

## Studies on Leukotaxine and Leukocytosis-Promoting Substances

By

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Recently many investigators came to take a serious view of Menkin's factors and Duran Reynal's spreading factor produced in an inflammatory area. The authors have also studied on Menkin's factors, especially the actions, properties and the essential character of leukotaxine. Studies have been made, on the other hand, on the relationship between Menkin's leukocytosis-promoting factor (L.P.F.) and Muto's neutrophin or Komiya's neutropoetin.

Menkin published his studies on leukotaxine in 1937, and on a leukocytosis-promoting factor several years later.

In Japan, however, Muto and Komiya made neutrophin and neutropoetin known in 1935 and 1936, respectively. But both of them have studied without having any connection with each other.

### I. On Leukotaxine.

1) *Method* Menkin's method was adopted, together with the following improvements:

- a) Leukocytic migration was observed by supravital staining of extended subcutaneous tissues of rabbits (Amano's method).
- b) The essential character of leukotaxine was shown by paper chromatography.

2) *Actions of Leukotaxine* Observations of increased capillary permeability and leukocytic migration were made by means of subcutaneous injections of exudates taken from rabbits, which had been brought about through turpentine inoculations in the pleural cavities of the animals, moreover, the observations of the same kind were also carried out on leukotaxine. The results obtained were the same with those by Menkin. Such actions are not recognizable on healthy human serum, ascites, distilled water, saline and histamine. The data presented by Menkin, therefore, can be admitted. Lewis (1927) accentuated considerable importance to histamine for increasing capillary

**Table 1.** *Comparison Between the Leukotaxine-Action through Exudate, Saline, Serum of Healthy Human Beings, Turpentine and Histamine*

Time	5'		20'		40'	
	Accumulation of Trypan Blue in Skin of Rabbit	Leukocytic Migration	Accumulation of Trypan Blue	Leukocytic Migration	Accumulation of Trypan Blue	Leukocytic Migration
Exudate	±	— —	+	### +	++	## ++
Saline	—	— —	—	— —	—	— —
Serum of healthy human beings	—	— —	—	+ —	+	++ —
Turpentine	+	— —	++	— —	++	— —
Histamine	±	— —	±	— —	±	— —

permeability, while, after the results obtained by the authors, the actions of histamine were weaker than those of leukotaxine. The Hanke Koessler test of leukotaxine is negative. Hence it follows that the identity of leukotaxine does not appear to be affirmed. Turpentine is very interesting, because, in spite of an increased capillary permeability, it does not induce any leukocytic migration. According to Menkin, the action of aleuronat is also just the same with those of turpentine. Due to these reasons the action of leukotaxine does not depend on the strength of stimuli, since leukotaxine is quite different from turpentine or aleuronat. Referring to the action of leukotaxine, Menkin mentioned that leukocytic migration is induced after increased capillary permeability in the treated cutaneous areas of rabbits. The authors also reached the same results.

Existing records throw light on the fact that there are several substances, which, without any leukocytic migration, may induce capillary permeability, while converses do not exist. Accordingly one of the factors of leukocytic migration is ascribed presumably to increased capillary permeability at least.

Menkin pointed out the branched areas of a small vein as areas of increased capillary permeability and leukocytic migration. Recently Amano and Yasuhira also insisted on this point strongly.

Menkin declares he cannot recognize leukocytic migration within 60 minutes in a treated area of rabbits through serum of the same animal and saline, whereas the authors recognize it to a certain extent. This may, of course, be caused from the differences of individuals, but the authors want to give prominence to the fact that their method and that of Menkin's are strikingly different. Their method can afford an easy observation of leukocytic migration, and the technique for it is also simple. Especially this method is serviceable for the purpose of observing the process in the course of time.

3) *The Properties and the Essential Character of Leukotaxine* Menkin's method was adopted for the extraction of leukotaxine. The properties of leukotaxine extracted by the authors are almost the same with those shown in Menkin's results, too. The color of leukotaxine is slightly yellow

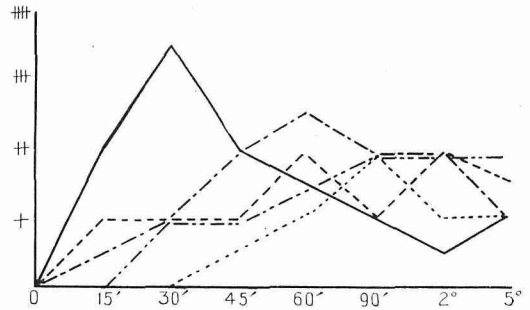
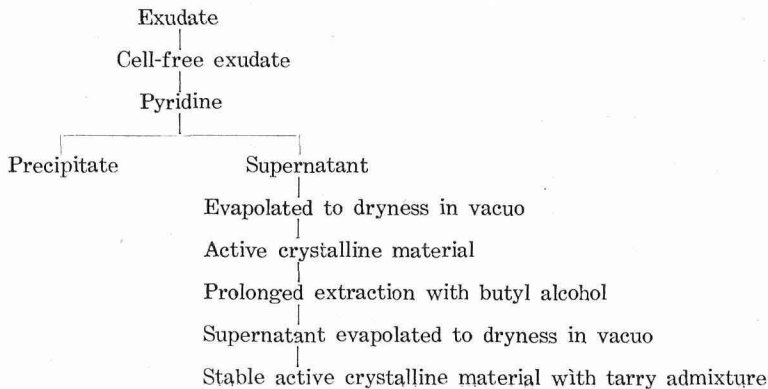


Fig. 1 Leukocytic migration through  
 exudates of rabbits ———  
 rabbit serum .....  
 human serum - - - -  
 human ascites - · - ·  
 saline - - - - -

Table 2 Scheme of Extraction of Leukotaxine



brownish, and its appearance is of hygroscopic crystalline. It is readily soluble in acid, obtaining soluble in water. They carried out several tests concerning proteins and amino saline, and less the following results:

The biuret, the Millon, and the Pb.S. tests are negative, but the ninhydrine, the xantho-proteic and the Adamkiewicz tests are positive. Because of the precipitation of leukotaxine with ammonium sulfate, Menkin supposed that leukotaxine was a relatively simple intermediary product of protein catabolism and stated, "It is interesting to note in this connection that, of dialyzable proteolytic breakdown products, polypeptides seem to be the only ones that are precipitated by concentrated ammonium sulfate." But the authors cannot always recognize these data. Because, according to Menkin, the melting point of leukotaxine fails to show a sharp point, and the authors discovered the positiveness of the Sakaguchi test, besides that of the xantho-proteic and the Adamkiewicz reaction. Accordingly, if leukotaxine is regarded as a kind of polypeptides, it would also be a tripeptide at least. So the authors came to doubt Menkin's maintenance that leukotaxine is a relatively simple intermediary product of protein catabolism.

**Table 3** *Properties of Leukotaxine, Extract of Rabbit Serum Injected with Typhoid Vaccin by Menkin's Method, Some Kind of the Serum of Human Beings, L.P.F. and Rabbit Serum Containing Neutrophilin.*

Properties	Leukotaxine	E.R.S. i.w. T.V.*	S.K.o.t.S.o.H.B.**	L.P.F.	R.S.C.N***
Effect of heat	Thermostable	Thermostable	Thermolabile	Thermolabile	Thermolabile
Diffusibility	Dialyzable	Dialyzable	Dialyzable	Non dialyzable	Non dialyzable
Effect of acid and alkali	Resistant	Resistant	Not resistant		
Ninhydrine test	+	+			
Xantho-proteic t.	+	+			
Adamkiewicz t.	+	+			
Sakaguchi t.	+	+			
Biuret t.	-	-			
Millon t.	-	-			
Pb. S. t.	-	-			
Hanke Koessler t.	-	-			

\* E.R.S. i.w. T.V. .... Extract of rabbit serum injected with typhoid vaccine by Menkin's method.

\*\* S.K.o.t.S.o.H.B. .... Some kind of the serum of human beings.

\*\*\* R.S.C.N. .... Rabbit serum containing neutrophilin.

Then paper chromatography of leukotaxine was employed by the authors, and then, they discovered phenylalanine, tryptophane, arginine and lysine in the material. They understood thereupon that those chemical reactions on leukotaxine depending on these several amino acids according to these data. After injecting these amino acids compounds in subcutaneous area of rabbits, the authors observed increased capillary permeability and leukocytic migration, but they did not succeed to recognize a leukotaxine reaction so far as through such level of amino acid contained in leukotaxine. So they attempted paper chromatogram (solvent butanol) again after the hydrolysis of leukotaxine, and obtained amino acid-like substances at Rf. 0.12, 0.10.

The activity of hydrolyted leukotaxine is weaker than that of leukotaxine itself. Therefore the authors suppose that the essential character of leukotaxine will be a previous substance to amino acid which is stained at Rf. 0.12, 0.10 through ninhydrine on paper chromatogram. It will be

Table 4 *Rf. of Leukotaxine and Several Amino acids*

Rabbits No.	1		2		3		4			5		
	L	C	L	C	L	C	L	H.L	C	L	H.L	C
Penylalanine	0.31	0.31	0.33	0.32	0.32	0.31	0.33	0.33	0.33	0.30	0.30	0.31
Tryptophane	0.18	0.18	0.21	0.21	0.20	0.22	0.19	0.25	0.21	0.21	0.22	0.20
Arginine	0.02	0.01	0.02	0.02	0.01	0.01	0.04	0.04	0.03	0.03	0.06	0.02
Lysine	0.01	0.01	0	0.01	0	0.01	0.02	0.02	0.01	0.01	0.02	0.01
								0.11 0.10			0.11 0.09	

Rabbits No.	6			7			Average		
	L	H.L	C	L	H.L	C	L	H.L	C
Penylalanine	0.30	0.38	0.32	0.33	0.33	0.32	0.31	0.34	0.31
Tryptophane	0.20	0.27	0.20	0.21	0.22	0.20	0.19	0.24	0.19
Arginine	0.03	0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.02
Lysine	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.01
		0.11 0.08			0.13 0.11			0.12 0.10	

L ... Leukotaxine H.L ... Hydrolyted leukotaxine C ... Control

difficult to determine this material only by paper chromatography, but, if a conjecture is allowed, the above mentioned previous substance will be one of the peptids. The authors therefore want to designate it as leukotaxine containing several amino acids "Crude leukotaxine", and "pure leukotaxine", of which the latter is the previous substance to amino acid at Rf. 0.12, 0.10 with paper chromatograms after hydrolysis. The extract of the latter will be a future problem.

Table 5 *Comparison Between the Action of Leukotaxine and Hydrolyted Leukotaxine (by trypan blue accumulation and leukocytic migration in and out of small veins)*

Rabbit No.	Time	Leukotaxine	Hydrolyted Leukotaxine	Distilled water
132	5'	—	—	—
	10'	±	—	—
	20'	+	+	—
	40'	++	++	—
133	5'	—	—	—
	10'	+	—	—
	20'	++	—	—
	40'	++	—	—
134	20'	migration out ++ in ++	out ++ in +	out — in —
	40'	+++ ++	++ ++	— —
135	20'	++ ++	+ —	— —
	40'	+++ +++	++ ±	— —

We discovered a leukotaxine-action in acute inflammatory serum of human beings and in rabbit serum injected with typhoid vaccine. According to Kayukawa the leukotaxine-action of human serum in tuberculous pleurisy is not so strong. So the existence of the leukotaxine-action suggests the degree of a disease.

Some kind of healthy human serum shows a leukotaxine-action, but this does not depend on leukotaxine, because this material is not thermostable and destroyed both through acid and alkali.

## II. Leukocytosis-Promoting Substances.

Muto (1935) and Komiya (1936) injected typhoid vaccine intravenously into rabbits and indicated that typhoid vaccine induced leukocytosis in the circulating blood of rabbits and this serum contained also a leukocytosis-promoting substance. Muto called therefore this substance "neutrophilin" and Komiya "neutropoetin." But its essential character, however, is not sufficiently known.

The authors have discovered, on the other hand, leukocytosis in the circulating blood of rabbits by injecting intravenously human serum suffering from acute appendicitis, peritonitis, and pneumonia. In the meantime they obtained rabbit serum containing neutrophin through intravenous injection of typhoid vaccine. They induced also leukocytosis in rabbits by injecting this serum cutaneously 1.0 cc per kg. 30 minutes after this injecting serious leukopenia was observed and 6 hours after that leukocytosis appeared suddenly in blood. On the other hand, L.P.F. was confirmed by Ishida, one of the author's university. The L.P.F. is thermolabile; rabbit serum containing neutrophilin is, according to the authors of the present article, also thermolabile. According to Menkin, a leukocytosis-promoting action through acute inflammatory serum which was taken from human bodies is due to L.P.F. Because acute inflammatory exudate contains such a leukocytosis-promoting substance, it is quite natural that a leukocytosis-promoting substance is produced from an inflammatory

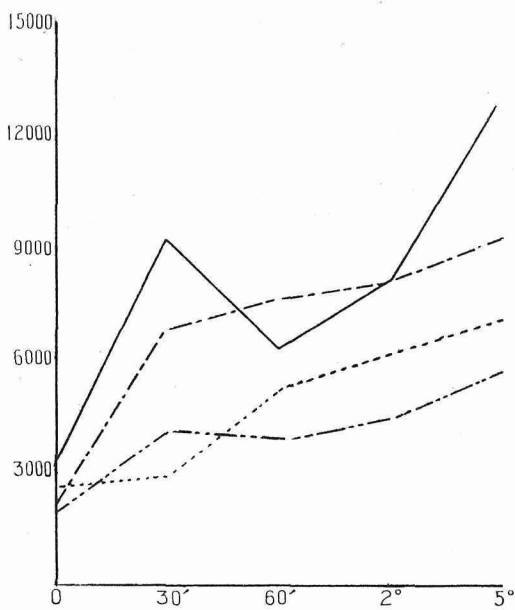


Fig. 2 Leukocytosis through

acute inflammatory serum of human beings ———  
 neutrophilin containing rabbit serum - - - - -  
 rabbits serum .....  
 human serum .....

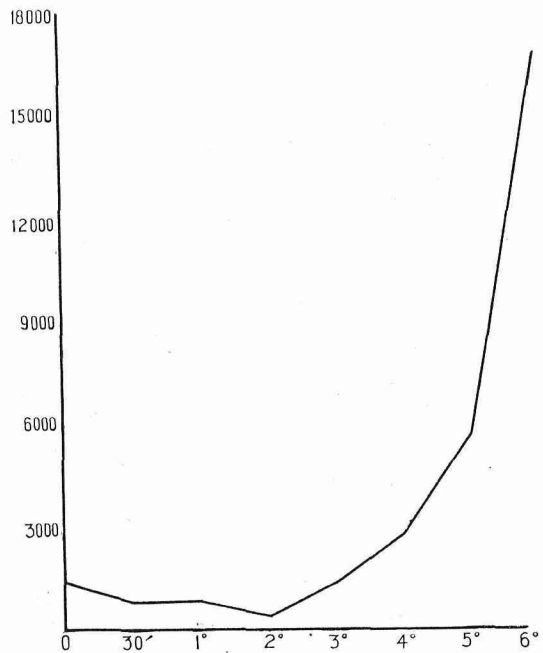


Fig. 3 Leukopenia and leukocytosis by intravenous injection of typhoid vaccine into rabbits

area induced through intravenous injection of typhoid vaccine. Therefore L.P.F. and neutrophilin will be the same substance. This was proved by Wada of the authors' laboratory in his study on the eosinophilic leukocytosis-promoting factor (E.P.F.). According to his study, notwithstanding the cause any serum of patients suffering from eosinophilic leukocytosis contain E.P.F., and its properties are very similar to L.P.F. The distinction between L.P.F. and E.P.F. depends upon merely their actions, inducing leukocytosis (L.P.F.) or eosinophilic leukocytosis (E.P.F.).

As for the factors which may induce leukocytosis, there are neutropoetin, eosinopoetin, mono-poetin, basopoetin (Komiya) as well as L.P.F. (Menkin) and E.P.F. (Wada). If so, are there not eosinotactic, monotactic, lymphotactic and basotactic substances in participation in the phase of taxis, just as there is leukotaxine concerning polymorphonuclear leukocytes? These studies are believed to be very important in the mentioned direction in future.

The distinction between neutrophilin (or L.P.F.) and leukotaxine is obvious in their actions, effect of heat, diffusibility, producing area, and revealing time of production caused with the injections of typhoid vaccine into rabbits, nevertheless, the mentioned three kinds of substances exist in rabbit serum treated with typhoid vaccine (neutrophin) and exudates of rabbits (L.P.F. and leukotaxine).

According to Menkin, the leukopenic factor exists in the euglobline fraction of exudates of rabbits and dogs. But we could not recognize this factor during the leukopenic period after the injection of typhoid vaccine into rabbits.

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#### Summary and Conclusion.

- 1) The authors recognize that Menkin's leukotaxine theory is true.
- 2) The essential character of leukotaxine, however, must be studied in future.
- 3) It is not difficult to suppose that increased capillary permeability and leukocytic migration are not only sustained with leukotaxine but also with turpentine, aleuronat and extract of organs. (Yasuhira).
- 4) The authors wish to point out that there are many important problems in future about the relationship between rabbit serum injected with typhoid vaccine, some kinds of human serum from healthy bodies, leukocytosis-promoting factor, reciprocally.