mitochondria[19]. Such a possibility has also been critically discussed by SIEKEVITZ and PALADE[20]. As mentioned in our description, the localization of these mitochondria with altered morphology in the absorptive intestinal cells of germfree rats suggests a relationship to the surrounding structures, especially those just beneath the terminal web where freshly absorbed materials first come into contact with the cytoplasm to be metabolized[1]. The limited biochemical data on germfree rats available does not permit us to draw now any definite interpretation of the changes in mitochondrial morphology. Consideration should be given to their possible relation to cell necrosis, but the type of mitochondrial swelling is different and none of the other changes related to cell necrosis[21] were encountered in these cells[22].

Zusammenfassung. Die Mitochondrien der Dünndarm-Epithelzellen 3 Monate alter keimfreier Ratten zeigten elektronenmikroskopisch auffallende Formunterschiede im Gegensatz zu Vergleichstieren desselben Wurfes, die mit Coecum-Inhalt gewöhnlicher Ratten kontaminiert wurden.

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Specificity of the Macrophage Reaction in vitro

BOYDEN[1] demonstrated that macrophages, from guinea-pigs with delayed hypersensitivity to sheep red cells, give mixed agglutination in vitro, forming a 'rosette' with these red cells. Sera of these animals contain a cytophilic antibody similar to the one shown in sera of immunized rabbits[1–5]. These sera confer in vitro, to macrophages of normal guinea-pigs, ability to absorb on their surface the soluble or cellular antigens used for immunization. Analogous results were reported by JONAS et al.[6].

We have investigated the species- and the group-specificity of this phenomenon. For this purpose groups of guinea-pigs were inoculated with human red cells (A, B, O), human sera (A, B, O) and sheep red cells respectively. The red cells and the sera mixed with complete Freund Adjuvant (Difco) were inoculated into the footpads. Skin tests, subsequently performed with urea extract of the appropriate red cells[1] or with human sera, showed a delayed reaction of hypersensitivity.

Macrophages were obtained from the peritoneal cavity, 2 weeks after inoculation, by injecting i.p. 15 ml of 199*M*[7] with 1% heparin; the abdomen was massaged for 3 min, the peritoneal fluid sucked up in sterile siliconized pipettes, transferred to siliconized tubes, washed 3 times and resuspended[8] in fresh 199*M*. About 70% of the cell population consisted of macrophages.

Macrophages from guinea-pigs inoculated with different red cells were brought into contact in vitro with the erythrocytes used for immunization or with heterologous ones. Macrophages from guinea-pigs inoculated with human sera (A, B, O) were divided into 2 groups: the first was incubated in vitro with these sera and, after washing, brought into contact with human red cells from different blood groups; the second was brought into contact, in vitro, with human red cells (A, B, O), without being previously exposed in vitro to any of these sera.

Examination using phase-contrast microscopy, showed the following: macrophages from guinea-pigs inoculated with red cells invariably gave a strong positive reaction in vitro with the red cells used for inoculation (Figure 1a). Macrophages from guinea-pigs inoculated with sheep red cells mostly gave strong in vitro agglutination with sheep red cells but some provoked adherence of a few rabbit and human red cells. Agglutination of rabbit and human red cells was slow (12–20 h), whereas that of sheep red cells was more rapid (2–3 h). Goat, horse, ox and swine red cells were not adsorbed.

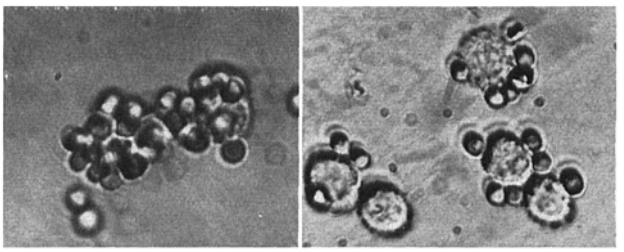

Fig. 1. Left: Adherence in vitro of sheep red cells, on macrophages of guinea-pig inoculated with sheep red cells. Right: Adherence of human red cells on macrophages of guinea-pig inoculated with pool of human sera, after previous in vitro sensitization of macrophages with human sera.

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Macrophages from guinea-pigs inoculated with human red cells provoked adherence, in vitro, of human red cells, irrespective of blood group or Rhesus factor. These macrophages adsorbed sheep and rabbit red cells on their surface to a lesser degree than macrophages of guinea-pigs inoculated with sheep red cells adsorbed human or rabbit erythrocytes. Controls performed with macrophages from normal guinea-pigs were negative. Macrophages from guinea-pigs inoculated with human sera (A, B, O) or pools of human sera, when sensitized in vitro with A or B serum, gave weak agglutination with the respective cells, according to the groups of the adsorbed sera and of the red cells (Figure 1b).

The groups of the inoculated sera had no bearing on the reaction. When sensitized in vitro with O serum, these macrophages agglutinate human red cells (A, B, O). This adherence was sometimes followed by phagocytosis of the erythrocytes attached on the surface of the macrophages.

The same macrophages provoked adherence of human red cells (A, B, O), without previous in vitro sensitization with any human sera.

Macrophages from non-inoculated guinea-pigs, brought into contact, in vitro, with human sera (A, B, O), did not provoke adherence of the corresponding human red cells, nor were they directly able to provoke adherence of human erythrocytes.

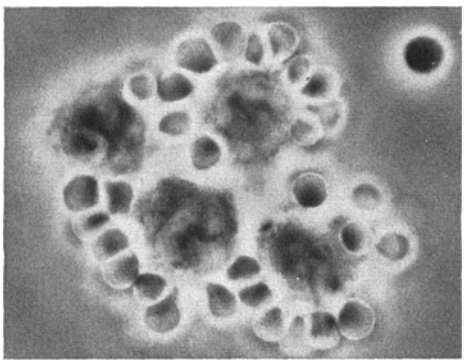

Fig. 2. Lysis of sheep red cells, when complement is added to the 'rosettes'.

On addition of complement (pool of fresh guinea-pig sera, absorbed with sheep red cells and diluted $^1/_{20}$) to 'rosettes' formed by macrophages of guinea-pigs inoculated with sheep red cells and by these red cells, lysis of the red cells attached to the macrophages occurred and red cell ghosts were observed around the macrophages (Figure 2). When lysed red cells were numerous, several macrophages seemed to be affected and to undergo lysis and disruption. Controls performed with unsuitable sera or red cells, or with normal or non-sensitized macrophages, were always negative.

Our experiments suggest that the macrophage reaction is species- but not group-specific. Weak cross reactions for sheep, human and rabbit red cells are attributable to the presence of a heterophile antigen other than FORSSMAN.

Experiments on the relationship of cytophilic antibody to other antibodies in different serum fractions are now in progress in our laboratory[9].

Résumé. Les macrophages de cobayes immunisés provoquent in vitro l'adhérence des hématies utilisées comme antigènes, selon une spécificité d'éspèce et non de groupe. Les sérums humains s'adsorbent in vitro sur les macrophages de cobayes immunisés par ces sérums, indépendemment du groupe (mais non sur des macrophages de cobayes normaux) causant l'adhérence et la phagocytose des hématies correspondantes. Les hématies adhérant aux macrophages sont hémolysées en présence du complément.

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Carcinogenesis in Different Animal Species by Diethylnitrosamine

Only a few carcinogenic compounds have been tested comparatively in different animal species. Mostly the experiments were carried out in rats and mice only and seldom in rabbits or dogs. Therefore many people are doubtful about the possibility of relating the results of experimental work to human beings, saying that the conditions for instance in rodents are quite different from those in men. To verify these objections we have tested diethylnitrosamine (DENA), whose carcinogenic actions were first observed by us in rats[1,2], also in mice[3,4], guinea-pigs[3], rabbits[5], dogs[6], monkeys[7], grass parakeets[8] and pigs[9]. The question was not only to check DENA in these animals but also to investigate the organotropy of the action and doses necessary to provoke cancer in all these animals. For that reason the experiments were done quantitatively.

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