

antigen, demonstrated that splenectomy in the chicken only delayed the day of peak titre, but did not diminish the total response.

In the present study, a weak antibody response was noticed when splenectomy was performed 7 days after immunization. However, the low antibody titres were within the range produced by normal animals of this species on the 7th day following immunization, suggesting that the observed antibody may have been produced prior to splenectomy and simply maintained in the blood in the ensuing 2 weeks. The presence of PFC in the spleen at the time of splenectomy supports this interpretation.

Our observations on the total ablation of reaction to SRBC after splenectomy is reinforced by the observation of KANAKAMBIKA<sup>6</sup> who could detect PFC only in the blood and spleen of immunized lizards, but not in any other tissue tested. Similar observations have been made by ROTHE and AMBROSUS in *Testudo*<sup>14</sup>. Further studies involving other reptilian species, other antigens, and alternate routes and schedules of immunization are required, however, before generalizations concerning reptilian spleen functions can be attempted<sup>15</sup>.

**Zusammenfassung.** Nach Entfernung der Milz kann die Eidechse *Calotes versicolor* nicht mehr gegen Immunisierung mit Schafserthrozyten reagieren. Wenn die Milz aber nur teilweise entfernt wird, ist noch eine völlig normale Reaktion vorhanden.

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<sup>14</sup> F. ROTHE and H. AMBROSUS, *Acta biol. med. germ.* 21, 525 (1968).

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## Inhibition of the Primary Immune Response Against Sheep Red Blood Cells by Short-Term Reticulo-Endothelial System Blockage Experiments in Mice

The possible suppressive effect of the immune response by 'blockage' of the reticulo-endothelial system with particles is known (CRUCHAUD<sup>1</sup>). It is debatable, however, whether the inhibition is caused by the uptake of the particles by the RES cells or whether it is due to direct action on the surface membrane. Electron microscopic studies have shown that thorotrast is taken up by spleen cells about 20 min after the i.v. injection and most of the thorotrast at that time still covers the surface membrane (WEISS<sup>2</sup>). We studied the question in short-term experiments using thorotrast as blocking agent and sheep red blood cells (SRBC) as the antigen.

**Methods.** 0.2 ml of thorotrast were injected i.v. into adult female and male mice. The thorotrast injection was related to the antigen injection in the following way: A) 15 sec, 30 sec, 1, 2, 5, 15, 45, 60, 90 and 120 min prior to the antigen injection,  $8 \times 10^8$  SRBC. B) Together with the antigen. C) 15 sec, 30 sec, 1 min, 5 min and 2 h after the antigen application. The animals were sacrificed 72 or 96 h after the immunization and the immune response evaluated by counting the plaque forming cells in the Jerne test; 11 different experimental groups were set up, each of them involving 12-92 mice. The results were reproduced at least 3 times. The findings were statistically evaluated by variance analysis in single classification according to the model  $\gamma_{ij} = \mu + \alpha_i + \epsilon_{ij}$  and by linear comparison of the different experimental groups with the control mice, which were immunized against SRBC without any thorotrast injection. This linear comparison was performed according to the test of Scheffe. The numeric calculations were done on IBM-7040-computer of the Calculation Center at the University of Freiburg, West Germany, Dr. Bloedhorn.

**Results.** The injection of thorotrast 15-30 sec, 1-5 min and 30-90 min prior to the injection of antigen inhibited the appearance of plaque-forming cells. The inhibition was statistically highly significant,  $p \leq 0.001$ , corresponding to a probability of error of 0.1%. The injection of thorotrast simultaneously with the antigen or 15 sec to 2 h

after the antigen injection did not influence the number of plaque-forming cells compared with the control animals which obtain only SRBC and no thorotrast at all.

These results are compatible with the assumption that the inhibitory effect observed is due to an action of thorotrast on the surface of the immune competent cells in the spleen of mice. Because of this, the antigenic determinants are not able to meet their membrane receptor sites. Whether the action of thorotrast is merely a physical coating of the cell or a chemico-physical interaction with the cell surface membrane cannot be deduced from these experiments. The experimental model described can be used for studies on cell membrane in its functional relation to the antibody response<sup>3</sup>.

**Zusammenfassung.** Mäuse wurden gegen Schaferythrozyten immunisiert und die Immunreaktion im Plaque-Test von Jerne gemessen. Erhalten die Tiere unmittelbar vor der Antigeninjektion Thorotrast, so kommt es zur statistisch hochsignifikanten Hemmung der Antikörper bildenden Zellen.

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<sup>1</sup> A. CURCHAUD, *Lab. Invest* 19, 15 (1968).

<sup>2</sup> L. WEISS, *Bull. Johns Hopkins Hosp.* 115, 99 (1964).

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