Experimental Studies on the Standard of Evaluating the Defence Reaction of the RES against Inflammation

By

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I. INTRODUCTION

The series of studies on the reticuloendothelial system (RES) at this laboratory parts in the defensive reactions and these undergo proliferation and transformation to various extents to suit the required functional conditions. The present authors were led to infer that a quantitative evaluation of the extent of proliferation of RES cells might enable us to estimate the defensive capacity of RES against inflammation and thence to deduce the defensive resistance of the living organism in concreto, by morphological examination of the cells. The following experiments were carried out under this postulation.

II. EXPERIMENTAL METHODS

Pieces of small cover glasses (4 mm × 6 mm) were introduced into the subcutaneous tissue of rabbits, and at stated intervals, these pieces of glass were extracted together with a part of the surrounding subcutaneous tissue and distended preparations were made of the latter. Both the glass specimen and the distended preparations were stained with neutral red, Janus green or by May-Giemsa method and examined under a microscope, for observation of the transformation and proliferation of RES cells in response to the invasion of the glass piece.

First, normal rabbits were thus experimented upon, for the purpose of determining the normal range of the reaction of RES cells, then similar experiments were made upon variously pretreated rabbits.

III. EXPERIMENTAL RESULTS

1. Reaction of the subcutaneous histiocytes in normal rabbits

In the distended preparations of the subcutaneous tissue of normal rabbits sampled before the insertion of glass, histiocytes of fixed form alone are found always sporadically but never in proliferated or in groups, and no basophilic
elongated spindle-form histiocytes or any leucocyte is ever present. After the
glass insertion, the subcutaneous histiocytes proliferate and are liberated from
the tissue and begin to be found adhered to the surface of the extracted glass
pieces. Mononuclear cells appeared on the glass surface to the rate of 10-15% in
12 hours, 25% in 24 hours and 45-55% on the second day; giant cell formation
from these mononuclear cells began to be observed on the third day.

In a normal hare similarly treated, the appearance of histiocytic cells on the
glass surface was somewhat more marked than in normal rabbits.

### Table I. Subcutaneous Findings in Normal Rabbits

<table>
<thead>
<tr>
<th></th>
<th>Before glass insertion</th>
<th>After glass insertion</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>12h</td>
</tr>
<tr>
<td>No. 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (-)</td>
<td>A</td>
<td>#</td>
</tr>
<tr>
<td>P.H (-)</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.5%</td>
</tr>
<tr>
<td>No. 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (-)</td>
<td>A</td>
<td>#</td>
</tr>
<tr>
<td>P.H (-)</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.0%</td>
</tr>
<tr>
<td>No. 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (-)</td>
<td>A</td>
<td>#</td>
</tr>
<tr>
<td>P.H (-)</td>
<td>B</td>
<td>±</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12.5%</td>
</tr>
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</table>

### Normal Hare

<table>
<thead>
<tr>
<th></th>
<th>Before glass insertion</th>
<th>After glass insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12h</td>
</tr>
<tr>
<td>L (-)</td>
<td>A</td>
<td>#</td>
</tr>
<tr>
<td>P.H (±)</td>
<td>B</td>
<td>±</td>
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<tr>
<td></td>
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<td>14.3%</td>
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</table>

A: On distended preparations  B: On inserted glass surface
L: Leucocytes                  P.H: Prehistiocytes
F.H: Free histiocytes          M.C: Mononuclear cell
G.C: Giant cell

2. Ditto in pretreated rabbits

Normal rabbits were subjected to various pretreatments for observing the
changes in the activation or inhibition of the function of subcutaneous histiocytes
after such pretreatments. The pretreatments consisted in administration of
heterogeneous protein (typhoid vaccine, communin and horse serum), antibiotics
(streptomycin, PAS, INAH, sarkomycin and promin), lipoids (cholesterin,
cholesterin plus lecithin, and hydrocarpus oil), pigments (Trypan-blue and Evans-
blue), and X-ray irradiation (200 r, 400r and 800r), as specified in Table II.
1) After heterogeneous protein
In the animals pretreated with heterogeneous protein, the proliferation of RES cells was found more rapid and next after typhoid vaccine. In the distended preparations sampled before the insertion of glass, prehistiocytes not observed in the intact rabbits were discovered in all the cases of this group.

2) After antibiotics
INAH and promin caused the strongest proliferative reaction, followed by streptomycin and sarkomycin, but PAS administration was followed by a somewhat subnormal reaction only

3) After lipoids
The animals pretreated with lipoids showed strongest reactive proliferation without exception

4) After pigments
While Trypan-blue called forth a strong proliferation of the RES cells, the reaction after injection of Evans-blue was rather feeble, showing that the latter causes a hypofunction of the RES

5) After X-ray irradiation
Upon irradiation of 200r or 400r of X-ray, the reactive proliferation was
above normal, but irradiation of 800 r was followed by a mere subnormal reaction.

IV. DISCUSSION

As the promising indicators in morphologically evaluating the intensity of the reaction of RES cells, the author first selected the velocity of and the number in appearance of the histiocytes, the variability of the size of their cell bodies and nuclei, their basophilia, the increase of mitochondria, the rapidity of appearance and the number of newly formed histiocytes, especially, rounded histiocytes, and in particular, took up the quantitative change of the prehistiocytes and the new histiocytes produced from them as reflected on the surface of the inserted pieces of glass, as listed in the foregoing tables, as criteria for evaluating the reaction. In
Standard of Defence Reaction of RES

the comparative study of my experimental results, the following points were given special attention:

a. The relative proliferation per hour of the mononuclear cells appearing on the inserted glass piece between 12 and 48 hours after the insertion.

**Table IV**

Percentage of Mononuclear Cells Detected on the Glass Surface

<table>
<thead>
<tr>
<th>Condition</th>
<th>% of Detection</th>
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<tbody>
<tr>
<td>Cholesterin</td>
<td>90</td>
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<tr>
<td>X-200 r</td>
<td>80</td>
</tr>
<tr>
<td>INAH</td>
<td>70</td>
</tr>
<tr>
<td>Trypan-blue</td>
<td>60</td>
</tr>
<tr>
<td>Communin</td>
<td>50</td>
</tr>
<tr>
<td>Hydrocarpus oil</td>
<td>40</td>
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<tr>
<td>Streptomycin</td>
<td>30</td>
</tr>
<tr>
<td>Typhoid vaccine</td>
<td>20</td>
</tr>
<tr>
<td>Horse serum</td>
<td>10</td>
</tr>
<tr>
<td>Sarkomycin</td>
<td></td>
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<tr>
<td>PAS</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>X-800 r</td>
<td></td>
</tr>
<tr>
<td>Evans-blue</td>
<td></td>
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</tbody>
</table>

Grade of Detection of Giant Cells on the Glass Surface

\[ h : \text{hour} \]
\[ d : \text{day} \]
b. The relative frequency of the giant cells found on the test glass on the third day.

c. Comparative study of the findings on the distended preparation of the subcutaneous tissue sampled before the insertion of the glass sheets and the picture of proliferation of the mononuclear cells on the inserted glass.

d. Comparative examination of the findings on the distended preparations of the subcutaneous tissue sampled from animals subjected to different pretreatments.

Summing up such findings, the proliferation of RES cells was evaluated as having been the most rapid and strongest after INAH, cholesterin, communin, Trypan blue and 200 r of X-ray, owing to conspicuous activation of the histiocytes (Grade +3), next after typhoid vaccine and streptomycin (Grade +2), rather weak after horse serum and sarkomycin (Grade +1), but a little weaker than normal after PAS and somewhat still feeble after 800 r of X-ray (Grade -1) and Evans-blue caused a rather remarkable hypofunction of the RES at the dose used in the experiment (Grade -2).

In short, it was ascertained that the percentage of the mononuclear cells appearing on the surface of the inserted glass sheets on the second day and the number of giant cells appearing there on the second and the third days after the insertion will enable us to make inferences on the intensity of the RES cell reaction to an extent.

V. SUMMARY

1. In consideration of the important part played by the RES in dealing with inflammatory foci, an attempt was made in finding a method of quantitatively evaluating the defense reaction of this system. The authors succeeded in demonstrating the possibility of evaluating the intensity of the reaction by cytological examination at stated intervals of the infantilization of the RES cells, their numerical increase and the number of detected round histiocytes appearing in the inflammatory foci against foreign bodies induced by insertion of slide-glasses beneath the back skin of rabbits, and that the actual state of the cytological reaction is reflected on the surface of the inserted glass pieces.

2. It is herewith proposed that for evaluating the defense capacity of the RES by this method, the count of the mononuclear cells on the glass on the second day and the comparative observation of the number of giant cells detected on the glass surface on the second and the third days after the insertion of glass give serviceable criteria.

3. A general parallelism was observed between the findings on the subcutaneous histiocytes before and their extent of proliferation after the glass insertion.
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References

5) Kojima, Nikketsukaishi., 1957, 20, 75.
10) Ikuta, Nikketsukaishi., 1949, 12, 211, 1950, 13, 1.

Fig. 1. Normal rabbit

Distended preparation of subcutaneous tissue before insertion of glass. The preexisting typical histiocytes are scattered about. No exudation of leucocytes. No basophilic elongated fusiform histiocyte in appearance.

Fig. 2. Normal rabbit

Glass specimen on 2nd day after insertion. The proliferated histiocytes appearing on the glass surface changed into free round cells of ununiform size, forming the so-called mononuclear cells, detected in the mean rate of 50% per field of vision (45-55%).
3rd day glass specimen. The mononuclear cells now detected in a large number, including 2 smallest-sized giant cells and some small-sized cells of newly medium size. Grade of detection of giant cells (+).

Fig. 4. 2nd day glass specimen from communin-pretreated rabbit

The mononuclear cells on the glass surface much more numerous than in the specimen from normal rabbit, already grouped here and there, in the mean detection rate of 75%.

Fig. 5. 3rd day specimen from INAH-pretreated rabbit

Giant cells of smallest to medium-size detected in many places, larger both in size and in number than in unpretreated cases. One medium-sized giant cell observed. Grade of detection (++).
Fig. 6. 2nd day specimen from Evans-blue-pretreated rabbit

Mononuclear cells sporadic and very subnormally few. Detection rate 16\%.

Fig. 7. 3rd day specimen from Evans-blue-pretreated rabbit

Giant cell not yet detected, mononuclear cell less grouped than in the other specimens.