Moreover, there is some reason to question the validity of the conclusion of the above investigators because the main difference that we found between the action of barbiturates at a high and at a low vitamin C level is not so much concerned with the prolongation of sleeping time as with the magnitude of the initial depression. We are inclined to believe that a given hypnotic has a greater initial effect in animals at a lower physiological level than in animals at a higher physiological level. These initial differences were very readily observed in the individual animals.

SUMMARY

1. There is a correlation between the vitamin C level and the response produced by phenobarbital and by pentobarbital in guinea pigs. The higher the vitamin level, the less the depression produced by the barbiturates.

2. The frequent administration of pentobarbital and phenobarbital to guinea pigs did not cause a depletion of vitamin C from the tissues.

3. It is suggested that the most likely cause for the effect of vitamin C level on barbiturate depression is the altered general metabolism produced by the lack of this vitamin rather than an effect produced in major degree by direct conjugation.

The authors wish to thank Dr. C. G. King of the University of Pittsburgh for suggesting this problem, Dr. T. Koppanyi of the Georgetown University School of Medicine for guidance in the selection of the barbiturates and advice on the measurements of barbiturate depression, and Dr. R. D. Shaner, Medical Director of the Hoffmann La Roche, Inc., for his constant interest in the problem and the generous supply of vitamin C.

REFERENCES CITED

(1) Longenecker, H. E., Musulin, R. R., Tully, R. H., and King, C. G., *J. Biol. Chem.*, 129 (1939), 445.

(2) Longenecker, H. E., Fricke, H. H., and King, C. G., *Ibid.*, 135 (1940), 497.

(3) Ritz, N. D., Samuels, L. T., and Addis, G., *J. Pharmacol.*, 70 (1940), 362.

(4) Gruber, C. M., *et al.*, *Ibid.*, 71 (1941), 268.

(5) Sherman, H. C., La Mer, V. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 44 (1922), 165.

(6) King, C. G., and Luck, J. M., *Annual Review of Biochemistry*, Stanford University, 8 (1939), 392.

(7) Onoyama, T., *Mitt. med. Akad. Kioto*, 27 (1939), 1187.

(8) Koike, M., *et al.*, *Osaka Igk. Z.*, 35 (1936), 1705; *Chem. Abstr.*, 32 (1938), 658.

(9) Holmes, H. N., Campbell, K., and Amberg, E. F., *J. Lab. Clin. Med.*, 24 (1939), 1119.

(10) Beyer, K. H., *J. Pharmacol.*, 71 (1941), 394.

(11) "Methods of Analysis—A. O. A. C.," 4th Edition, page 478, Washington, D. C., 1935.

(12) Wright, S. T., Callaghan, T. J., and Minnich, V., *Proc. Soc. Exptl. Biol. Med.*, 47 (1941), 490.

(13) Hjort, A. M., de Beer, E. J., and Fassett, D. W., *J. Pharmacol.*, 65 (1939), 79.

(14) Richards, R. K., *et al.*, *J. Biol. Chem.*, 140 (XXXV, Proceedings) (1941), lxxii.

(15) Koppanyi, T., Linegar, C. R., and Dille, J. M., *J. Pharmacol.*, 58 (1936), 199.

(16) Musulin, R. R., and King, C. G., *J. Biol. Chem.*, 116 (1936), 409.

Marihuana Investigations. IV. A Study of Marihuana Toxicity on Goldfish Applied to Hemp Breeding*

By Brittain B. Robinson†

INTRODUCTION

It has been shown in previous articles (1, 2, 3) of this series that individual plants or varieties of hemp, *Cannabis sativa*, react differently to the chemical Beam tests and that the resins vary in amount and reaction to the Beam tests with hemp of different varieties, different ages, or hemp grown under different climatic conditions. The results presented suggested a possibility of obtaining by plant breeding methods a variety of hemp that might contain a low amount of the resin. It was not proved in the previous articles that the resins in the different lots of hemp were different in their physiological activity as measured upon animals.

Although significant progress has been recently made in elucidating the chemistry of marihuana resins, the material is of such complex nature that it may require consider-

* In collaboration with the Treasury Department, Bureau of Narcotics, Washington, D. C.

† Agronomist of the U. S. Department of Agriculture, Division of Cotton and Other Fiber Crops and Diseases.

able additional work before any simple chemical assay is available that would be useful in plant breeding work.

The purpose of this article is to discuss studies which have been made to determine if the degree of toxicity of hemp extracts to goldfish was of value for plant breeding work in attempting to obtain varieties with little or no active marihuana content. Although the dog is recognized as the standard test animal for marihuana biological assay work, Duquenois (4) reported using fish in aqueous solutions. If goldfish can be used the ease and expense in making numerous tests needed in plant breeding work would be greatly simplified. Results are presented to indicate that dog and goldfish react somewhat similarly to the same extracts. Further, resinous extracts of varieties grown at one location or in different regions of the United States produce different degrees of toxic activity upon fish.

A calibration of toxicity of varied doses of the same preparation of marihuana was not made but it would be desirable in future investigations to make such a calibration.

EXPERIMENTAL

Method of Testing.—The following method was used to obtain the resinous extracts from the hemp leaves. Eight grams of air-dried powdered leaves were placed in an adapter tube. The lower end of the adapter tube was plugged with a half-inch high plug of cotton to act as a filter. The lower end below the cotton was then closed with a stopper and 40 cc. of acetone were used to immerse the leaves. The upper end of the tube was then closed with a stopper. The tube and similar prepared ones of other samples were then allowed to stand over night or for 17 hours at room temperature which probably varied between 70° and 90° F. during several months of experimentation.

The stoppers were removed from the tubes at the end of 17 hours and the acetone extract filtered through the cotton plug. This acetone was immediately filtered with charcoal (Darco Corporation, New York City, brand) and placed in closely stoppered bottles until used.

It was determined that 2 cc. of the acetone extract was the proper amount to use with 1000 cc. of water for a fish test. Two cc. of pure acetone in 1000 cc. of water did not affect fish when tested as controls. All experiments were conducted in an incubator with full width glass doors which allowed observations. The temperature of this incubator was kept at 28° C. The actone extracts of occasional samples of leaves that did not prove toxic to the fish for various reasons were concentrated by allowing slow evaporation of the acetone from measured test-tubes at room temperatures and by this means much greater concentrations of the extract were obtained. This permitted a concentration of the resinous extracts without using more than 2 cc. of acetone per 1000 cc. of water, thus eliminating the effect of acetone which would have appeared in greater acetone concentrations. Goldfish acclimated to 28° C. temperature varying in weight from 3 to 5 Gm. were used in these experiments.

Studies upon leaf material that was first extracted with acetone by the above procedure and then by petroleum ether distillation showed that the acetone failed to remove about 33% of the extractable resins. However, the remaining third of the resins which were only removed by petroleum ether distillation were much less toxic upon fish than the first two-thirds of the total resins extracted.

Purified Resin Fractions.—In preliminary tests the Bureau of Narcotics, Treasury Department, furnished sample fractions of highly purified resins obtained by molecular distillation and of known physiological activity. These were diluted in the ratio of 1 to 10 with acetone. A fraction which was known to be physiologically active on dogs was found to be toxic to fish, and a fraction, not physiologically active on dogs, was not toxic to fish. The activity shown by the fish when affected by these fractions was similar to that produced on fish when affected by extracts of leaf material.

It may be interesting to record that the extracts made up of the purified fractions were theoretically much stronger than extracts prepared from leaves with cool acetone. However, the toxic affect of the strong fractions was not much more rapid than acetone extract of certain leaves. This may have been due to the fact that it was difficult to emulsify the purified fractions in the water.

Toxicity Differences Due to Variety, Maturity and Region of Growth.—Robinson and Matchett (3) reported results on four varieties of hemp grown in 1939 in Mississippi, Nebraska, Virginia and Wisconsin. Three separate harvests representing progressive stages in the growth of the plants were made on each variety in each state. Acetone extracts of available leaf material from these harvests were tested to determine their toxicity to goldfish. The actual results are shown in Table I. In this table the results presented for the earliest harvest are incomplete as the material from the first harvest was not sufficiently toxic to affect the fish in most instances in the first 24 hours at the concentration used throughout the experiment. As a result only one test was made as it was thought that in relation to the other harvest periods the results were conclusive in showing the general trend of the toxicity in reference to the age of the plants. In other words, the toxicity increases with the age of the plant and this is positively correlated with the petroleum ether extraction made from this same material the results of which were presented in a previous article (3).

Table I.—Showing the Number of Minutes Elapsing before Death Occurred When Goldfish Were Placed in Water Solutions Containing Acetone Extracts of Leaves of Four Different Varieties of Hemp Grown in Four States and Harvested at Three Periods of Growth. The Experiment Was in Triplicate for the Second and Third Harvests

Variety / Test number	Virginia 1st	Virginia 2nd	Virginia 3rd	Virginia Total	Nebraska 1st	Nebraska 2nd	Nebraska 3rd	Nebraska Total	Mississippi 1st	Mississippi 2nd	Mississippi 3rd	Mississippi Total	Wisconsin 1st	Wisconsin 2nd	Wisconsin 3rd	Wisconsin Total	Variety Totals
Third harvest:																	
Manchuria "A"	97	152	196	445	156	230	202	588	1440[a]	420[b]	420[b]	2280	134	68	122	324	3637
Manchuria "B"	161	140	203	504	105	161	190	456	1440[a]	141	288	1869	233	167	233	633	3462
African "C"	135	143	227	505	88	93	158	339	93	77	153	323	90	107	112	309	1476
Kentucky "D"	177	83	112	372	113	96	96	305	90	135	99	324	180	127	155	462	1463
State totals				1826				1688				4796				1728	
Second harvest:																	
Manchuria "A"	249	198	177	624	376	336	321	1033	1440[a]	1440[a]	1440[a]	4320	346	208	166	720	6697
Manchuria "B"	307	249	347	903	420[b]	268	420[b]	1108	1440[a]	1440[a]	1440[a]	4320	420[b]	331	233	984	7315
African "C"	155	305	203	663	308	257	199	764	266	195	346	807	180	113	174	467	2701
Kentucky "D"	103	94	86	283	135	127	101	363	115	141	296	552	149	164	170	483	1681
State totals				2473				3208				9999				2654	
First harvest:																	
Manchuria "A"	No material				420[b]				1440[a]				420[b]				
Manchuria "B"	available for tests				420[b]				1440[a]				420[b]				
African "C"					420[b]				1440[a]				176				
Kentucky "D"					118				1440[a]				420[b]				

[a] Alive at end of experiment—24 hours.
[b] Alive at 420 minutes but dead at 24 hours.

Table I shows the marked differences of toxicity obtained with different varieties. Manchurian "A" and "B" were decidely not as toxic as African "C" and Kentucky "D." As these differences were rather consistent at different locations and at different times of harvest, it indicates that hereditary differences exist in varieties. This is important from a practical control method as it indicates the possibility of obtaining by intensified breeding methods a variety of low marihuana content.

Table I further shows that environment plays an important role in the production of the toxicity. This had been expected and it confirms to some extent the belief that the production of the drug is more pronounced in dry elevated regions than in ones of abundant rainfall. This is shown in the table as Mississippi, the most moist location where the hemp was grown, produced the least toxic plants and Nebraska was selected for the driest and most elevated region and it produced the most toxic plants.

Variety Testing.—In 1940, 60 lots of hemp representing original seed from Roumania, Russia, China, Turkey, Tunisia, Hungary, Italy, Germany, Malta, Manchuria, Kentucky, Wisconsin, wild Connecticut, and selections of imbred material from many of these sources were planted in small plots side by side at Arlington, Virginia. Twice during the growing period leaves were collected from plants growing on these plots. Acetone extracts were made upon the leaves and these extracts were tested upon fish. From these tests two plots representing varieties very toxic to fish and two representing varieties only mildly toxic to fish were selected for further study. Additional material of these four varieties was collected and presented to the Bureau of Narcotics, Treasury Department, for alcoholic and petroleum ether extractions. The ether extracts were submitted to Dr. S. Loewe, Cornell University Medical School, who tested their activity upon dogs. The results upon the four selected varieties are presented in Table II.

This table presents results that indicate that the fluidextracts of the Tunisia variety of hemp are from four to eight times as potent on dogs as the fluidextracts of the other varieties. It is not believed that the differences in the stage of maturity of the varieties accounted for the different degrees of toxicity or potency. The Tunisia and Kentucky varieties were the latest in maturing. Further, it is interesting to note in the table that the two varieties, Tunisia and Wisconsin, both are very toxic to fish,

Table II.—Comparison of the Resins Extracted from Four Varieties of Hemp in Reference to Their Potency on Dogs and Their Toxicity upon Fish

Original Source of Seed	Generations Imbred, Brother × Sister	Toxicity upon Fish: Concentration	Toxicity upon Fish: Life Span	Extractions: Alcohol, %	Extractions: Ether, %	Dog (5) Potency	Solid Basis
Tunisia, Africa	2	1 conc.	Dead in 68 Min.	9.4	7.67	0.12	0.70
Wisconsin (Ferramington)	0	1 conc.	Dead in 267 Min.	8.1	6.39	0.03	0.20
Kentucky	1	3 conc.	Alive 24 Hrs.	6.5	3.51	0.02	0.17
Manchurian	2	3 conc.	Alive 24 Hrs.	5.0	3.61	0.014	0.15

both yielded high in alcohol and ether extractions in comparison with the other two varieties of low toxicity to fish, and low active potency to dogs.

The varieties of hemp listed in Table II would hardly be considered pure genetically. It would appear likely that varieties of hemp of even lower potency upon dogs and with less toxicity to fish might be obtained by further selection and imbreeding the Kentucky and Manchurian varieties.

CONCLUSIONS

Acetone extracts of leaves of different varieties of hemp produce different degrees of toxicity in goldfish. The region of growth and the age of the hemp plants affect the degree of the extract toxicity upon goldfish. Fractions of purified resins of different potency acted upon dogs in a somewhat similar manner as they did on fish. Of four varieties used for both fish and dog tests, the degree of toxicity upon fish and potency to dogs was in the same relationship. It is believed that goldfish testing of hemp resins may facilitate hemp breeding.

REFERENCES

(1) Wollner, *et al.*, JOUR. A. PH. A., 27 (1938), 29–36.

(2) Matchett, *et al.*, JOUR. A. PH. A., SCIENTIFIC EDITION, 29 (1940), 399–404.

(3) Robinson, *et al.*, *Ibid.*, 29 (1940), 448–453.

(4) Duquenois, P., *Bull. sci. pharmacol.*, 46, No. 5, May, 1939.

(5) Loewe, S., JOUR. A. PH. A., 28 (1939), 427; *J. Pharmacol.*, 66 (1939), 23.

Aliphatic Amines I—A Review*

By Melvin F. W. Dunker† and Walter H. Hartung

An extensive survey of the literature on the simple aliphatic amines has been made in order to determine whether it is possible to make a correlation between structure and biological properties. A vast amount of material has been published, by far the most of it pertaining to chemical and physical properties and to methods of syntheses. The references relating to the physiological effects of the simple aliphatic amines, while numerous, represent only a small part. Since the publication of Trendelenburg's comprehensive review in 1923 (1), two partial reviews have appeared, namely, Hartung's tabulation of toxicity and pressor effects (2) and Tainter's discussion of pressor and autonomic nervous system effects (3). Many of the original reports in the literature are difficult or impossible to correlate because of differences in species, in the state of the experimental animal, dosage, technique, mode of administration and the like.

In view of the increasing commercial importance and availability of many simple aliphatic amines, it seems desirable to be better informed on their physiological properties. Furthermore it would seem presumptuous to attempt to correlate the effect of chemical structure of complex compounds with physiological action, a field in which much of the current research is being done, when the simple bases have been inadequately studied or neglected.

The one property on which good comparative data are available is the effect of amines on the blood pressure on intravenous injection. A few generalizations may be repeated here. The lower members, up to the butylamines according to Barger and Dale (4), or to isopropylamine according to Tainter, are inactive or give no constant response on blood pressure in experimental animals. As the chain is increased beyond this point, the pressor activity steadily increases to a maximum in *n*-hexylamine and then steadily decreases as the chain is lengthened to 13 carbon atoms. From propyl to hexyl, it has been shown that the normal radical is more active than the corresponding iso-chain, although Tainter (3) reports the isopropylamine to have pressor activity while the normal propylamine is inactive. Except for the sec-butylamine which was found to be indifferent (3) and the tertiary amylamine for which pressor activity is claimed (5) no other saturated branched chain amines have apparently been investigated. The primary amines seem to be somewhat more active than the secondary and tertiary amines (1).

The amines as a class appear to stimulate the tone and movement of the isolated intestinal and uterine tissues, the effect increasing somewhat with increasing length of the chain up to C_6 (6). Isoamylamine has an effect which parallels that of adrenalin on smooth muscle (7) although it is much less active, that is to say, it produces an inhibition of the intestine, contraction of the rabbit uterus in all states, an inhibition of the non-pregnant cat uterus and

* From the Department of Pharmaceutical Chemistry of the School of Pharmacy, University of Maryland.

† William R. Warner Co. Fellow, 1939–1940; present address, University of Wisconsin, College of Pharmacy, Madison, Wis.