Mobilization of Leukocytes into the Peritoneal Fluid[1]

Much work has been done on the mechanisms involved in the diapedesis, chemotaxis, and phagocytosis by leukocytes with the use of saline solutions of undetermined purity. DeHaan[2] was perhaps the first to report that the intraperitoneal injection of a 0·9% NaCl solution causes large numbers of neutrophils to enter the peritoneal fluid in rabbits. Hamburger[3] described this method in detail, and it has become a popular one for harvesting neutrophils for morphological, physiological, and biochemical studies[4]. In these investigations it has been assumed or implied that the salt solution itself has the power to evoke a local neutrophilia. However, there remains a measure of uncertainty, because the water used as a solvent in such experiments has been of unknown or of unspecified purity. Distilled water of the sort ordinarily used in laboratories often contains pyrogenic substances, and even nonpyrogenic water will become pyrogenic if it is left exposed to the air[5], probably through bacterial contamination. It is noteworthy that Harris, Menkin, and Yoffey[6] found 'virtually no exudate or cells' in the peritoneal fluid of guinea pigs that were injected intraperitoneally with a substance extracted from an inflammatory exudate and suspended in sterile, pyrogen-free saline. These investigators conjectured that the absence of an exudate might be the result of (a) the small amount of material injected, (b) the pyrogen-free nature of the solvent, or (c) the type of animal used. The experimental results which follow offer evidence that a 0·9% NaCl solution injected intraperitoneally into rats has little or no neutrophil-mobilizing power provided that the solutions used are free from certain trace contaminants. Moreover, minute amounts of a bacterial extract, when added to such solutions, produce results which mimic the effects seen after the injection of ordinary laboratory saline.

Male rats of the Sprague-Dawley strain (Holtzman) weighing 180–250 g were used. All rats were given an initial peritoneal lavage with 30 ml of saline. Sterile, nonpyrogenic 0·9% NaCl[7] was used for Groups I and III. Group II received 0·9% NaCl made with water which had been distilled a single time in the laboratory. The rats were challenged immediately by an intraperitoneal injection of one of the following solutions: (a) 10 ml of 0·9% sterile, nonpyrogenic saline (Group I), (b) 10 ml of 0·9% NaCl prepared with ordinary single-distilled water (Group II), or (c) 10 ml of sterile, nonpyrogenic saline to which was added 0·1 μg of a bacterial polysaccharide preparation derived from Pseudomonas[8] (Group III). The animals were sacrificed 5 h later and a second peritoneal lavage was performed. The numbers and types of cells removed by each washing were determined, and the total influx of new cells was calculated. This is especially easy to do for the neutrophils since the peritoneal fluid of rats contains few or no neutrophils in the unstimulated state[9].

The Table summarizes the results. It is evident that sterile, nonpyrogenic saline is not effective in evoking a neutrophilia of the peritoneal fluid. This is in sharp contrast to either ordinary saline or a solution of nonpyrogenic saline to which 0·1 μg of a bacterial extract has been added. These solutions cause millions of neutrophils to pour into the peritoneal fluid. Another interesting fact is that a significant influx of mononuclear cells also occurs. Such cells are primarily macrophages, but some cells resembling lymphocytes are present. We have chosen to group macrophages and lymphocytes together, because numerous transitional forms are seen, making it difficult to distinguish clearly between these cells.

The observation that a nonpyrogenic salt solution fails to evoke a neutrophil response renders untenable the idea that almost any substance introduced intraperitoneally will cause the appearance of large numbers of neutrophils. Indeed, glucose which often is described as a powerful chemotactic agent for neutrophils, is also impotent in mobilizing these cells in the peritoneal fluid. The relevant data pertaining to this finding will be published elsewhere. It may suffice here to point out that

[1] Supported by Grant No. CY-3071 from the U.S.P.H.S.

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[5] E. C. Holt and W. J. Penfold, Brit. med. J. *2*, 1589 (1911). – K. E. Darrow, Lancet *54*, 65 (1934).

[6] P. F. Harris, V. Menkin, and J. M. Yoffey, Blood *11*, 243 (1956).

[7] Obtained from Baxter Laboratories, Inc., Morton Grove, Ill.

[8] 'Piromen' obtained from Travenol Laboratories, Morton Grove, Ill.

[9] J. Padawer and A. S. Gordon, Anat. Rec. *124*, 209 (1956).

Net Influx of Leukocytes into the Peritoneal Fluid Measured after 5 h

Group	Treatment	No. of Rats	Neutrophils, Millions	Mononuclear Cells, Millions
I	10 ml nonpyrogenic saline	15	0·10 ± 0·05*	3·0 ± 0·5
II	10 ml 0·9% NaCl in laboratory distilled water	15	38·3 ± 2·8***	14·5 ± 1·7***
III	10 ml nonpyrogenic saline + 0·1 μg bacterial extract	6	48·5 ± 7·3***	8·5 ± 2.3**

* Means ± Standard error of the mean.

** $P < 0.05$
*** $P > 0.01$
} compared with Group I.

some of the currently held concepts regarding leukocyte mobilization need re-evaluation. G. J. Fruhman

Department of Anatomy, Albert Einstein College of Medicine, Yeshiva University, New York, April 11, 1959.

Zusammenfassung

Es gibt zahlreiche Berichte darüber, dass intraperitoneale Injektion von physiologischer Kochsalzlösung bei Säugetieren eine lokale Mobilisierung neutrophiler Leukocyten verursacht. Diese Reaktion bleibt bei Verwendung nicht-pyrogener NaCl-Lösung aus, tritt jedoch bei Zusatz von Bakterienextrakt zu dieser Lösung wieder auf. Zellzählungen ergeben ebenfalls eine erhöhte Zahl von mononukleären Leukocyten (Makrophagen und Lymphocyten) in der Peritonealflüssigkeit. Geringste Verunreinigungen der verwendeten Kochsalzlösungen dürften in zahlreichen früheren Untersuchungen zu falschen Schlussfolgerungen geführt haben, weshalb eine Reihe allgemein anerkannter Hypothesen über die Leukocytenmobilisierung neu zu bearbeiten wären.

Gastrulation in the Housefly, *Musca vicina*, Macquart

Among the muscids, the gastrular movements begin after the cytoplasmic layer ('innere blastemma') has become incorporated in the blastoderm and the delimitation of the blastoderm cells has been completed. In other insects also (where the 'innere blastemma' does not develop), gastrulation takes place after delimitation of the blastoderm cells. In the case of the housefly studied here, however, the gastrular invagination begins even before the cell furrows have fully developed and the blastoderm is still syncytial (Fig. 1). This feature in *M. vicina* would indicate that the morphogenetic forces responsible for gastrulation are not localised in the individual cells but are spread over the whole mid-ventral strip of the blastoderm. It may indeed be that the forces involved originate in the cortex of the egg, as shown in amphibian eggs (Holtfreter[1]).

Another noteworthy feature is that the 'innere blastemma' (whose incorporation in the blastoderm is responsible for the great thickness of the blastoderm seen in Figure 1) is rich in ribonucleic acid and glycogen, as demonstrated by histochemical techniques used along with the appropriate controls. The 'innere blastemma' also has basophil granules (probably derived from yolk globules) which become localised in the inner portion of the blastoderm after the incorporation of the former in the blastoderm. The outer portion of the blastoderm, however, is composed of the original egg cortex. This orientation is retained in the ectodermal cells but, in the mesoderm cells, the granule bearing areas begin to face outwards due to gastrulation (Figure 2).

Details of the morphological and histochemical study of the early embryology of the housefly would be published elsewhere.

N. I. Bhuiyan and S. A. Shafiq

Department of Zoology, Dacca University, Dacca (Pakistan), January 8, 1959.

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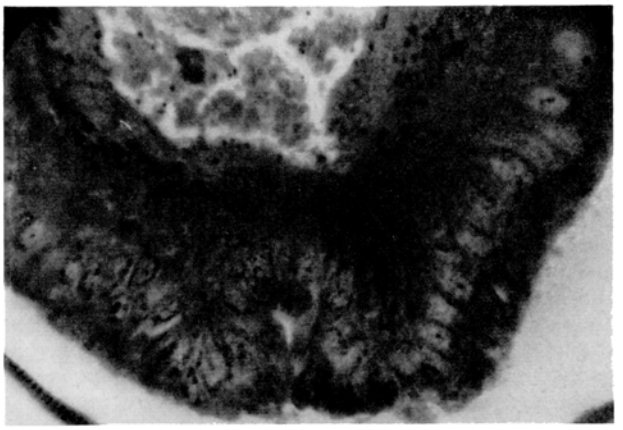

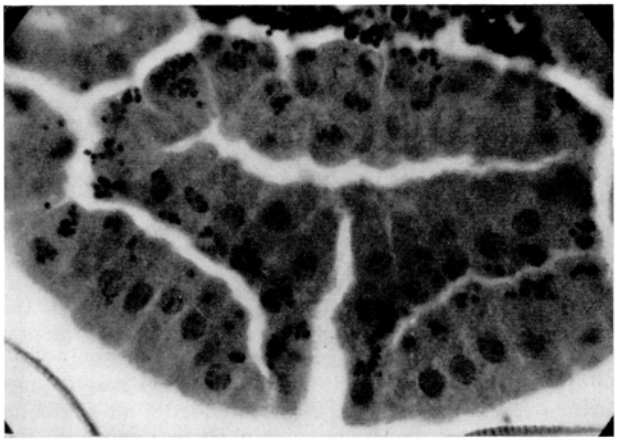

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Fig. 1.—Transverse section of the egg showing the beginning of the gastrular invagination even though cell furrows have not fully developed.

Fig. 2.—Transverse section of the egg showing the cells of the ectoderm and the mesoderm tube. In the former, the granules are seen in the inner ends of the cell; in the latter, they are in the outer portions.

Résumé

L'«innere blastemma» de l'œuf de la mouche domestique est riche en acide ribonucléique et en glycogène. Ce stratum s'incorpore dans le blastoderme et le mouvement gastrulaire commence peu après, avant même que la délimitation des cellules soit complète. La signification de ce caractère est soulignée.

Etude immuno-électrophorétique de l'uromucoïde

On sait que l'urine normale renferme une mucoprotéine, dite *substance de* Tamm et Horsfall[1] ou *uromucoïde* (Boyce[2]), qui est réputée être particulièrement insoluble dans des solutions salines, même faibles.

Il nous a paru intéressant de vérifier, au moyen d'un antisérum spécifique, si une précipitation saline par le

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