respective silicone controls.) On the other hand, the control PGT performed in glass showed an increase of the plasminoplastin generation only in normal plasma, not in the Hageman deficient.

It seems, therefore, that when Hageman factor is activated, this triggers both systems: (*a*) the clotting mechanism; (*b*) the fibrinolysis. What can be demonstrated in the test-tube could very well occur *in vivo*[11]. Since XI, X, IX and VIII deficient plasmas, in which factor XII is normal, behave in the PGT like normal plasmas, we may conclude that factors XI, X, IX and VIII have no fibrinolytic activity and only factor XII is the link between coagulation and fibrinolysis.

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IMMUNOLOGY

Immunological Significance of Lysosomes within Lymphocytes *in vivo*

RECENT reports[1,2] have demonstrated the presence of lysosome-like particles in human peripheral blood lymphocytes and have shown that these increase in size and number in tissue culture following stimulation with phytohaemagglutinin. These changes appear to precede the transformation of the small lymphocytes into cells which synthesize RNA and eventually undergo mitosis. In both these reports[1,2] it is suggested that these lysosomes are in some way involved in the initiation of cell division.

In this work we have investigated the relative number of small lymphocytes *in vivo* which contain lysosomes, and the average number of lysosomes within lymphocytes in lymph nodes draining the site of application of a chemical sensitizing agent, at various stages during the initiation of delayed-type hypersensitivity. The development of delayed-type hypersensitivity has been shown to be associated with massive proliferation of small lymphocytes in the cortex of the draining lymph node[3,4]. This has been shown to occur through an intermediate large pyroninophilic cell which has many features in common with the transformed lymphocyte found following stimulation with phytohaemagglutinin *in vitro*. It was therefore essential to find out whether lymphocyte lysosomes responded *in vivo* to an immunological stimulus which would induce lymphocyte proliferation in the lymph node. An investigation was also undertaken to see whether any changes could be detected in the lysosomes within small lymphocytes in the circulation and in an inflammatory exudate in an established hyperimmune state. The system chosen was one where the animals were in an exquisitely sensitive delayed hypersensitive state to tuberculin, following the injection of dead tubercle bacilli in water-in-oil emulsion.

Guinea-pigs of the Hartley strain bred in the Institute of Dermatology were used throughout. They were examined either fresh from stock at a weight of between 400 and 500 g, or following sensitization either by one application of the chemical sensitizing agent 2-phenyl-4-ethoxymethylene-5-oxazolone (oxazolone) or by the injection of heat-killed *Mycobacterium tuberculosis* (tubercle bacilli) in water-in-oil emulsion.

Sensitization to oxazolone was with 0·2 ml. of 10 per cent oxazolone dissolved in ethanol and painted on the left ear[3]. Sensitization with tubercle bacilli was by injection of 1 mg of heat-killed dried bacilli (obtained from the Ministry of Agriculture Central Veterinary Laboratory, Weybridge) suspended in water-in-oil emulsion using Freund's incomplete adjuvant (Difco). Guinea-pigs were injected intramuscularly with a total of 0·8 ml. divided equally between four sites in the thighs and the nape of the neck between three and four months before the experiment. They were selected because they gave a necrotic reaction 24 h after the intradermal injection of 1·3 μg tuberculin (human PPD, obtained from the Ministry of Agriculture Central Veterinary Laboratory, Weybridge). Imprints of the left auricular lymph nodes were made from normal guinea-pigs and 2, 4 and 6 days after the application of oxazolone to the left ear.

Peritoneal exudates containing large numbers of mononuclear cells were obtained by washing out the peritoneal cavity with Hanks's balanced salt solution four days after the intraperitoneal injection of 30 ml. of sterile light liquid paraffin B.P. The cells were washed twice, diluted to a concentration of 3×10^6 cells/ml. in 5 per cent bovine albumin (Armour, fraction V) and centrifuged lightly on to glass slides[5].

Peripheral blood lymphocytes were prepared from the blood of guinea-pigs injected intravenously with 10 mg of heparin. The erythrocytes were allowed to settle following the addition of an equal volume of 5 per cent polyvinylpyrrolidone ('Polyvidone', May and Baker) in 0·15 M sodium chloride. The supernatant was first centrifuged at 150*g* to remove polymorphonuclear leucocytes and the residual cells in the supernatant ($\sim$ 90 per cent mononuclear cells) were centrifuged on to glass slides in 5 per cent bovine albumin as outlined here.

Acid phosphatase was demonstrated by the Gomori method as modified by Holt[6,7] using sodium-β-glycerophosphate as the substrate. The incubation medium was prepared as follows: 0·1 M sodium-β-glycerophosphate (B.D.H.), 0·003 M lead nitrate and 0·05 M acetate buffer (*p*H 5). The mixture was kept in the incubator at 37° C for 18 h and the precipitate was filtered. Fresh unfixed cell preparations were incubated in the substrate solution at 37° C for varying periods up to 2 h. An incubation time of 1–2 h was found to be necessary to obtain definite reactions in small lymphocytes. The slides were then immersed in a saturated solution of hydrogen sulphide prepared by bubbling hydrogen sulphide gas from a Kipp's apparatus into distilled water. They were then mounted in 8 per cent gelatine in an equal volume of glycerol and water. Control slides were incubated in the same substrate medium but containing 0·01 M sodium fluoride to inhibit the enzyme.

Lysosomes within cells of the lymphoid series were identified as granules which stained for acid phosphatase. These could be activated by pre-incubation in an acid buffer at *p*H 5·0 and the enzyme activity was stripped off by treating the preparation with 'Triton *X* 100'. Pre-treatment with distilled water increased the enzyme activity of the granules, which was also inhibited by 0·01 M sodium fluoride. The granules were therefore found to possess the property of latency of enzyme activity. By these criteria it was considered that these granules could definitely be identified with lysosomes which have been defined previously[8] by biochemical criteria.

An investigation was undertaken of cells in imprints of the auricular lymph nodes of normal guinea-pigs and those which had been painted with oxazolone on the left ear. Three types of cells were seen containing acid

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phosphatase staining granules. One group consisted of large cells containing granules which stained after only 5-min incubation with the substrate mixture. These would appear to be cells of the macrophage series and were similar to cells in the peritoneal exudate which had ingested oil; the cytoplasm of these cells was not basophilic. A second group of large cells which had an intensely basophilic staining cytoplasm and corresponded to the large pyroninophilic cells in previous studies[3,4] had a much smaller number of granules which only began to stain for acid phosphatase after 20 min incubation with the substrate mixture. Finally, there were small lymphocytes which contained granules which stained optimally for acid phosphatase following 2-h incubation. As can be seen in Table 1, there was a marked increase in the proportion of small lymphocytes containing lysosomes in the lymph nodes draining an area of application of a chemical sensitizing agent. The average number of lysosomes per cell also increased, reaching a peak 4 days after sensitization, falling off on the sixth day after sensitization.

Table 1. Effect of Sensitization to Oxazolone on the Activity of Lysosomes in Lymphocytes within the Draining Lymph Node

Auricular lymph node	Small lymphocytes: Maximum percentage of cells containing lysosomes	Small lymphocytes: Mean number of lysosomes per cell	Large lymphoid cells: Per cent cells containing lysosomes
Normal	10	3	19·5
2 days after sensitization	78	6·3	77
4 days after sensitization	89	8·1	93·5
6 days after sensitization	85	5·1	77·5

All figures are the mean of those found on examination of the material from six different animals. The 'large lymphoid cells' were found to have a strongly basophilic cytoplasm. Highly active macrophages which were not basophilic were not included in this series.

About 35 per cent of small lymphocytes in the peripheral blood or an oil-induced peritoneal exudate from normal guinea-pigs were found to contain lysosomes (Table 2). In guinea-pigs which had been sensitized with dead tubercle bacilli in water-in-oil emulsion 3–4 months previously, the number of small lymphocytes containing lysosomes had increased to between 80 and 90 per cent. Cells from sensitized animals contained about 10–11 lysosomes per cell as compared with 3·5–5 lysosomes per cell in normal animals. Lysosomes in small lymphocytes from sensitized animals were seen to be markedly swollen when compared with those from normal animals. Incubation of cells from peritoneal exudates with the substrate mixture for only 5 min showed that macrophages from tuberculin-sensitive animals had more active lysosomes than macrophages from normal animals.

Table 2. Effect of Sensitization with Tubercle Bacilli on the Activity of Lysosomes within Small Lymphocytes in the Peripheral Blood and in a Peritoneal Exudate

Source	Normal guinea-pigs: Per cent active cells	Normal guinea-pigs: Mean No. of lysosomes per cell	Tuberculin sensitized guinea-pigs: Per cent active cells	Tuberculin sensitized guinea-pigs: Mean No. of lysosomes per cell
Peripheral blood	36 (3)	5·1	80·6 (3)	11·5
Peritoneal exudate	35·2 (5)	3·5	92·2 (5)	9·8

Figures in parentheses indicate the number of animals examined.

In an investigation of this type it is important to differentiate cells of the lymphocyte series from macrophages. Macrophages can be distinguished easily because their lysosomes appeared to have a more fragile lysosomal membrane and consequently the lysosomes stain for acid phosphatase after a shorter incubation time than cells of the lymphocyte series. Lymphocyte lysosomes would appear to be more stable than those of macrophages. Large cells of the lymphocytes series with a basophilic cytoplasm (immunoblasts) were seen in lymph nodes draining the area of application of a chemical-sensitizing agent, reaching a peak in concentration on the fourth day after sensitization. A high proportion of these cells contained lysosomes. However, these lysosomes were present in smaller numbers and were far less fragile than those seen in macrophages. These cells would appear to be similar to those described by previous authors[1,2] in studies on the lysosome content of lymphoid cells in tissue cultures containing phytohaemagglutinin.

On the days immediately following the application of a chemical sensitizing agent to the skin, there was a marked increase in the number of small lymphocytes containing lysosomes in the draining lymph node. The number of active lysosomes in an active cell also increased, reaching a peak on the fourth day after sensitization. It is not known whether these cells were precursors of the immunoblasts, or were derived from them by cell division. However, they were still present in large numbers on the sixth day after sensitization, at a time when it was known that the number of immunoblasts had fallen considerably[3,4]. It would therefore be unlikely that these cells were about to differentiate into immunoblasts.

It may also be that small lymphocytes containing lysosomes are immunologically competent cells. It is therefore interesting that there is an increased number of small lymphocytes with lysosomes in animals injected with tubercle bacilli in water-in-oil emulsion and that these are also much larger in size and increased in number. This may also have some bearing on the increased immunological responsiveness of animals injected with Freund's adjuvant. One might also speculate on whether the small lymphocytes which contain lysosomes are the effector cells in delayed-type hypersensitivity. However, in preliminary experiments in which tuberculin has been injected intraperitoneally in a dose sufficient to cause aggregation of macrophages (10 μg PPD)[9] no significant change was found in the lysosomes of either small lymphocytes or macrophages.

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Precocious Anti-diphtheria Response induced by RNA Immuno-carrier

In previous papers an increase of the RNA content in the serum of rabbits immunized with horse serum albumin and rat or guinea-pig red blood cells was observed; the greatest increase was in the gamma-globulin fraction. The RNA extracted from immune rabbit sera (RNA Immuno-Carrier or RNA-I-C) was able to induce in normal rabbits a precocious antibody production against the same antigens used for immunizing the animals' source of the RNA[1,2].

It was then found that RNA-I-C from the serum of animals immunized with heterologous RBC was able to induce rapid antibody production in normal animals of different species[3]. The possibility was also demonstrated of obtaining a precocious auto-haemantibody response in normal rats treated with a suitable amount of RNA-I-C from serum of rabbits immunized with rat RBC[4].

The following investigations were undertaken to ascertain whether it is possible to induce a precocious antibody response in normal animals with RNA from serum of animals immunized against diphtheria toxin. Further-

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