MSE Centriscan 75 analytical ultracentrifuge at 55,000 rpm).
3. Viscosities were measured at 37 °C in a Chaix-Meca coaxial-cylinder viscosimeter (CNRS-Anvar license).
4. The electrophoretic migration was measured on cellulose-acetate (Cellogel) at pH 9.2 and at 150 V, and the electropherograms were stained with Ponceau red and scanned on a Chromoscan (Joyce Loebl). Electrofocusing was conducted for 1.5 h at 1200 V on a 3.5–9.5 pH gradient established with Ampholine (LKB), and the gels were stained with amido-black.
5. The susceptibility to oxidation was determined by measuring the methemoglobin level after polymerization (Evelyn and Malloy's method[6]).
6. The dissociation curve was established spectrophotometrically by the method of Labie and Byckova[7] at pH 7.1 with a DBGT Beckman spectrophotometer. The binding capacity and p 50 were measured as described by Teisseire[8].

Results and discussion. Both the chromatographic behavior (figure 1) and the calculated sedimentation rate show that the mol.wt has not been multiplied and that no polymerization took place. However, carbodiimide reacted with the polypeptide chains, as the shape of the dissociation curve suggests.

The viscosity was not higher than that of unreacted hemoglobin at the same concentration: 1.06 CP versus 1.00 CP (10 determinations). This finding also favors the conclusion that the molecules obtained had an unchanged molecular weight.

When electrophoresis was done at pH 9.2 on Cellogel, the 'polyhemoglobin' migrated less far towards the anode than did hemoglobin, which means that its negative charge is lower. This result was confirmed upon isoelectric focusing, where the band extended from pH 7 to pH 9. This finding may be due to a variable total charge arising from the many opportunities that hemoglobin polypeptide chains offer for the creation of peptide bonds. The methemoglobin levels remained below 4%.

The dissociation curve for oxyhemoglobin reveals an increase in the affinity: The p 50 was therefore very low: 2 to 4 mm Hg in standard conditions instead of 26.6 for normal human blood and 17.5 for 2–3 DPG depleted stored human blood or hemolysates (pH 7.4, pCO_2 40 mm Hg and 37 °C). Also, the oxygen-binding capacity decreased from 1.34 to 0.9 at 1 ml O_2/g hemoglobin. This show a partial denaturation of hemoglobin.

In conclusion, the carbodiimide that we used, like other reagents recently tried (divinylsulfone, 1-4 butanediol diglycidyl ether), did not really polymerize hemoglobin (unpublished results). The unsuccessful attempt reported here shows once more that hemoglobin, because of its unusual structure and properties, resists attempts to modify its polypeptide chains. The change in shape of the hemoglobin dissociation curve resembles that observed when the pigment is coupled with albumin or other macromolecules. Interestingly, all these compounds are more stable than in the free state. Other agents likely to induce the formation of high-mol.wt polyhemoglobins with a less distorted dissociation curve should be tried.

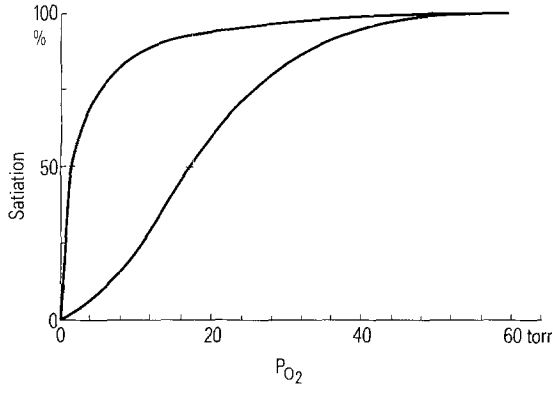

Fig. 2. Barcroft's curve obtained with hemoglobin before treatment with EDCI (right) and after treatment (left). p 50 are respectively 17,4 and 1,7 Torr. Curves are obtained in standard conditions with the Dissociation Curve Analyzer described by Duvelleroy (in Teisseire et al.[8]).

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Immunological recovery of thymectomized and sham-thymectomized lethally irradiated mice reconstituted with syngeneic bone marrow cells

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Summary. Immunological functions of lethally irradiated mice reconstituted with syngeneic bone marrow cells recover after 5–6 weeks. In mice that had been thymectomized before irradiation and reconstitution, T-cell function is deficient but the B-cell function is preserved.

The bone marrow cells given to lethally irradiated mice require the presence of the thymus in order to reconstitute the host immune potential[3]. Animals that had been thymectomized before irradiation and reconstitution have substantially impaired T-cell-dependent immune reactivity[4]. Accordingly, thymectomized, irradiated, bone marrow reconstituted (TIR) mice were used by numerous investigators as T-cell deficient animals for various purposes, e.g. to grow xenogeneic tumors[5], to study chemical carcinogenesis[6], T-B cell cooperation[7], etc. The purpose of the present study was

to determine the time interval required for the post-irradiation recovery of the TIR mice, i.e. the earliest time at which the TIR mice can be used in experiments as stable T-cell deficient animals.

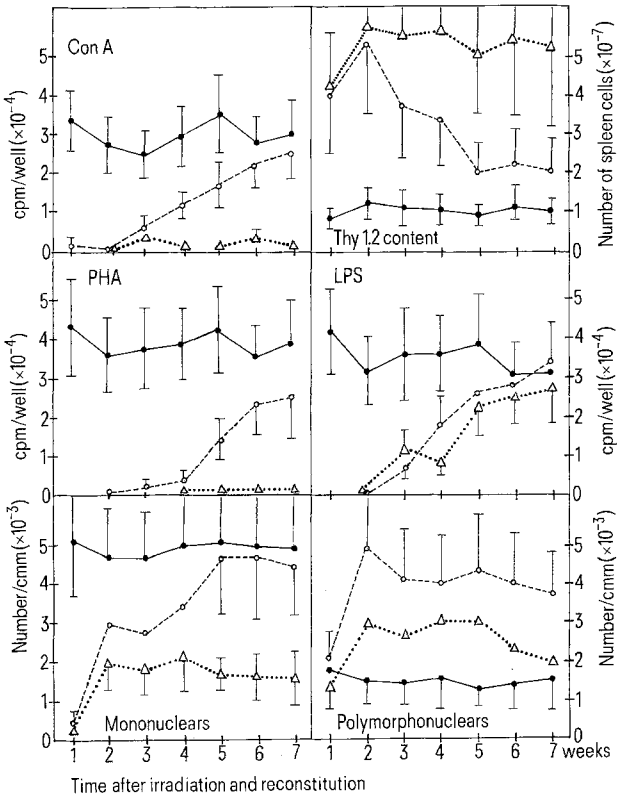
Material and methods. Male mice of CBA strain were thymectomized and sham-thymectomized at 8 weeks of age and were irradiated 5 weeks later. Thymectomy was performed using a method described by Miller[8]. Mice were exposed to a dose of 800 R of X-rays. Irradiation constants have been described[9]. Within 4 h after irradiation, mice were reconstituted i.v. with 7×10^6 Thy 1.2 antiserum-treated syngeneic bone marrow cells. The preparation and use of Thy 1.2 antiserum have been described[9]. For mitogen stimulation, washed spleen cells were resuspended in RPMI-1640 (Gibco, Grand Island, USA) with 7% fetal calf serum (Reheis Chem., Phoenix, USA) to a concentration of 5×10^6 cells/ml. Mitogens were diluted in the same medium to the following concentrations: Concanavalin A (Con A, Welcome, Beckenham, England), 20 µg/ml; phytohemagglutinin (PHA, Welcome, Beckenham, England), 2.5 µg/ml; and *Escherichia coli* lipopolysaccharide (LPS, Difco Labs, Detroit, USA), 400 µg/ml. Equal volumes (0.1) of spleen cell suspension and mitogen solutions were put in microtiter plates (Falcon, Oxnard, USA). Each sample was done in triplicate. Samples were cultured at 37 °C in a humidified atmosphere containing 10% CO_2 for 48 h. After the first 24 h, 0.5 µCi of [^{3}H] thymidine (Schwarz/Mann, Orangeburg, USA) (spec. act. 6 Ci/mmole) in 0.025 ml RPMI-1640 was added to each well. The cultures were harvested with an automatic multiwell harvester, and counts per min were determined. The number of spleen cells required to absorb 50% Thy 1.2 antiserum activity was determined as described elsewhere[9]. Peripheral blood cells were stained with May Grünwald-Giemsa stain.

Results and discussion. The response of spleen cells to T-cell mitogens Con A and PHA, the number of spleen cells required to absorb 50% Thy 1.2 antiserum activity (as an estimation of Thy 1.2 antigen content in the spleen), and the number of mononuclear and polymorphonuclear leucocytes in the peripheral blood was determined weekly for 7 weeks in thymectomized and sham-thymectomized irradiated and bone marrow reconstituted mice. Age-matched normal mice were always used as a control. Each analysis was done on 3–6 mice.

As seen in the figure, the response of spleen cells from sham-thymectomized, irradiated, bone marrow reconstituted (SIR) mice to T-cell mitogens Con A and PHA, reached normal value 6 weeks after irradiation and reconstitution. In contrast, the response of spleen cells from TIR mice was absent or minimal throughout the observation period. Similarly, whereas the number of spleen cells from TIR mice neccessary to absorb 50% Thy 1.2 antiserum activity was always significantly higher than that of normal spleen cells, the number of spleen cells from SIR mice required to absorb 50% Thy 1.2 antiserum activity reached normal value approximately 5 weeks after irradiation and reconstitution. 6 weeks after irradiation and reconstitution, the response to LPS of neither SIR nor of TIR mice was different from the response of spleen cells from normal mice.

During the 7-week observation period, the number of mononuclear cells in the peripheral blood of TIR mice was significantly lower than that in normal mice. In contrast, the number of mononuclears in the peripheral blood of the SIR mice did not differ significantly from the normal values since the second postirradiation week, and reached normal value at approximately 5 weeks after irradiation and reconstitution. Whereas the number of polymorphonuclear cells in the peripheral blood of TIR mice did not differ significantly from that in normal mice, the number of polymorphonuclears in the peripheral blood of SIR mice was significantly higher than that in normal mice. This is in contrast to the normal number of polymorphonuclears in the peripheral blood of SIR mice 3 months after irradiation and reconstitution[4].

Taken collectively, the present data show that the immunological functions of irradiated, bone marrow reconstituted mice recover at approximately 5–6 weeks after irradiation and reconstitution. In the mice that had been thymectomized before irradiation and reconstitution, both T-cell number and function are deficient, but B-cell function is preserved.



Immunological recovery of lethally irradiated mice reconstituted with syngeneic bone marrow cells. Normal mice (●——●), sham-thymectomized, irradiated and reconstituted mice (○——○), thymectomized, irradiated and reconstituted mice (△·····△). Vertical bars indicate 95% confidence limits.

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