higher dose to obtain the same lethal effect than the normal bacteria.

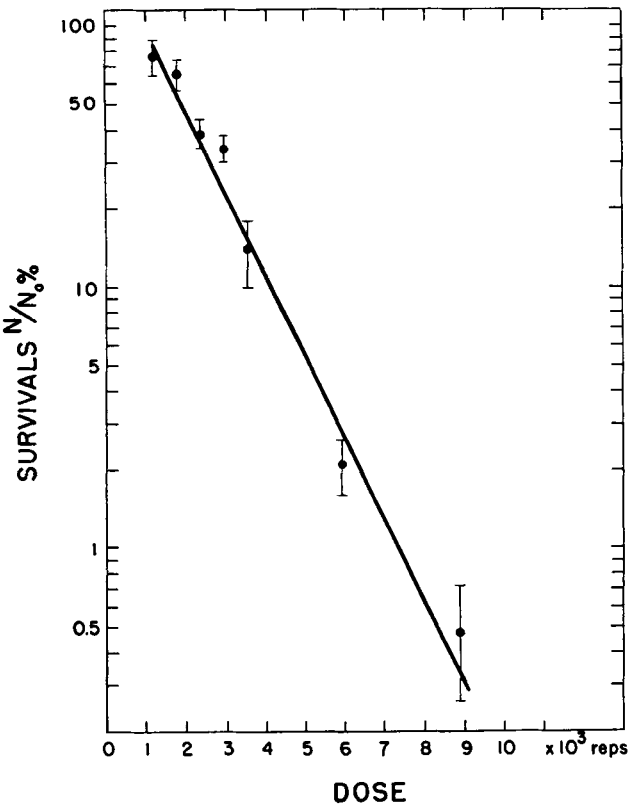

Fig. 3.—Survival curve of bacteria irradiated with 2 Mev cathode-rays.

The results obtained with cathode ray irradiation (88 reps/10^{-8} amp per s at 44 cm window-sample distance, 0·037 cm Al scatterer) of normal bacteria are given in Figure 3. Irradiation of the bacteria in the dose range between 0 and 500 reps did not give reproducible results and therefore left some doubts whether the killing effect in these experiments has been exponential as it might be suspected by the stright form of the rest of the curve.

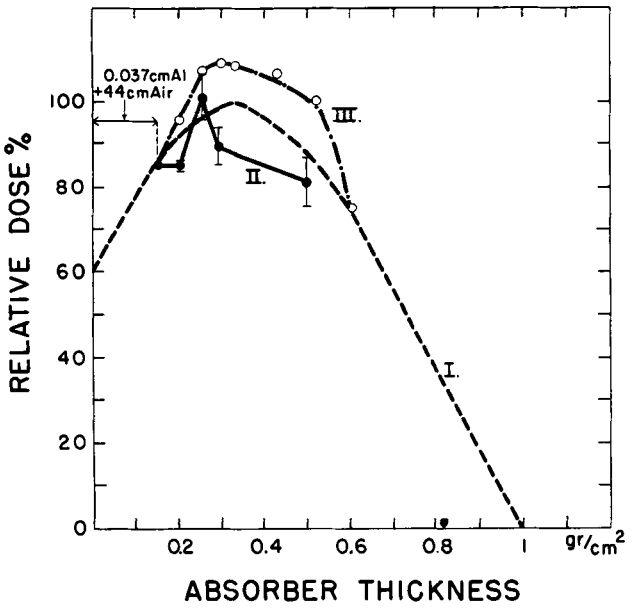

Fig. 4.—Absorption curves of 2 Mev cathode-rays measured with: I. Ionization chamber, II. Bacteria, III. Photographical method.

Application of bacteria as a biological dose indicator was also investigated in form of absorption measurements of cathode rays. This was performed by placing different absorbing layers of aluminium foils on top of the petri dishes. Figure 4, curve II was the result of these experiments and is compared with the results of physical measurements, curve I[1]. The amount of X-rays produced by the scatterer and absorbing Al foils was neglegible. The dose causing the smallest amount of survivals (N/No) in the absorption measurements was arbitrarely adopted as 100% relative dose value. For every number of survivals obtained at different absorption depths the proper dose value could be evaluated from Figure 3, and transformed in reference to the 100% value. The curve indicates a definite dose maximum at the depth of 0·251 g/cm² and follows approximately the physically measured absorption curve, which has a peak at about 0·3 to 0·33 g/cm². With essentially the same conditions, an additional check with photographic films (Kodak, Technical X-ray film, Type A) was performed, curve III. In these experiments the relative dose was calculated in the same manner as with the bacteria taking the highest film density obtained in the absorption measurements as the 100% reference point and using a film calibration for the conversion of density in relative dose. The location of the greatest relative dose and the form of the curve can be considered the same as curve I. The somewhat increased value of relative dose may be due to overlapping exposure caused by scattered radiation.

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The help and advice of Dr. J. G. TRUMP and K. A. WRIGHT is gratefully acknowledged.

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Zusammenfassung

Der Effekt von 2-Mev-Röntgen- und -Kathodenstrahlen auf Bakterien wurde untersucht. Die eventuelle Verwendung von Mikroorganismen für Dosismessungen wurde in Betracht gezogen. Die physikalisch bestimmte Absorptionskurve der 2-Mev-Kathodenstrahlen konnte trotz der mit biologischen Objekten zu erwartenden Ungenauigkeit relativ gut dupliziert werden. Als Strahlenquelle diente ein druckisolierter Van-de-Graaff-Generator.

[1] J. G. TRUMP *et al.*, J. appl. Phys. *21*, 345 (1950).

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Discrimination entre l'inactivation de la virulence et du développement bactériens par le rayonnement ultra-violet de 2537 Å

(Etude sur *Bacillus cereus alesti* Tou. et V.).

Si les recherches sur la photosensibilité des microorganismes sont nombreuses, les études comparatives de cette action sur les différentes propriétés physiologiques des microbes sont encore peu connues.

Ainsi, la discrimination entre la virulence et la faculté de développement d'un germe, en rapport avec sa photosensibilité, restait à entreprendre sur un nombre important d'éléments permettant une interprétation statistique satisfaisante.

Dans ce but, nous avons choisi un germe peu photosensible qui dans nos essais n'a été détruit qu'après avoir été soumis a une dose globale de 0,828 W d'énergie ultraviolette par cm². Il s'agit du *Bacillus cereus alesti* Tou. et V. dont la virulence se mesure aisément sur la chenille de *Bombyx mori* (Lepidoptera); ceci nous a permis à l'inverse des travaux sur les petits mammifères de laboratoire, d'expérimenter sur un grand nombre d'individus de souche commune et d'une homogénéité parfaite correspondant à notre but (7500).

Méthodes et techniques utilisées:

Nous avons employé des lampes à vapeur de mercure à basse pression et à basse tension, de 25 W (type germicide) émettant sur plusieurs longueurs d'onde, notemment 1850, 2537 et 3129 Å avec une prédominance de la raie 2537 Å.

Les préparations bactériologiques placées à une distance de 5 cm de l'axe des lampes et parallèlement à celles-ci étaient irradiées avec une densité d'énergie de 2300 W par cm²/min. Celles-ci étaient préparées de la manière suivante:

Ensemencement homogène de plaques de gélose nutritive avec *Bacillus cereus alesti*, incubation pendant 48 h à 30°C jusqu'à formation complète de spores.

Lavage des cultures avec de l'eau stérile.

Mise de lames de verre de dimensions égales dans la suspension bactérienne et dessèchement lent de celle-ci.

Irradiation des lames sèches aux rayons ultra-violets pendant: 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min.

Lavage des lames irradiées dans des quantités égales d'eau stérile.

Ensemencement de 0,1 cm³ de chaque suspension pour numération de germes.

Contamination de feuilles de mûrier de surfaces égales avec 1 cm³ de chaque suspension.

Etablissement de courbes de mortalité des chenilles de *Bombyx mori* L. nourries avec ces feuilles.

Résultats. Le contrôle du développement des spores irradiées (moyenne de 10 examens de chaque cas) donne les chiffres groupés dans le tableau ci-dessous.

Temps d'irradiation en minutes	Energie ultra-violette reçue par cm² en mW	Nombre de colonies dans l'unité
60	138	67
90	207	64
120	276	65
150	345	60
180	414	61
210	483	66
240	552	59
270	621	67
300	690	67
330	759	64
360	828	65
390	897	44
420	996	14
450	1035	3
Témoins (souche non irradiée)		64

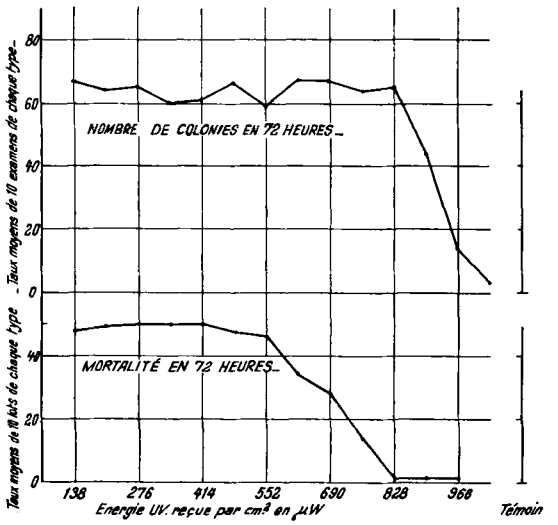
On constate qu'il n'y a pas de variation significative de la faculté de développement des spores pour des doses d'irradiation inférieures à «828 mW/cm²». Ensuite une diminution brusque de cette faculté après 6 h d'irradiation.

La virulence des spores irradiées s'exprime dans les moyennes des mortalités de vers dans 10 lots de 50 individus infectés pour chaque temps d'irradiation:

Temps d'irradiation en minutes	Mortalité des chenilles après				Mort par d'autres maladies
	6 h	24 h	48 h	72 h	
60	32	39	45	48	2
90	34	42	46	49	1
120	41	46	47	50	
150	40	46	46	50	
180	40	48	49	50	
210	34	43	47	47	
240	28	39	45	46	1
270	15	19	30	34	
300	11	16	28	28	
330	2	5	14	14	
360	0	0	0	1	
390	1	1	1	1	1
420	1	1	1	1	2
450	0	0	0	0	0
Témoins (souche non irradiée)	35	45	49	49	1

Une diminution progressive et très notable de la virulence des spores se manifeste après 4 h d'irradiation; la virulence de *Bacillus cereus alesti* est détruite après 6 h d'irradiation.

Conclusion. L'examen statistique basé sur 300 individus montre que la faculté de développement et la virulence des spores de *Bacillus cereus alesti* répondent d'une façon parallèle au rayonnement ultra-violet de 2537 Å, mais l'inhibition du développement est retardée par rapport à celle exercée sur la virulence.



Les rapports numériques entre les deux effets sont exprimés dans le graphique. On doit souligner l'inflexion qui se produit après 4 h d'irradiation, temps nécessaire pour la perte de virulence mais où les individus conservent intacte leur faculté de développement, celle-ci n'étant altérée qu'après 6 h d'irradiation.

Nous exprimons notre vive gratitude à Monsieur R. C. Geslin pour l'aide qu'il a apporté à nos travaux.

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Summary

A statistical study founded on 300 elements (*Bacillus cereus alesti*, with a test: *Bombyx mori* larvae. Lepidoptera). Capacity for development and virulence respond in a parallel manner to ultraviolet radiation 2537 A; but the inhibition of virulence is more rapid than that of development. Irradiation for 4 to 5 hours gives avirulent elements which are, however, capable of development.

Studies on a Non-Heritable Physiological Modification in *Aerobacter aerogenes*

Heritable and non-heritable modifications arise in the ability of the bacterium *Aerobacter aerogenes* to grow in media containing *slow-adaptive substrates* as sole carbon sources[1]. These adaptive substrates include certain Krebs cycle compounds, such as citrate, alpha-ketoglutarate, succinate, fumarate, and acetate. This communication deals with some studies made on the induction of the non-heritable changes. In these studies populations were grown in a synthetic medium containing inorganic salts[2] plus d-glucose or an equivalent concentration (on a carbon-for-carbon basis) of one of the adaptive substrates. The number of cells capable of growing on a specific carbon source was determined by plating a suitable dilution of the culture in appropriate media containing 2% by weight of washed agar[3]. Populations to be irradiated were diluted in physiological saline solution to a density of about 500 cells per ml. From 4 to 8 ml of the diluted culture were irradiated with constant stirring in open beakers by a low pressure mercury arc (steri-lamp), about 90% of whose energy output is at a wavelength of 254 mμ.

The following observations can be made by plating cells from a liquid glucose culture in two different agar media, one containing glucose and the other citrate: (1) if the plated cells are in their logarithmic phase of growth or if their growth has been stopped due to glucose-exhaustion of the liquid culture, then the number of colonies formed in glucose and citrate agar is the same; (2) if the growth of the plated cells has been stopped due to the production of low pH in an unaerated culture containing an excess of glucose, then the number of colonies formed in glucose agar is greater than that formed in citrate agar, and the magnitude of the discrepancy between the two colony counts is a function of the length of time the cells are exposed to the culture conditions existing in the stationary phase (see Fig. 1); (3) if logarithmic phase cells are irradiated with ultraviolet prior to plating, then a similar difference in colony counts is found, which, however, depends upon the dose of ultraviolet employed (see Fig. 2). From these observations it is clear that specific culture conditions and ultraviolet irradiation can both induce heterogeneity in populations of *Aerobacter aerogenes* with respect to the ability to grow in media containing citrate as sole carbon source. In the case of stationary phase cultures, it has been found that the induction of heterogeneity is specific for the class of slow-adaptive substrates[4]. In the case of irradiated cultures the ability to grow in succinate, as well as citrate media is known to be affected (Fig. 2).

[1] A. W. RAVIN, J. Gen. Microbiol. (in press); (Ph. D. thesis, Columbia University, 1951).

[2] A. W. RAVIN, J. Gen. Microbiol. (in press).

[3] F. J. RYAN, Methods Med. Research *3*, 51 (1950).

[4] A. W. RAVIN (Ph. D. thesis, Columbia University, 1951).

The cells that have thus lost their ability to grow in media containing slow-adaptive substrates as sole carbon sources do not inherit their physiological alteration. This is in contradistinction to the genetic modification that is observed in certain types of noncitrate-utilizing mutants that appear spontaneously at a low frequency in glucose cultures of *Aerobacter aerogenes*[1]. Presumptive evidence of the non-heritable nature of the modification was obtained in tests of samples of glucose agar colonies derived from heterogeneous cultures, stationary phase or irradiated. Although the number of colonies tested was always far in excess of that necessary to include the colonies developing from originally non-citrate-utilizing (or non-succinate-utilizing) cells, every colony tested was capable of growth in media containing citrate (or succinate) as the sole carbon source. Proof was furnished by experiments in which heterogeneous stationary phase populations were inoculated into fresh glucose medium. It was observed that such populations become homogeneous again in such a brief period of time that selection of the unaffected cells cannot be invoked as the homogeneity restoring mechanism. For example, Table I furnishes data from an experiment in which a heterogeneous population was washed and aliquots were then inoculated into unaerated media, which either contained glucose or lacked any carbon source. The population became completely homogeneous after having been allowed to grow four hours in the presence of glucose. Since *Aerobacter aerogenes* has a generation time of 43 minutes in unaerated glucose medium[1] and, therefore, can increase at most fourfold in 1½ hours and about 32-fold in 4 hours, then the observed increases in the citrate- and succinate-utilizing components of the population can only be explained by some process wherein non-citrate-(or non-succinate-)utilizing cells are reconverted into utilizers. The appropriate calculations based on this experiment show that the probability of such a reconversion is from 0·53 to 0·86 within 1½ hours, depending on whether an exogenous carbon source is present. The presence of a carbon source apparently speeds and allows the completion of the process. Other experiments performed with an auxotrophic (prolineless) mutant indicate that the restoration of homogeneity is correlated with the active metabolism of the initially heterogeneous population, since the absence of the required nutrient (proline) in the medium acted to considerably reduce the rate of the restorative process.

Table I. Restoration of Homogeneity in a Heterogeneous Culture

Aliquots from a heterogeneous stationary phase culture were inoculated into media under the following conditions, and the restoration of homogeneity to the populations was followed by platings in glucose (G), citrate (C), and succinate (S) agars. Values shown (except for ratios) are expressed in cells $\times 10^5$ per ml. The ratios indicate the degree of heterogeneity in the culture at the time of sampling; ratios greater than 1·5 are significant.

Medium	Time (hours)	G	C	S	G:C	G:S
Salts minus glucose	0	51·1	9·7	0·13	5·3	389·7
	1½	84·0	52·0	–	1·6	–
	4	75·0	39·0	9·0	1·9	8·3
Salts plus glucose	0	51·1	9·7	0·13	5·3	389·7
	1½	88·0	60·0	–	1·5	–
	4	160·0	180·0	173·0	0·9	0·9

[1] A. W. RAVIN, J. Gen. Microbiol. (in press).