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.

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 It is interesting that glucose stimulates uptake of ^{14}C-oleic acid (unpublished data).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.

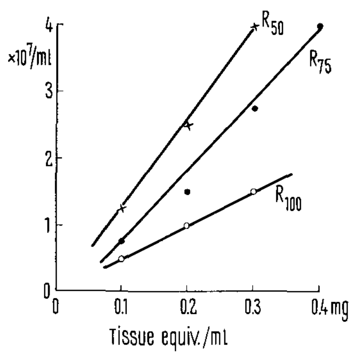

Fig. 2. Relationship between platelet count and dose of the purified alkaline kidney extract to induce a certain extent of serotonin release. Various counts of platelets (ordinate) were incubated with specified doses of the extract (abscissa). R_{50}, R_{75} and R_{100} indicate 50, 75 and 100% release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

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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dehydrase (DALAD) activity, as a measure of erythropoietic activity, was determined following MAUZERALL and GRANICK[4]. Hemoglobin and hematocrit measurements supplemented DALAD activity measurements to follow the relative change in erythrocyte level in the hypoxic and normal chicken.

Results and discussion. Altitude exposure in the embryonic age resulted in a drastic suppression of the immune response in the young chicks (Table I). The suppression was as effective as that caused by the administration of passive Ab to normally hatched and immunized chicks. In contrast, when the altitude stress was applied to 1-week-old chicks, immune response was not suppressed, although decreased compared to ground-level immunized chicks (Figure).

Table I. Immune response of chicks exposed to a hypoxic stress during embryonic development

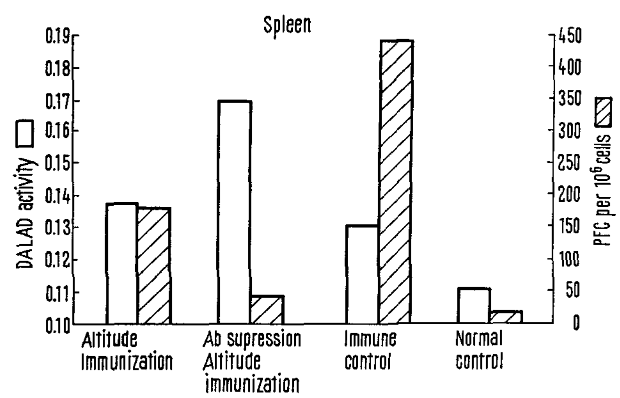
Group	Treatment	PFC/10^6 cells Spleen	Bursa
1	Altitude	2 ± 2.0	2 ± 2.0
2	Suppressive Ab	10 ± 8.0	4 ± 4.0
3	Immunized Control	440 ± 10.1	102 ± 160
4	Normal Control	–	16
5	Altitude Control	4 ± 2.0	20

PFC = Ab plaque forming cells. 7-day-old chicks were immunized.

Table II. DALAD activity in chicks exposed to a hypoxic stress during embryonic development

Group	Treatment	DALAD activity Spleen	Liver
1	Altitude and immunized	0.150 ± 0.005	0.512 ± 0.008
2	Suppressive Ab	0.112 ± 0.04	0.388 ± 0.03
3	Immunized Control	0.130 ± 0.02	0.520 ± 0.05
4	Normal Control	0.110 ± 0.04	0.414 ± 0.02
5	Altitude Control	0.145 ± 0.005	0.520 ± 0.05

DALAD activity = μM porphobilinogen/g tissue in 1.5 h.



Comparison of the immune response and erythropoietic response of 1-week-old chicks, subjected to hypoxia.

It is interesting to note the apparent homeostatic balance between the cellular immune response and DALAD activity. When the immune response was suppressed, either by the apparent lack of immunocompetence in the chicken embryo or by passive Ab in the young chick, the hypoxic stimulus increased DALAD activity as much as in the altitude control group (Table II, Figure). However, when the simultaneous altitude treatment and immunization produced an immune response (in the 1-week-old chick), the increase in DALAD activity was much reduced (Figure).

These results provide an indirect support for the theory that the cells of the erythropoietic and immune responses may develop from a common pluripotential stem cell[5–8]. In the 16-day-old chicken embryo immunocompetence is lacking. This may be due to the lack of differentiation of a pluripotential stem cell into immunocyte. The hypoxic stimulus applied at this stage of development may have committed cell differentiation into the route that had already been developed (erythroid cell differentiation), thus temporarily blocking or delaying immune maturation.

The homeostatic balance observed between immune response and erythropoietic response in the young chick also appears to support the common origin of erythrocytes and immunocytes. The simultaneous immunogenic and hypoxic stimuli may have caused a competitive demand for repopulating various differentiated stages of the 2 cell lines from the common pluripotential stem cell pool. This may have caused the relatively reduced increase in immune response and DALAD activity under the simultaneous hypoxic and immunogenic stimuli as compared to the single stimuli.

In summary, a hypoxic shock can suppress the immune response of chicken embryos and reduce the immune response of 1-week-old chicks. Hypoxia (altitude treatment) thus may provide means for regulating the immune response[9].

Zusammenfassung. Die Immunreaktion von Hühnerembryonen konnte durch stimulierte Höhenbehandlung (hypoxischer Stress) unterdrückt werden, während die Immunreaktion von Junghühnern nicht beeinflusst wurde. Dies macht wahrscheinlich, dass Hypoxia die Differenzierung pluripotentieller Stammzellen nach Immunozyten verhindert.

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