epimastigotes[17,18]. Similar results were obtained with the microsomal fraction, although specific H_2O_2 production rates were about 50% of those with the mitochondrial fraction. The highest rates of H_2O_2 generation were observed with the supernatant, but at variance with the other assayed fractions, NADPH was the more effective electron donor. The high H_2O_2 production rates with the soluble fraction suggests the presence of autoxidizable flavoproteins of the flavodoxin[19] type in that fraction. Taking into account the role of superoxide dismutase in H_2O_2 generation[6] (reaction 3),

$$O^-_2 + O^-_2 + 2H^+ \rightarrow H_2O_2 + O_2. \quad (3)$$

T. cruzi homogenates were investigated for this enzyme. The experimental results are presented in figure 2. Trace A shows the control activity, while trace B shows the inhibition of adrenochrome formation by the homogenate. Addition of cyanide abolished the inhibition, thus indicating the presence of a cyanide-sensitive cupro-zinc superoxide dismutase in the homogenate. The quantitative data in figure 2 (lower graph) enable one to calculate the dismutase concentration in the homogenate, which was equivalent to 0.28 µg bovine superoxide dismutase per mg homogenate protein.

Discussion. Measurement of H_2O_2 generation by T. cruzi homogenates accounts for about 4% of the endogenous oxygen uptake by the epimastigotes (see De Boiso et al.[17] for rates of respiration). This may be a minimum value considering the dilution of endogenous substrates and coenzymes presumably involved in peroxide generation under physiological conditions. On the other hand, the antimycin and cyanide-insensitive respiration of T. cruzi is about 15% of the overall aerobic respiration[17], a value from which one can infer that a large proportion of the antimycin- and cyanide-insensitive oxygen consumption is due to H_2O_2 formation.

Distribution of protein in the subcellular fractions as described in the table, as well as the specific values for H_2O_2 generation by those fractions, allow one to calculate the relative contribution of fractions to total cellular generation of H_2O_2. This contribution is as follows: supernatant, 63%; mitochondrial membranes, 31%, and endoplasmic reticulum, 6%. It must be noted, however, that this calculation is based on the assumption that in physiological conditions the peroxide generators are fully saturated with reductant.

In contrast to the results obtained with homogenates, the CCP assay failed to demonstrate H_2O_2 production by epimastigotes. This failure is presumably due to the inaccesibility of the extracellular CCP to the intracellular H_2O_2, as well as to the negligible diffusion of intracellular H_2O_2 to the extracellular medium.

Since T. cruzi epimastigotes contain peroxidase (not catalase) as H_2O_2 metabolizing enzyme[8], the intracellular steady state level of H_2O_2 must depend on both a) the rate of H_2O_2 generation and b) the supply of hydrogen donors for the peroxidase reaction. Unbalance of these processes may prove lethal for the parasite since H_2O_2 is toxic for Trypanosomatidae[9,10]. Consequently, drugs that should either prevent H_2O_2 utilization or stimulate H_2O_2 generation may be regarded as possible trypanocide agents.

The control of melanin dispersion in the hypomere of *Xenopus* larvae[1]

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Summary. Studies on melanophores cultured in vesicles derived from Xenopus hypomeric tissues suggest production of a diffusible melanin-concentrating substance by ventral hypomeric tissues and melanin-dispersing properties in lateral hypomeric tissues.

During normal development and under normal levels of incident illumination the reticulate melanophores in the lateral tissues of the hypomere of early larval Xenopus undergo a progressive concentration of melanin granules which begins at stage 40 in the most ventrally situated melanophores then spreads dorsally, culminating in a dorsoventral gradation of reticulate, stellate and punctate melanophores. The dispersion of melanin in melanophores is controlled principally by the level of MSH (melanophore stimulating hormone) in the tissues[4,5]. However, Pehlemann[6] found that the dispersion of melanin in melanophores on the peritoneum (formerly hypomeric melanophores) of Xenopus larvae is largely independent of MSH and suggested some control by environmental tissues. Recent work[7] has suggested the possibility that the dispersion of melanin in hypomeric melanophores might be influenced by a melanin-concentrating substance produced by the ventral tissues of this region. In the present study the dispersion of melanin in melanophores isolated in vesicles derived from tissues of various regions of the hypomere was examined in order to investigate this possibility.

Methods. Eggs of Xenopus laevis were obtained by standard methods[8]. The techniques used in preparing and culturing vesicles have been described previously[9]. Uniform portions of neural crest and underlying dorsal sector of neural tube were excised from the anterior trunk of stage 22 embryos (staging according to Nieuwkoop and Faber[10]). Such explants were cultured in vesicles derived from sheets of a) lateral, b) ventral and c) lateral and ventral hypomeric epidermis and all subadjacent mesoderm obtained from stage 22 embryos. 50 vesicles of lateral, 50 vesicles of ventral and 46 vesicles of lateral and ventral hypomeric tissues (composite vesicles) were cultured for up to 2 weeks under normal indoor illumination and the dispersion of melanin in melanophores which

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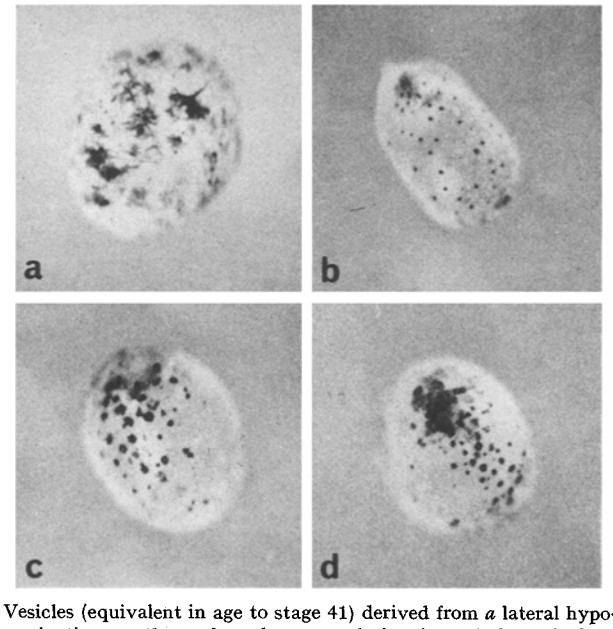
subsequently differentiated in the vesicles was examined. Other stage 22 embryos were allowed to develop normally for use as controls. The effects of bright light and darkness on melanin dispersion were tested when vesicles were of an age which corresponded to stage 41 control larvae. Vesicles of each type were illuminated from a distance of 30 cm by an ordinary bench lamp or placed in a light-tight box. Melanophores were observed at intervals over a period of 45 min.

Results. Melanophore differentiation began after about 2 days at stage 33–34 of control larvae. All vesicles until stage 38 possessed similar-sized populations of reticulate melanophores. In vesicles derived from lateral tissues almost all melanophores remained reticulate for the duration of the experiment. In contrast, in vesicles derived from ventral tissues almost all melanophores became punctate at stage 39 or 40. Most composite vesicles at stage 39 or 40 possessed relatively discrete areas of predominantly reticulate, stellate or punctate melanophores.

Number (expressed as a percentage) of vesicles of lateral (L), ventral (V) and lateral + ventral (L + V) hypomeric tissues and the type of melanophore population

Type of vesicle	Type of melanophore population				
	All reticulate	Mostly reticulate	Mostly stellate or punctate	All stellate or punctate	All punctate
Stages 33–38					
L	100	0	0	0	0
V	100	0	0	0	0
L + V	100	0	0	0	0
Stages 39–40					
L	96	4	0	0	0
V	0	0	14	16	70
L + V	0	17.4	47.8	21.8	13
Stage 41					
L	90	10	0	0	0
V	0	0	4	18	78
L + V	0	0	34.8	47.8	17.4



Vesicles (equivalent in age to stage 41) derived from *a* lateral hypomeric tissues; the melanophore population is entirely reticulate, *b* ventral hypomeric tissues; the melanophore population is entirely punctate, *c* and *d* lateral and ventral hypomeric tissues; the melanophore population is entirely (*c*) or mainly (*d*) stellate or punctate; reticulate melanophores (*d*) are confined to a small area of the vesicle.

However at stage 41 $^2/_3$ of these vesicles exhibited only stellate or punctate melanophores; the remainder retained a small number of reticulate melanophores which were restricted to a small area of the vesicle.

Bench lamp irradiation experiments revealed that following a period of illumination of 20 min most punctate melanophores became reticulate. After a period of 45 min in darkness most reticulate melanophores became punctate.

Discussion. The transformation of melanophores from reticulate to punctate which took place in vesicles of ventral tissues was not observed in vesicles of lateral tissues, suggesting that ventral tissues either possessed melanin-concentrating properties which were absent from lateral tissues or lacked melanin-dispersing properties which were present in lateral tissues. However in most composite vesicles the entire melanophore population exhibited some degree of melanin concentration. In a composite vesicle diffusible substances capable of controlling melanin dispersion produced by the tissues of one region would be able to influence all melanophores in the vesicle. Accordingly these results appear consistent with the production of a diffusible melanin-concentrating substance by ventral tissues.

Only a small number of composite vesicles possessed entirely punctate melanophore populations. Most composite vesicles exhibited discrete areas of stellate and, less frequently, reticulate melanophores, suggesting the existence of localized regions of the lateral tissue component of the vesicle which possessed properties capable of modulating or countering the melanin-concentrating properties of the ventral hypomeric substance. Evidence that lateral mesoderm possesses properties capable of effecting melanin dispersion is provided by the characteristic presence of reticulate melanophores in vesicles of lateral tissues (epidermis + mesoderm) and by Obika and Bagnara's[11] finding that vesicles composed solely of the lateral trunk epidermis of Xenopus were characterized by punctate melanophores.

Melanophores contained in vesicles of ventral tissues and in composite vesicles first exhibited melanin concentration at stage 39 or 40, an age which coincided with the onset of melanin concentration in the most ventrally placed hypomeric melanophores of normal larvae, suggesting that the factors controlling melanin dispersion in vesicles also operate in vivo. Hence it is proposed that the dorsoventral gradation of reticulate, stellate and punctate melanophores observed on the hypomere of normal larvae after stage 40 is dependent on a) the production of a diffusible melanin-concentrating substance by the ventral tissues giving rise to punctate melanophores, b) melanin-dispersing properties of the lateral mesoderm giving rise to reticulate melanophores and c) competition between both these factors giving rise to stellate melanophores.

The mode of action of substances capable of influencing melanin dispersion is unknown. In the present study reticulate melanophores became punctate in darkness and punctate melanophores reticulate on exposure to bright light. A similar light sensitivity has been observed in Xenopus melanophores in hanging drop cultures[12], suggesting the possibility that the hypomeric factors described above as controlling melanin dispersion might act by modulating intrinsic processes of the melanophores, e.g. a photochemical reaction[13] taking place within the melanophore.

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