Tolerance of Red Cell Antigens and Transplantation Immunity in Chickens

"Acquired tolerance" has been defined[1] as the specific inhibition of immunological response which is caused by the exposure of embryonic or very young animals to antigenic stimuli. HAŠEK[2] has shown that the synchorial anastomosis ("parabiosis") of chicks from the 10th day of embryonic life until hatching leads to a prolonged and radical impairment of their power to form iso-agglutinins when injected in later life with the blood from the parabiotic partner. We ourselves[1] had shown that the injection of 10-day old chicken embryos with 0·1 ml whole blood from an adult chicken will cause a high proportion to become tolerant of skin homografts later transplanted from the donor of the injected blood. The purpose of the present communication is to show that these two forms of tolerance depend upon the inhibition of different and experimentally separable immunological reactions.

We have lately shown[3] that even the period of birth in chicks is not too late for the induction of tolerance. Chicks were injected intravenously, within 12 h of hatching, with 0·5 ml whole blood from an adult donor of different breed; when tested with skin homografts from the blood donor 14 days later, 40–45% were found to tolerate their homografts for more than a month instead of for less than 10 days. It has furthermore been proved[3] that the power of whole blood to confer tolerance of skin homografts resides wholly in the leucocyte fraction; it may be abolished either by removing the leucocytes or by heating the blood to 49°C for 20 min. These treatments, which remove or inactivate the antigens responsible for transplantation immunity, need not be expected to affect the red cell iso-agglutinogens.

In the present experiment, heparinized venous whole blood from an adult Rhode Island Red (RIR) hen was freed from leucocytes by differential centrifugation and filtration through glass wool; then–to be doubly certain of the removal of "transplantation" antigens–it was heated for 20 min to a temperature between 49°C and 50°C. The red cells were finally suspended in Ringer-phosphate at the concentration at which they were originally present in whole blood. Within 6 h of hatching, 10 members of a homogeneous batch of 20 White Leghorn chicks were injected intravenously with 1·0 ml of this essentially "pure" preparation of red cells. When the 20 chicks were 7 weeks old, each was injected intramuscularly with 1·0 ml whole blood from the original RIR donor; four further injections were given at 5-day intervals. 5 days after the last injection, sera from all the chicks were tested for the presence of red cell iso-agglutinins against a 2% suspension in Ringer's solution of the donor's washed red cells. The agglutination tests were carried out by incubating equal mixtures of antibody and cell suspension in miniature test-tubes for 1 h at 37°C; the degree of agglutination was then read microscopically on slides. Instead of titrating each of the antisera individually, it was found adequate to read the scores cross-sectionally at serum dilutions of ½ (Table I) and 1/10 (Table II). In these two Tables, ± represents an agglutination just perceptible under the microscope, and + an agglutination just perceptible to the naked eye; +++ or ++++ represent a bold and coarse aggregation.

These results show that, as a group, the chickens injected with adult homologous red cells at hatching (EXP) responded much more feebly to active immunization than their untreated controls (CON). Some, indeed, did not react at all; that tolerance should have been incomplete in the others is not surprising, for the injection conferring tolerance was confined to a single dose administered relatively late in development. It follows that tolerance of red cell iso-antigens in chickens may be experimentally distinguished from tolerance of the antigens responsible for transplantation immunity, for blood freed from leucocytes or heated to 49–50°C contains no active transplantation antigens and is incapable of producing a perceptible tolerance of homografts of skin[1].

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Résumé

L'injection d'érythrocytes de la poule adulte à poussins récemment éclos abaisse sérieusement leur capacité de repondre dorénavant à l'immunisation active par la formation d'iso-agglutinines.

La tolérance à l'égard d'homéogreffes de peau n'est en rien due aux érythrocytes, mais exclusivement aux leucocytes.

Nous tirons de ces faits la conclusion que la formation d'isoagglutinines et la réaction conduisant à la destruction d'homéogreffes cutanées représentent deux modes de réponse immunologique nettement différents.

[1] R. E. BILLINGHAM, L. BRENT, and P. B. MEDAWAR, Nature *172*, 603 (1953); Ann. N. Y. Acad. Sci. *59*, 409 (1955).

[2] M. HAŠEK, Cesk. Biol. *2*, 25, 265 (1953); Nature *175*, 765 (1955).

[3] R. E. BILLINGHAM, L. BRENT, and P. B. MEDAWAR, Philos. Trans. Roy. Soc. [B], in the press.

Table I.–Dilution ½.

EXP:	0	0	±	+	+	+	+ +	+ +	+ + +	+ + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
CON:				+			+ +	+ +	+ + +		+ + + +				

Table II.–Dilution 1/10.

EXP:	0	0	0	±	±	±	+	+	+	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + + +
CON:				±	±		+			+ +						

Iso-agglutinin formation in chickens in response to the injection of whole blood. Both groups of animals (EXP and CON) were immunized in exactly the same way, but those marked EXP had been injected with 1·0 ml of homologous red cells within 6 h of hatching.