TABLE 3

THE EFFECT OF IRRADIATION IN HYDROGEN ON CHROMOSOME ABERRATION FREQUENCY IN *Tradescantia* MICROSPORES (400 r at 50 r/min)

Pressure in exposure chamber	No. cells	No. interchanges	Interchanges /cell	Interstitial deletions	I.D./cell
Normal atmospheric (*ca.* 740 mm Hg)	707	149	0.21 ± 0.017	166	0.23 ± 0.018
Three atm above normal	498	100	0.20 ± 0.020	124	0.25 ± 0.022

frequencies of chromosomal interchanges at various pressures in 5% and 10% oxygen have been plotted against the standard curve from Fig. 1, on the assumption that the amount of dissolved oxygen in the microspores is directly proportional to the pressure, and that this value determines the aberration yield at the standard dose of 400 r at 50 r/min. As can be seen from the graph, the agreement with previous results at comparable partial pressures of oxygen is very good. These results thus support earlier views that the amount of dissolved oxygen present in cells is an important factor in determining aberration frequency. They indicate that pressure alone, at least within the range up to 3 atm above normal, does not influence this type of radiation-induced change.

Several different types of evidence (*3*) support the view of Thoday and Read (*4*) that the effect of oxygen in increasing chromosome aberration frequency may result from an indirect action of x-radiation to decompose water with the production of hydrogen peroxide. However, although very little hydrogen peroxide appears to be produced by x-rays in oxygen-free water (*5, 6*), it will be recalled (Table 1) that there is still a substantial aberration frequency in inflorescences exposed in purified helium to 400 r. The question naturally arises as to the mechanism by which these aberrations are produced. It seems quite probable (*3*) that the effect of a substance such as hydrogen peroxide on aberration frequency would arise from an increased production of chromosome breaks, rather than from an influence on the reunion behavior of broken ends. If this is the case, aberrations induced by x-rays in the absence of oxygen might result (at least in part) from breaks produced by another substance, the OH radical, which is formed by the radiodecomposition of oxygen-free water. On the other hand, all the aberrations produced in the absence of oxygen might arise from the *direct* action of the radiation in ionizing the molecules of the chromosomes themselves.

A preliminary attempt has been made to distinguish between these two possibilities by means of experiments designed to remove the OH radical by promoting, during x-irradiation, the back reaction to form water. Allen (*6*) has shown that the presence of molecular hydrogen markedly accelerates this back reaction. Consequently, inflorescences were irradiated in pure hydrogen at normal atmospheric pressure and at 3 atm above normal. These data are presented in Table 3. Although there is some decrease in aberration frequency compared to earlier results obtained with irradiation in helium, the differences for the most reliable observations, those of interchanges, are not significant. In this experiment it was not possible to obtain evidence that hydrogen was actually present in the cells during irradiation. However, previous experiments with oxygen (*2*) have indicated that under similar conditions this gas diffuses into microspores very rapidly. Thus, despite the fact that hydrogen is less soluble in water than is oxygen, it seems reasonable to conclude that a considerable amount of hydrogen would be present in the microspores during irradiation, and that this should react to remove the OH radical. Consequently, the failure to find a significant decrease in aberration frequency when x-radiation is performed in hydrogen may mean that all the residual aberrations induced by x-rays in the absence of oxygen arise as a result of a *direct* effect of the radiation on the chromosomes. It should be pointed out, however, that this conclusion is based on the assumption that reactions leading to H_2O_2 production or suppression in cells from which oxygen has been removed as completely as possible are similar to those occurring in oxygen-free pure water. There is as yet little experimental evidence on this point, and it is quite possible that the complexity of the cellular environment may cause very different reactions to occur.

References

1. GILES, N. H., JR., and RILEY, H. P. *Proc. Nat. Acad. Sci.*, **35**, 640 (1949).
2. *Ibid.*, **36**, 337 (1950).
3. GILES, N. H., JR. Paper presented at Oberlin Symposium on Radiobiology, June 1950.
4. THODAY, J. M., and READ, J. *Nature*, **163**, 133 (1949).
5. BONET-MAURY, P., and LEFORT, M. *Nature*, **162**, 381 (1948).
6. ALLEN, A. O. *J. Phy. Coll. Chem.*, **52**, 479 (1948).

The Detection of Internally-borne Bacterial Pathogens of Beans by a Rapid Phage-Plaque Count Technique[1]

H. Katznelson[2]

Division of Bacteriology and Dairy Research, Science Service, Department of Agriculture, Ottawa, Ontario

The use of specific bacteriophages for identifying bacterial species or strains is a valuable aid in medical and epidemiological work; phage typing of enteric pathogens, staphylococci, and streptococci is extensively employed (*1*). Phage has also been widely used to separate closely related groups, species, and strains of bacteria for purposes of classification (*1, 2*). Within recent years bacterial viruses have been recognized as very useful tools in the rapid identification of plant

[1] Contribution No. 302.

[2] The author gratefully acknowledges the cooperation of M. D. Sutton, Division of Botany and Plant Pathology, in preparing the bean samples and carrying out the pathogenicity tests

pathogenic bacteria (*3, 4*); organisms distinguishable only by pathogenicity may be readily separated by specific phages (*5*).

No satisfactory rapid method is available for detecting internal infection of seed by phytopathogenic bacteria such as *Pseudomonas phaseolicola* and *Xanthomonas phaseoli,* which cause halo and common blight, respectively, of beans. Serological techniques may hold some promise, but plant pathological laboratories are not generally equipped to use them. In the present work typical phages were isolated for these two organisms from a mixture of soil, compost, and sewage and were found to lyse all strains of these species tested but no other species in the same genera. The phage for *X. phaseoli,* for example, did not lyse *X. phaseoli* var. *fuscans* or *X. phaseoli* var. *sojensis.* Such specificity has also been reported by Thornberry *et al.* (*4, 5*) working with *X. pruni* phage. The above phages were used successfully in identifying isolates from suspected bean samples.

If the pathogen were present in a macerated bean sample in any number, and if a known number of phage particles were added to a given volume of this sample, a significant increase in number of phage particles should occur after a suitable interval, if conditions are favorable and contaminating organisms and the bean tissue itself do not interfere. Such conditions will eliminate the need for plating, isolating, and phage-testing. This theory was based on the well-established fact that one susceptible bacterial cell infected with one phage particle may liberate as many as 20–100 or more particles ("burst size"), depending on the bacterial species and the conditions of growth (*6*). Experiments along these lines gave results that supported the above hypothesis.

TABLE 1

PRESENCE OF *Pseudomonas phaseolicola* IN BEANS AS INDICATED BY MULTIPLICATION OF SPECIFIC PHAGE ADDED TO MACERATED TISSUE

Series	Plaque count/0.1 ml	
	When phage added	After 6-hr incubation
1. Nutrient broth	30	35
2. 1. + cells of *P. phaseolicola*	25	Confluent plaques
3. Sterile bean tissue	24	12
4. 3. + cells of *P. phaseolicola*	20	Confluent plaques
5. Bean samples		
RT 1	24	1,200
RT 4	26	Confluent plaques
RT 5	26	" "
RT 8	25	1,150
RT 10	40	1,450
RT 13	22	3,200

The method finally evolved consists of surface-sterilizing 50 bean seeds (with chlorine), washing, and grinding for 5 min in 250 ml sterile nutrient broth in a Waring Blendor. The mixture is retained at room temperature overnight, after which it is shaken and 10 ml of the turbid supernatant removed aseptically into a sterile flask; 1 ml of a suitable dilution of phage containing a known number of virus particles (about 4,500 in most of our experiments) is then added to the flask. Plates are poured immediately with 0.1 ml of the mixture in the flask, 0.6 ml of a nutrient broth suspension of cells from a 24-hr slant culture of the homologous organism ("substrate cells"), and 10 ml yeast-beef agar. Plaques may be counted after 5–8 hr at room temperature. The contents of the flask are allowed to stand at room temperature for 5–6 hr, after which 0.1 ml or smaller amounts of the mixture (obtained by dilution) are again plated and plaques counted after 5–8 hr. Where the specific organisms are present in a sample, the number of plaques increases strikingly (Table 1), frequently producing one confluent lytic area over the entire plate—unless, of course, suitable dilutions are made. Plaques show up despite rapid growth of saprophytic bacteria, which frequently are present in the sample; these may be suppressed to some extent if necessary by increasing the volume of the cell suspension used when plates are poured or by means of a suitable antibiotic. The number of phage particles added to the original flask is arbitrary; a sufficient number should be used to permit ready contact with the susceptible cells in the sample, allowing for some nonspecific adsorption by constituents of the ground beans, but not so many as would necessitate time-consuming dilutions of the sample prior to plating. In the above procedure 0.1 ml of the phage-bean mixture gives about 20–40 plaques per plate at zero time and usually from 20–100 times that number after 5–6 hr if the host cells are present.

By this method 11 out of 14 samples of beans were shown to be infected with *P. phaseolicola.* The positive and negative samples were also plated out, colonies on representative sections of suitable plates picked, and the resulting cultures tested for phage lysis and for pathogenicity. To date only the isolates from positive samples, which were susceptible to lysis, were found to be pathogenic to bean seedlings.

The size of sample was found to be of great importance in these tests. This is of course conditioned by the degree of infection in the field: in a heavily infected sample 50 seeds are sufficient; in a lightly infected sample 200 or more may be required to give a positive result. Where 200 were required they were divided into 4 lots of 50 seeds each in 250 ml of broth before grinding.

Specificity of the virus is obviously of prime importance in this procedure, nor can the possible occurrence and development of resistant strains in the sample during the preliminary incubation be dismissed. However, the isolation of phage-susceptible pathogenic cultures from samples giving a positive reaction by the plaque-count technique and their absence in negative samples indicate that for this species at least, resistant strains are not a serious obstacle. The possible presence of a specific phage in the sample has also been considered, and in fact phage has been encountered several times. By pouring plates containing susceptible "substrate" cells immediately after grinding the beans and after the preliminary overnight incubation, such phages can be detected. If they have increased in amount significantly overnight they themselves may serve as indicators of in-

fection of the beans, provided their specificity is established.

Preliminary tests with beans infected with *X. phaseoli* have indicated that this method holds promise in the diagnosis of common blight as well. This procedure may be applicable to the rapid detection of both plant and animal pathogens or saprophytic organisms in various complex substrates or in simple mixtures of closely related forms.

Details of the experiments discussed above will be published elsewhere.

References

1. CRAIGIE, J. *Bact. Rev.*, **10**, 73 (1946).
2. SMITH, N. R., GORDON, R. E., and CLARK, F. E. *USDA Misc. Pub. 559*, 1946.
3. THOMAS, R. C. *Ohio Agr. Expt. Sta. Tech. Bull. 11*, 1947.
4. THORNBERRY, H. H., BRAUN, A. C., and ELROD, R. P. *Phytopathology*, **39**, 152 (1949).
5. THORNBERRY, H. H., EISENSTARK, A., and ANDERSON, H. W. *Phytopathology*, **38**, 907 (1948).
6. DELBRÜCK, M. *Adv. Enzymol.*, **2**, 1 (1942).

The Potentiation of Muscular Contraction by the Nitrate-Ion[1]

Arthur J. Kahn and Alexander Sandow

Department of Biology,
Washington Square College of Arts and Science,
New York University, New York City

It has long been known that frog skeletal muscles that have been exposed to various anions of the lyotropic series yield potentiated contractions when stimulated by such agents as cold, potassium, or electric shock (*1–5*). There is general agreement in this earlier work that the ions cause a "sensitization" of the excitatory membranes of the muscle fibers, which is then effective in increasing the mechanical response of the whole muscle. Thus, in work most directly pertinent to our own research, Chao (*4*) studied the behavior of frog sartorii exposed to a Ringer's solution normal with respect to potassium and calcium chlorides, but having all the NaCl replaced by an equivalent amount, e.g., of $NaNO_3$. Such muscles were found to quickly develop a reduction in rheobase for electric stimulation; and, furthermore, if they were stimulated with twitch shocks of a *submaximal* intensity, as determined for these muscles when previously equilibrated to ordinary Ringer's solution, their isotonic contractions became greatly augmented after only a few minutes' contact with the nitrate medium. Chao attributed the mechanical potentiation to recruitment of fibers; i.e., the lowering in threshold of the fibers of the nitrate-treated muscle permitted response in those fibers that were not excited by the submaximal shock in normal Ringer's solution, and thus, by adding their outputs to the total twitch response, caused the observed potentiated contractions. In this investigation the effects of other anions were similarly tested, and it was found that the degree of lowering of the rheobase and of the associated enhancement of shortening by submaximal stimulation was a function of the particular anion, the relative effects falling into the usual lyotropic series, $Cl < Br < NO_3 < I < CNS$.

In our research, which is reported here in preliminary form, we have used a procedure rather similar to Chao's, with the important exception that stimulation was effected by originally slightly *supermaximal* shocks. Under these conditions, irrespective of the lowered threshold of the NO_3-treated muscle, no recruitment of fibers is possible; yet such muscles produce greatly potentiated twitches approaching, or even equalling, the strength characteristic of full tetanus output. Full analysis of our results proves that the nitrate ion not only results in an increase in excitability but also causes, among other things to be discussed below, augmentation of the maximal mechanical response of which each fiber is capable.

We have studied the isometric twitch responses of excised curarized[2] frog sartorii when supermaximally shocked by the massive, transverse stimulation procedure (*6*). Records have been made by piezoelectric, cathode-ray methods (*7*) of the latent period changes and, using optical myography, of the associated developed peak tension outputs. All experiments have been done at 25°C. After 1 hr of equilibration in oxygenated phosphate buffered (pH 7.2) normal Ringer's solution, the behavior of the muscle was recorded for 2 responses separated by an interval of 1 min. This chloride-Ringer's solution was then withdrawn from the muscle chamber and replaced by a similarly buffered nitrate-Ringer's solution, through which oxygen was bubbled. In our test medium *all* the chloride was replaced by nitrate, and it is in this sense that we shall refer to our experimental medium as a nitrate-Ringer's solution. In this new environment the muscle was tested at intervals, the stimuli being again massive, transverse electric shocks of the same supermaximal strength as those applied to the muscle in normal Ringer's.

The results of a representative experiment are presented in Fig. 1. The inset at the top of this figure is a diagrammatic sketch of a typical photographic record obtained with our apparatus. The curved line, corresponding to the deflection of the cathode-ray beam in a single sweep, gives the mechanical changes that appear during the twitch latent period. The arrow at the left indicates the instant of stimulation. Following this moment 2 time intervals are presented: L_R, which measures the duration of a mechanically quiescent period —i.e., the latency of the immediately following latency relaxation (LR); and L, which measures the time interval of the mechanical latent period for positive tension development. After the termination of L, the record shows in the upward deflection the very beginning of the contraction period. Also on this sketch are indicated 2 tension variables: R, measuring the depth of the LR;

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and the New York University (NR113-300).

[2] We are very grateful to E. R. Squibb & Sons for generously supplying us with the *d*-tubocurarine chloride used in these experiments.