

THE EFFECTS OF INTRAVENOUS INJECTIONS OF DI-
CHLOROETHYLSULFIDE IN RABBITS, WITH SPECIAL
REFERENCE TO ITS LEUCOTOXIC ACTION.*

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PLATE 10.

(Received for publication, September 9, 1919.)

INTRODUCTION.

There exists evidence pointing towards the general toxicity of dichloroethylsulfide both when administered by inhalation and when injected subcutaneously or intravenously.

Lynch¹ found that dogs gassed with high concentrations of dichloroethylsulfide (0.3 mg. per liter for 1 hour) exhibited symptoms similar to those produced by injection, and not referable to the primary irritation of the respiratory tract. These symptoms were salivation, vomiting, bloody diarrhea, hyperexcitability, and convulsions, with a slow irregular pulse which became rapid before death, and were attributed to vagal paralysis. Furthermore, the absorption of dichloroethylsulfide during inhalation was shown by the appearance of the hydrolysis product, dihydroxyethylsulfide, in the urine.

* The experiments were performed at the Experimental Field of the Chemical Warfare Service, American Expeditionary Forces, and were continued by one of us at the Brady Laboratory of Pathology of the Yale Medical School.

We are indebted to Lieutenant Colonel H. C. Clark, Chemical Warfare Service, for advice, to Professor M. C. Winternitz for the privilege of his laboratory, and to Professor F. P. Underhill for procuring samples of nitrobenzene and chlorobenzene.

A preliminary note of this work was presented at the meeting of the Society of Experimental Biology and Medicine, New York, March 19, 1919.

¹Lynch, V., American University Experiment Station, Monograph No. 1, Washington, 2nd edition, 1918, 317. See also Lynch, V., Smith, H. W., and Marshall, E. K., Jr., Dichlorethylsulphide (mustard gas). I. Systemic effects and mechanism of action, *J. Pharmacol. and Exp. Therap.*, 1918-19, xii, 265.

Severe symptoms, ending usually in death within 24 hours, followed the intravenous injection of approximately 0.01 gm. per kilo in dogs. At autopsy intense congestion, often with extensive hemorrhage into the intestinal mucosa, was the only lesion noted.²

Muratet and Fauré-Fremiet³ reported upon blood examinations in a series of six rabbits, four of which were poisoned by inhalation and two by subcutaneous injection. In all the animals the following changes were noted. The red blood cells were augmented in number soon after exposure (increase to 6,000,000 to 10,000,000), and then gradually diminished. Nucleated forms were sometimes seen. There was no polychromatophilia or other degenerative change. The leucocytes showed a rise following the intoxicating dose, but later diminished progressively. There was a relative lymphocytosis. Morphologically, degenerative changes were found in the leucocytes. The polymorphonuclears showed abnormal lobulation of the nucleus, dissolution of the granules, and, finally, a breaking up of the chromatin into spherical masses. The lymphocytes also showed degenerative changes, and many abnormal cells which could not be identified were present in the films. Jolly⁴ repeated the experiments of Muratet and Fauré-Fremiet, using both dogs and rabbits, and administering the dichloroethylsulfide both by inhalation and subcutaneous injection. His results were less consistent than those of the previous workers, but a study of the figures obtained seems to show a rather constant diminution of leucocytes after injection; with inhalation the results were variable.

Zunz⁵ observed in severely gassed human cases a leucopenia with relative lymphocytosis developing after several days.

Stewart,⁶ in a study of the blood changes in six fatal cases of poisoning with mustard gas, found an initial polymorphonuclear leucocytosis, followed by a rapid fall in the total and neutrophil count, beginning on the 3rd or 4th day, and reaching an extremely low figure (200 to 500) before death. The leucopenia is ascribed to acute failure of the leucoblastic function, without associated erythroblastic failure. No histological study of the bone marrow is included in the report.

² Winternitz, M. C., Finney, W. P., and Wislocki, G. B., American University Experiment Station, Monograph No. 1, Washington, 2nd edition, 1918, 389.

³ Muratet, L., and Fauré-Fremiet, Confidential Report of the French Gas Service.

⁴ Jolly, Confidential Report of the French Gas Service.

⁵ Zunz, Report to the Interallied Gas Conference, Paris, 1918.

⁶ Stewart, M. J., Report on cases of poisoning by "mustard gas" (dichloroethylsulfide) with special reference to the histological changes and to alterations in the leucocyte count, Report of the Chemical Warfare Committee, *Great Britain Med. Research Com. Rep., No. 17*, 1918.

Krumbhaar⁷ also reported a series of human mustard gas cases in whom there developed, after the initial leucocytosis, a marked leucopenia, persisting even in the presence of the complicating bronchopneumonia. Moderate anemia, without blast formation, and an apparent decrease in the number of platelets were also observed. Postmortem examination of the bone marrow showed a "greater or less disappearance of normoblasts, myelocytes and adult forms," only "primordial cells and megaloblasts" remaining. Krumbhaar's observations offer convincing evidence of a systemic effect in severe cases of mustard gas inhalation. It is highly improbable that the leucopenia and other blood changes were due to complicating influenzal infections, inasmuch as practically all these gas cases occurred previous to the development of the influenza epidemic.

Hermann,⁸ in his study of the clinical pathology of mustard gas poisoning, finds "no leucopenia at any time," the changes observed being slight secondary anemia with a well marked polymorphonuclear leucocytosis, a definite eosinophilia, and the appearance of myelocytes and young forms of leucocytes. The value of Hermann's observations is lessened by the fact that in none of the eight cases upon which his report of the blood changes is based, was the blood examined before the 10th day after gassing, and that only one of his cases was fatal, and the majority mild in character.

Warthin and Weller⁹ studied the effects of subcutaneous and intravenous injection of dichloroethylsulfide in a series of rabbits and dogs. The toxic symptoms described are salivation, diarrhea, depression of temperature, and increased rapidity of respiration followed by slowing. With large doses animals, after a short period of nervous excitement, die within a few hours in coma with gradual failure of respiration. The gross lesions, apart from the local effect, consist of a general passive congestion of all organs with minute hemorrhages, emboli, and infarctions. The only specific changes are more or less severe catarrhal enteritis with corresponding microscopic findings. A striking microscopic feature is the presence of hemorrhages and pigmented phagocytes in great numbers in the splenic tissue. Intravenous injections (dose of 0.0075 to 0.18 cc.) are followed, after a quiescent period, by general convulsions, opisthotonos, irregular jerking movements, salivation and lacrimation, and fall of temperature. The animal passes into coma, and there is gradual respiratory and cardiac failure. Patho-

⁷ Krumbhaar, E. B., Rôle of the blood and the bone marrow in certain forms of gas poisoning. I. Peripheral blood changes and their significance, *J. Am. Med. Assn.*, 1919, lxxii, 39.

⁸ Hermann, G. R., The clinical pathology of mustard gas (dichlorethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1918-19, iv, 1.

⁹ Warthin, A. S., and Weller, C. V., The general pathology of mustard gas (dichlorethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1919, iv, 265; also in *Researches on the pathology of mustard gas (dichlorethylsulphide) poisoning*, Contributions from the Pathological Laboratory, University of Michigan, 1918-19, ix.

logically, there are found dilatation of the right side of the heart, congestion, petechia, hemorrhages of the lungs, and general congestion of other viscera. We have found no reference in Warthin and Weller's papers as to changes in the circulating leucocytes or bone marrow except the general statement¹⁰ that animals exposed to direct application in the gassing chamber may show, among other effects, "secondary anemia, leucocytosis or leucopenia."

The following report deals with the effects of the intravenous injection of dichloroethylsulfide in rabbits. We have given special attention to the alterations in the blood picture and in the blood-forming organs, since these were the most striking of the results observed.

Technique.

Method of Injection.—Difficulty was at first experienced in obtaining suitable emulsions for injection. In the earlier experiments the given amount of a 10 per cent alcoholic solution by weight of dichloroethylsulfide was suspended in 0.85 per cent salt solution, shaken vigorously, and immediately injected, after allowing the larger globules to settle out. The dose administered was, therefore, considerably less than the total amount taken. Later it was found that a more satisfactory suspension could be made by emulsifying in 30 per cent alcohol in distilled water. A slightly milky emulsion was obtained which after shaking did not separate out in the time necessary for injection. The suspension was prepared from a recently made, accurately weighed 10 per cent solution in absolute alcohol, and immediately injected to avoid hydrolysis. The dichloroethylsulfide used was a distillate from the contents of a German yellow cross shell and was actively vesicant.

Dose.—We have not attempted to determine accurately the minimum lethal dose. It was found in the earlier experiments that the limit of tolerance was in the neighborhood of 0.01 gm. per kilo, and in the last six rabbits used a uniform dose of 0.005 gm. per kilo was chosen.

¹⁰Warthin, A. S., and Weller, C. V., The general pathology of mustard gas (dichloroethylsulphide) poisoning, *J. Lab. and Clin. Med.*, 1919, iv, 266.

Symptoms.

Emaciation and Loss of Weight.—These symptoms were noted in all the rabbits which survived for 2 days or more after the injection. In all but one animal (Rabbit 1, loss of weight only 100 gm.) this was associated with diarrhea, and perhaps was due to it.

Nervous Symptoms.—These were observed only in Rabbits 2 to 4, which died 1 hour, 1½ hours, and during the night following injection. The animals showed extreme restlessness, incoordinate movements, retraction of the head, and transient spasticity, but no definite paralyzes or convulsions.

Respiratory Symptoms.—No definite or characteristic respiratory symptoms were observed even in animals dying a few hours after the injection, in which the occurrence of pulmonary embolism might have been suspected.

Intestinal Disturbances.—Diarrhea occurred in six animals, in all but one associated with the finding of gross lesions of the intestinal tract at autopsy. The feces were copious, fluid, dark brown, and not grossly admixed with blood or mucus.

Edema.—Edema of the ears of wide extent invariably followed injection, even when great care was taken to avoid introducing the material outside the vein. It would appear that the dichloroethylsulfide diffused readily through the vessel wall. It was also observed that after the injection was begun an area of blanching, involving the neighboring skin over a width of several centimeters, at once appeared. This persisted for a few minutes after the injection, after which the normal circulation was restored.

Pathology.

Respiratory Tract.—Of twelve rabbits injected, four showed definite pulmonary lesions (Nos. 2 to 5). All these animals died or were killed within a period of from 1 to 22 hours following the injection, and none of the eight animals surviving over 24 hours showed significant gross or microscopic lesions.

The changes observed were (1) irregular areas of edema, in part fibrinous; (2) areas of atelectasis and emphysema; and (3) accumula-

tions of leucocytes in the capillaries, often showing caryorrhexis and fragmentation, and slight emigration into the alveoli. The trachea and bronchi were normal except that they contained a homogeneous coagulum. No thrombi were found in the capillaries or larger vessels. The small pulmonary arteries were thick walled and appeared contracted. Clear vacuoles were seen beneath the endothelium. The significance of this finding is not clear as somewhat similar pictures may be seen in normal animals.

It is, of course, not possible to conclude from the histological findings that dichloroethylsulfide is eliminated by the pulmonary epithelium; on the other hand, no support is found for the view that the lesions are the result of capillary embolism due to impaction of dichloroethylsulfide globules. The fact that animals which survived for a longer period showed no pulmonary lesions might suggest that the edema is the result of a direct and immediate action of the dichloroethylsulfide upon the pulmonary capillaries.

Alimentary Tract.—No lesions were found in the esophagus. Rabbit 5 showed hemorrhages into the pyloric portion of the stomach and in the duodenum. Of the remaining rabbits, three (Nos. 6, 7, and 8) had a severe diphtheritic enteritis affecting the middle or lower portion of the intestine. Rabbit 6 showed also patches of membranous inflammation in the large intestine. Rabbit 9 also had a diphtheritic colitis, but this proved to be coccidial in origin. Seven rabbits were free from lesions of the intestinal tract.

Liver.—The liver showed no significant changes.

Nervous System.—No detailed study has been made. Neither capillary thrombosis nor hemorrhage was found.

Kidneys.—Definite changes were present only in the kidneys of Rabbits 2 and 5. The capsular spaces contained hyaline globules and occasionally red blood cells. Hyaline material was also present about the blood vessels in the intermediate zone between cortex and pyramids. Blood cells and hemoglobin (?) casts were found in the collecting tubules. The urine was not examined.

Blood and Hematopoietic System. General Considerations.—Since the observations of previous workers upon the blood changes following the administration of dichloroethylsulfide had yielded somewhat conflicting results, it was decided to undertake a detailed study of a

small series of rabbits, eliminating as far as possible incidental factors which might influence the blood picture. Of these incidental factors, variations in the surrounding temperature were found to be the most disturbing. When rabbits were exposed to a temperature of from 40–50°C. for $\frac{1}{2}$ hour, the total leucocytic count showed a tendency to fall, although there were individual exceptions. Counts made shortly after removing the animals from the warm chamber to room temperature (15–20°C.) invariably showed an abrupt and striking rise (Table I). After this point had been established, the rabbits were kept in the laboratory during the observation period and following the injection in order to avoid sudden chilling or abrupt temperature change. Daily counts were made at approximately the same time (9 to 11 a.m.) before food was given. The differential counts are based upon an enumeration of 500 cells, except when the extreme leucopenia made this impracticable. In spite of the usual precautions unexplained variations occurred which made it difficult to draw conclusions as to the percentile fluctuation. In making the counts blood was always taken from the ear not used for injection. When both ears had been used, a small cut was made in the skin of the abdomen and blood taken from one of the superficial abdominal veins. At least two counts were made on each rabbit before injection.

Erythrocytes.—Stress of other work prevented a detailed study of the numerical variation in the erythrocytes. Preliminary observation (Rabbit 9) showed no significant change. There were no striking morphological changes pointing to a marked anemia, except, perhaps, the occurrence of a moderate anisocytosis in the terminal stages. Polychromatophilia is not infrequently seen in films from normal rabbits. Rabbit 13, during the period in which recovery from the effects of an injection was taking place, showed numerous normoblasts—an indication that the erythroblastic tissue had suffered injury, as well as the leucoblastic. Histological study of the bone marrow and spleen affords further evidence of the toxic action of dichloroethylsulfide upon the formation of red blood cells, which will be described.

Leucocytes.—In all but two rabbits (Nos. 8 and 12) of those surviving more than 24 hours, there occurred after a single injection of 0.005

to 0.01 gm. of dichloroethylsulfide per kilo a pronounced fall in the number of circulating leucocytes. In these two rabbits a second injection of the same dose after 7 and 8 days respectively was followed by the typical reaction. The leucopenia was preceded by a transient rise in only one rabbit (No. 7), but it should be noted that no counts were made at short periods following the injection. Although degenerated leucocytes with poorly staining and fragmented nuclei and vacuolated cytoplasm were occasionally found in films, they were infrequent, and even in the presence of an extreme leucopenia the rare leucocytes present in the films were usually normal morphologically. Blood platelets were found at all stages and showed no alterations. As regards the behavior of the different types of leucocytes, a study of these data shows that in some instances the injection is followed by an absolute and percentile increase in the polymorphonuclears, which fall rapidly with the onset of the leucopenia. In the terminal stages the polymorphonuclears practically disappear from the peripheral blood. On the other hand, sometimes an initial fall in the number and percentage of polymorphonuclears is followed by an absolute and relative increase. This secondary rise is associated with the appearance of unripe forms in considerable numbers (Rabbits 12 and 13), and coincides with regenerative activity of the bone marrow, as shown by a study of sections from rabbits killed at this stage. The leucopenia is accompanied by a relative lymphocytosis. The absolute number of lymphocytes is diminished in the later stages and lags behind that of the granular cells in the cases in which regeneration is occurring. There is a percentile increase in the large mononuclear cells, but their absolute number is unchanged or diminished.

The data showing the alterations in the blood count are presented in Table I and in Text-figs. 1 to 3.

Bone Marrow.—Although the appearance of the marrow varies in different animals, there is evidence of the destructive effect of the dichloroethylsulfide upon the blood-forming elements. The variations observed can be correlated with different stages of injury and repair, and these again are reflected more or less closely in the blood picture during life. The following brief descriptions will illustrate the different phases observed.

Rabbit 1 died 4 days after injection. At the time of death the leucocyte count had fallen to 800, of which 46 per cent were polymorphonuclears. The marrow of the femur contains a large amount of adipose tissue, the fat cells being separated by a loose edematous tissue containing less than the normal number of cells. The leucocytes of the granulocyte series are almost without exception degenerated. The cytoplasm in sections stained with Wright's stain (normal control) shows no granules. The nuclei stain diffusely and are frequently fragmented. The megacaryocytes also show degenerative changes. Some contain clumps of pink-staining, hyaline material in their cytoplasm. There are islands of apparently normal erythroblasts. The blood sinuses are wide and intensely congested with normal appearing red blood cells. They contain practically no nucleated elements. The appearances are interpreted as indicating an active injury to the bone marrow. The toxic action seems to have affected especially the granular cells.

Rabbit 8 died 4 days after a second injection of dichloroethylsulfide. Leucocytes on the last 2 days had fallen to 800, a film of the peripheral blood showing very few nucleated cells, which were almost exclusively large and small mononuclears. Sections of the marrow show an extreme aplasia, comparable with that seen in experimental benzene poisoning. Myelocytes, polymorphonuclears, and megacaryocytes have practically disappeared. There are loose collections of normoblasts scattered through the edematous fat tissue. Occasional globular fragments of chromatin, often enclosed in phagocytes, represent the remains of the destroyed cells. The sinuses are congested and contain no leucocytes (Fig. 1).

Rabbit 12 was killed on the 7th day following a second injection. The leucocytes which had fallen to 1,000 on the 3rd day after injection then rose to 1,400 and 2,100, and at the same time large numbers of myelocytes appeared in the peripheral blood. The histological picture shows the effect of a previous injury and at the same time an active regeneration. The predominant cell type is the myelocyte, the granules of which are definite and well stained in Wright's preparations. There are also a fair number of adult polymorphonuclears. The myelocytes are congregated in islands, as are also the erythroblasts. Megacaryocytes are numerous and are not

	3.00 p.m.					4,600	24	430	58	1,050	18	320		
	Sept. 17, 9.00 a.m.					4,900								
	" 18					6,400								
	" 19					1,800								
	" 20, 10.30 a.m.					900								
	3.00 p.m.					400								
4	Sept. 9				5,680,000	70								
	" 10					17,400	56	9,750	39	6,780	5	870		
	" 12, 9.00 a.m.					15,300	11	1,680	70	10,710	19	2,910		
	10.35 "													
	11.30 "					17,500	54	9,450	40	7,000	6	1,050		
	" 13, 9.00 "					8,800	41		53		6			
	10.30 "					24,200	72		22		6			
	" 14, 9.00 "					8,000	43	3,440	50	4,000	7	560		
	10.30 "					13,600	68		25		7			
	11.00 "					20,000	52		42		6			
	" 15, 9.00 "					11,700								
	10.30 "					6,800								
	11.30 "					26,200								
	" 16, 2.00 p.m.					12,400								
	2.30 "					19,200								
						10,400								
						12,300								
						15,400								
						8,200								
6	Sept. 12, 9.00 a.m.					12,000	58	6,960	40	4,800	2	240		
	10.35 "					5,800	70		27		3			
	11.30 "					18,200	61		37		2			

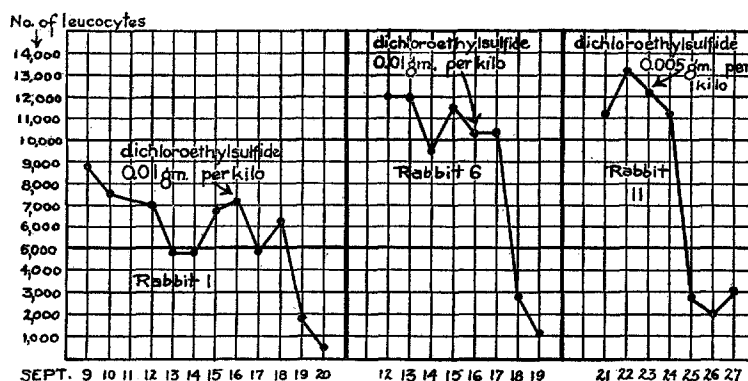
Where the hour is not given the blood counts were made at 9 to 9.30 a.m.

TABLE I—Continued.

Animal No.	Date.	Temperature. °C.	Dose per kilo.	Red blood cells.	Hemoglobin per cent	Total No. of leucocytes.	Polymorpho-nuclears.		Lymphocytes.		Large mono-nuclears.		Other forms.
							Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	Per cent.	No. per c.mm.	
6	1918		gm.										
	Sept. 13, 9.00 a.m.	11				12,000	62	7,440	34	4,080	4	480	
	" 10.30 "	30-40				12,800	55		35		10		
	" 11.30 "	13				13,800	66		31		3		
	" 14, 9.00 "	13				9,400							
	" 10.30 "	50				10,200							
	" 11.30 "	16				21,600							
	" 15, 9.00 "					11,600							
	" 10.30 "					8,400							
	" 11.30 "					14,100							
" 16, 2.00 p.m.			0.01		10,200								
" 2.30 "													
11	3.00 p.m.					8,200							
	Sept. 17, 9.30 a.m.					10,200							
	" 18, 10.30 "					3,100	27	850	59	1,830	14	420	
	" 2.30 p.m.					1,700	7	120	83	1,410	10	170	
	" 19					1,100	10	110	69	760	21	230	
								Died.					
	Sept. 21, a.m.					11,200	35	3,900	59	6,600	6	700	
	" 22, "					13,460	44	5,900	45	6,030	11	1,470	
	" 23, 9.00 a.m.					12,200							
	" 10.00 "			0.005	6,160,000	90							

degenerated. There are many mitoses. The marrow on the whole is less cellular than normal marrow, and new fat cells are in process of formation. There is much hemosiderin pigment, chiefly intracellular, which may be taken as evidence of previous blood destruction.

After the first injection in Rabbit 13 there ensued a fall in the leucocytes from 37,000 to 2,700 on the 4th day. This was apparently followed by an active regeneration. The count rose again to 32,400, followed by a slight drop to 25,400. At this point a second injection was given, which again was followed by a marked leucopenia, the leucocytes falling to 300 per c.mm., at which point the rabbit was

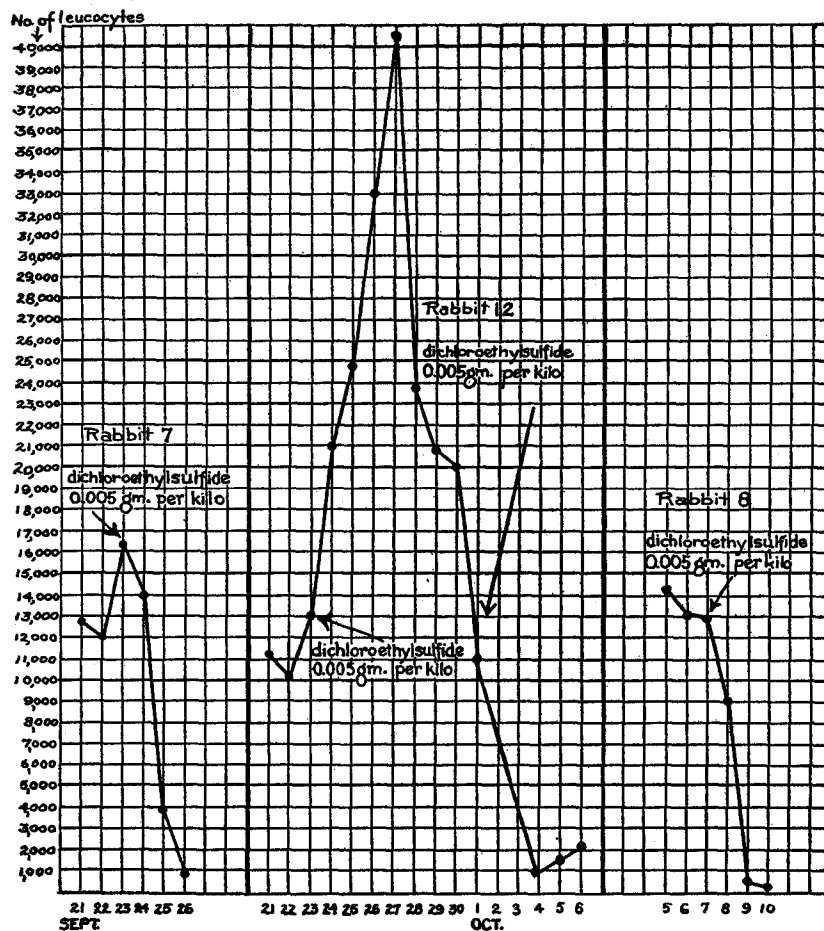


TEXT-FIG. 1. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.¹¹

killed. The marrow histologically shows evidence of an initial injury, followed by repair and a fresh destruction of the regenerating cells. There are large areas of almost complete aplasia, similar to that described in Rabbit 8. But there are also hyperplastic areas, composed of aggregates of large cells with poorly staining nuclei, which, under the high power, are found to be distorted and obviously degenerating. It would seem that these hyperplastic foci have been again injured by the second injection of the toxic substance.

¹¹ The text-figures include only the animals in which blood counts were made during a preliminary period. The fluctuations produced by abrupt change in the surrounding temperature are omitted.

Spleen.—Changes in the follicles—fragmentation of lymphoid cells with phagocytosis of chromatin particles—were seen only in Rabbits 4 and 5. Both these animals died within less than 24 hours after

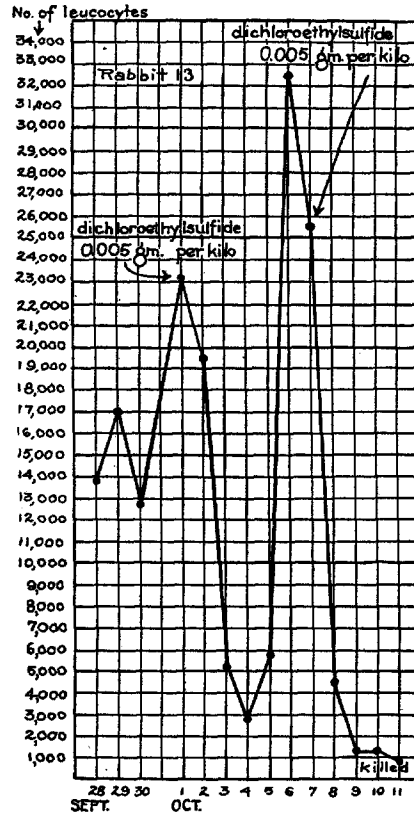


TEXT-FIG. 2. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.

injection. In the remaining rabbits the follicles were normal, or at least showed no signs of either active destruction or excessive proliferation. The sinuses in the majority of the rabbits contained large mononuclear cells laden with blood pigment. The most striking

change, however, was the paucity of free cells in the meshes of the reticulum. The sinuses were separated by strands of cells with pale oval nuclei, evidently belonging to the reticular elements.

Lymphoid Tissue.—The two rabbits (Nos. 4 and 5) which showed acute destruction of the lymphoid cells of the splenic follicles also



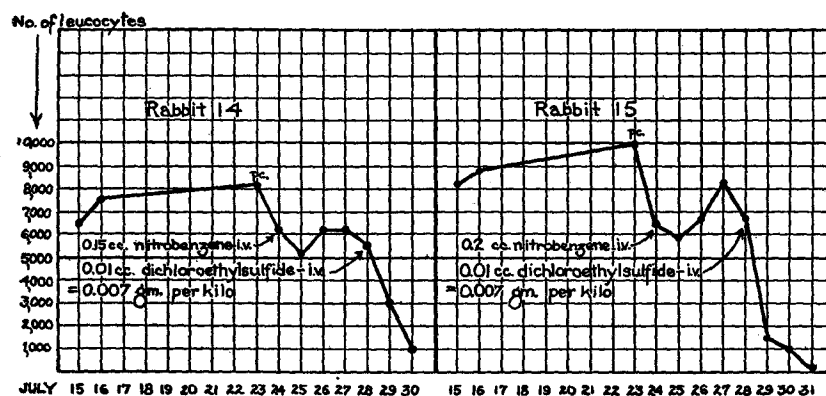
TEXT-FIG. 3. Variations in the total leucocyte count after the intravenous injection of dichloroethylsulfide.

showed cytolysis of the lymphocytes of the thymus and of the intestinal lymphoid tissue.

Because of the great susceptibility of the small thymus cells and of the tissue lymphocytes generally to injurious agents, we are not inclined to regard this as a specific effect of the dichloroethylsulfide,

particularly as it was not present when the destruction of the bone marrow elements was extreme.

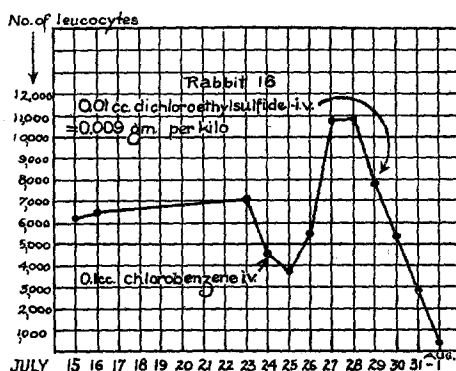
The injury to the blood-forming tissues described bears a close resemblance to the well known action of benzene, with the possible difference that the erythroblastic tissues appear to be less seriously injured than they are with the latter substance. It had been ascertained that the Germans were using nitrobenzene and chlorobenzene as solvents for dichloroethylsulfide in shell fillings, and, indeed, the distillate used by us in our experiments was said to have contained a proportion estimated at roughly from 10 to 20 per cent of these benzene derivatives. Although we were not able to find state-



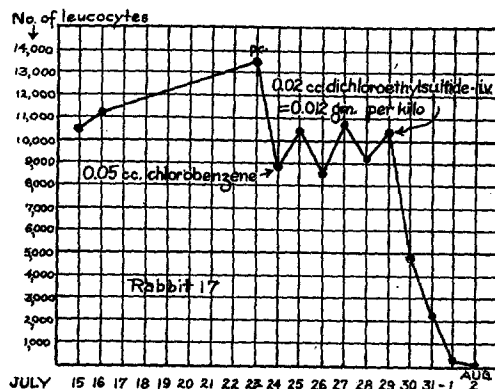
TEXT-FIG. 4. Variations in the total leucocyte count after the intravenous injection of nitrobenzene and dichloroethylsulfide.

ments in the literature as to the leucotoxic action of chlorobenzene and nitrobenzene, doubt naturally arose as to whether the effects which we observed might not have been attributable to the admixture of these solvents, rather than to the dichloroethylsulfide itself, although the minute amounts contained in the doses injected made this rather improbable. The armistice prevented further control experiments at that time. Recently, however, one of us has been able to perform additional experiments on a small series of rabbits, which make it clear that the toxic action upon the leucocytes and bone marrow is due to the dichloroethylsulfide, and not to a possible admixture of chlorobenzene and nitrobenzene.

After a preliminary period of observation two rabbits were injected intravenously with 0.15 and 0.2 cc. of nitrobenzene respectively—doses approximately fifteen and twenty times as large as the total dose of dichloroethylsulfide used in the original experiments. In



TEXT-FIG. 5. Variations in the total leucocyte count following the intravenous injection of chlorobenzene and dichloroethylsulfide.



TEXT-FIG. 6. Variations in the total leucocyte count following the intravenous injection of chlorobenzene and dichloroethylsulfide.

neither of the rabbits did a leucopenia develop. After 4 days, during which time the animals gained in weight and showed no symptoms, they received an intravenous injection of dichloroethylsulfide.¹² In

¹² The sample used was obtained by Dr. Winternitz from the American University, and was stated to be free from nitrobenzene and chlorobenzene.

TABLE II.

Animal No.	Date.	Substance injected.	No. of leucocytes.	Weight.
	<i>1919</i>			<i>gm.</i>
16	July 15, 1.00 p.m.		6,175	
	" 16, 2.10 "		6,600	
	" 23, 1.25 "		7,500	
	" 24, 11.10 a.m.		4,750	1,600
	11.15 "	0.1 cc. of chlorobenzene intravenously.		
	" 25, 10.30 "		3,680	1,650
	" 26, 10.00 "		5,500	1,530
	" 27, 10.00 "		10,250	1,670
	" 28, 10.15 "		10,400	1,540
	" 29, 10.00 "		7,900	1,500
	10.50 "	0.01 cc. of dichloroethylsulfide intravenously (0.009 gm. per kilo).		
	" 30, 10.30 "		4,800	1,470
	" 31		2,850	1,450
	Aug. 1		450	1,430
" 2			Found dead.	
14	July 15, 11.30 a.m.		6,500	
	" 16, 1.30 p.m.		7,400	
	" 23, 1.15 "		8,100	
	" 24, 9.50 a.m.		6,250	
	10.05 "	0.15 cc. of nitrobenzene intravenously (1.5 cc. of 10 per cent solution in absolute alcohol plus 3 cc. of distilled water).		
	" 25, 10.00 "		5,300	1,630
	" 26, 9.30 "		6,200	1,730
	" 27, 9.30 "		6,300	1,790
	" 28, 9.30 "		5,650	1,620
	2.35 p. m.	0.01 cc. of dichloroethylsulfide intravenously (0.007 gm. per kilo).		
" 29, 9.30 a.m.		3,500	1,620	
" 30, 9.30 "		800	1,550	
2.30 p.m.			Killed.	

TABLE II—*Concluded.*

Animal No.	Date.	Substance injected.	No. of leucocytes.	Weight.		
				<i>gm.</i>		
15	1919 July 15, 12.30 p.m.	0.2 cc. of nitrobenzene intravenously.	8,300			
	" 16, 1.45 "		8,850			
	" 23, 1.00 "		10,300			
	" 24, 10.30 a.m. 10.35 "		6,400	1,720		
	" 25, 10.15 "		5,800	1,770		
	" 26, 9.45 "		6,700	1,900		
	" 27, 9.45 "		7,800	1,860		
	" 28, 9.55 "		6,100	1,770		
	" 29, 9.45 "		1,500	1,720		
	" 30, 9.45 "		1,050	1,720		
	" 31, 11.00 "		>200 (counted from heart's blood).	?	Killed.	
	17		" 15, 12.45 p.m.	0.05 cc. of chlorobenzene intravenously.	10,450	
			" 16, 2.45 "		11,300	
" 23		13,900				
" 24, 11.30 a.m. 11.35 "		8,900	2,000			
" 25, 10.45 "		10,400	2,120			
" 26, 10.10 "		8,000	2,270			
" 27, 10.20 "		10,700	2,430			
" 28, 10.30 "		8,700	2,140			
" 29, 10.30 " 11.00 "		10,400	2,040			
" 30, 10.45 "		4,900	1,980			
" 31, 10.00 "		2,400	1,940			
Aug. 1, 10.15 "		300	1,900			
" 2, 10.00 "		>200	1,760		Killed.	

each rabbit the injection was followed by the typical leucopenia (Text-fig. 4, Table II).

Two rabbits also were injected with chlorobenzene, the doses used being 0.1 and 0.05 cc. respectively. One of these rabbits before injection showed a slight leucopenia (4,750 per c.mm.), and on the following day the leucocytes fell to 3,680; they then rose to slightly above normal and remained so. The other rabbit showed no significant fluctuation. 5 days after the first injection each rabbit received an intravenous injection of dichloroethylsulfide, the dose being 0.009 and 0.012 gm. respectively, and this was followed by the typical reaction. The leucocyte counts are shown in Text-figs. 5 and 6 and Table II.

These experiments, though not numerous, are clear-cut, and seem to rule out definitely the possible part played by the benzene derivatives in producing these striking effects upon the blood-forming organs.

CONCLUSIONS.

1. The lethal dose of dichloroethylsulfide (distilled from a German yellow cross shell), when injected intravenously into rabbits, is from 0.005 to 0.01 gm. per kilo.

2. Rabbits dying within 24 hours showed extensive hemorrhages and edema of the lungs.

3. Severe lesions of the intestinal tract were present in about one-third of the rabbits.

4. Dichloroethylsulfide injected intravenously is specifically poisonous for the hematopoietic tissues. Severe lesions are caused in the bone marrow, and the number of circulating leucocytes is markedly diminished. In animals surviving the injection regeneration occurs. The granular cells of the bone marrow seem to be more sensitive than the lymphoid cells and the erythrocytes.

5. The effect upon the blood and hematopoietic tissues is not due to the admixture of nitrobenzene or chlorobenzene in the shell filling. Injection of these substances in animals in amounts many times greater than the total dose of dichloroethylsulfide used produced no changes in the blood picture, and the subsequent injection of dichloroethylsulfide free from these solvents produced a typical reaction.

EXPLANATION OF PLATE 10.

FIG. 1. Rabbit 8. Bone marrow of femur 4 days after the second injection of 0.005 gm. of dichloroethylsulfide. The sinuses (*bl. ves.*) are widely dilated and filled with erythrocytes. They contain practically no nucleated cells. The endothelial cells (*end.*) are preserved. There are scattered megacaryocytes (*mg.*) with darkly stained nuclei. There are also degenerating cells with fragmented chromatin (*pyc.*). Myeloblasts, myelocytes, and adult granulocytes of all types have disappeared, and there are only occasional small mononuclear elements. The space between the sinuses is occupied by adult and immature fat cells (*f.*).

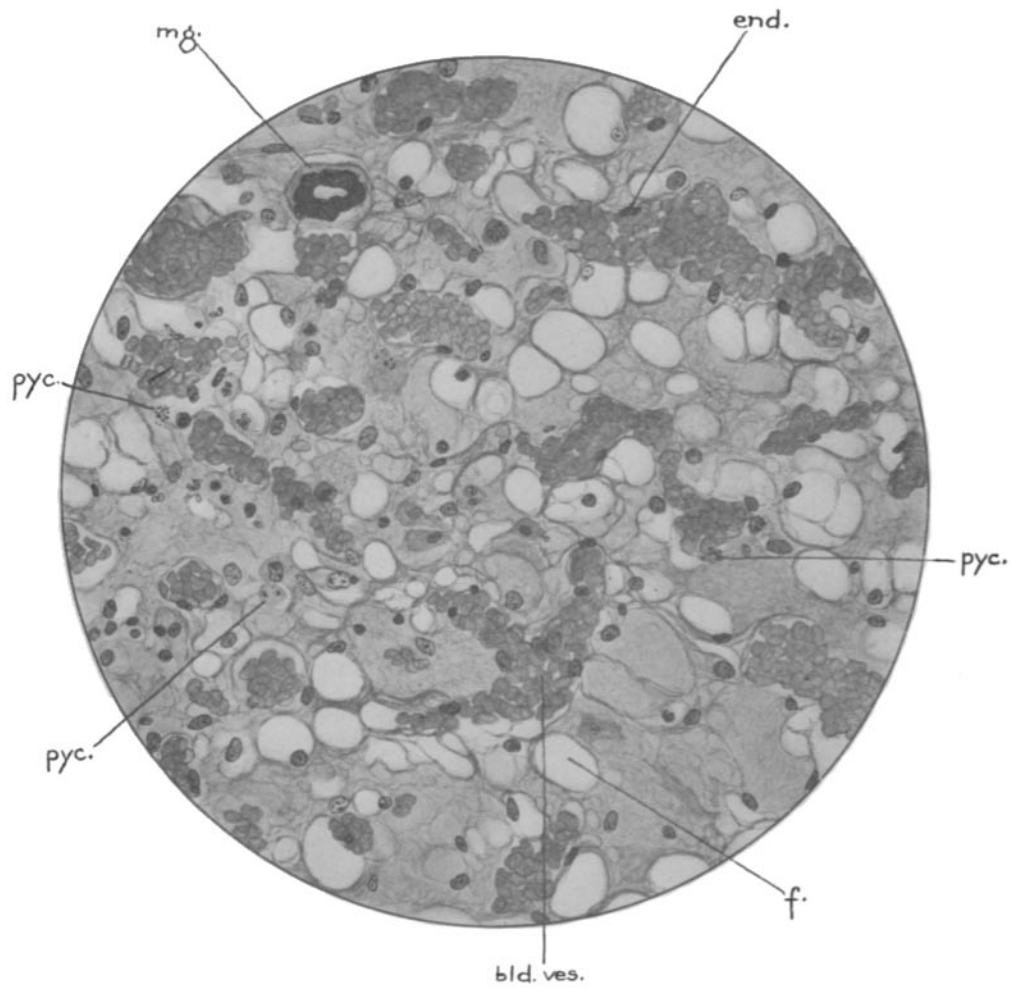


FIG. 1.

(Pappenheimer and Vance: Intravenous injections of dichloroethylsulfide.)