No kinin activity was detected in 9 healthy persons, and only 1 showed the value of 2 mμg/ml. In the asthmatic patients, various kinin contents were observed: no significant difference was found in the kinin levels between healthy persons and the patients belonging to the groups 1 and 2. In group 3, however, 5 cases showed extremely high values of blood kinin and the remaining 6 patients showed relatively higher kinin levels.

Mean values of kinin contents in each group were calculated; they were: 0.20 ± 0.60 (standard deviation) for healthy persons, 0.32 ± 0.66 for group 1, 0.33 ± 0.66 for group 2 and 2.9 ± 2.7 mμg/ml for group 3. Almost 10 times greater values than that for the normal group were found in group 3.

Our experiments have shown that the circulating plasma kinin is significantly increased in most patients with severe bronchial asthma. It was an interesting finding that higher contents of kinin were obtained in the more severe forms of asthma. These results strongly suggest that kinin release is somehow involved in bronchial asthma[7].

Zusammenfassung. Bei 33 Patienten mit Asthma bronchiale von verschiedenem Erkrankungsgrade wurde der Kiningehalt im zirkulierenden Blut bestimmt. Erhöhte Blutkininwerte wurden in den Patienten gefunden, und zwar im allgemeinen mit der Schwere der Krankheit korrelierbar. Daraus folgt, dass das «Kinin» aetiologisch mit Asthma bronchiale verknüpft ist.

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Relation of ATPase Activities to Iron Uptake in Rabbit and Cat Erythroid Cells

Mammalian erythrocytes are known to differ in 'sodium pump' activity. For example, cat erythrocytes, in contrast to rabbit erythrocytes, are unable to extrude Na^+ and accumulate K^+ against concentration gradients. Also cat erythrocytes are relatively low in Na^+-K^+ stimulated adenosinetriphosphatase (NaKA)[1]. Genetically significant differences in 'sodium pump' activity have been found within individuals of a given species. Tosteson obtained a direct correlation of active transport of cations in sheep erythrocytes, known to be high (HK) and low (LK) in potassium, with NaKA fractions[2]. Baker and Simmons have shown that individuals of the Australian marsupial species, the possum (*Trichosurus vulpecula*) differ in sodium and potassium concentrations[3]. The LK possum has a low erythroid K^+ characterized by absence of NaKA in the cell membrane.

Since preliminary experiments indicate that ouabain retards accumulation of iron in the rabbit reticulocyte speculation is raised as to whether NaKA somehow is involved in iron transport[4]. Recently Sheeler and Barber followed iron incorporation in rabbit and turtle reticulocytes[5]. Cells were produced by injection of phenylhydrazine. They found that more iron was retained in the stroma fraction of the red cell hemolysate of the turtle than the rabbit. In this respect turtle and avian reticulocytes are alike[6]. However, it is known that the red cell stroma of birds, reptiles and amphibians, in contrast to mammals, is comprised of cell nuclei as well as membrane[7].

In the present study rabbit and cat erythrocytes and reticulocytes have been analyzed for ATPase, total and NaKA, and these activities correlated with cellular iron uptake. The experimental animals were New Zealand rabbits and mixed breeds of cats. Reticulocytosis was induced by injecting 0.25 ml/kg of 2.5% aqueous phenylhydrazine daily 4 consecutive days. Blood was drawn by cardiac puncture on the 7th day. Cats were less refractory to the compound as several did not survive the treatment. The reticulocytes, identified with New Methylene Blue, increased to 55–65% of the blood cells. For simplicity, the blood cells are referred to as cells, or reticulocytes.

Cell Iron Fractionation: Cells were washed 3 times with isotonic (0.28 *M*) tris [2-amino-2-(hydroxymethyl)-1,3 propanediol], brought to pH 7.2–7.4 with HCl. 5.0 ml of packed cells were then suspended in 95.0 ml of the *tris*-Cl solution. A 1.0 ml aliquot of this cell suspension was introduced into each of a number of test-tubes which were centrifuged 1 min at 750 *g*. The cells in each tube were resuspended in 5.0 ml of solution containing 125 m*M* NaCl, 5 m*M* KCl, 1 m*M* $MgCl_2$, 19 m*M* *tris*-Cl buffer and 10^{-8} to 10^{-7} *M* iron (Fe^{59} labeled[8]). Each preparation was then agitated in a water bath 4 h at 37 °C, after which it was centrifuged 1 min. Again the cells were resuspended, this time in 2.0 ml of isotonic NaCl solution containing non-radioactive $FeCl_3$ in identical molar concentration. After a repeated centrifugation the cells were hemolyzed with 2.0 ml water. This preparation was finally centrifuged 3 min. Radioactivity was assayed with a Nuclear-Chicago DS5 well-scintillation detector, attached to a 181A decade scaler. Fe^{59} was counted in the hemolysate (total) and supernatant fraction separately. Stroma Fe^{59} was considered as the difference in counts.

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Measurement of ATPase activity: the preparation of blood cell ghosts and evaluation of enzyme activity was based on the methods of POST et al., described for human erythrocytes[9]. However, 20 mOsm *tris*-Cl buffer, pH 7.4, was added to hemolyze the cells. DODGE et al. found this treatment removed more hemoglobin from the cell than water alone[10]. For determination of alkali ion-dependent activity the concentration of Na^+ was $0.08M$ and K^+ was $0.033M$. ATP-liberated phosphate was quantified by the method of FISKE and SUBBAROW[11].

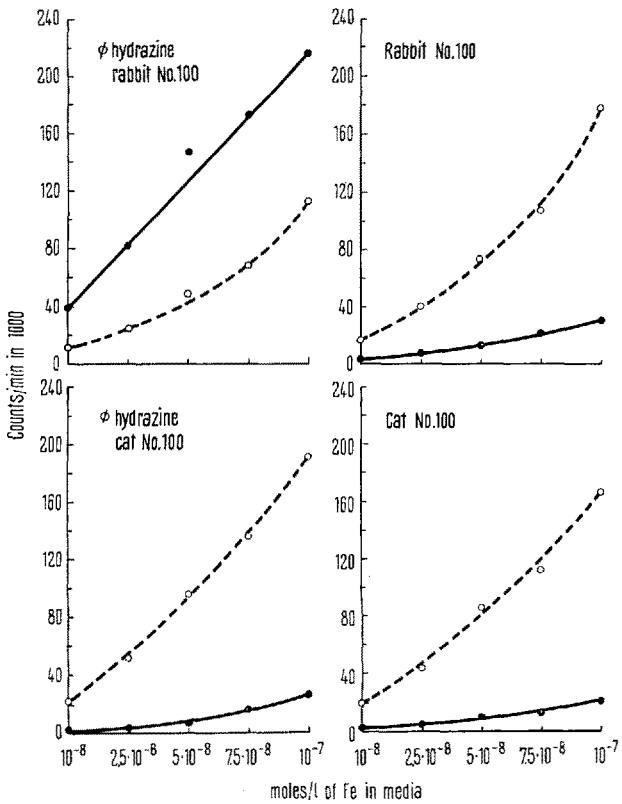

Fig. 1. Fe^{59} in stroma and water soluble supernate from rabbit and cat reticulocytes and erythrocytes after 4 h incubation in solutions containing several concentrations of iron. ●–● supernate, o–o stroma.

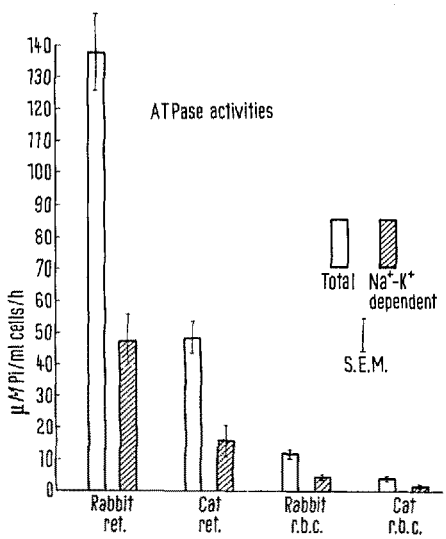

Fig. 2. ATPase activities, total and NaKA, in rabbit and cat reticulocytes (ret.) and erythrocytes (r.b.c.) (at least 8 animals in each group).

Fe^{59} in the stroma and supernate, as a function of several concentrations of iron, is shown in Figure 1. Reticulocytes are represented on the left, erythrocytes on the right of the figure. The specific activity of the radioactive compound was identical in the 4 experiments. It should be emphasized that the animals' sera were not present in the bathing fluids. JANDL et al. observed that human erythrocytes took up practically no iron when the metal was bound as transferrin by serum[12]. On the contrary, reticulocytes readily took up iron in this form.

Clearly the kinetics of iron movement was different in the rabbit reticulocyte. This cell, in contrast to the other rabbit and cat cells, delivered considerably more Fe^{59} from the stroma to the cell interior, as shown in the Figure. Other experiments, not recorded here, indicate the metal was largely in the water-soluble supernate independent of mitochondria or microsomes. In the cat reticulocyte and the rabbit and cat erythrocytes the Fe^{59} seemed to be relatively immobilized in the stroma. These findings suggest that the membrane of the rabbit reticulocyte was either less of a physical barrier or that it attached the free iron and removed it more actively into the cell interior. Iron in the stroma and supernate was practically equivalent in the cat reticulocyte and the rabbit and cat erythrocytes, as reflected in the figure. Finally it seems the amount of Fe^{59} taken up by all cells was positively correlated with the amount of Fe^{59} in the bathing fluid.

ATPase activities, total and NaKA, are shown in Figure 2. At least 8 animals comprised each group. The values for the rabbit and cat reticulocytes are higher actually than shown since these cells were admixed with reticulocytes. Only the rabbit reticulocytes were capable of delivering more iron from the membrane to the cell interior, as evinced by Fe^{59} in the stroma and supernate. Other studies show that cat marrow cells have higher concentrations of potassium and lower of sodium than cat blood cells, indicating the possibility of 'sodium pump' action in developing cells[13]. If ATPase or NaKA participates in any way with iron movement in the cell a comparatively higher level of activity seems to be required[14].

Zusammenfassung. Fe^{59}-Aufnahme in Kaninchen-Retikulozyten ist durch erhöhte Werte des Isotops in der wasserlöslichen Fraktion und erniedrigten Werten im Stroma der Katzen-Retikulozyten charakterisiert. Bei Kaninchen- und Katzen-Erythrozyten ist dieses Verhältnis umgekehrt. Na^+ und K^+ aktivierte ATPase zeigt die höchsten Werte in den Kaninchen-Retikulozyten.

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