

laboratory provide strong evidence that the extirpation of bursa brings about the depletion of plasmocytic cells in the spleen, whereas the density of small lymphocytes is only slightly affected. The deficiency in the antibody-forming mechanism of the bursectomized chickens⁸⁻¹² may conceivably be concerned with such a lack in plasma cells. On the other hand, birds also have thymus, and it is tempting to assume, therefore, that this organ plays a part in the development of hypersensitive capacity. The immunological competence of genuine thymic lymphocytes is conjectural. Nevertheless, it is widely regarded that those cells seed other lymphoid organs. Since the lymphocytes are considered as the essential cells in the expression of delayed hypersensitivity¹⁷, it seems quite possible that thymus in birds may represent a primary source of small lymphocytes responsible for delayed reactive capacity. This possibility is at present under investigation.

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Synthesis of Antibodies by Blood Leucocytes of the Rabbit

AFTER injection of heterologous antigen, antibody formation has been shown to occur in lymphoid tissues. Among the different lymphoid cell types, plasma cells contain a large amount of specific antibody as shown by the fluorescent antibody technique of Coons *et al.*¹ It is therefore assumed that plasma cells form and secrete antibody into lymph and blood². The buffy coat also contains immunologically competent cells. This fact is shown by the ability of these cells to induce a homograft reaction or to transfer delayed type hypersensitivity. Another form of immunological competence, antibody synthesis by peripheral blood leucocytes against a heterologous antigen, has not been demonstrated.

The work described in this preliminary note was undertaken to investigate whether blood leucocytes from immunized rabbits can form antibody against a soluble heterologous antigen *in vitro*. A differential cell count was carried out from the buffy coat. For comparison spleen and lymph nodes were studied in the same way.

The methods and techniques have been described elsewhere³. Essentially the following method was used:

Table 1. ANTIBODY SYNTHESIS BY RABBIT BLOOD LEUCOCYTES *in vitro*

Origin of rabbit cells	Exp. No.	No. of nucleated white cells	No. of plasma cells and plasmoblasts (per cent)	Antibody synthesis (counts/min)
Immune blood leucocytes	1	66 × 10 ⁶	7.5	3,519
	2	67 × 10 ⁶	4.5	3,726
	3	58 × 10 ⁶	9.0	4,751
	4	40 × 10 ⁶	8.0	2,897
Immune spleen	1	80 × 10 ⁶	26.5	6,171
	2	80 × 10 ⁶	43.0	19,619
	3	80 × 10 ⁶	40.5	32,652
	4	80 × 10 ⁶	29.5	3,393
Immune popliteal lymph node	1	80 × 10 ⁶	—	2,129
	2	80 × 10 ⁶	15.0	10,378
	3	80 × 10 ⁶	15.5	26,489
	4	80 × 10 ⁶	9.0	2,070
Controls	1-4	50-84 × 10 ⁶	0-5.5	164-394

normal rabbits were hyperimmunized by 7 intravenous injections of 1 ml. human serum during a period of 4 weeks. 4-5 weeks after the last injection the animals received a booster injection of human serum. Four days later they were bled through the carotid artery. The blood was immediately heparinized and cooled. Leucocytes were concentrated by centrifugation and the buffy coat was removed and again concentrated in a narrow tube. This procedure was repeated until the layer of contaminating erythrocytes had been eliminated. Suspensions of blood leucocytes and spleen and popliteal lymph nodes were prepared. They contained about 5-10 × 10⁷ nucleated white cells and were incubated with 5 μc. algal protein hydrolysate labelled with carbon-14 (obtained from the Radiochemical Centre, Amersham, Buckinghamshire, England) for 3 h at 37° C. Radioactive antibody was then isolated by specific precipitation with carrier antigen and antibody. The radioactivity of the carefully washed precipitates was counted in a gas-flow counter.

Four typical experiments are shown in Table 1. The results demonstrate that in hyperimmunized animals circulating blood leucocytes produce surprisingly large amounts of antibody if taken 4 days after a booster injection. In fact, if the total number of circulating leucocytes is taken into account, the role of these leucocytes in antibody production is as important as that of the spleen, the organ usually considered to be the main contributor to antibody formation following intravenous injection of antigen.

As can be seen in column 4 of Table 1, the number of plasma cells and plasmoblasts is not only high in spleen and lymph nodes of immunized animals, but also in peripheral blood. In comparing the different cell types, we observed that also in blood a high degree of antibody synthesis was associated with a high percentage of plasma cells.

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BIOLOGY

Bioassay of Oestrogen and Recurring Oestrus in Ovariectomized Ewes

EARLIER work¹ has indicated that oestrogenic hormones increased the dilatibility of the ovine vagina. In those experiments, the increase in dilatibility was smaller at a dose-level of 25 μg than at 100 μg stilbestrol dipropionate. It was decided, therefore, to test this method as a bioassay of oestrogenic hormones in sheep.

In March 1961, 20 half-bred (Romney × Border Leicester) parous 7 year-old ewes which had been ovari-