a lowered work load, is in agreement with those of others based on acute observations in man[16–18].

GORLIN et al.[16,17] reported that nitroglycerin increased coronary blood flow in normal subjects, but not in patients with myocardial insufficiency. These authors proposed that the beneficial effect of this agent in these patients was due to a reduction in cardiac work. In addition ROWE et al.[18] concluded that the primary effect of a longer acting agent, erythrol tetranitrate is to reduce coronary vascular resistance and cardiac work while permitting sufficient coronary flow to sustain the reduced cardiac work load.

However, these results are not in agreement with the findings of others in acute experiments in animals[5,6] in which pentaerythritol tetranitrate produced an increase in coronary blood flow. However, we have reported in a separate communication[7] that in the dog this compound decreased coronary flow, but also decreased cardiac work.

Using a modified radioisotope technique JOHNSON et al.[12] measured myocardial blood flow before and after a single dose of pentaerythritol tetranitrate in 8 angina patients receiving the drug for 2–3 weeks prior to the hemodynamic studies. Myocardial blood flow increased significantly at 2 and 4 h after the last dose when compared to the placebo. The lack of agreement between this and the present study may be related to the animal employed, the administration of anesthesia, the method of evaluating the myocardial circulation, or to the state of the myocardium before drug administration. Nevertheless, the present results point to the difficulty in evaluating agents in experimental animals, by presently useful methods, which are or may be clinically useful in the treatment of myocardial insufficiency.

Zusammenfassung. Die Untersuchungsergebnisse stützen die Vorstellung, dass die gute Wirkung der prophylaktischen Anwendung von Pentaerythritol-tetranitrat bei Patienten mit Coronarinsuffizienz auf Abnahme der Herztätigkeit und Zunahme der Herzmuskelleistung, nicht aber auf eine Zunahme des Coronarkreislaufes zurückzuführen ist.

A. D. BENDER,
F. J. SULLIVAN, and S. M. HORVATH

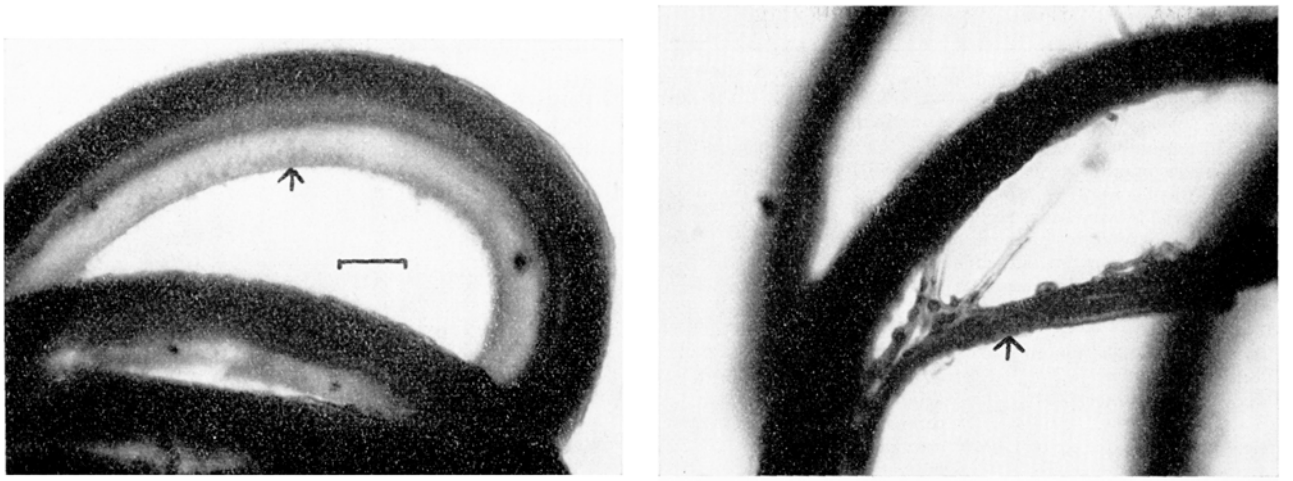
Departments of Physiology, The Jefferson Medical College, Philadelphia (Pennsylvania) and the University of California, Santa Barbara (California, U.S.A.),
January 2, 1963.

[16] R. GORLIN, N. BRACHFELD, C. MACLEOD, and P. BOPP, Circulation *19*, 705 (1959).
[17] N. BRACHFELD, J. BOZER, and R. GORLIN, Circulation *19*, 697 (1959).
[18] G. G. ROWE, C. J. CHELIUS, S. AFONSO, H. P. GURTNER, and C. W. CRUMPTON, J. clin. Invest. *40*, 1217 (1961).

Masked Polyphenols in the Cuticle of a Cyst Forming Nematode

The cuticle of the mature female of the potato-root eelworm *Heterodera rostochiensis* Wollenweber is hardened to form a resistant cyst by a tanning process involving a polyphenol oxidase[1]. In histochemical investigation of isolated cuticles, already briefly described[2], the process appears to begin in the outer, fibrous layer, and to proceed inwards to the hyaline, innermost layer ('endocuticle'[3]). Although the tanning agents seem to diffuse inwards, the endocuticle will tan even when isolated. The presence of polyphenols can invariably be demonstrated in the outer region, but it was puzzling that these substances cannot always be detected in the endocuticle; indeed, in 'white cysts' (mature females), it is exceptional for their presence to be demonstrable. MONNÉ[4] has recently shown that

[1] C. ELLENBY, Nature *157*, 302 (1946).
[2] C. ELLENBY, 14th Int. Congr. Zool. (Copenhagen 1953), p. 373.
[3] W. WIESER, Medd. Vaxtskyddsanst. Stockh. No. 65 (1953), p. 15.



a b

Isolated cuticles held at 60°C for 24 h in water (a), or in methanol-HCl (b); both specimens then treated with ammoniacal silver nitrate for 4 h. → indicates the endocuticle. Scale = 10 μ.

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.

C. Ellenby

Department of Zoology, King's College, University of Durham, Newcastle upon Tyne (England), December 27, 1963.

[4] L. Monné, Arkiv Zool. *13*, 287 (1960).

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

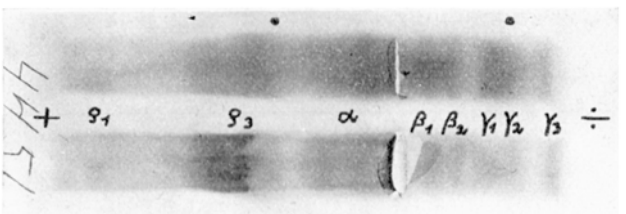


Fig. 1. Electrophoretic pattern of two normal leucocyte populations with about 80% neutrophils. The anodic dot indicates the mobility of serum albumin, the cathodic dot that of dextran (M = 150000, Pharmacia).

[1] J. W. Athens, A. M. Mauer, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe, Blood *14*, 303 (1959).
[2] A. M. Mauer, J. W. Athens, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe, J. clin. Invest. *39*, 1481 (1960).
[3] V. Andersen, *X Colloquium on Protides of the Biological Fluids* (Bruges 1962).
[4] P. Grabar, M. Seligmann, and J. Bernard, Ann. Inst. Pasteur *88*, 548 (1955).
[5] Ö. Ouchterlony, Ark. Kemi Mineral. Geol. *26* B, 14 (1949).
[6] R. J. Wieme, *Studies on Agar Gel Electrophoresis* (Arscia, Brussels 1959).
[7] J. J. Scheidegger, Intern. Arch. Allergy *7*, 103 (1955).
[8] J. Uriel, in P. Grabar and P. Burtin (ed.), *Analyse immunoélectrophorétique* (Masson, Paris 1960), p. 33.
[9] J. Clausen and T. Munkner, *Protides of the Biological Fluids*. Proceedings of VIII Colloquium (Elsevier, Amsterdam 1961), p. 147.