Neben der Bestätigung unserer im Phasenmikroskop erhobenen Befunde haben somit diese Untersuchungen gezeigt, daß durch die künstliche Schwellung in destilliertem Wasser mittels des Elektronenmikroskops Befunde erhoben werden können, die an nichtgeschwellten, kompakten Körpern eventuell nicht sichtbar sind.

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Summary

It is shown that mitochondria suspended in distilled water are particularly well adapted to electron-microscopic examination, since the membrane can be loosened from the mitochondrial body by this method. The membrane is composed of short protein fibrils and globular protein molecules, and has a thickness of approximately 200 Å. The body cannot be further illuminated, but parts of the periphery show that the protein is here much more compact. It is assumed that this is dependent upon its lipoid contents.

SH Poisoning and Mutation

BACQ[1] includes mustard gas in his list of "substances thioloprives", that is of substances whose toxic effects are caused primarily by action on –SH groups. British workers[2], although recognizing an action of mustard gas on –SH groups, nevertheless consider that this is not its main-effect, and find that it does not in general affect –SH enzymes. It is, however, extremely effective in its action on one particular –SH enzyme, hexokinase, and on a number of related phosphate transferring –SH enzymes, the so-called phosphokinases. These are readily inactivated by small amounts of mustard gas. Lewisite, like other arsenical vesicants, reacts strongly, but reversibly, with –SH groups, including those of hexokinase. The observation that lewisite does, not like mustard gas, produce mutations in *Drosophila*[3] suggests that neither –SH poisoning in general, nor phosphokinase inhibition in particular are the primary cause of the mutagenic action of mustard gas. In view of the somewhat exceptional position of the arsenicals, confirmation with a typical –SH poison and inhibitor of phosphokinases seemed desirable. Chloropicrin was chosen because, together with bromopicrin, it heads the list of –SH poisons arranged by BACQ[1] in order of efficiency. According to BACQ[1], $N/100$ chloropicrin at 37°C destroys 50% of all –SH groups in denatured ovalbumen in less than one minute, it blocks irreversibly the masked –SH groups in native ovalbumen, and it rapidly inactivates papaine. No experiments with hexokinase or other phosphokinases are reported; but it seems very probable—and is assumed also by BACQ—that phosphokinases will be inactivated by chloropicrin.

Three series of tests were carried out. In the first young males of *Drosophila melanogaster* were kept in an exsiccator together with a small open dish of chloropicrin. The longest exposure tolerated by a minority of the flies was three minutes. A test for sex-linked lethals was carried out on the survivors from exposures for 2, $2^1/_2$ and 3 minutes: only 1 lethal was found in 1318 *X*-chromosomes. As the period of exposure may have been too short to ensure penetration to the chromosomes of the germ cells, a second series of tests was carried out in such a way that exposures could be prolonged to at least 5 minutes, a time which is amply sufficient for the production of mutations by mustard gas. The flies were exposed to air which had passed through a mixture of chloropicrin and liquid paraffin. By altering the proportion of the two fluids, the tolerance threshold of exposure could be shifted at will. In all tests, the dose was near the threshold of tolerance and lethal to a proportion of the males. Only 2 sex-linked lethals were found in 463 chromosomes from males which had been exposed for periods from 6 to 9 minutes. The results of these two series confirm the findings with lewisite and show that –SH poisoning does not produce mutations in mature sperm.

Frequency of sex-linked lethals in successive broods of males after treatment with

Brood	(*A*) Chloropicrin			(*B*) Mustard Gas		
	No. tested chromosomes	lethals No.	lethals %	No. tested chromosomes	lethals No.	lethals %
1	1226	4	0·3	465	10	3·9
2	1206	1	0·1	458	21	4·6
3	1205	0	0·0	386	36	9·3
4	817	2	0·3	566	31	5·5
Total	4454	7	0·2	1875	98	5·2

It still seemed possible that inhibition of enzymes, especially of phosphokinases, might affect the chromosomes in younger, more actively metabolizing and dividing germ cells. This, in fact, has been suggested as a possible interpretation of the observation that the male germ cells of *Drosophila* exhibit their greatest sensitivity to the genetical effects of mustard gas before they have reached maturity[1]. In order to test this possibility, a third series of tests was carried out, using the same method as in the second. After treatment, the males were given a succession of fresh virgin females every 3–4 days, and lethals were scored separately in successive broods representing germ cells of successibly younger ages at the time of exposure. The Table presents the pooled results of four such tests; exposures were given for periods of 5–7 minutes. For comparison data are shown from a similar experiment with mustard gas.

In all broods mutation frequencies after treatment with chloropicrin are those usually found in untreated laboratory stocks. No lethal at all was obtained in the third brood which, as shown in the right half of the Table, represents germ cells treated during their most sensitive stage to mustard gas. All attempts, therefore, to show a mutagenic action of –SH poisoning have failed. Moreover, if it can be assumed that chloropicrin inactivates phosphokinases *in vivo*, these experiments suggest that phosphokinase inhibition is not concerned in the production of mutations by mustard gas. C. AUERBACH

Institute of Animal Genetics, University of Edinburgh, October 8, 1949.

[1] Z. M. BACQ, Exper. *2*, 1 (1946).

[2] M. DIXON and D. M. NEEDHAM, Nature *158*, 432 (1946). – A. WORMALL et al., Biochem. J. *40*, 734 (1946). – R. A. PETERS, Nature *159*, 149 (1947).

[3] C. AUERBACH and J. M. ROBSON, Proc. Roy. Soc. Edinburgh, *62*, 284 (1947).

[1] C. AUERBACH, Proc. Eighth Int. Congr. Gen. *1948*, 128 (1949).

Zusammenfassung

Männchen von *Drosophila melanogaster* wurden subletalen Dosen von Chloropikrindampf ausgesetzt. Die Behandlung war ohne mutagene Wirkung sowohl auf reife als auch auf unreife Keimzellen.

The Effect of Raphanin on the Colonies of Common Pathogenic Fungi

IVÁNOVICS and HORVÁTH[1] produced in 1947 from the seed of radishes (*Raphanus sativus*), a thermostabile, water-soluble, syrup-like substance which has been termed raphanin. In a dilution of 1 in 20,000 the substance inhibited the growth of a tissue culture made up of rabbits' testicle; its solution of 1 in 1,000 impeded the germination of vegetable seeds. When applied as in HEATLEY's[2] procedure it inhibited, in areas of various diameter, the growth of various gram-positive and gram-negative bacteria. 10 mg administered intravenously kills a mouse.

IVÁNOVICS was of the opinion that the substance might, despite its toxic effect on *per os* administration, be occasionally applied to the skin in cases of dermatomycoses. Therefore, we had to study the effect of raphanin *in vitro*, i.e., on the growth of such fungi as produce diseases in man.

The fungi to be studied were selected so that the main-groups responsible for the most common mycotic diseases were represented (*Trichophyton gypseum asteroides*, *Epidermophyton* KAUFMANN-WOLF, *Cryptococcus hominis*, *Cryptococcus* GILCHRIST, *Achorion* GRUBY-SCHÖNLEIN, *Microsporon* AUDOUIN, *Nocardia bovis*). All the selected individuals were pathogenic strains derived from early clinical manifestations.

The following culture media were employed: malt soup (Wander's malt 4·0 g, peptone 1·0 g, dist. water up to 100 g); malt agar (malt 4·0 g, peptone 1·0 g, agar 1·8 g, dist. water ad 100 g).

The most suitable form of medium seemed the oblique agar in test tubes. Raphanin was mixed with the medium before sterilization, whereby the fungi were exposed to a continuous and stable raphanin action. HEATLEY's original procedure was but rarely applied. The procedure, consisting in placing a glass cylinder in the centre of the medium spread upon a flat plate, may give erroneous results because the solution escaping from the cylinder is unevenly diluted by the medium.

The spores were placed on the medium with a platinum loop. The growth of all colonies inoculated onto malt soup showed the following features: the 1 to 2 weeks old colonies, both those swimming in the liquid and those forming a superficial membrane, gradually decreased, proportionally with the increase of the raphanin concentration. At a certain concentration of raphanin no growth at all took place. On the solid medium, the colonies of the same age were in an inverse proportion to the concentration of raphanin, and there was a raphanin concentration at which the fungi failed to grow (Fig. 1). The raphanin concentration which thoroughly inhibited the growth of the fungi was, by the fungi, 1 in 4,000 to 1 in 100.

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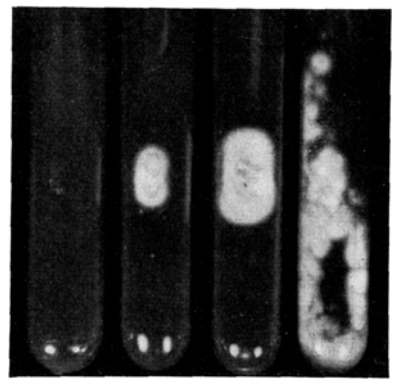
[2] N.G. HEATLEY, Bioch. J. *38*, 61 (1944).

Trichophyton gypseum was cultivated in a plate by HEATLEY's procedure. In the centre there was a strip free of fungi. Close to this strip there was another in which the concentration of raphanin was the greatest. In this area the fungi developed pleiomorphism.

When *Trichophyton gypseum* and *Penicillium glaucum* grow beside each other on a solid medium, the *Trichophyton* displays a greater vitality, which is manifested by its growing into the zone of the other and forming a concavity in the marginal zone of the *Penicillium*, where the growth of the latter was inhibited by the *Trichophyton*. However, such diluted solutions as e.g. 1 in 10,000 of raphanin suffice to change this relationship. The *Trichophyton* loses vitality under the effect of raphanin and becomes incapable of inhibiting the growth of *Penicillium*. On the contrary, the *Penicillium* will enter into the colony of the *Trichophyton*.



1:1,000, 1:2,000, 1:3,000, K.
Fig. 1. – *Epidermophyton* KAUFMANN-WOLF.

In malt-soup microculture, high dilutions of raphanin (1 in 8–10,000) suffice to diminish the reproductiveness of *Trichophyton gypseum asteroides*. If the concentration of raphanin is raised to 1 in 5,000 the inoculated spores remain unchanged. The substance inhibits their growth in diluted solutions to a less degree, in more concentrated solutions thoroughly (Fig. 2). Generally, the thorough inhibition of growth requires more concentrated solutions of raphanin in the case of fungi than in the case of bacteria.

IVÁNOVICS believes that raphanin acts like other antibiotic drugs, i.e., it enters into a reaction with the –SH radical existing in the bacterial body.

We observed that those fungi exhibiting a more rapid development needed, for the thorough inhibition of their growth, more concentrated raphanin solutions than those developing slowly.

FREY[1] claimed that there was a very close correlation between the development of the micro-organisms and the intensity of their oxidation. Probably this correlation is true of the fungi also. I believe further that the converse also is true: the decrease of development may represent an inhibition of oxidation.

All the strains examined in the experiments were, at the same time and with the same method, inoculated separately on oblique malt agar. One week later the tubes were arranged according to the intensity of the growth of the inoculated fungi. In this way an order of the strains was obtained which was identical with the other obtained by arranging the strains according to the order of the raphanin concentration needed for the

[1] W. FREY, Schweiz. med. Wschr. *77*, 23 (1947).