5. Cannabis has a profound effect upon the imaginative faculties. The lower or animal faculties are apparently neither stimulated nor dulled or paralyzed. There is nothing in the tests herein recorded which would throw light on the cases of homicidal mania observed in Hindoo addicts. One of the constant manifestations of Cannabis action is the idea that the sensations are not real, that they are a "bluff," but invariably the reality of the sensations is admitted.

6. The following statements are offered as to the sex emotions due to Cannabis. Wood in his single test has this to say—"At no time was there any aphrodisiac effect produced." On the other hand nearly all commentators on Cannabis action refer to the sex imagery as described by the Hindoo addicts to this drug. The writer's own experience with the drug would indicate that there is a distinct and unmistakable sex tinge to its action, more especially in the association with the effects of exaltation and emotions of grandeur. The subjective nature of the sex imagery is unmistakable, but it is never objectively materialistic. The sex imagery is very difficult to describe and is most apparent during the first half of the period of drug action.

7. The specific effects of Cannabis have been set forth in the text and are not summarized.

THE UNIVERSITY OF NEBRASKA,
COLLEGE OF PHARMACY,
LINCOLN, NEBRASKA, MARCH 13, 1922.

TOXICITY OF NEOARSPHENAMINE. II.*

BY FREDERICK W. HEYL, MERRILL C. HART AND WILBUR B. PAYNE.

In the course of some work on mixed arseno derivatives, which will be reported later, we have prepared condensation products in which the amino groups in both benzene rings were substituted. We have been interested in comparing the toxicities of these preparations with neoarsphenamine.

As we have previously shown[1] such a comparison can only be made by eliminating the error introduced through the variations in the test rats. We therefore prepared fresh solutions of neoarsphenamine on a number of occasions, and were surprised to find the tolerance much higher than we had previously reported. Instead of 320 mg./Kg., maximum tolerated doses of 440 mg./Kg. were almost always withstood, due simply to variation in the rats.

During this work 3-amino-4-hydroxyphenylarsinic acid has been used as starting material, and the reduction yielding mixed arseno compounds have been made with sodium hydrosulphite of high purity. We have incidentally determined the influence of these apparent improvements upon the toxicity of the resultant neoarsphenamine. It amounts to 40–60 mg./Kg. in the tolerance test. The rats used were uniformly from one strain.

In a series of papers[2] Christiansen has shown that where the synthesis of arsphenamine is carried out, using 3-amino-4-hydroxyphenylarsinic acid, instead of

* Received January 14, 1923.

[1] Hart and Payne, *J. Am. Chem. Soc.*, 44, 1150, 1922.

[2] *J. Am. Chem. Soc.*, 42, 2402, 1920; 43, 370 and 2202, 1921.

the corresponding nitro derivative, with which all our previous work had been done, there is a conspicuous decrease in the toxicity of the resultant product. This method in fact gives material which is frequently tolerated in doses of 160 mg./Kg., and thus shows a conspicuous improvement over the average samples prepared from the nitro body. It was thought that the greater purity and lower toxicity of this arsphenamine might have a favorable influence on the toxicity of the neoarsphenamine prepared from it. We have shown in a previous paper[1] that the toxicity of the arsphenamine from 90 to 130 mg./Kg. from "nitro acid" has no appreciable effect on the neoarsphenamine prepared from it. Our results in this case also show that even arsphenamine of a toxicity of 160 mg./Kg. from pure "amino acid" has no measurable effect on the toxicity of the neoarsphenamine derived from it.

Voegtlin and Miller[2] recently reported the lethal dose of this drug as varying from 360–520 mg./Kg. and concluded that the product is now considerably less toxic than heretofore, because of improved methods of production. This conclusion is not justified unless the rat variation is taken into consideration. The early usefulness of this test in detecting accidental variation due to oxidation remains of practical value, but the refinements of production have no doubt been carried to the point where the test animals may vary more than the perfected drug itself.

Dale and White[3] go so far as to practically discard this test and do not calculate therapeutic efficiency.[4] Instead they are inclined to a new laboratory test in which the effectiveness in parasiticidal action on infected mice is demonstrated. On rats the effective dose is shown to vary from 8.5 to 28.1 mg./Kg. and these maximum and minimum values were found for samples having the same toxicity (480 mg./Kg.). Obviously the toxicity and efficiency do not vary simultaneously. However, even this range of effectiveness does not appear to establish the limits, for in an earlier paper[5] a sample is shown to have a maximum lethal does of 560 mg./Kg. and a minimum effective does of 3.75 mg./Kg. This pharmacological test would therefore incline one to accept the idea that one sample of neoarsphenamine might be 7.5 times as efficient as another.

From the chemical point of view it is practically impossible to attach to this conclusion the importance which it appears to have pharmacologically. When a sample of neoarsphenamine is prepared starting from the pure 3-amino-4-hydroxyphenylarsinic acid so that the side reactions resulting from the interaction of sodium bisulphite on nitro derivatives[6] are excluded, and when toxicity and composition are found practically constant, there appears to be no chemical basis for these findings, so far as the drug is concerned. Whether further pharmacological work will prove helpful to clinicians is questionable. It appears doubtful to us whether further pharmacological control can replace or assist the essential clinical work of the trained syphilographer.

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[1] Hart and Payne, *Loc. cit.*

[2] "Public Health Reports," 37, No. 27, 1632, 1922.

[3] *The Lancet*, CCII, 779, 1922.

[4] The minimum lethal dose in mg./Kg. divided by the minimum dose which is effective in killing all the parasites in an experimentally infected rat in 24 hours.

[5] Voegtlin, Dyer & Miller, *J. Pharmacolog.*, XX, 148, 1922.

[6] Weil and Moser, Ber., 55, 732, 1922.

EXPERIMENTAL.

1. *Arsphenamine.*—The material used in the following experiments was prepared by the Ehrlich process, using highly purified crystalline 3-amino-4-hydroxyphenylarsinic acid to replace the corresponding nitro acid. The hydrosulphite had a purity of approximately 95% and was prepared by the method previously described[1] except that the filtration and desiccation were carried out in the specially designed apparatus in which these operations are conducted in an inert atmosphere on one operation.[2] The special usefulness of this apparatus is demonstrated in the preparation of hydrosulphite. We used 3.6 times the calculated quantity, thus decreasing the amount of this reagent by about 50%.

The resultant arsphenamine is correspondingly freer from sulphur, containing not more than 0.25% and is considerably less toxic. The M. T. D. varies from 140 mg./Kg. to 170 mg./Kg. The product which is chemically purer than that derived from the nitro body gives considerably more difficulty in the subsequent condensation due to peculiar colloidal properties exhibited.

When the nitro acid was converted into arsphenamine hydrochloride using the purer hydrosulphite no decrease in toxicity was observed. This averaged about 110 mg./Kg. The substance tended to a high sulphur content (S = 1.9%) but dissolved more readily than that prepared from the amino acid.

Typical samples were used in the following experiments.

All toxicity determinations were made by the official method of the Hygienic Laboratory on white albino rats. The same rats used in all the subsequent experiments did not have a higher resistance to arsphenamine, but toward neoarsphenamine they showed a greatly increased tolerance. For example, using the same stock of rats, a sample of arsphenamine prepared from "amino acid" showed a tolerance equivalent to 160 mg./Kg. They tolerated arsphenamine from nitro acid at only 110 mg./Kg. and then tolerated neoarsphenamine at doses of 440 mg./Kg. We thus satisfied ourselves that the arsphenamine from amino acid was actually less toxic than that from the nitro acid. Upon now using a different stock of rats we obtained results of 160, 110 and 320 mg./Kg., respectively. These values especially emphasize the cause of variability in toxicity of neoarsphenamine, and demonstrate uniformity of the action of arsphenamine.

It is further remarkable that arsphenamine which is tolerated at 160 mg./Kg. gives a neo-derivative which is not significantly less toxic than the derivative prepared from samples of arsphenamine which are much more toxic. There are other factors which have a much greater significance, and these appear to be related, not to slight differences in structural configuration but to physical differences.

2. *Neoarsphenamine from Arsphenamine Prepared from the Pure 3-Amino-4-hydroxyphenylarsinic Acid.*—(*a*) 2.8659 Gm. arsphenamine hydrochloride (As = 29.3%) for which the M. T. D. was 160 mg./Kg. were dissolved in 15 cc absolute methyl alcohol. This material presents exceedingly colloidal properties and dissolves with difficulty. There was some question as to the completeness of solution. The reaction was carried out exactly as described[3] in the previous paper, using

[1] Heyl and Greer, *Am. J. Pharm.*, 94, 80, 1922.

[2] Heyl and Miller, JOUR. A. PH. A., 11, 432, 1922.

[3] *Loc. cit.*

1.95 cc formaldehyde sulphoxylate solution (100%). Time, 18 minutes. Temperature, 25–26°. The methyl alcohol was removed at 15 mm. pressure. For toxicity of this neoarsphenamine see Table I (*a*). It will be observed that the results indicate a greater tolerance than previously reported, but that the results are irregular. (*b*) It was considered that this irregularity was due to colloidal properties of the arsphenamine hydrochloride, for not only was the solution obtained unsatisfactory, but the reaction was delayed and possibly not quite completed. For this experiment we used arsphenamine hydrochloride (31.15% As) having M. T. D. of 140 mg./Kg. The alcoholic solution presented even poorer properties than (*a*). It was centrifuged clear, and the neoarsphenamine solution prepared as before. Time, 20 minutes. Temperature, 27°. This product gave more regular results, and was less toxic. See Table I (*b*). (*c*) Experiment (*b*) was repeated, omitting the centrifugation. See Table I (*c*).

3. *Neoarsphenamine from Arsphenamine Prepared from 3-Nitro-4-hydroxyphenylarsinic Acid.*—(*d*) From the results obtained in the above experiments it is established that the maximum tolerated dose of neoarsphenamine might appear to be much higher than previously reported (320 mg./Kg.). This might be presumed to be due to the use of pure 3-amino-4-hydroxyphenylarsinic acid. Other possibilities present themselves however. The rats used might be more resistant. The use of purer hydrosulphite might be an influence. The experiment (*b*) was repeated using arsphenamine hydrochloride which had been prepared from nitro acid by the use of 95% hydrosulphite. (Analysis showed As = 31.1; S = 1.9%; M. L. D. = 110 mg./Kg.) The result is astonishingly high. See Table I (*d*). The same experiment, using the same arsphenamine and omitting centrifugation, gave results in (*e*).

(4). *Determination of the Resistance of the Test Animals.*—For this purpose we started with a sample of arsphenamine hydrochloride M used in our previous work and which had been prepared using cruder hydrosulphite. The methyl alcoholic solution was centrifuged. Time, 20 minutes. Temperature, 26°. The results of the rat test are found below (Table I (*f*)).

TABLE I.

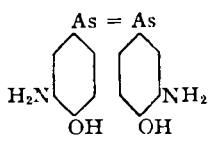
Expt. No.	As %.	Doses: mg./Kg. 360.	400.	440.	480.	520.
2 *a*	20.2	++−−	++−−−	+++−−		
b	19.0			++++−	+++−−	+++−−
c	19.65	10 L.; 0 D.	+++++			−−−−−
3 *d*	20.0	10 L.; 0 D.	+++++			
	20.25			++++−	+++++	+−−−−
e	20.0		++++−	+++−−	++−−−	
4 *f*	18.0	+++++	+++++	++++−	+++++	+−−−−

\+ Lived. − Died.

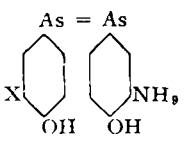
These results are readily interpreted and the relative importance of various factors which yield high test material may be estimated. The most significant factor is the variation in the resistance of the rats. This amounts to 120 mg./Kg.; the same sample to which previously a tolerance of 320 mg./Kg. could be established is now survived at doses of 440 mg./Kg. (at 20% As). We attribute a decrease in toxicity to the use of purer hydrosulphite of not more than 40 mg./Kg. The

value of the use of amino acid is exceedingly questionable, the centrifugation being more important.

5. *Sulphur Content of Neoarsphenamine.*—The neo-derivative which was prepared from amino acid might be expected to show some differences in composition. If we accept Christiansen's hypothesis there will be less of the mixed arseno derivative II, or perhaps of III, since the pure product I is derived from the pure amino acid.



I.



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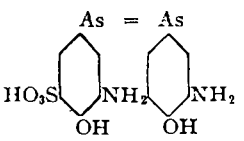

III.

As a matter of fact the derived neoarsphenamines show very slight variation due to this factor, since arsphenamine derived from the nitro acid, and containing almost 2% of sulphur, gave a neoarsphenamine which was withstood in approximately the same doses as that prepared through arsphenamine (0.25% S) from the amino acid. The analyses for sulphur distribution, carried out as previously described,[1] gave the following results, reported as atomic ratios. Theory for a mono-substituted neoarsphenamine requires 2 arsenic: 1 sulphur.

TABLE II.—DISTRIBUTION OF SULPHUR IN NEOARSPHENAMINE.

Expt.	Arsphenamine.	Source.	Procedure.	Total S.	"Neo" S.	Nuclear S.
2 *a*	52	"Amino acid"	95% $Na_2S_2O_4$	1.33	1.24	0.09
*b**	69	"Amino acid"	95% $Na_2S_2O_4$	1.39	1.27	0.12
c	69	"Amino acid"	95% $Na_2S_2O_4$	....	....	....
3 *d**	181	Nitro acid	80% $Na_2S_2O_4$	1.32	1.16	0.16
	181	Nitro acid	80% $Na_2S_2O_4$	1.40	1.26	0.14
	65	Nitro acid	95% $Na_2S_2O_4$	1.37	1.00	0.37
e	65	Nitro acid	95% $Na_2S_2O_4$	1.35	1.10	0.25
4 *f**	M	Nitro acid	80% $Na_2S_2O_4$	1.49	1.27	0.22

* Centrifuged.

Arsphenamine 52 and 60 contained 0.25% S (0.04 atomic ratio).

No. 65 contained 1.9% S (0.29 atomic ratio), No. 181 contained 1.0% S.

It is evident that the variations in the sulphur distribution vary to such an extent that any alteration due to the use of the amino acid cannot be detected.

SUMMARY.

1. The decrease in toxicity of neoarsphenamine that results from the use of 3-amino-4-hydroxyphenylarsinic acid is slight.

2. Similar slight improvement results from purified hydrosulphite.

3. The variability of different test rats toward the same neoarsphenamine is shown to have large dimensions. On the other hand test rats do not exhibit this behavior toward arsphenamine.

4. The maximum toxicity of neoarsphenamine was found to be 480 to 500 mg./Kg.

5. Taking the variability of the test animals into consideration, the value 360–380 mg./Kg. would have resulted had rats been used of the same resistance as those previously reported.

[1] *Loc. cit.*

6. Since the previous work gave M. T. D. = 320 mg./Kg. it has been shown that the maximum decrease of toxicity due to amino acid, pure hydrosulphite and centrifugation is 40–60 mg./Kg.

CONTRIBUTION FROM THE
LABORATORIES OF THE
UPJOHN COMPANY,
KALAMAZOO, MICHIGAN.

MISCELLANEOUS CHEMICAL PAPERS.*

4. Fractionation of Turpentine Oil.[1]

BY W. F. SUDRO.

A quantity of approximately five gallons of turpentine was steam-distilled, the distillate being received in approximately liter fractions, sixteen in number. The distillates were immediately placed into bottles filled to the stopper in order to prevent oxidation or other changes. The data on the steam-distillation are given in Table I.

TABLE I.

Steam distillation fraction.	Sp. gr. 20° C. (Westphal).	Refractive index 20° C.	Polarization 23° C.
1	0.862	1.4660	+15.25
2	0.863	1.4675	+14.85
3	0.863	1.4671	+15.1
4	0.864	1.4682	+14.7
5	0.863	1.4680	+14.6
6	0.863	1.4682	+14.5
7	0.864	1.4661	+14.1
8	0.863	1.4668	+13.9
9	0.863	1.4670	+13.8
10	0.864	1.4685	+13.3
11	0.864	1.4684	+13.1
12	0.864	1.4687	+12.4
13	0.865	1.4690	+11.7
14	0.865	1.4690	+10.5
15	0.868	1.4698	+ 9.4
16	0.870	1.4708	+ 7.4

Upon inspection of the table it will be observed that the specific gravity gradually increases from the first fraction to the last, the constants running as one would expect. The polarization constants are also in strict agreement, the angle of rotation gradually diminishing with an increase in the number of the fractions, the only exception noted being either with fraction number two or three.

The indices of refraction show a relative increase with the exception of Fractions 7, 8 and 9. This will be discussed later.

* From the Laboratory of Edward Kremers. Read before Scientific Section, A. Ph. A., Cleveland meeting, 1922.

[1] Part of thesis submitted for the degree of Master of Science at the University of Wisconsin, 1919.

The fractionation here recorded was carred out by Mr. Sudro for the purpose of gaining a better insight into the boiling temperature conditions of a so-called hydrocarbon oil. The results of a carefully performed series of steam and direct distillations of a commercial American turpentine oil may prove of interest to others, hence should be worth recording. The immediate incentive to perform such a "tedious" task was to obtain as pure a pinene fraction as obtainable by this means for chemical experiments that will be reported in another paper.—E. K.