PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 575–577 (1978)

Platelet Hypoaggregability in Hypothyroid Rats (40249)

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Several hematologic abnormalities have been noted in patients with hypothyroidism (1–3, 6). The occurrence of easy bruising, menorrhagia and severe bleeding tendencies have been observed in hypothyroid patients. Depression of concentrations of coagulation factors (3) and abnormalities of platelet adhesiveness have been recently reported (4). In the latter report the initiation of platelet aggregation by epinephrine appeared to be blunted.

Lipid abnormalities with hypercholesterolemia, hypertriglyceridemia and hyperphospholididemia have been regularly observed in these patients. Since platelet function and platelet lipid metabolism are affected by plasma lipid contents (7, 8) abnormal platelet function noted in these patients may be the result of a disturbed lipid metabolism secondary to the deficient thyroid hormone. Surgically induced hypothyroid and hypoparathyroid rats do not show significant changes in their lipid metabolism. Such an animal model may be used to study platelet function in hypothyroid state without the participation of altered serum lipids. This is a report of our studies with this experimental model.

Materials and methods. Nine male Sprague-Dawley rats weighing 200 g underwent thyroparathyroidectomy under pentothal anesthesia. A similar number of animals of the same strain and weight underwent sham operation and served as controls. Four to eight months later the presence of hypothyroidism, clinically evident by disparity in size, activity and hair condition from controls, was confirmed in survivors by repeated low serum T_4 levels (done by radioimmunoassay). Cholesterol, triglyceride and lipoprotein profiles were also done in order to assess the influence of any alteration of lipid metabolism due to hypothyroidism on platelet aggregation.

All animals were anesthetized with an intraperitoneal injection of pentobarbital. While asleep animals were exsanguinated via cardiac puncture. Samples of blood were collected in polystyrene tubes containing 0.1 *M* citrate buffer (pH 6.5) in 2.5% (w/v) of dextrose. Platelet-rich plasma (PRP) was obtained by centrifugation of blood at 300*g* for 20 min at room temperature. Platelet-poor plasma (ppp) was prepared by centrifugation of blood at 1000*g* for 20 min at 4°. Platelet aggregometry was performed using a Chrono-log platelet aggregometer coupled to a Fisher Recordall recorder (5). 0.45 ml of PRP was added to a cuvette with a magnetic stirring bar. This was placed in the aggregometer and the light transmission adjusted to about 10%; that for PPP was adjusted to about 90%. At zero time 50 μl of one of the aggregating agents were added. Platelet aggregation resulted in an increase in light transmission and an upward deflection of the recording pen. The following were used for induction of platelet aggregation: adenosine diphosphate (ADP), lipopolysaccharide (LPS) (endotoxin) and collagen (Sigma Chemical Co., St. Louis, MO). The final cuvette concentrations of aggregating agents were 10^{-4} *M* for ADP and 1 mg/ml for LPS. The characteristic of LPS used has been described previously (10). The collagen suspension was prepared from bovine tendon (5) and used at a minimal dilution that gave maximal platelet aggregation response.

Results. Of the nine rats that were thyroparathyroidectomized, only two rats had T_4 levels that exceeded the one standard deviation value (Tables I and II). Comparing the T_4 levels of the thyroparathyroidectomized and sham-operated rats, it is apparent that they are significantly different with $P < 0.001$.

Normal aggregometry tracings were obtained when the aggregating agents were added to PRP from control animals. However, there was a marked depression of ag-

0037-9727/78/1584-0575$01.00/0

gregation when PRP from hypothyroid animals (except number 4 which had a T_4 level of 2.2 μg/dl) was used (Table I). The response was blunted for all the aggregating agents used (Table II). The differences in platelet response from the two groups of animals were most marked when LPS was used as the aggregating agent (Table II). LPS produced a biphasic response in control animal platelets. However aggregation was uniformly depressed in experimental animals indicating that both the first- and second wave of aggregation were depressed.

The serum cholesterol and triglyceride in these animals changed minimally in the presence of hypothyroidism (Table II).

In vitro addition of T^4 to PRP from a hypothyroid rat caused minimal improvement in response to aggregating agents. Intravenous injection one half hour prior to exsanguination into a hypothyroid rat of T_4, in a volume calculated to increase the serum T_4 level to supranormal levels, failed to correct the platelet hypoaggregability.

Discussion. Previous platelet studies in hypothyroid patients have demonstrated a decrease in adhesiveness (3, 4) but normal response to aggregating agents in standard concentration. However, the response to lesser concentrations of epinephrine demonstrated absence of response below a concentration of 1.375×10^{-7} *M*. However, all these studies were performed with platelets from hypothyroid patients who, as part of the disease process, also had disturbed lipid metabolism. Thus the reported abnormalities of platelet dysfunction might result from the aggregated effect of altered thyroid and lipid

TABLE I. AGGREGOMETRY STUDIES OF HYPOTHYROID RATS.

	Collagen		ADP 10^{-4} *M*		LPS 1 mg/ml		
Animal	Slope[a]	dT[b]	Slope	dT	Slope	dT	T_4 (μg/dl)
1	1.2	10.6	3.8	4.3	0.6	7.2	1.7
2	1.2	17.5	0.5	3.1	0.3	0.9	0.0
3	1.8	21.3	3.7	7.3	0.7	6.9	0.8
4	4.3	37.5	27.5	76.2	6.8	21.3	2.2
5	2.2	21.9	0.6	4.3	0.5	6.3	0.0
6	1.0	13.1	8.8	41.8	0.6	8.1	1.0
7	1.3	24.4	2.5	15.6	0.0	0.0	0.0
8	2.5	20.3	1.0	4.4	1.0	6.5	0.4
9	1.3	8.6	1.5	11.9	0.6	6.9	0.6

[a] Slope: Expressed as the maximal change in percentage light transmission at a time interval of 12 sec.
[b] Dt: Delta-T is expressed as the maximal change in percentage light transmission as a result of platelet aggregation.

TABLE II. COMPARISON OF PLATELET AGGREGOMETRY AND SERUM LIPID LEVELS IN CONTROL AND HYPOTHYROID RATS.[a]

Aggregating agents Aggregation tracing	Collagen		ADP 10^{-4} *M*		LPS 1 mg/ml	
	Slope[b]	dT	Slope	dT	Slope	dT
Hypothyroid—mean	1.9	19.5	5.5	18.8	1.2	7.1
SD	1.0	8.7	8.6	24.7	2.1	6.0
Control—mean	3.6	38.9	17.3	71.1	6.7	28.5
SD	0.6	9.5	4.3	7.3	2.1	3.9
Comparison $P <$	0.01	0.01	0.01	0.001	0.001	0.001

	Cholesterol (mg/dl)	Triglyceride (mg/dl)	T_4 (μg/dl)
Hypothyroid—mean	66.0	53.1	0.74
SD	13.7	14.8	0.78
Control—mean	80.2	77.3	3.48
SD	16.3	35.3	0.44
Comparison $P <$	0.1	0.2	0.001

[a] Each group has nine rats.
[b] Units used for both slope and dT of aggregometry tracings are identical to those in Table I.

metabolism. Recent studies suggested that haemostatic changes in hypothyroid patients were not correlated to alterations in lipid metabolism (9). The present experimental study was performed in rats which had no significant changes in lipid metabolism following the surgical induction of hypothyroidism. Furthermore, the use of hypothyroid rats allowed the assessment of platelet aggregability in the presence of T_4 levels below 0.5 μg/dl, a lower level than that observed in human hypothyroid subjects. This revealed marked hypoaggregability of platelets. The failure of *in vitro* correction with addition of T_4 suggested a qualitative defect in the platelets, possibly a reflection of the general depression of metabolic activity in hypothyroidism.

Summary. The induction of aggregation of platelets obtained from hypothyroid rats was investigated. Using adenosine diphosphate, collagen and endotoxin as aggregating agents, a uniform depression of aggregability was noted. Since hypothyroid rats do not have concomitant changes in lipid metabolism, the hypoaggregability of platelets in hypothyroidism is directly related to the disturbed endocrine function and can be demonstrated in the absence of changes in lipid metabolism.

1. Orr, F. R., Lancet **2**, 1012 (1962).
2. Egeberg, O., Scand. J. Clin. Lab. Invest. **15**, 1 (1963).
3. Simone, J. V., Abildgaard, C. F., and Schulman, I., N. Engl. J. Med. **273**, 1057 (1965).
4. Edson, J. R., Fecher, D. R., and Doe, R. P., Ann. Intern. Med. **82**, 342 (1975).
5. Fong, J. S. C., White, J. G., and Good, R. A., Blood **44**, 399 (1974).
6. Hellem, A., Segaard, E., and Solem, J. H., Acta. Med. Scan. **197**, 15 (1975).
7. O'Brien, J. R., Etherington, M. D., Jamieson, S., Vergroesen, A. J., Tenhoor, F., Lancet **11**, 995 (1976).
8. O'Brien, J. R., Etherington, M. D., Jamieson, S., Lancet (i) **878**, (1976).
9. Nordøy, A., Vik-Mo, H., and Berntsen, H., Scand. J. Heamatol. **16**, 154 (1976).
10. Fong, J. S. C., and Good, R. A., J. Exp. Med. **134**, 642 (1971).

Received December 12, 1977. P.S.E.B.M. 1978, Vol. 158.