are desired the method given here is applicable as the product is obtained pure.

5. The preparation of the phenylenediamines from nitroanilines by this method is extremely slow, due to the slight volatility of the nitroanilines.

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[CONTRIBUTION FROM THE DEPARTMENT OF NUTRITION, OHIO AGRICULTURAL EXPERIMENT STATION.]

A STUDY OF THE ACTION OF 10% THYMOL-CHLOROFORM PRESERVATIVE ON THE CHLORINE CONTENT OF URINE.[1]

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Received December 20, 1918.

The effect of thymol-chloroform as a urinary preservative on the chlorine content, both in acid and alkaline urines, was studied over longer and shorter periods of time.

The advantages of chloroform thymol over the alcoholic thymol as a urinary preservative lies in its convenience, the small quantities necessary and the avoidance in the case of toluol of an insoluble layer on the surface of the urine. Thymol-chloroform is being more widely used.

What effect the presence of chloroform would have on the chlorine content was not known. It was desirable to keep the urines liquid near freezing temperature rather than frozen and hence it was deemed advisable to ascertain what this effect would be.

TABLE I.—THE EFFECT OF CHLOROFORM AS A PRESERVATIVE ON THE CHLORINE CONTENT OF SWINE URINE PER 100 CC.

Sample.	Det. No.	Control. Gms. Cl.	Preserved. Gms. Cl.	Acidity. Cc. 0.1 *N* NaOH.	Added preservative. Cc.
1	1	..	0.3369	117	0.4
	2	0.3375	0.3372	...	0.8
2	3	0.3170	0.3165	112	0.4
	4	..	0.3159	...	0.8
3	5	0.2672	0.2685	101.1	0.5
4	6	0.2326	0.2319	92.1	0.5
5	7	0.2515	0.2502	82.2	0.5
6	8	0.2437	0.2415	84.8	1.0
7	9	0.2584	0.2591	72.1	1.0
8	10	0.2672	0.2700	70.1	1.0
9	11	0.1655	0.1773	85.0	1.0
10	12	0.1617	0.1689	17.5	1.0
11	13	0.1090	0.1129	22.1	1.0
12	14	0.2343	0.2395	88.8	1.0
13	15	0.3155	0.3232	24.7	1.0
14	16	0.4072	0.4108	30.9	1.0

[1] An abstract of this work was presented at the Cleveland meeting of the American Chemical Society, September 10, 1918.

It is well known that strong bases will disrupt the chloroform molecule splitting off chlorine. Whether this action was possible, over longer periods of time, in urines having a *physiological* alkalinity was not known. This possible effect in acid urines over shorter periods of time at laboratory temperatures was also studied. Six alkaline urines of the cow stored slightly above freezing (32–36° F.) for twenty-three months, while sixteen acid urines (swine) kept at laboratory temperatures four to twenty-one days were investigated.

The results are given in Tables I and II.

Decreased acidity of urines 10, 11, 13 and 14 are due to the precipitated calcium carbonate fed.

Urines 1 and 2 stood six days in cool laboratory window.

Urines 3, 4 and 5 stood 5 days in cool laboratory window.

Urines 6, 7 and 8 stood $4^1/_2$ days in cool laboratory window.

Urines 9–14 stood 21 days in cool laboratory window.

TABLE II.—THE EFFECT OF CHLOROFORM AS A PRESERVATIVE ON THE CHLORINE CONTENT OF COW URINE PER 100 CC.

Sample.	Det. No.	Control kept frozen. Gms. Cl.	Preserved.[1] Gms. Cl.	Preservative added. Cc.
15	17	0.0187[2]	0.0190	0.25[3]
16	18	0.0160	0.0160	0.25
17	19	0.0538	0.0577	0.25
18	20	0.0739	0.0812	0.25
19	21	0.2739	0.2778	0.25
20	22	0.3150	0.3192	0.25

Urines No. 15 and 16 from cows receiving basal ration; No. 17 from cow receiving 70 g. precipitated bone flour daily.

Urine No. 18 from cow receiving 42 g. calcium lactate daily.

Urines 19 and 20 from cows receiving 40 g. calcium chloride daily.

Discussion and Conclusion.

Chlorine was determined according to the A. O. A. C. methods[4] ashing in the presence of Na_2CO_3, after evaporation to dryness; $N/20$ normal solutions were used in titration of the $AgNO_3$. All determinations were done in duplicate.

It could not be demonstrated that there is any appreciable effect of the chloroform on the chlorine content of urine either in that of cows or of swine when the cow urine was kept near freezing temperature for long

[1] The preserved cow urines were stored in bottles at 0–2.2° C. (32–36° F.) for 23 months while the control urines without preservatives were kept frozen in tin cans for the same length of time.

[2] Average of sets 0.0211 and 0.0163 each of which was run in duplicate; this discrepancy not accounted for.

[3] Approximately 5 cc. per $2^1/_2$ liter acid bottle.

[4] *Revised Bull.* 107, Bur. of Chem., U. S. Dept. Agr., p. 24.

periods of time (23 months) nor in swine urine kept at laboratory temperature for shorter periods of time (4 to 21 days).

In determinations 8, 9, and 10 (Table I) where twice as much preservative was used as in determinations 5, 6 and 7, the increase in chlorine content was slight (1.04%) being within the experimental error. These urines were taken from the same animals on successive days.

In one set only is there any evidence of a slight increase (3.6 to 7.1%) in the chlorine content and that occurred when the preserved acid urine was kept at laboratory temperature for 21 days. The slightly higher chlorine content in several of the preserved alkaline cow urines may be due to analytical variations rather than to actual increased chlorine content.

The authors wish to express their appreciation to Dr. E. B. Forbes, who made this work possible.

[CONTRIBUTION FROM THE DIVISION OF LABORATORIES AND RESEARCH OF THE NEW YORK STATE DEPARTMENT OF HEALTH, ALBANY.]

THE PREPARATION OF ARSPHENAMINE (SALVARSAN).[1]

BY PHILIP ADOLPH KOBER.

Received December 21, 1918.

I. Introduction.

The synthesis of an arsphenamine or salvarsan suitable for therapeutic purposes, in spite of the work of Ehrlich and Bertheim[2] and their collaborators, is still a vital problem. It is fairly well known that the toxicity of arsphenamine varies and that batches made by individual manufacturers vary more than can be accounted for by the differences in their procedures. Furthermore, since it seems fairly well proven that even Ehrlich's own manufacturers are unable to maintain a uniformly high standard,[3] it is evident that there are some factors which are not understood or not under control. I am informed by manufacturers of arsphenamine that about 50% of the arsphenamine made does not meet the Surgeon General's requirements[4] and therefore is not distributed.

In studying the subject, I came to the conclusion that the toxicity of arsphenamine or at least the variation of the toxicity is largely due to the use of methyl alcohol and ether in the final precipitation of the base as the dihydrochloride. While most chemists use Ehrlich and Bertheim's methyl alcohol and ether method or some modification of it for precip-

[1] Read in part before the Society of Experimental Biology and Medicine, New York City, November 20, 1918.

[2] *Ber.*, **45**, 756 (1912).

[3] Roth, *Hygienic Laboratory Bulletin* **113**, 7 (1918).

[4] The U. S. Public Health Service, *P. H. Reports*, **33**, 540 (1918), requires an M. L. D. of 0.060 g./kilo body weight, while the Surgeon General (recently) raised the requirement to 0.080 g./kilo, and to 0.100 g./kilo.