erosions were seen, but nothing comparable with the deep, extensive ulcers which developed in the guinea pigs was observed. When the rats' adrenals were stained with silver nitrate, the darkening was immediate and marked. At present we are unable to say definitely whether this resistance of the rat to ulcer production through bile salt injection is due to their ability to synthesize vitamin C or to some other species difference.

7544 P

Differential Reduction of Methylene Blue by Living Organisms.

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With the oxidized dye and with a leucobase prepared by adding small amounts of HCl and $Na_2S_2O_3$ to dye solutions axial differentials or gradients in rate of reduction of dye have been observed in various unicellular and multicellular organisms. *Paramecium* is able to reduce methylene blue in mixtures of culture fluid and dye exposed to air, provided the animals are numerous in proportion to volume of fluid or gather in aggregations and decrease oxygen locally, or provided other organisms which take up oxygen are present, but reduces more rapidly in sealed preparations with small amounts of fluid. With high oxygen content of solutions the anterior ectoplasm stains more rapidly than other parts. In low concentrations of dye with somewhat lower oxygen content permitting some reduction stain appears first in the deepest part of the posterior entoplasm, extends anteriorly in the entoplasm and the ectoplasm does not stain or stains more slowly, also from posterior to anterior, except for the extreme posterior tip which often stains less rapidly than adjoining regions. Apparently rate of staining under these conditions varies inversely as reducing power of different regions. In stained but uninjured animals reduction first becomes evident in the ectoplasm of the anterior end and progresses posteriorly, somewhat more rapidly along the peristome than on the aboral side and the extreme posterior tip of some individuals shows early reduction. In high concentrations the anterior ectoplasm stains more rapidly and more deeply than other regions at first and with sufficient staining it is injured and its reducing power is decreased or lost while more posterior regions are still able to reduce the dye in low oxygen.

Other ciliates examined, *Frontonia, Spirostomum, Dileptus,* show differentials in staining and reduction essentially similar to those of *Paramecium,* but with different ranges of concentration and different degrees of decrease in oxygen. The animals and other material from the infusion decrease the oxygen sufficiently to permit more or less reduction in *Frontonia* and *Dileptus* in open preparations, while *Spirostomum* requires several hours in sealed preparation for complete reduction after slight staining.

In *Hydra* tentacles stain first with concentrations used thus far and if staining is not carried to the point of injury each tentacle shows a basipetal reduction gradient in low oxygen. With further staining before reduction loss of reducing power occurs first at the tentacle tip and progresses basipetally as staining proceeds and cytolysis follows basipetally. In the body ectoderm reduction occurs most rapidly in the hypostome region and progresses basipetally, provided staining has not progressed to the point of injury and loss of reducing power. The basal stalk region of *Pelmatohydra* reduces more rapidly than the body after light staining, but is also more susceptible than the body and injury and loss of reducing power occur with comparatively little staining. In *Stenostomum* chains of zooids the head regions of the developing zooids beyond a certain stage of development reduce the dye more rapidly than more posterior levels with light staining, but are more susceptible to loss of reducing power after deeper staining. The ventral body wall also reduces more rapidly than the dorsal. In the microdrilous oligochetes, *Tubifex* and *Nais* the body wall of the anterior region and the posterior growing region consisting of a large number of developing segments do not stain or stain less rapidly than the middle region and the ventral body wall does not stain or stains less rapidly than the dorsal in low concentrations of dye in the oxygen content of the cultures of decaying vegetation, bacteria, protozoa, etc., in which the animals are maintained in the laboratory. After staining up to a certain point anterior and posterior regions reduce more rapidly than the middle in low oxygen and the ventral body wall reduces more rapidly than the dorsal. With high dye concentrations the anterior end stains more deeply than the middle and its reducing power is decreased or lost. The posterior region is so susceptible that it is usually killed before it becomes very deeply stained.

Concentrations used in these experiments range from 1/5000 to 1/300000 methylene blue. Since the leucobase stains and injures some forms (*e. g., Paramecium*) much more rapidly than the oxidized dye the range of concentrations for certain results is lower

than with the latter. The dye is more toxic in light, even the light of the microscope condenser, than in darkness.

That the results described are due to reduction, not to diffusion outward of the dye is shown by the fact that after reduction the color returns within a few seconds with increase in oxygen content of the fluid. It may also be noted that in all cases the reduction gradient in animals not irreversibly injured is essentially identical with the gradient of susceptibility to a large number of chemical and physical agents in gradually lethal concentration or dosage.

The anteroposterior reduction gradient in *Paramecium* was noted in an earlier paper.[1] Recently Roskin and Semenoff,[2] using a leucobase only, have concluded from the observed course of reduction that oxidation occurs most rapidly in the posterior region of *Paramecium*. Their results can be duplicated by following their procedure but this procedure results in deeper staining of the anterior ectoplasm and less rapid or no reduction there while more posterior regions are still able to reduce, consequently they have failed to observe the normal anteroposterior reduction gradient.

7545 C

Effect of 1-2-4 Dinitrophenol on Cellular Respiration.

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Numerous investigators (Plantefol,[1] Field, Martin and Field,[2] Ehrenfest and Ronzoni[3]) have reported that 1-2-4 dinitrophenol added *in vitro* to plants, yeast cells and frog tissues increases their respiration. This increase seems to be associated with an increased aerobic fermentation in the case of yeast cells (Cutting and Tain-

[1] Child, C. M., and Deviney, E., *J. Exp. Zool.*, 1926, **43**, 257.

[2] Roskin, G., und Semenoff, W., *Z. f. Zellforschung u. mikr. Anat.*, 1933, **19**, 150.

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[1] Plantefol, L., *Ann. Physiol. Physicochim. Biol.*, 1933, **8**, 127.

[2] Field, J., 2nd, Martin, A. W., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 56.

[3] Ehrenfest, E., and Ronzoni, E., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 318.

[4] Cutting, W. C., and Tainter, M. L., *J. Pharm. and Exp. Ther.*, 1933, **48**, 410.