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7996 C

Non-Production of Granulocytopenia with an Amidopyrine Compound in Some Acute Infections.

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The experimental production of Agranulocytosis in rabbits by subcutaneous injections of benzene and olive oil was reported by Kracke.[1] Later Madison and Squire[2] emphasized the fact that in their analysis of 13 consecutive cases of granulocytopenia, all of the patients had received drugs containing barbiturate compounds with amidopyrine. Since then numerous reports[3] by physicians seem to indicate that amidopyrine is an important factor in the production of granulocytopenia. However, the attempts to produce granulocytopenia with amidopyrine have not been very gratifying. Madison and Squire's observation of an anti-mortem drop in the granulocytes of 1 rabbit in a series of 11 experimental animals receiving amidopyrine with allylisopropyl-barbituric acid, may have unduly suggested the possibility of a specific agranulocytic property of this drug, thus leading to erroneous conclusion as to the real cause of the disappearance of the granulocytes and placing a ban on the use of drugs hitherto considered relatively free from untoward symptoms, and very effective as analgesics and antipyretics.

The present speculative nature, both as to the real etiology of granulocytopenia and the rôle that these drugs play in this disease give evidence of a timely need for more experimental investigation and accurate clinical observation, which may further our information on these obscure but significant factors.

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1 Kracke, Roy R., *Am. J. Clin. Path.*, 1932, **2**, 11.

2 Madison, F. W., and Squires, T. L., *J. A. M. A.*, 1933, **101**, 2076.

3 Reznikoff, Paul (Special Report of the Council on Pharmacy and Chemistry), *J. A. M. A.*, 1934, **102**, 2183; Johnson, Wingate M., *J. A. M. A.*, 1934, **103**, 1299.

The following experiments were performed at a time when 2 acute diseases were endemic among the rabbits of our laboratory, and occurred at frequent and irregular intervals, *viz.*, (1) an acute respiratory infection commonly called snuffles, from which rabbits frequently recover spontaneously and (2) an acute intestinal disturbance characterized by diarrhoea, loss in weight, and death. Before beginning the experiments we observed that rabbits infected with either of these diseases develop a pronounced leucocytosis.

The plan of our experiment consisted in administering cibalgine "Ciba" [Amidopyrine (dimethyl-amino-phenyl-dimethyl-pyrazolone) and dial (diallyl malonyl urea)] by mouth, in large daily dosages, to rabbits housed in adjacent cages, or in the same cage with infected rabbits, and comparing the leucocyte count of those which became spontaneously infected while receiving the drug, to those which received the drug but escaped infection.

Thirty-five rabbits were used. Nine of these were thyroidectomized 4 to 6 weeks before beginning the use of the drug. The object of the thyroidectomy was to impair the activity of the bone marrow; it having been previously shown that thyroidectomy in the rabbit is followed after a period of time by a histological picture of the bone marrow suggestive of damage to this tissue (Kunde[4]). The experimental production of such a bone marrow seems pertinent at this time, inasmuch as it has been generally considered that individuals with granulocytopenia have a defective bone marrow.

Table I contains a summary of the data taken from our protocols. The 35 rabbits used were divided into 7 groups. Group A consisted of 9 normal animals. Thirty-nine leucocyte counts were made of this group (3-6 counts on each animal always on different days) to establish variations in the number of leucocytes occurring in rabbits under normal conditions, apparently free from infection, and receiving no drug. The variation in number of leucocytes ranged from 3,200 to 10,536 per c.mm. with an average of 6,887. It is our opinion at this time, however, that the leucocyte count in normal young rabbits entirely free from infection rarely exceeds 6,000 and that counts exceeding this are indicative of mild infection giving no detectable gross signs. Group B represents a comparable group of normal animals, *i. e.* apparently free from infection but receiving dosages of the compound (1 to 4 tablets) daily, over a period of time 7 to 17 days in duration to establish the effect of this compound on the leucocyte count of normal rabbits. In this group, the variation in number of leucocytes ranges from 8,938 to 17,575 per

[4] Kunde, M. M., *et al.*, *Am. J. Physiol.*, 1932, **99**, 469.

c.mm. These results clearly indicate that large dosages of the compound caused no suppression of leucocytes in normal rabbits when administered for 17 consecutive days.

Groups C and D consisted of infected animals. In Group C the infected animals received no drug whereas in Group D, the infected animals received the compound daily. No rabbits in these groups weighed more than 1100 gm. and varied in age from 3 to 4 months. A significant leucocytosis always preceded gross signs of either disease in the infected animals and as the severity of the disease progressed and became grossly obvious, the leucocytes frequently exceed 27,000 per c.mm. In the infected animals of Group D the compound was administered daily in dosages varying from 1 to 2 tablets for 21 to 30 days. Leucocyte counts made on the 20th to the 30th day of drug administration in infected animals revealed a leucocytosis of more than 34,000, showing that the drug has no tendency to depress the leucocytosis which accompanies these infections although it was administered in relatively large dosages for 21 to 30 days, at which times the animals died. Groups E, F and G were made up of rabbits with thyroids removed 3 to 4 weeks after birth. The leucocyte counts of these animals were made 4 to 6 weeks after extirpation of the thyroids. The data of the rabbits of Group E (Table I) gave no evidence of infection. They received no drug. The data show that in this stage of thyroid deficiency (4 to 6 weeks after thyroidectomy) with no infection, the range of variation in leucocytes is approximately the same as in normal rabbits. In Group E, the thyroidectomized rabbits became infected. Infection in these thyroidectomized rabbits produced a severe leucocytosis, as occurs in infected rabbits with thyroids intact. The rabbits of Group G, as in Group E, were thyroidectomized and infected but these animals received large daily dosages (1-4 tablets) of the compound (10 to 22 days). In the rabbits of this group, the infection was characterized by a severe leucocytosis (more than 26,000 per c.mm.) comparable to that which occurs in infected animals receiving no drug.

Many differential counts were made from time to time, of animals to which the drug was administered. The percentage of granulocytes of the total leucocyte count was at all times within range of normal variations.

Summary. Cibalgine (Ciba) does not diminish the number of leucocytes in the circulating blood of normal rabbits when given in large dosages for 17 consecutive days. The pronounced leucocytosis which occurs in rabbits with sniffles or a certain gastro-

TABLE I.
Summary of Leucocyte Counts under Conditions of This Experiment.

Condition of animal	No. of animals	No. of counts	No. of counts each animal	Daily dose in *Tablets	No. of daily dosages	Leucocyte Counts			Remarks
						Lowest	Highest	Average	
A Thyroid intact. No drug, no infection. (Normal)	9	39	3-6	—	—	3,200	10,536	6,887	Normal animals
B Thyroids intact. No infection. Rec. drug daily	6	15	2-3	1-4	7-17	8,938	17,575	13,306	Killed between the 7 and 17 day.
C Thyroids intact. No drug. Infected.	6	12	2	—	—	11,150	27,200	20,180	Died either of respiratory or intestinal infection.
D Thyroids intact. Infection. Rec. drug daily.	3	15	3-6	1-2	21-30	6,650	34,550	12,835	Died either of respiratory or intestinal infection.
E Thyroids removed. No drug. No infection.	3	15	3-9	—	—	6,200	9,408	7,577	Uncomplicated hypothyroidism
F Thyroids removed. No drug. Infected.	4	9	2-3	—	—	8,350	26,989	19,207	Died either of respiratory or intestinal infection.
G Thyroids removed Rec. drug daily Infected.	4	12	1-4	1-4	10-22	7,712	26,500	13,544	Died either of respiratory or intestinal infection.

* Each tablet contains:
Amidopyrine (Dimethy-amino-phenyl-dimethyl-pyrazolone.....3½ grains
Dial, "Ciba" (diallylmalonylurea)..... ½ grain

intestinal infection is not depressed by the above mentioned amidopyrine barbiturate compound administered from 17 to 30 days. Rabbits thyroidectomized 6 weeks previously and receiving cibalgine develop a leucocytosis in response to the infections comparable to that which occurs in rabbits with thyroids intact.

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Isolation of Bacterium Typhosum When Mixed With Anaerobic, Non-Spore Forming, Gram-Negative Rods (Bacteroides)

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The enormous numbers of anaerobic non-spore forming, gram-negative rods reported in the intestinal contents of man (Sanborn[1] and Eggerth and Gagnon[2]) have not been considered in the routine isolation of pathogenic bacteria, or in the false presumptive tests in water analyses. *Bacteroides* are small gram-negative or gram-positive rods, some of the gram-negative forms being indistinguishable morphologically from the gram-negative pathogenic bacteria. Although they are strict anaerobes, the possibility that they might find conditions favorable for multiplication in mixed cultures with aerobes was considered in this investigation.

Bacterium typhosum (Rawlings) and 2 strains of *Bacteroides,* one isolated from a colon specimen from man and the other from a monkey colon, were studied. The biochemical reactions of the 2 strains of *Bacteroides* are listed in Table I.

TABLE I.

Strain	Gas from peptone	Gas from glucose	Glycerol	Mannitol	Sorbitol	Arabinose	Salicin	Trehalose	Amygdalin	Cellobiose	Glycogen	Rhamnose	Xylose	Lactose	Levulose	Glucose	Gelatin liquefaction	Milk	Indol	Lead acetate
Monkey origin	—	—	—	—	—	a	—	—	a	—	a	a	a	a	a	a	+	a	—	—
Human origin			—	—	—		—	—	a	a	a	—	a	a	a	a	—		—	—

— = negative test. a = acid production only. + = positive test.

[1] Sanborn, A. G., *J. Infect. Dis.*, 1931, **48**, 541.

[2] Eggerth, A. H., and Gagnon, B. H., *J. Bact.*, 1933, **25**, 398.