percentage of male-mothers and male-female-mothers. We used the line 2K of *Heteropeza pygmaea* (Itonididae, Dipt.)[7]. The substrate for the fungus *Peniophora albula* consisted of 5% maltextract, 4% agar and 91% distilled water. The Petri dishes were inoculated with the fungus in the centre of the plates. To obtain female-mothers, 2.5 days after inoculation 8–10 4-mm-sized mother larvae with daughter larvae about to hatch were placed in a Petri dish on the edge of the growing fungus. For the production of male-mothers and male-female-mothers, the mother larvae were placed in the centre of the Petri dish after 7 days of fungus growth. All other parameters such as temperature, etc., were held constant[9].

Results. From the 8–10 mother larvae which were placed in a Petri dish, between 200 and 300 daughter larvae hatched within a few hours. In both groups (group 1: larvae placed on plates with 2.5-days-old fungus; group 2: the same on 7-day-old fungus) the larvae started feeding immediately after hatching from the maternal cuticle and hardly crawled around during larval development. In both cases food was abundant and the increase in amount of the food on account of the fungus growth was even larger than the decrease caused by the feeding of the larvae. The Petri dishes were clearly not overpopulated and the larvae consumed only a fraction of the food supply provided. Another indication that in both groups the larvae consumed the same (i.e. the maximum) amount of food, is that they reached the same size at the end of development.

In the Petri dishes of group 1, almost 100% female-mothers developed. Occasionally, a larva started feeding only some time after hatching and developed into a male-female-mother, or did not feed at all. In the Petri dishes of group 2, nearly 2 out of 3 larvae developed into male-mothers or male-female-mothers. In 10 dishes the medium value was 63.9 $\pm$ 3.6%; the minimum value in a single Petri dish was 49% and the maximum value 74%, the standard deviation of the individual value was 8.9%.

Discussion. For our investigations on development of *Heteropeza pygmaea*, a well-functioning male-mothers- and male-female-mothers-producing culture method is needed: in attempts to culture ovaries of this gall midge in vitro, i.e. in haemolymph taken from sterile, full-grown larvae[10–12], we intend to compare the influence on the ovaries of haemolymph from larvae grown on a female-mother-inducing medium with that from larvae grown on a male-mother- and male-female-mother-inducing medium. To this end we must be sure that these sterile (progeny-less) donor larvae indeed comprise either a female- or a male-determining internal milieu. Moreover, for studies on the aberrant processes at meiosis of the male-determined eggs, male-mothers and male-female-mothers have to be at our disposal in sufficient numbers. The present article describes such a reproducible method and contributes to the controversial discussion on the role of the food quantity versus quality in the determination of the reproductive direction of the daughter larvae. It now seems clear that the quality of the food is the decisive factor, since only the age of the fungus was altered. In the Petri dishes of group 2, the amount of nutrition was, of course, larger than in the Petri dishes of group 1. However, in both cases, there was far more food than necessary for every larva, especially since the larvae can naturally incorporate only a limited amount of food. Since the population density was low, there was no crowding-effect; even in the Petri dishes of group 1, the larvae did not have to crawl around looking for food. Thus, feeding larvae were not disturbed.

Perhaps it should be mentioned that a fungus usually alters its metabolism in ageing (production of toxins, etc.)[13]. Different species of fungus, of course, differ in composition of their hyphae contents representing the food supply for the larvae. Since for the production of female imago larvae (which develop into female imagos) the daughter larvae in the laboratory must be grown on a different fungus species[7], it seems probable to us that an alteration of the food quality is also responsible for this switch-over to an additional developmental pathway[14].

Summary. The decisive role of food quality in determining the developmental fate and the sex of progeny of female larvae of the paedogenetic gall midge *Heteropeza pygmaea* is established.

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Lactate Dehydrogenase Isozymes of Mouse Epidermis

MARKERT and URSPRUNG[1] described in detail the ontogeny of the adult lactate dehydrogenase (LDH) isozyme patterns in a variety of tissues and organs of the mouse. In nearly all cases, they found 5 electrophoretically distinct forms, but in proportions that were highly tissue-specific. MARKERT and URSPRUNG did not examine the LDH isozyme patterns of the skin. This organ, particularly the mitotically-active epidermis, exhibits marked regional morphological variation in the mouse and in other mammals. PAPACONSTANTINOU[2] has related the LDH isozyme profiles of tissues to their mitotic activity.

In the present study, answers have been sought to the following questions: First, do the epidermal LDH isozymes in the adult mouse vary between the different anatomical regions of the integument? Second, will irritants that stimulate hyperplasia of the epidermis influence the patterns of LDH isozymes? Third, do genes that alter dermal-epidermal interactions also influence epidermal isozyme patterns?

Materials and methods. To identify regional differences in the LDH isozymes of the epidermis, 8-week-old, male and female mice of the C57BL/(sublines: 6J and St) (*a*/*a*), C3HB/St (mi^{bw}/mi^{bw}), and C57HR/Ch (*a*/*a*; *hr*/*hr*)

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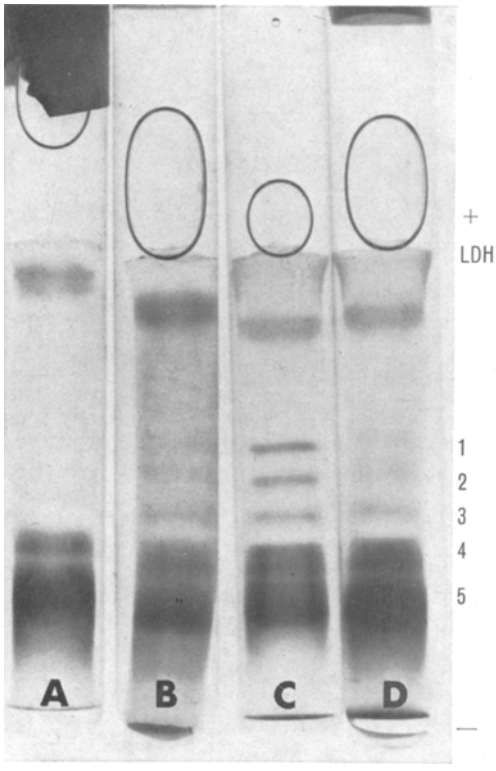

Fig. 1. Electropherograms illustrating regional differences in patterns of LDH isozymes in the epidermis of C57BL mice: A) tail; B) ear pinna; C) trunk dorsum; D) hind foot. LDH-5 predominates in all regions; the ratio of LDH-5 to LDH-1 varies.

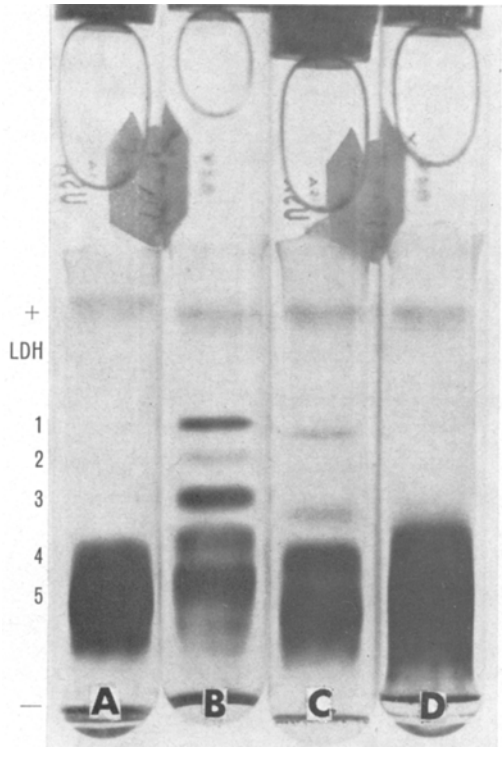

Fig. 2. Electropherograms demonstrating regional differences in patterns of LDH isozymes in the epidermis of C57HR (hairless) mice: A) hind foot; B) trunk dorsum; C) ear; D) tail. LDH-5 predominates in all regions; the ratio of LDH-5 to LDH-1 varies (compare with Figure 1). In ear and trunk, LDH-1 stains more intensely than LDH-2.

strains were sacrificed by cervical dislocation. The quiescent (telogen) hair[3,4] was plucked from an extensive area of the trunk dorsum. In each experiment, the skin from the ear pinnae, tails, entire hind feet (less phalanges) and trunk dorsa of approximately 8 mice was removed and incubated for $1^1/_2$–2 h in 2 *M* NaBr at 37 °C. The epidermis was split from the underlying dermis and rinsed in cold physiological saline. In haired skin the epidermal sheets included substantial numbers of whole and/or fragments of hair follicles. All skin components were recovered from the rinse solution by low speed centrifugation, frozen immediately in a petri dish chilled on dry ice, and stored at −20 °C. To induce epidermal hyperplasia, the tails and hind feet of 12 female C57BL/6J mice were dipped thrice weekly in a 1:1 solution of turpentine and acetone for a total of 4 weeks. At sacrifice, the tails and hind feet were removed and treated as above.

Each frozen preparation was homogenized in a Potter-Elvehjem all-glass homogenizer in chilled 30% sucrose (wet weight/volume = 0.33 g/ml). The homogenate was centrifuged at 31,000–35,000 × *g* for 30 min at 0 °C. Aliquots of the supernatant (0.05 ml) were subjected to disc polyacrylamide gel electrophoresis and LDH activity visualized according to the method of Dietz and Lubrano[5]. Gels were removed from the staining mixture at appropriate intervals with an incubation period usually of 2 h or less at 37 °C. A Photovolt 'Densicord' Model 542 recording electrophoresis densitometer was used to scan the stained gels for an estimate of relative activities of the LDH isozymes.

Results. Five isozymes of LDH were present in electropherograms of extracts of the epidermis from the ear pinnae, tails, hind feet, and trunk dorsa of C57BL, C57HR, and C3HB mice. LDH-5 and, to a lesser extent, LDH-4 predominated in all types of epidermis; generally very small amounts of LDH-3, LDH-2, and LDH-1 were detected (Figures 1 and 2). There was usually, but not always, a progressive decline in staining intensity from LDH-5 to LDH-1. In one of three cases, the LDH-1 band appeared to be slightly more prominent than the LDH-3 and LDH-2 bands in the trunk epidermis of C57BL mice. In C57HR mice the LDH-2 band was clearly less evident than the LDH-1 and LDH-3 bands in the electropherograms of back and ear epidermis (Figure 2). The hairless back epidermis was an exceptional case in which LDH-3 activity exceeded that of LDH-4. The general impression for mice of the three strains was that the ratio of activity of LDH-1 to LDH-5 for the ear and back epidermis was greater than that for the tail and hind foot. Both visual inspection and densitometric scans of the stained gels were fully consistent with this conclusion.

Repeated dipping of the tails and hind feet of C57BL mice in turpentine-acetone to induce epidermal hyperplasia resulted in prominent, but somewhat reduced, LDH-5 in both types of epidermis; LDH-4 was markedly reduced and LDH-3, LDH-2, and LDH-1 were completely absent (Figure 3). Overnight incubation of the gels in the staining mixture did not result in their appearance, although the LDH-5 and LDH-4 bands were enhanced.

Discussion. The region-specific LDH isozyme patterns in mice are not correlated with the melanin pigmentation of the epidermis, for they are the same in mice with pigmented (C57BL, C57HR) and nonpigmented (C3HB) integuments. The greater proportions of LDH-1 to LDH-5 in ear and trunk epidermis might reflect region-

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specific differences in the synthesis of the A(M) and B(H) subunits[1] or rates of catabolism of the various isozymes[6–8].

Regional differences in epidermal morphology have been shown to be regulated in adult guinea-pigs and hamsters by the local dermis[9,10]. Overall, it would appear that the varying patterns of LDH epidermal isozymes derive mainly from the activity of local populations of keratinocytes with possibly some contributions by other cell types such as Langerhans cells. It remains to be shown whether the relative amounts of LDH isozymes synthesized depend on information intrinsic to the epidermis or are programmed by the underlying dermis throughout life. In hairless (C57HR) mice, the altered dermal-epidermal interactions culminating in hair loss[10] produce at most a small departure from the LDH isozyme patterns found in the haired mice.

The significance of a multiplicity of forms of LDH in skin is uncertain, for the precise roles played by the various isozymes have not been established[11–17]. PAPACONSTANTINOU[2] has proposed that the passage of mitotically-active cells into the stationary (postmitotic) phase is characterized by a change from an isozyme pattern where LDH-5 predominates to one where LDH-1 predominates. The high level of LDH-5 in mitotically-active normal mouse epidermis and the apparent loss of LDH-1 in epidermis treated with a hyperplasia-producing chemical agent are consistent with PAPACONSTANTINOU's hypothesis. Nonetheless, neither the available data on the LDH isozymes nor on regional mitotic activities in the epidermis of the mouse[18] permit any final conclusions regarding their possible relationship. This study does suggest that in whatever capacity LDH isozymes function, all areas of the epidermis do not have precisely the same requirements.

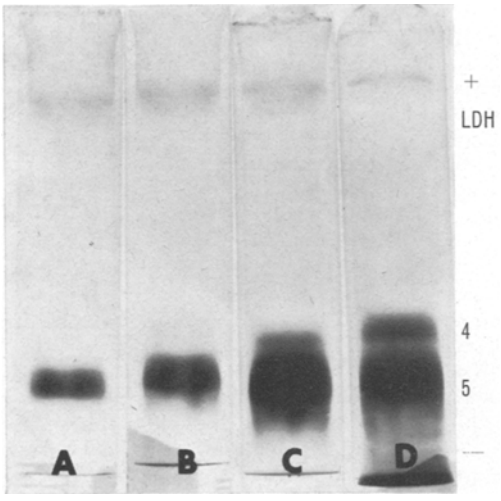

Fig. 3. Electropherograms of LDH isozymes in epidermis of C57BL/6J mice treated repeatedly with a mixture of turpentine and acetone: A) hind foot (treated); B) tail (treated); C) hind foot (nontreated); D) tail (nontreated). LDH-5 predominates in treated and nontreated mice. Although LDH-5, LDH-4 and traces of LDH-3, LDH-2 and LDH-1 are detectable in skin of nontreated mice, only LDH-5 and LDH-4 are demonstrable in treated skin.

Summary. Five isozymes of LDH are demonstrable in the epidermis of the ear pinnae, hind feet, trunk dorsa, and tails of adult C57BL, C57HR, and C3HB mice by polyacrylamide gel electrophoresis. LDH-5 activity predominates in electropherograms. The ratio of LDH-1 to LDH-5 is greater in the epidermis of ear pinna and trunk dorsum than in that of tail and hind foot. The region-specific patterns of epidermal LDH isozymes are not correlated with melanin pigmentation or 'hairiness' of the skin.

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Peroxidase Uptake Through the Tegument of the Cestode *Taenia hydatigena*

One of the important attributes of the cestode tegument is the absorption of food materials from the host intestine, as they lack a mouth and digestive tract. By applying autoradiographic techniques KING and LUMSDEN[1] observed the passage of labelled linolic acid through the tegument of *Hymenolepis diminuta*, suggesting that the tegument is capable of transporting macromolecular substances from the host intestine. Similarly, in the same cestode species, ROTHMAN[2], employing different colloidal substances, reported the absorption of those colloidal substances through the tegument by electron microscope. However, hitherto no experimental evidence has been provided to substantiate the entry of protein molecules through the tegument of cestodes. Hence an attempt has been made in the present investigation to study the nature of the permeability of the tegument of the cestode *Taenia hydatigena* to protein molecules by using plant peroxidase, which has been extensively used recently as a

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