The Estimation of Chloroform in the Blood of Anæsthetised Animals.

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(From the Physiological Laboratory of the University of London.)

In a former paper* we expressed doubt whether the methods employed by Dr. Maurice Nicloux and other French observers for accurately determining the percentage of chloroform in the blood of anæsthetised animals were capable of yielding such satisfactory results as those obtained by Nicloux when known quantities of chloroform were added to blood *in vitro;* on the ground that the mixing of blood and chloroform *in vitro* is an essentially different process from that which takes place during the physiological process of inhalation, since it has been shown that, during anæsthesia, chloroform associates itself particularly with the red corpuscles.

In order to test the validity of our criticism, and to enable the results obtained by the French observers to be correlated with our own, and also to gain an idea as to how far the method employed by Nicloux is capable of giving reliable results in the case of tissues to which our method may not be readily applicable, we have made a series of parallel estimations by the two methods on the blood of anæsthetised animals.

In our experiments with the method of Nicloux, we followed closely the procedure which he has detailed†, and we take this opportunity to express our indebtedness to Dr. Nicloux for his kindness in supplying us with the distillation-apparatus which he uses, and in giving us a demonstration of his method. As Nicloux points out,‡ the chief difficulty in his method consists in determining exactly the end-point of the reaction which occurs in titrating with silver nitrate, using potassium chromate as an indicator. While taking every care to hit the end-point as closely as possible, we uniformly slightly over-stepped rather than under-stepped the mark. In carrying out the method, we found it necessary to use caustic soda made

* 'Roy. Soc. Proc.,' B, vol. 78, p. 418, 1906.

† "Dosage de petites quantités de chloroforme," 'Extraits du Bulletin de la Société Chimique de Paris,' 3rd series, vol. 33, p. 321, 1906.

‡ *Ibid.*

from sodium instead of caustic potash as recommended by Nicloux, as we were unable in this country to obtain potash absolutely free from chloride.

I. *Analyses of Chloroform by the Carius and by the Nicloux Method (Dumas' Reaction).*

The chloroform used in these experiments was kindly distilled for us by Dr. J. Wade of Guy's Hospital. It had a boiling-point of 61·14°—61·15° C. at 760 mm. and a specific gravity 1·5088 15°/15°. The results of the analyses are given in Table I and Table II.

Table I (Carius' determinations).

Amount of chloroform taken.	Percentage of chlorine.	Theoretical percentage of chlorine.	Percentage of chloroform found.
gramme.			
0·1177	89·172	89·102	100·07
0·2721	89·139	89·102	100·03
0·2683	89·084	89·102	99·97

Table II (Nicloux' determinations).

	Amount of chloroform taken.	Percentage of chlorine found.	Theoretical percentage of chlorine.	Percentage of chloroform found.
	gramme.			
I. Made with a solution of 0·92 per 1000 c.c. of alcohol	0·0184	89·262	89·102	100·18
II. Made with a solution of 1·575 per 1000 c.c. of alcohol	0·0315	88·041	89·102	98·8
III. Made with a solution of 1·6764 per 1000 c.c. of alcohol	0·0335	87·935	89·102	98·69
IV. Made with a solution of 3·3556 per 1000 c.c. of alcohol	0·06785	86·66	89·102	97·26
V. Made with a solution of 9·3675 per 1000 c.c. of alcohol	0·018735	90·54	89·102	101·6
VI. Do. do.	0·04684	87·378	89·102	98·06

From the second table it will be seen that the average percentage of chlorine found by the Nicloux method from the sample of chloroform is 88·3, a figure which is 0·8 per cent. below the theoretical value. The figures

are comparable with those given in the following table taken from Nicloux' paper.

	Poids de chloroforme.		Quantité de chloroforme retrouvé.
	Mis.	Trouvé.	
	millegrammes.	millegrammes.	par cent.
I. Faite avec une solution alcoolique de chloroforme à 10 grammes par litre	5	5·0	100·0
	10	9·8	98·0
	20	19·4	97·0
	50	48·6	97·0
	100	96·0	96·0
II. Faite avec une solution alcoolique de chloroforme à 2 grammes par litre	4	4·0	100·0
	10	9·9	99·0
	20	19·7	98·5
	40	39·8	98·5

II. *Determination of Chloroform in the Arterial Blood of Anæsthetised Animals by our Method and that of Nicloux (Dumas' Reaction).*

The experiments were conducted on cats. The animals were anæsthetised by ether, and the necessary operations for introducing cannulæ into the trachea and carotid artery were performed. The tracheal cannula was fitted to a Chauveau's valve and chloroform administered by a Woulff's bottle. Samples of blood were taken before the administration of chloroform, and at some selected period in the course of the anæsthesia a measured quantity of blood (20 to 30 c.c.) was withdrawn, and this was *at once* divided up into various proportions for analysis by the two methods. For the purpose of the Nicloux estimations some samples were allowed to clot before estimation,* some were treated with sodium oxalate to prevent clotting, others with hirudin, and others were defibrinated by shaking with mercury. In the case of blood withdrawn at the asphyxial point it is, in the case of cats, very difficult to weigh any sample before clotting takes place, since this occurs with great rapidity.

1. *Experiments in which the Blood was allowed to clot before treatment with acidified Alcohol.*

Varying weights of blood were taken on which to carry out duplicate experiments. During distillation, the clotted blood was found to become detached as a disc of leathery consistence, about the size and thickness of

* Dr. Nicloux has kindly informed us, by letter, of a detail that we did not gather from his paper, viz., that in his observations the blood is withdrawn with a syringe and immediately mixed with alcohol so that it does not clot *en masse*.

a four-shilling piece, which persisted to the end of the process. The formation of this leather-like mass was only partially prevented by breaking up the clot with a rod before distillation. The results of these experiments are given in the following table.

Table III.

Number of experiment.	Weight of blood taken.	State of anæsthesia.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.			
1	5·954	Asphyxia	0·053	
	7·925		—	0·0324
	7·6908		—	0·0354
2	5·1381	Near asphyxia	0·0575	
	9·7735		—	0·0269
	5·4656		—	0·0404
3	7·0302	Between vanishing of reflexes and asphyxia	—	0·0333
	9·5823		—	0·0236

2. *Experiment in which Blood was defibrinated by shaking with Mercury before the addition of Alcohol.*

Only one determination was made by this method, since it might be objected that some chloroform was lost in the shaking process.

Table IV.

Number of experiment.	Weight of blood taken.	State of anæsthesia.	Chloroform by Nicloux.
	grammes.		
4	9·4017	After 55 minutes' inhalation of 2 per cent. chloroform	0·021
	5·9302		0·025

3. In this series of experiments a comparison was made, using oxalated and clotted blood.

Table V.

Number of experiment.	Weight of blood.	Condition of the blood.	State of anæsthesia.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.				
5	5·0748	—	Between disappearance of reflexes and asphyxia	0·0298	
	7·02	Oxalated	—	—	0·0237
	5·5657	„	—	—	0·0244
6	5·2192	—	After 47 minutes with 3·7 per cent. chloroform	0·034	
	3·616	—		0·0358	
	6·883	Oxalated	—	—	0·0285
	8·2588	„	—	—	0·0256
	10·97	Clotted.....	—	—	0·017
7	4·6723	—	After 40 minutes' inhalation of 2 per cent. chloroform	0·0256	
	8·2447	Oxalated	—	—	0·022
	6·3256	„	—	—	0·0248
8	4·5388	—	After 35 minutes' inhalation of 2 per cent. chloroform	0·016	
	7·0821	Oxalated	—	—	0·0235
	7·7917	„	—	—	0·0206
9	4·1422	—	After half an hour inhalation of 3 per cent. chloroform	0·0447	
	7·9383	Oxalated	—	—	0·0338
10	4·2004	—	After a prolonged anæsthesia. Chloroform, given intermittently, about 3 per cent.	0·0485	
	6·6885	Clotted and clot broken up with glass rod	—	—	0·0333
	5·9814	Oxalated	—	—	0·031
11	4·2491	—	—	0·0354	
	5·2041	—	—	0·0339	
	11·6862	Clotted.....	—	—	0·0236
	5·7553	„	—	—	0·03
	9·4907	Oxalated	—	—	0·027

4. In the last series of experiments the cat was treated with hirudin in such proportion as to render the blood incoagulable for some minutes. We find that this is an excellent substance for checking the tendency to clotting, which is so liable to occur in cats at the moment of asphyxia.

Table VI.

Number of experiment.	Weight of blood.	Chloroform by Carius.	Chloroform by Nicloux.
	grammes.		
12	4·579	0·0319	
	4·1432	0·0327	
	14·9269	—	0·0334
	13·879	—	0·0359

Conclusions.

1. The method of chloroform determination, based on Dumas' reaction, though not possessing a very high degree of precision as an exact chemical method, as is apparent from a comparison of Tables I and II, is sufficiently accurate for most practical purposes and, as used by Dr. Nicloux, is a simple and rapid method, and one capable of giving satisfactory results for the estimation of small quantities of chloroform in air and in simple solution in such liquids as urine, etc.

2. In the case of the blood of anæsthetised animals, if the blood for analysis is allowed to clot before admixture with acid alcohol, the results by Nicloux' method are uniformly too low. An inspection of Tables III and V will show that results concordant amongst themselves are obtained when sensibly equal quantities of blood are taken, but, as might be expected, the figures decrease the larger the amount of blood used. In cases where smaller amounts of blood are taken and, consequently, the amounts of chloroform dealt with are very small, the results are often near the truth; but this, we think, is largely due to the inherent difficulty in the method as regards the determination of the end-point.

3. In cases where the clotting of blood was prevented by oxalate, the results more nearly approach those given by the methods we have previously adopted, but still are somewhat lower.

If we take the results given in Table V, the average percentage of chloroform indicated by our method is 0·0319, and by that of Nicloux in the case of oxalated blood is 0·0259. In our method we are satisfied that the average maximum error is not more than 5 per cent., generally considerably less. We find the difference between the results given by our method and that of Nicloux' method averages 19 per cent.

4. In the single experiment quoted with hirudin, the figures by the two methods are remarkably concordant.

5. On account of the variable and low results obtained with clotted blood, it may be inferred that the method of Nicloux may give low results when applied to organs or tissues which do not disintegrate when boiled with acid alcohol. We take this opportunity of stating that the expenses of the foregoing work were defrayed out of a grant made by the Royal Society.

Cyanogenesis in Plants. Part VI.—On Phaseolunatin and the Associated Enzymes in Flax, Cassava, and the "Lima Bean."

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In a previous paper of this series* it has been shown that the production of prussic acid by the seeds or beans ("Lima beans") of *Phaseolus lunatus* is due to the interaction of a cyanogenetic glucoside, phaseolunatin, with an enzyme, both these substances being proved to exist in the seeds.

Phaseolunatin was proved to have the composition and constitution of a dextrose ether of acetonecyanohydrin, but it was not then obtained in sufficient quantity to ascertain precisely the structure of the dextrose residue in the glucoside. Recently, however,† large supplies of "Java beans" (the beans produced by *Phaseolus lunatus* grown in Java) have been imported into this country, and we are indebted to Dr. Bernard Dyer for a small consignment of these beans, which has constituted the raw material from which the considerable quantities of phaseolunatin required in the course of the present investigation have been prepared.

Since the publication of our previous paper, it has been asserted by Kohn-Abrest‡ that these "Java beans" contain not one, but several cyanogenetic glucosides, and that none of these yield acetone on hydrolysis by hot dilute mineral acids or by the glucosidolytic enzymes present in the beans.

We have considered it necessary, therefore, to examine carefully the glucosidic product obtained from "Java beans" by the process originally used by us§ in the investigation of the beans of *Phaseolus lunatus* obtained from Mauritius and we have been unable to detect the presence of any other cyanogenetic glucoside in Java beans except phaseolunatin, identical in all

* Dunstan and Henry, 'Roy. Soc. Proc.,' 1903, vol. 72, p. 285.

† 'Bulletin of the Imperial Institute,' 1905, vol. 3, p. 373, and 1906, vol. 4, p. 329.

‡ 'Comptes rendus,' 1906, vol. 143, p. 182.

§ Dunstan and Henry, *loc. cit.*