polyphenols are frequently present in animal tissues in a masked form; using his techniques, I have now shown that these substances may be present in this manner in the endocuticle of the eelworm cyst wall.

White cysts were embedded in 10% gelatin and sectioned at 20 μ with a freezing microtome. Cuticles, separated from all other tissues, were treated with methanol-HCl at 60°C for 24 h; controls were similarly maintained in water. When tested with potassium dichromate and with the azo-coupling dyes Fast Red Salt B and Fast Garnet Salt G.B.C., differences between methylated and control cuticles were not sufficiently definite for confidence. However, clear differences between treatments were shown up by KIO_3, a very specific reagent, and by ammoniacal silver nitrate used as recommended by Fontana. Results with the latter reagent, the most sensitive of those employed, were particularly striking: in control cuticles, only the outer layers reduced silver appreciably, the endocuticle remaining almost clear (Figure a); in the methylated cuticles silver is reduced in all regions (Figure b). (The distortion shown by both specimens is due to the effect of heat on the collagen of the cuticle.)

The treatment demonstrated then, that polyphenols may be present in the endocuticle in a masked form. Although it is not certain that, in the intact animal, the endocuticle is tanned by substances already present in a masked form *in situ*, the results show that this, at least, is possible; they certainly help to explain the tanning of the isolated endocuticle.

Zusammenfassung. Nach Methanol-HCl-Behandlung wurde gefunden, dass sich die Polyphenole im allgemeinen in der Endocuticula der Cyste der Kartoffelnematode *Heterodera rostochiensis* Wollenwerber finden.

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Esterase Activity of Leucocyte Proteins and their Labelling with Radioactive Diisopropylfluorophosphate

Radioactive diisopropylfluorophosphate (DFP^{32}) has been used as a label for leucocytes by a group from Utah[1,2] in order to determine the rate of removal of these cells from the circulation. These workers have presented evidence that the label is primarily attached to neutrophilic granulocytes. In the present work, the esterase activity and the DFP^{32} binding capacity of the proteins extracted from normal and myeloid leukaemic human leucocytes have been investigated by means of agar-gel electrophoresis and immunoelectrophoresis followed by staining for esterases and autoradiography.

Methods. The procedures used in the isolation of leucocytes and extraction of their proteins have been described in detail earlier[3]. The isolated cells were washed four times in an isotonic solution[4] and suspended in 0.1 *N* $NaHCO_3$. Disintegration of the cells was effected by homogenization in a Potter–Elvehjem apparatus followed by ultrasonic treatment (5 min with ice cooling). The suspensions were then centrifuged and the clear, slightly yellowish supernatants concentrated by vacuum dialysis to a protein concentration of 3–4%. Purity was ascertained by immunodiffusion according to OUCHTERLONY[5], using antisera against human serum, erythrocytes, and thrombocytes.

Electrophoresis in agar-gel was performed by the micro-technique described by WIEME[6] in a barbital buffer of pH 8.2; the proteins were stained with Amidoschwarz 10 B.

Immunoelectrophoresis was performed according to SCHEIDEGGER's micro-modification[7], in which the electrophoresis and the immunodiffusion take place in agar-gel on an object slide. Antisera against leucocyte antigens were obtained by immunizing rabbits subcutaneously with suspensions of whole white cells + Freund's adjuvant.

Esterase activity was visualized in the gel after electrophoresis or immunodiffusion by the azo-coupling method as described by URIEL[8]. The substrate was β-naphthyl acetate (0.001 *M*, pH = 7.4), and the liberated β-naphthol was coupled to diazo-ortho-dianisidine (40 mg/100 ml). After staining for 1 h, the active fractions showed a stable purple colour.

DFP^{32} was delivered from The Radiochemical Centre, Amersham, Great Britain, dissolved in propylene glycol; the concentration of DFP was 0.1% and the specific activity in 1 ml 0.3 mc at the time of delivery.

Autoradiography was performed as described by CLAUSEN and MUNKNER[9]. Extracted proteins dissolved in 0.1 *N* $NaHCO_3$ were incubated with DFP^{32} for 2–3 h at room temperature; the concentration of DFP varied from

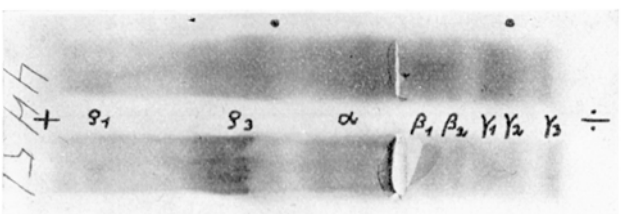

Fig. 1. Electrophoretic pattern of two normal leucocyte populations with about 80% neutrophils. The anodic dot indicates the mobility of serum albumin, the cathodic dot that of dextran (M = 150000, Pharmacia).

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$10^{-3}M$ to $5 \times 10^{-6}M$. After incubation, the solutions were submitted to electrophoresis and immunoelectrophoresis as usual.

The radioactivity of the slides was estimated by means of a Geiger–Müller detector, and the dried agar-gel placed in direct contact with the emulsion of a Kodirex X-ray film. Exposure took place for varying lengths of time according to the activity measurements (generally 3 h to 6 days), whereupon the films were developed and the slides stained for proteins.

Results. The electrophoretic pattern of two normal leucocyte populations with about 80% neutrophilic granulocytes is seen in Figure 1. Five fractions are found in the cathodic area, two corresponding to the β-area and two to the γ-area of serum; the third γ-fraction is situated just cathodically to serum γ-globulin, and as a rule this is the most prominent fraction. In the anodic area three main fractions are seen. The two migrating faster than serum albumin contain nucleoprotein[8]; the third occupies the α-region. – The pattern of myeloid leukaemic leucocytes does not differ from the one described.

Esterase activity is seen both in the α- and γ-regions (Figure 2); some blurring of the active fractions takes place during the staining procedure because of diffusion of the proteins, as these are not fixed until after staining. In the γ-area two active fractions can be distinguished,

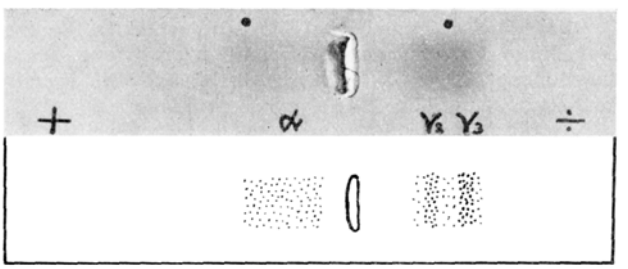

Fig. 2. Esterase activity in leucocyte extracts, revealed after electrophoresis.

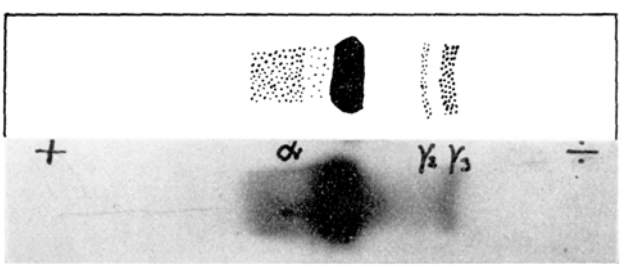

Fig. 3. Autoradiogram of leucocyte extracts incubated with DFP^{32} and submitted to electrophoresis.

corresponding to the γ-2 and γ-3 fractions of Figure 1. Both in the α- and γ-region, the activity was abolished in $10^{-3}M$ DFP and just distinguishable in a $2 \times 10^{-4}M$ solution.

In immunoelectrophoresis, two precipitation lines possess esterase activity, one in the α- and one in the β-γ-region.

Autoradiography of proteins incubated with DFP^{32} showed (Figure 3) blackening of the film in the α-area; in the cathodic part the γ-3 fraction was distinct, γ-2 faint. In immunoelectrophoresis, a distinct precipitation line was developed in the α-area, and a weaker bow in the β-γ-region corresponding to the ones possessing esterase activity; in a few preparations, a third very faint bow was seen in the anodic part of the γ-region.

Neither enzyme coloration nor autoradiography revealed differences between normal and leukaemic leucocytes.

Discussion. Diisopropylfluorophosphate inhibits esterases and certain other enzymes, being irreversibly bound to the hydroxyl group of a serine residue[10] with liberation of hydrogen fluoride. The diisopropylphosphate liberated by the degradation of the labelled proteins does not react with proteins. DFP^{32} is thus suitable for the labelling of cells, although this labelling means inhibition of several important cellular enzymes. As regards erythrocytes, the life span as measured by DFP^{32} corresponds to the values found by other methods.

In our electrophoretic studies on leucocyte proteins, we have found close agreement between esterase activity as visualized by a staining reaction in the agar-gel and DFP^{32} binding capacity of the leucocyte extracts as revealed by autoradiography. That the main activity of each of the three active fractions visualized by agar-gel electrophoresis is due to one immunologically homogeneous protein is suggested by the immunoelectrophoretic results; two precipitation bows possess esterase activity, and three are labelled with DFP, their centers being in the α-, late β-, and γ-area, respectively, corresponding to the fractions obtained by simple electrophoresis.

Résumé. Après électrophorèse en gélose des protéines extraites de leucocytes humains, l'activité estérasique des fractions fut comparée à leur affinité pour DFP^{32}. Les deux méthodes montraient trois fractions actives, tant en leucocytes normaux qu'en leucocytes de leucémie myéloïde.

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The Effect of Inhalatory Adaptation to Histamine on Histamine Shock and the Anaphylactic Shock

The effect of adaptation to histamine (administered in the form of aerosols) on the course of histamine shock and of the anaphylactic shock has been studied in guinea-pigs.

There are few data concerning the influence of adaptation to histamine on the course of the anaphylactic shock[1]. No data were found in the bibliography as to the influence of adaptation produced by a prolonged administration of histamine aerosols on the course of the anaphylactic shock. The effect of adaptation to histamine, as produced by different methods, on the course of hista-

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