These results are compatible with the presence of 2 different amino acid chains in each type of erythrocyte enzyme. It is also possible to explain the appearance of these bands in terms of a development of polymers containing 1 rat unit and 3 bovine units on the one hand, but 3 bovine units and 1 rat unit on the other. In such an instance, however, a fifth band containing 2 bovine and 2 rat units could be anticipated. We thus consider it most probable to be that the enzyme normally contains 2 different polypeptide chains. If the enzyme is normally a dimer, the rat enzyme might then be designated $\alpha^R\varepsilon^R$, the normal bovine enzyme $\alpha^B\varepsilon^B$, and the 2 hybrids $\alpha^R\varepsilon^B$ and $\alpha^B\varepsilon^R$. Since all known mutations involving G-6-PD a e sex-linked and no hybrid enzyme is formed through interspecific matings, it would appear that both genes are on the X-chromosome and probably very closely linked[4].

Zusammenfassung. Die Hybridisierung von Ratten- und Rinder-Glukose-6-Phosphat Dehydrogenase ergibt 2 Hybride, was darauf hindeutet, dass das Enzym in beiden Gattungen ein Polymer von verschiedenen Aminosäureketten ist.

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[4] This work was made possible by a supporting fund established in the name of the Barry T. Leithead Research Fellowship, and was supported, in part, by Public Health Service Grant No. HE 07449 from the National Heart Institute, National Institutes of Health.

Occurrence of Microvilli-Like Structures in the Gut of Digenetic Trematodes

During a comparative survey of nutrition in the Trematoda it was found that the gastrodermis of 7 species of Digenea examined bear filamentous outgrowths, reminiscent in most instances of the microvilli present in the vertebrate gut. These outgrowths may be of varying length from 1–15 μ, as in *Haematoloechus medioplexus*, *Haplometra cylindracea*, *Opisthioglyphe ranae*, and *Schistosoma mansoni*, or they may be more uniform in appearance and organized into a definite striated border, 15–20 μ high, as in *Diplodiscus subclavatus* (Figure 1), *Gorgoderina vitelliloba* and *Gorgodera cygnoides*.

Similar structures have been described in *Dasymetra villicaeca*[1], *Fasciola hepatica* (various authors summarized by DAWES[2]), *Cleptodiscus reticulatus* and *C. kyphosi*[3], so that they would appear to be a characteristic feature of the Digenea.

In all the specimens of the 7 species examined the outgrowths stain very poorly with most counterstains, such as eosin or light green, often to the extent that they give the appearance of being separate from the underlying gut cells. In addition they assume a deep pink colouration with the PAS technique, and also give a positive reaction for acid phosphatase, whereas the cells hardly stain at all. On the other hand, with Mallory's trichrome stain, the outgrowths are clearly visible, under oil immersion, as individual structures the surfaces of which are covered by a thin blue staining layer surrounding a yellowish core which is directly continuous with the cytoplasm of the rest of the cell.

In *D. subclavatus*, electron microscope observations on the outgrowths confirm those made with Mallory's stain under oil immersion, and show the projections to be uniform in appearance, orientated parallel to each other and perpendicular to the cell surface (Figure 2). Each projection is seen to be composed of a core of undifferentiated cytoplasm surrounded by a true outer membrane which is continuous with the plasma memb ane of the cell. Electron micrographs also confirm the absence of basal bodies, or blepharoplasts, from the cytoplasm beneath the processes, indicating that these cellular extensions are not actively motile cilia and therefore not concerned with creating currents for movement of the gut contents. This is only to be expected in view of the infrequent occurrence of ciliated structures throughout the adult Trematoda. The electron micrographs also make possible approximate estimates of the number of processes present,

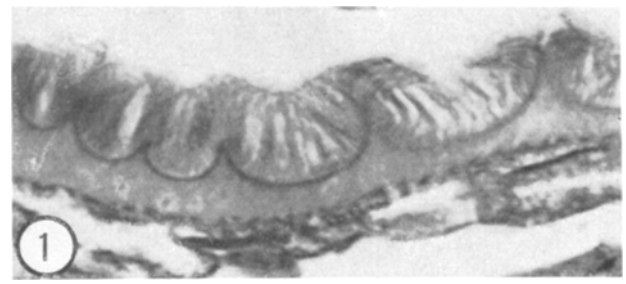

Fig. 1. A longitudinal section through part of the gastrodermis of *Diplodiscus subclavatus* showing the microvilli-like outgrowths. Periodic acid-Schiff (PAS) and light green. × 600.

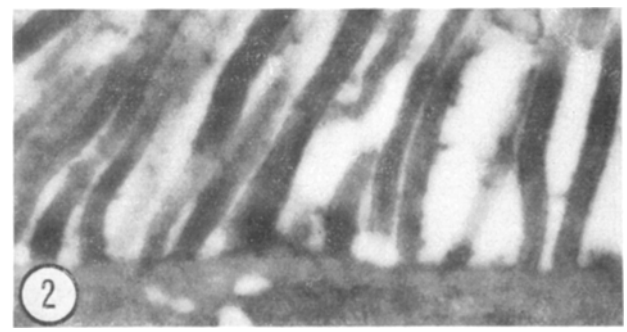

Fig. 2. An electron micrograph of part of a gastrodermal cell in *D. subclavatus* showing the origin and basal region of the microvilli. × 50,000.

[1] E. E. BYRD, Trans. Am. micr. Soc. *54*, 196 (1935).
[2] B. DAWES, Parasitology *52*, 483 (1962).
[3] R. M. WOTTON and F. SOGANDARES-BERNAL, Parasitology *53*, 157 (1963).

together with some idea of the effective increase in internal surface area. A single gut cell in *D. subclavatus*, for example, supports approximately 1000 of these processes, and a square millimetre of gut surface something of the order of 26,000,000. These numbers bring about an increase in internal surface area of the gut of about 115–120 times. Although no electron microscope observations were made on any of the other species, estimates with the light microscope indicate a similar increase in internal surface area.

The most obvious conclusion as to the function of these microvilli-like processes in the gut of digenetic trematodes is that, by virtue of the enormous internal surface area they provide, they greatly enhance absorption of food from the gut lumen. This is supported by the presence in the processes of acid phosphatase, an enzyme which is commonly associated with sites of active carbohydrate transfer. In addition, a non-specific esterase has been detected in the gut and in association with the processes of at least 3 of the trematodes, *D. subclavatus*, *G. vitelliloba* and *G. cygnoides*, so that it is possible the structures may also have a role in digestion. In this connection, supporting evidence comes from the work of UGOLEV[4] who has demonstrated the importance of the intestinal surface on the rate of hydrolysis of starch in vertebrates, and who believes there to be hydrolytic enzymes adsorbed on the surface of the microvilli which provide an intermediate link between cavital hydrolysis and absorption.

Microvilli-like structures have never been observed in the gut of the Monogenea, *Polystoma integerrimum*, *Diplozoon paradoxum*, *Discocotyle sagittata*, *Diclidophora merlangi* and *Octodactylus palmata*, during the present study, nor are there any reports of such in the literature. Indeed any variations in gastrodermal structure that exist in this group can be related to differences in diet[5]. The gut in the Monogenea is generally a much branched and diffuse structure, however, whilst that of the Digenea is characterized by its apparent simplicity, the only exception being in certain members of the Fasciolidae where the gut caeca resemble those of a typical monogenean trematode. This difference in the gross structure of the gut within the Trematoda may well be related to the differences in gastrodermal structure, the microvilli of the Digenea greatly increasing the internal surface area and thereby compensating for the lack of branched and subdivided diverticula so characteristic of the Monogenea.

Résumé. Etude microscopique de la structure du tube digestif de 7 espèces de Digenea (Trematoda). Dans toutes les espèces, les cellules du gastroderme possèdent des microvillosités nombreuses qui donnent une réaction positive avec PAS et la phosphatase acide. Ces structures augmentent considérablement la surface cellulaire de l'intestin simple. Leur rôle dans la digestion et l'absorption est discutée.

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[4] A. UGOLEV, Nature, Lond. *188*, 588 (1960).
[5] D. W. HALTON and J. B. JENNINGS, Biol. Bull. *129*, 257 (1965).
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A Comparison of Lymph Collected from the Thoracic Duct in CBA and A Strain Mice[1]

Increasing attention has been given in recent years to the use of isolated lymphocytes for immunological studies, and different methods for collecting lymph from the thoracic duct of mice have been described (SHREWBURY[2], GESNER and GOWANS[3], and BOAK and WOODRUFF[4]). This paper compares the results of collecting lymph from the thoracic duct of 2 strains of mice over several days under sterile conditions.

Material and methods. Male white mice of the 'A strain', weighing 25–30 g and male and female CBA mice, weighing 23–28 g were used.

About 2 h before operation 0.2 ml of olive oil was given orally. The animals were anaesthetized with injections of nembutal given s.c. and supplemented with ether. The operation technique described by BOAK and WOODRUFF[4] was used but we did not give s.c. injections of saline with heparin after operation. The cannulae (mouse cannula, Portex Plastic Ltd.) were sterilized with 70% ethanol for 30–60 min.

The lymph was collected in sterile tubes containing Hank's solution with 14 U of heparin/ml or 20% normal mouse serum in Tyrode solution with 200 U of heparin/ml. For special purpose the tubes were placed in an ice bath.

The cells were counted in chambers and the differential cell counts were made on smears stained with Leishman's stain. For sterility tests blood agar plates, Sabouraud's agar plates and broth were used.

The mice were fixed in a comfortable position on rotating drums (GESNER and GOWANS[3]), and the food hopper and drinking bottle were adjusted so as to be within easy reach. The animals were kept clean and warm. Clots which formed in the cannulae were removed with a fine stainless-steel wire and the cannulae were washed carefully with a small volume (0.05 ml) of Hank's solution.

Results. A strain mice: The mean volumes of lymph and the mean total of lymphocytes collected during successive 24 h intervals are summarized in the Table. The samples of lymph were sterile. About 90–95% of the cells in the lymph produced during 24–48 h were morphologically typical small lymphocytes; the remaining cells were medium size and large lymphocytes. During the third and fourth days, usually a higher percentage of medium or larger cells was found. Most of the mice appeared well and lively during the experiment.

CBA mice: The results in CBA mice are summarized in the Table. The samples of lymph were found sterile.

[1] This work was supported by the National Institute for Medical Research, Mill Hill, London N.W.7, England.
[2] M. M. SHREWBURY, Proc. Soc. exp. Biol. N.Y. *101*, 492 (1959).
[3] B. M. GESNER and J. L. GOWANS, Brit. J. exp. Path. *43*, 424 (1962).
[4] J. L. BOAK and M. F. A. WOODRUFF, Nature *205*, 396 (1965).