Antibody Formation by Isolated Cells of Hen Spleen after mixing with Antigen *in vitro* and Transfer to Chicks

RECENTLY, Šterzl[1] used newborn animals as recipients for the transfer of tissues producing antibodies as infant animals cannot produce antibodies after administration of antigens during the early postnatal period[2]. This experimental model has been used successfully by Mitchison[3], who evoked antibody production in chicks after transfer of spleen tissue from strongly immunized hens. Dixon and Weigle[4], however, were unable to demonstrate the usefulness of infant animals for the demonstration of antibody formation after transfer of tissues from animals immunized 24 hr. previously. In further work, when cells mixed with antigen *in vitro* were transferred, results were again negative[5]. In the work reported here, the possibility of using infant animals soon after birth as recipients was tested.

Normal white Leghorn hens (weight 1·5–2 kgm.) were used as donors of spleen cells. No antibodies to *Salmonella paratyphi* B and *Brucella suis* could be found in their serum by agglutination. These were used as antigen. Spleen cells were isolated according to Šterzl[6]. Isolated spleen cells were diluted quantitatively (6×10^7) according to counts in a Bürcker chamber either with phosphate physiological saline or directly into a suspension of heat-inactivated bacteria (*Salmonella paratyphi* B 12×10^7; *Brucella suis* 36×10^7). After 10 min. of incubation at room temperature they were injected by the intraperitoneal route into Leghorn chicks 48–56 hr. after hatching. 1 ml. of suspension was administered to each bird. Blood from the chicks was obtained by cardiac puncture 3, 5, 7, 9, 12, 16, 24 and 31 days after transfer of a mixture of spleen cells and bacterial antigen. Further details of the lay-out of the experiment and results are apparent from Table 1. Positive results were obtained with the mixtures; but results were negative when only a suspension of bacteria or spleen cells alone was given.

Cells must be transferred to animals of the same species. Results were negative when spleen cells from rabbits mixed *in vitro* with antigen *Brucella suis* was given to chicks (Table 1).

The results of Dixon and Weigle[4,5] are not in agreement with ours. These authors conclude that infant animals are not suitable as recipients and that the environment of infant animals cannot provide conditions necessary for the realization of the first phase of antibody formation. On the other hand, the results presented here, together with those of Šterzl[1,6], are supported by the work of Simonsen[7], who injected reticulo-endothelial cells from adult hens into chick embryos and young chicks. Using the Coombs test he detected antibodies produced by adult cells against the antigens of the recipient.

It is concluded that infant animals incapable of an antibody reaction to an antigenic stimulus are suitable for studying the conditions for antibody formation by isolated mesenchymal cells.

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Presence of Plasma Globulins in the Hyaline Tissue in Cases of Silicosis

PREVIOUS chemical results[1] obtained by one of us show that the hyaline tissue in cases of silicosis contains appreciable amounts of non-collagenous proteins. In fact the determination of the amino-acid composition, and the ultra-violet absorption spectrum of the residue left after extraction of collagen by autoclaving, suggested the presence of proteins chemically related to β- or γ-plasma globulins. These proteins constitute up to one-half of the total protein content of the original tissue. In order to ascertain beyond all doubt the presence in silicosis hyaline tissue of plasma globulins, it was decided to investigate whether proteins having the immunological properties of plasma globulins could be identified in it. Hyaline tissue was therefore isolated carefully from the pulmonary nodules of three cases of typical silicosis immediately after autopsy. The tissue was washed and homogenized in saline with a

Table 1

Source of cells	Amount of cells injected into recipient	Amount of antigen	Age of chicks (in hr. after hatching)	No. of chicks in experiment	No. of chicks surviving at least 12 days after transfer	Maximum titre 5–9 days after transfer*	Beginning of antibody formation after transfer (in days)*
Spleen of normal adult hen	6×10^7	*S. paratyphi* B 12×10^7	48–56	19	10	16, 8, 16, 16, 8, 16, 16, 16, 8, 8	5, 5, 5, 5, 5, 5, 5, 5, 7, 7
Spleen of normal adult hen	6×10^7	—	48–56	5	2	0, 0	negative until 31st day
—	—	*S. paratyphi* B 12×10^7	48–56	8	5	0, 0, 0, 0, 0	16, >31, >31, >31, >31
Spleen of normal adult hen	6×10^7	*Brucella suis* 36×10^7	48–56	31	20	128, 64, 128, 64, 128, 32, 64, 128, 128, 32, 32, 8, 8, 32, 8, 16, 8, 8, 0, 0	3, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 7, 7, 7, 9, 9, >31, >31
Spleen of normal adult hen	6×10^7	—	48–56	10	5	0, 0, 0, 0, 0	Negative until the 31st day
—	—	*Brucella suis* 36×10^7	48–56	7	4	0, 0, 0, 0	>12, >12, >12, 16
Spleen of normal adult rabbit	6×10^7	*Brucella suis* 36×10^7	48–56	10	7	0, 0, 0, 0, 0, 0, 0	12, 31, 31, >31, >31, >31, >31
Spleen of normal adult rabbit	6×10^7	—	48–56	6	4	0, 0, 0, 0	Negative until the 31st day

* Figures are arranged in such a way that those situated in the same place in each column always correspond to one experimental animal.