Length of lag phase and duplication time of *Trypanosoma equiperdum* in control and endotoxin treated rats

Control						Endotoxin treated[a]		
Serial No.	Lag phase (h)	Duplication time (h)	Serial No.	Lag phase (h)	Duplication time (h)	Serial No.	Lag phase (h)	Duplication time (h)
1	0	5.6	14	0	5.4	1	11.1	4.7
2	0	6.0	15	0	5.9	2	20.8	5.2
3	0	5.2	16	0	5.7	3	17.1	6.1
4	0	5.4	17	0	6.0	4	13.9	5.5
5	0	6.0	18	0	4.7	5	9.7	5.1
6	0	5.6	19	0	4.9	6	17.8	5.8
7	0	5.1	20	0	5.3	7	15.1	5.6
8	0	5.5	21	0	5.3	8	16.8	5.3
9	0	4.9	22	0	5.6	9	12.5	5.7
10	0	6.0	23	0	5.4	10	13.5	5.3
11	0	5.4	24	0	5.9			
12	0	6.1	25	0	5.4			
13	0	5.5	26	0	5.9			
				Average:	5.5		Average: 14.8	5.4
				S.E.:	0.025		S.E.: 1.06	0.05

[a] Received a total of 6 doses of endotoxin at 2-day intervals.

Serial pretreatment with endotoxin resulted in a characteristic alteration of the trypanosome growth curves. Infection was generally followed by a 14.8 h lag phase, and the logarithmic phase started only afterwards.

The Table shows the lengths of lag phases and duplication times measured on endotoxin-treated and control rats. The duplication times measured in the logarithmic phase did not differ in the experimental and control group. This implies that in the logarithmic phase the growth rate of trypanosomes was the same in rats treated and not treated with endotoxin.

The lengths of the lag phases were graphically extrapolated to the abscisse by means of points plotted out during the logarithmic phase[8].

With trypanosome count of 2×10^7/ml, the counts read at 1 h intervals during the logarithmic phase fluctuated from 1.9×10^7 to 2.1×10^7 owing to the $\pm$ 5% error.

The experimental animals died, similarly to the controls, at a trypanosome count of 2–2.5 $\times 10^9$/ml blood, thus the delay caused by the 14.8 h lag phase was realized also in the time of exit.

It appears that the length of the lag phase is an appropriate marker of the endotoxin-induced increase of non-specific resistance. Investigations on this basis are scheduled to be carried further along 2 lines: as a method, the above procedure seems to be suitable for the assessment of the resistance-increasing effect of various preparations; on the other hand, though we are aware that the experiment described above is just an approximate model, we believe that the clarification of the mechanism of the appearance of lag phases will help to disclose the mechanism of action of endotoxin induced resistance.

Zusammenfassung. In mit Endotoxin chronisch behandelten Ratten verändert sich die Vermehrungskurve des *Trypanosoma equiperdum* charakteristisch.

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Studies on Lysosomes in Rat Heart Cell Cultures. II. The Effect of Exogenous Lysosomes[1]

Since DE DUVE's[2] discovery of the lysosomes, there has been a dispute about their function and significance in cell physiology and pathology[3–8]. FRIEDMAN et al.[9] were unable to show lysosomal or any other cellular alterations in beating rat heart cell cultures, using 10 µg/ml of vitamin A. When greater concentrations of the vitamin (100 µg/ml and 1000 µg/ml) were used, a cytopathic effect, which appeared to be due to a primary alteration of the cell membrane rather than to lysosomal damage, was obtained. The present study deals with the effect of exogenous lysosomes and the synergistic effect of chlorpromazine or dimethylsulphoxide and vitamin A on heart tissue culture.

Materials and methods. Beating rat heart cell cultures were prepared according to the technique described previously[9].

Preparation of lysosomal fraction. Rat, guinea-pig and mouse liver lysosomes were prepared according to the method described by WEISSMANN and THOMAS[10]. 2 ml of 20% of the large granule fraction in lactalbumin hydrolysate (LAH) medium[9], supplemented with 15% inactivated new-born calf serum, were added to each heart cell culture.

Rat liver lysosomal enzymes. The large granule fraction was ultrasonicated twice, for 20 sec, at 4 °C in a MSE ultrasonicator. The fluid was examined for lysosomes

using a phase microscope. When necessary, the suspension was sonicated for a third time. Thereafter, the suspension was centrifuged at 17,800 *g* for 20 min. 2 ml of a 20% of the supernatant were added as before.

Guinea-pig PMNL lysosomes. Guinea-pigs weighing 500 g were injected i.p. with 10 ml of 6% sodium caseinate (Eastman Organic Chemicals)[11]. The rest of the procedure was performed according to COHN and HIRSCH[12]. The differential count after 18 h was 96–98% PMNL. The PMNLs were sonicated 3 times for 5 sec at 4 °C in order to release the granules from the cells. 2 ml of 10^7 PMNL/ml or 2 ml of granules, equivalent to 10^7 cells/ml in LAH, were added to each Petri dish.

Guinea-pig monocytes' lysosomes. The procedure was identical with that described above, but the cells were collected after 96 h.

Vitamin A. Vitamin A acid was used as described previously[9]. PMNL and monocyte lysosomes were suspended in the medium containing vitamin A, supplemented with 15% serum of new-born calf. The final concentration of vitamin A was 10 µg/ml.

Synergism. The cultures were incubated with CPZ $10^{-4}M$ or 5% DMSO for 8 h. Thereafter, the cultures were washed with a fresh medium; 2 ml of the LAH, containing 10 µg/ml of vitamin A, were added to each culture.

Cytotoxicity was determined by vital staining with 0.1% eosin Y and counting of the cells.

Results. The results are summarized in the Table. Synergistic effect of $10^{-4}M$ CPZ or 5% DMSO and vitamin A 10 µg/ml: In an attempt to assess whether there is any synergistic action of agents known to increase cell permeability, CPZ $10^{-4}M$ or DMSO 5% were added to the medium for 8 h, followed by the addition of vitamin A 10 µg/ml. No changes were observed after 5 days.

Discussion. In the present study heterologous liver cell lysosomes, lysosomal enzymes as well as guinea-pig PMNL, monocytes and their granules did not cause any damage to cultured heart cells. Nor was any cytopathic effect achieved after the addition of vitamin A 10 µg/ml. MOVAT et al.[13] showed the cytopathic effect of intact or lysed granules of PMNL after the ingestion of antigen antibody complexes on human derived tissue cultures.

In an attempt to determine whether the slightly basic pH of the medium was responsible for the lack of activity of the lysosomes, the pH of the medium was decreased to 6.2. However, there was no alteration in the growth of the cells, nor was there any cell damage. $10^{-4}M$ CPZ and 5% DMSO, substances known to cause cell destruction after 24 h[9], were added to the medium for 8 h, a period of time sufficient to produce membrane alteration. However, after the removal of these substances, the vitamin A which was added failed to cause any lysosomal or cellular alterations and the cultured cells continued to grow normally.

JANOFF and SCHAEFER[14], MOVAT et al.[13] and THOMAS[15] showed enhanced permeability of blood vessels, local hemorrhages and necrosis in the vascular system produced by PMNL lysosomes and lysosomal materials.

Despite the fact that we could not demonstrate cell destruction, and although the cell cultures in our model are devoid of a vascular system, it is possible that the above mechanism may take place in vivo. The lysosomal enzymes released following the necrosis may in turn change the antigenicity of heart tissue. The formation of circulating antibodies and sensitized lymphocytes to these 'newly' formed antigens, as has been earlier suggested by LAUFER and DAVIES[16], would be able to perpetuate the process, even after the removal of the primary cause.

Zusammenfassung. Isolierte Lysosomen wie auch intakte Monozyten hatten keinen zytopathischen Einfluss auf Herzmuskelzellen von Ratten, gezüchtet im Medium mit pH 7,2. Ein erniedrigtes Medium wie auch Nachbehandlungen der Kulturen mit Vitamin A nach Chlorpromazin 10^{-4} konnten keinen synergistisch zerstörenden Einfluss auf die Herzkulturen hervorrufen.

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Effect of lysosomes and lysosomal enzymes from various sources on rat heart and kidney cell culture

Source of lysosomes	Lysosomes from mg organ (wet weight)	Cytopathic effect[a]
Rat liver	80	–
Guinea-pig liver	80	–
Mouse liver	80	–
Guinea-pig PMNL lysosomes equal to	10^7 cells	–
Guinea-pig monocytes' lysosomes equal to	10^7 cells	–
Guinea-pig monocytes (whole)	10^7 cells	–
Guinea-pig monocytes (whole) and vitamin A 10 µg/ml	10^7 cells	–
Guinea-pig PMNL (whole)	10^7 cells	–
Guinea-pig PMNL (whole) and vitamin A 10 µg/ml	10^7 cells	–
Rat liver (after sonication)	80	–
Rat liver (after sonication) and vitamin A acid 10 µg/ml	80	–

[a] After 5 days.

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