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Formation of Specific Antibody by Circulating Cells

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Summary. The rate of antibody synthesis as measured by ^{14}C-amino acid incorporation was estimated in thoracic duct lymph, buffy coat and spleen cells from rabbits hyperimmunized and boosted with human serum albumin (HSA). Cells from thoracic duct and buffy coat synthesized antibody in most experiments in small amounts, in two experiments in larger amounts. Spleen cells gave the highest values but also showed considerable variation from animal to animal.

The comparison of antibody synthesis with the cellular composition of the different cell preparations led to the conclusion that the small percentage of pyroninophilic cells in lymph and buffy coat are responsible for the antibody synthesis and not the bulk of lymphocytes.

Cell-bound antibody measured by fixation of radioactive HSA was found in spleen lymph and buffy coat cells. The major part of the detected cell-fixed antibody is presumably present on the surface of and within antibody-producing cells and not due to passively adsorbed cytophilic antibody on non-antibody-producing cells.

INTRODUCTION

The accumulated evidence that antibodies are formed primarily in cells of the plasmocytic series and within lymphoid organs is impressive. However, recent experiments (Hulliger and Sorkin, 1963) have also demonstrated the capacity of peripheral blood leucocytes to produce specific antibody. These findings have been confirmed quite separately by Landy, Sanderson, Bernstein and Jackson (1964) and by Forbes (1965). The purpose of this communication is to bring further data supporting antibody production by circulating cells and to evaluate in addition whether small lymphocytes also have antibody-forming capacities. Wesslén (1952), Holub (1960) and Hallander and Danielsson (1962) presented results which suggested antibody production by thoracic duct lymphocytes *in vitro* and *in vivo*. In these experiments, however, no detailed reference was made to the composition of the cells obtained from the thoracic duct. Wesslén and also Hallander and Danielsson attributed antibody formation to the small lymphocytes since they could measure antibodies in the medium after incubation of thoracic duct lymphocytes *in vitro*. Gowans, McGregor, Cowen and Ford (1962) have definitely established that thoracic duct cells are immunologically competent, that is if transferred into an adequate host, they give rise to antibodies. Gowans *et al.* (1962) have given good evidence that the small and not the large lymphocytes are responsible for both the homograft reaction and the development of the primary response to heterologous proteins.

Several issues remain unresolved. First, are the small lymphocytes themselves capable of producing antibodies or is it necessary that they first transform into plasmocytic cells, cells

rich in endoplasmic reticulum? Secondly, lymphocytes and other cells of the lymphoid series are known to carry antibody on their surfaces. It has been shown that such cell-fixed antibody can be released during incubation of cells *in vitro*. Consequently, it was felt that methods measuring *de novo* synthesis of antibody *in vitro* would be capable of differentiating between transported antibody and that actually synthesized. Thirdly, demonstration of antibody-synthesizing cells in thoracic duct lymph draining into the blood would be of great importance in that these cells are generally considered to be a 'pure' population of lymphocytes. It could then mean that antibody-producing cells in the form of lymphocytes undergo extensive recycling.

The present report compares antibody synthesis and cell-fixed antibodies of thoracic duct cells and blood leucocytes and spleen cells of the same animals hyperimmunized to human serum albumin. Evidence for circulation of antibody-forming cells in blood and lymph is presented and the possible function of different cell types and the significance of circulating antibody-producing cells are discussed.

MATERIALS AND METHODS

Animals. Randomly bred male and female rabbits weighing 2000–3000 g were used in the immunization and control experiments.

Course of immunization of rabbits. A group of rabbits received eight intravenous injections of 1 ml human serum spaced over 4 weeks. Between 6 and 12 weeks after the last injection the rabbits received an intravenous booster injection of 1 ml human serum. Four or 5 days later the thoracic duct cells, blood leucocytes and spleen cells were isolated and tested for antibody synthesis *in vitro* and uptake of isotope labelled antigen (^{131}I-HSA) as shown below.

Medium. Ten per cent normal rabbit serum (NRS) in Gey's solution containing 25 units penicillin and 25 units streptomycin per ml.

Technique for cannulation of thoracic duct. The technique used for the cannulation of the thoracic duct in its abdominal part has been described in detail (Hulliger, 1956). The animals were starved for 12–24 hours before the operation. During the operation and collection of lymph they were fully anaesthetized with a barbiturate (Vetanarcol, Veterinaria AG, Zurich) by the intravenous route. About 8 ml of lymph was allowed to flow into a flask containing 0·5 ml normal rabbit serum (NRS) and 0·5 ml heparin solution (2000 units/ml). When a sufficient amount of lymph had been collected (normally after 2–3 hours), the animals were bled by carotid artery cannulation.

Preparation of cell suspensions

Lymph was centrifuged at 1000 rev/min for 5 minutes. The supernatant lymph fluid was collected for determination of cytophilic antibody titres. Cells were resuspended to make 150–180 $\times$ 10^6 cells/ml.

Buffy coat. After cannulation of the thoracic duct and collection of lymph for 2–3 hours the animals were bled by carotid artery cannulation. Blood was immediately mixed with heparin to a final concentration of 50 units heparin per ml of blood. The leucocytes were concentrated by centrifuging the blood in the cold at 2000 rev/min for 8 minutes. The concentrated buffy coat was removed and centrifuged a second time in a narrow tube at the same speed. Leucocytes were finally suspended in the same concentration as thoracic duct lymph cells.

Spleen was cut into pieces in 10 per cent NRS in Gey's salt solution (medium) and then gently pressed through a metal sieve. The cell suspension was centrifuged and resuspended ($150–180 \times 10^6$ cells/ml) in medium.

Technique for the estimation of antibody synthesis in vitro. The method used was similar to that described by Sorkin, Rhodes and Boyden (1961) and Hulliger and Sorkin (1963). About $5–10 \times 10^7$ nucleated white cells were incubated with 2·5 μc algal protein hydrolysate labelled with carbon-14 (obtained from the Radiochemical Centre, Amersham, England). The cells were suspended in 5 ml of a mixture containing 4·5 ml Gey's solution, 0·5 ml NRS and 250 units penicillin and 135 units streptomycin. To the mixture were also added synthetic carrier amino acids in the form of 0·1 ml Parker's solution. The cells were gently shaken in 50 ml Erlenmeyer flasks for 3 hours at 37°. The mixture was then centrifuged for 15 minutes at 20,000 ***g***, the supernatant withdrawn and once more centrifuged. To the supernatant fluid was added 0·5 ml carrier amino acid solution, 0·5 ml rabbit anti-human serum and 500 μg human serum albumin (very slight antigen excess). After 1 hour's incubation at 37° and standing overnight at 4° the mixture was centrifuged. The antigen–antibody deposit was washed four times in a neutralized mixture of 4 ml saline containing 0·5 ml of the carrier amino acids. The washed precipitate was dissolved in 2 N $(NH_4)OH$ and transferred to discs and air-dried before determining the radioactivity in a gas-flow counter.

As in our earlier work (Sorkin *et al.*, 1961) a number of control experiments was performed to ascertain that no non-specific radioactive material was co-precipitated with the specific HSA–anti-HSA complex. For this purpose an ovalbumin–rabbit anti-ovalbumin precipitate was prepared prior to the HSA–anti-HSA precipitation. Only very low radioactivity values, similar to the normal controls, could be detected in the ovalbumin–anti-ovalbumin precipitates. These findings agree with our earlier observations (Sorkin *et al.*, 1961).

Technique for the estimation of cell-bound antibody. The method used was essentially the same as described by Sorkin *et al.* (1961). 0·5 ml of 20 per cent cell suspensions in medium was incubated with 0·5 μg ^{131}I-human serum albumin (^{131}I-HSA) for 1 hour at 37° and the cells then washed four times in medium. The cells were dried and the radioactivity counted.

In other experiments the technique differed from the above. The pellet of cells was treated with 1 ml methanol for 5 minutes prior to exposure to labelled antigen. The mixture was centrifuged, the cells washed twice with medium and then resuspended to 20 per cent in medium. 0·5 μg of ^{131}I-HSA was added to the methanol-treated cells, the mixture incubated and further treated as described above for non-methanolized cells.

Technique for estimation of cytophilic antibody (Boyden and Sorkin, 1960, 1961). One ml of test serum was added to 0·5 ml of 20 per cent normal spleen cell suspension in medium. After 1 hour's incubation in ice water the cells were washed four times in medium. They were resuspended in 0·5 ml of medium and incubated with 0·5 μg ^{131}I-HSA for 1 hour at 37°. The radioactivity was determined after washing and drying the cells four times.

^{131}I-Human serum albumin. Trace labelled ^{131}I-HSA was obtained from the Radiochemical Centre, Amersham, England.

Measurement of radioactivity. A gas-flow counter was used to measure the radioactivity of the samples. The values given for cell-bound and cytophilic antibody are corrected for background (usually about 30 counts/min). They are also corrected for infinite thinness for antigen–^{14}C-antibody precipitates.

Preparation of antiserum against human serum (carrier serum) for isolation of ^{14}C*-antibody.* A group of rabbits received eight intravenous injections of 1 ml normal human serum three times a week for 3 weeks. Six weeks after the last injection a booster dose of 1 ml human serum was injected intravenously. Ten days later the rabbits were bled out, and sera with a high precipitin titre were pooled.

Cytological observations

Smears were prepared from lymph, spleen and buffy coat both before and after preparation of cell suspensions. Slides were stained with May–Grünwald–Giemsa or with methyl-green/pyronin. The following differentiation of cells was made.

1. Non pyroninophilic cells: small and large lymphocytes, reticulum cells and macrophages.
2. Granulocytes.
3. Pyroninophilic cells:

(a) *Large pyroninophilic cells* (blast type), cells with a more or less eccentric nucleus, one or more definite nucleoli and a more or less large amount of pyroninophilic cytoplasm (in Giemsa stained smears basophilic).

(b) *Plasma cells* smaller than (a) above, with an eccentric nucleus, paranuclear vacuole and deeply pyroninophilic cytoplasm.

(c) *Small pyroninophilic lymphocytes*, size of the small lymphocytes with a narrow rim of strongly pyroninophilic cytoplasm and sometimes a slightly eccentric nucleus.

Two hundred to five hundred cells were counted.

Blood picture. Daily blood smears were made from six animals starting before the injection of the booster dose of HSA until the day of the operation. Cells were differentiated in Giemsa preparations in the way described above. Blood pictures of two immunized and boosted animals are described in detail in Table 4.

RESULTS

ANTIBODY SYNTHESIS *in vitro* BY CELLS FROM THORACIC DUCT LYMPH, BLOOD AND SPLEEN

The thoracic duct was cannulated successfully in eleven immunized and in four control animals.

The values for ^{14}C-antibody synthesized *de novo* by these cells *in vitro* are summarized in Fig. 1. Antibody synthesis by 80×10^6 cells is expressed as counts/min found in the isolated carrier antigen–antibody precipitates (see Methods). The results were as follows.

In the hyperimmunized animals spleen cells produced varying amounts of antibody with values from 800 to 12,000 counts/min. In two of the eleven animals spleen cells had very high values (above 6000 counts/min). Five other animals had values between 2000 and 4000 counts/min. The remaining animals displayed values higher than those for cells from control animals.

Thoracic duct cells and buffy coat cells showed a similar picture. The two animals with very high spleen values had the highest values in the same range for both lymph and buffy coat cells. Of the five animals with middle values for spleen cells, thoracic duct cells were not elevated but one of these animals showed a very high value for buffy coat.

The percentages of pyroninophilic cells are listed in Table 1. All the hyperimmunized animals displayed an increased number of pyroninophilic cells in spleen. There was how-

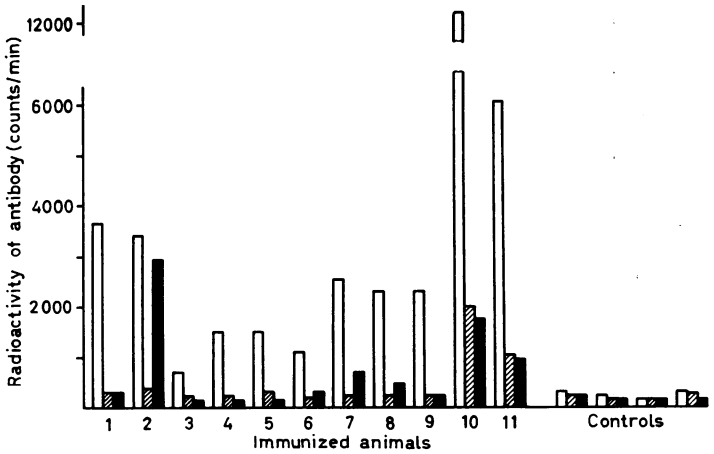

FIG. 1. Antibody production *in vitro* by blood leucocytes (solid columns), thoracic duct cells (shaded columns) and spleen cells (open columns) of rabbits hyperimmunized with HSA.

ever, no correlation between the percentage of pyroninophilic cells and the level of antibody synthesis for individual animals, neither was there any correlation as regards cells from buffy coat and lymph.

The increase in pyroninophilic cells in spleen is due mainly to an increase in proplasmocytes and plasma cells, the increase in blood is due to plasma cells, whilst that in lymph is mainly due to the small pyroninophilic cells.

TABLE 1

SUMMARY OF VALUES FOR PERCENTAGE PYRONINOPHILIC CELLS IN THORACIC DUCT LYMPH, BUFFY COAT AND SPLEEN

Immunized animals	Spleen	Lymph	Buffy coat
1	13·1	4·7	0
2	12·3	6·4	8
3	4·4	3·6	4
4	23·1	4·1	7
5	10·7	2·6	0
6	14·0	2·0	1
7	10·4	2·7	2
8	16·0	2·0	7
9	5·3	4·7	–
10	16·6	5·9	3·7
11	18·0	1·3	9
Controls	2·0	1	1
	1·0	6·3	0
	0·7	5·4	0
	1·7	1·3	0

On the other hand, in cell suspensions of control animals small lymphocytes are present in larger numbers than was found in hyperimmunized animals.

SPECIFIC ADSORPTION OF ANTIGEN (^{131}I-HSA) BY CELLS (= CELL-BOUND ANTIBODY)

The results which are summarized in Fig. 2 show that cells from normal spleen and thoracic duct lymph do not adsorb antigen (^{131}I-HSA) whilst cells from immunized

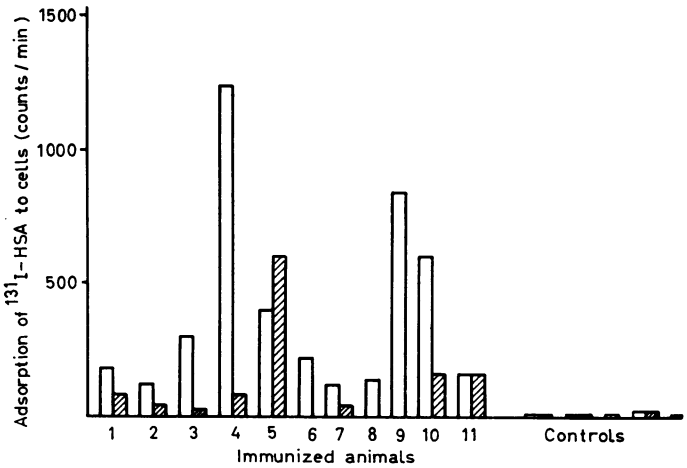

FIG. 2. Cell-bound antibody in thoracic duct cells (shaded columns) and spleen cells (open columns) of rabbits hyperimmunized with HSA.

animals do so to a varying degree. Spleen cells gave the highest values, thoracic duct cells which were assayed in seven experiments, gave lower values, but in five experiments these values were nonetheless definitely above controls. In one experiment thoracic duct cells adsorbed more antigen than spleen cells. Antigen adsorption was not due to the erythrocytes present in the cell preparations, since it was shown that even 5×10^8 erythrocytes did not bind appreciable amounts of antigen.

TABLE 2

CELL-BOUND ANTIBODY IN THORACIC DUCT CELLS: THE EFFECT OF METHANOL PRETREATMENT OF CELLS ON SPECIFIC ADSORPTION OF ANTIGEN (^{131}I-HSA)

	Number of cells $\times 10^6$	Pyronino-philic cells (per cent)	Antigen adsorbed	
			No methanol (counts/min)	Methanol pretreatment (counts/min)
Immunized animals	80	4·8	487	904
	80	11·0	—	6646
	85	4·7	104	76
	77	7·0	92	572
	65	5·8	73	256
	60	1·6	39	50
	76	6·3	201	504
	80	1·3	185	200
Normal animals	75	1·0	37	37
	80	6·5	33	38
	74	5·4	47	46

0·5 μg ^{131}I-HSA varying from 4350 to 14,285 counts/min were applied.
Only the antigen adsorption values for a single animal should be compared with each other.

The presence of cell-bound antibody on circulating blood leucocytes has recently been demonstrated by means of specific adsorption of labelled antigen (Sorkin, 1964). A number of other experiments was also carried out to test for cell-bound antibody in thoracic duct cells after previous methanol treatment of the cells. The number, composition of the lymph cells and the values for specific adsorption of isotope-labelled antigen are summarized in Table 2.

The results demonstrate that appreciable quantities of antibody were bound to thoracic duct cells. As already found earlier in the case of spleen cells and blood leucocytes, methanol pretreatment did not change the very low nonspecific fixation of antigen onto such cells. With cells from immunized animals the values increased in general several fold. These experiments attest to the fact that in the present immunological test system thoracic duct cells carry antibody. Whether these antibodies were actually produced by these circulating cells as could be supposed on the basis of the above incorporation results, or whether these cells passively carried cytophilic antibody can not be decided. However, the results on cytophilic antibody titres in the thoracic duct fluid, shown below for some animals, suggest in part at least passive adsorption of antibody onto cells.

CYTOPHILIC ANTIBODY TITRES IN LYMPH FLUID AND SERUM FOR A NUMBER OF EXPERIMENTS

The results are summarized in Table 3. The levels of cytophilic antibody in lymph fluid and in serum of all animals are in the same range, serum titres being in general somewhat higher than lymph titres. The values for cell-fixed antibody in thoracic duct cells, some of which are represented in Table 2 above, show no apparent relationship to the titres of cytophilic antibody in the lymph fluid, neither did the values for antibody synthesis by thoracic duct cells.

TABLE 3

CYTOPHILIC ANTIBODY IN SERUM AND THORACIC DUCT LYMPH OF IMMUNIZED RABBITS

	Cytophilic antibody in	
	Serum (counts/min)	Lymph fluid (counts/min)
Immunized animals	1111	775
	793	684
	1315	1020
	769	97
	2272	746
	1265	456
Normal animals	35	47

Cytophilic antibody was measured by the standard technique (see Methods) using 0·5 μg ^{131}I—HSA = 4446 counts/min.

CHANGES IN COMPOSITION OF PERIPHERAL BLOOD CELLS OF HYPERIMMUNIZED RABBITS AFTER A BOOSTER INJECTION OF ANTIGEN

Changes in cellular composition of blood leucocytes from two animals are given in Table 4.

In all the hyperimmunized animals in which daily blood smears were made after the booster-injection, pyroninophilic cells appeared in varying numbers with a peak between 2 and 5 days after boosting. In animal No. 10 the peak of pyroninophilic cells occurred on the day of cannulation of the thoracic duct. This animal displayed very high values for antibody synthesis by both blood and thoracic duct cells. Animal No. 11 had a very pronounced peak of pyroninophilic cells as early as 2 days after the booster injection. On day 4, when the thoracic duct lymph was collected, the peak reaction had already occurred however, antibody synthesis in blood and thoracic duct cells was still fairly active.

TABLE 4

CHANGES IN THE PERCENTAGE COMPOSITION OF PERIPHERAL BLOOD LEUCOCYTES PRODUCED BY RE-ADMINISTRATION OF ANTIGEN

Immunized animal No.	Time of collecting cells	Lympho-cytes	Plasmo-blasts	Plasma cells	Small pyronino-philic lymphocytes	Granulo-cytes
10	Before injection	63·0	—	—	0·5	36·5
	10 minutes after injection	76·0	—	—	3·0	21·0
	1 day after injection	66·0	1·0	1·0	0·5	31·5
	2 days after injection	54·5	1·5	—	1·0	43·0
	3 days after injection	35·5	2·0	0·5	1·5	60·5
	4 days after injection	55·0	4·5	2·0	2·0	36·5
11	Before injection	70·5	—	—	0·5	29·0
	10 minutes after injection	90·0	1·0	1·0	—	8·0
	1 day after injection	84·0	—	—	2·0	14·0
	2 days after injection	65·0	4·0	8·0	10·0	13·0
	3 days after injection	84·0	1·5	—	5·0	9·5
	4 days after injection	31·5	0·5	1·5	1·5	65·0

DISCUSSION

The data obtained in this investigation attest the synthesis of antibody by blood leucocytes and thoracic duct cells of immunized rabbits. They confirm our preliminary experiments (Hulliger and Sorkin, 1963) with blood leucocytes, although buffy coat leucocytes gave somewhat lower results in this series than reported in our preliminary note. Besides the animal variation this might be explained by the fact that the thoracic duct lymph was drained for a few hours before the collection of the buffy coat. As lymphocytes and some other mononuclear cells are now known to circulate fairly rapidly, the cannulation may have induced a decrease in the number of such peripheral blood cells. Leucocyte counts did not, however, reveal any significant difference before and after cannulation.

In the present and previous experiments twenty hyperimmunized rabbits were examined for antibody production by splenic and peripheral blood cells. Appreciable synthesis of specific antibody by blood leucocytes was demonstrated in twelve of these animals. In each of the reactors the values for antibody production by spleen cells were considerably higher than those for peripheral cells. In this study thoracic duct cells were also shown to be capable of producing antibody. Only two animals gave high values for thoracic duct cells and they had correspondingly high values in spleen and buffy coat cells.

The findings on antibody production by circulating blood leucocytes have meanwhile been confirmed by Landy *et al.* (1964). These authors also worked with rabbits, but their experimental situation differed strikingly in many ways from ours. While we gave a total of eight intravenous injections about 300 *milligrams* of a relatively low molecular weight serum protein, Landy and his colleagues gave one intravenous injection of only 5 *micrograms* of the high molecular weight *S. enteritidis* polysaccharide. Production of antibody was demonstrated in our experiments by *de novo* synthesis of antibody *in vitro* by incubation of blood leucocytes with ^{14}C-amino acids. The other workers showed production of specific antibody by a modification of the Jerne procedure for localized haemolysis in gel (Jerne, Nordin and Henry, 1963). Related results with a similar technique have also been obtained by Bussard and his colleagues (Bussard, personal communication).

Elves, Roath, Taylor and Israels (1963) have also produced suggestive evidence by fluorescence techniques for γ-globulin, possibly specific immunoglobulin production by human blood leucocyte cultures *in vitro*. Thorbecke, Asofsky, Hochwald and Jacobson (1964) have reported synthesis of immunoglobulins *in vitro* by blood leucocytes of rhesus monkeys immunized with type II pneumococci. In addition these authors stated that 'pure lymphocyte' suspensions from the thoracic duct of humans and rats were extremely active in synthesizing immunoglobulins, but unfortunately no data on the cellular composition were given.

Taking all these data together it seems a reasonable assumption that the appearance of circulating antibody-forming cells may represent a significant aspect of the overall immune response.

Many questions which are more fully discussed elsewhere (Sorkin and Landy, 1965) may be posed.

(1) Have these antibody-forming cells in blood been antigenically stimulated while in the peripheral circulation? Or alternatively did these cells originate from classical antibody-forming lymphoid organs, e.g. spleen or lymph nodes? The recent work on transformation of peripheral lymphocytes *in vitro* by means of specific antigen and the resultant blast cell and γ-globulin formation provides some indication for the former assumption (see for example Hirschhorn, Bach, Kolodny, Firschein and Hashem, 1963).

(2) Is the chemical nature of the specific immunoglobulins produced by peripheral cells similar to that synthesized within the architecture of the lymphoid organs?

(3) The nature, destination and fate of these circulating antibody-forming cells poses still another problem. The relocation of these cells, perhaps in a morphologically different form, in other sites must be considered.

(4) The demonstration of antibody-producing cells in unfamiliar sites should now perhaps be viewed with some caution as such cells may well have originated in other sites, although they might have undergone some local proliferation (Askonas and Humphrey, 1958).

(5) What is the overall contribution of circulating blood leucocytes to the total antibody production? In our preliminary report (Hulliger and Sorkin, 1963) values were presented which showed that the total of all blood leucocytes produced approximately the same amount of specific anti-HSA antibody as did spleen.

It is important to know the types of cells in blood which are responsible for antibody production. Detailed studies on the composition of blood leucocytes were performed and are shown in Tables 2 and 4. There was found consistently a great increase in the numbers of pyroninophilic cells in blood at the time when antibody synthesis occurred (0·5–9 per cent). Some of these cells were plasmablasts and plasma cells while others were of the type of small pyroninophilic lymphocytes.

The question as to which cell type is responsible for antibody production by thoracic duct lymph cells could not be answered as clearly as had been hoped. In our immunized rabbits thoracic duct cells consisted not only of small lymphocytes, as has been claimed by Wesslén (1952) and by Hallander and Danielsson (1962). Thoracic duct lymph also contained a small percentage of pyroninophilic cells, mostly around 5 per cent. Only few of these are typical plasma cells, most of them are best categorized as small pyroninophilic lymphocytes. As in the present work Wesslén and Hallander and Danielsson also used a hyperimmunization procedure and challenged the animals 3–4 weeks later. They were, however, unable to demonstrate antibodies in the lymph cells immediately after collection.

Antibodies were released only after incubation *in vitro* for 6–24 hours. Since these authors did not measure antibody formation *de novo* it could well have been that the antibodies measured after incubation were carried by lymphocytes and slowly released rather than actually synthesized.

As regards the cell type responsible for the antibody synthesis measured in thoracic duct lymph and peripheral blood leucocytes we have obtained no evidence to support the hypothesis that small lymphocytes are involved. The increase in pyroninophilic cells is definitely related to the response to the challenging injection of antigen. It is most probable that the pyroninophilic cells are responsible for the synthesis of antibody. However, it is emphasized that our findings are not in conflict with the concept of Gowans *et al.* (1962) that the small lymphocyte is an immunologically competent cell. It may well be that under different conditions than in lymph or blood small lymphocytes can produce antibodies.

As shown in Table 2 and recently in work with blood leucocytes (Sorkin, 1964) lymph cells also carry cell-bound antibody on their surfaces. But in the above experiments, although cytophilic antibody titres were high in certain sera and lymph fluid, no direct correlation existed between the cytophilic titres of the fluid medium and the fixation of antigen onto cells of these animals. It is therefore rather improbable that the amount of cell-fixed antibody measured is distributed evenly onto the surface of all the cells. We rather suggest that the major amount of cell-bound antibody as measured by antigen binding is present on the surface of and within antibody-producing cells in blood and lymph. This assumption is strengthened by the fact that methanol treatment of these cells prior to antigen adsorption increases the extent of antigen adsorption significantly. We assume that methanol treatment increases the surface permeability so antigen can interact with intracellular antibody.

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