The sample was prepared as follows: 16 cc. of the extract were rapidly boiled to dryness without scorching. To the residue was added enough distilled water to bring the volume to 16 cc.; this sample was again boiled to dryness and the residue dissolved in enough distilled water to make the volume 16 cc.

After the normal base line was obtained the same procedure of injection was carried out as in the previous experiments. The response produced by the injection of the heated sample was definite, but less than that created by the unmodified sample (Table I, animals number 5 and number 6). The heating did not destroy the activity, but may have reduced the potency somewhat. Additional study may clarify this point.

In Table I the reticulocyte responses following the injection of liver extract are given. The erythrocyte counts did not change significantly, and are therefore omitted to conserve space.

From the data thus far obtained, we feel that the method described compares favorably with the other methods presented in the literature and is, therefore, worthy of further study, particularly with reference to its specificity.

In a personal communication from Dr. H. W. Rhodehamel, of Eli Lilly and Co., we have learned that the samples of liver extract used had shown clinical activity.

CONCLUSIONS.

1. A method suggesting the possibility of using the normal, healthy guinea pig as a hematopoietic test animal is introduced.

2. Experimental test animals that did not respond to injections of iron and ammonium citrate later gave a reticulocyte response when injected with liver extract.

3. The injection of liver extract over a given period caused a definite rise in the reticulocyte count without significantly effecting the erythrocyte count or the weight.

4. A second period of injection produced a response in the reticulocyte count similar to that produced in the first period suggesting the possibility that a given animal may be repeatedly used for test purposes.

5. The active constituent of liver extract which produced a reticulocyte response in the guinea pig is not readily destroyed by heating.

6. This report is of a preliminary nature and is presented merely as a possibility worthy of further study.

BIBLIOGRAPHY.

(1) J. P. McGowan, *Edinburgh Med. J.*, 37 (1930), 330.
(2) J. P. McGowan and R. D. Sinclair, *Ibid.*, 38 (1931), 405.
(3) Vaughan, Muller and Zetzel, *Brit. J. Expil. Path.*, 11 (1930), 456.
(4) C. W. Edmunds, H. H. Brueckner and A. I. Fritzell, JOUR. A. PH. A., 22 (1933), 91.
(5) W. A. Peabody and R. C. Neale, *Ibid.*, 22 (1933), 1232.
(6) M. O. Schultze and C. A. Elvehjem, *J. Biol. Chem.*, 102 (1933), 357.

ALKALOIDAL REAGENTS V. THE ACONITE ALKALOIDS.*

BY JAMES C. MUNCH AND HARRY J. PRATT.

The reactions of the aconitine group of alkaloids with the usual alkaloidal reagents, as well as with certain special reagents reported in the literature, contain

* Scientific Section, A. PH. A., Madison meeting, 1933.

conflicting statements regarding colors and precipitate formations (1–30). Data on aconitine, pseudoaconitine and benzoylaconine are given in Table I.

TABLE I.—REACTION OF ACONITE ALKALOIDS WITH ALKALOIDAL REAGENTS.

(Reported in the Literature.)

No.	Name.	Reagents. Composition.	Aconitine. Color or Ppt.	Threshold, Mg./Liter.	Pseudoaconitine.
1	.....	HCl conc.	Colorless	...	.....
2	.....	HNO_3 conc.	Colorless; heated, brick-red	...	.....
3	Vitali	HNO_3 + KOH alc.	Colorless; odor EtOBz	...	Purple-red
4	Alvarez	Br + HNO_3 + NaOH alc., then 10% aq. $CuSO_4$	Red or brown; deep green	...	.....
5	.....	H_2SO_4 conc.	Colorless	...	Colorless
6	Erdmann	H_2SO_4 + HNO_3	Colorless, changing to violet	...	.....
7	.....	H_2SO_4 + $K_2Cr_2O_7$	Yellow to light green	...	.....
8	Mandelin	H_2SO_4 + NH_4 meta-vanadate	Light brown to orange	...	.....
9	Froehde	H_2SO_4 + Na molybdate	Yellow–yellowish–brown–blue	...	.....
10	Mecke	H_2SO_4 + SeO_2	Yellowish; lt. brown on heating	...	.....
11	Marquis	H_2SO_4 + HCHO	Colorless	...	.....
12	Mǫnti	H_2SO_4 + resorcinol	Yellow-red to red-violet	...	.....
13	Schneider	H_2SO_4 + sucrose	Red	...	.....
14	.....	Phosphoric acid conc.	Red; violet on heating	...	Colorless
15	.....	Phosphomolybdic acid	White; turning blue (A)	200	.....
16	Palet	H_3PO_4 + Na molybdate	Violet	...	.....
17	DeVry's-Sonnen-schein	NH_4 phosphomolybdate	Yellowish white ppt. (A)	...	.....
18	Scheibler	Phosphotungstic acid	White ppt. (A)	200	.....
19	.....	Phosphoantimonic acid	White ppt. (A)	1000	.....
20	Ecolle	Silicotungstic acid	White ppt. (A)	...	.....
21	Wormley	Br + KBr	.....	2	.....
22	.....	KBr	.....	10	.....
23	.....	HIO_3	Colorless	...	.....
24	Wagner	IKI[a]	Red, brown crystals	2	Precipitate
25	Jurgens	KI + CH_3COOH	Tabular rhomboids	50	Needle druses
26	Dragendorff	$BiI_3.4KI$	Yellow (A)	...	.....
27	Marmę	$CdI_2.2KI$	Orange-red (A)	100	.....
28	Mayer	$HgI_2.2KI$	Yellowish white (A)	100	Ppt. dilute soln.
29	.....	$NaClO_4$	.....	2	.....
30	.....	$K_2Cr_2O_7$	Yellow ppt.	2	.....
31	Dunstan and Carr	$KMnO_4$	Dense purple-red crystals	250	.....
32	.....	Na or NH_4CNS	(A)	10	.....
33	.....	K_3FeCN_6	Ppt. conc. soln.	...	.....
34	Cole	K_4FeCN_6	Ppt. conc. soln. (A)	...	.....
35	Mellaneh	K_4FeCN_6 + HCOOH	Green	...	Green
36	.....	Na nitroprusside	(A)	...	.....
37	Fehling Solution	Alkaline copper compd.	Granular white ppt.	...	.....
38	.....	$AuCl_3$[a]	Whitish yellow (A)	200	Ppt. dilute soln.
39	.....	$PtCl_4$	Ppt. conc.	500	Ppt. conc. soln.
40	.....	$FeCl_3$	Yellow (A)	...	.....
41	.....	$HgCl_2$	Ppt. conc. (A)	...	.....
42	Millon	$HgNO_3$	White (A)	...	.....
43	.....	$AgNO_3$	(A)	10	.....
44	.....	$ZnCl_2$	(A)	...	.....
45	.....	$PdCl_2$	(A)	...	.....
46	.....	CH_3COOH	Red crystals	...	.....
47	.....	Gallic acid	Colorless	...	.....
48	Hager	Picric acid	Yellow concn. soln. (A)	250	.....
49	Knorr	Picrolonic acid	(A)	...	.....
50	Rosenthaler	Rufianic acid	(A)	5000	.....
51	.....	Tannic acid	.....	300	Ppt. dilute soln.
52	.....	$NaHCO_3$	Characteristic rosettes	10000	.....
53	.....	KOH	(A)	5000	.....
54	.....	KCN	(A)	5000	.....

[a] Benzoylaconine gives a yellow ppt. with $AuCl_3$ (38) and granular crystals with IKI (24).

A = Amorphous ppt.

The series of standard alkaloidal reagents used in our previous investigations (13, 19, 20, 21) were supplemented by a number of special reagents, a total of 71 being considered. Our results are given in Table II. Deviating somewhat from our previous practice, one drop of solution was placed on a watch crystal and mixed

TABLE II.—REACTIONS OF ACONITE ALKALOIDS WITH ALKALOIDAL REAGENTS.

No.	Reagent.	Aconitine.	Benzoylaconine.	Aconine.
1	HCl	0	0	0
2	HNO_3	0	0	0
5	H_2SO_4	0	0	0
6	$H_2SO_4 + HNO_3$	0	0	0
7	$H_2SO_4 + K_2Cr_2O_7$	0	0	0
8	$H_2SO_4 + NH_4$ meta-vanadate	Yellow soln.; large specks	Yellow soln; large specks	Yellow soln.; large specks
9	Froehde	Cold, 0; heated, 0	Cold, 0; heated, blue-gray	Cold, 0; heated, blue-gray
10	Mecke	0	0	0
11	Marquis	0	0	0
12	H_2SO_4 + resorcinol	Cold, 0; heated, lt. brown granules (A)	Cold, 0; heated, orange granules (A)	Cold, 0; heated, red-violet bundles of tabloids (C) ?
13	Schneider	Cold, 0; heated, black	Cold, 0; heated, black	Cold, 0; heated, black
55	H_2SO_4 + saccharose	Cold, 0; heated, brown	Cold, 0; heated, black	Cold, 0; heated, black
14	H_3PO_4	Red-violet	Black	Dark violet
16	Palet	Dark purple, changing to black	Red-purple, changing to dark brown	Purple-violet, changing to green, then dark green
18	Schiebler	Dense white (A)	Whitish yellow (A)	White (A)
20	Ecolle	Fluffy white (A)	Clabber white (A)	Curdy white (A)
56	HNO_3 + Br	0	0	0
23	$H_2SO_4 + KIO_3$	0	0	0
24	Wagner	Curdy light brown (A)	Staining; no ppt.	Dark brown (A)
26	Dragendorff	Dense white flocc. (A): later dark	Amorphous white ppt. and tables	Cloudy white (A)
28	Mayer	Dense white flocc. (A)	Sparse white warts (A)	Sparse white warts (A)
57	$KClO_3$	0	0	0
30	$K_2Cr_2O_7$	Yellow (A)	Yellow (A)	Yellow (A)
31	$KMnO_4$	Purple (A)	Purple (C)	Purple (C)
33	K_3FeCN_6	Green-white (A)	Green-white (A)	Green-white (A)
34	K_4FeCN_6	White granular (A)	Whitish yellow (A)	White granules (A)
35	Mellaneh	Greenish blue granules (A)	Greenish blue granules (A)	Greenish blue granules (A)
36	Na nitroprusside	White rods (A)	White (A)	White (A)
37	Fehling's soln.	White granules (C)	Sparse tablets (A)	Sparse tablets, bundles (C)
39	$PtCl_4$	Yellow white plates (A)	Yellow-white plates (A)	Yellow white plates (A)
40	$FeCl_3$	Yellow (A)	Sparse yellow (A)	Sparse yellow (A)
41	$HgCl_2$	Black (A)	Specks (A)	Gray (A)
42	Millon	White (A)	Soln., later white needles	White warts (A)
45	$PdCl_2$	Specks (A)	Lardy mass white (A)	Specks (A)
46	CH_3COOH	0	0	0
48	Picric acid	Dense yellow (A)	0	0
54	KCN	White (A)	Large tables (C)	Tables, prisms (C)
58	Dimethylglyoxine	Amorphous, later rods	Amorphous	Feathery sheaves
59	HCl + vanillin + Br	0	0	0
60	$HNO_3 + CuSO_4$	0	0	0
61	H_2SO_4 + hexylresorcinol	Fine air bubbles; heat, 0	Bubbles; heat, 0	Bubbles; heat, 0
62	K_2CrO_4	Yellow (A)	0	0
63	$Co(NO_3)_2$	Pink pinholes (A)	Greenish large tables (C)	Changes to purplish brownish pink (C) ?
64	$Cr(NO_3)_2$	0	0	0
65	$CuSO_4$	Specks	Brownish tables	Brownish (C) ?
66	$AlK(SO_4)_2$	0	0	0
67	$MnSO_4$	0	0	0
68	$SnCl_2$	White (A)	White (A)	White (A)
69	$SrCl_2$	0	0	0
70	Uranium acetate	Yellow strings (A)	Yellow granules (A)	Yellow granules (A)
71	KOH + rochelle salts	White (C)	Sparse ppt. tablets (A)	Sparse tablets and bundles (C)

0 = No color or ppt. A = Amorphous ppt. C = Crystalline ppt.

with one drop of the reagent. The development of colors and/or precipitates was followed by a hand lens or under a microscope. In case a positive reaction developed, quantitative tests were made, using 10 millimolar solutions. Since most of the precipitates were white, such reactions are recorded without differentiating between chalky-white, greyish white, yellowish white, brownish white or other shades of "white."

Little information regarding the chemical reactions of benzoylaconine and aconine were found. Accordingly, 10 millimolar solutions were prepared and tested simultaneously with aconitine (Table II). In case positive results were obtained, confirmatory tests were conducted with other dilutions. The 10 millimolar solutions were the most favorable for color tests. Benzoylaconine may be differentiated from aconitine by confirmatory tests with a number of reagents, although no single reaction may be considered as absolutely definitive. In further distinction, aconine may be differentiated from aconitine or benzoylaconine. Only negative reactions were obtained with aconitic acid.

TABLE III.—SENSITIVITY OF TESTS FOR ACONITINE.

No.	Reagent.	Concentration in Millimols. 4.0.	0.4.	0.04.	0.004.	0.0004.	0.00004.
	Taste test	0.02 cc. bitter; saliv., tingling tongues and cheeks	Bitter; tingling	Bitter; tingling, saliv.	Bitter; slight tingling	Bitter; no tingle	No effect
18	Schiebler	Dense white (A)	Slight white (A)*	0	0	0	0
20	Ecolle	Fluffy white (A)	Slight white (A)*	0	0	0	0
24	Wagner	Curdy brown (A)	Lt. brown ppt.*	0	0	0	0
26	Dragendorff	Immed. dense white flocc. (A)	Immed. sparse white flocc. (A)*	0	0	0	0
28	Mayer	Immed. dense white flocc. (A)	Immed. sparse white flocc. (A)	Slowly turbid opalescent, white (A)*	0	0	0
31	$KMnO_4$	Purple (A)	Slight purple (A)*	0	0	0	0
48	Picric acid	Dense yellow (A)	Slight yellow (A)*	0	0	0	0
58	Dimethylglyoxine	White (A)*	0	0	0	0	0
68	$SnCl_2$	Curdy white (A)*	0	0	0	0	0

* Limit of sensitivity. A = Amorphous ppt.

Very little information was found in the literature regarding the sensitivity of various tests for aconitine (9, 14, 16, 18, 23). Those reagents which gave promising results were tested with various dilutions of aconitine, ranging from 4 millimolar down to 0.00004 millimolar (Table III). No positive reactions were obtained with 0.04 millimolar or weaker solutions except Mayer's reagent, which gave a positive reaction at 0.04 millimolar, and thus proved the most sensitive of the reagents studied. The limiting concentrations for the other reagents are reported. No efforts were made to study intermediate concentrations. In making taste tests, 0.02–0.05 cc. of solution was placed upon the tongue: one minute later the mouth was thoroughly rinsed with tap water. Taste phenomena were recorded for fifteen minutes (bitterness, tingling, etc.).

In order to determine the toxicological applicability of these reagents (5, 15, 23), a post-mortem examination was conducted upon a dog which had died following the oral administration of Tincture of Aconite, U. S. P. X. The stomach and contents, the intestine and contents, a portion of muscle of the thigh, the liver, one kidney and the spleen were removed and examined separately. Each tissue was chopped fine and extracted with alcohol containing 1 per cent of tartaric acid.

When extraction was complete, the material was filtered, the alcohol evaporated off on a water-bath at a low temperature, and the residue taken up with water. After standing until solution appeared complete, this was filtered and the solvent evaporated off on a water-bath. This process was repeated with alcohol and water a second time, yielding eventually a residue which was completely soluble in alcohol and water. This was dissolved in 5 cc. of distilled water and tested (Table IV). Since tincture of aconite had been administered to this dog daily over a period of several months, it might be anticipated that distribution in the cadaver would differ from that to be expected following acute poisoning. Positive results were obtained in tests conducted on extracts of the stomach and intestine, indicating that absorption was not complete in the interval of ten hours since the administration of the last dose. Strongly positive results were obtained in testing the liver, but negative results were obtained from the kidney, spleen and the muscles. Following the administration of a single dose of an aconite preparation to dogs, positive reactions have been obtained in extracts of the liver, and doubtful or negative reactions in extracts of the kidneys. It would appear that the aconite alkaloids are excreted largely into the liver.

TABLE IV.—TOXICOLOGICAL TESTS FOR ACONITINE IN VISCERA.

No.	Reagent.	F. E. Aconite.	Stomach.	Intestine.	Liver.	Kidney.	Spleen.	Muscle.
18	Schiebler	White (A)	Brown (A)	Brown (A)	0	0	0	0
20	Ecolle	White (A)	Brown (A)	Brown (A)	Brown (A)	Brown (A)	0	0
24	Wagner	Lt. brown (A)	Lt. brown (A)	Lt. brown (A)	0	0	0	0
26	Dragendorff	Dark brown curdy (A)	Lt. brown curdy (A)	Lt. brown (A)	Flocculent (A)	Flocculent (A)	0	0
28	Mayer	Lt. brown (A)	Lt. brown (A)	Lt. brown (A)	Lt. brown (A)	0	0	0
31	$KMnO_4$	Brown (A)	Brown (A)	Brown; no ppt.	Brown (A)	0	0	0
48	Picric acid	Yellow (A)	Yellow (A)	Yellow (A)	Yellow (A)	0	0	0
58	Dimethylglyoxine	White (A)	Brown (A)	Brown (A)	Brown (A)	0	0	0
68	$SnCl_2$	0	Brown (A)	Brown (A)	Brown (A)	0	0	0

A = Amorphous ppt.

CONCLUSION.

1. The behavior of aconitine, benzoylaconine and aconine have been determined with 71 alkaloidal reagents. Characteristic reactions for the differentiation of these three alkaloids have been developed.

2. Mayer's reagent, which was the most sensitive chemical test for aconitine, had a limiting threshold at a concentration of 0.04 millimolar; the taste test detected tingling at 0.004 millimolar, and bitterness at 0.0004 millimolar concentrations.

3. A substance giving the chemical reactions of aconitine has been detected in the liver, stomach and intestines of dogs, but not in the kidneys, spleen or muscle.

AUTHORS' NOTE: We wish to express our appreciation to Richard I. Grantham and the late Dr. Herman Engelhardt for the benzoylaconine and aconine used in this investigation.

BIBLIOGRAPHY.

(1) E. P. Alvarez, "Sopra un nuova reattivo dell'aconitina," *Gazz. chim. ital.*, 39 (1905), 429–430.

(2) W. Autenrieth, "Laboratory Manual for the Detection of Poisons and Powerful Drugs" (1928), 6th Am. Edition from 5th German.

(3) E. Barillot, "Traité de chimie legale" (1894), 202.

(4) G. Baudran, "Wirkung von Calciumpermanganat auf die Alkaloide, speziel auf das Strychnin," *Compt. rend. acad. sci.*, 139 (1904), 1000–1002.

(5) M. W. Blyth, "Poisons: Their Effects and Detection" (1906), 364.

(6) F. C. Carr, "Aconite Alkaloids," in "Allen's commercial organic analysis," 7 (1929), 227–268.

(7) H. W. Cole, "Potassium Ferrocyanide as a Reagent in the Microscopic Qualitative Chemical Analysis of the Common Alkaloids," *Philippine J. Sci.*, 97 (1923), 101.

(8) G. Dragendorff, "Plant Analysis" (1909), 179.

(9) W. R. Dunstan and F. H. Carr, "Aconitine: a Characteristic Reaction Suitable for Its Detection," *Pharm. J.* (Feb. 15, 1896), 122–123.

(10) Ecolle, "Determination of Aconitine by Silicotungstic Acid," *J. pharm. chim.*, 14 (1901), 97.

(11) N. Evers and G. D. Elsdon, "Aconitine: the Analysis of Drugs and Chemicals" (1929), 126.

(12) E. H. Hankin, "Tests for Certain Narcotic and Anesthetic Drugs," *Ind. J. Med. Res.*, 4 (1916), 237–255.

(13) W. H. Hartung, F. C. Crossley and J. C. Munch, "Alkaloidal Reagents IV. Quelques Remarques sur la Reaction Colorimetriques de Sivadjian pour Distinguer l'Ephedrine," *J. pharm. chim.* (8), 13 (1931), 474–478.

(14) L. Lanoy, "Sensitiveness of the General Method for Extracting Alkaloids from Water," *Compt. rend.*, 165 (1917), 360–362.

(15) Mecke, "Über ein dem Aconitin ähnliches Leichenalkaloid," *Z. Öffent Chem.*, 5 (1899), 204–206.

(16) S. Mellaneh, "Color Reaction for Aconite," *Analyst*, 46 (1921), 193–194.

(17) Merck's Index (1930), 560.

(18) N. Monti, "Una nuova reazione dell'Aconitina," *Gazz. chim. ital.*, 36 (1906), 477–480.

(19) J. C. Munch, F. C. Crossley and W. H. Hartung, "Alkaloidal Reagents I. Introduction," JOUR. A. PH. A., 20 (1931), 1037–1041.

(20) J. C. Munch, F. C. Crossley and W. H. Hartung, "Alkaloidal Reagents II. Aromatic Monohomocyclic Derivatives," *Ibid.*, 21 (1932), 341–348.

(21) J. C. Munch, F. C. Crossley and W. H. Hartung, "Alkaloidal Reagents III. Propadrin, Its Homologs and Derivatives," *Ibid.*, 21 (1932), 463–465.

(22) B. E. Nelson, "Variations in Quality of Vegetable Drugs," *Drug. Circ.*, 47 (1903), 137.

(23) L. P. J. Palet, "A Reaction for Aconitine," *J. pharm. chim.*, 19 (1919), 295–296.

(24) F. Peterson, W. S. Haines and R. W. Webster, "Legal Medicine and Toxicology," 2nd Edition, Vol. 2 (1923).

(25) C. Reichard, "Color Reactions of Aconitine," *Pharm. Zentralh.*, 46 (1905), 476.

(26) H. Ribait, "Sur le Dosage de l'Aconitine par l'Acide Silicotungstique," *Bull. sci. pharmacol.*, 17 (1910), 634.

(27) A. Richaud, "Sur la Teneur en Aconitine de Quelques Echantillons d'Alcoolature de Feuilles d'Aconit," *J. pharm. chim.*, 23 (1921), 15.

(28) L. Rosenthaler, "Value of Rufianic Acid as an Alkaloidal Precipitant," *Pharm. Zentralh.*, 71 (1930), 561. Through YEARBOOK, A. PH. A., 19 (1930), 187.

(29) F. O. Taylor, "Aconite," *Ind. & Eng. Chem.*, 1 (1909), 549–567.

(30) M. Wagenaar, "Microchemical Reactions of Aconitine," *Pharm. Weekbl.*, 67 (1903), 165. Through YEARBOOK, A. PH. A., 19 (1930), 180.

LABORATORIES SHARP AND DOHME,
PHILADELPHIA, PA.

GAMMA RAYS.

Discovery of a way to make sodium give out gamma rays has been announced by Prof. Ernest O. Lawrence at the University of California on October 20th. The radiation, it is believed, will open a promising field for cancer research and a further study of how radiation acts on living tissues.