virus antiserum was able to neutralize not only the homologus virus but also over 100 MLD of the Japanese B virus and partially 10 MLD of the St. Louis virus.

Conclusion. A virus (Peiping encephalitis virus) capable of producing encephalitis in Swiss mice and a monkey was isolated from a case of acute encephalitis. On the basis of animal pathogenicity and virus neutralization tests, the virus is considered to behave like the Japanese type B virus, but to be serologically distinguishable from the St. Louis encephalitis virus.

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Comparative Anticonvulsant Activity of 1-Diethylacetyl-5, 5-Cyclopentamethylene Biuret, Sodium Diphenyl Hydantoinate and Sodium Pentobarbital in Mice.*

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The structural features which appear to account for the hypnotic action of the barbiturates are the presence of a –CONH– group[1] and an alkyl or acyl group replacing both hydrogen atoms in position 5.[2] Recently Hill and Degnan[1] have emphasized that the alkyl biurets have 2 –CONH– groups within their structure. They postulated that an acyl biuret containing branched chain alkyl groups might have hypnotic properties and low toxicity. Although Hesse and Taubmann[3] had previously reported on the pharmacology of certain biurets and some of their guanyl and thio derivatives, the use of these agents as hypnotics had not been proposed. They reported that guanyl-thiourea lowered the body temperature of rabbits in which artificial fever had been produced. The antipyretic action was believed to be due to increased heat loss and was abolished when animals were kept in a warm environment. The presence of the thio group seemed necessary for antipyretic action. When oxygen or an amino group was substituted for the thio group antipyretic action

* Sodium pentobarbital was supplied through the courtesy of Eli Lilly and Company, Indianapolis, Indiana.

1 Hill, A. J., and Degnan, W. M., *J. Am. Chem. Soc.*, 1940, **62**, 1595.

2 Tatum, A. L., *Physiol. Rev.*, 1939, **19**, 472.

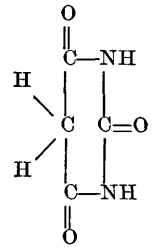
3 Hesse, E., and Taubmann, G., *Arch. f. Exp. Path. u. Pharmakol.*, 1929, **146**, 113.

was lost but lowering of blood pressure occurred. Oral doses of the ethoxyphenyl derivatives of guanyl urea in 0.2 g amounts given 3 times a day to man were reported to relieve headache and other pains. Apparently analgesic action accompanied antipyretic effects.

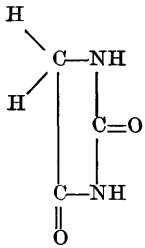
A series of acyl biurets synthesized by Hill and Degnan[1] indicated that these compounds compared favorably with sodium barbital in effectiveness as hypnotics. They reported that the biurets "do not show any anesthetic (depressor) action. The toxicities of these compounds, with the exception of those of the thiobiurets, are remarkably low; doses as high as 500 mg/kg have no permanent ill effects on the test animals. The presence of branched chains in the acyl radical increases effectiveness. Alkylation in the 5-position of the biuret chain also increases effectiveness, 1-diethylacetyl-5, 5-cyclopentamethylene biuret being the most active of the compounds tested."

Recently Ch'eng, Anderson and P'an[4] compared the hypnotic properties of this compound with those of paraldehyde and sodium barbital in white mice. Doses of 800 mg per kg of paraldehyde, 300 mg per kg of sodium barbital and 450 mg per kg of the 1-diethylacetyl-5, 5-cyclopentamethylene biuret were found to produce narcosis of longer duration with the dialkylacetyl biuret than with paraldehyde but of shorter duration than with sodium barbital. Dialkylacetyl biuret caused death in approximately half of the animals given 600 mg per kg intraperitoneally.

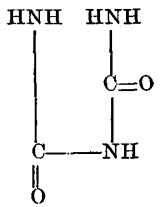
In view of the definite hypnotic activity of this dialkylacetyl biuret,† it was believed desirable to determine whether the compound was anticonvulsant as well. A comparison was made with sodium diphenyl hydantoinate (sodium dilantin) and sodium ethyl-1-methyl-butyl barbiturate (sodium pentobarbital). Dilantin, pentobarbital and biuret have analogous structural formulae of their molecules. Barbiturates are malonylurea derivatives while dilantin



Barbituric acid



Hydantoin



Biuret

4 Ch'eng, C. H., Anderson, H. H., and P'an, S. Y., in press.

† Prepared by Dr. Charles H. Ch'eng, Yenching University, Peiping, China.

is a derivative of glycolyl urea and biuret is composed of 2 urea molecules linked together with the loss of one molecule of ammonia. In contrast to the other two, it is open chained. It will be noted that all of these agents have –CONH– groups in their molecules.

Putnam and Merritt[5, 6] have reported the anticonvulsant properties of dilantin and this agent has proved to be useful in the treatment of epilepsy. Pentobarbital has been shown by Swanson and others to be effective in antidoting cocaine and strychnine poisoning.[7, 8, 9] The antagonism between picrotoxin and pentobarbital has also been demonstrated by Koppanyi, *et al.*[10]

Convulsants which act on different levels of the central nervous

TABLE I.
Comparative Anticonvulsant Activity of Dialkylacetyl Biuret, Sodium Dilantin and Sodium Pentobarbital in Mice.

Convulsant		Hypnotic					
Name	Dose= LD_{100} mg/kg	Name	Dose= LD_{50} mg/kg	No. of animals	Avg time of death, min.	No. having convulsions	No. dying
Cocaine*	150‡	—	—	10	55	10	10
,,	,,	Alkyl biuret	600§	12	207	3	12
,,	,,	Sodium dilantin	200[13]	6	194	6	6
,,	,,	Na pentobarbital	150[14]	6	7	2	6
Picrotoxin	9.75[11]	—	—	10	10	5	10
,,	,,	Alkyl biuret	600	10	—	0	0
,,	,,	Sodium dilantin	200	5	19	5	5
,,	,,	Na pentobarbital	150	10	12	0	5
Strychnine	2[12]	—	—	10	6	5	10
,,	,,	Alkyl biuret	600	10	66	0	1
,,	,,	Sodium dilantin	200	10	1560	8	5
,,	,,	Na pentobarbital	150	5	13	1	5

*Larger doses were required to kill all animals, but no animal given this amount plus an hypnotic survived.

‡ Determined in this laboratory.

§ Determined in this laboratory.

5 Putnam, T. J., and Merritt, H. H., *Science*, 1937, **85**, 525.

6 Merritt, H. H., and Putnam, T. J., *Arch. Neurol. and Psych.*, 1938, **39**, 1003; *J. A. M. A.*, 1938, **111**, 1068; *Arch. Neurol. and Psych.*, 1939, **42**, 1053.

7 Swanson, E. E., and Shonle, H. A., *J. Lab. and Clin. Med.*, 1931, **16**, 1056.

8 Barlow, O. W., *J. A. M. A.*, 1932, **98**, 1980.

9 Swanson, E. E., *J. Am. Pharm. Assn.*, 1935, **24**, 959.

10 Koppanyi, T., Linegar, C. R., and Dille, J. M., *J. Pharm. and Exp. Therap.*, 1936, **58**, 199.

11 Chakravarti, M., *J. Pharm. and Exp. Therap.*, 1939, **67**, 154.

12 Schwartze, E. W., *J. Pharm. and Exp. Therap.*, 1922, **19**, 273.

13 Gruber, C. M., Haury, V. G., and Drake, M. E., *J. Pharm. and Exp. Therap.*, 1940, **68**, 433.

14 Gates, W. H., *Science*, 1932, **76**, 349.

system were chosen for study. These were cocaine, picrotoxin and strychnine. Except with cocaine, single doses equivalent to the LD_{100} (a surely fatal dose) for each convulsant were given subcutaneously to inbred white mice. Immediately before or after this injection the hypnotic was given intraperitoneally. Single or repeated doses equivalent to one or more LD_{50}s were administered. There were 24 or more animals in each treated group. Thirty mice served as controls and were given the convulsants alone. Animals were observed for a period of 48 hours and mice that lived longer were considered as "survived".

Table I summarizes the results of this study. Convulsions developed early and persisted until death in 5 animals which had been injected with strychnine and treated with dilantin and in 3 of the other 5 mice which survived this treatment. Administration of an hypnotic either before or after the convulsant was given did not alter the protective action significantly. While none of the hypnotics antagonized cocaine completely, the dialkylacetyl biuret and pentobarbital usually antagonized the convulsant action of cocaine. This result agrees with the findings of Dragstedt and Lang[15] who found that barbital was effective in preventing or relieving cocaine convulsions but it did not decrease the mortality percentage in rabbits.

Under the conditions of this experiment the biuret was superior to pentobarbital or dilantin in antagonizing and detoxifying the action of lethal doses of picrotoxin and strychnine. In view of the frequent occurrence of toxic side-actions in epileptic patients treated with sodium dilantin, even under careful supervision and adjustment of dosage[16, 17] the anticonvulsant activity of the dialkylacetyl biurets and their derivatives certainly deserves further study.

Summary. The anticonvulsant activity of 1-diethylacetyl-5, 5-cyclopentamethylene biuret was compared in inbred mice with that of sodium dilantin and sodium pentobarbital. The biuret and pentobarbital protected the majority of animals given cocaine but they did not prevent death. All cocaine-treated mice given dilantin died from convulsions. Against surely fatal doses of picrotoxin and strychnine the dialkylacetyl biuret was effective in 19 of 20 animals. Dilantin had no effect against picrotoxin but saved half of the strychnine-treated animals. Sodium pentobarbital prevented convulsions in mice given picrotoxin and saved 5 of 10. It was anticonvulsant in the majority of strychnine poisoned mice but did not prevent death.

[15] Dragstedt, C. A., and Lang, V. F., *J. Pharm. and Exp. Therap.*, 1928, **32**, 215.
[16] Fetterman, J. L., *J. A. M. A.*, 1940, **114**, 396.
[17] Lennox, W. G., *J. A. M. A.*, 1940, **114**, 1351.