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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in these 2 experimental groups were bled 7, 14, 28 and 87 days after the last injection, when they were killed.

Experiment 3 was designed to investigate the persistence of the antigen. 8 animals received a single injection of 10 mg BSA in saline into the dorsal lymph sac. 26 weeks after this injection blood samples were taken to test for the persistence of the antigen. These animals are still living and further tests will be made.

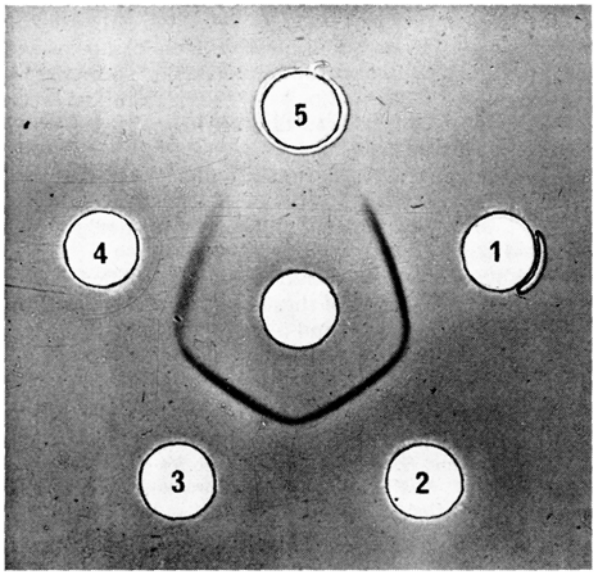
All animals were kept at 23 ± 1 °C. Blood samples were obtained by puncturing the Sciatic vein and the serum tested by means of double diffusion on agar micro plates, using Hyland immuno plates pattern B. *Xenopus* serum was tested against (a) rabbit anti-bovine albumin (Hyland) for the presence of BSA and (b) various dilutions of BSA for the presence of precipitating antibody to this protein. All animals were tested before initial injections of BSA and then again at the times mentioned above.

Results. The tests on control animals and the pre-injection tests on experimental animals showed that there were no proteins in *Xenopus* serum that cross-react with antiserum to BSA produced in rabbits and that there were no naturally occurring precipitins to BSA. No precipitins to BSA were detected in the sera of experimental animals.

Samples from all 3 experimental groups when tested against rabbit anti-BSA consistently gave strong precipitation lines indicating that BSA was present in the serum. Such lines never occurred in control animals.

Animals of groups 1 and 2 were killed at 87 days after the last injections. At this time their sera still showed strong precipitation lines to anti-BSA. In the third experiment, in which toads were intentionally kept for 26 weeks before testing, the precipitation lines were likewise still strong. This shows that BSA was still present in the circulation 6 months after it had been injected (Figure).

Discussion. The phenomenon of long-term persistence of injected proteins of low molecular weight has been previously noted in several lower vertebrates. KASTER and SCHECHTMAN[9] found that human serum albumin persists in the anuran, *Rana pipiens*, for 2–3 months.



Precipitation lines after immunodiffusion on agar microplate. Experiment 3. Wells 1–4 contain serum of 4 individual *X. laevis*, taken 26 weeks after injection of 10 mg BSA into the dorsal lymph sac. Well 5 contains serum from a control, non-injected toad. In the central well is antiserum to BSA (produced in a rabbit). × 4.

CHING and WEDGWOOD[4] working on the Axolotl, *Siredon mexicanum*, showed that human gamma globulin (HGG) persists for 45 weeks. In another lower vertebrate, the rainbow trout, BSA persists for 138 days[10]. MAUNG[11] in his work on reptiles showed that the albumin fraction of normal rabbit serum persists for 7 months.

It is quite possible that the doses of protein used in all these experiments paralysed the animal's immune system. CHING et al.[4] mentioned this point in their work on the Axolotl, but found that persistence of HGG occurred when as little as 1 mg of protein was injected. Even if paralysis is occurring in these animals, the time the antigen remains circulating is remarkable when compared with mammals. If the latter are injected with heterologous proteins of low molecular weight their half-life during non-immune elimination, prior to antibody synthesis, is only a few days[12] as it is when BSA is injected into neonatal and tolerant adult rabbits[13].

Previous authors working on *Xenopus* have also been unable to demonstrate antibody formation to BSA (ALCOCK[1]) or to bovine gamma globulin (ELEK, REES and GOWING[3]), although these toads are capable of antibody production to high molecular weight proteins[8] and to particulate antigens[1,3]. ALCOCK also showed that the common frog, *Rana temporaria*, could not produce antibodies to BSA. Nevertheless some anurans[2,5–7] are capable of responding to proteins of low molecular weight administered in doses comparable to those used in the present experiment. This difference in response given by different anurans is of interest. One possible explanation may lie in the differences in antigen-trapping mechanisms that these animals possess. The marine toad, *Bufo marinus*[6,7], and the larvae of the bullfrog, *Rana catesbeiana*[5], are both able to produce antibodies to BSA and in both lymph nodes occur, namely the jugular bodies of *B. marinus*[14] and the lymph glands and ventral cavity bodies of *R. catesbeiana*[15]. To my knowledge such lymphoid organs have not been described in *Xenopus*. In fact some earlier work by STERBA[16] suggests that jugular bodies do not occur in adult *Xenopus*.

In view of the long-term persistence of BSA demonstrated in this experiment, antigen-trapping mechanisms of *Xenopus* require further investigation.

Résumé. Dans ces expériences le *Xenopus laevis*, maintenu à 23 °C, n'a pas formé d'anticorps en présence de l'albumine du sérum bovin (BSA). La BSA se conserve plusieurs mois dans le sérum de tous les animaux expérimentaux.

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