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The Detection of Strychnine in Carcasses and Corpses.

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In a previous paper (Steyn, 1935) the tests employed in the detection of strychnine, the length of the period after death during which strychnine is detectable in corpses and carcasses, and recommendations as to the most reliable method of diagnosing strychnine poisoning were fully discussed. This discussion was based on experiments conducted by the author upon twenty-four dogs killed with strychnine and five control dogs which were shot and buried on 7th September, 1933. The remains of these twenty-nine dogs were again exhumed on the 1st and 2nd November, 1937, that is, approximately four years and two months after they were killed and buried. The bones were found to be dry, devoid of fat, brown in colour, and most of them were brittle. The method of extracting the bones was the same as that employed with the organs collected on previous exhumations (Steyn, 1935), namely the Stas-Otto process. The acidified extracts of the bones varied from light yellow to dark reddish brown in colour. No emulsions formed during the process of shaking out with chloroform. As recommended by the author in his previous paper (Steyn, 1935) the taste, potassium bichromate-sulphuric acid (Otto test), and biological tests were applied to the evaporated chloroform residues.

The results of the tests conducted with the bones of the twenty-four dogs killed with strychnine are incorporated in the following table (Table I):—

TABLE I.

Carcasses of Dogs Killed with Strychnine on 7th September, 1939.

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1247	150 gm.	Otto's test—yellowish-green discolouration which changed to brown Taste—tasteless. Injected mouse developed slight transient apathy and paresis.
1253	260 gm.	Otto's test—slight violet discolouration as in strychnine. Taste—tasteless. Injected mouse developed no symptoms of poisoning.
1309	200 gm.	Otto's test—yellowish-green discolouration. Taste—slightly bitter, not persistent. Injected mouse developed no symptoms of poisoning.
1310	150 gm.	Otto's test—yellowish-green discolouration which changed to dark brown. Taste—tasteless. Injected mouse developed transient symptoms of paresis in hind-quarters.
1311	400 gm.	Otto's test—slight, but definite, violet colour as in strychnine. Taste—slight transient bitter taste. Injected mouse developed symptoms of paresis within 20 minutes after injection and was paralysed within a further 30 minutes. Death occurred 1½ hours after injection.
1312	400 gm.	Otto's test—slight indefinite violet colour which changed to reddish-brown. Taste—not bitter. Injected mouse developed no symptoms of poisoning.
1313	220 gm.	Otto's test—light green colour reaction which changed to light brown. Taste—tasteless. Injected mouse developed dyspnoea, paresis and progressive paralysis within 3 minutes after injection. Died within 1 hour after injection.
1314	400 gm.	Otto's test—dark-green colour. Taste—slightly bitter taste. Injected mouse developed clonic spasms of the muscles of the fore-quarters which was followed by paresis and complete paralysis. Death within 50 minutes.
1317	400 gm.	Otto's test—slight transient violet discolouration as in strychnine. Taste—faint, but definitely bitter. Injected mouse developed dyspnoea and progressive paralysis within 2 minutes after injection. Complete paralysis set in 5 minutes after injection. Died within 45 minutes.
1318	400 gm.	Otto's test—light green discolouration which changed to dark brown Taste—faint bitter taste. Injected mouse developed paresis and general paralysis within 10 minutes after injection. Death occurred within 65 minutes after injection.

TABLE I (*continued*).

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1319	550 gm.	Otto's test—light green colour. Taste—very slight bitter taste which persists for a while. Injected mouse developed symptoms of general paralysis, and died within 1 hour.
1320	400 gm.	Otto's test—light green discolouration. Taste—bitter taste. Injected mouse developed transient symptoms of paresis in the hindquarters.
1321	350 gm.	Otto's test—dark green discolouration which changed to brown. Taste—faint bitter taste. Injected mouse reacted as in No. 1320.
1322	550 gm.	Otto's test—dark green discolouration which changed to brown. Taste—tasteless. Injected mouse reacted as in No. 1320.
1323	600 gm.	Otto's test—dark-green colour. Taste—slight bitter taste. Injected mouse developed no symptoms of poisoning.
1324	400 gm.	Otto's test—brick-red colour reaction which changed to light green. Taste—intense, persistent bitter taste. Injected mouse remained normal for about 3 minutes; subsequently it showed pronounced hypersensitivity with symptoms somewhat resembling those of strychnine.
1325	700 gm.	Otto's test—persistent yellow colour. Taste—bitter taste which persists for about 1 minute. *N.B.*—Extracted with amyl alcohol by mistake. Injected mouse developed symptoms of paresis within 10 minutes and then continuous, quick clonic strychninelike spasms of the whole body. It died within 1½ hours after injection.
1328	320 gm.	Otto's test—dark-red colour which changed to dark-brown. Taste—slight bitter taste. Injected mouse developed no symptoms of poisoning.
1329	410 gm.	Otto's test—yellow colour reaction. Taste—intense persistent bitter taste. Injected mouse developed dyspnoea and progressive paralysis within 2 minutes after injection and died 20 minutes after injection.
1330	550 gm.	Otto's test—dark green discolouration. Taste—tasteless. Injected mouse became hypersensitive and developed transient paresis of the hindquarters within 10 minutes after injection.
1331	500 gm.	Otto's test—yellowish-green discolouration which passed into brown. Taste—intense persistent bitter taste. Injected mouse developed slight paresis and recovered 3 hours after injection.

TABLE I (*continued*).

Dog No.	Weight of dry bones exhumed and extracted.	Tests for Strychnine.
1332	550 gm.	Otto's test—dark green discolouration. Taste—tasteless. Injected mouse became apathetic and developed transient paresis of the hindquarters.
1333	500 gm.	Otto's test—yellowish-green discolouration which changed to brown. Taste—not bitter. Injected mouse developed progressive paresis and paralysis within 3 minutes and died after 1 hour.
1334	400 gm.	Otto's test—light green discolouration which changed to brown. Taste—tasteless. Injected mouse developed no symptoms of poisoning.

From the above table it is evident that no strychnine is detectable in the remains of any of the twenty-four dogs killed with strychnine and exhumed four years and two months after burial. In four cases (dogs 1253, 1311, 1317 and 1312) the chloroform residue showed a slight violet discolouration with the potassium bichromate-sulphuric acid test. This colour reaction was, however, most probably not due to the presence of strychnine, as in each case the biological test was negative for strychnine. With the exception of six mice (1253, 1309, 1312, 1323, 1328 and 1334) all of them developed symptoms of paresis and general paralysis, which is probably due to the presence of ptomaines in the injected solutions. In a number of cases the chloroform residue contained a fair amount of an amorphous white powder (ptomaines?). The author has again confirmed the results of previous experiments in regard to the relative sensitivity of the potassium bichromate-sulphuric acid test and the biological test, in that he was able to detect as little as 0·004 mg. of strychnine* by means of intraperitoneal injections into white mice. The chloroform residues were taken up in 1·0 c.c. of physiological saline solution slightly acidified with hydrochloric acid and injected intraperitoneally into 14 day old white mice weighing 5 to 6 gm. With the potassium bichromate-sulphuric acid test no violet colour was detectable with quantities of strychnine smaller than 0·007 to 0·01 mg. It is therefore evident that the biological test, in which 14 day old white mice are employed is approximately twice as sensitive as the chemical test (Otto's test).

* In the previous paper (Steyn, 1935) page 172, paragraph (2) "strychnine sulphate" should read "strychnine."

Control Dogs.

The results of experiments conducted upon the five control dogs are quoted in the following table:—

TABLE II.

Carcasses of Control Dogs Shot on 7th September, 1933.

Dog No.	Weight of dry bones exhumed and extracted.	Effects of extracts of the bones injected intraperitoneally into 14 day old white mice (5–6 gm. in weight).
1251	300 gm.	Otto's test—dark green discolouration. Taste—pronounced transient bitter taste. Injected mouse developed no symptoms of poisoning.
1315	500 gm.	Otto's test—yellowish-green discolouration. Taste—slightly bitter. Injected mouse developed general paresis within 5 minutes after injection and was paralysed within a further 5 minutes. When prostrate the mouse suddenly developed repeated attacks of clonic spasms of the whole body musculature. This lasted until death which occurred 3¼ hours after injection.
1316	500 gm.	Otto's test—greenish discolouration, which passed into brown. Taste—tasteless. Injected mouse developed slight transient apathy and paresis.
1326	600 gm.	Otto's test—dark-green discolouration. Taste—fairly pronounced bitter taste. Injected mouse developed slight paresis of all the muscles and recovered within ½ hour
1327	410 gm.	Otto's test—slight violet discolouration which changed to light brick-red. Taste—not bitter. Injected mouse became paretic 3 minutes after injection, but recovered after 1½ hours.

It is of great interest and extreme importance to note that the evaporated chloroform residue prepared from the bones of dog 1327 yielded a colour reaction with potassium bichromate and sulphuric acid which bore a marked resemblance to that seen in positive tests for strychnine. The result of the more sensitive biological test, however, excluded the possibility of strychnine being present. The slight positive colour test for strychnine was therefore probably due to the presence of ptomaine(s).

Also in the case of the control dogs the symptoms of paresis and paralysis, induced in the injected mice by the evaporated chloroform residues were probably due to the presence of ptomaines.

Discussion and Conclusions.

In the course of experiments conducted upon twenty-four dogs killed with strychnine and upon five control dogs, and of analysis of specimens of animal organs submitted in cases of suspected

strychnine poisoning the author had occasion to analyse hundreds of specimens for the presence of strychnine. From the results of these investigations the following conclusions can be drawn:—

(1) Serious mistakes in the detection of strychnine can be made if only colour tests are applied to the material to be tested. It is absolutely essential that three different tests, namely the taste test, a colour test (potassium bichromate-sulphuric acid test) and the biological test be applied. The biological test at the same time allows of a fairly accurate quantitative determination of strychnine. It has repeatedly been experienced by the author that extracts of animal organs submitted for analysis have yielded a fairly distinct positive Otto test for strychnine, whilst it was definitely proved by means of biological tests upon white mice and by the taste test that no strychnine was present in the extracts. From Tables I and II it is evident that extracts prepared from the bone of four dogs (1253, 1311, 1317 and 1312) killed with strychnine and from the bones of one control dog (1327) yielded colour reactions with the Otto test which could easily be mistaken for those of strychnine. *Our one and only safeguard is the biological test.* The author therefore cannot but agree with Gadamer (1924) and Wrede (1937) who state that the colour tests for strychnine, when only small quantities of material are available, cannot be exclusively relied upon but should be confirmed by the biological test. Van Itallie and Bylsma (1930) refer to repeated statements made in the literature *that ptomaines in decomposing carcasses and corpses may yield colour reactions, especially with the Otto test, which very closely resemble those seen in strychnine.* Van Itallie and Bylsma further state that no ptomaines are known which yield both a positive chemical and biological test. Extensive experience gained by the author in the course of the last twelve years definitely confirms the above statements made by various authors.

In order to conduct both the Otto test (potassium bichromate-sulphuric acid test) and the biological test, using 14 day old white mice, it is possible to detect quantities of strychnine as small as 0·011 mg.

In the extraction of strychnine from human and animal organs, Stewart, Chatterji and Smith (1937) recommend that the protein- and fat-containing material be precipitated by grinding the material to be tested with an equal volume of a 10 per cent. trichloracetic acid solution. The filtrate is then shaken with kaolin which absorbs the strychnine. It has, however, as yet to be established whether it would be possible to isolate quantities of strychnine as small as can be done with the Stas-Otto process carefully executed. There is a possibility of a certain amount of the strychnine present in the specimen being removed with the proteins and fats during the process of precipitation by means of trichloracetic acid and this would certainly be undesirable in cases where only minimal amounts of strychnine are present in the specimens to be analysed.

Wrede (1937) states that he found his method of determining and weighing strychnine as the aurochlorate more satisfactory than the Stas-Otto process. He was able to determine quantities of

strychnine as small as 0·1 mg. From facts stated above it is evident that the method suggested by the author, namely, the Otto colour test and the biological test, allows of the determination of much smaller quantities of strychnine (±0·011 mg.). In the course of routine analysis of specimens of animal organs submitted for the strychnine test the entire specimens were frequently found to contain less than 0·1 mg. of strychnine.

(2) It appears that the view that strychnine is still detectable in carcasses and corpses for periods up to twelve years and longer, after death, is fallacious. In twenty-four dogs killed with approximately 5 m.l.d. of strychnine sulphate it was impossible to detect strychnine in any one of them four years and two months after death and burial. No strychnine was detectable in some of these carcasses within eighteen weeks after death, and in four out of eight carcasses exhumed eleven months after death it was impossible to detect strychnine.

It therefore appears that strychnine, like so many other organic poisons, disappears from carcasses and corpses in due course of time, although it appears to resist destruction to a fair extent during the processes of decomposition.

Summary.

1. A description is given of the results of experiments conducted upon twenty-nine dogs in order to ascertain (*a*) for what period after death strychnine is still detectable in carcasses and corpses, and (*b*) what tests are essential in the testing of materials for the presence of strychnine.

2. *It has been definitely established that it is absolutely essential to apply the taste test, colour test (Otto's test) and biological test to materials (purified extracts) to be tested for the presence of strychnine, especially when only minimal quantities of strychnine are present in such materials.* This statement is made with special reference to the detection of strychnine in carcasses and corpses, as it has been established that there is a possibility of decomposition products (ptomaines) being present which yield colour reactions similar to those seen with strychnine.

It is obvious that in forensic medicine it is of the utmost importance to bring absolute proof of the presence or absence of strychnine in carcasses and corpses, hence the necessity for applying all three the above-mentioned tests.

3. Strychnine, like so many other organic poisons, disappears from carcasses and corpses in the course of time. The view so generally held that strychnine is still detectable in carcasses and corpses for periods up to twelve years, or longer, after death appears to be fallacious.

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