sank die Konzentration von Pyruvat und «Acetaldehyd». Man kann daraus folgern, dass das Acetylmethylkarbinol bei diesen Aktinomyzeten aus Pyruvat und Acetaldehyd synthetisiert wird, ähnlich wie wir bei *S. erythreus* (auch aus freiem Acetaldehyd allein) *in vitro* festgestellt haben[1].

Im Falle von *S. rimosus* und *S. coelicolor* liess sich die Acetylmethylkarbinolbiosynthese ausser durch den Zusatz von Arsenit auch durch die Wegnahme von Ca-Karbonat aus dem Fermentationsmedium induzieren. Die dadurch beeinflusste Herabsetzung des pH-Wertes wurde von einer Erhöhung der Acetylmethylkarbinolproduktion begleitet. Eine ähnliche wesentliche Erhöhung der Acetylmethylkarbinolsynthese, jedoch ohne Sinken des pH-Wertes, wurde im Falle von *S. aureofaciens* durch einen Zusatz von 0,03 bis 0,3% sekundären Kaliumphosphates zum Kulturmedium erreicht (Tabelle). Dieses Resultat bietet eine biochemische Erklärung für den schon früher bekannten[3] negativen Einfluss von Phosphat auf die Biosynthese von Chlortetrazyklin.

Unsere Resultate zeigen, dass die Fähigkeit, das Acetylmethylkarbinol zu produzieren, bei den Aktinomyzeten offensichtlich beträchtlich verbreitet ist. Die Acetylmethylkarbinolbiosynthese lässt sich dabei durch entsprechende Kulturbedingungen induzieren oder mehrfach erhöhen. Aus diesen Befunden ergibt sich vielleicht die Möglichkeit, dass, ähnlich wie bei den Bakterien, auch bei den Aktinomyzeten ihre Fähigkeit zur Acetylmethylkarbinolbildung zur systematischen Kennzeichnung benutzt werden könnte. Die Erhöhung der Acetylmethylkarbinolbiosynthese durch Arsenit ist sehr wahrscheinlich die Folge der Störung der oxydativen Dekarboxylierung von Pyruvat zu Acetat, die durch eine adaptive Intensivierung der anoxydativen Umwandlung von Pyruvat unter anderem auch zu Acetylmethylkarbinol ersetzt wird.

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Summary

The production of acetylmethylcarbinol by various strains of actinomycetes was established. The biosynthesis of acetylmethylcarbinol depends largerly on the composition of the fermentation medium and on the presence of arsenite, which regulates the metabolism of pyruvic acid. In the case of *Streptomyces aureofaciens* the stimulating effect of phosphate on the production of acetylmethylcarbinol gives a partial explanation of the well-known negative effect of phosphate on the biosynthesis of chlortetracycline.

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A Study of the Temperature Dependence of Radiation Sensitivity of Dry Spores of *Bacillus Megaterium* Between 5° K and 309° K[1]

The temperature dependence of effects of X-rays on biological systems has been the subject of several recent investigations[2]; however, none of these studies included temperatures below 77° K. In view of the theoretical importance of the temperature dependence relationship, the temperature range was extended in this study to 5° K to provide a complete description of it. A brief report has appeared previously[3].

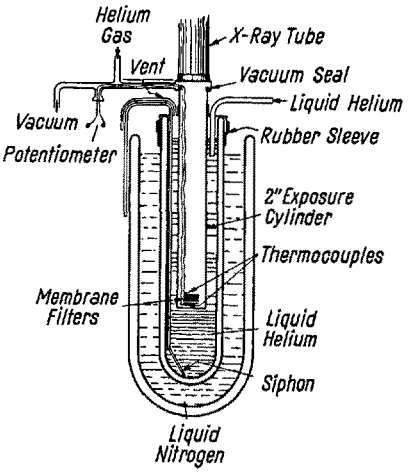

Fig. 1.—Diagram of the apparatus for controlling temperature during irradiation.

Spores from two nonlysogenic strains of *Bacillus megaterium* (No. 337, Welshimer and ATCC No. 8245) were mounted in known concentrations on membrane filters and dried *in vacuo* according to the method of POWERS *et al.*[4]. The X-ray source was a Machlett OEG 60 tube, with a tungsten target and a beryllium window, operated at 50 kvp and 45 ma without added filtration (HVL 0·070 mm Al). The irradiation chamber was a gas tight stainless steel cylinder, 5 cm by 41 cm, attached directly to the X-ray tube (Fig. 1). The membranes carrying the spores on their surfaces were irradiated in stacks of five at a target distance of 45 cm at a dose rate of 2800 r/min (measured in air with a windowless ionization chamber) to the first filter (2400 r/min to the fifth filter). Anaerobic conditions were achieved in the exposure chamber by evacuating to 1 mm Hg pressure and flushing with purified helium twice before sealing the chamber at one atmosphere of He at 25° C. The temperature of the spores was fixed by immersing the exposure chamber in appropriate liquid and slush baths. The double Dewar apparatus illustrated in Figure 1 was designed to meet the requirements of liquid helium, but was applicable to the other baths. The temperature of the membrane filters was monitored during all irradiations with two thermocouples in contact with the spore-bearing filters. The temperatures at which experimental points were obtained were observed to be as follows: 309° K (water bath); 299° K (water bath); 274° K (ice water); 253° K (NaCl-saturated ice water slush); 196° K (dry ice-acetone); 152° K (95% ethanol slush); 79° K (liquid nitrogen); 64° K (nitrogen slush); 21° K (liquid hydrogen); 6° K (liquid helium); and 5° K (liquid helium).

[1] This work was performed under the auspices of the U. S. Atomic Energy Commission. – The assistance and advice of Dr. DARRELL W. OSBORNE, Chemistry Division, Argonne National Laboratory, and his colleagues in producing and handling liquid He and liquid H are gratefully acknowledged.

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[3] R. B. WEBB, C. F. EHRET, and E. L. POWERS, Radiation Res. *1*, 459 (1957).

[4] E. L. POWERS, C. F. EHRET, and A. BANNON, Appl. Microbiol. *5*, 61 (1957).

The membrane filters were returned rapidly to about 25°C following the completion of the X-ray exposure and were maintained at this temperature until plated 1–8 h later on a buffered peptone glucose medium (double strength Difco M.R.-V.P.). Colony counts were made after 16–18 h incubation at 35°C.

The biological effect measured was loss by the irradiated spores of colony-forming capacity. Using direct spore counts as the comparative measure, we ascertained that control (zero dose) preparations of spores demonstrated statistical 100% colony-forming capacity (= survival) at all temperatures. This observation of survival of the spores at the very low temperatures at prolonged times is of interest in itself, and is similar to the one other observation of this kind known to us in which spores of bacteria and molds were exposed *in vacuo* to the temperatures of liquid hydrogen and liquid helium without loss of germinating power[5].

The dose-survival curves at all temperatures are sigmoid[4] with a small shoulder at high survival values. Of a number of possible ways of describing them, we have chosen

$$N/N_0 = ne^{-kD} \qquad (1)$$

in which D is radiation dosage and k is the slope of the exponential portion of the inactivation response curve. In this case n is the y-axis intercept of the extrapolated exponential curve. Use of this function, which is convenient because of the ease of estimating values of k and n, yields values of k and n very close to those given by the preferred and more complete description

$$N/N_0 = 1 - (1 - e^{-kD})^n. \qquad (2)$$

In these experiments no survival values above 50% were used in evaluating k and n and, since the shoulder exists only at values higher than these, the constants in equation (1) are very close to those of equation (2).

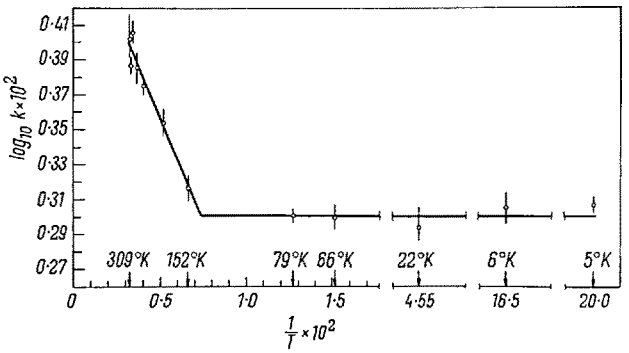

Fig. 2.—Relationship between the logarithm of the X-ray inactivation constant (k) and the reciprocal of the temperature during irradiation for dry spores of *B. megaterium*.

In Figure 2, the log of k, the slopes of the response curves, is plotted against the reciprocal of the temperature at which the curve was obtained. It is obvious that the slope is temperature dependent at higher temperatures, and temperature–independent at lower temperatures. The log of the slope is proportional to the reciprocal of the temperature ($1/T$) from 309°K down to 152°K. If we assume the meaningful applicability of an Arrhenius plot of these data, then the straight-line portion indicates an activation energy of 110 cal. (If the temperature independent background is subtracted the activation energy becomes about 1060 cal.) While this is a small value, it seems to be definite and real, since, as shown in the Figure, the errors about the individual slopes are small in relation to the total change.

From 79°K down to liquid helium temperatures it is equally obvious that the Arrhenius component is negligible. Complete temperature independence seems to be indicated since the inactivation constants observed at 79°K and below are well within one standard error.

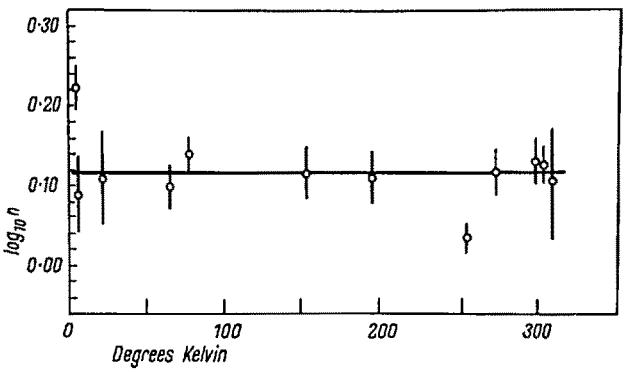

Fig. 3.—The relationship between the value of log n in equation (1) and the temperature at the time of X-irradiation of spores of *Bacillus megaterium* exposed in one atmosphere of Helium at 2800 r/min. The values and their errors were estimated from the response curve in the form: $\log(N/N_0) = \log n - 2{\cdot}303\ kD$.

The temperature at which the two sets of points appear to intersect is about 130°K. However, no measurements were made in this region, and a sharp change from a temperature independent condition to a temperature dependent condition is not required by the data—the change may occur gradually over a range of temperatures.

The other constant in the equation used in the numerical analysis of these results is invariant with temperature. Figure 3 presents the value of n of equation (1) as related to temperature, our interpretation of the relationship being that n is varying randomly about a mean value of 1·30 as the temperature at the time of irradiation changed.

These experiments show for the first time in a biological system the existence of a transition region in the temperature dependency of radiation inactivation at about 130°K; this is characterized by temperature dependence above the transition region, and temperature independence below it. It is probably significant that these relationships are consistent in two respects with observations made previously in another biological system in this laboratory by BACHOFER *et al.*[6]. First, the relationship of the X-ray inactivation slopes to temperature for the dry bacteriophage T 1 is such that there may be a temperature-dependent region and a temperature-independent region with the transition occurring at approximately 130°K. Second, recalculation of the activation energy for the dry virus (assuming the 130°K transition point) yields a value of about 200 cal, a value in the order of the 110 cal reported in this paper for the dry bacterial spore. Observations on the temperature dependence of dry spores of *B. subtilis* reported by HOUTERMANS[7] may also be consistent with those of this paper. The phenomena, then, probably occur in more than one kind of spore, and the

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The constants n and k and their standard errors of the expression $N/N_0 = n\,e^{-kD}$ at indicated temperatures

T (°Kelvin)	$k\left(=\frac{1}{\text{kiloroentgens}}\right)$ ± standard error	Intercept (n)	log n ± standard error
5	0·0203 ± 0·0002	1·67	0·223 ± 0·024
6	0·0202 ± 0·0004	1·21	0·083 ± 0·058
22	0·0197 ± 0·0006	1·29	0·111 ± 0·062
66	0·0199 ± 0·0004	1·25	0·097 ± 0·029
79	0·0200 ± 0·0002	1·38	0·140 ± 0·022
152	0·0208 ± 0·0004	1·30	0·114 ± 0·034
195	0·0226 ± 0·0004	1·26	0·100 ± 0·035
253	0·0237 ± 0·0003	1·08	0·034 ± 0·025
274	0·0243 ± 0·0005	1·31	0·117 ± 0·031
299	0·0255 ± 0·0004	1·35	0·130 ± 0·029
304	0·0244 ± 0·0003	1·34	0·127 ± 0·023
309	0·0252 ± 0·0009	1·27	0·104 ± 0·073

virus data indicate that they are probably not peculiar to spores.

Because of the unusually inert properties of this biological system, the thermal conditions imposed on it during irradiation must influence the initial (or, at least, the very early) events in photon-matter interaction that lead eventually to loss of colony-forming ability. It is logical, then, to search for parallels in studies of temperature effects on radiation damage in nonliving systems. GHORMLEY and STEWART[8] reported that the rate of disappearance of H_2O_2 in H_2-saturated ice induced by γ-radiation may be independent of temperature below about −175°C, and dependent upon temperature above this. Not only are these results similiar in type, but the temperature at which the change occurs is close to the approximate −145°C temperature for the beginning of temperature dependence seen in these studies. Several investigators have reported stabilization of free radicals in solid systems at very low temperatures[9], and other have noted that the depolymerization of plastic materials by X-rays shows a temperature dependence over the short range investigated of a magnitude like that reported here[10].

Unfortunately these resemblances in response patterns between living and nonliving systems do not allow for the singling out of any one of the several possible general causes of thermal dependency that might have been enumerated *a priori* for either the physical or the biological results. These formally include effects arising from thermally dependent variations (1) in probability of primary absorption, (2) in probability of 'reversal' of damage by 'repair', and (3) in probability of induction of damage either (A) by energy transfer towards or away from sensitive sites, (B) by thermal supplementation of energies at threshold values for inactivation, or (C) by thermal influence over the formation of unusual electrostatic conditions as postulated by PLATZMAN and FRANCK[11]. While we cannot at this time choose with confidence one or a combination of causes, the fact that there is a definite and distinct change in the biological response in the neighborhood of 130°K is probably an important clue that will make the identification of the physical processes easier.

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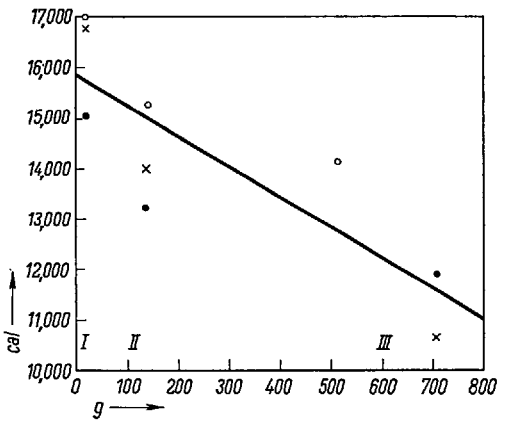
Division of Biological and Medical Research, Argonne National Laboratory, Lemont (Illinois), May 28, 1958.

Zusammenfassung

Die Temperaturabhängigkeit der Röntgenstrahlenempfindlichkeit trockener Sporen von *Bacillus megaterium* wurde über einen Temperaturbereich von 5° K bis 309° K gemessen. Die Empfindlichkeit ist temperaturunabhängig unterhalb 130° K; sie entspricht der van-t'Hoff-Arrhenius-Regel zwischen 130° K und 310° K bei einer Aktivierungsenergie von 0,110 kcal.

Zur Abhängigkeit der Aktivierungsenergie der Gewebsatmung bei Säugetieren von der Körpergrösse

In der letzten Zeit wurde die Beobachtung gemacht, dass nicht nur die Temperaturaktivierung der Gesamtatmung poikilothermer Tiere von der Körpergrösse abhängt[1], sondern dass sich auch an der Gewebsatmung poikilothermer Wirbeltiere die Zunahme der Körpergrösse im Sinne einer Drosselung der Temperaturaktivierung bemerkbar macht[2]. Um zu prüfen, ob dieses Phänomen nur auf poikilotherme Organismen beschränkt ist oder auch für Warmblütergewebe gilt, wurden in der vorliegenden



Abhängigkeit der Aktivierungsenergie (μ) der Atmung und aeroben Glykolyse der Leber von der Körpergrösse bei Maus (I), Ratte (II) und Meerschweinchen (III) im interspezifischen Vergleich. Ordinate: Aktivierungsenergie in cal, Abszisse: Körpergewicht in g. • Sauerstoffverbrauch der Leber normal ernährter Tiere, ○ dasselbe für hungernde Tiere, × aerobe Glykolyse normal ernährter Tiere.

Untersuchung die Aktivierungsenergien (μ) nach ARRHENIUS der endogenen Atmung und aeroben Glykolyse der Leber von Mäusen, Ratten und Meerschweinchen im interspezifischen Vergleich mit der Körpergrösse in Beziehung gebracht. Die Atmung und aerobe Glykolyse der Leber wurde in substratfreier Krebs-Bikarbonat-Ringerlösung nach dem indirekten Verfahren von WARBURG über einen Zeitraum von 3 h gemessen. Sonstige methodische Einzelheiten wie in vorangegangenen Untersuchungen[3]. Der

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[2] A. LOCKER, Z. vergl. Physiol. 1958 (im Druck).

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