depend for their development on stimuli always present in the normal environment. Others, depending on a new or occasional stimulus, do not appear in the next generation unless the stimulus is present". The point of my communication was to show that in certain circumstances the last sentence of this quotation is not necessarily true.

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[1] Waddington, C. H., *Nature*, **169**, 278 (1952).
[2] Goodrich, E. S., "Living Organisms" (Oxford, 1924).

Validity of the *Neurospora* Back-Mutation Test

In a recent communication, Grigg[1] has discussed the possibility of an interconidial inhibition in *Neurospora*, conidia of biochemically deficient mutant strains inhibiting the growth of wild-type conidia. It was demonstrated that large numbers of mutant conidia inhibit the growth of wild-type conidia by mixing varying concentrations of mutant conidia with small aliquots of wild-type conidia and subsequent plating on 'minimal' medium. The mutant conidia will not grow on minimal medium, although they germinate; the wild-type conidia should grow normally. Using, for example, an adenineless mutant (*W*40) of a macroconidial strain, 100 per cent inhibition of wild-type conidia was observed in the presence of 4×10^7 adenineless conidia per plate, whereas no inhibition was found in the presence of 2×10^7 adenineless conidia.

On the basis of this observation, the validity of the *Neurospora* back-mutation test for investigating the mutagenic effect of chemical and physical agents is questioned. This test has been used in our laboratory, and in a number of other laboratories, in recent years for studying the mutagenic effect of various treatments (for references see Grigg[1]). The increased number of wild-type colonies which is observed after treatment with mutagens is explained by Grigg as a "dilution effect": the treatment kills so many spores that the interconidial inhibition is overcome.

Grigg, however, has overlooked the fact that the experimental conditions under which the interconidial inhibition is observed are different from those under which the back-mutation test is carried out. In Grigg's experiments the conidia are plated on a minimal medium containing 0·2 per cent sucrose and 0·4 per cent sorbose. The latter is not used as a source of carbon by the organism. The medium used in the back-mutation experiments contains 1·5-2·0 per cent glucose or glycerol, and no sorbose.

We have repeated the reconstruction experiments by mixing varying concentrations of adenineless conidia (*W*40) with small aliquots of wild-type spores originating from a reverted *W*40 strain. The conidia were plated both on a sorbose medium similar to Grigg's and on our standard medium with 2 per cent glucose. The results of one experiment are shown in the accompanying table. The experiments have consistently shown that the inhibition effect observed by Grigg is observed on the sorbose medium only, whereas no inhibition is found on the glucose medium. We believe this discrepancy is due to the fact that the sorbose medium is a starvation medium,

	Number of colonies					Strain and medium
ad.−	0	$1{\cdot}1 \times 10^7$	$2{\cdot}2 \times 10^7$	$4{\cdot}4 \times 10^7$	$8{\cdot}8 \times 10^7$	
ad.+ colonies	53	55	40	0	0	*W*40, sorbose
	41	41	47	42	43	*W*40, glucose

containing a limiting amount of glucose. When the mutant spores germinate, they may use up all the glucose, and no more carbon is available for the further growth of the wild-type conidia. This limiting factor does not occur in the standard glucose medium. Since the latter medium is used exclusively in the back-mutation experiments, we conclude that Grigg's observation, although interesting in itself, has no relation to the back-mutation experiments, and that his criticism of this test is invalid.

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[1] Grigg, G. W., *Nature*, **169**, 98 (1952).

Fertilizing Capacity of Bull Spermatozoa after Freezing at −79° C.

In 1949, Polge, Smith and Parkes[1] reported that glycerol had remarkable properties in protecting spermatozoa against the effects of low temperatures. The most promising results were obtained with fowl spermatozoa, which, frozen rapidly to − 79° C. (solid carbon dioxide) or − 192° C. (liquid air) in 15 per cent glycerol, resumed full motility on thawing. Later, it was found that fowl spermatozoa so treated were capable of effecting fertilization of the egg[2]. Mammalian spermatozoa have proved more refractory. The spermatozoa of several species—rabbit, guinea-pig and horse[3], bull and goat[4]—can be revived to show some degree of motility after freezing at − 79° C. in 15 per cent glycerol. However, repeated experiments with the rabbit, the only laboratory animal suitable for artificial insemination, have, as yet, resulted in the fertilization of only one egg by spermatozoa so treated[3], and early tests with frozen bull spermatozoa carried out at Shinfield were unpromising, though one calf was reported to have been produced[5].

The object of the present communication is to report the results of two series of experiments carried out on bull spermatozoa at Cambridge. In the autumn of 1950, semen samples were divided into three parts and diluted 1 : 1 at + 30° C. with (1) Wellcome citrate buffer containing 50 per cent egg yolk; (2) and (3) a similar yolk-buffer mixture containing 30 per cent glycerol. Samples (1) and (2) were then stored for 24 hr. in the refrigerator at + 5° C. Sample (3), shortly after dilution, was cooled slowly overnight from + 30° C. to − 79° C., and thawed at + 40° C. the following day. Rates of revival from the frozen semen were poor, and were not much improved by adding the glycerol slowly or by varying the rate of freezing. About twenty cows were used for testing semen of each of the three categories, and those not returning to œstrus within