INACTIVATION TEST OF THE LUPUS ERYTHEMATOSUS (L.E.) PLASMA FACTOR

BY

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The existence of two types of lupus erythematosus (L.E.) plasma factor—transferable and non-transferable—has been suggested by our experiments with lupus erythematosus plasma transfer *in vivo* (Bencze, Kovács, and Cserháti, 1959; Bencze, Lakatos, and Ludányi, 1960).

Our further experiments *in vitro* showed differences between the two types of lupus erythematosus factor. These investigations, performed simultaneously with leucocytes from the subject and from other persons have shown that the L.E. factor of the non-transferable type does not produce the L.E. phenomenon, unless the subject's own leucocytes are used; this phenomenon could not be observed at all, or occurred to a much less extent, with leucocytes from other subjects (Bencze and Lakatos, 1960).

The present paper reports a further series of experiments *in vitro*, having in view the inactivation of the L.E. plasma factor. Earlier observations have shown that the inactivation of the L.E. plasma factor occurs at 59 and 65° C. respectively (Hargraves, 1949; Haserick, 1950).

Methods

(1) Plasma obtained from ten patients with L.E.-positive systemic lupus erythematosus (S.L.E.) was heated for 30, 60, 120, and 240 min. at + 56° C. 2 ml. of each batch of this plasma was added to the subjects' own leucocytes (washed six times in 240-ml. physiological saline) and to leucocytes obtained from normal persons. Subsequently the L.E.-cell test was performed by the rotatory method of Zinkham and Conley (1956).

(2) The usual L.E.-cell examination was done simultaneously in every case as a control test by Zinkham and Conley's rotatory method. Additional L.E.-cell tests were made with the patient's own washed leucocytes not supplemented with plasma, by Zinkham and Conley's rotatory method, to check the proper washing of the leucocytes.

(3) 2 ml. transferable plasma collected from each of three S.L.E. patients was kept for 30 and 60 min. respectively at +65° C.; they all coagulated within 30 min. The coagulated plasma was broken up with a glass stick and added to normal leucocytes. Subsequently the

L.E.-cell test was done by Zinkham and Conley's rotatory method.

(4) Plasma obtained under sterile conditions from three S.L.E. patients was heated at +56° C. for one hour and transfused to dogs in doses of 1 and 2 ml./kg. body weight respectively, as described in our earlier report (Bencze and Ludányi, 1960).

Results

Several inactivation tests were performed with plasma obtained from ten different S.L.E. patients, and with the pleural effusion of one such subject. The tests were repeated at least six times with plasma from each patient. The L.E.-cell phenomenon was seen when three batches of L.E. plasma factor of transferable type were used, both with the subject's own leucocytes and leucocytes from other persons, even with plasma kept at 56° C. for 4 hours. In all cases identical amounts of L.E. cells were present, as with plasma not exposed to heat. These three batches of S.L.E. plasma produced the L.E.-cell phenomenon, even when heated at +65° C. for 30 and 60 min. respectively, when the plasma also coagulated with leucocytes from other persons. One of these patients developed polyserositis. The L.E.-cell phenomenon was also seen when the pleural effusion was added to leucocytes from other persons. This pleural effusion was not inactivated when heated at 56° C. for 2 hours; the L.E. cells were seen in unchanged amounts when it was added to leucocytes from other persons.

Plasma from the other seven S.L.E. patients was inactivated, however, in 30 min. at 56° C.; no L.E. cells were seen even when it was added to the subject's own washed leucocytes (Table, opposite, Cases 4 to 10).

Plasma transfer experiments were carried out on dogs with plasma from five of the seven S.L.E. patients; all proved to be non-transferable (Table, Cases 4 to 8). *In vitro* L.E.-cell investigations, however, showed the presence of the L.E.-cell phenomenon in two patients only with their own leucocytes (Table, Cases 9 and 10). Using other leucocytes, no L.E. cells, or hardly any, were pro-

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TABLE
TRANSFER OF L.E. CELLS AFTER EXPERIMENTAL INACTIVATION BY HEATING

Experiments		In vitro												In vivo Transfer to Dogs	
Source of Leucocytes		Plasma							Pleural Effusion	Plasma		Fresh Plasma		Heated Plasma	Fresh Plasma
		Subject's Own				Others			Others	Others		Own	Others		
Temperature (°C.)		56				56			56	65		Nil		56	Nil
Heating Time (min.)		30	60	120	240	30	120	240	120	30	60	Nil		60	Nil
Case No.	1	+	+	+	+	+	+	+	—	+	+	+	+	T	T
	2	+	+	+	+	+	+	+	—	+	+	+	+	T	T
	3	+	+	+	+	+	+	+	+	+	+	+	+	T	T
	4	0	0	0	—	0	—	—	—	—	—	+	±	—	NT
	5	0	0	0	—	0	—	—	—	—	—	±	±	—	NT
	6	0	0	0	—	0	—	—	—	—	—	+	±	—	NT
	7	0	0	0	—	0	—	—	—	—	—	±	±	—	NT
	8	0	0	0	—	0	—	—	—	—	—	±	±	—	NT
	9	0	0	0	—	0	—	—	—	—	—	±	0	—	—
	10	0	0	0	—	0	—	—	—	—	—	+	0	—	—

— = No experiment.
+ = L.E. cells seen.
0 = L.E. cells not seen.

T = Transferable.
NT = Non-transferable.
± = L.E. cells sometimes seen, sometimes not seen.

duced in the other five patients, even when a large amount of plasma was added.

When we transfused three batches of S.L.E. plasma of the transferable type kept at 56° C. for 60 min. into dogs, we succeeded in producing L.E. cells in the same way as with plasma not exposed to heat (Table, Cases 1 to 3).

of the non-transferable type became inactivated, however, when heated at 56° C. for 30 min.

REFERENCES

Bencze, G., Kovács, J., and Cserhádi, I. (1959). *Brit. med. J.*, 2, 864.
—, Lakatos, L., and Ludányi, M. (1960). *Ibid.*, 1, 1707.
—, — (1960). *Ibid.*, in the press.
— and Ludányi, M. (1960). *Ann. rheum. Dis.*, 19, 48.
Hargraves, M. M. (1949). *Proc. Mayo Clin.*, 24, 234.
Haserick, J. R. (1950). *Arch. Derm. Syph. (Chicago)*, 61, 889.
Zinkham, W. H. and Conley, C. L. (1956). *Bull. Johns Hopk. Hosp.* 98, 102.

Discussion

Additional differences between the two types of L.E. plasma factor were demonstrated by L.E. plasma factor inactivation tests. We failed to confirm findings reported in the literature concerning the inactivation of the L.E. plasma factor. Our investigations showed that the transferable type of L.E. plasma factor was not inactivated even when heated at 65° C., whereas the L.E. plasma factor of the non-transferable type was destroyed when heated at 56° C. for 30 min.

Summary

In vitro inactivation experiments were carried out with plasma from ten different patients with systemic lupus erythematosus. Three batches of L.E. plasma factor of the transferable type failed to become inactivated, even when heated at 65° C. for 60 min. Seven batches of L.E. plasma factor

Réaction d'inactivation du facteur L.E. du plasma

RÉSUMÉ

On effectua des expériences d'inactivation *in vitro* avec du plasma provenant de dix malades atteints de lupus érythémateux généralisé. Trois lots du facteur plasmatique L.E. du type transferable n'ont pas pu être inactivés, même à une température de 65° C. pendant 60 minutes. On est arrivé, cependant, à inactiver sept lots du facteur plasmatique L.E. du type non-transferable en les soumettant à une température de 56° C. pendant 30 minutes.

Reacción de inactivación del factor L.E. del plasma

SUMARIO

Se llevaron a cabo experimentos de inactivación *in vitro* con plasma procedente de diez enfermos con lupus eritematoso generalizado. Tres muestras del factor L.E. del plasma del tipo transferible no llegaron a inactivarse, aun siendo sometidas a una temperatura de 65° C. durante 60 minutos. Siete muestras del factor L.E. del plasma del tipo no transferible resultaron inactivadas sin embargo, cuando se sometieron a una temperatura de 56° C. durante 30 minutos.