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Leucocyte aggregation and lymphocyte transformation induced by mercuric chloride

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SUMMARY

Leuco-aggregation tests carried out on normal subjects using the non-specific mitogen mercuric chloride produced micro-aggregates in the concentration range 2–100 μg/ml with a peaking effect at about 10 μg/ml. These results showed good correlation with lymphocyte transformation test thymidine uptake ratios which were maximal at 2–10 μg/ml.

INTRODUCTION

Aggregation of leucocytes in human whole blood in the presence of specific antigen has been described by Nicholls (1974). He demonstrated aggregation of buffy coat leucocytes with the addition of tuberculin PPD in Mantoux-positive subjects and suggested that this represented a simple test of cell-mediated immunity. We have subsequently shown this test to differentiate between nickel-sensitive and control subjects (MacLeod, Hutchinson & Raffle, 1976), and report here our findings using a non-specific mitogen—mercuric chloride.

MATERIALS AND METHODS

Blood samples were obtained from fourteen normal volunteer subjects without history of sensitization to mercuric salts. The technique employed and assessment used was that described previously (Nicholls, 1974; MacLeod, Hutchinson & Raffle, 1976). Mercuric chloride $HgCl_2$ (AR) was employed in concentrations of 0·1–1000·0 μg/ml of final culture and leucocyte aggregation assessed macroscopically and microscopically after 24 hr. Lymphocyte transformation tests (LTT) on gravity-sedimented samples from four subjects were also carried out using tissue culture plates (Dynatech). The final autologous serum concentration was 20% and the cell concentration 3×10^6 per ml. The mercuric chloride concentrations ranged from 2–200 μg/ml of final culture. Transformation was assessed by the addition of [^{3}H]thymidine 24 hr before harvesting 6-day cell cultures.

RESULTS AND DISCUSSION

The microscopic leuco-agglutination results obtained from the fourteen subjects are shown in Table 1 and the mean values are shown graphically in Fig. 1.

Macroscopic assessment proved unreliable for reasons discussed elsewhere (MacLeod, Hutchinson & Raffle, 1976).

LTT results for the controls are shown as thymidine uptake ratios (TURs) in Table 2.

The maximum concentration of $HgCl_2$ quoted in Table 1 is 100 μg/ml since higher concentrations produced marked RBC haemolysis and proved toxic. The results (Fig. 1) are similar to those obtained with nickel in that there is a marked increase in the number of leuco-aggregates produced with increasing concentration. Nine out of the fourteen subjects however show a peaking effect at concentrations lower than 100 μg/ml.

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TABLE 1. Mercury leuco-aggregation in normal subjects (microscopic evaluation)

Subject no.	Concentration of $HgCl_2$ (μg/ml)							
	0	0·1	1	2	5	10	20	100
1	1	1	2	4	13	13	0	38
2	0	4	9	0	27	7	17	0
3	0	2	0	1	11	1	8	1
4	0	2	1	8	14	47	28	95
5	3	0	3	11	27	52	34	11
6	0	0	0	0	11	11	10	32
7	2	0	1	4	4	2	12	18
8	0	n.d.	n.d.	n.d.	5	22	13	9
9	4	n.d.	n.d.	n.d.	5	14	18	3
10	0	n.d.	n.d.	n.d.	15	15	32	45
11	7	n.d.	n.d.	n.d.	10	27	31	42
12	0	n.d.	n.d.	n.d.	18	31	43	22
13	2	n.d.	n.d.	n.d.	12	18	24	41
14	0	n.d.	n.d.	n.d.	13	23	32	25
$\bar{x}$	1·3	1·3	2·3	3·9	13·2	20·2	21·6	27·3
s.d.	2·0	1·5	3·1	3·6	7·1	15·1	12·2	25·2

n.d. = Not done.

TABLE 2. Thymidine uptake ratios in lymphocyte transformation test

$HgCl_2$ μg/ml	Subject			
	1	2	3	4
200	<1	<1	<1	<1
150	<1	<1	<1	<1
100	<1	<1	1	<1
20	1	1	19	16
10	53	5	62	29
5	51	6	41	25
2	26	22	32	16

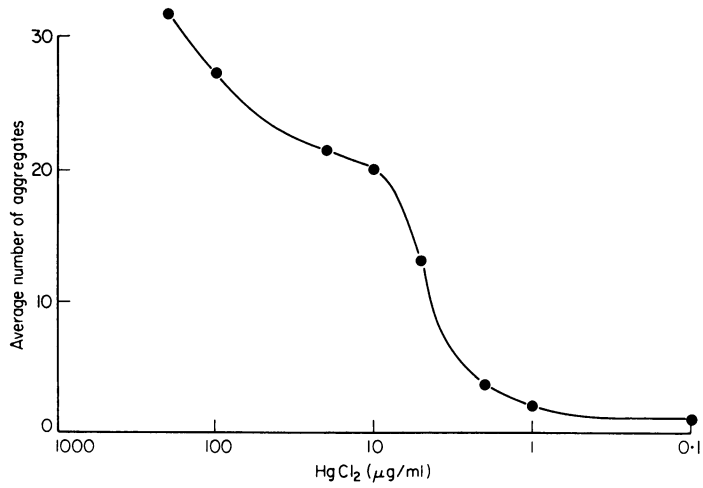

FIG. 1. Variation of average number of micro-aggregates with antigen concentration.

The concentrations of mercuric chloride used for the LTT ranged from 2 to 200 μg/ml (Table 2). In earlier experiments concentrations of 0·001, 0·1 and 10 μg/ml were employed but this wide range with 100-fold gaps produced negative results in the majority of samples tested. The concentration range required to induce a positive TUR is critical and suggests that other elements may be mitogenic if adequate serial concentrations up to toxicity levels are tested.

Positive LTT TURs were obtained using similar concentrations (i.e. 2–10 μg $HgCl_2$/ml) to those of other workers (Caron, Poutala & Provost, 1970; Schopf, Schultz & Gromm, 1967). In Table 2 we have concluded that TURs <1 indicate toxicity although no viability tests were carried out on the samples. If we assume that, on average, WBC counts on whole blood are 6×10^6/ml, i.e. double the WBC concentration used in the LTT, then positive TURs are obtained in equivalent mercuric chloride concentrations of 4–20 μg/ml in whole blood. This range corresponds to the peaking effect in the leuco-aggregation results shown in Fig. 1. There may thus be a correlation between the two tests. It is probable that a critical concentration of stimulant is required to influence the characteristics of cell membranes and thus trigger cellular interactions.

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