Body weight loss, feed intake, organ weights and gonadotropin levels in male and female rats treated with saline or T_3

	Male		Female	
	Saline	T_3	Saline	T_3
Number of rats	7	8	8	8
Body weight loss (g) [a]	−0.6 ± 3.5 [b]	30.3 ± 5.7	2.6 ± 3.7	27.6 ± 6.9
Total feed consumed (g) [c]	404 ± 11	406 ± 24	251 ± 8	250 ± 10
Testes (g)	3.38 ± 0.02	3.50 ± 0.08	—	—
Ovaries (mg)	—	—	78 ± 6	86 ± 11
Seminal vesicles (mg)	329 ± 16	333 ± 9	—	—
Pituitary gland (mg) [c]	13.3 ± 0.6	12.8 ± 0.3	18.7 ± 1.8	19.5 ± 0.8
Pituitary FSH conc. (μg/mg) [c]	5.52 ± 0.62	5.74 ± 0.44	1.13 ± 0.05	1.23 ± 0.14
Pituitary LH conc. (μg/mg) [c]	8.2 ± 2.0	10.7 ± 1.7	6.5 ± 0.6	4.8 ± 1.0
Serum FSH conc. (ng/ml)	372 ± 25	316 ± 26	331 ± 30	320 ± 33
Serum LH conc. (ng/ml) [d]	15.7 ± 2.4	22.0 ± 3.1	22.8 ± 4.5	12.6 ± 2.3

[a] T_3 treatment $P < 0.01$. [b] Mean ± S.E. [c] Sex $P < 0.01$. [d] T_3 treatment X sex $P < 0.05$.

in rats of either sex and did not alter the weight of seminal vesicles in males. The ovaries of female rats receiving T_3 contained numerous large corpora lutea. The prolonged maintenance of corpora lutea in hyperthyroid rats has been reported previously[4]. T_3 treatment had no effect on the concentration of gonadotropins in the pituitary gland or on the concentration of FSH in the serum. A significant ($P < 0.05$) T_3 treatment X sex (or strain) interaction was noted for serum LH concentration. This resulted from T_3 causing a reduction in LH concentration in females while in males it tended to elevate LH levels.

The dose of T_3 employed in this study was approximately 13-fold higher than the dose required to restore metabolic rate to normal in thyroidectomized rats[11]. The hyperthyroid state was confirmed by the body weight loss in treated animals. The reason for the lack of effect on food intake is unknown. Hsieh and Ti[12] have reported increased feed consumption in rats treated with thyroxine.

The results obtained in this study provide no evidence that the hyperthyroid state adversely affects gonadotropin secretion or sex organ weights in male rats. In hyperthyroid female rats however, serum levels of LH appear to be reduced. Although ovarian weight was not affected by treatment, we have assumed that the maintenance of ovarian weight in treated rats was due to the presence of maintained corpora lutea and therefore was not indicative of normal function.

In this study we have assumed that the differences between the males and females represented sex differences however the possibility that some of the apparent sex differences may have been the result of strain differences should not be overlooked.

Résumé. Chez des rats adultes, mâles et femelles, traités à la triidothyronine (T_3), on a remarqué une perte de poids qui n'est pas dûe à une diminution de consommation de nourriture. Les niveaux sériques de l'hormone lutéinisante semblent être réduits par le traitement au T_3 chez les rats femelles, mais non pas chez les rats mâles. Le niveau sérique de l'hormone stimulante de la follicule et le niveau pituitaire de gonadotropines ne sont pas affectés de façon significative par le traitement au T_3.

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Effect of Lactic Acid on the Aggregation of Human Platelets Induced by ADP, Adrenaline and Collagen

Intravascular coagulation is reported in connection with hypoxaemia and anoxaemia under various clinical and experimental situations[1-4]. As a consequence of tissue hypoxaemia and/or anoxia, lactate might be increased in tissues and blood[5-7]. In animal experiments, thrombosis was observed after i.v. injection of lactic acid[8]. Lactic acid has even been reported to increase platelet adhesiveness in vitro[9]. This suggests that under these conditions lactic acid plays an intermediary role between hypoxaemia and intravascular coagulation. Considering this evidence, we have investigated in vitro the effect of hyperlactacidaemia on the human platelet aggregation, induced by ADP, adrenaline and collagen.

Materials and methods. Human blood was collected from the antecubital vein of healthy volunteers (who had not taken medication within the previous week) into 1/10 its volume of 3.1% trisodium citrate. Platelet rich plasma (PRP was prepared by centrifugation of the blood at $180 \times g$ for 15 min at room temperature, and the platelet count was determined using an automatic

particle counter (Celloscope 101, Linson, Stockholm, Sweden). Aggregation of platelets was studied turbidimetrically at 37 °C with continuous recording of light transmission (EEL Titrator, Evans Electroselenium Ltd, Essex, England and Recorder Beckman, Model 1005, USA). 0.1 ml of lactic acid (AnalaR, England) at various concentrations, or other test substances were added to 0.9 ml of PRP (containing $2.5–3.5 \times 10^5$ platelets/μl), incubated for 3 min at 37 °C and platelet aggregation was initiated by the addition of 0.1 ml of one of the following agents: ADP sodium salt (Sigma, St. Louis, USA) dissolved in *tris*-buffered saline (0.12 *M* NaCl, 0.03 *M* *tris*-HCl, pH 7.4); adrenaline dissolved in *tris*-buffered saline[10], and collagen from tendons (Sigma) prepared as described by HOLMSEN et al[10], and diluted with 0.1% acetic acid. Platelet aggregation was assessed by measuring: 1. maximum aggregation (MA) i.e. the maximum change in light transmission expressed as a percentage of the difference in light transmission between PRP and PPP for each determination, 2. initial rate of aggregation (IRA), increase in light transmission (mm chart paper) during the first 30 sec and 3. *r* value, the time elapsing between addition of agent and start of secondary wave of aggregation. The mean and range of 7 experiments are recorded.

Results and discussion. Lactic acid added alone to citrated PRP at a final concentration in plasma of 0.2 to 0.8 mg/ml (a concentration reached during conditions of metabolic acidosis), did not induce platelet aggregation. These observations are in contrast to the results obtained by the in vitro experiments of LABORIT and ORNELLAS[9].

Lactic acid in vitro produced a concentration-dependent inhibition of the ADP-induced platelet aggregation

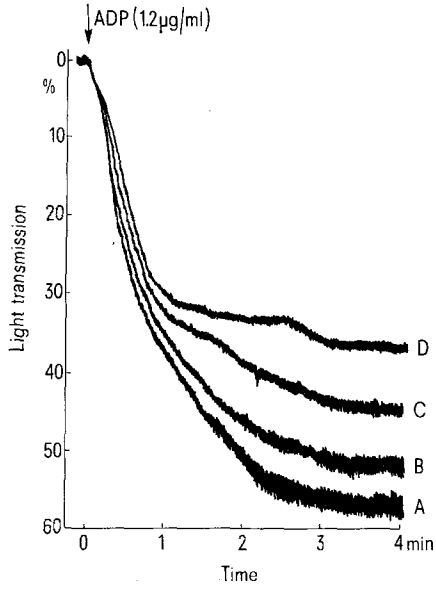

Fig. 1. Effect of lactic acid on platelet aggregation induced by ADP (final concentration 1.2 µg/ml) ADP added at zero time to PRP which had been preincubated at 37 °C for 3 min with 0.15 *M* NaCl or lactic acid in increasing concentrations. Final concentration of lactic acid were: A) 0.0; B) 0.2; C) 0.4; D) 0.8 mg/ml. Final pH were: A) 7.8; B) 7.6; C) 7.4; and D) 7.2.

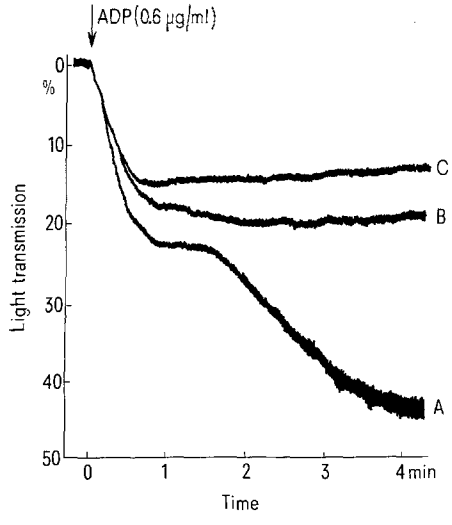

Fig. 2. Effect of lactic acid on platelet aggregation induced by ADP (final concentration 0.6 µg/ml). ADP added at zero time to PRP which had previously been preincubated at 37 °C for 3 min with 0.15 *M* NaCl or lactic acid. Final concentrations of lactic acid were: A) 0.0; B) (♀)0.4; C) 0.8 mg/ml. Final pH were: A) 7.8; B) 7.4 and C) 7.2.

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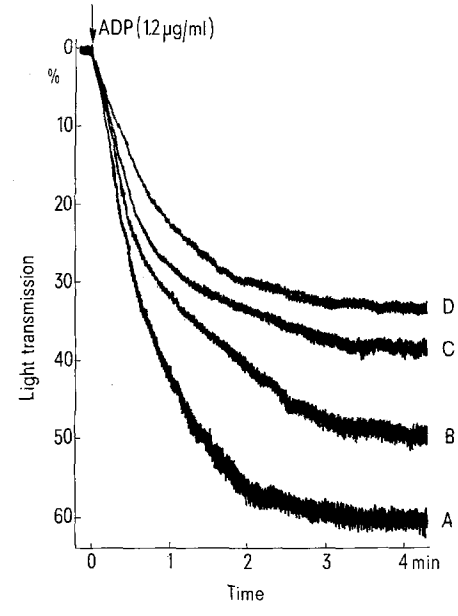

Fig. 3. Effect of HCl on platelet aggregation induced by ADP (final concentration 1.2 µg/ml). ADP added at zero time to PRP which had been preincubated at 37 °C for 3 min with 0.15 *M* NaCl or various concentrations of hydrochloric acid (10^{-2} *N*) in 0.15 *M* NaCl. Final pH were: A) 7.8; B) 7.6; C) 7.4 and D) 7.2.

Table I. Influence of free lactic and hydrochloric acid as compared to Na-lactate on platelet aggregation induced by ADP, adrenaline and collagen

Test reagent (final concentration)	Final pH	Platelet aggregation							
		ADP (1,2 μg/ml)		ADP (0,6μg/ml)		Adrenaline (3.6 μ*M*)		Coll. (2 μg/ml)	
		IRA (mm)	MA (%)	IRA (mm)	MA (%)	*r* (sec)	MA (%)	*r* (sec)	MA (%)
Saline control	7.8	22.4	41.9	17.0	35.5	73.2	45.5	48.2	40.3
Lactic acid (0.8 mg/ml)	7.2	15.3	23.0	14.6	17.4	96.6	40.2	48.5	40.8
HCl ($5\times10^{-3}N$/ml)	7.2	15.7	25.5	14.8	19.4	92.4	40.6	45.9	39.1
Lactic acid (0.8 mg/ml) + NaOH	7.8	19.7	38.0	15.4	35.4	73.2	44.6	48.4	40.1

The values represent means from 7 experiments.

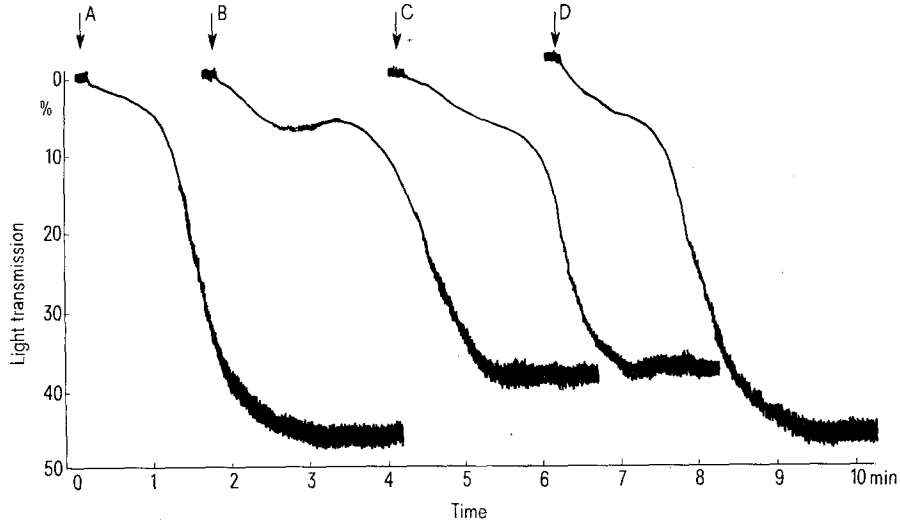

Fig. 4. Effect of lactic acid and pH on platelet aggregation induced by adrenaline. PRP had been preincubated with A) 0.15 *M* NaCl (final pH 7.8); B) lactic acid (final concentration 0.8 mg/ml, final pH 7.2); C) hydrochloric acid (final concentration 5×10^{-3} *N*, final pH 7.2) and D) lactic acid neutralized with 0.1 *M* NaOH (final concentration of lactate 0.8 mg/ml, final pH 7.8). At ↓ adrenaline was added (final concentration 3.6 μ*M*).

(Figure 1). This inhibition seems essentially to effect the second phase of the platelet aggregation (Figure 2), and thus probably the release of ADP from the platelets. In the experiments of Figure 1 and 2 the final pH varied from 7.8 to 7.2, according to lactic acid concentration. Addition of various concentrations of hydrochloric acid to the PRP to vary the pH between 7.8 and 7.2 caused an inhibition of ADP-induced aggregation similar to that induced by lactic acid (Figure 3). These results suggest that the effect of lactic acid on ADP-induced platelet aggregation is a due to the pH lowering effect of the acid. Evidence for this is that lactic acid neutralized to pH 7.4 with 0.1 *M* NaOH and preincubated with PRP (final concentration of lactic acid 0.8 mg/ml, final pH 7.8) has no influence on platelet aggregation as compared to the control (Table).

The results of experiments with adrenaline as the aggregating agent are shown in Figure 4. The lactic acid and/or acidosis prolonged the lag phase between primary and secondary aggregation, indicating inhibition of the release reaction. The neutralized lactic acid did not influence the adrenaline-induced platelet aggregation.

Collagen-induced platelet aggregation was not influenced by lactic acid (final concentration between 0.2–0.8 mg/ml) and/or the concomittant change in pH (final pH between 7.8–7.2) (Table).

The described phenomena suggest that hyperlactacidaemia alone does not play a role in the induction of intravascular coagulation by influence of platelet aggregation.

Zusammenfassung. Nachweis, dass die Milchsäure die durch ADP und Adrenalin induzierte Aggregation der menschlichen Thrombozyten hemmt und keinen Einfluss auf die Kollagen-induzierte Plättchenaggregation in vitro hat. Die Hemmwirkung ist unspezifisch, kommt sie infolge der pH-Verschiebung in saurer Richtung zustande.

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