as described elsewhere[13]. Pools of plasma were made from rats similarly treated. The total dose was divided into 2 halves, and were injected s.c. 24 h apart. First injection was made on 7th day of the post hypoxia period.

Results and discussion. Table I shows the results of the erythropoietic activity of young rats and Table II of the adult rats. The 48 h ^{59}Fe uptake of mice injected with plasma of normal rats did not differ from saline injected controls. The highest erythropoetic activity was, as expected, found in the plasma of normal stimulated rats (kidney present).

The most important and new finding is the marked reduction in extrarenal EP titers when submandibular glands were removed: The average ^{59}Fe uptakes were 1/4 in the case of young rats and 1/3 in the adults, compared to anephric rats but with submandibular glands left intact. Since the relationship between ^{59}Fe incorporation and EP titers is logarithmic, it means that a several time reduction on EP production after removal of those salivary glands had occurred.

Therefore, a reasonable interpretation would be that submandibular glands are a site of extrarenal EP production, either directly or through a mechanism similar to the renal erythropoietic factor (REF) described by GORDON et al.[14]. In the present study, we are not able to differentiate the mechanism of the hormone production[15].

Resumen. La extirpación de las glándulas submandibulares en ratas machos nefrectomizadas produjo una marcada disminución en la actividad eritropoyética del plasma, medida por la incorporación de ^{59}Fe en ratones policitémicos. Este hallazgo es compatible con la formación de eritropoyetina extra renal en esas glándulas salivares.

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Enhancement of 'Memory Cell' Pool by Polyanions in Mice

It has been shown that polyanions, (PA) enhance the primary immune response to sheep red blood cells (SRBC) in mice[1,2]. Experiments reported previously[3] concerning the effects of polyanions on the secondary response to SRBC in mice indicated that PA may enhance not only the number of antibody forming cells, but possibly also the pool of memory cells. For instance, mice primed with an optimal dose of antigen and injected with dextran sulfate (DS) at the time of priming, give a slightly but significantly higher secondary response than animals primed with antigen alone[3]. On the other hand, it has also been reported that both anti-SRBC IgG and anti-SRBC IgM injected together with SRBC enhance the ImR to SRBC[4], and that the mechanism of stimulation of the ImR by antibodies might be based on specific accumulation of the antigen in the spleen[4]. Since mice primed with SRBC and injected with DS at the time of priming showed enhanced anti-SRBC titers at the time of antigen challenge in comparison to mice primed with antigen alone[3], it could not be decided whether enhancement of the secondary ImR in the DS pretreated mice was due to an acceleration of the antigen uptake by an antibody-mediated mechanism suggested by DENNERT et al[4] or to an increase in the number of 'memory cells' in lymphoid tissues.

The present experiment was designed to determine wheter polyanions really influence the memory cell pool in mice primed with SRBC.

Groups of C57 B1/6J, mice, 6 animals per group, 8–10 weeks old, were injected either with polyanion (dextran sulfate, molecular weight 5×10^5, 1 mg/mouse

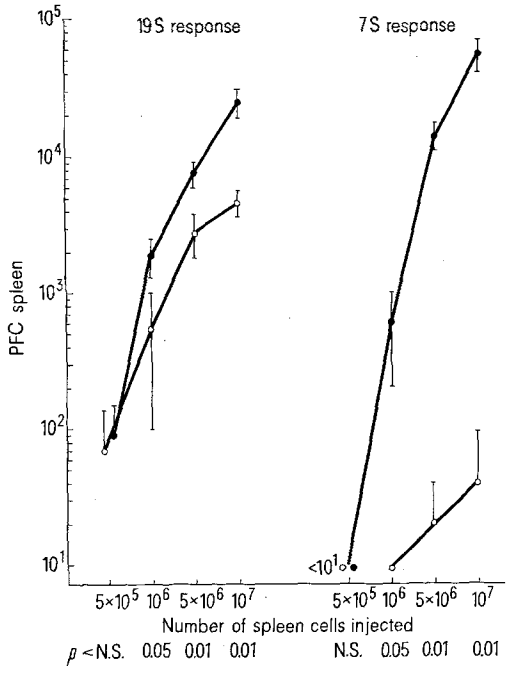

Fig. 1. PFC response to SRBC in irradiated C 57 B1/6J mice injected with graded numbers of spleen cells derived either from dextran sulfate and SRBC primed (●) or from SRBC primed syngeneic mice (○). PFC response assayed 7 days after cell transfer and antigen inoculation. Each point represents average values obtained in 6–8 mice. The standard errors are shown by vertical bars. N.S. = not significant.

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given 1/2 h prior to antigen i.p.) and then primed with a sub-optimal dose of antigen (2×10^6 SRBC i.p.) or primed with a sub-optimal dose of antigen (2×10^6 SRBC i.p.)alone. The mice were killed 21 days later and the spleens of each group pooled separately. Graded numbers of spleen cells, derived either from polyanion-injected and antigen-primed, or from antigen-primed animals, were injected i.v. together with an optimal dose of antigen (4×10^8 SRBC) into irradiated, syngeneic 9-11-week-old recipients. The recipient mice had been irradiated with 600 r (80 r/min) and injected, within less than 4 h after irradiatin, with a mixture of spleen cells and SRBC. Seven days later the number of direct (19 S) and indirect (7 S) plaque-forming cells (PFC) in the spleen, as well as the total and 2-mercapto-ethanol-resistent hemolytic titers in the serum of the recipient mice, were assayed as describetd earlier[2]. For statistical analysis the Wicoxontest was used[5]. The effects were considered significant when $p < 0.05$.

As shown in the Figures 1 and 2, irradiated recipients injected with adequate numbers of spleen cells derived from DS-treated and antigen-primed animals gave a significantly higher ImR to SRBC than recipients injected with the same number of spleen cells derived from animals primed with SRBC alone. Moreover, a typical secondary response (predominantly 7 S antibody formation) could be obtained only in recipients injected with an adequate number of spleen cells derived from DS-treated and antigenprimed mice. Similar results (data not given here) were obtained using spleen cells derived from donors 10 or 42 days after antigen priming or when polyacrylic acid was used instead of DS. It is generally accepted that cooperation of thymus-dependent antigen-carrying lymphocytes (T-cells) and bone marrow-derived lymphocytes (B-cells) is required for primary and for secondary humoral response to SRBC. It has also been shown that memory cells are present in both T-cell and B-cell populations. (for review see[6]). In the present report it has been demonstrated that polyanions enhance the number of memory cells, and it must be assumed that both T- and B-cell memory is enhanced by polyanions, since a profond 'T-cell'-dependent 7 S memory seems to be present only in polyanion-treated mice. If this assumption is correct, polyanions must act on both T- and B-cell populations. Indeed we reported previously that polyanions in general are mitogenic for spleen cells[7]. Moreover it has recently been shown that polyanions activate DNA-Synthesis in B-cells[8] and, to a lesser degree in T-cells[9] in vitro. It seems possible that polyanions act on ImR by increasing the rate of proliferation of immunocompetent B- and T-cells.

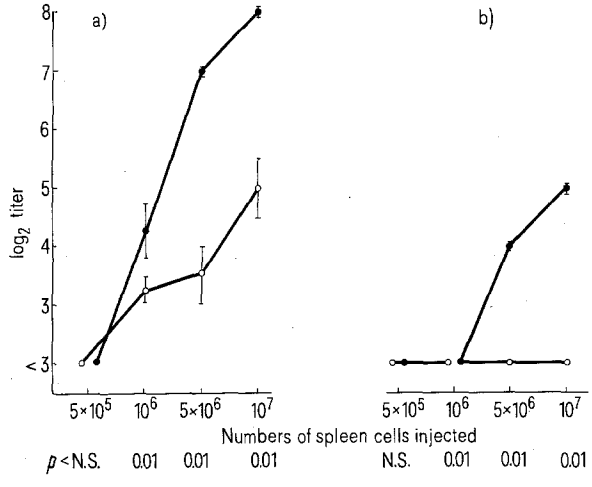

Fig. 2. Reciprocal hemolytic anti-SBRC-titers in irradiated C 57 B1/6J mice injected with graded numbers of spleen cells derived either from dextran sulfate and SRBC primed (●) or form SRBC primed syngeneic mice (○). a) total antibody titers (19S + 7S). b) 2-mercaptoethanol resistent (7S) antibody titers. Antibody titers were assayed 7 days after cell transfer and antigen inoculation. Each point represents average values obtained in 6–8 mice. The standard errors are shown by vertical bars. N.S. = not significant.

Zusammenfassung. Mit 600r bestrahlte Mäuse wurden mit Milzzellen von Spendermäusen, die entweder mit Antigen allein (Kontrollen) oder mit Antigen und einem Polyanion vorbehandelt wurden, injiziert. In den Recipienten, die mit Milzzellen von mit Polyanion vorbehandelten Mäusen injiziert wurden, konnte eine gegenüber Kontrollen signifikant erhöhte (vorwiegend 7 S) Immunantwort gemessen werden. Die Ergebnisse lassen vermuten, dass Polyanionen sowohl auf die B- als auch auf die T-Zellen wirken.

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An Improved Method for the Study of Reagin-Mediated Mast Cell Degranulation in Rats

There are three main methods for demonstrating homocytotropic or reagin-mediated antigen-antibody reactions and their inhibition in rats: Passive cutaneous anaphylaxis (PCA)[1], histamine release[2], and degranulation of mast cells[1] can be studied. The latter method is particularly useful in separating the inhibitory actions on mediator release (antiallergic actions) of drugs from simple antihistaminic ones. Drugs with antiallergic properties are valuable in antigen-induced asthma; they inhibit the PCA reaction as well as the degranulation of mast cells. Antihistaminic drugs inhibit the PCA reaction without

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