A SUPRAVITAL STUDY OF LEUCOCYTES IN ALLERGIC STATES
A COMPARISON OF DELAYED AND IMMEDIATE INTRA-PLEURAL ANAPHYLACTIC REACTIONS *

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It has long been appreciated that the delayed skin reaction to proteins in rabbits (Arthus phenomenon) possesses some features in common with the tuberculin reaction in guinea-pigs in that both are delayed in onset and are characterized by similar macroscopic lesions, edema, erythema, induration and often necrosis. The authors, with Bradley,¹ have recently studied by supravital staining methods the behavior of reacting leucocytes during intra-pleural tuberculin reactions in guinea-pigs. It has seemed desirable to apply these same methods to the study of delayed and immediate anaphylactic skin reactions, the first being the type of allergy seen in rabbits and the second being the variety found in the guinea-pig.

Consequently a series of rabbits were sensitized by three intraperitoneal injections of horse serum at four day intervals, a total of 10 cc. of antigen. Twenty-one days after the initial injection, all of the rabbits gave strong precipitin reactions up to 1:10,000 dilution of antigen. Pleural exudates were induced in six animals, three sensitized and three controls, by intrapleural injections of 5 cc. of sterile hormone broth to which was added 0.3 cc. of horse serum. The cells of the resultant exudates were examined twenty-four hours later by supravital neutral red and Janus green staining in a constant temperature box. Cell counts are indicated in the following protocols.

RABBIT 774 (sensitized):

living polymorphonuclear neutrophiles, 41 per cent; dead, 44 per cent;
living lymphocytes, none; dead lymphocytes, 2 per cent;
living monocytes, none; dead, 8 per cent;
living clasmatocytes, none; dead, 5 per cent.

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RABBIT 1 (control):
- living polymorphonuclear neutrophiles, 88 per cent; dead, none;
- living polymorphonuclear basophiles, 1 per cent; dead, none;
- living monocytes, 4 per cent; dead, none;
- living clasmatoocytes, 7 per cent; dead, none.

RABBIT 775 (sensitized):
- living polymorphonuclear neutrophiles, 2 per cent; dead, 75 per cent;
- living polymorphonuclear eosinophiles, none; dead, 6 per cent;
- living lymphocytes, none; dead, 1 per cent;
- living monocytes, none; dead, 13 per cent;
- living clasmatoocytes, none; dead, 3 per cent.

RABBIT 2 (control):
- living polymorphonuclear neutrophiles, 87 per cent; dead, none;
- living monocytes, 12 per cent; dead, none;
- living clasmatoocytes, 1 per cent; dead, none.

RABBIT 776 (sensitized):
- living polymorphonuclear neutrophiles, none; dead, 90 per cent;
- living lymphocytes, none; dead, 2 per cent;
- living monocytes, none; dead, 8 per cent.

RABBIT 3 (control):
- living polymorphonuclear neutrophiles, 90 per cent; dead, none;
- living small lymphocytes, 1 per cent; dead, none;
- living monocytes, 9 per cent; dead, none.

Examination of the peripheral blood of these rabbits revealed no dead cells.

Three guinea-pigs were sensitized to horse serum by doses similar to those used for the rabbits. Eighteen days after the last injection attempts were made to induce exudates in these and in three control guinea-pigs by intrapleural injection of 3 or 4 cc. of hormone broth. The following day all animals received additional intrapleural injections of 0.3 cc. horse serum to note its immediate effect on the cells of the pre-existing exudates. The results were not satisfactory. Two of the three sensitized guinea-pigs failed to show fluid; the third one gave a doubtful result, since many leucocytes in the exudates of both the sensitized and the corresponding control guinea-pig were injured, probably from over concentration of the dye but possibly from some normal toxic effect of the horse serum on guinea-pig cells. Two other control guinea-pigs, however, showed no dead cells in their exudates. To avoid any non-specific toxic action of horse serum, a second series of five guinea-pigs were sensitized by
two intraperitoneal injections of chemically pure crystalline egg albumen dialyzed free from ammonium sulphate. The total dose of antigen was 6 cc. of an approximately 10 per cent solution. Eighteen days after the last injection, pleural exudates were produced in the usual manner in four of the animals and twenty-four hours later 0.8 cc. of egg albumen solution was added to the pre-formed exudate. Two control animals were similarly treated. Cell examinations were made both before and after intrapleural injection of antigen. No dead cells were found before administering the antigen. The cell counts after antigen injection appear in the following protocols.

**Guinea-pig 431 (sensitized):**
- living polymorphonuclear neutrophiles, 46 per cent; dead, none;
- living polymorphonuclear eosinophiles, 10 per cent; dead, none;
- living lymphocytes, 2 per cent; dead, none;
- living monocytes, 42 per cent; dead, none.

**Guinea-pig 1 (control):**
- living polymorphonuclear neutrophiles, 57 per cent; dead, none;
- living polymorphonuclear eosinophiles, 4 per cent; dead, none;
- living lymphocytes, 2 per cent; dead, none;
- living monocytes, 34 per cent; dead, none;
- living clasmaticocytes, 3 per cent; dead, none.

**Guinea-pig 432 (sensitized):**
- living polymorphonuclear neutrophiles, 66 per cent; dead, none;
- living polymorphonuclear eosinophiles, 2 per cent; dead, none;
- living monocytes, 28 per cent; dead, none;
- living clasmaticocytes, 4 per cent; dead, none.

**Guinea-pig 2 (control):**
- living polymorphonuclear neutrophiles, 66 per cent; dead, none;
- living polymorphonuclear eosinophiles, 1 per cent; dead, none;
- living monocytes, 28 per cent; dead, none;
- living clasmaticocytes, 5 per cent; dead, none.

**Guinea-pig 433 (sensitized):**
- living polymorphonuclear neutrophiles, 64 per cent; dead, 3 per cent;
- living polymorphonuclear eosinophiles, 4 per cent; dead, none;
- living lymphocytes, 2 per cent; dead, none;
- living monocytes, 17 per cent; dead, none;
- living clasmaticocytes, 10 per cent; dead, none.

**Guinea-pig 3 (control):**
- living polymorphonuclear neutrophiles, 67 per cent; dead, none;
- living polymorphonuclear eosinophiles, 2 per cent; dead, none;
- living monocytes, 30 per cent; dead, none;
- living clasmaticocytes, 1 per cent; dead, none.
In order to verify the sensitization, guinea-pig 432 was given 1 cc. of antigen intravenously after the pleural tap had been made; the animal died a typical anaphylactic death in about five minutes. Guinea-pig 433 died an anaphylactic death about forty-five minutes after receiving the antigen intrapleurally; necropsy showed distended, rigid lungs, a heart still beating and small gastric hemorrhages; microscopically lungs were markedly edematous. The cells of the exudates of both of these animals were alive and motile three hours after the death of the guinea-pig and of course were all that time in contact with antigen.

One guinea-pig received 0.3 cc. of antigen in 4 cc. of hormone broth in the right pleural cavity in order that any delayed reaction might be studied. In twenty-four hours the fluid contained only living cells. The cell count was as follows:

- living polymorphonuclear neutrophiles, 77 per cent; dead, none;
- living polymorphonuclear eosinophiles, 1 per cent; dead, none;
- living small lymphocytes, 1 per cent; dead, none;
- living monocytes, 16 per cent; dead, none;
- living clasmatocytes, 5 per cent; dead, none.

The question was then raised as to whether the rabbits sensitized to horse serum would show any immediate reaction. Consequently, using the same rabbits employed for the study of the delayed reaction, the precipitin titer was brought back to a high level (1:10,000) by two additional injections of horse serum intraperitoneally. Pleural fluids were produced with hormone broth and in twenty-four hours 1 cc. of horse serum was added to the exudate. The rabbits were tapped twenty to thirty minutes later. One animal gave no fluid; the other two yielded an exudate containing only living cells.

**RABBIT 774 (sensitized):**

- living polymorphonuclear neutrophiles, 42 per cent; dead, none;
- living polymorphonuclear basophiles, 1 per cent; dead, none;
- living polymorphonuclear eosinophiles, 1 per cent; dead, none;
- living lymphocytes, 1 per cent; dead, none;
- living monocytes, 23 per cent; dead, none;
- living clasmatocytes, 32 per cent, dead, none.

**RABBIT 775 (sensitized):**

- living polymorphonuclear neutrophiles, 50 per cent; dead, none;
- living lymphocytes, 6 per cent; dead, none;
- living monocytes, 15 per cent; dead, none;
- living clasmatocytes, 26 per cent; dead, none;
- living serosal cells, 3 per cent; dead, none.
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Summarizing the above results, it will be seen that in rabbits the leucocytes are killed after contact with the antigen for twenty-four hours but not after a shorter period, i.e., thirty minutes. In guinea-pigs, on the other hand, the leucocytes are not injured at either period. The differences in the results in these two animals are interesting and significant in view of the difference in their skin reactions. As is well known, a sensitized guinea-pig gives an immediate skin reaction on reinjection of the antigen intradermally; this skin reaction is transient and causes no lasting injury such as necrosis. The guinea-pig under ordinary conditions, at least, does not give a delayed skin reaction. A sensitized rabbit, on the other hand, never gives an immediate skin reaction but does give a delayed skin reaction which is more than transient and in a highly sensitized animal leads to definite injury such as necrosis. The cause for the variation in the two animals is not yet clear; it has been suggested by some that the difference is dependent on the inequality in amount of circulating antibodies, for in guinea-pigs the titer of these is usually low in comparison to the titer in rabbits. However, whatever the explanation of this difference in type of reaction may be, in the above experiments an analogous condition as regards their leucocytes apparently holds true, i.e., the leucocytes of the guinea-pig are uninjured by antigen whereas those of the rabbit are killed. This apparently is a fundamental difference and may throw some light on the dissimilarity in the types of skin reactions, for it seems improbable that these cells alone, the leucocytes, should be the only cells that become sensitive to the antigen. Here again the presence of a large amount of circulating antibody can conceivably be the cause of this difference; further work along this line is in progress.

On comparing these results with those obtained by the authors in working with tuberculin sensitization in guinea-pigs, a striking similarity will be noted. In a tuberculin reactive guinea-pig the leucocytes are killed on contact with tuberculin for twenty-four hours. The skin tuberculin reaction in a guinea-pig is of course a delayed reaction and the analogy with a rabbit sensitive to horse serum, giving a delayed reaction, whose leucocytes are killed by the antigen, is evident.

If we consider the death of leucocytes on exposure to antigen as due to some definite inherent cause dependent on factors other than circulating antibody content, then we are faced with the following
situation. In protein sensitized guinea-pigs that give only an immediate reaction, the leucocytes are uninjured by antigen. In protein sensitized rabbits that give only a delayed reaction, the leucocytes are killed by the antigen. In guinea-pigs reactive to tuberculin, that give only a delayed reaction, the leucocytes are killed by the antigen. How leucocytes of tuberculin sensitive rabbits behave in contact with tuberculin is a question which will be dealt with in the near future.

Two small histologic details, although wholly apart from the general subject of this communication, nevertheless deserve reporting. The first of these concerns the question of phagocytized polymorphonuclears. The writers had always assumed from study of fixed preparations that when once a polymorphonuclear had been taken up by a monocyte or clasmacocyte, the phagocytized cell died. Probably in most instances this is true although death is not immediate and many living polymorphonuclears are engulfed to be destroyed later. On one occasion during the present study, a phagocytized polymorphonuclear neutrophile was observed to free itself completely from a clasmacocyte and to escape. The other point of note may be described briefly as follows.

While studying the pleural effusions of rabbits for evidence of an immediate type of reaction, we observed what appeared to be direct cell division in two polymorphonuclear leucocytes; one of these divisions was seen too late to follow the method, but the other was observed in fair detail: a polymorphonuclear neutrophile with a triple nuclear mass was seen to segment partially, one of the nuclear masses passing through the constricted zone and two remaining behind; granules could be observed passing through the constricted portion which gradually became greatly attenuated and finally ruptured, the cells moving off in opposite directions. No evidences of mitotic phenomena were seen. So far as we are aware, neither of the above observations has hitherto been recorded although no survey of the literature has been made.

**Summary and Conclusions**

In the delayed intrapleural anaphylactic reaction in rabbits sensitive to horse serum, reacting leucocytes are largely killed.

In this respect, the reaction parallels the intrapleural tuberculin reaction in guinea-pigs.
Attempts to induce an immediate intrapleural anaphylactic reaction in rabbits resulted in no apparent cell injury.

In sensitized guinea-pigs no cell injury either immediate or delayed was observed when antigen was introduced into the pleural cavity. Evidence gleaned from this study accords well with facts already known about delayed and immediate anaphylaxis.

REFERENCE