tion of the fungus from liver, spleen, mesenteric nodes, heart blood, as well as from the lungs and bronchial nodes. Detailed description of the gross and microscopic pathology, clinical and laboratory data, including serologic results, will be reported in a separate communication.

Little is known about immunity, or reinfection in histoplasmosis. In these studies it has been demonstrated that although intratracheal inoculation with mycelial phase induced no clinical disease a relative immunity to subsequent challenge to mycelial phase. plus cortisone, was observed. However. challenge with yeast phase did produce a severe disease. Since this latter inoculum was large, similar studies with lesser dosages are indicated to clarify the nature of immunity in histoplasmosis.

Summary. Yeast phase *H. capsulatum* induced acute, progressive, fatal histoplasmosis in all of 9 dogs when inoculated intratracheally, but no clinical disease in all of 7 receiving the same inoculum by stomach tube. Mycelial phase produced no clinical evidence of infection in 9 and 10 normal dogs receiving intragastric and intratracheal inoculations, respectively. Cortisone treated dogs, however, were susceptible to intratracheal but not intragastric mycelial phase inoculations. Dogs previously receiving mycelial phase were resistant to reinfection with the same phase even when given cortisone; they succumbed to challenge with intratracheal yeast phase in the absence of cortisone.

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Transfusion of Separated Leukocytes into Irradiated Dogs with Aplastic Marrows. (20539)

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The consistent finding of bone marrow aplasia and agranulocytic ulcerations in dogs dying from lethal doses of x-ray suggested a causal role of neutropenia in death from radiation injury(1,2). To test this thesis, concentrated suspension of homologous white blood cells were transfused into irradiated leukopenic dogs. The method used was not successful in maintaining a normal level of circulating granulocytes. However, significant elevations of the white blood cell counts in dogs with totally aplastic marrows were obtained. This report describes preliminary results on the functional activity and fate of the transfused granulocytes.

Material and methods. Dogs were exposed to whole body irradiation of 600 r (cobalt 60 source, measured in air by a Victoreen rate meter in 4 π geometry), a uniformly lethal dose which resulted in complete bone marrow aplasia. Donor dogs were given an i.m. or s.c. injection of 0.2 to 0.5 ml of a 1:10 solution of turpentine in alcohol which generally elevated their circulating WBC level. All donor dogs were negative for the canine A hemagglutinogen(3). Donor dogs were bled from the femoral artery into siliconed 230 ml bottles, each containing 40 ml of 5% dextran and 0.5% di-sodium ethylenediamine tetraacetate (EDTA) in Hanks' solution. The dextrans† used were 2 lots (No. 237 and No. 649) that

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† Generously supplied by Commercial Solvents, Terre Haute, Ind.

TABLE I. Transfusion of Separated WBC into Irradiated Dogs.

Dog. No.	Day after x-ray	Leucocytes $\times 10^8$ inj.	Circulating leucocytes $\times 10^3/mm^3$ Before	1 hr after transfusion	4-6 hr after
510	5	102	8	18	
	6	74	6	28	49
	7	47	11	22	41
	8	52	10	22	44
	9	73	12	32	42
	10	77	3	19	
	11		12	16	
	12	46	9	12	
	13	126	3	18	
	14	57	4	8	
	15	59	7	6	4
	16	62	2	6	4
	17	52	1	4	2
	18		0.2	3	
	19	59	1	4	4
	20	69	1	2	3
518	5	94	22	34	52
	6	98	12	8	13
	7	57	2	3	4
	8	159	2	23	35
	9		6		

Martin(4,5) found to interfere but little, if at all, with WBC respiration. A third lot of dextran (No. 546) used from the 15th-20th post-irradiation day in dog No. 510 (Table I) was later found to interfere with WBC respiration. Thirty minutes were allowed for sedimentation of red blood cells. The plasma, which contained 80-90% of the leucocytes and platelets originally present in the whole blood, was siphoned through siliconed glass and polyvinyl tubing into a second set of siliconed bottles. The plasma was centrifuged at room temperature at 30x*g* for 20 minutes or 150x*g* for 30-45 minutes. This resulted in sedimentation of 70-90% of the leucocytes which were resuspended in a small amount of plasma and injected. 760 ml of whole blood were processed for each daily transfusion, yielding 30-40 ml of a suspension of 100000-300000 leucocytes per cu mm, representing 50-80% of the leucocytes originally present in the blood drawn. The cell suspensions prepared by centrifugation at 150x*g* also contained one to 5 million platelets per cu mm. Thirty to 40 ml of supernatant plasma which was poor in WBC's and platelets were injected into irradiated control dogs.

Results. In untreated irradiated dogs, the leucocytes generally fell to levels below 2000/cu mm by the 5th post-irradiation day. The WBC count remained below 1000, and usually below 500 per cu mm from the 7th day on. Four irradiated dogs receiving daily transfusions of plasma poor in leucocytes and platelets showed the same low levels of circulating cells. Four dogs received a total of 30 transfusions of WBC concentrates between the 7th and 20th post-irradiation day. Data on 20 of these transfusions are presented in Table I. The weights of the recipient dogs varied from 10 to 13.6 kg and their estimated blood volume from 800 to 1100 ml. In dog No. 518, weighing 13.6 kg, the maximum expected rise on day 5 was 9000 WBC's per cu mm, and on day 8 it was 14000 leucocytes/cu mm. The circulating cells actually rose by 3000 cells on day 5 and by 3300 cells on day 8, representing 30% and 25%, respectively, of the expected rise. In most of the transfusions, however, much smaller proportions of the transfused leucocytes circulated. Elevation of the recipient's platelet level was similar to that in previously reported experiments when platelet rich suspensions were used(6).

About 80% of the leucocytes in the concentrates were polymorphonuclear cells, and rises in circulating leucocyte levels were primarily due to increases in granulocytes. Even when

transfusions were followed by only small rises in the leucocyte count, there was a clear-cut shift from a predominantly lymphocytic differential blood picture to one with a relatively high percentage of polymorphonuclear cells.

One of the 4 dogs receiving leucocyte transfusion died on the 20th post-irradiation day. The others were killed 24 hours after the last transfusion. Dog No. 510 had developed a folliculitis during the last 10 days of life and polymorphonuclear leucocytes were found infiltrating hair follicles in sections taken at autopsy. Polymorphonuclear infiltration of a small ulcer of the tonsil was present in dog No. 518. The other 2 dogs showed polymorphonuclear infiltration of localized skin lesions. No polymorphonuclear leucocytes were seen in the lung or spleen of these dogs. Granulocytes were present in the sinuses of some of the lymph nodes, although no infectious lesions were noted in the region drained by these nodes. In contrast, none of the 4 dogs receiving WBC-poor plasma showed granulocytes in any organ of the body. Three were killed 24 hours after the last transfusion. One died on the 9th day and showed the granulocytic tonsillar lesion characteristic of dogs dying spontaneously from exposure to lethal doses of x-ray(1).

Discussion. The elevation of the peripheral leucocyte count resulting from transfusion of fresh leucocyte suspension, although variable, appears significant, since similar results have not been obtained previously in the presence of marrow aplasia, except on cross-circulation(7). In unpublished experiments, 50 dogs were exposed to 600 r whole body x-irradiation and died or were killed between the 6th and 23rd post-irradiation day and sections taken of all organs. In none of these were granulocytes seen. The presence of granulocytes in tonsillar ulcers and skin lesions of animals receiving WBC transfusions indicates that the transfused leucocytes were able to migrate to sites of infection. The accumulation of granulocytes in lymph node sinuses in the absence of anatomic evidence of infection in the region drained by these nodes suggests the possibility that lymph nodes may participate in the removal of granulocytes from the circulation in irradiated dogs. However, it cannot be excluded that the leucocytes in the lymph node sinuses represent the response to a minor local infection which may have been overlooked.

Summary. The feasibility of recirculating separated leucocytes has been demonstrated in dogs with complete bone marrow aplasia induced by x-irradiation. The transfused granulocytes were shown to migrate to sites of infection.

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