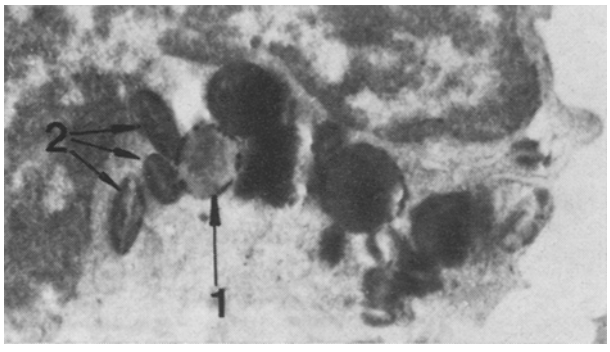

Fig. 4. Fragment of eosinophilic myelocyte. The double-reaction on peroxidase and acid phosphatase. Immature granule (1) is seen in which, on the background of a less dense reaction product on peroxidase near the periphery of the organelle, there is a more dense reaction product on acid phosphatase. Mature eosinophilic granules (2) contain a homogeneous reaction product on peroxidase. The crystal remains negative. ×14,500.

Jemieson and Palade[3,4] and also with autoradiographic data on the path of granule formation in granulocytes[5]. Our results on peroxidase localization in mice eosinophils coincide with the data obtained by other authors on rats, rabbits and guinea pigs[6–10].

We demonstrated a heterogeneous peroxidase reaction in specific granules. It is likely that this phenomenon dependends on the absence of granular permeability of DAB and H_2O_2. Cotran and Litt[7] showed a positive reaction in some granules on the incubation of guinea-pig eosinophils in a medium lacking H_2O_2. We also demonstrated a weak positive reaction on some granules on the incubation without exogenous H_2O_2. Preincubation with absolute acetone does not inhibit this reaction but, on the contrary, promotes it. This depends on a small destruction of membranes and an increase in substrate permeability. Perhaps eosinophilic granules are not homogeneous in regard to the presence of endogenous peroxides. The granules in which endogenous peroxide is present are more reactive and their role in cellular metabolies seems to be more important.

It is likely that the incubation with the omission of H_2O_2 permits the characterization of the granules not only on the basis of enzymatic activity when, as noted above, a certain heterogeneity in the activity in the matrix of granule was found, but also on the concentration of endogenous peroxides.

Previously[1], we presented the results on a simultaneous demonstration of peroxidase and acid phosphatase on developing mice neutrophils. Acid phosphatase was present in the Golgi apparatus and the peroxidase in azurophil granules. Our results obtained on eosinophils have confirmed our assumptions that the peroxidase-containing granules are not lysosomes, but special peroxidase-containing antimicrobial organelles[11,12].

Summary. The peroxidase and acid phosphatase activity in developing mice eosinophils has been demonstrated. Both peroxidase and acid phosphatase are localized in various cellular organelles.

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Role of Food Quality Versus Quantity in Determining the Developmental Fate of a Gall Midge Larva (*Heteropeza pygmaea*) and the Sex of its Paedogenetically-Produced Eggs

Depending on rearing conditions, the larval ovaries of the viviparous paedogenetic gall midge *Heteropeza pygmaea* can produce 2 types of eggs which both develop through embryogenesis in the haemocoele of the larvae[1–3]. These eggs can be male- or female-determined[4] and, in accordance with the sex of their progeny, the growing mother larvae with female and/or male embryos are called female-mothers, male-female-mothers or male-mothers.

For a long time the role of the nutritive conditions which determine the type of mother larvae and – indirectly – the sex of its eggs, has been discussed[1,5,6]. The larvae feed on fungus which in the laboratory is easy to grow on a malt-agar substrate. Since, under defined culture conditions for the fungus, male-mothers and male-female-mothers appeared only when the density of population of the feeding larvae in the Petri dishes was relatively high (and especially much higher than was required for the production of female-mothers[4,7]), it was assumed that the quantity of the food was the determining factor for the developmental fate of the larvae and for the sex of their progeny[1,8]. Looking for a method which would yield a reproducible high percentage of male-mothers and male-female-mothers in the cultures, we came upon a simple formula for the culture medium of both female-mothers and male-mothers or male-female-mothers respectively. In this culture method merely the age of the fungus is altered; the population density of the larvae is held constant.

Material and method. In this context we give only the above-mentioned formula for the cultivation of the fungus, which yielded either female-mothers or a high

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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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