

Recovery of the Leukocytosis-Promoting Factor in Exudates of Rabbits.*

VALY MENKIN. (With the assistance of Arnold A. Swanson.)

From the Department of Surgical Research, Temple University School of Medicine, Philadelphia, Pa.

Earlier studies have demonstrated the presence of a leukocytosis-promoting factor in inflammatory exudates.¹⁻⁵ This factor, which has been abbreviated as the LPF has been found in canine exudates and human exudates. Canine material has been found to be active in guinea pigs and in human beings, and the factor present in human exudates was found to be active on dogs.

The factor has not been successfully isolated from rabbit exudate although its presence has been demonstrated by the author,⁶ and has been confirmed by Reifstein and his collaborators.⁷ Nevertheless attempts to extract the factor from rabbit exudates resulted in failure. The rabbit is well known to be somewhat atypical as to the type of polymorphonuclear cells it contains in exudates, the type of intracellular enzymes, and the changes in the hydrogen ion concentration of the exudate, with the progress of inflammation. In the present communication observations have been collected which indicate that the factor can be extracted from rabbit exudates. Its effect on the absolute white count is not very striking when a study of normal variations is made on rabbits. However, when one studies the types of leukocytes which are being discharged as a result of

the injection of the LPF, it is clear that the leukocytosis-promoting factor operates primarily in inducing a rise in the percentage of immature leukocytes. It is interesting in this connection that Danzer⁸ showed that the injection in rabbits of various extracts of organs deflects the polymorphonuclear count. The extracts were prepared from normal muscle, liver, brain, and testis by shaking the minced tissue in saline. Ponder and MacLeod⁹ demonstrated that the repeated injections of an irritant into the peritoneal cavity is correlated with a showering into the blood stream of very young polymorphonuclear leukocytes of Class I.

The method employed to extract the LPF from rabbit exudates can be outlined as follows:

1. Inflammatory exudates are recovered from the pleural cavity by injection of 0.2 cc of 5% croton oil in olive oil. A day or 2 later variable amounts of exudates are obtained from such region.

2. Such exudates are immediately treated with benzene in a proportion of 1:1.

3. The mixture of benzene and exudate is stirred under a hood with a stirring apparatus for a period of about 20 minutes.

4. The well-mixed contents are poured into a separatory funnel and held in such a container until good separation occurs.

5. The benzene-free fraction is now treated with ammonium sulfate at half saturation.

6. The precipitate is centrifuged. The supernatant fluid is discarded and the precipitate is dialyzed until free of sulphate ions. It is then injected in variable amounts into the marginal vein of a normal rabbit. Counts

* Aided by a grant from the Agnes Barr Chase Foundation for Surgical Research.

This communication represents Paper No. 36 of a series entitled "Studies on Inflammation."

¹ Menkin, V., *Am. J. Path.*, 1940, **16**, 13.

² Menkin, V., *Arch. Path.*, 1940, **30**, 363.

³ Menkin, V., and Kadish, M. A., *Am. J. Med. Sci.*, 1943, **205**, 363.

⁴ Menkin, V., *Am. J. Path.*, 1943, **19**, 1021.

⁵ Menkin, V., *Arch. Path.*, 1946, **41**, 376.

⁶ Menkin, V., *Dynamics of Inflammation*, Macmillan Co., New York, 1940.

⁷ Reifstein, G. H., Ferguson, J. H., and Weiskotten, H. G., *Am. J. Path.*, 1941, **17**, 233.

⁸ Danzer, M., *Quart. J. Exp. Physiol.*, 1930, **20**, 141.

⁹ Ponder, E., and MacLeod, J., *J. Exp. Med.*, 1938, **67**, 839.

TABLE I.
Effect of Leukocytosis-promoting Factor of Rabbit Exudates on Number of Circulating Leukocytes.

Rabbit No.	Amt of LPF injected, cc	Basal white cell count, per mm ³	Basal No. of immature WBC (one lobe), %	Max. No. of white cells within 6 hr following injection of LPF, per mm ³	Max. No. of immature WBC (one lobe), %
1-53	5	9,325	2	13,950	18
1-73	22	9,600	6	17,500	38
1-59	13	8,875	2	15,400	38
19-77	20	6,850	—	16,300	30
19-73	6	10,975	4	15,400	30
1-78	9	7,600	10	21,600	35
Avg		8,871	4.8	16,692	31.5

TABLE II.
Effect of Saline and Other Inert Material on Number of Circulating Leukocytes in the Rabbit.

Rabbit No.	Amt of material inj. cc	Basal white cell count, per mm ³	Basal No. of immature WBC (one lobe), %	Maximum No. of white cells within 6 hr after injection, per mm ³	Maximum No. of immature WBC (one lobe), %
2-5	5 Saline	8,400	4	14,850	4
19-92	5 "	7,825	—	9,750	—
2-5	5 "	6,350	—	17,400	—
18-24	6 Serum (rabbit)	5,350	0	9,250	2
19-97	15 Saline	6,625	—	11,550	—
1-82	5 Benzene in horse serum	8,100	—	13,650	—
1-88	10 Saline	9,075	4	16,100	14
19-73	20 "	8,225	4	12,150	2
1-61	8 Horse serum	9,325	4	13,400	10
Avg		7,697	3.2	13,122	6.4

are made every hour for several hours and blood smears are likewise taken.

The results of such a series of experiments appear in Table I. It is clear that although the rise in the circulating leukocytes is not excessive, yet the percentage of immature leukocytes is markedly increased as a result of such an injection.

The results obtained when the normal variation in the leukocytes of rabbits is studied indicate that the striking effect of the LPF is primarily on the differential count. The

same fails to hold when various inert materials are injected into the circulating blood of normal rabbits. The results appear in Table II.

Summary. The leukocytosis-promoting factor is present in rabbit exudates, and it has been extracted from such fluids. The extracted LPF has primarily an effect in inducing the discharge of immature leukocytes. The effect on the absolute level of leukocytes is to increase it, but this effect is not pronounced.