would be non-toxic (low haemagglutination and platelet agglutination) and of moderate potency in vivo. These experiments indicate that lymphocyte antigen-antibody complexes are immunogenic. Our results also suggest that by precipitation with an antiserum raised against cell membranes we have selected a fraction of the cellular antigens which leads to more potent and less toxic antisera than are obtained against whole cells. A small amount of antiserum raised against highly purified membrane or soluble antigen[2,4,5] could be used to prepare enough complexed antigen to immunize several large animals, and the antigen need not be recovered from the complex. Further immunological procedures which might prove useful in the preparation of ALS are illustrated by the sequential agglutination of erythrocytes and thymocytes in the first experiment and by the prior absorption of the agglutinating antiserum with an immunoabsorbent prepared from erythrocytes in the second experiment.

Résumé. Des complexes antigène-anticorps préparées par réaction du sérum antilymphocytaire (ALS) avec des lymphocytes ou avec des broyats lymphocytaires furent injectées aux lapins. Les ALS obtenu ainsi des lapins furent immunodépresseurs. Cette technique peut être utilisée pour obtenir des ALS plus specifique et moins toxique.

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Heme Synthetase in Thalassemia

Heme synthetase (HS) is the mitochondrial enzyme which introduces iron into protoporphyrin and thereby participates in the last stage of heme synthesis. A clinically useful test for measurement of HS activity on cell lysates was recently described by Bottomley[1] and closely followed in our laboratory. The test is based on the progressive incorporation of radioactive iron into heme, using optimal amounts of protoporphyrin and ferrous iron as substrates. The increase of specific radioactivity of extracted and crystallized heme[2] is linear during the first 30 to 60 min, and the incorporation of radioactivity may therefore be expressed per minute and number of cells originally present. The current investigations were performed on peripheral blood rather than bone marrow. After removal of plasma and the buffy coat, HS activity of washed and lysed red cells was measured. Prior to hemolysis red cells and reticulocytes were counted.

First we examined blood from 8 hematologically normal, healthy donors. The incorporation of radioactivity into heme per min and 5×10^9 red cells, including about 1.8% reticulocytes, was approximately 0.04% of initial radioactivity added. In contrast, incorporated radioactivity reached 0.5% in the blood of these same normal individuals when we attributed the HS activity to 10^9 reticulocytes. Both values appeared normal to markedly increased in the blood obtained from 6 patients with thalassemia major, and the measured activities were more variable (Table).

The ratio of iron incorporation into heme attributed to 10^9 reticulocytes (R) over that calculated per 5×10^9 red cells, including reticulocytes in the percentages indicated (E), again was more variable in the thalassemia group. A decrease in the ratio would indicate that relatively more HS activity resides with red cells which have matured beyond the stage of the reticulocyte, i.e. HS activity remains longer with maturing red cells or, alternatively and more likely, the proportion of young red cells is increased.

Our results do not reflect mechanisms controlling heme synthesis by normal and thalassemic reticulocytes or red cells. Heme synthesis is indeed influenced by the availability of the protein moiety of hemoproteins[3], although the mechanism of reduction of heme synthesis in thalassemia is not well understood[4]. Earlier studies of Steiner et al.[5] on HS of bone marrow suggested that this enzyme also was reduced in thalassemia. However, HS values expressed per number of bone marrow erythroblasts include the pool of ineffective erythropoiesis in this disorder. Our present results indicate that HS activity of *circulating* red cells and/or reticulocytes of thalassemic blood is normal or increased, rather than reduced as a consequence of impaired globin synthesis[6].

Zusammenfassung. Hämsynthetase (HS)-Aktivität zirkulierender Retikulozyten und Erythrozyten von Normalspendern und Patienten mit Thalassämie wurde in vitro bestimmt. HS war normal oder erhöht bei Thalassämie. Jugendliche, über das Stadium des Retikulozyten hinaus gereifte Erythrozyten enthalten wahrscheinlich noch einen signifikanten Anteil der im peripheren Blut messbaren HS-Aktivität.

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Heme synthetase activity of normal and thalassemic hemolysates

^{59}Fe incorporated into heme/min/number of cells (%)	Normal donors (mean ± standard deviation)	Thalassemia (range)
5×10^9 erythrocytes (E)	0.037 ± 0.017	0.09 — 4.0
10^9 reticulocytes (R)	0.530 ± 0.244	0.70 — 7.3
reticulocyte number	av.: 1.8%	1 — 14%
ratio *R/E*	av.: 14.3	1.3 — 20.4

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