Omission of thromboplastin increases the coagulation time 4.5–5.5 times both for normal and diluted plasma. Addition of PGE_1 or PGE_2 instead of thromboplastin has no effect. We can therefore conclude that PGE_1 and PGE_2 do not interfere with thromboplastic activity and, second, that they do not possess such an activity.

The calcium clotting times of rat plasma (Table III) are not affected by PGE_1 at any of the $CaCl_2$-concentrations used for recalcification of the plasma. FERRI et al.[12], who examined the calcium clotting times of citrated rat blood dialysed against saline for 7–8 h at various $CaCl_2$-concentrations, found shorter calcium clotting times in the presence of PGE_1 (1 µg/2 ml blood) than in its absence at any of the $CaCl_2$-concentrations used. They postulate that Ca-ions can be substituted by PGE_1 in a ratio exceeding 1000:1. The discrepancy between our results and those obtained by FERRI et al.[12] can be explained by assuming that a dialysable factor – not the Ca-ions – can be substituted by PGE_1[13].

Table III. Effect of PGE_1 on calcium clotting time (sec), using cPRP of rats ($n = 6$)

Substance added	$CaCl_2$-concentration (mM)								
	25	17.5	15	13.75	12.5	11.25	10	8.75	7.5
Saline (control)	60	68	72	79	84	98	124	162	260
1 µg PGE_1	61	65	74	77	86	99	126	161	264

Zusammenfassung. Die Prostaglandine E_1 (PGE_1) und E_2 (PGE_2) haben keinen Einfluss auf die Gerinnung von Rattenblut. Im Gegensatz zu früheren Postulationen können die Ca-Ionen im Plasma nicht von PGE_1 ersetzt werden. PGE_1 beeinflusst die maximale Amplitude des Thrombelastogramms in vitro nach Zugabe von 0.3–6 µg/ml, während PGE_2 diesen Effekt nicht aufweist.

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Unsaturated Fatty Acids; Platelet-Serotonin Releasers in Tissue Extract

A factor is present in alkaline extract of mammalian tissue which releases serotonin from blood platelets[1]. Partition with organic solvents and thin-layer chromatography[2] indicated that a mixture of naturally occurring unsaturated fatty acids such as oleic and linoleic is responsible for the serotonin-releasing activity of the alkaline tissue extract and in consequence, these acids may be added to the family of serotonin releasers. In this communication, the action of partially purified tissue extract on platelet cells and isolated amine-containing granules is explored.

Methods. As reported in a previous paper[2], the active material(s) could be obtained more effectively by total lipid extraction and subsequent alkaline treatment of minced hog kidney. Purification of active principle(s) was carried out with 2 steps of thin-layer chromatography and finally the zone corresponding to mono- and di-enoic fatty acids was eluated with acetone[2]. Rabbit platelets were washed and resuspended in Tullis-Toh's solution[3]. Isolated amine-containing granules were prepared by sonication (100 W, 10 kc for 1 min) and differential centrifugation[4] and suspended in the same solution. Serotonin release from platelet cells and granules was estimated by fluorimetrical procedures[2,5].

Results and discussion. More than 90% of the platelet serotonin was released by the purified material derived from 1–2 mg original tissue per ml cell suspension fluid (ca. 10^8 cells per 4 ml) in 60 min at 37 °C. The spontaneous release of serotonin ranged from 5–10%. The action of the serotonin-releasing material of such extract was found to be markedly inhibited by addition of glucose (0.1–5.5 mM) to the incubation media and ATP (1–5 mM) had little effect.

Certain saturated fatty acids like palmitic, stearic and behenic release platelet serotonin and histamine as well and cause adhesion of the cells to the vessel wall and cell damage[6]. However, these acids require the presence of blood plasma for their platelet effect. In contrast, even when all the serotonin was released by the alkaline tissue extract no diminution in the platelet count or significant adhesion and aggregation was observed. Electron microscopy revealed morphological changes such as degranula-

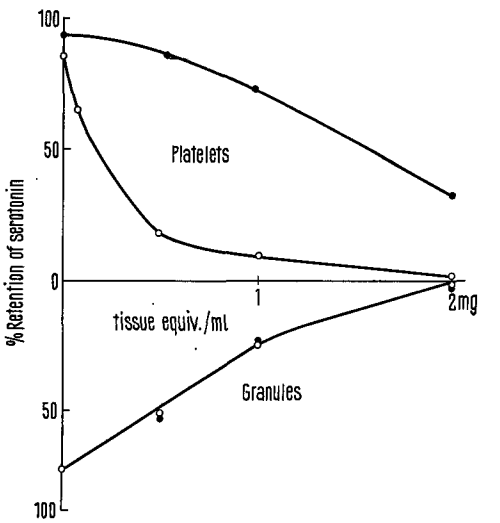

Fig. 1. Effect of the purified alkaline kidney extract on the serotonin content of platelets (top) and of granules (bottom). Incubation, with glucose (closed circle) or without glucose (open circle) in the medium, was carried out at 37 °C for 60 min and at 24 °C for 30 min, for platelets and for granules respectively.

tion without membrane damage, similar to that caused by reserpine, amphetamine or Ro-4-1284[7], which was minimized by adding glucose (5 mM) to the medium. As illustrated in Figure 1, not only the platelets but also the amine-containing granules isolated from platelets were found to liberate serotonin in the presence of tissue extract or oleic acid. However, in contrast to the situation with platelets, such a release of serotonin from isolated granules was not inhibited by glucose (5.5 mM). With platelet suspensions ($1–4 \times 10^7$ cells per ml), there exists a stoichiometric relationship between platelet count and the concentration of the tissue extract or of oleic acid needed to release serotonin (Figure 2) while the dose-effect curve on the isolated granules of different population demonstrated that isolated granules, corresponding to $10^7–10^8$ platelets per ml, showed nearly complete loss of serotonin even by low concentration of the extract or oleic acid which cause less than 50% liberation of the platelet serotonin. The serotonin release by the extract or oleic acid from platelets is temperature-dependent, the greater release occured on incubation at 37 °C, far less at 20 °C and practically no release at 4 °C. In contrast, isolated granules show no such temperature-dependency.

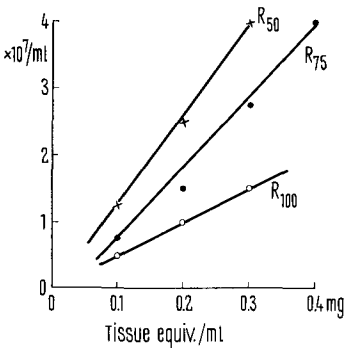

Fig. 2. Relationship between platelet count and dose of the purified alkaline kidney extract to induce a certain extent of serotonin release. Various counts of platelets (ordinate) were incubated with specified doses of the extract (abscissa). R_{50}, R_{75} and R_{100} indicate 50, 75 and 100% release of serotonin respectively.

Mannose was found to be the only sugar which showed an inhibitory effect like glucose on serotonin release from platelets; galactose, xylose, fructose, 2-deoxyglucose, sodium pyruvate, glucose-6-phosphate, 2,4-dinitrophenol and monoiodoacetate had little effect. There appeared to be no metabolic basis for the action of glucose in inhibiting the serotonin-releasing material and oleic acid since little variation in the glucose inhibitory effect was observed between pH 6 to 8. However, the glucose inhibition effect is very likely to be a competitive one ($Ki = 1.4 \times 10^{-4} M$). It is interesting that glucose stimulates uptake of ^{14}C-oleic acid (unpublished data). Clearly, this effect of glucose and the interaction of incorporated fatty acids with both amine-containing granules and the platelet membrane requires detailed study.

Zusammenfassung. Der Hauptanteil Serotonin freisetzenden Materials in alkalischen Gewebsextrakten erweist sich als ein Gemisch ungesättigter Fettsäuren, welche die Anregung der Serotoninausschüttung von Blutplättchen bewirken.

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The Immune Suppressive Effect of Hypoxia on Chicken Embryos

In a previous report we presented data suggesting that an erythropoietic stimulus (hypoxia) and an immunogenic stimulus may affect directly the differentiation of a common pluripotential stem cell in adult rabbits, and that a homeostatic balance may exist between the two differentiated cell lines, erythrocytes and immunocytes[1]. Based on these experiences, we reasoned that if the effect is indeed a cell differentiation stimulus, one should find interesting responses in a developing embryo whose immune system is still immature. I report here some observations on the drastic suppression of the immune response in the chicken embryo subjected to hypoxia in a hypobaric chamber.

Materials and methods. White Leghorn chicken-embryos, 16 days old, were exposed for 30 h to a 12,500 ft (3800 m) simulated altitude in a hypobaric chamber. The altitude tolerance (hatchability) of the embryos was determined in a preliminary experiment[2]. Under the above conditions, 70% of the eggs hatched. For comparison, 1-week-old chicks were also exposed to the same altitude for 72 h. Another group of chicks received a suppressive antiserum, made against the *E. coli* antigen, 5 days prior to immunization.

The chicks were immunized at age 7 days with a somatic *E. coli* antigen. 10 chicks from each group were sacrificed at age 10 days for assays. The cellular immune response was measured by the Jerne plaque-forming cell (PFC) test in the spleen and bursa[3]. The highest PFC numbers were found consistently on the third day following immunization, therefore, in the results below the PFC tests are all compared at that time. A more detailed account of the temporal relationship of the immune and erythropoietic responses in chicken embryos will be presented in another publication. Delta amino levulinic acid

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