It is known that the differentiation of enzymes is directly related to the development of function[3], and this is especially true with the oxidative enzymes in the metanephros of the rat[4,5].

Résumé. L'iodoacétate retarde histologiquement et histo-chimiquement le développement du métanéphros de l'embryon de poule.

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The Mass and Size of Normal and Activated Macrophages - Studies with a Scanning Interferometer

Physico-chemical stimulation of peritoneal macrophages results in the production of a morphologically distinct population of cells[1–3] which often displays greater functional activity. Activated macrophages may, for instance, exhibit enhanced bactericidal ability[4]. One morphological characteristic of the new, activated population is a greater mean size. This change in cell volume has obvious effects on the relationship between the volume of the macrophage and its membrane surface area. However, it does not provide a complete explanation of the altered volume-to-surface ratio shown by the cells of the activated population[5,6].

The ultrastructural studies of NORTH and MACKANESS[7,8] led them to suggest that the cellular hypertrophy might result from a 'hydration' of the macrophage cytoplasm rather than from an increase in cytoplasmic mass. The present investigation was designed in order to determine whether the increase in macrophage size is accompanied by an alteration in cellular dry mass. To our knowledge the use of the scanning interferometer employed in this study has not been described until now.

Population of normal peritoneal macrophages, together with populations from rats challenged 5 or 8 days previously with a single i.p. injection of Freund's Complete Adjuvant (FCA), were harvested by a standardized procedure[6]. Cells were spotted on to slides to avoid the excessive flattening of cells which accompanies smearing[9]. Fixation was in 10% neutral formalin. Air-dried preparations were scored with a series of lines (1–2 mm apart) to provide reference areas, free of cells, in which the interferometric measurements could be made. Unstained preparations were mounted in water and the cover-slips sealed with paraffin wax.

Estimates of the dry mass of individual macrophages were obtained using a prototype scanning interferometer loaned to this Department by Vickers Instruments, Ltd. The machine is now available commercially as the Vickers M86 combined scanning interferometer and integrating microdensitometer[10]. The instrument measures automatically the integrated optical path difference (IOPD) of individual cells. This parameter is directly proportional to cellular dry mass. Measurements were made using a 100 × water-immersion objective (shearing distance of the optical system, 83 μm) on macrophages bordering the scored areas of the preparations. This simple routine eliminates errors due to 'ghosting'.

Machine fluctuation over long periods was negligible. The IOPD of macrophages in the scanning field were

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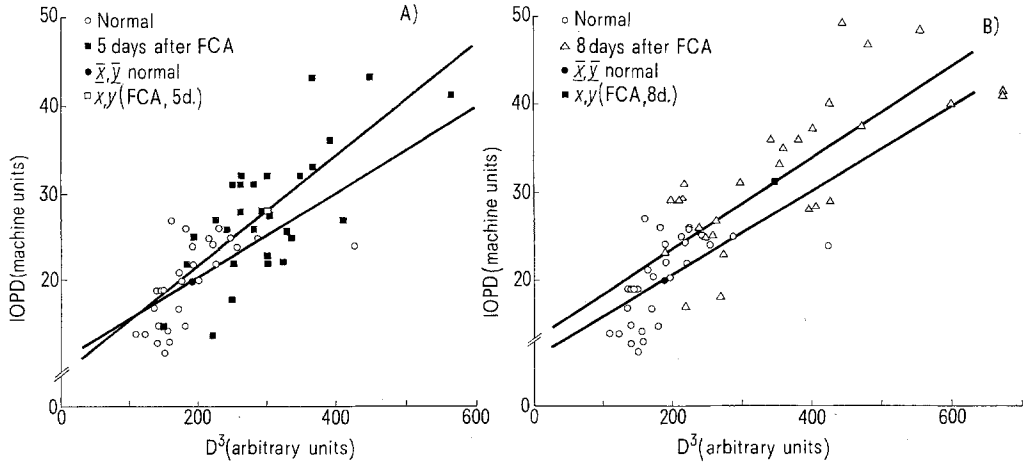

Regression lines for 3 experimental populations of peritoneal macrophages showing the relationship between integrated optical path difference (IOPD) and cell size (D^3). Mean values $\bar{x}$ and $\bar{y}$ are shown. A) Regressions for normal and 5 day-stimulated cells. B) Regressions for normal and 8 day-stimulated cells.

measured, together with 'background' readings for adjacent, cell-free regions. Estimates of macrophage size (maximum diameter, D) were made on the same cells using a PZO (Warsaw) vernier eyepiece (15 ×). Macrophages were identified on the basis of relative cell size and interference contrast. The latter facilitated the appreciation of nuclear morphology and nucleocytoplasmic ratio.

From the raw data, graphs were plotted showing the relationship between the two important descriptive parameters, IOPD (measured in machine units) and the cube of the maximum diameter (i.e., D^3) given in arbitraty units. Regression lines for the 3 population samples were computed by covariance analysis[11], using IOPD as the dependent variable. Differences between population regression coefficients were tested for significance by a comparison of their variance ratios. Sample differences in elevation were assessed by the same procedure. Standard errors for the regression coefficients were tested for significance using 't'values.

The statistical analyses were designed to ascertain whether a) regression lines were parallel, b) the regressions coincided, and c) there was a significant, positive relationship between the dependent (IOPD) and independent (D^3) variables for the normal and stimulated experimental populations.

Regressions for normal and FCA-induced macrophage populations are shown in the Figure. The regression coefficients (i.e. ,the slopes of the graphs) were found to be 0.045 (normal), 0.061 (5 days after FCA), and 0.044 (8 days after FCA). There was no significant difference between these values ($P > 0.05$), the population regression lines being parallel. Thus, the increase of cellular dry mass for a unit increase in D^3 was the same for normal and stimulated cells.

Adjusted means for the normal and 5 day-stimulated groups were not significantly different ($P > 0.05$). The regression lines for these populations (Figure A) were, therefore, one and the same. However, the variance ratios of the adjusted means for normal and 8-day-stimulated samples showed that the difference in sample elevations was significant ($P < 0.05$). This difference is related to differences in initial cellular dry mass: macrophages obtained 8 days after injection had a higher IOPD than normal cells of the same size (Figure B). Differences between 5- and 8-day-samples were not significant ($P > 0.05$). Finally, standard errors for the 3 regression coefficients pointed to a significant and positive relationship between cell size and cell mass, and for all populations the relationship was the same. Thus, the increase in size of activated macrophages is not due to any 'hydration' or loss of cytoplasmic density.

The difference in sample elevation for normal and 8-day-stimulated populations may be explained by ultrastructural changes of macrophage shape. FCA-induced peritoneal macrophages are rounder than normal[6] and diameter estimates for these cells may be open to smaller errors. The values for normal cells may be overestimates. This explanation is supported by the observation that the sample differences in elevation between the two FCA-induced populations were not significant.

Experiments using Feulgen photometry and tritiated thymidine labelling indicate that the increased mass of the activated cells does not originate from preparations for mitosis. Indeed, cells in G_2 and S phases of the mitotic cycle are less frequent in stimulated preparations than in normal ones[12].

Résumé. Les dimensions et la masse sèche de macrophages normaux et activés ont été mesurées en utilisant le «Vickers M86 scanning interferometer and integrating microdensimeter». Une analyse statistique a montré un rapport positif entre les deux paramètres descriptifs, ce qui infirme une suggestion précédente selon laquelle une augmentation des dimensions d'un macrophage est accompagnée d'une perte de densité cytoplasmique.

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Effect of Alkoxyglycerols on the Frequency of Injuries Following Radiation Therapy

Alkoxyglycerols occur in small quantities in many natural products. In the haemopoietic organs of mammals, particularly the bone marrow, they are relatively abundant. They also occur in relatively high concentrations in human mother's milk. They occur most abundantly in nature in the liver oil of certain species of shark[1–3]. The general formula for alkoxyglycerols is $CH_2OH \cdot CHOH \cdot CH_2O \cdot R$, where R is a longchain aliphatic radical.

The alkoxyglycerols have proved to be of medical interest[1,4–6]. To some extent they prevent leucopenia and thrombocytopenia. The administration of alkoxyglycerols to patients with cancer of the uterine cervix results in higher survival rates than if radiation treatment alone is given[1,4]. Furthermore the alkoxy-glycerols promote the growth of *Lactobacillus lactis*[1] and the formation of antibodies[4,6].

In order to throw light on the effect of alkoxyglycerols on the frequency of injuries following radiation therapy, we have studied 3 groups of patients with cancer of the uterine cervix (Table), one group which received alkoxyglycerols the week before (prophylactically), during, and 3 months after radiation treatment(I) a second one where administration of alkoxyglycerols occurred only during and 3 months after radiation treatment(II) and a third one, the control group, which received radiation treatment but no alkoxyglycerols(III). The groups I and II are from 1.1. 1964–15. 2. 1966, during which period 99% of the patients with cancer of the uterine cervix received alkoxyglycerols in connection with radiation treatment. The groups I and II are compared with the control group (III) composed of

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