THE ISOLATION OF THE CONIUM ALKALOIDS FROM ANIMAL TISSUES, AND THE ACTION OF LIVING CELLS AND DECOMPOSING ORGANS ON THESE ALKALOIDS

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The literature on the isolation of the conium alkaloids is very limited, and the only treatises requiring mention are those of Harley¹ and Zalewsky.² Zalewsky's method consisted in extracting the organs with acidified water, followed by alcohol, and in shaking out the final residue with petroleum ether after making it alkaline with ammonia. This author has given no quantitative results, and from the fact that he frequently describes scaffold-like crystals as occurring in the final residue, one is inclined to believe that the residue in many cases consisted of ammonium chloride, which gives dense precipitates with phospho-molybdic acid, as he describes.

PRELIMINARY OBSERVATIONS

On account of the volatility of pure coniine, it is impossible to obtain a satisfactory result unless the base is converted into one of its salts which are not volatile at 100° C. The hydrochlorate of coniine is very suitable for the purpose, as it crystallises in long, double-refracting, silky needles, which are easily recognisable. Coming to the estimation of the amount of coniine present, there is a choice of three methods, namely:—(1) The weight of the residue; (2) estimation of pure coniine by titration with normal acid using suitable indicators, such as iodeosin, haematoxylin, cochineal, lacmoid, or congo red; (3) estimation of the alkaloid or its salts by titration with some precipitating reagent, such as Mayer's solution. In the following experiments I have used a combination of the first and third methods, and, in applying the latter, I have estimated the amount of the alkaloid by the number of drops from a capillary tube required to

1. *Old Vegetable Neurotics*, pp. 19 and 80, 1869.

2. *Untersuchungen über das Coniin. Dissert., Dorpat*, p. 17, 1869.

titrate the residue which was acidulated with a definite quantity of dilute hydrochloric acid. This method gave in test cases results which were correct to the fourth decimal place in grams. It will be noted that in many of the experiments the weight of the residue and the amount of alkaloid present, as estimated by Mayer's reagent, do not agree; this is due to the fact that one cannot get a pure alkaloidal residue without going through processes which would entail serious loss of the alkaloid. In such cases the amounts estimated by Mayer's reagent may be taken as correct.

I. ISOLATION OF CONIINE BY DISTILLATION WITH AN ALKALI

The distillation method I have used was that ordinarily employed for such purposes; I have not adopted the method of distilling in hydrogen gas, as I found this unnecessary, also it is not readily applicable to the distillation of animal organs. When coniine hydrochlorate is distilled with sodium hydrate, I have found that a certain amount of ammonia is present in the distillate, and also that the crystals of coniine hydrochlorate re-obtained from the distillate are not nearly so strongly double-refracting as they usually are. If 10 mg. of coniine hydrochlorate is used, the whole of the coniine distils over in the first 12 to 20 c.c. of fluid, and the alkaloid, when estimated by Mayer's reagent, was present to the amount of 9.5 mg. On the other hand, by distilling the coniine hydrochlorate with sodium carbonate, almost no ammonia is produced, and the crystals obtained from the distillate are strongly double-refracting, and, again, when using 10 mg. of the salt the whole of the coniine is contained in the first 20 c.c. of fluid distilled, and this amounted in test cases to exactly 10 mg. Experiments done with sodium bicarbonate showed a more marked production of ammonia but no effect on the refraction of the crystals.

When, however, coniine has to be isolated from organic matters there is always found in the distillate a large amount of ammonia, and, on evaporating the distillate with hydrochloric acid, ammonium chloride forms the greatest proportion of the residue. This salt I have removed by treating the residue with a mixture of two parts of absolute alcohol and one part of ether, or with pure chloroform, which is much more satisfactory. The organic matters were distilled immediately after the addition of the alkaloid.

(1) *Isolation from Urine*

The residue, left after evaporation of the neutralised distillate, is usually so large that it requires to be treated two or three times with the above solvents.

		200 c.c. human urine with 10 mg. coniine hydrochlorate		200 c.c. human urine with 10 mg. coniine hydrochlorate
Residue from chloroform	...	Double-refracting, needle-like crystals		Strongly double-refracting, needle-like crystals and irregular crystals, not double-refracting ¹
Weight of residue	...	0.007 gms.	...	0.013 gms.
Estimated alkaloid by Mayer	...	0.0054 gms.	...	0.0083 gms.
On boiling Mayer's ppt. with sodium-hydrate	...	Distinct smell of coniine	...	Strong smell of coniine

From the above experiments it will be seen that 83 per cent. of 10 mg. of coniine hydrochlorate can be regained from urine.

(2) *Isolation from Blood*

Forty c.c. of calf's blood with 5 mg. of coniine hydrochlorate was distilled with 100 c.c. of water and excess of sodium carbonate. The crystals of the residue being not quite typical, they were made alkaline by sodium hydrate and the freed base shaken out with ether and re-converted into the hydrochlorate. The crystals obtained were long, transparent, double-refracting needles. A solution of these in acidified water gave dense precipitates with Dragendorff's and Rohrbach's reagents, and also with phospho-wolframic and phospho-molybdic acids, and, on boiling with lime water, a strong smell of coniine was given off. This shows that a sufficient amount of 5 mg. can be regained from 40 c.c. of blood for identification purposes.

(3) *Isolation from Liver*

		100 gms. horse's liver with 10 mg. coniine hydrochlorate	100 gms. horse's liver with 10 mg. coniine hydrochlorate	100 gms. horse's liver with 10 mg. coniine hydrochlorate
Residue from chloroform		Long, needle-shaped crystals, strongly double-refracting	Long, needle-shaped crystals, strongly double-refracting	Pure, long, needle-shaped crystals, strongly double-refracting
Weight of residue	...	0.0045 gms.	0.005 gms.	0.0085 gms.
Estimated alkaloid by Mayer	...	0.00416 gms.	0.005 gms.	0.0083 gms.
On boiling ppt. with sodium hydrate	...	Strong smell of coniine	Strong smell of coniine	Strong smell of coniine

These results demonstrate that one can obtain back from liver not less than about half and not more than 83 per cent. of the coniine salt.

1. As to what the other crystals present were, one cannot give any opinion. Certainly they did not seem to affect the titration by Mayer's reagent.

(4) *Isolation from Spleen*¹

				100 gms. horse's spleen with 10 mg. coniine hydro- chlorate
Residue from chloroform	...	...	...	Long, transparent, double- refracting needles
Weight of residue	...	...	...	0.0085 gms.
Estimated alkaloid by Mayer	...	...	...	0.00833 gms.
On boiling Mayer's ppt. with sodium hydrate	...	...	...	Strong smell of coniine

Thus 83 per cent. of alkaloid can also be regained from spleen.

II. EXTRACTION OF CONIINE BY TREATMENT WITH ALCOHOL AND
SUBSEQUENTLY SHAKING OUT WITH ETHER

The finely minced organs containing the alkaloidal hydrochlorate were extracted three times with fresh portions of absolute alcohol. After filtering, the alcohol was evaporated off and the residue again extracted with fresh alcohol; this was filtered, evaporated, and the residue treated with water, any insoluble matter being removed. The watery solution was then made alkaline with sodium hydrate, and the freed base removed by shaking out with ether. In earlier experiments, the ether was evaporated at a low temperature, but in later cases it was acidified with hydrochloric acid before evaporation, in order to obtain the crystalline hydrochlorate. Urine was treated by the same process except that it was evaporated to syrupy consistence with excess of hydrochloric acid, before adding alcohol. When dealing with fatty organs, the ether frequently separated as a muddy layer. This trouble has been avoided either by previous shaking of the still acid water with ether or by evaporating the muddy ether which has separated from the alkaline water with hydrochloric acid, and extracting the residue with water, filtering, and evaporating the watery solution.

(1) *Extraction from Urine*

		100 c.c. human urine with 10 mg. coniine hydrochlorate		100 c.c. human urine with 10 mg. coniine hydrochlorate
Residue from ether	...	Double-refracting, needle- like crystals and some resinous matter	*	Double-refracting, needle- like crystals and some resinous matter
Weight of residue	...	0.010 gms.	...	0.015 gms.
Estimated alkaloids by Mayer	...	0.0046 gms.	...	0.0054 gms.
On boiling Mayer's ppt. with sodium hydrate	...	No smell of coniine ²	...	Distinct smell of coniine

1. The minced spleen containing the alkaloid was first treated with tannic acid in presence of HCl to precipitate with albumin as much of the fatty matter as possible. The filtrate was then rather easier to distil. Tannic acid in presence of HCl does not precipitate coniine salts.

2. The probable explanation of this is that since I had been working with pure coniine just before and as the nose becomes rapidly insensible to this smell, I had failed to detect the slight odour which must have been present.

(2) *Extraction from Blood*

Fifty c.c. of calf's blood with 5 mg. coniine hydrochlorate. The blood was first acidified and coagulated by heat, the coagulum was filtered off, and the filtrate evaporated to a syrup and treated as with urine.

Residue from ether.—Long, double-refracting, needle-like crystals, which, when dissolved in acidulated water, gave a dense precipitate with Dragendorff's reagent, but only slight precipitates with phospho-molybdic and phospho-wolframic acids, and Rohrbach's reagent; on boiling the solution with excess of lime water a distinct smell of coniine was evolved.

(3) *Extraction from Liver*

The following table will give some idea of the results which are obtained when the coniine is isolated as a free base. It also shows that by the method of extraction one may isolate substances from liver which show alkaloidal reactions.

	Dragendorff's reagent	Phospho-wolframic acid	Phospho-molybdic acid
Pig's liver 80 gms. without alkaloid	Slight yellow ppt.	Faint white ppt.	Faint yellow ppt.
„ „ + 10 mg. coniine HCl	Good yellow ppt.	Slight white ppt.	Slight yellow ppt.
„ „ + 10 mg. coniine HCl	Good yellow ppt.	Faint white ppt.	Faint yellow ppt.

In the following results the ether was acidified before evaporation.

	100 gms. horse's liver with 10 mg. coniine hydrochlorate	100 gms. horse's liver with 10 mg. coniine hydrochlorate
Residue from ether	Transparent, needle-like crystals, slightly double- refracting, along with some resinous matter	Long, transparent, double- refracting needles, arranged in sheaves
Weight of residue	0.003 gms.	—
Estimated alkaloids by Mayer...	0.0021 gms.	0.00125 gms.
On boiling Mayer's ppt. with sodium hydrate	Faint smell of coniine	Slight smell of coniine

(4) *Extraction from Spleen*

One hundred grams horse's spleen with 10 mg. coniine hydrochlorate.

Residue.—Long, double-refracting, needle-like crystals with some resinous matter.

Weight of residue.—0.009 grams.

Estimated alkaloid by Mayer.—0.0029 grams.

On boiling Mayer's precipitate with sodium hydrate.—Distinct smell of coniine.

(5) *Extraction from Kidney*

Fifteen grams rabbit's kidney with 10 mg. coniine hydrochlorate.

Residue.—Long, double-refracting needles, which, when dissolved in acid water, gave dense precipitates with alkaloidal reagents and gave off a strong smell of coniine when boiled with lime water.

III. PRECIPITATION OF THE ALKALOID BY MEANS OF PHOSPHO-WOLFRAMIC ACID

As phospho-wolframic acid gives precipitates with coniine hydrochlorate in presence of hydrochloric acid up to dilutions of 1 : 10000, the following method was adopted for the isolation of the alkaloids by this means:—The organs containing the alkaloid were coagulated by heat, the coagulum removed, and the filtrate treated with phospho-wolframic acid and some dilute hydrochloric acid till complete precipitation had occurred. The precipitate was filtered off, washed with water containing some phospho-wolframic acid, and then drained free of excess of fluid. The partially dried precipitate was rubbed up in a mortar with excess of barium hydrate and the freed alkaloid extracted with absolute alcohol. After being acidified with hydrochloric acid, the alcohol was evaporated off and the residue extracted with chloroform, filtered, and evaporated, when one ought to obtain the hydrochlorate of coniine pure. Any barium hydrate which dissolves in the alcohol may be removed by means of carbon dioxide or by extracting the residue of chlorides as above with chloroform. Urine was treated directly with the acids, but it is safer to precipitate it twice, since the first precipitate is very copious.

(1) *Precipitation from Urine*

	100 c.c. human urine with 10 mg. coniine hydrochlorate	100 c.c. human urine with 10 mg. coniine hydrochlorate
Residue from chloroform ...	Double-refracting needles ...	Double-refracting needles
Weight of residue ...	0.008 gms. ...	0.006 gms.
Estimated alkaloid by Mayer ...	0.0042 gms. ...	0.0046 gms.
On boiling Mayer's ppt. with sodium hydrate ...	Faint smell of coniine ...	Distinct smell of coniine

(2) *Precipitation from Blood*

Fifty c.c. calf's blood with 5 mg. coniine hydrochlorate.

Residue.—Long, needle-like crystals, strongly double-refracting.

Weight of residue.—0.004 grams.

Estimated alkaloid by Mayer.—0.0017 grams.

On boiling Mayer's precipitate with sodium hydrate.—Faint smell of coniine.

(3) *Precipitation from Liver*

	100 gms. horse's liver with 10 mg. coniine hydrochlorate	100 gms. horse's liver with 10 mg. coniine hydrochlorate	100 gms. horse's liver with 10 mg. coniine hydrochlorate
Residue from chloro- form	Long, needle-shaped crystals in sheaves; strongly double- refracting	Double-refracting, needle-like crystals	Few, irregular, double- refracting crystals and some fatty matter
Weight of residue ...	—	0.008 gms.	0.005 gms.
Estimated alkaloid by Mayer	0.0037 gms.	0.0025 gms.	0.0005 gms. ¹
On boiling Mayer's ppt. with sodium hydrate	Distinct smell of coniine	Smell of coniine	Faint, but recognisable smell of coniine

(4) *Precipitation from Spleen*

One hundred grams horse's spleen with 10 mg. coniine hydrochlorate.

Residue from chloroform.—Long, double-refracting, needle-like crystals.

Weight of residue.—0.008 grams.

Estimated alkaloid by Mayer.—0.0021 grams.

On boiling Mayer's precipitate with sodium hydrate.—Distinct smell of coniine.

(5) *Precipitation from Kidney*

Fifteen grams rabbit's kidney with 10 mg. coniine hydrochlorate.

Residue from chloroform.—Double-refracting, needle-like crystals which, dissolved in acid water, gave dense precipitates with alkaloidal reagents and gave off a strong smell of coniine when boiled with lime water.

IV. PRECIPITATION BY KRAUT'S REAGENT

This process proved in my hands quite useless for coniine, as the precipitate was of such a nature that it passed readily through a filter, and, again, it was found impossible to get rid of the iodine completely.

CONCLUSIONS ON THE ISOLATION OF CONIINE

It will be observed that, in the case of the distillation, the best results are in three cases 83 per cent., while with the alcohol and ether process and precipitation method the results vary considerably, and in most instances the results are considerably below 50 per cent. If one makes an average of all the figures one finds that the average return by distillation is 65.7 per cent., while the other two methods only show half

1. This liver proved very difficult to filter after boiling.

this amount. Again, the distillation method can be carried through in at most three hours, while the other two occupy eight hours at least, and the alkaloidal salt obtained in the end is much purer in the case of distillation, as may be surmised by the small differences between the weights of the residues and the amounts of alkaloid found by Mayer's reagent. Taking everything into account, only one conclusion is possible, that, for practical purposes and satisfactory results, distillation is the most valuable method for isolating coniine from tissues.

ACTION OF LIVING CELLS ON CONIINE

In order to ascertain whether living cells had any power of decomposing coniine or in any way interfering with its recognition in the animal organism after death, finely minced liver containing coniine hydrochlorate was mixed with 100 c.c. normal saline solution, to which had been added 1 c.c. of chloroform and 1 c.c. of toluol to prevent decomposition, and the whole left in a water bath at a temperature of 38° C. for a definite period. The alkaloid was re-isolated by distillation.

	60 gms. rabbit's liver with 20 mg. coniine hydro- chlorate	60 gms. rabbit's liver with 10 mg. coniine hydro- chlorate	30 gms. rabbit's liver with 10 mg. coniine hydro- chlorate	100 gms. horse's liver with 10 mg. coniine hydro- chlorate	100 gms. horse's liver with 10 mg. coniine hydro- chlorate
Time on water bath ...	16 hrs.	18 hrs.	24 hrs.	12 hrs.	18 hrs.
Residue from chloro- form ...	Long, double- refracting, needle-like crystals	Long needles, slightly double- refracting	Some long, double- refracting needles	A few needle- like crystals, not double- refracting	A few long, double- refracting crystals
Weight of residue ...	0.0065 gms.	0.003 gms.	0.010 gms.	0.002 gms.	0.002 gms.
Estimated alkaloid by Mayer ...	0.0065 gms.	0.0021 gms.	0.0037 gms.	No ppt.	0.0004 gms.
Boiling ppt. with sodium hydrate ...	Distinct smell of coniine	Faint smell of coniine	Distinct smell of coniine	No smell	Faint smell of coniine

All these livers were re-distilled with dilute sulphuric acid, the distillates neutralised with sodium carbonate and evaporated to dryness. To the residue absolute alcohol and excess of concentrated sulphuric acid were added, and in all the above cases a very strong smell of butyric ether was evolved, mixed in some cases with the smell of acetic ether. A test experiment, done with liver alone, gave a distinct smell of butyric acid, but not so powerful as that of the above cases.

Conclusions.—If one takes the average amount of alkaloid which may be regained from liver by distillation as 58 per cent.—a low estimate—it will be seen from the table that there is a remarkable loss of alkaloid

after the cells of the liver have been allowed to act on it even for a short time. The powerful smell of butyric acid would seem to suggest that the coniine may probably be broken up into this substance. Whether this suggestion is correct or not, I am unable as yet to say, but I hope to follow this up shortly.

ACTION OF DECOMPOSING TISSUES ON CONIINE

To ascertain whether decomposing tissues had any influence on coniine, minced liver with the alkaloidal hydrochlorate was mixed with 100 c.c. of normal saline solution and left in a water bath at 38° C. for a definite period. The alkaloid was isolated by distillation.

	60 gms. rabbit's liver with 10 mg. coniine hydro- chlorate	160 gms. horse's liver with 10 mg. coniine hydro- chlorate	100 gms. horse's liver with 10 mg. coniine hydro- chlorate	100 gms. horse's liver with 10 mg. coniine hydro- chlorate	100 gms. horse's liver with 10 mg. coniine hydro- chlorate
Time on water bath ...	18 hrs.	24 hrs.	24 hrs.	24 hrs.	24 hrs.
Residue from chloro- form ...	Some trans- parent double- refracting crystals, which were very deli- quescent	Double- refracting needles, not typical of coniine hydro- chlorate, and very deliquescent	Deliquescent needles, some double- refracting, others not	Needle-like crystals, not double- refracting and not deliquescent	Double- refracting needles, not typical of coniine hydro- chlorate, and very deliquescent
Weight of residue ...	0.009 gms.	0.004 gms.	0.01 gms.	0.003 gms.	0.009 gms.
Estimated alkaloid by Mayer ...	0.005 gms.	0.0021 gms.	0.0025 gms.	0.0021 gms.	0.0008 gms.
Boiling ppt. with sodium hydrate ...	— ²	Peculiar smell, resembling ammonia	— ¹	Peculiar smell, resembling ammonia	Strong and peculiar smell, resembling ammonia

Conclusions.—It will be apparent that the maximum amount of alkaloid found after twenty-four hours was 2.5 mg., but the crystals of the residue were only partially double-refracting and, at the same time, very deliquescent, while the smells evolved in all cases were ammonia-like. In Experiment III it should have been easily possible to obtain the uranium nitrate reaction for coniine, but this failed; in Experiment I, however, a positive reaction was got, probably since the quantity of liver was small and the time for decomposition short. I am, therefore, of the opinion that no coniine was present in the distillate from the latter four experiments, but that the substance obtained was a body possessing

1. On treating the Mayer's precipitate in this case with sodium carbonate, carbon bisulphide, and uranium nitrate, and shaking up with toluol—no red colour was obtained in the liquid.

2. The same reaction in this case, however, gave a red colour in the toluol which would indicate that coniine was present.

some of the characters of coniine hydrochlorate, while lacking others. The suggestion is possible that many of these crystals were cholin hydrochlorate, which is double-refracting and very deliquescent. This salt also crystallises in needles, and when boiled with sodium hydrate a smell of trimethylamine is given off which would correspond closely to the smell perceived above. The cholin hydrochlorate must have passed through the filter in a deliquesced state along with the chloroform extract.

ISOLATION OF CONHYDRINE

The isolation of conhydrine proved more difficult, on account of the fact that it cannot be distilled from watery solutions, even when these are saturated with calcium chloride or under pressures as low as 10 mm. of mercury. Conhydrine is also less soluble in ether than in water, and chloroform, in which it is readily soluble, gave unsatisfactory results, so that it was discarded in favour of repeated shakings with fresh ether. Again, conhydrine forms a very deliquescent hydrochlorate, and was therefore isolated as the free base, care being taken to evaporate solutions of this at low temperatures. With reference to the process of precipitation, phospho-wolframic acid is again the only suitable method, but its delicacy only reaches to dilutions of 1 : 1000. The extracts of the organs were therefore evaporated to small bulk before adding the reagent, and urine was first precipitated with lead acetate in presence of hydrochloric acid, in order to avoid bulky precipitates with the reagent. For the estimation of the amount of alkaloid recovered, phospho-wolframic acid was substituted for Mayer's reagent, as the latter is not sufficiently delicate.

(1) *Extraction from Urine by Alcohol and Ether*

	50 c.c. human urine with 10 mg. conhy- drine	100 c.c. human urine with 10 mg. conhy- drine	100 c.c. human urine with 10 mg. conhy- drine	100 c.c. human urine with 10 mg. conhy- drine	200 c.c. human urine with 10 mg. conhy- drine
Residue from ether ...	Double- refracting needles and plates, the latter having one angle cut out	Needle-like, double- refracting crystals	Double- refracting, needle-like crystals	Double- refracting needles and flat, oblong crystals, having an angle cut out	Double- refracting, needle and plate-like crystals, the latter having an angle cut out
Weight of residue	0-008 gms.	0-005 gms.	0-012 gms.	0-015 gms.	0-014 gms.
Estimated alka- loid by phospho- wolf. acid ...	0-0012 gms.	0-0017 gms.	0-0045 gms.	0-0075 gms.	0-0037 gms.
On boiling ppt. with sodium hydrate ...	Distinct smell of conhydrine	Distinct smell of conhy- drine	Strong smell of conhydrine	Very strong smell of conhydrine	Strong smell of conhydrine

(2) *Extraction from Liver by Alcohol and Ether*

	100 gms. horse's liver with 10 mg. conhy- drine	100 gms. horse's liver with 10 mg. conhy- drine	100 gms. horse's liver with 10 mg. conhy- drine
Residue from ether ...	No crystals	Needle-like crystals, slightly double- refracting	Double-refracting crystals of oblong form with one of the angles cut out
Weight of residue ...	0.002 gms.	0.003 gms.	0.009 gms.
Estimated alkaloid by phosp.-wolf. acid ...	0.0005 gms.	0.00075 gms.	0.0037 gms.
On boiling ppt. with sodium hydrate ...	Faint smell of conhydrine	Distinct smell of conhydrine	Strong smell of conhydrine

(1) *Precipitation from Urine by Phospho-wolframic Acid*

	100 c.c. dog's urine with 10 mg. conhy- drine	100 c.c. human urine with 10 mg. conhy- drine	100 c.c. human urine with 10 mg. conhy- drine
Residue from chloroform	Colourless, imperfect, double-refracting crystals	Imperfect, double- refracting crystals	Irregular, double- refracting crystals
Weight of residue ...	0.004 gms.	0.006 gms.	0.009 gms.
Estimated alkaloid by phosp.-wolf. acid ...	0.0015 gms.	0.0025 gms.	0.006 gms.
On boiling ppt. with sodium hydrate ...	Faint smell of conhy- drine	Distinct smell of conhydrine	Strong smell of conhy- drine

(2) *Precipitation from Liver by Phospho-wolframic Acid*

	100 gms. horse's liver with 10 mg. conhy- drine	100 gms. horse's liver with 10 mg. conhy- drine	100 gms. cow's liver with 10 mg. conhy- drine
Residue from chloroform	Irregular, double- refracting crystals	No crystals	Transparent needles, double-refracting and very deli- quescent
Weight of residue ...	0.004 gms.	0.003 gms.	0.005 gms.
Estimated alkaloid by phosp.-wolf. acid ...	0.0012 gms.	0.0025 gms.	0.0025 gms.
On boiling ppt. with sodium hydrate ...	Distinct smell of conhydrine	Distinct smell of conhydrine	Strong smell of conhydrine

CONCLUSIONS ON THE ISOLATION OF CONHYDRINE

The figures given above show that, by neither of these two methods are results got which are in any way constant. The higher figures are those obtained from later experiments and show probably about the limit attainable by these processes. One can, however, say that conhydrine can be isolated from both urine and liver in quite appreciable amounts, even when the quantity added consists of only 10 mg. in 100 c.c. or grams of substance.

ACTION OF LIVING CELLS ON CONHYDRINE

The method used was the same as that described for coniine. The alkaloid was extracted by alcohol and ether.

		100 gms. cow's liver with 10 mg. conhy- drine	100 gms. horse's liver with 10 mg. conhy- drine	100 gms. horse's liver with 10 mg. conhy- drine
Time on water bath	...	18 hrs.	18 hrs.	12 hrs.
Residue from ether	...	Thin gummy layer, no crystals	No crystals	A few double- refracting needle- like crystals
Weight of residue	...	0.011 gms.	0.003 gms.	0.012 gms.
Estimated alkaloid by phosp.-wolf. acid	...	0.00076 gms.	0.001 gms.	0.0037 gms.
On boiling ppt. with sodium hydrate	...	Doubtful smell of conhydrine	Faint smell of conhy- drine	Distinct smell of conhydrine

ACTION OF DECOMPOSING TISSUES ON CONHYDRINE

The alkaloid was treated in the way described for coniine and isolated by means of the alcohol and ether process.

		100 gms. horse's liver with 10 mg. conhydrine		100 gms. horse's liver with 10 mg. conhydrine
Time on water bath	...	18 hrs.		18 hrs.
Residue from ether	...	Gummy skin, no crystals	...	Resinous residue and some double-refracting, oblong crystals
Weight of residue	...	0.008 gms.	...	0.008 gms.
Estimated alkaloid by phosp.-wolf. acid	...	0.0015 gms.	...	0.002 gms.
On boiling ppt. with sodium hydrate	...	Faint smell of conhydrine	...	Distinct smell of conhydrine

Conclusions.—The above results show that, on account of the fact that the method of isolation does not give constant returns, it is impossible to draw any definite conclusion as to whether or not conhydrine is affected by the action of living cells or decomposed tissues.

ISOLATION OF PSEUDO-CONHYDRINE

On account of the small amount of this alkaloid which I possess, I have limited this research to a very few experiments, which, however, show fairly satisfactory results. The difficulties encountered in the isolation of pseudo-conhydrine are the same as those detailed under conhydrine, and they were avoided by the same methods. Pseudo-conhydrine cannot be distilled from watery solutions.

(1) *Extraction from Urine by Alcohol and Ether*

	60 c.c. human urine with 10 mg. pseudo- conhydrine	100 c.c. human urine with 10 mg. pseudo- conhydrine	200 c.c. human urine with 10 mg. pseudo- conhydrine
Residue from ether ...	Double-refracting, needle-like crystals	Double-refracting, small, needle-like crystals	Double-refracting, small, needle-like crystals
Weight of residue ...	0.014 gms.	0.006 gms.	0.007 gms.
Estimated alkaloid by phosp.-wolf. acid ...	0.0062 gms.	0.0035 gms.	0.0017 gms.
On boiling ppt. with sodium hydrate ...	Strong smell of pseudo-conhydrine	Distinct smell of pseudo-conhydrine	Faint smell of pseudo-conhydrine

(2) *Extraction from Liver by Alcohol and Ether*

	100 gms. horse's liver with 10 mg. pseudo- conhydrine	100 gms. horse's liver with 10 mg. pseudo- conhydrine
Residue from ether ...	No distinct crystals	Small hair or needle-like crystals, double-refracting
Weight of residue ...	0.002 gms.	0.007 gms.
Estimated alkaloid by phos.- wolf. acid ...	0.0005 gms. ¹	0.004 gms.
On boiling ppt. with sodium hydrate ...	No smell of alkaloid	Distinct smell of pseudo- conhydrine

(1) *Precipitation from Liver by Phospho-wolframic Acid*

100 grams horse's liver with 10 mg. pseudo-conhydrine.

Residue from chloroform.—No crystals.

Weight of residue.—0.002 grams.

Estimated alkaloid by phospho-wolframic acid.—0.0015 grams.

On boiling precipitate with sodium hydrate.—A faint smell of pseudo-conhydrine.

Conclusions.—The results indicate that, with urine, it is possible to isolate 35 per cent. of the alkaloid added, while, with liver, about 40 per cent. can be regained. The single experiment done with phospho-wolframic acid shows a fairly good return for that method, namely, 15 per cent.

ACTION OF DECOMPOSING TISSUES ON PSEUDO-CONHYDRINE

One experiment was done with this alkaloid by leaving 10 mg. in 100 grams of horse's liver in a water bath for twelve hours, as described for coniine. The alkaloid was extracted by alcohol and ether.

Residue from ether.—A few, irregular, double-refracting crystals in a gummy matrix.

1. Liver difficult to filter.

Weight of residue.—0.003 grams.

Estimated alkaloid by phospho-wolframic acid.—0.0007 grams.

On boiling precipitate with sodium hydrate.—No smell which resembled pseudo-conhydrine.

No definite conclusion can be drawn from this, since, in one case, as small a quantity was regained from fresh liver, although that was due to difficulties in carrying out the process.

SUMMARY

1. The most satisfactory method for the isolation of coniine from animal tissues is that of distillation.

2. Coniine appears to be decomposed both by the action of living cells and by decomposing tissues.

3. Conhydrine and pseudo-conhydrine can be isolated from animal tissues by extraction with alcohol and by precipitation with phospho-wolframic acid, but these methods do not give sufficiently constant results to allow of any definite conclusions being drawn as to the action of living cells or decomposing tissues on these poisons.

I have, finally, to thank Professor Kobert of Rostock for procuring for me the materials used in this research and for his kind assistance and advice. I wish also to acknowledge my indebtedness to Professor MacWilliam and Professor Cash for their courtesy in advising me with regard to the arrangement of the results.