THE RELATION OF BARBITAL AND PHENOBARBITAL TO GRANULOCYTOPENIA.*

BY J. C. KOPET AND F. J. GOODRICH.

Evidence obtained by reviewing the literature indicates that amidopyrine is an important factor in the production of granulocytopenia. Since barbituric acid derivatives are commonly prescribed with amidopyrine, and since the cause of many of the cases of syndrome have been traced to certain proprietary combinations of amidopyrine and barbituric acid derivatives, it might be expected that barbiturates could be responsible for the causation of a decrease of granulocytes in peripheral blood. An investigation was conducted to clarify the rôle of the barbiturates in the experimental production of granulocytopenia. The question of whether the presence of a barbiturate with amidopyrine results in a synergistic action, or otherwise influences the effect of amidopyrine remains to be determined.

Agranulocytosis was first named and described by Schultz (2) in 1922. Kracke (3) reported that the term granulocytopenia more correctly expressed the actual condition existing in that disease known more commonly as agranulocytosis than any that has been suggested. The name of granulocytopenia is consistent with the accepted nomenclature describing variations in numbers and percentages of white blood cells and denotes a decrease in granular cells. The term "agranulocytic angina" should be abandoned as many of the patients having a definite granulocytopenia have shown no oral lesions.

Granulocytopenia seems to be a disease which may be acute or chronic, severe or mild, depending upon the extent to which the granular cells are decreased. The condition known as agranulocytosis, meaning "an increase in the number of immature granular cells," is probably the most extreme state of the disease in which the granular cells almost completely disappear from peripheral blood; this, in turn, being followed by local or general sepsis and usually by death. Agranulocytosis is apparently a disease entity in which an unknown agent produces a depression of the bone marrow, this resulting in loss of neutrophilic resistance with subsequent overwhelming infection in those areas of the body normally inhabited by bacteria. The type of case which is characterized by loss of neutrophiles and death without evidence of infection is also classified as agranulocytosis.

Kracke (3) felt that the peculiar distribution of granulopenia could well be correlated with the use of certain drugs containing the benzene ring which are commonly used for therapeutic purposes. Benzene poisoning has been recorded as a process in which hematopoieses is completely inhibited in all of the three bone marrow elements. Certain drugs of the coal-tar series have been constantly associated with cases of granulocytopenia. Kracke points out that these drugs all have as a nucleus the benzene ring with an attached amine ($-NH_2$) group, making them substituted primary amines. This basic structure sets them apart as far as their reactions are considered from other coal-tar derivatives, such as aspirin, and the like, which do not contain the primary amine. For this reason, he has arbitrarily designated these compounds as "benzamine drugs" to facilitate reference to them as a group. The drugs contained in this group are amidopyrine, phenacetin, acetanilid and arsphenamine.

Amidopyrine appears on the market in proprietary compounds containing it and a barbiturate and is commonly prescribed this way. In many cases of granulocytopenia, this type of sedative had been administered previous to the appearance of the clinical picture of the disease, and it could not be stated definitely that either amidopyrine or the barbiturate alone was responsible for the condition.

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EXPERIMENTAL.

Barbital (diethyl barbituric acid) and phenobarbital (phenyl ethyl barbituric acid) were administered to rabbits in an attempt to produce granulocytopenia experimentally. Barbital was used because it consisted of two straight chain groups linked to the malonyl-urea group, and phenobarbital was administered because it contained a benzene group substituted for an ethyl group of barbital. In this way it might be possible to indict either the barbituric acid nucleus or the phenol group, if a condition of granulocytopenia could be produced in either case.

Since the interest lay in determining whether or not a daily administration of the drug, rather than an acute poisoning, could produce a decrease in granulocytes, no change in the amount administered was made during the period that the animals were subjected to the experiment. It was decided to administer orally an amount equal approximately to one-half of the minimum lethal dose of the drug for the body weight of the animal. There was a possibility that the accumulation of the drug in the blood stream could cause a poisoning, since the barbiturates are excreted slowly, but this in itself might cause a condition of granulocytopenia, which was the object of the experimentation. Taking 300 mg. (4.626 grains) per Kg. of body weight as being nearly the minimum lethal dose for barbital, it was decided to administer 5 grains (0.324 Gm.) to the rabbits weighing 2 Kg. In a like manner, 1.5 grains (.098 Gm. of phenobarbital were selected as a convenient dose for 2-Kg. rabbits, since the M. L. D. is approximately 100 mg./Kg. It is merely coincidental that the amount of these doses correspond to the average human dose.

The drug was administered orally to allow the substance to undergo any changes in the alimentary tract that might alter its toxicity before absorption, since, as it has been explained previously, there is a suspicion that the harmful effect of the benzamine class of drugs is due to various derivatives that might be formed within the body. Such could possibly be the case with the barbiturates. It would not be right to seek to obtain data from the effect of hypodermic administration of the substances when the majority of the cases of granulocytopenia from chemicals were traced to the oral administrations. Furthermore, these particular barbiturates are universally prescribed to be taken orally. A total white cell count was performed following the daily oral administration of the drugs and a differential count was also obtained. For convenience, the differential count was recorded on the basis of granulocytes and nongranulocytes; polymorphonuclear neutrophiles, basophiles and eosinophiles being classed as granulocytes, and the lymphocytes and monocytes falling into the other classification. The total number of granulocytes was obtained by calculation from the total white cell count and the percentages obtained by the differential count.

SUMMARY.

1. Granulocytopenia is a condition of a decreased number of circulating granulocytes in the peripheral blood, probably affected by a chemical agent causing the bone marrow to fail to produce granulocytes.

2. Drugs similar to amidopyrine, having an amine group attached to a benzene group in their structural formula, are apparently the cause of this condition.

3. Barbital and phenobarbital do not cause a permanent decrease in the number of circulating granulocytes in the peripheral blood of normal rabbits.

REFERENCES.

(1) Council on Pharmacy and Chemistry, "The Relation of Amidopyrine and the Barbituric Acid Derivatives to the Granulocytopenia," *Journal of American Medical Association*, 102 (26), 2076–2077 (June 30, 1934).

(2) Schultz, Werner, *Deut. med. Wochschr.*, 48, 1495 (November 3, 1922).

(3) Kracke, Roy R., "A Review of Granulocytopenia (Agranulocytosis)," *J. Lab. Clin. Med.*, 17 (10), 993–1003 (July 1932).

Reference numbers not given in text.

(4) Stein-ab, *J. A. M. A.*, 104 (24), 2218 (June 15, 1935).

(5) Piersol, G. M., and Steinfield, E., "Granulopenia," *Arch. Int. Med.*, 49 (1932).

(6) Pepper, O. H. P., "Leukopenia—a Review; with special reference to Agranulocytic Angina," *Calif. and West. Med.*, 35 (September 1931).

(7) *Society Proceedings, J. A. M. A.*, 101 (26), 2076–2077 (1933).

(8) Benjamin, J. E., and Biederman, J. B., "Agranulocytic Leukopenia," *J. A. M. A.*, 103 (3), 161–163 (1934).

(9) Kracke, Roy R., "The Etiology of Granulopenia," *J. Lab. Clin. Med.*, 19 (8), 799–817 (May 1934).

(10) Kracke, Roy R., and Parker, F., "Agranulocytosis," *J. A. M. A.*, 105 (12), 960–966 (September 21, 1935).

(11) Madison, F. W., and Squier, T. L., "The Etiology of Primary Granulocytopenia," *Ibid.*, 102 (10), 755–759 (March 10, 1934).

(12) Jackson, W. J., and Stovall, W. D., "The Normal Blood Count of the Rabbit," *J. Lab. Clin. Med.*, 16 (21), 1–5 (October 1930).

A STUDY OF METHYLENEDISULFONIC ACID AND ITS DERIVATIVES.*

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Chemotherapeutic studies of the barbital and sulfonal groups of hypnotics have established definitely a relationship between their chemical structure and their therapeutic action.

The two drugs, barbital and sulfonal, may be considered the precursors of two important series of hypnotics, the methylenedisulfonic acid group and the malonylureide group. The chemotherapeutic concepts of these compounds undoubtedly link their physiological activities with their characteristic molecular structures, and it would seem rational to conceive of a compound having a structure which included the hypnophore groups of both of these series. The basic structure of such a barbituric acid sulfonal hybrid would be $:C(SO_2.NH.CO.NH.SO_2)$, and the relation of methylenedisulfonic acid to it would be analogous to the relation of malonic acid to the barbiturates.

A survey of the literature revealed only one attempt to prepare such a hybrid compound, and this was unsuccessful and offered little encouragement to future investigators. However, the synthesis and pharmacological study of such a series of compounds would add much information to our present knowledge of the chemotherapy of hypnotics, and might result in the production of some valuable therapeutic agents. The work reported here has as its object the study of the problems involved in the preparation of derivatives of methylenedisulfonic acid and as its hope the preparation of the hybrid methylenedisulfonureide structure shown above.

EXPERIMENTAL.

The basic compound methylenedisulfonic acid has been prepared by Schroeter (1) who obtained it as its barium salt in yields of 15 per cent and by Backer (2) who obtained it as the potassium salt in yields of 85 per cent.

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[1] Abstracted, in part, from a thesis submitted by John C. Bauer to the Faculty of the Graduate School of the University of Maryland in partial fulfilment of the requirements for the degree of Doctor of Philosophy.