THE PERIODIC ACID-SCHIFF REACTION
IN NEUTROPHIL LEUKOCYTES—INFLUENCE OF FIXATION
AND AMYLASE DIGESTION
A QUANTITATIVE MICROSCOPIC STUDY
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Summary. The periodic acid-Schiff (PAS) reaction in normal human neutrophil leukocytes
was studied morphologically and microspectrophotometrically after different fixation methods
and after treatment with α-amylase and β-amylase.

The best fixation methods with respect to the morphological distribution of the stain and
to the quantitatively measured amount of PAS positive material preserved in the cells were
fixation with absolute methanol, absolute ethanol, acetic alcohol formalin and Rossmann's
fluid as well as fixation by freeze substitution. The amount of PAS reactive material in the cells
after these fixation methods was in the same range as in unfixed cells covered with a semi-
permeable formvar membrane. The PAS positive material could be removed by treatment with
α-amylase after most fixatives, the most important exception being acetate alcohol formalin,
after which fixed material was obtained in the amount of PAS positive material. This
may be due to the fixative creating an α-amylase resistant protein-glycogen binding. β-amylase
digestion gave only a slight reduction in the amount of PAS reactive material.

The results indicated that the amount of PAS reactive material in normal human neutrophil
leukocytes consists of glycogen. It is probably preserved to about 90 per cent or more
by the above mentioned fixatives.

It has been shown by biochemical methods that human neutrophil blood
leukocytes contain considerable amounts of glycogen (WAGNER, 1946; VALENTINE
et al., 1953; ESMAN, 1961; DAHLQVIST et al., 1962; LUGANOVA and SEITS, 1962;
OLSSON et al., 1963). It is thus quite logical that they have a strong periodic
acid-Schiff (PAS) reaction, as shown in a great many investigations (GIBB and STOWELL, 1949; WACHSTEIN, 1949; WISLOCKI et al., 1949; ASTALDI et al., 1952; GÄRT-
PAS Reaction in Neutrophils — Fixation and Amylase Digestion

Most authors claim that the PAS reactive material is completely digestible with saliva or malt diastase (GIRB and STOWELL, 1949; WACHSTEIN, 1949; WISLOCKI, 1949; ASTALDI et al., 1952; MAHR, 1964) and consider that it consists of glycogen. PEARSE (1960), however, has repeatedly found considerable amounts of diastase or salivary resistant PAS positive material in neutrophil leukocytes both in Susa fixed blood smears and in formalin fixed tissue specimens. He, therefore, considers that the finding of diastase resistant material could be due to "the presence of carbohydrate-containing substances other than glycogen, to the presence of lipid substances of the phosphatide class, or to incomplete removal of glycogen due to the presence of a protein bound diastase-fast fraction".

In view of the contradictory results obtained after diastase and saliva digestion of the PAS reactive material in neutrophils, it seemed worthwhile to make a more systematic investigation of factors influencing the digestion. Most authors who have obtained removal of the PAS positive material with saliva and diastase have used alcoholic fixatives, mainly methanol, while PEARSE has used formalin and Susa fixation. This suggested that the fixative might be important for the effectiveness of the removal of the PAS reactive material by α-amylase. As the fixative itself might influence the PAS reaction both qualitatively and quantitatively, this investigation is concerned with (a) this possible influence of the fixative per se on the PAS reaction and (b) the quantitative influence of various fixatives on the α-amylase digestion of the PAS reactive material.

Material and Methods

Sampling and preparation of smears. Normal human neutrophil blood leukocytes were obtained from the buffy coat of venous blood after sedimentation with a 1 per cent fibrinogen solution (Kabi AB, Stockholm, Sweden) (6 ml venous blood + 3 ml fibrinogen solution) for 20–30 min at room temperature (±20 °C) (DAHLQVIST et al., 1962; GAHRTON, 1964a and 1964b). About 0.5 ml of the buffy coat material was gently removed with a Pasteur pipette and smears were made by the cover slip method and air-dried.

Fixation. The fixatives used were absolute methanol (AM), absolute ethanol (ice cold) (AE), acetic alcohol formalin (AAF) (glacial acetic acid 5 ml, 90 per cent ethanol 85 ml, 40 per cent formaldehyde 10 ml), Rossman's fluid (RF) (absolute ethanol, saturated with picric acid 9 parts, 40 per cent formaldehyde 1 part), Carnoy's fluid (CF) (absolute ethanol 60 ml, chloroform 30 ml, glacial acetic acid 10 ml), alcohol acetone (AA) (absolute ethanol 1 part, acetone 1 part), Bouin's fluid (BF) (1.4 per cent picric acid in distilled water 150 ml, 40 per cent formaldehyde 50 ml, glacial acetic acid 10 ml), and Lillie's neutral buffered formalin (LNF).

Freeze substitution (FS) was also applied according to the following method. The whole supernatant was removed after fibrinogen sedimentation and centrifuged at 300 g for 7 min. The pellet, containing a high proportion of neutrophil leukocytes, was resuspended and washed twice in saline and centrifuged at 300 g for 7 min each time. A few drops of saline were added to the final pellet, which was then dropped with a Pasteur pipette onto a hemocytometer cover glass (BÜRKER) and immediately exposed to liquid propane (−196 °C) for 30 sec and then to absolute ethanol (−70 °C), cooled by CO₂ ice in 85 per cent ethanol. The preparations were left for 48 h to reach room temperature. They were then passed through graded alcohols to water before staining.

Formvar treatment. For the study of the PAS reaction in unfixed air dried cells, these were covered by a semipermeable formvar membrane (Formvar 15/95 E for wire enamels, Shawinigan Resins Corporation, Springfield 1, Mass., USA) as described before (GAHRTON, 1964a). The smears were dipped in an 0.5 per cent formvar solution in ethylene chloride and air-dried before staining.