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AN ASSESSMENT OF THE VALUE OF SUGGESTED THERAPIES FOR LEUCOPENIA

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The subcutaneous injection of methyl-bis(β-chloroethyl)amine (a nitrogen mustard) into rabbits consistently produces a leucopenia, and the degree of this leucopenia is readily varied by variation in the dosage of methyl-bis(β-chloroethyl)amine. This phenomenon has been used as a method of assessment of the value of three suggested therapies for leucopenia. The therapies tested were :

(i) Twenty-five per cent *p*-chloroxylenol in methylacetamide (CXM), which Zondek and Bromberg (1943) claim produces a leucocytosis lasting about four days in normal human subjects. In patients with typhoid fever the white cell count was maintained at a normal level by intramuscular injections of CXM, the count falling within 36 hours of discontinuing the injection.

(ii) The leucocytosis promoting factor (LPF) of Menkin and Kadish (1943). The latter state that 100 mg. of this preparation in saline or phosphate buffer at *p*H 7.4 when given to animals either by cardiac puncture or by subcutaneous injection causes leucocytosis.

(iii) 1 g./100 c.c. aqueous sodium succinate, 0.1 c.c. of which, when injected subcutaneously into normal subjects or phthisic patients, is said to raise the white cell count (Hammett, Vessler, and Browning, 1917).

METHOD OF ASSAY

The leucopenia was produced by the subcutaneous injection of methyl-bis(β-chloroethyl)-amine hydrochloride solutions (1 mg./c.c.). Rabbits (circa 2 kg.) and occasionally goats (circa 30 kg.) were used as the test animals. The therapies under test were given at various intervals after the methyl-bis(β-chloroethyl)amine hydrochloride injections, the efficacy of the therapies being judged by daily white cell counts performed between 10 a.m. and noon and before feeding.

RESULTS

(i) *CXM.*—This was given by intramuscular injection. Zondek and Bromberg's dosage for man (i.e., two doses of 10 c.c. the first day and 10 c.c. daily for the next three days) was given to goats. In normal goats only a slight rise in the white cell count was produced, while in methyl-bis(β-chloroethyl)amine hydrochloride-poisoned animals leucopenia was not prevented and the mortality was actually doubled (Table I).

A similar picture was obtained with rabbits. In normal rabbits a slight leucocytosis was produced with small doses, but raising the dosage caused all

TABLE I

EFFECT OF INTRAMUSCULAR CXM ON THE WHITE BLOOD CELL COUNTS OF GOATS

Number of animals	Dosage nitrogen mustard	Therapy dosage	Average white cell counts in thousands/cu. mm. on days						
			Z–1	Z	1	2	3	4	5
8		20 c.c. day Z 10 c.c. days 2 and 3	7.0	7.0	9.6	10.4	10.1	8.7	7.7
4	1 mg./kg. day Z	—	9.9	11.0	10.0	3.0*	3.7	3.7	2.4*
4	1 mg./kg. day Z	20 c.c. day 1 10 c.c. days 3 and 4		11.0	5.4	7.8*	4.6	2.9*	—**

* = one animal dead.

the rabbits to die. With methyl-bis(β-chloroethyl)amine-poisoned rabbits leucopenia was not prevented and the mortality was increased (Table II).

TABLE II

EFFECT OF INTRAMUSCULAR CXM ON THE WHITE BLOOD CELL COUNTS OF RABBITS

Number of animals	Dosage nitrogen mustard	Therapy dosage	Average white cell counts in thousands/cu. mm. on days								
			Z–1	Z	1	2	3	4	5	6	8
4	—	0.5 c.c./kg. day Z 1 c.c./kg. day 1	9.2	9.3	12.3	12.2	9.3*	6.3			
3	—	5.0 c.c./kg. day Z	7.9	7.1	6.9*	6.2*	3.0*				
4	1 mg./kg. day Z	1 c.c./kg. day 1 0.5 c.c./kg. day 2		8.4	6.7	6.2*	1.2	2.4*	2.9	7.4	
5	1 mg./kg. day Z	5 c.c./kg. day 1		7.4	8.1	7.0	*** **				
5	1 mg./kg. day Z	2 c.c./kg. day 1 1 c.c./kg. day 2	7.4	7.5	7.4	6.8	6.5	4.4**	3.1	3.6**	
5	1 mg./kg. day Z	1 c.c./kg. days 1 and 2	6.5	7.0	6.5	6.4	5.8	4.9	3.4	3.4*	3.8
10	1 mg./kg. day Z	—	10.4	11.5	9.3	7.7*	6.3	5.4	4.8	8.3	9.2

* = one animal dead.

(ii) *LPF.*—This was prepared from the inflammatory pleural exudates (produced by injection of turpentine) of rabbits and goats (LPF(R) and LPF(G)). When injected subcutaneously into normal rabbits or goats there was some evidence of the production of a slight leucocytosis, but with methyl-bis(β-chloro-

ethyl)amine hydrochloride-poisoned animals the leucopenia was not prevented and the death-rate was actually increased (Table III).

TABLE III

EFFECT OF LPF ON THE WHITE BLOOD CELL COUNTS OF GOATS AND RABBITS

Number of animals	Dosage nitrogen mustard	Therapy and dosage	Average white cell counts in thousands/cu. mm. on days								
			Z–1	Z	1	2	3	4	5	6	7
(a) RABBITS.											
5	—	100 mg. LPF(R) day Z and 10 mg. day 1	10.5	11.0	11.0	9.6	10.0	10.4			
4	—	100 mg. LPF(G) day Z	7.0	6.8	9.1	11.3	8.9	13.2	14.9	11.6	12.3
5	1 mg./kg. day Z	100 mg. LPF(R) day 1	9.6	9.7	9.6	4.8	4.6*	8.4**	8.2	10.9	10.0
3	1 mg./kg. day Z	100 mg. LPF(R) day 1 and 2		8.1	8.7	5.9	1.2	1.3	5.0**	10.2	
5	1 mg./kg. day Z	100 mg. LPF(G) day 1	9.1	10.2	9.6	8.9	9.2** *	9.8	13.0*	9.8	8.6
4	1 mg./kg. day Z	100 mg. LPF(G) day 1 and 2		7.5	7.0	3.9	1.0*	0.5	1.2	5.3	
10	1 mg./kg. day Z	—	10.4	11.5	9.3	7.7*	6.3	5.4	4.8	8.3	9.2
(b) GOATS.											
2	—	300 mg. LPF(G) day Z and 1	7.3	5.6	12.9	11.5	10.8	9.0	9.8	8.4	
6	1 mg./kg. day Z	300 mg. LPF(G) day 1 and 2	8.1	7.9	14.9	9.9	7.3	5.5**	5.8**	5.4	
4	1 mg./kg. day Z	—	9.9	11.0	10.0	3.0*	3.7	3.7	2.4*	2.5	6.1

* = one animal dead.

(iii) *Sodium succinate.*—Various quantities and concentrations of sodium succinate in aqueous solution were injected subcutaneously into normal rabbits

TABLE IV

EFFECT OF SODIUM SUCCINATE ON THE WHITE BLOOD CELL COUNTS OF NORMAL RABBITS

Number of animals	Therapy dosage	Average white cell counts in thousands/cu. mm. on days								
		Z–1	Z	1	2	3	4	5	6	7
5	0.1 c.c. 1% solution day Z	8.6	8.9	10.9	11.9	12.0	9.3	9.2		
5	0.5 c.c. 1% solution day Z	9.2	9.6	11.9	13.1	12.1	9.5	9.2		
5	1 c.c. 1% solution day Z	9.8	8.1	11.1	11.9	12.0	13.1	9.2		
5	1 c.c. 1% day Z; twice daily, days 1, 2, 3	9.1	9.8	11.8	14.2	20.8	10.8	14.2	15.0	9.8
5	1 c.c. 10% solution day Z		15.5	14.3	12.4	12.2	13.4	8.9	11.9	13.6

and there was definite evidence of the production of a leucocytosis, a 1 g./100 c.c. solution being better than a 10 g./100 c.c. concentration (Table IV). However, the sodium succinate solutions did not prevent the occurrence of leucopenia in methyl-bis(β-chloroethyl)amine hydrochloride-poisoned animals (Table V).

TABLE V

EFFECT OF SODIUM SUCCINATE ON THE WHITE BLOOD CELL COUNTS OF NITROGEN MUSTARD POISONED RABBITS

Number of animals	Dosage nitrogen mustard	Therapy dosage	Average white cell counts in thousands/cu. mm. on days								
			Z–1	Z	1	2	3	4	5	6	7
10	1 mg./kg. day Z	—	10.4	11.5	9.3	7.7*	6.3	5.4	4.8	8.3	9.2
15	1 mg./kg. day Z	1 c.c. 1% day 1	9.6	9.4	11.4	10.2	5.7	6.0*	10.2	10.5	
5	1 mg./kg. day Z	1 c.c. 1% at Z + 6 hours	—	12.0	13.9	10.2	6.9	6.6	12.7	—	—
10	1 mg./kg. day Z	0.5 c.c. 1% day 1	9.6	10.3	10.7	11.5	10.0*	11.3	11.8	12.0	10.6
5	1 mg./kg. day Z	1 c.c. 10% day 1	8.7	9.0	4.5	4.2	5.7	3.3**	—	—	—
10	2 mg./kg. day Z	—	10.5	14.0	12.5*	11.1*	9.2	3.9	11.7**	11.5	15.3
5	2 mg./kg. day Z	1 c.c. 1% days 1, 2, 3 and 4	13.2	13.6	14.9	16.7	6.7	10.4	16.8	12.9	—
10	2 mg./kg. day Z	1 c.c. 1% days 1–6	9.5	12.7	8.9*	6.9	5.1	2.7**	13.4*	11.9	7.9
5	2 mg./kg. day Z	5 c.c. 10% day 1	8.4	10.1	8.8	3.8	0.4	0.3*	3.7		
5	2 mg./kg. day Z	1 c.c. 1% at Z + 6 hours	9.2	8.4	14.5	4.2	0.9*	3.5**			

* = one animal dead.

SUMMARY

When assayed on rabbits or goats poisoned with methyl-bis(β-chloroethyl)-amine hydrochloride (given by subcutaneous injection), the following substances were ineffective in preventing leucopenia:

1. *p*-chloroxylenol in methylacetamide—this preparation, indeed, was found to be toxic in the recommended dosage.
2. The leucocytosis-promoting factor of Menkin.
3. An aqueous solution of sodium succinate.

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