4 min values. On the assumption that the plasma volume in the skin did not change during the 2 h interval, the radioactivity due to the transvascular-passed albumin-I^{131} in this organ was calculated. These values were 15,285 cpm, 13,977 cpm and 12,041 cpm, respectively. This indicates that the transvascular-passed labeled albumin of the experimental groups compared to the normal control is 91.4% for the amyloidic and 78.8% for the strep-antigen group. Thus, the data suggest that plasma clearance is delayed and transvascular passage of the labeled albumin into the skin is reduced at 2 h. Since the radioactivity in the sera of immunized animals at 2 h is even slightly higher than that of the normal control, any uptake of the radioactivity from the pool by other organs could not have decreased the transvascular passage of albumin-I^{131} into the skin. The results are consistent with the original hypothesis that immunization may have affected the architecture of the interstitial space-ground substance[5].

Zusammenfassung. Mäuse wurden mit Kasein- und Streptokokkus-Antigen injiziert. In den immunisierten Tieren ist die Ausscheidungsgeschwindigkeit von i.v. injiziertem Albumin-I^{131} aus dem Plasma erniedrigt, und ebenso ist im Vergleich zu den Kontrolltieren der transvaskuläre Transport in die Haut reduziert.

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An Attempt at Colonization of W^vW^v Anemic Mice by Rat Hemopoietic Tissue

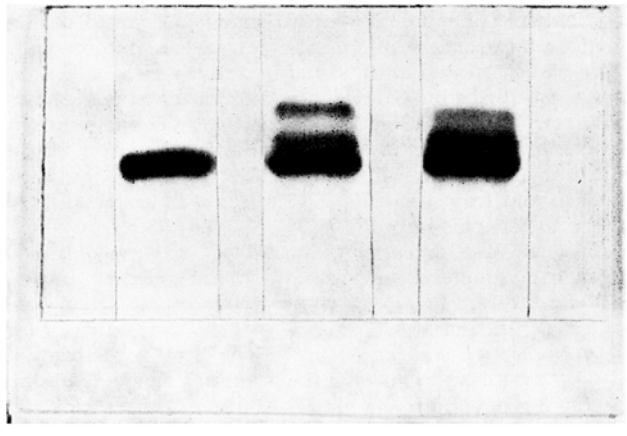
Anemic mice of the W series can be transplanted with normal mouse hemopoietic tissue. BERNSTEIN and RUSSELL[1] showed that syngeneic tissue would implant without previous irradiation of the host, and SELLER and POLANI[2] later used allogeneic tissue. This was administered during the immediate neonatal period before immunological competence is established. Red cells of donor origin could be demonstrated in the circulation of the transplanted animals for the rest of their lives[3], and when hemopoietic tissue bearing chromosome markers was used, the donor cells could be directly observed in the host[4].

In irradiation experiments in mice, hemopoietic tissue is administered to circumvent the death which normally follows a high dose of irradiation. It has been shown that this is effective not only with mouse hemopoietic tissue but also with rat[6] and hamster[6] tissue. Recovery, although often only temporary, was due to repopulation of the host by the donor tissue[7].

Thus, if the immunological barrier is overcome and the environmental conditions in the host are suitable, hemopoietic tissue from one species will live and proliferate in a member of another species. It was wondered whether rat hemopoietic tissue would implant in the W series anemic mice following administration immediately after birth. If successful, ways would exist for testing the chimerism established which are not available when mouse hemopoietic tissue is used.

The method used was as previously described[2]. A suspension of liver cells obtained from 19- to 20-day-old foetal Wistar or Sprague-Dawley rats was injected i.v. into mice of the genotype W^vW^v within the first day of life. When these animals were adult, various studies were made on them for evidence of implantation of the rat hemopoietic tissue. Red blood cell counts were made by conventional hemacytometry, using Hayem's fluid as the diluent. The hemoglobins were studied by electrophoresis on cellulose acetate paper, as previously described[3]. The presence or absence of alkaline phosphatase activity in the granulocytes was detected histochemically on blood smears[8]. Plasma proteins were separated by electrophoresis on a horizontal starch gel according to the method of SMITHIES[9].

Initially 10 to 12×10^6 rat foetal liver cells were injected into the new-born W^vW^v recipients, but no evidence of implantation was observed in the first 6 treated mice which survived to maturity. Experiments on the induction of tolerance to foreign tissue in mice suggest that the wider the antigenic disparity between the donor and the host, the greater the number of cells



Electrophoresis on cellulose acetate paper of hemoglobins of a W^vW^v mouse, a rat and a W^vW^v mouse successfully transplanted with rat hemopoietic tissue.

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required to confer the tolerance[10]. Consequently the dose was raised to 17 to 23 × 10^6 cells, but larger numbers of cells were generally lethal to new-born mice. Following this treatment, 24 mice survived to maturity, and only 1 of these was successfully colonized by the rat hemopoietic tissue.

The red blood cell count of this mouse was increased from the anemic level of approximately 7 × 10^6/mm^3 to a count of up to 10.9 × 10^6/mm^3. Electrophoresis of red cell lysates revealed the presence of rat hemoglobin (Figure). The granulocytes when stained histochemically showed high alkaline phosphatase activity in the cytoplasm, typical of the rat. Mouse granulocytes do not have this enzyme.

Since serum proteins, with the exception of the γ-globulins, are manufactured by the liver[11], a study of these may indicate whether, in addition to the stem cells in the foetal liver cell inoculum, any parenchyma cells had also implanted. Electrophoresis of plasma samples, however, showed no evidence of any rat plasma proteins. This method, using starch gel, is not highly sensitive, and trace amounts of rat protein may well have been present, but it was felt that the existence of any fairly large functioning implants of rat liver tissue could be excluded. No immunoelectrophoretic studies were made of the γ-globulins.

Clinically, the animal appeared runt. Its growth was stunted and it developed dermatitis. It was sacrificed for chromosome studies at the age of $3^1/_2$ months. At autopsy it had gross splenomegaly, and the thymus was small, but all the other organs appeared normal to macroscopic examination.

Mitotic chromosome studies were made following Colcemid (CIBA) treatment using an air dried method[12]. These revealed that the bone marrow consisted exclusively of rat cells. The spleen, too, had a large proportion of rat cells (99%), and the lymphoid tissue (obtained from cervical, brachial, axillary and inguinal lymph nodes) was composed of 40% rat cells. The minute thymus yielded few cells, and no mitoses could be found.

It would appear, therefore, that a xenogeneic hemopoietic tissue graft, though difficult to achieve, can exist in the W^vW^v anemic mice. However, the gross antigenic difference between donor and host is not entirely without consequence and runt disease ensues. In the bone marrow the foreign cells have such a selective advantage over the defective host that they completely replace them. It is of interest that granulopoiesis has been completely taken over by the donor cells. This suggests that, in addition to erythropoiesis, granulopoiesis is also defective in the W^vW^v mice[13].

Zusammenfassung. Speziesfremdes, hämopoietisches Gewebe wurde neugeborenen, anämischen W^vW^v-Mäusen implantiert. Die Folgen davon werden analysiert.

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Long-Term Persistence of Bovine Serum Albumin when Injected into the Amphibian *Xenopus laevis* Daudin

The use of pure proteins to stimulate antibody production in amphibians has met with varying success. Proteins of low molecular weight, such as albumins and globulins, have produced negative results when injected into some amphibians[1-4], whereas other amphibians respond by producing circulating antibodies[2,5-7]. Positive results are also obtained when proteins of high molecular weight, such as hemocyanins, are used[8].

Investigations in this laboratory on furthering knowledge of the immune response in *Xenopus laevis* (South African clawed toad) show that toads of our colony readily produce agglutinins to sheep red blood cells, but attempts to stimulate antibody production to bovine serum albumin (BSA) have failed. During these experiments the observation was made that the injected BSA was not cleared from the circulation but persists for many months.

Materials and methods. 8 male and 8 female adult *Xenopus* (average weight 50 and 100 g respectively) were injected with BSA in these experiments.

Three experiments were set up. In experiment 1 the antigen was administered by different routes. 6 toads (3 males and 3 females) received a series of 3 injections of BSA (Armour bovine albumin powder, fraction V from bovine plasma) in 0.85% saline at weekly intervals. Each injection contained 2 mg BSA for male animals and 4 mg for females. 2 animals (1 male, 1 female) were injected into the dorsal lymph sac, 2 (1 male, 1 female) i.p. and the last 2 i.m. As a control to this experiment 3 animals were given a series of 3 injections of 0.85% saline at weekly intervals. Each animal was injected throughout by one of the routes mentioned above.

In experiment 2, antigen was administered initially with complete Freund's adjuvant into the dorsal lymph sac of 1 male and 1 female animal. The former was given 2 mg and the latter 4 mg BSA. This initial injection was followed by 2 booster injections of BSA in saline at weekly intervals given i.p. Each of these injections contained 2 mg BSA for the male animal and 4 mg for the female. Animals

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