, bile, milk, saliva, etc. In the case of large doses with death soon after the poison had been taken per os a high percentage of the amount taken will be found in the stomach contents provided no vomition occurred.

In regard to the absorption of strychnine from the stomach Ryan (1912-13) found that strychnine nitrate in alcoholic solutions (10 to 20 per cent. alcohol) is not absorbed as readily as in aeqeous solution. He also found that strychnine is fairly readily absorbed by the gastric mucous membrane.

If a dose of strychnine equivalent to the M.L.D. or slightly more, had been administered no strychnine, or mere traces, will be detectable in the stomach contents. The comparatively long period that elapses between administration and death allows of complete, or almost complete, absorption of the poison from the gastrointestinal tract.

If the M.L.D. of strychnine for the human being be calculated on the generally accepted basis of 1·0 mgm. per Kg. body weight i.e. $\frac{1}{1{,}000{,}000}$ part of the body weight) a human being weighing 70 Kg. will require 0·07 gm. (=approximately 1 grain) of strychnine to cause death. If it be assumed that the strychnine taken is evenly distributed throughout the body, and that no excretion or destruction of the poison has occurred, then the corpse will contain 0·1 mgm. strychnine per 100 gm. This amount of strychnine will allow of precipitation and colour tests and the biological test being conducted.

It appears, however, that strychnine is not evenly distributed in corpses and carcasses but that apart from the stomach contents, which should be analysed, the greatest proportion of strychnine is to be found in the liver, spleen, kidneys, lung and brain and spinal cord [Autenrieth (1928) and others]. In cases of acute and peracute strychnine poisoning the urine (if any is present in the corpse or carcass) should also be analysed. When decomposition of corpses and carcasses sets in the strychnine, which is present in the stomach, will diffuse through the stomach wall and permeate the other organs.

Weiss and Hatcher (1922) experimenting upon cats found that "strychnine sulphate leaves the blood stream rapidly, and after two minutes as much as 30 per cent. may have left the circulation; within five minutes more than 50 per cent. and after forty minutes the blood may contain only about 4 per cent. of that injected." It therefore appears that the blood is not a suitable specimen for the isolation of strychnine.

As strychnine has cumulative effects it is quite possible that small amounts of strychnine may be detected in the body (especially liver, spleen, lungs, bones, and central nervous system) of persons, and animals receiving strychnine as a tonic for a certain period before death due to some cause other than strychnine poisoning.

In cases of suspected strychnine poisoning where the following information is at our disposal we may be able to discriminate between

cases, which have received strychnine as a tonic and have died from some other cause, and cases of strychnine poisoning:—

(i) The evidence of the doctor or veterinary surgeon, who attended the patient or animal concerned;

(ii) The symptoms of poisoning and post-mortem appearances;

(iii) The amount of strychnine present in the gastro-intestinal tract and in the organs.

In the case of a person or animal receiving strychnine as a tonic the drug will be completely absorbed within a few hours after administration. It is, however, possible that strychnine may be detectable in minute quantities in the gastrointestinal contents and wall in cases which received strychnine as a tonic, owing to the fact that the drug is partially excreted in the gastrointestinal tract and in the bile.

It is obvious that the weight of the specimens analysed for the presence of any poison is of the utmost importance. The heavier the specimen the more reliable the results will be.

The author conducted the following experiment (see Table VI) in order to ascertain whether strychnine taken as a tonic, that is, in very small (non-toxic) amounts, is detectable in carcasses or not.

From Table VI it is evident that strychnine was detectable in the liver and stomach of two dogs, which had received non-toxic amounts of strychnine daily for twenty-two days, and which were killed three hours after administration of the last dose of strychnine. It is apparent that a very large proportion of the amount of strychnine administered in the last dose had at the time of death (3 hours after administration) already disappeared from the stomach wall and contents. The kidneys of dog 1438 showed an advanced degree of cirrhosis. No strychnine was detectable in the organs of dog 1408, which was killed three days after administration of the last dose of strychnine. Sufficient time had evidently elapsed for the excretion and destruction of the poison.

The detection of strychnine in carcasses and corpses of individuals and animals, which had received a tonic containing strychnine and which died from a cause other than strychnine poisoning, depends on (1) the amount of strychnine administered, (2) the period that elapsed between the last dose and death, (3) the period that elapsed between death and the time of analysis of the corpse or carcass, (4) the age of the individual or animal, (5) the state in which the organs of excretion (especially liver and kidneys) were, (6) whether or not embalming fluids were used, and (7) on the rainfall.

It is interesting to note that the purified extracts prepared from the lungs and heart of dogs 1438 and 1439 contained a paralytic poison(s).

(2) *The treatment applied.*—It is obvious that the amount of strychnine present in corpses and carcasses especially in the stomach depends on the treatment administered to the victims. A certain proportion of the strychnine present in body will be removed or destroyed by (i) stomach lavage, (ii) emetics, and (iii) oxidising agents (e.g. potassium permanganate).

TABLE VI.

Strychnine tonic Experiment.

Dog No.	Weight.	Amount of strychnine sulphate administered.	Killed on.	Result of analysis of organs and remarks.
1408 (aged).	22·8 Kg.	0·066 mgm.* per Kg. body weight daily from 28/2/34 to 24/3/34	27/3/34	At no time did the animal exhibit any symptoms of poisoning. No strychnine was detectable in the following organs: Lungs and heart (300 gm.), liver (500 gm.), spleen (40 gm.), stomach wall plus contents (500 gm.), brain and spinal cord (100 gm.), and kidneys 80 gm.).
1438 (aged).	22·5 Kg.	0·1 mgm.* per Kg. body weight daily from 11/4/34 to 2/5/34	2/5/34	At no time did the animal exhibit any symptoms of poisoning. Killed three hours after administration of the last dose of strychnine. (1) Brain and spinal cord (80 gm.): No strychnine detectable. (2) Lungs and heart (400 gm.): The white mouse, which was injected with the extract, developed pronounced apathy and paresis, but recovered after two hours. (3) Liver (500 gm.): 0·025 mgm. strychnine sulphate present. (4) Spleen (30 gm.): No strychnine detectable. (5) Stomach wall and contents (700 gm.): 0·015 mgm. strychnine sylphate present.
1439 (6 years old)	16 Kg.	0·1 mgm.* per Kg. body weight daily from 11/4/34 to 2/5/34	2/5/34	At no time did the animal exhibit any symptoms of poisoning. Killed three hours after administration of the last dose of strychnine. (1) Brain and spinal cord (60 gm.): No strychnine detectable. (2) Lungs and heart (250 gm.): No strychnine detectable. The white mouse, which was injected with the extract, developed symptoms of paralysis and died two hours after injection. (3) Liver (400 gm.): 0·016 mgm. strychnine sulphate present. (4) Spleen (30 gm.): No strychnine detectable. (5) Kidneys (50 gm.): No strychnine detectable. (6) Stomach wall and contents (500 gm.): 0·014 mgm. strychnine Sylphate present.

* 0·2 gm. strychnine sulphate was dissolved in 1,000 c.c. distilled water and the requisite amount administered.

Tannic acid will combine with the strychnine still present in the gastrointestinal tract forming the insoluble tannate.

(3) *Destruction of Strychnine by the Body Tissues and Fluids intra vitam.*—According to Autenrieth (1928) the largest proportion of strychnine administered is destroyed " in the living organism as a result of animal metabolism ".

(*c*) *After the Corpses and Carcasses are in a state of advanced decomposition.*

In perusing the literature (see III) dealing with the persistence of strychnine in corpses and carcasses it appears that this poison is very resistant to the processes of decomposition. In some cases strychnine was detected a few years after death. These records should, however, be regarded with a critical eye as the methods used in the detection of strychnine are open to criticism.

In many cases only taste and colour tests (Otto's test, Mandelin's test) were used whilst other investigators applied the biological test only. In the discussion on ptomaines (IV. D) it will be seen that ptomaines resembling strychnine, either chemically or biologically are known. It has already been stated that both the colour and biological tests for strychnine should be applied in order to come to definite conclusions.

The amount of strychnine present in the body at the time of death may in the course of time decrease in the following ways:—

(1) *Method of Burial or Disposal of Corpse or Carcass.*—It is obvious that cremation completely destroys the poison. Embalming retards processes of decomposition, hence it is probable that strychnine will persist in such corpses for longer periods than in unembalmed corpses. The possibility of the embalming fluid destroying, or chemically changing strychnine, should also be considered.

Low temperatures inhibit processes of decomposition, hence the possibilities of detecting strychnine in corpses and carcasses buried in ice- and snow-ridden areas are more favourable than in warm areas.

A certain amount of strychnine escapes with the fluid (decomposed blood and liquefied tissues) flowing out of the corpse or carcass. It is therefore obvious that in cases in a state of advanced decomposition specimens of the garments and coffin (unless tin-lined) should also be taken as these were saturated with the fluid referred to. If there is evidence of the fluid having percolated through the coffin specimens of the soil concerned should also be analysed. In the case of tin-lined coffins containing bones only it is advisable to thoroughly rinse out the coffins with a small quantity of 96 per cent. alcohol acidified with acetic acid.

In the case of carcasses, which are decomposed it is essential that specimens of underlying soil be analysed for strychnine. Carcasses are sometimes covered with quicklime, which cause rapid disintegration of the tissues with consequent loss of fluid which soaks into the underlying soil.

The author conducted a number of experiments in order to determine whether processes of decomposition have a destructive effect on strychnine. Bovine livers were minced, placed in tins and definite amounts of strychnine (dissolved in water) added. These tins were stored at room temperature during summer. After a month practically no decrease in the amount of strychnine added had occurred, whilst after four months only about 1/50 of the original amount of strychnine added was present.

It was noticed that according to the method of biological assay (intraperitoneal injections into three weeks old white mice) much larger amounts (6 to 10 times) of strychnine were detectable in the extracts prepared from the decomposed livers if these extracts were not thoroughly purified before injection. This phenamenon was most probably due to the presence of ptomaines, which have actions similar to those of strychnine. We also have to consider the possibility of ptomaines, which have effects opposite to those of strychnine, being present in the extracts examined. In this case the biological test may not reveal the presence of strychnine in spite of the fact that it is present in the extract.

In one case the extract prepared from a control liver (to which no strychnine was added), which was analysed after having been allowed to decompose for four months, yielded a positive potassium bichromate-sulphuric acid test, which was indistinguishable from that of strychnine.

(2) *Rainfall.*—It is obvious that the presence of water in graves will dissolve out some of the strychnine present in corpses provided they are not enclosed in coffins (tin-lined and sealed) to which water has no access. In the case of corpses treated with acid embalming fluids large amounts of, if not all, the strychnine present in the corpse will be dissolved and removed by the water as strychnine salts are easily soluble in water. The less decomposed the carcass the less strychnine will be dissolved by the water as the fatty substances present will to a certain extent prevent the entrance of water into the organs and bones.

In the Onderstepoort experiment it was found that the boxes containing the carcasses of the twenty-nine dogs were floating in water after about 12 in. of rain had fallen in less than five weeks. A month later, after another 6·28 ins. of rain had fallen, the boxes were still submerged. It was interesting to note that after 6 inches of rain had fallen in the course of three weeks the soil (black clay) was wet only about 18 inches deep and there was no water in the holes.

(C) The most Suitable Tests for the Detection of Strychnine.

It has already been stated that for reasons mentioned above the taste test and at least one colour test (Otto's or Mandalin's test, etc.), and the biological test should be employed for strychnine before a definite opinion can be expressed. The author achieved very satisfactory results with the Otto test in conjunction with a biological test conducted upon three weeks old white mice.

The greater the number of tests for strychnine the more reliable the results will be.

(D) Substances resembling Strychnine.

The substances resembling strychnine chemically (colour tests, etc.), and biologically could be discussed under (*a*) chemical substances other than ptomaines, and (*b*) ptomaines.

(*a*) *Chemical Substances other than Ptomaines.*

(i) Kobert (1906), Witthaus (1911) and Gadamer (1924) refer to a substance (pellagrozein) which is sometimes present in decomposing maize and which resembles strychnine both chemically and biologically.

(ii) The colour reactions seen when oxidising agents are added to strychnine are not specific for this drug, but are also seen in all ethyl derivatives of aniline and tetrahydrochinolin, provided that the position *para* to the nitrogen atom is unsubstituted [Seka (Klein, 1933)]. Methyl- and ethyl-strychnine, which differ in action from strychnine, also give a positive Otto test.

(iii) The following alkaloids when injected in comparatively large amounts also induce tetanus in frogs: brucine, thebain, morphine, hydrastine and caffeine [Koffer (Klein, 1933)].

(iv) Brucine, quinine, corchorin, picric acid, and many other substances also have a bitter taste.

(v) Poe and Bailey (1933) studied the colour reactions of the Otto test on a large number of chemical substances and found twenty-three compounds which gave some shade of violet, lavender, or purple. Some of the colours seen were very similar to those given by strychnine. The alkaloids, cryptopine and papaverine, gave violet colours, so also did phenylglocine (amino acid derivative). Allylphenylthiocarbamide bave a brown colour with streaks of violet and the glucosides, aesculin and arbutin, brown with a trace of violet and a reddish violet colour respectively. Furthermore, the following substances gave traces of, or, a distinct violet colour: aniline derivatives + ortho- anisidine, benzanilide, ortho-benztoluide, para-bromoaniline, ortho-phenetidine, para-phenetidine; ortho-aminophenol; anisic acid (aromatic acid); naphthalene and anthracene derivatives + alizarin, alpha-naphthylaminoazobenzene, miscellaneous aromatic compounds: benzylphenylhydrazine, tetrabromophenolphthlalein, and triphenylmethane.

(vi) The alkaloids *geissospermin* and *hypaphorin* are said to cause a colour reaction like that seen in strychnine with sulphuric acid and potassium bichromate.

(*b*) *Ptomaines.*

Ptomaines (animal alkaloids, putrefactive alkaloids, cadaveric alkaloids) are defined as bases " formed under the action of bacteria or of metabolism by the splitting of carbon dioxide from an amino-acid."

In 1865 Aeby and Schwarzenbach extracted from corpses substances which induced tetanus (Kobert, 1906). Selmi prepared from corpses a substance which resembled strychnine chemically (Kobert, 1906). Kobert also states that Maas isolated from muscle and brain in the first stages of decomposition a ptomaine which caused tetanus; and that Amthor (see also Gadamer, 1924, p. 593) found a ptomaine which resembled strychnine chemically.

Ranke (1879) refers to ptomaines isolated from carcasses in a state of advanced decomposition which have a narcotic (paralytic) effect on frogs. He correctly remarks that the presence of such ptomaines masks the pharmacological effects of strychnine.

According to Witthaus (1911) Baumert states that " in one case the ptomaine in question not only gave various chemical reactions, including the identifying reactions of strychnine, but also possessed the tetanizing action of that alkaloid ". Unfortunately no particulars about this case are available.

Mecke isolated a faintly bitter, non-tetanizing ptomaine which gave a positive Otto test and was coloured yellow by Erdmann's reagent, and dirty violet by Fröhde's reagent (Witthaus, 1911; Gadamer, 1924).

Gadamer (1924) groups the ptomaines according to their solubility in organic solvents. He remarks that the ptomaines which are capable of being dissolved by chloroform out of an alkaline solution are said to have a pungent bitter taste and that they reduce iodic acid to iodine. " They are also said to give a red colour with sulphuric acid and Fröhde's reagent? "

According to Kippenberger many ptomaines mentioned in the literature are nothing else but peptones (Gadamer, 1924).

Onderstepoort Experiments with Ptomaines.

(i) *Unidentified ptomaines isolated from the carcasses of dogs killed as controls in the strychnine experiment.*—Whenever carcasses of the dogs killed with strychnine were exhumed, specimens for analysis were also collected from the carcasses of the control dogs which were shot. The extracts from the organs were prepared by the same method which was used in the isolation of strychnine. It is therefore obvious that only those ptomaines, which have the same solubility characters as strychnine are concerned in Onderstepoort experiments. When organs in an advanced state of decomposition are analysed for the presence of strychnine it is advisable to take up the chloroform residue in a small quantity of acidulated (H_2SO_4) distilled water and then shake out once with ether. The ether, in which strychnine sulphate is practically insoluble, will remove from the aequeous solution those ptomaines which are soluble in this organic solvent.

Table VII embodies the results of the extracts prepared from the organs of the control dogs.

TABLE VII.

Control Dogs—Shot on 7 September, 1933.

Dog No.	Approximate age.	Weight in Kg.	Degree of decomposition of carcass.	Effects of extracts of organs injected intraperitoneally into three weeks old white mice.	
				Organs examined 6 days after death.	*Organs examined 18 weeks after death.*
1326	5 years	28	*Six days after death:* Carcass very decomposed; skin very moist and almost hairless; internal organs very decomposed but still recognisable. *Eighteen weeks after death:* Box full of water. Decomposed matter floating on water in box; bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry.	*Liver:* 0·1 c.c extract (16·6 gm. liver) caused death in a mouse 5 minutes after injection. No spasms. Animal collapsed and died. 1·0 c.c. extract (166·6 gm. liver) induced a few clonic spasms of the hind-legs. The legs were, however, not rigidly extended backwards as in strychnine poisoning. Extract not bitter. *Stomach-wall and stomach contents:* 0·1 c.c. extract (13·3 gm. stomach wall and contents) induced no symptoms in a mouse. 1·0 c.c extract (133·3 gm. stomach-wall and contents) induced clonic spasms of whole body closely resembling those seen in strychnine poisoning. Death occurred 5 minutes after injection. Extract not bitter. *Spleen:* 0·1 c.c. extract (2 gm. spleen) induced no symptoms in a mouse. 1·0 c.c. extract (20 gm. spleen) induced pronounced dyspnoea, collapse and death 12 minutes after injection. No spasm. Extract not bitter. *Bone (left tibia and fibula and marrow):* 0·1 c.c. extract (6·6 gm. bone) induced no symptoms in a mouse. 1·0 c.c. extract (66·6 gm. bone) induced no symptoms in a mouse. Extract not bitter. *Lung:* 0·1 c.c. extract (6·6 gm. lung) induced no symptoms in a mouse. 1·0 c.c. extract (66·6 gm. lung) induced collapse and death 4 minutes after injection. The animal gasped for breath but showed no spasms. Extract not bitter. *Kidney:* 0·1 c.c extract (6·6 gm. kidney) induced collapse and death without spasms 6 minutes after injection. 1·0 c.c. extract (66·6 gm. kidney) induced collapse and death without spasms 2 minutes after injection. Extract not bitter. *Heart:* 0·1 c.c. extract (6·6 gm. heart) induced dyspnoea, clonic convulsions of whole body and death in a mouse 20 minutes after injection. The hind-legs were, however, not extended backwards as in strychnine poisoning. 1·0 c.c. extract (66·6 gm. heart) induced death in a mouse 4 minutes after injection. The head was thrown backwards but hind-legs not extended backwards as in strychnine poisoning. *Muscles of posterior aspect of humerus:* 0·1 c.c. extract (10 gm. muscle) induced no symptoms in a mouse. 1·0 c.c. extract (100 gm. muscle) induced no symptoms in a mouse. Extract not bitter.	*Bone (right tibia and fibula plus vertebrae):* N.B. Extract had a slightly bitter taste. 0·1 c.c. extract (10 gm bone) induced no symptoms. 1·0 c.c. extract (100 gm. bone) induced repeated clonic spasms of hind-legs and of body 10 minutes after injection. The convulsions closely resembled those seen in cases of strychnine poisoning. *Extract of a slight bitter taste.*

Dog No.	Approximate age.	Weight in Kg.	Degree of decomposition of carcass.	Effects of extracts of organs injected intraperitoneally into three weeks old white mice.	
1316	4 years	19 Kg.	*Six weeks after death:* Carcass in advanced state of decomposition; bones exposed and almost dry; nothing left of internal organs. *Eighteen weeks after death:* Box full of water. Decomposed matter floating on water in box; bones at bottom of box. After bones had been wiped and pounded they appeared fairly dry.	*Organs examined 6 weeks after death.* *Hair and remains of skin:* 0·1 c.c. extract (10 gm. hair and skin) induced no symptoms in a mouse. 1·0 c.c. extract (100 gm. hair and skin) induced pronounced dyspnoea in a mouse 4 minutes after injection. The animal jumped into the air, fell down on left side, and showed spasms of the hindlegs very much resembling those seen in strychnine poisoning. The hindlegs were, however, not as rigidly extended backwards as in strychnine poisoning. Death occurred 7 minutes after injection. Extract not better. *Bone (skull and femur):* 0·1 c.c. extract (20 gm. bone) induced no symptoms in a mouse. 1·0 c.c. extract (200 gm. bone) induced dyspnoea in a mouse 4 minutes after injection. Then spasms of all 4 legs. The animal made short quick jumps for about 5 minutes. It then went down showing spasms of hindlegs resembling those seen in strychnine poisoning. The animal died 10 minutes after injection. Extract not bitter.	*Organs examined 18 weeks after death.* *Remaining bones of hind-legs a vertebrae:* 1·5 c.c. extract (100 gm. bone) induced no symptoms. Extract not bitter.
1315	6 years	23 Kg.	*Six weeks after death:* Slightly less decomposed than dog 1316. Internal organs not recognisable. *Eleven months after death:* Bones almost dry but still covered by a certain amount of fatty material.	*Organs examined 6 weeks after death.* *Abdominal contents:* 0·1 c.c. extract (20 gm. abdominal contents) induced excitement in a mouse 10 minutes after injection. The animal was running about in the cage; it then became paretic and crawled with head dangling about. It appeared normal 1½ hours after injection. 1·0 c.c. extract (200 gm. abdominal contents) induced collapse and death in a mouse 8 minutes after injection. The hindlegs were rigidly extended with spasms as in strychnine poisoning. There were, however, no general convulsions as in strychnine poisoning. Extract not bitter. *Bone (skull and femurs):* 0·1 c.c. extract (20 gm. bone) induced no symptoms in a mouse. 1·0 c.c. extract (200 gm. bone) induced collapse and death in a mouse 4 minutes after injection. No spasms present. Extract not bitter.	*Organs examined 11 months after death.* *Bones (almost dry):* 1·0 c.c. extract (270 gm. bone) induced symptoms of paralysis and gasping for breath three minutes after injection. Death occurred 5 minutes after injection. No spasms were seen. Extract bitter. Otto's test slightly positive.
1251	5 years	19 Kg.	*Ten weeks after death:* Only skeleton, which is almost dry, and hair left. *Eleven months after death:* Bones almost dry but still covered by a certain amount of fatty substance.	*Organs examined 10 weeks after death.* *Bone (skull, left tibia and fibula):* 0·1 c.c. extract (20 gm. bone) induced no symptoms in a mouse. 1·0 c.c. (200 gm. bone) induced restlessness (running about) and accelerated respiration 4 minutes after injection. The animal performed short quick jumps (clonic spasms of legs?). There were clonic spasms of the hindlegs, which were fairly rigidly extended but not quite to such an extent as in strychnine poisoning. Died gasping for breath 7 minutes after injection. Extract not bitter.	*Organs examined 11 months after death.* *Bones (almost dry):* 1·0 c.c. (170 gm. bone) induced transient symptoms of paresis and laboured respiration. Two hours after injection the animal appeared normal again. Extract not bitter.
1327	6 years	18 Kg.	*Ten weeks after death:* Only skeleton, which is still fairly moist, and hair left. *Eleven months after death:* Bones almost dry but still covered by a certain amount of fat.	*Organs examined 10 weeks after death.* *Bone (skull, left tibia and fibula:* 0·1 c.c. Extract (20 gm. bone) induced no symptoms in a mouse. 1·0 c.c. extract (200 gm. bone) induced rigidity of the tail, accelerated respiration and clonic spasms of the whole body 2 minutes after injection. Soon the animal was unable to move about and exhibited clonic spasms of the hindlegs very similar to those seen in strychnine poisoning Died gasping for breath 5 minutes after injection. Extract intensely bitter.	*Organs examined 11 months after death.* *Bones (almost dry):* 1·0 c.c. extract (200 gm. bone) induced laboured respiration and symptoms of paresis 15 minutes after injection. The animal gave continuous short jumps and was completely paralysed when it died 30 minutes after injection. Extract slightly bitter.

The following is evident from Table VII.

(1) Dog 1326—the extract prepared from the stomach (plus contents) collected six days after death induced symptoms in white mice very closely resembling those seen in cases of strychnine poisoning. The extract was, however, not bitter and gave a negative Otto test.

The extract prepared from the bones collected eighteen weeks after death also induced strychnine-like spasms. The extract had a slight bitter taste and yielded a negative Otto test.

(2) Dog 1316—the extract prepared from the bones collected six weeks after death induced strychnine-like spasms. The Otto test was negative and the taste not bitter.

(3) Dog 1315—the extract prepared from the "abdominal contents" exhumed six weeks after death yielded strychnine-like spasms and a negative Otto test and was not of a bitter taste.

The extract of the bones exhumed eleven months after burial induced no strychnine-like spasms. The result of the Otto test very much resembled that seen in strychnine poisoning and the extract also had a bitter taste.

(4) Dog 1251—the extract prepared from the bones exhumed ten weeks after death induced strychnine-like spasms. The Otto test was, however, negative and the taste of the extract not bitter.

(5) Dog 1327—the extract of the bones exhumed ten weeks after death induced strychnine-like spasms and the taste was intensely bitter. The Otto test was, however, negative.

The extract of the bones exhumed eleven months after death induced no strychnine-like spasms. The extract was slightly bitter in taste but yielded a negative Otto test.

The remaining extracts had no chemical or biological characters which could be mistaken for those of strychnine.

A number of the extracts contained narcotic ptomaines.

(ii) *Identified ptomaines.*—The undermentioned ptomaines were obtained from Messrs. Fraenkel and Landau, Berlin- Oberschöneweide, Germany, and were submitted to the following tests. (Table VIII.)

For the colour test 0·2 gm. of the above ptomaines was used. For the biological test the amounts of ptomaines mentioned in the above table were dissolved in 0·5-1·0 c.c. physiological saline solution (sterilised) and injected intraperitoneally into three weeks old white mice.

Not in a single case did the result of the colour tests resemble the play of colours seen in strychnine. On the other hand neurin-bromide, guanidine carbonate, cholin chloride (0·001 gm.) and trimethyl-pyridine induced spasms which resembled those seen in cases of strychnine poisoning.

Cadaverine and putrescine in the doses mentioned above appear to be typical paralytic poisons for white mice.

TABLE VIII.

Tests conducted with Identified Ptomaines.

Ptomaine.	Potassium chromate-sulphuric acid test.	Biological test (conducted upon 3 weeks old white mice).
(1) Neurinbromide (colourless crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of a few small crystals of potassium bichromate a dark brown colour, which changes to a dark green, appears.	*Mouse No.* 1 (7 *gm.*) : 0·0125 gm. neurinbromide. The animal collapsed and died a few seconds after injection. Spasms slightly resembling those seen in cases of strychnine poisoning were seen. The hindlegs were, however, not very rigidly extended backwards. *Mouse No.* 2 (8 *gm.*) : 0·005 gm. neurinbromide. Same result as above. *Mouse No.* 3 (9 *gm.*) : 0·001 gm. neurinbromide. Convulsions of the whole body were seen but the hindlegs were not extended. Death occurred one minute after injection. *Mouse No.* 4 (10 *gm.*) : 0·0002 gm. neurinbromide. No symptoms appeared.
(2) Guanidine carbonate (small white crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of a few crystals of potassium bichromate a dark yellow colour, which then became slight greenish, appeared.	*Mouse No.* 5 (9 *gm.*) : 0·05 gm. guanidine carbonate. Three minutes after injection the animal became very excited, and showed extremely laboured respiration. It made movements as if chewing and retching. After a further three minutes it fell into convulsions, extending the hindlegs. Death occurred seven minutes after injection. *Mouse No.* 6 (10 *gm.*) : 0·025 gm. guanidine carbonate. The symptoms described in Mouse No. 5 set in five minutes after injection. Death occurred seven minutes after injection.
(3) Cadaverine dihydrochloride (small white crystals)	The crystals dissolved with effervescence in concentrated sulphuric acid. Upon the addition of potassium bichromate a yellow colour, which changed to a dark green, appeared.	*Mouse No.* 7 (10 *gm.*) : 0·025 gm. cadaverine dihydrochloride. Two minutes after injection the animal walked with a stiff gait. Dyspnoea and paralysis (no spasms) then set in. Two hours after injection the animal appeared normal again. *Mouse No.* 8 (8 *gm.*) : 0·05 gm. cadaverine dihydrochloride. Progressive paralysis and dyspnoea set in a few minutes after injection. No spasms were seen, Death occurred three hours after injection. *Mouse No.* 9 (10 *gm.*) : 0·1 gm. cadaverine dihydrochloride. The animal was completely paralysed three minutes after injection. It appeared dead but gave an occasional gasp (every few seconds). Death occurred about two and a half hours after injection.
(4) Cholin chloride (small white crystals)	The crystals were dissolved in concentrated sulphuric acid and upon the addition of a few potassium bichromate crystals a yellow colour, which changed into grec, appeared.	*Mouse No.* 10 (10 *gm.*) : 0·025 gm. cholin chloride. Within a few seconds after injection the animal gasped for breath ; and it fell into convulsions and died almost at once. No strychnine-like spasms were seen. *Mouse No.* 11 (10 *gm.*) : 0·005 gm. cholin chloride. The results were the same as in mouse No. 10. *Mouse No.* 12 (10 *gm.*) : 0·001 gm. cholin chloride. Symptoms of paresis (weakness) set in five minutes after injection. After a further ten minutes the animal was paralysed and showed strychnine-like spasms of the hindlegs.
(5) Cholin (base). (Brownish fluid)	The fluid was mixed with a few drops of concentrated sulphuric acid and a few crystals of potassium bichromate added. The result was a dark green colour.	*Mouse No.* 13 (7 *gm.*) : 0·065 gm. cholin. Death occurred three minutes after injection after the animal had exhibited laboured respiration (gasping for breath), and symptoms of paralysis. No spasms were seen. *Mouse No.* 14 (9 *gm.*) : 0·013 gm. Cholin. Symptoms very similar to those described in mouse No. 13 set in 2 minutes after injection. In addition, there were clonic spasms of the whole body and legs. There were, however, no spasms, which resembled those seen in strychnine poisoning. *Mouse No.* 15 (10 *gm.*) : 0·0026 gm. cholin. No symptoms of poisoning were seen
(6) Putrescine dihydrochloride (white crystalline powder)	The crystals dissolved in concentrated sulphuric acid with effervescence. Upon the addition of potassium bichromate a dark orange colour, which changed to a green, appeared.	*Mouse No.* 16 (9 *gm.*) : 0·025 gm. putrescine dihydrochloride. The animal died wh symtoms of progressive paralysis twenty minutes after injection. No spasms were seen. *Mouse No.* 17 (9 *gm.*) : 0·15 gm. putrescine dihydrochloride. Died with symtoms of progressive paralysis fifteen minutes after injection.
(7)2 – 4 – 6 – Trimethylpyridine (colourless fluid)	A few drops of concentrated sulphuric acid added to the fluid caused the formation of a white precipitate, which soon redissolved, forming a colourless liquid. Upon the addition of a few crystals of potassium bichromate to this colourless liquid a dark green colour appeared.	*Mouse No.* 18 (9 *gm.*) : 0·065 gm. trimethyl-pyridine. Within a few seconds after injection the head was thrown back violently and repeatedly. There were clonic spasms of the hindlegs very closely resembling those seen in strychnine poisoning. Death occurred half-a-minute after injection. *Mouse No.* 19 (10 *gm.*) : 0·013 gm. trimethyl-pyridine. Death occurred one minute after injection. The symptoms were similar to those described in mouse No. 18, but no strychnine-like spasms were seen. *Mouse No.* 20 (9 *gm.*) : 0·004 gm. trimenthyl-pyridine. Within half-a-minute after injection the animal collapsed with clonic spasms of the hindlegs, resembling those seen in strychnine poisoning. *Mouse No.* 21 (10 *gm.*) : 0·0008 gm. trimethyl-pyridine. A few minutes after kinjection dyspnoea and symptoms of paresis set in. For twenty-five minutes the animal lay breathing quietly, then violent convulsions of the whole body and legs occurred. The hindlegs were slightly extended backwards, but not as rigidly as in strychnine poisoning. Death occurred twenty-seven minutes after injection.

V. SUMMARY.

(1) In order to express a definite opinion as to the presence or absence of strychnine in *purified* extracts of specimens of organs, etc., it is essential that the following tests be conducted: (*a*) taste test, (*b*) colour test, and (*c*) a biological test. Immature white mice are for various reasons more suited to the biological test than frogs, (*Rana esculenta*, *Rana pipiens*, *Rana palustris*, *Rana aqualensis*).

It is definite that very *unreliable* and *inaccurate* results will be obtained if both the colour and biological tests for strychnine are not applied to extracts as a large number of chemical substances, including ptomaines, are known which yield positive results either with the colour test, or with the biological test for strychnine. Many of these substances also have a bitter taste. The greatest care should be exercised in expressing an opinion as to the presence of strychnine in decomposed carcasses and corpses. The author isolated a strychnine-like ptomaine(s) from a decomposed liver, which was known not to contain any strychnine. This ptomaine(s) had a bitter taste and gave a positive sulphuric acid-potassium bichromate test for strychnine. The results of taste, chemical and biological tests with unidentified and identified ptomaines are recorded.

(2) If three weeks old white mice are used in the biological test at least 0·008 mgm. strychnine sulphate is required in order to produce recognisable strychnine spasms in a mouse weighing 10 to 12 gm. With 14 day old white mice weighing 5 to 6 gm. 0·004 mgm. strychnine sulphate is detectable. In order to achieve reliable results in the detection of strychnine in *purified* extracts of organs, etc., at least 0·011 mgm. strychnine sulphate should be present as approximately 0·007 mgm. is required for the Otto test and 0·004 mgm. for the biological test if this is conducted upon 14-day old white mice weighing from 5 to 6 gm. If three-weeks-old white mice are employed the least amount of strychnine detectable in extracts is 0·015 mgm. if both the Otto and biological tests are conducted.

(3) The symptoms of strychnine poisoning in white mice and in the frog (Rana aqualensis) are described.

(4) The taste test and chemical and biological tests for strychnine are discussed.

(5) Factors responsible for the disappearance of strychnine from corpses and carcasses are discussed. Of four dogs killed with strychnine and exhumed ten weeks after death strychnine was detectable in three carcasses, whilst of four carcasses exhumed eighteen weeks after death only one was positive for strychnine. Eleven months after death eight carcasses of dogs killed with strychnine were exhumed and strychnine was detectable in only four of these. Subsequent exhumations of carcasses of dogs killed with strychnine and of control dogs are to be conducted.

(6) Methods of extracting strychnine from carcasses and corpses and of purifying these extracts are discussed.

(7) In fresh carcasses and corpses the most suitable organs for analysis for the presence of strychnine are liver, stomach, spleen, lung and the central nervous system; also the urine.

(8) In two out of three dogs, which had received strychnine as a tonic, strychnine was detectable in the liver and stomach (plus contents).

(9) A large number of chemical substances, which resemble strychnine chemically and biologically, are discussed.

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