nachzuweisen (Figur 1). Aufgrund der unterschiedlichen elektrophoretischen Wanderungsgeschwindigkeiten im Agargel lässt sich die Auftrennung von Insulin, Proinsulin und Intermediär erreichen (Figur 2). In Humaninsulinpräparaten, die nach den oben erwähnten Verfahren hergestellt werden, konnte aus disk- und immunoelektrophoretischen Untersuchungen (Figur 3) auf das Vorhandensein weiterer, noch nicht beschriebener Komponenten mit antigener Wirkung geschlossen werden.

Diskussion. Kreuzreaktionen von Insulinantikörpern mit Proinsulin, Intermediär und Dimer konnten bisher nur durch kompetitive Hemmung nachgewiesen werden. Mit einem mit Rinderinsulin stark präzipitierenden Ziegen-anti-Rinderinsulinserum war es möglich, die Kreuzreaktionen von Rinderproinsulin und Intermediär durch Immunpräzipitation nachzuweisen. Aus Figur 1 sind keine visuellen Unterschiede in der Stärke der Präzipitationslinien zwischen den einzelnen Komponenten zu erkennen. Durch Anwendung der Immunoelektrophorese im Agargel lassen sich antigene Komponenten in Insulinpräparaten analytisch nachweisen. Während bisher ausschliesslich diskelektrophoretische Verfahren zur Überprüfung von Insulinen auf ihren Gehalt an Proinsulin und den genannten Komponenten eingesetzt wurden, kann die hier beschriebene Methode als spezifischer und empfindlicher Nachweis für Insulin-verwandte Proteine verwendet werden. Die diffusen Präzipitationsbögen der proinsulinfreien Humaninsulinpräparate zeigen eine Insulin-verwandte Komponente mit geringerer elektrophoretischer Mobilität. Auch in der Diskelektrophorese liess sich diese Komponente nachweisen[9].

Schon STEINER et al.[5] hatten bei der Isolierung von Rinderproinsulin auf die Existenz von basischen, höhermolekularen Proteinen hingewiesen, die offenbar mit dem Proinsulin verwandt sind.

Die zur Zeit gebräuchlichen Methoden der kombinierten Gelfiltration und Ionenaustauschchromatographie sind zeitaufwendig und mit grösserem Substanzverlust verbunden. Die elektrophoretische Auftrennung im Agargel bietet eine Alternative zur Gewinnung hochgereinigter Insuline, analog dem Prinzip zur Glukagon-Insulin-Trennung[10].

Summary. Bovine insulin, proinsulin, and intermediate form were prepared by Sephadex gelfiltration and chromatography on DEAE-cellulose. Using a goat anti-insulin serum, the cross-reactions of proinsulin and intermediate form could be shown by immuno-precipitation. A method is described for the separation of proinsulin from insulin in agar gel. New compounds related to proinsulin or insulin were found in human insulin preparations by means of immunoelectrophoresis.

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Inhibition by Lanthanum Ions of ADP-Induced Platelet Aggregation

ADP-induced platelet aggregation requires extracellular divalent cations, especially calcium[1,2]. The function of this metal ion is poorly understood, although several mechanisms might be considered: formation of an active CaADP complex[3], Ca-uptake into the cells during aggregation, binding of Ca to the membrane, involvement in bridges between adjacent platelets in aggregates[4,5], and participation in an ecto-ATPase system[6]. It has been claimed that in muscle[7,8] and mitochondria[9,10] the rare earth metal ions interfere strongly with Ca binding and movements. We therefore investigated the effect of lanthanum ions on ADP-induced platelet aggregation.

Material and methods. Blood was drawn by venipuncture from healthy volunteers into citrate (0.11 *M* final concentration) or heparin (10 units/ml blood). Platelet-rich plasma (PRP) was prepared by centrifugation of the blood at room temperature for 15 min at $180 \times g$. Platelet aggregation was measured as the fall in optical density (O.D.) in PRP at 37 °C using an EEL platelet aggregation meter, model 169. ADP (sodium salt, from Sigma, St. Louis) was made up in 0.15 *M* NaCl (400 μM), kept frozen and diluted with 0.15 *M* NaCl to desired concentration before use. $LaCl_3$ (analytical grade) was made up in H_2O to a concentration of 132 m*M*, and was diluted to desired concentrations with 0.15 *M* NaCl.

Results and discussion. Unlike ions of the alkaline earth and transition group metals[11], La^{3+} did not cause platelet aggregation when added to either citrate or heparin PRP. In both heparin and citrate PRP platelet aggregation by ADP was markedly inhibited by La^{3+} in a concentration dependent manner (Figure). About 4 times as much La^{3+} was required to produce a given degree of inhibition with citrate as anticoagulant than with heparin. This most probably was caused by chelation of

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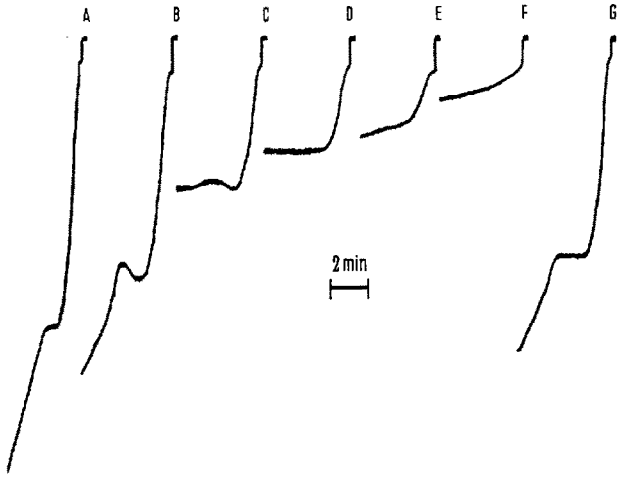
La^{3+} by citrate. Irrespective of anticoagulant used, the inhibition was always revealed by a reduction of the maximal change in optical density (O.D.$_{max}$) and only in a few experiments were the initial velocity of the change in O.D. affected. It should be noted that O.D.$_{max}$ refers to maximal degree of primary and not secondary aggregation[12,13], the latter phenomenon being caused by release of ADP from the platelets[13,14]. As seen from the Figure reduction of the magnitude of primary aggregation by La^{3+} beyond a certain value completely abolishes the release reaction. Whether La^{3+} inhibits the release reaction per se cannot be judged from these experiments, although the results are in accordance with the view that a certain degree of platelet aggregation is a prerequisite for release induction[15,16]. Preliminary results in our laboratory show that La^{3+} completely inhibits the thrombin-induced release of serotonin and acid hydrolases from washed platelets. Irrespective of platelet number, the release was only inhibited above 2 mM La^{3+}; no effect was observed below this concentration.

Addition of Ca^{2+} (up to 5 mM) to heparin PRP had no effect on the inhibition of aggregation produced by La^{3+} (up to 3 mM). This was expected, since La^{3+} and other rare earth metal ions are regarded[17] to have far greater affinity for anionic binding sites than Ca^{2+}. The affinity constant for $Y(ATP)_2^{3-}$ is about 10^6 times[18] that for $CaATP^-$. We have not been able to find any information in the literature as to affinity constants of complexes between adenosine phosphates and rare earth metals but that of Y and ATP. The great similarity between physical and chemical properties of Y^{3+} and La^{3+}, however, renders it highly probable that the affinity constant for $La(ADP)_2^-$ is much greater than that for CaADP and MgADP. The concentration of La^{3+} used in our experiments was 300 times or more that of ADP used, and of the same order of magnitude as the Ca concentration in plasma. Even if we assume that as little as 10% of the La^{3+} added to PRP remain freely ionized, 30 times more free La^{3+} than ADP^{2-} should be present at the lowest concentration of La^{3+} used in heparin PRP. With this concentration ratio almost all the ADP should be in the $La(ADP)_2^-$ form, and increase in La^{3+} above 300 μM should consequently not influence the concentration of $La(ADP)_2^-$. However, inhibition of aggregation continued to increase as the La^{3+} concentration was raised above 300 μM. Thus, the inhibition did not correlate with the formation of $La(ADP)_2^-$, nor with disappearance of CaADP or any other divalent metal-ADP complexes in PRP. In heparin PRP addition of 0.3 mM La^{3+} gave only 15–20% inhibition, indicating that CaADP is not a requirement for aggregation. This is in accordance with the findings[2] that the amount of Ca required for maximal aggregation was 150 times that necessary to convert 99.9% of ADP to the CaADP form. Therefore, Ca appears to serve other functions in ADP-induced platelet aggregation than merely providing CaADP; our experiments indeed show that the very unusual complex, $La(ADP)_2^-$, is able to induce aggregation.

The per cent inhibition of aggregation did not vary significantly with the ADP concentration (0.3–1.5 μM) at constant levels of La^{3+}. This could indicate that La^{3+} acted directly on the platelet membrane. If this occurred in a reversible manner, the inhibition should be dependent on the concentration ratio of La^{3+} to platelets. However varying the platelet concentration by dilution of PRP with autologous plasma at different La^{3+} and ADP concentrations had no effect on the per cent inhibition. This rather indicates that La^{3+} irreversibly destroys the aggregability of the cells in a way that at a given level of La^{3+} a certain percentage of the platelets became functionless.

The effect of La^{3+} was immediate, no preincubation was required. At La^{3+} concentrations above 3 mM heparin-PRP became increasingly turbid as the metal concentration was raised, suggesting protein precipitation. However, massive inhibition of platelet aggregation was present below 3 mM, and these La^{3+} concentrations did not alter the O.D. of PRP or plasma. Citrate PRP did not become turbid with La^{3+} concentrations up to 12 mM, nor did it clot.

In summary, platelet aggregation by ADP is inhibited by La^{3+}, but not in a way that suggests 1. interference with binding of Ca^{2+} to the platelet membrane, 2. interference with Ca^{2+} transport across the platelet membrane, 3. formation of $La(ADP)_2^-$ or 4. removal of CaADP. This lends support to the view that Ca does not participate in aggregation by uptake into or binding onto the cells, nor by formation of CaADP as the active aggregating agent. La^{3+} might inhibit by interference with a plasma factor necessary for aggregation, and the mechanism could as well be through interference with binding of



Effect of addition of $LaCl_3$ in increasing concentration on ADP-induced platelet aggregation. To 0.9 ml citrate PRP was added 0.1 ml of 0.15 M NaCl or $LaCl_3$ in 0.15 M NaCl followed by 0.1 ml ADP solution. Final concentration in mM of $LaCl_3$ were: A, 0.0; B, 2.4; C, 4.8; D, 7.2; E, 9.6; F, 12.0 and G, 0.0. The samples were aggregated in the direction from A through G. Final ADP concentration: 1.2 μM.

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Ca to such a cofactor. However, the strong, concentration-dependent inhibition by La^{3+} of release reaction in washed cells makes it more likely that La^{3+} does act on the cell, perhaps intracellularly. Quite recently O'BRIEN[19] also has shown La^{3+} induced inhibition of platelet aggregation.

Zusammenfassung. La^{3+}-Ionen zeigten eine konzentrationsabhängige Hemmung der ADP-induzierten Aggregation menschlicher Blutplättchen. Diese Hemmung ist nicht von der Zahl der Zellen oder der Konzentration des ADP abhängig und lässt sich nicht durch Zusatz von Ca^{2+}-Ionen neutralisieren.

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Effect of Normal and Irradiated Marrow Grafts on the Haemopoietic Recovery After a Sublethal Irradiation

A transitory accumulation of lymphoid-like cells in the bone-marrow after a sublethal irradiation has been reported by several authors[1–3]. These peculiar cells are called transitional cells[2,3] or X-cells[4].

In a previous report, we have shown that the lymphoid overshoot observed in mice after a 500 R irradiation[5] is not found in animals exposed to a lethal irradiation followed by a normal bone marrow graft[6]. This difference can be related either to the increase in the X-ray dose or to the presence of the injected marrow. In order to discriminate between these two possibilities, we decided to study the influence of a bone marrow graft in mice recovering from a 500 R irradiation.

Methods. Male RIII mice, 75 days old, were exposed to a 500 R X-irradiation under the following conditions: Siemens Stabilivolt apparatus, 190 KV, 18 mA, 0.5 mm Cu filter, 35 cm F.D., 210 R/min.

The mice were divided into 3 groups. 20 h after irradiation, the mice of the first group were injected i.v. with a suspension of normal isogeneic bone-marrow. The second group was treated as the first, except that the bone-marrow was harvested from donors irradiated at 500 R the day before. Each mice received 5×10^6 nucleated cells in 0.5 ml sterile Hanks solution. No additional treatment was given to the third group. In each experimental group, 4–6 animals were killed every other day from the 2nd to the 20th day. Additional mice were sacrificed at the 24th day.

The haematocrit and the leukocyte counts were measured. The total number of nucleated cells in 2 femurs was counted by means of an electronic counter. Marrow smears were prepared and stained by May-Grunwald-Giemsa method. The results of the myelograms were expressed in absolute number of cells for 2 femurs.

Results. The variations of the haematocrit, the leukocyte counts and the total number of marrow nucleated cells are closely similar in our 3 experimental models (Figure 1). The haematocrit is slightly decreased between the 4th and the 20th days. The leukocyte counts show a fast drop followed by a very slow recovery, the values found after 24 days being still below the control values. After a fall, during the first post-irradiation days, the total number of the marrow nucleated cells increases progressively; at the 20th day, normal values are reached again.

The results of the myelograms are plotted in Figure 2. The marrow injection does not influence the recovery of the myeloid and erythroid series. The differences concern the lymphoid-like cells. They rebound over the normal level from the 10th to the 14th day in the non-injected mice. This overshoot is also present in the animals grafted with irradiated marrow. No rebound can be detected after normal marrow injection.

Discussion. In 500 R irradiated animals, we reproduced the 'lymphoid' rebound already observed in previous experiments[15]. This overshoot was not modified by the administration of marrow irradiated 24 h prior to grafting. On the contrary, after a normal bone marrow graft, no lymphoid rebound could be produced.

Two mechanisms can be proposed to explain the absence of lymphoid overshoot. One could conceive that some cellular factor similar to the chalone exists in the normal

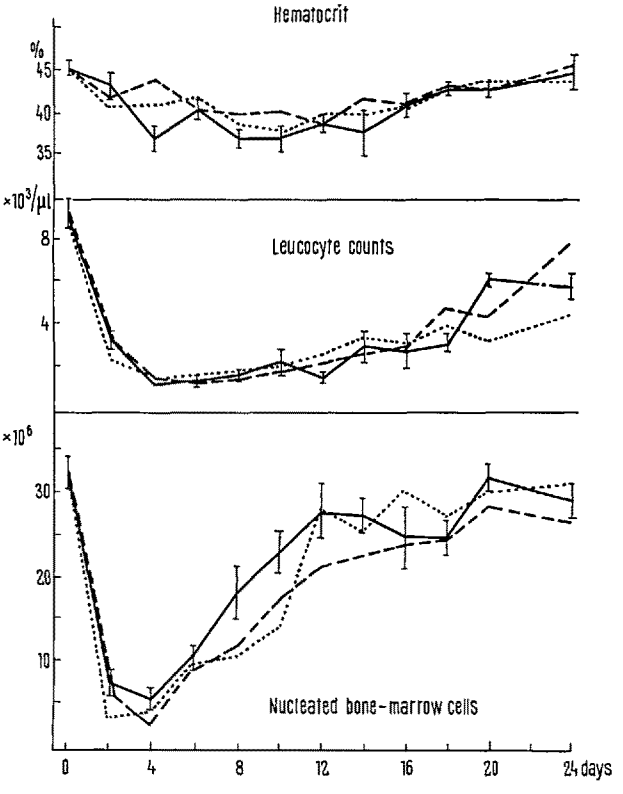

Fig. 1. Haematocrit values, leukocyte counts and bone marrow nucleated cell numbers from 2 femurs after a 500 R irradiation (——), a 500 R irradiation combined with a normal marrow graft (– – –) and a 500 R irradiation combined with an irradiated marrow graft (···).