VIROLOGY

Reactivation of Vaccinia Virus Inactivated by Mercury

It is generally recognized that the percentage kill of bacteria by mercuric chloride may be overestimated if residual mercury is not neutralized by the addition of –SH groups. When this is done bacteria are able to multiply after treatment with concentrations of mercury previously regarded as bactericidal.[1] I have recently noted a similar phenomenon with vaccinia virus.

Preparations of vaccinia virus exposed at 37° C. to mercuric chloride in concentrations of 1/10,000–1/40,000 lost all detectable infectivity for chick embryo in 60 min., as estimated by pock counts on the chorioallantoic membrane. The lower concentrations of mercuric chloride were effective only against highly purified virus suspensions. The results of 2 typical experiments with different concentrations of mercuric chloride (Tables 1 and 2)

Table 1.

Sample and Treatment		Infectious units/ml.
1. Virus + 0·05 *M* cysteine: (control)	37° × 60′	$\cdot 8 \times 10^7$
2. Virus + 10^{-4} mercuric chloride (= HgV):	37° × 60′	0
3. HgV + 12·5 moles cysteine/mole mercury:	37° × 30′	$4 \cdot 6 \times 10^2$
4. „ + 10·0 „ „ „ „	„ „	$9 \cdot 3 \times 10^2$
5. „ + 7·5 „ „ „ „	„ „	$8 \cdot 8 \times 10^2$
6. „ + 5·0 „ „ „ „	„ „	$1 \cdot 3 \times 10^2$
7. „ + 2·5 „ „ „ „	„ „	$3 \cdot 5 \times 10^2$

Table 2.

Sample and Treatment	Infectious units/ml.	
1. Virus + buffer:	37° × 60′	$4 \cdot 6 \times 10^7$
2. „ + „ + cysteine:	„ „	$3 \cdot 7 \times 10^7$
3. „ + $10^{-4 \cdot 6}$ mercuric chloride (= HgV)	37° × 60′	0
4. HgV + 125 moles cysteine/mole mercury:	37° × 30′	*c.*10^6
5. HgV + 12·5 moles cysteine/mole mercury:	„ „	$5 \cdot 3 \times 10^4$

show not only that vaccinia virus inactivated by mercuric chloride is reactivated by treatment with a –SH donor, but also that the degree of reactivation achieved is roughly proportional to the concentration of –SH available. This, in turn, is to some extent dependent on the inactivating concentration of mercuric chloride used. Similar results were obtained with sodium thioglycollate as –SH donor. Washing treated virus by centrifugation and resuspension to remove mercuric chloride made no difference to negative tests for infectivity, nor to the reactivation of infectivity. For convenience, therefore, washing before treatment with –SH donors was omitted.

Thiomersalate depresses the yield of vaccinia virus in tissue culture, and inactivates the virus slowly at 37° C. in concentrations of about 1/20,000.[2] Under conditions like those in the experiments with mercuric chloride, I was unable to reverse the inactivation of vaccinia virus induced by thiomersalate or by phenylmercuric borate.

These two substances presumably inactivate by a mechanism different from that of mercuric chloride.

The relative ease with which infectivity is restored suggests that the –SH groups attacked by the mercury are superficially placed in the virus, and may well be groups involved in the mechanism of attachment of virus to host cell.

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[1] Fildes, P., *Brit. J. Exp. Path.*, **21**, 67 (1940).
[2] [illegible], *Virology*, **6**, 775 (1958).

BIOLOGY

Thiol Groups and Morphogensis

We have recently shown[1,2] that β-mercaptoethanol (a strongly reducing —SH-containing substance) markedly inhibits morphogenetic movements (gastrulation, closure of neural plate) in amphibian eggs. The oxidized counterpart of β-mercaptoethanol (dithiodiglycol) produces a thickening of the neural plate; the same type of abnormalities can be obtained with other thiol reagents, iodoacetamide and chloropicrine for example[3]. Another interesting action of β-mercaptoethanol is the complete inhibition of cap formation in anucleate fragments of the alga *Acetabularia*, without appreciable effect on the growth in length of the stalks[4]. It is a surprising fact that β-mercaptoethanol exerts strongly inhibitory effects on morphogenesis in biological systems so different as amphibian eggs and *Acetabularia*; the purpose of this communication is to summarize the results obtained in further experiments made on *Acetabularia*, amphibian eggs and regenerating animals (axolotl tadpoles, planarians).

Acetabularia mediterranea. The inhibitory effects of β-mercaptoethanol (*M*/300) on cap formation in anucleate halves has been confirmed in many experiments. The action of dithiodiglycol (*M*/1,000) was tried on the same system: it was found that this —SS-containing substance, in all experiments, definitely stimulates cap formation in anucleate fragments. Cap formation (that is, morphogenesis) is thus strongly dependent on the —SH ⇌ —SS— equilibrium in anucleate fragments of *Acetabularia*.

Thanks to the kindness of Prof. D. Mazia, we were able to study the action of a derivative of β-mercaptoethanol on *Acetabularia*: this derivative is mercaptoethanol–ethylgluconamide. It has retained the —SH group and the reducing properties of β-mercaptoethanol, but its chemical structure makes it unlikely that it can penetrate inside the cells. This substance, at the *M*/300 concentration, did not prevent cap formation as effectively as β-mercaptoethanol: a number of caps were formed, but all became extremely abnormal later. These observations suggest that reduction of the —SS— groups present on the cell membrane ultimately results in abnormal morphogenesis.

Amphibian embryos. The effects of mercaptoethanol–ethylgluconamide (*M*/100) were studied on late gastrulæ or late neurulæ of the axolotl. In contrast to mercaptoethanol, this substance does not inhibit neural plate formation, and development is at first apparently normal. However, after 5–7 days, the embryos differ in general shape from the controls: in sections, the most conspicuous abnormality to be seen is a dilatation of the lumen of the nervous system. In the forebrain, the eyes retain the palisade appearance of the neural plate; the lenses are completely absent, perhaps as a result of a loss of competence of the ectoderm when it is treated with mercaptoethanol–ethylgluconamide.

Regeneration of the tail of tadpoles. As shown in Fig. 1*a* and *b*, β-mercaptoethanol (*M*/300) completely inhibits regeneration of the tail in axolotl tadpoles; in many experiments, dithiodiglycol (*M*/300) had, on the contrary, a stimulatory effect on tail regeneration, and it was observed, in sections, that the cells forming the regeneration blastema had a higher ribonucleic acid content