nic, Appleton-Century-Crofts, Inc., Publ., New York.
9. Hiatt, C. W., and Schneider, M. D., personal communication.
10. Yager, R. H., Gochenour, W. S., Warner, A. R., Wetmore, P. W., and Hall, H., *Fed. Proc.,* 1951, v10, 424.
11. Collier, W. A., and Mochtar, A., *Med. D.V.G.,* 1939, v28, 356.
12. Gsell, O., *Rev. Med. de la Suisse R.,* 1949, v69, 613.

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Presence of a Leukotaxine-like Substance in Extract of Severely Injured Muscles. (19577)

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Recently(1) the writer expressed the view that leukotaxine, the substance primarily responsible for the increased capillary permeability and the migration of leukocytes in inflammation, is formed whenever cells are severely injured; consequently one cannot really speak any longer of "normal tissue" as is done by Moon and Tershakovec(2,3) when one macerates tissues and prepares an extract from them. One would expect to recover either leukotaxine or a substance closely resembling leukotaxine in such extracts of severely injured cells. In 1937, (unpublished data) the writer has obtained such a leukotaxine-like substance from beef heart extract. The reason for not continuing and publishing these studies was neither to go on a tangent nor to reason by analogy, but only to center on his main theme which was inflammation and its natural product, namely the exudate. As stated in his discussion(1) leukotaxine and its polypeptide nature, has been frequently confirmed.

In the present communication, observations are reported on the presence of a leukotaxine-like substance in the saline extracts obtained from muscles of rabbits as a result of grinding such tissues in a mortar. Such injured cells yield essentially the same factors as obtained from inflammatory exudates(4,5).

Method. Rabbits were killed by injecting air intravenously. The muscles of the thigh were immediately removed and weighed. The tissue was cut up in small fragments and treated with physiological saline. The amount of saline utilized was approximately equal to a volume in cubic centimeters of twice the weight of the muscle tissue as measured in grams. Some of this whole saline extract was tested on the skin of normal rabbits by the intracutaneous injection of 0.5 cc of the material on the abdominal surface. Five to 6 cc of 1% trypan blue in saline was then injected intravenously. Readings on the accumulation of the dye in the treated area were recorded periodically for about 1 to 2 hours. The rest of the saline preparation was extracted for the presence of a leukotaxine-like substance by only slightly modifying the method described previously for the extraction of leukotaxine from inflammatory exudates (6). The scheme of extraction utilized may be stated as follows: the saline extract of the thigh muscles was treated with dioxan (1:1) and stirred for about two hours. The mixture was then centrifuged and the supernatant phase treated with an equal volume of acetone. A precipitate resulted. This was centrifuged off and the supernatant evaporated to dryness *in vacuo* at $\pm 50°C$. To the dried material an amount of butyl alcohol equal to ½ the volume of the acetone supernatant-treated fraction was added, and the mixture was stirred for about one hour. This was then concentrated *in vacuo* at $\pm 55°C$ to about 1/10th volume. At that point the butyl alcohol concentrate was divided into two parts. One part contained a granular-like precipitate

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TABLE I. Effect of a Leukotaxine-Like Substance from Muscle Extract on Capillary Permeability and on the Migration of Leukocytes.

Rabbit No.	Leukotaxine-like material inj. in skin	ACTH intrav., mg	Duration of inflammation, hr:min	Accumulation of trypan blue in the treated areas (permeability test)	Diapedesis of leukocytes in treated area (chemotactic response)
43–57	C		0:44	2+ to 3+	+ to 2+
–81	C–D		0:57	3+	+
–70	C		1:28	4+	2+
–48	C		1:30	4+	2+
–83	C	fraction	2:10	3+	3+
–80	C–D	20	0:57	4+	
–80	Same	20	1:07	4+	0
–69	C	30	1:25	2+*	
–82	C	20	2:09	3+	Faint trace
–97			1:04	3+	2+
–98			1:15	+ to 2+	3+
–96			1:29	Trace	2+
–94	Whole saline extr. of muscle		2:24	+	3+
–95		15	1:26	0	0
–93		15	2:25	0	3+

* At periphery.

which was centrifuged and the resulting precipitate dried in a desiccator. This was the so-called C-fraction. The other part of the butyl alcohol concentrate was evaporated to dryness, yielding a viscous and perhaps slightly tarry material. This was the so-called C-D-fraction.

These two fractions were suspended in 0.5 cc of saline and injected intracutaneously into the skin of the abdomen of rabbits. This was followed, as stated above, by the intravenous injection of trypan blue and readings on the accumulation of dye were accordingly made. At the termination of the observations, the skin areas were excised and fixed in 10% formaldehyde for subsequent microscopic examination.

Results and discussions. The results obtained are assembled in Table I. It is clear that the C-fraction or C-D-fraction of leukotaxine-like material, obtained in a manner similar to leukotaxine from exudative material, induce both an increase in capillary permeability and the migration of leukocytes into the treated skin area (Table I, Fig. 1.) Furthermore, it has been shown that ACTH does not suppress the increase in capillary permeability to trypan blue caused by leukotaxine(7), while at times it inhibits the diapedesis of leukocytes especially when the leukotaxine preparation is not too potent(7). The same finding has been observed with the leukotaxine-like fractions from muscle tissue extract following severe injury. This would support the contention that the fractions obtained are essentially not different from leukotaxine, as obtained from inflammatory exudates. Finally, these fractions likewise fail to contract the isolated segment of guinea pig intestine. This is precisely similar to the response of the intestine to leukotaxine from exudates(6). The only point of difference is the negative Biuret and ninhydrin tests which are found, however, to be positive in the case of leukotaxine(6). The amino nitrogen before and after hydrolysis suggests that perhaps one is dealing with a dipeptide. The nature of the prosthetic group, if present, is probably different in various tissues and from that in the leukotaxine of inflammatory exudates. The total nitrogen by Pregl's micro-Kjeldahl method is found in six determinations to average 5.52%. The whole saline extract tends to induce likewise an increase in capillary permeability and local migration of leukocytes. When ACTH is added, however, both the increase in capillary permeability and the migration of leukocytes in one instance seemed to be

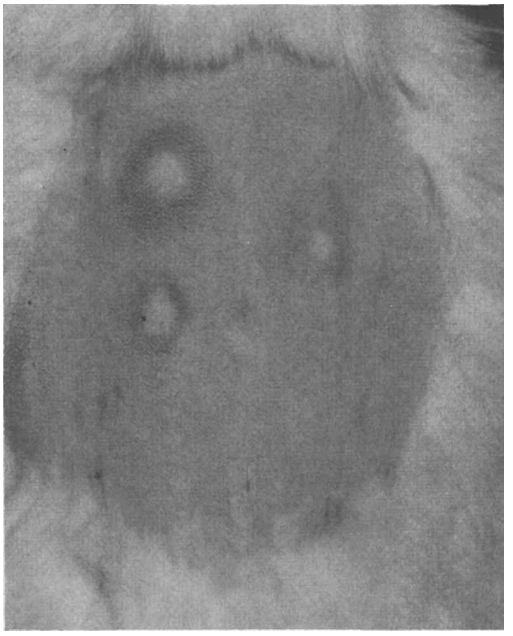

FIG. 1. Rabbit 4380. 20 mg of ACTH in 2 cc of saline inj. intrav. In upper left area .5 cc of the C–D–fraction from muscle saline extract inj. intracut. In lower left area .4 cc of the C–fraction has been inj. On the right side .4 cc of the C–D–fraction has been introduced, while physiological saline has been inj. intracut. in lower right side. 6 cc of 1% trypan blue in saline was then intrav. inj. About 40 min later present photograph taken. Note that intrav. inj. of ACTH failed to suppress increase in capillary permeability to trypan blue caused by the leukotaxine-like fractions C–D and C of muscle extract.

suppressed (Table I). The reason for this is not clear. It is possible that in the whole extract there is also an abundance of exudin which is in turn suppressed by ACTH(7); *i.e.*, as far as increased capillary permeability is concerned.

Moon in reply to the writer's comments(1) failed to answer the fundamental criticism, namely: do normal tissues contain leukotaxine? The writer carefully pointed out(1) that only injured cells, such as found in inflammation or by macerating tissues have their biochemistry so altered as to produce various common denominators, such as leukotaxine, which in turn are capable of accounting for some of the biological aspects of the very complex problem of inflammation. This does not mean that the writer believes that leukotaxine is present in all normal tissues, but rather it is his belief that it can only be formed during injury of cells. The present paper supplies further factual information toward the problem. Tershakovec and Moon (8) simply showed that numerous substances as well as protein split products increase capillary permeability and induce leukocytic migration. In the discussion, Moon questioned whether the present writer would infer that leukotaxine is present in all these substances. The answer is quite clear,(6,9). All these substances are essentially inflammatory irritants which injure the cells of the host. These cells, in turn, have their biochemistry altered by injury so that leukotaxine is formed which accounts for the standard reaction of local increased capillary permeability and leukocytic migration. It is also conceivable that perhaps some of the protein split products utilized contain, in addition, some leukotaxine-like material (10, 2nd reference), which thus accentuates the full permeability and chemotactic effect, but this possibility is not essential in order to elicit some degree of increased capillary permeability and chemotactic response by directly injuring the cells of the host.

As pointed out by the writer(10) when discussing the two factors in leukotaxine: "The analytical procedure has thus failed to dissociate the two factors. Although this type of evidence is strongly suggestive of a single substance, it is, however, to be borne in mind that only the certitude of complete chemical purity of the material can establish this as a definite fact." Thus far, although there may be two separate substances in leukotaxine, such a dissociation in exudates has not been accomplished. Spector has recently succeeded to do this in the digest of fibrin; but he has not been able to report any such dissociation in inflammatory exudates(11).

Finally, in regard to the criticism concerning the multiplicity of factors(1), this point has been discussed elsewhere(9,12). It simply reflects the complexity of the problem of inflammation. The analytical procedure employed has yielded in inflammatory exudates separate specific factors which are responsible for only specific biological processes in in-

flammation. These factors have been separated chemically and identified biologically. The future will yield, as in most biological entities, greater chemical accuracy in their identification. Thus far, pyrexin and the thermostable leukocytosis factor have been brought to the state of ready crystallization.† Many of the other factors have been identified as polypeptides. Their chemical structures, however, have yet to be identified by chemists.

Conclusions. Severely injuring muscle tissue of a rabbit by mechanical means induces the formation of a leukotaxine-like substance which has essentially the biological attributes of leukotaxine as obtained from inflammatory exudates. The fractions of leukotaxine-like material induce an increase in capillary permeability and cause leukocytic migration. The isolated guinea pig intestine fails to contract when treated with these fractions, while histamine added subsequently contracts the segment. This would indicate that the leukotaxine-like fractions obtained do not appear to be histamine.

† Studies now in progress indicate the possibility that the thermostable leukocytosis factor may be adsorbed on a non-specific crystalline structure, but this will be taken up *in extenso* in a subsequent communication.

1. Menkin, V., *Science,* 1952, v115, 382.
2. Moon, V. H., and Tershakovec, Geo. A., *Arch. Path.,* 1951, v52, 369.
3. ———, *Arch. Path.,* 1951, v52, 441.
4. Menkin, V., *Arch. Path.,* 1937, v24, 65.
5. ———, PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 350.
6. ———, *Dynamics of Inflammation,* Macmillan Co., New York, 1940.
7. ———, *Am. J. Physiol.,* 1951, v166, 518.
8. Tershakovec, Geo. A., and Moon, V. H., Reported before the Am. Assn. Path. and Bact., New York, April 10, 1952; *Am. J. Path.,* 1952, v28, 522.
9. Menkin, V., *Newer Concepts of Inflammation,* Springfield, Ill., C. Thomas, 1950.
10. ———, *J. Exp. Med.,* 1938, v67, 145; 1938, v67, 153.
11. Spector, W. G., *J. Path. and Bact.,* 1951, v63, 93.
12. Menkin, V., *Nature,* 1948, v163, 327.

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Efficacy of 1-Ethanesulfonyl-4-Ethyl-Piperazine Hydrochloride in Treatment of Controlled Hemorrhagic Shock.* (19578)

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Acute hemorrhage is of frequent occurrence from industrial and highway accidents and battle casualties. While its treatment has been facilitated greatly by the ready availability of supplies of plasma and whole blood, the time required to obtain them and for the proper matching of blood still may cause such a delay that successful reversal of the pathologic state is not produced quickly enough and death ensues. The development of a drug which would be satisfactory in delaying the onset of an irreversible state consequently is highly desirable. In preliminary studies, Bovet *et al.* reported that 1-ethanesulfonyl-4-ethyl-piperazine hydrochloride (R.P. 3885) apparently is successful in protecting rats from traumatic shock produced by the Noble-Collip drum(1). Later, these workers indicated that this compound was successful in increasing the survival time of dogs subjected to "traumatic-hemorrhagic"

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‡ The drug was synthesized and generously supplied to us as a pure crystalline compound by Dr. Thomas P. Carney, The Eli Lilly Co., and in a 25% solution, which was slightly amber in color, by Dr. Jacob W. Stutzman, of Smith, Kline & French, Inc.