Discussion. When human oxalated plasma is stored in glass, its prothrombin time becomes prolonged because of the disappearance of an agent which we have designated labile factor and which is probably the same as Factor V of Owren, the Ac-globulin of Seegers, the accelerator by Fantl, and the thromboplastin co-factor of Honorato. On restoring the labile factor by the addition of deprothrombinized rabbit plasma, which has a high concentration of this agent, the prothrombin time becomes 8 instead of 12 seconds, the normal for fresh human plasma. This finding can be interpreted as being due either to an increase in active prothrombin or to the formation of an agent which accelerates the conversion of prothrombin to thrombin as Alexander and his associates maintain(4). If such an accelerator exists, it is not identical with the labile factor, because the addition of the latter to fresh plasma does not increase prothrombin activity.

Aged human plasma with the labile factor restored requires definitely more thromboplastin for optimum prothrombin activity than does fresh plasma. This is independent of the labile factor since an excess added to fresh plasma has no effect. Stored human plasma behaves like fresh dog plasma in respect to thromboplastin requirement. When, however, the prothrombin level of dog plasma is reduced by Dicumarol or vitamin K deficiency, less thromboplastin is needed to obtain the constant minimum prothrombin time. This suggests that the thromboplastin requirement varies with the concentration of prothrombin. If this conclusion is correct, the increase in prothrombin activity in stored human plasma may be explained by an actual increase in active prothrombin. Quick and Stefanini(5) have postulated that the prothrombin in human blood is partly in an active form and partly in a precursor state which they designated prothrombinogen.

Summary. The amount of thromboplastin required for optimum prothrombin activity increases when human plasma is stored and the lost labile factor replaced. It decreases in dog plasma when the prothrombin level is reduced by means of Dicumarol or vitamin K deficiency. It is concluded that a direct relationship exists between the concentration of prothrombin and the amount of thromboplastin, for obtaining a minimal constant prothrombin time.

4. Alexander, B. and Landwehr, G., *Am. J. Physiol.*, 1949, v159, 322.

5. Quick, A. J. and Stefanini, M., *J. Lab. and Clin. Med.*, 1949, v34, 1203.

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Endothelial Damage by X-Rays Disclosed by Lymph Fistula Studies.* (18612)

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Studies of the rate of disappearance of varied substances from the blood of rabbits and mice(1) led to the assumption that capillary permeability is greatly increased in these species by exposure to large (LD-50) doses of X-rays. Tagged plasma, homologous and heterologous erythrocytes, and other tagged large-molecular substances when injected into the blood disappeared from the blood stream of X-rayed rabbits and mice (2 to 14 days after irradiation) faster than from that of normal control animals(1,2). In order to bring direct evidence for heightened capillary permeability and to ascertain its

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1. Wish, L., Furth, J., Sheppard, C. W., and Storey, R. H., *Am. J. Roentg.*, in print.

2. Furth, J., Andrews, G. A., Storey, R. H., and Wish, L., *S.M.J.*, 1951, v44, 85.

magnitude, lymph fistulas were prepared in dogs and rats and the cellular and chemical composition of the lymph was studied. In addition the rate of appearance in the lymph of intravenously injected tagged homologous plasma was assayed in both X-rayed and normal animals as will be described in the report which follows.

Technic. Lymph fistulas were prepared in dogs by a technic similar to that described by Brown and Hardenberch(3). Polyethylene† and polyvinyl‡ tubing was inserted into the thoracic duct at the left supraclavicular region and another into a large tributary of the external jugular vein. The connecting loop was exteriorized. In rats the procedure was that used by Bollman and associates(4). The dogs used were mongrels of both sexes. The rats were Wistar rats weighing approximately 240 to 370 g. By disconnecting the loops all injections and samplings could be done without further surgical intervention. Intravenous injections were made through the venous cannula. Aureomycin was given routinely on 3 consecutive days after operation. In the final phase of the work the cannulas were flushed with heparin but since this is inadequate to prevent clotting in normal dogs and exaggerates the bleeding tendency in irradiated dogs, it was abandoned.

Results. In normal dogs the lymph-venous anastomosis remained serviceable for about 2 to 3 days only, when clotting caused closure of the fistulas. In X-rayed animals the fistulas remained patent for as long as 13 days after operation. Closure of the lymph cannula was the usual cause of the cessation of flow in the anastomosis. The venous fistulas remained open or could be readily reopened. The maintenance of flow through the fistulous anastomosis in X-rayed dogs was due in part to the clotting disturbance caused by irradiation and in part to the increased lymph flow in X-rayed animals.

In normal dogs the rate of flow through the fistula was less than 1 cc per minute, while in several X-rayed dogs it exceeded 1 cc and in one dog reached 5 cc per minute. That increased flow, itself, is evidence of heightened permeability(5) was at first not appreciated by us and therefore exact flow rate determinations were not made except in the clearance studies.

The most striking finding was the presence of blood in the lymph of X-rayed rats (Table I) and dogs (Table II). In the lymph of normal rats the number of erythrocytes is a small fraction of that of lymphocytes. In X-rayed rats the lymph was grossly bloody, and this was noted before insertion of the cannula. In the normal dogs studied, the number of erythrocytes in the lymph was also much below that of lymphocytes. The slightly excessive number of erythrocytes occasionally caused by operative trauma dropped within a day. In the lymph of 6 irradiated dogs studied 3 to 17 days after exposure (two are detailed in Table II) the erythrocyte count was ten- to a hundredfold that of the lymphocyte count. At 2 days after irradiation there was little evidence of passage of erythrocytes into the lymph. Permeability as evidenced by the very high erythrocyte counts seemed to increase with time, reaching a maximum at about 7 to 14 days.

There is evidence that X-rays within the dose tolerated by a mammal do not injure erythrocytes(*cf.* 2,6). Yet, the anemia after massive irradiation is in excess of that caused by death of aging cells in absence of erythrogenesis(*cf.* 2,7). The findings here reported indicate widespread extravasation with flooding of the lymph-compartment by erythrocytes. The well-known finding of erythrophagocytosis and later massive hemosiderosis in lymph sinuses indicates that many of the extravasated erythrocytes are destroyed.

3. Brown, C. S., and Hardenberch, E., *Naval Medical Research Institute Project No.* NM 006 012.04.31, July 18, 1950.

† Purchased from Clay-Adams Company, New York, New York.

‡ Obtained through the courtesy of Irvington Varnish and Insulator Company, Irvington, New Jersey.

4. Bollman, J. L., Cain, J. C., and Grindlay, J. H., *J. Lab. Clin. Med.,* 1948, v33, 1349.

5. Drinker, C. K. and Yoffey, J. M., Lymphatics, Lymph, and Lymphoid Tissue, Harvard Univ. Press, Cambridge, Mass., 1941.

6. Kahn, J. B., and Furth, J., in preparation.

7. Dunlap, C. E., (Warren S.), *Arch. Path.,* 1942, v34, 562.

TABLE I. White and Red Cell Counts in Lymph and Blood of Normal and Irradiated Rats.

Rats	Lymph		Blood	
	W.B.C. × 10^3	R.B.C. × 10^3	W.B.C. × 10^3	R.B.C. × 10^6
		Normal (5)*		
Extremes	5.4-22.5	0-1.5	12.5-21.3	8.0-10.2
Avg	15.0	0.31	14.3	9.0
		X-rayed† (8)*		
Extremes	0.2- 1.5	23.5-1070.0	0.3- 3.2	4.6-10.0
Avg	0.7	523.2	1.0	7.6

* No. of animals in group.

† 6 to 11 days after 650-750 r. The factors of irradiation were 250 Kvp, 30 ma, 3 mm Al, TSD 93.7 cm, 55 r/min.

All irradiation and monitoring was done by John J. Lane.

TABLE II. White and Red Cell Counts in Lymph and Blood of Normal and Irradiated Dogs.

Dogs	Lymph		Blood	
	W.B.C. × 10^3	R.B.C. × 10^3	W.B.C. × 10^3	R.B.C. × 10^6
		Normal* (4)†		
Extremes	26.5-39.7	0.4-7.8	19.4-23.3	5.0-6.6
Avg	32.4	3.2	20.7	5.8
		Dog I		
8 days post 350 r	0.5	125.0	1.0	5.3
10 " " "	1.0	115.0	1.0	3.5
13 " " "	0.4	155.0	0.3	5.1
17 " " "	0.4	240.0	0.4	4.3
		Dog II		
2 days post 350 r	3.8	3.3	4.5	—
6 " " "	4.4	2.1	2.1	—
8 " " "	1.0	134.5	2.0	—
11 " " "	—	998.5	2.0	—
13 " " "	3.0	1060.0	0.3	—

* One or 2 days after establishment of fistula.

† No. of animals in group.

The factors of irradiation were 250 Kvp, 30 ma, 3 mm Al, TSD 11.7 cm. Proximal skin dose 49 r/min. Animal turned over at half-time exposure.

Postmortem examinations confirmed these long-known characteristic radiation changes. Every dog autopsied exhibited a hemorrhagic tendency. Red-colored lymph nodes were a common finding; the thoracic duct of several dogs dissected was red like a vein as were numerous lymphatics leading to lymph nodes.

Summary. The degree of capillary permeability was assayed in dogs and rats by studying the appearance of components of the blood in the lymph. Marked capillary permeability was indicated in dogs and rats exposed to approximately LD-50 doses of X-rays by the appearance of large numbers of erythrocytes in the lymph. The data indicate that the reduced red cell counts after irradiation are due to the flooding of the lymph compartments and tissue spaces by erythrocytes. The massive erythrophagocytosis and hemosiderosis in sinuses of lymph nodes of irradiated hosts indicate that many of the erythrocytes leaving the blood capillaries are destroyed. Loss of erythrocytes caused by capillary damage and death of aging erythrocytes with inhibition of erythrogenesis (*cf.* 2) are the main causes of radiation anemia

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