coverglass of the cultures were mainly copper K-rays.

The following procedure was adopted: Preliminary studies having shown that the X-ray dose necessary to inhibit completely cell multiplication is 10,000 r units, cultures of fibroblasts were irradiated with 25,000 r units, *i.e.*, a dose 2½ times greater than that required to arrest all mitoses. The cultures irradiated were divided into two equal parts: one fragment was planted into a medium which consisted of chicken plasma diluted with Tyrode's solution in the proportion 1:2; the other half was cultured in a medium of the same composition to which 30% embryo extract was added as a fluid phase.

In both fragments, the cells deprived by irradiation of their mitotic capacity migrated progressively and surrounded the explant. The growth rate of the culture that developed in a non-growth-promoting medium remained low. But the culture grown in a medium containing embryo extract showed considerable increase in area. After 6 days' cultivation the surface of cultures grown in a medium to which embryo extract was added exceeded by 200% (average of 17 experiments) the growth area of cultures developed in plasma alone. Fig. 1 represents this relation graphically.

The increase in area of a cell colony *in vitro* is the resultant of a number of factors of which the most important are cell multiplication and call migration. The culture in which no cell multiplication occurred enlarged by the migration of cells from explant into the culture medium. The fact that embryo extract promoted the rate of growth in cultures deprived of mitotic capacity proves that embryo extract not only possesses the property of stimulating cell divisions but also increases the migratory activity of the cells. These findings cast doubt on the validity of the idea that embryo extract activates the mitotic capacity of the cells selectively. Rather, they favor the view that embryo extract increases the general activity of the cells (probably by altering the cellular metabolism) and that this activation is reflected in both greater cell mobility and increased mitotic rate of the cells. The recent findings of Willmer and Jacoby[1] on the influence of various concentrations of embryo extract on cells *in vitro* would appear to lend support to this view. By means of cinematographic records, these workers were able to show that increased concentrations of extract caused an increase in the rate of migration as well as in the rate of mitosis of individual fibroblasts.

[1] Willmer, E. N., and Jacoby, F., *J. Exp. Biol.*, 1936, **13**, 237.

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Effect of Temperature on the Action of Penicillin* *In vitro.*

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Hobby *et al.*[1] reported that at low temperature penicillin showed very little bactericidal activity, but that this property was enhanced with a rise in the temperature, becoming very marked in that range favoring maximum growth of the organism. Their work showed the interrelation of the bactericidal action of penicillin and rate of bacterial growth. To support a hypothesis that penicillin acts upon organisms during the time of their division. Bigger[2] carried out almost the same type of experiment as the one of Hobby *et al.*, except that his technic of "one loop sterility" allowed the finding to be stated on a semi-quantitative basis. At almost the same time,

* Throughout this paper "penicillin" refers to material containing about 300 units per mg (Merck).

[1] Hobby, Gladys L., Meyer, Karl, and Chaffee, Eleanor, PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 281.

[2] Bigger, Joseph W., *Lancet*, 1944, **247**, 497.

Lee *et al.*[3] concurred with the above findings and pointed out that under a diversity of conditions, including variations in the temperature, a quantitative relationship was found to hold between the rate of growth of the organisms and the bactericidal activity of penicillin. For instance, if *S. aureus* FDA was kept at 14°C for 7 days, until growth had taken place in the control tubes, definite bactericidal action of penicillin (1 unit per ml) was noted. Eagle *et al.*[4] have found that the spirocheticidal activity of penicillin is greatly enhanced by an increase in temperature, no activity having been found at 8°C, whereas it became increasingly marked up to 39.4°C, the highest temperature studied. Garrod,[5] repeating the work of Hobby *et al.*, Bigger, and of Lee *et al.*, and using data difficult to interpret because of the lack of controls, attempts, on the basis of two observations, to discredit the concept of the relationship of the bactericidal activity of penicillin to the rate of growth of the organism. First, he notes the bactericidal activity of penicillin at 10° and even 4°C in the presence of 10 units per ml of penicillin. This finding, except for the slightly higher concentration of penicillin used (which might possibly alter the results), is at variance with all the work cited above. Secondly, he points out that even though growth ceases at 42°C, the bactericidal action of penicillin is greater at this temperature than at 37°C. We, too, have found that activity is very marked at this temperature, but we have also observed that with *S. aureus* FDA, the rate of growth of the organism may be very pronounced at 42°C. It has thus been clearly shown that with increasing temperature the bactericidal action of penicillin increases. It is now accepted also that by increasing the rate of growth of a susceptible organism, the other conditions remaining effectively constant, there results a faster killing of organisms by a given concentration of penicillin. Since growth rate is so dependent on the temperature of the medium, it has not been an easy experimental task to study the effect of temperature as a single variable on the action of penicillin. We wish to report this effect, among other findings, in this paper.

[3] Lee, S. W., Foley, E. J., and Epstein, Jeanne A., *J. Bact.*, 1944, **48**, 393.

[4] Eagle, Harry, and Musselman, Arlyne D., *J. Exp. Med.*, 1944, **80**, 493.

[5] Garrod, Lawrence P., *Brit. Med. J.*, 1945, Jan. 27, 107.

TABLE I.

Rate of Killing of *Str. agalactiæ* by Penicillin (5 units per ml) at 50°C.

Incubation of culture at 50°C (in min.)	Viable organism per ml in thousands	
	Control	Penicillin present
0	210	210
5	134	106
10	115	67
15	71	46
20	58	25
30	27	14
40	15	6

Direct action of penicillin on organisms at elevated temperatures. Several experiments have been carried out to measure the effect of penicillin on the rate of killing of organisms at temperatures above that at which growth takes place, and in this way to obtain a measure of the direct killing action of penicillin under such conditions. In Table I are shown the results of such an experiment carried out on a strain of *Streptococcus agalactiæ*. In this experiment, a 0.1 ml of a 1-20 dilution of an overnight culture was inoculated into 10 ml of brain-heart broth, giving an initial population of 210,000 organisms per ml. To this, sufficient penicillin Merck was added to give a final concentration of 5 units per ml. A control tube was prepared similarly, but without penicillin. The tubes were allowed to stand at room temperature for about 1 hour before the heating was begun in order to give time for any interaction which might possibly occur between the organisms and the penicillin.

A similar but more complete study of the action of penicillin at elevated temperatures has been carried out on *Staphylococcus aureus* FDA. The conditions were the same in this as in the preceding experiment, and the results are given in Table II.

It is seen from the table that at elevated temperatures 5 units of penicillin per ml kills

TABLE II.
Rate of Killing of *S. aureus* FDA by Penicillin (5 units per ml) at Elevated Temperatures.

Incubation of cultures at given temp. (in min.)	Viable organisms per ml in thousands Temperature of incubation					
	50°C		55°C		60°C	
	Control	Penicillin present	Control	Penicillin present	Control	Penicillin present
0	600	570	600	570	600	570
5	500	450	650	540	46	40
10	450	500	370	320	6.1	2.0
15	450	450	170	150	1.6	0.61
20	420	290	70	38	0.25	0.040
30	450	240	64	24	0	0

TABLE III.
Effect of Rate of Growth of *S. aureus* FDA and of Temperature on the Activity of Penicillin (1.5 units per ml) in Brain-Heart Medium. Effect of Age of Culture.

Age of organisms in inoculum	Incubation of culture at given temp. (in hrs)	Viable organisms in thousands Temperature of cultures					
		37°C		40°C		42°C	
		Control	Penicillin present	Control	Penicillin present	Control	Penicillin present
Overnight	0	1000	1000	1000	1000	1000	1000
	3	5120	540	2400	280	1280	640
3 hrs	0	900	900	900	900	900	900
	3	7840	160	4000	160	3200	145

S. aureus FDA at a much faster rate than does the heat alone. As might be expected, the killing action of the penicillin increases markedly with each rise in temperature.

It was thought of interest to study the effect of slight variation in the incubation temperature near that for optimum growth, as well as the possible effect of using inocula of an organism of different ages. In a typical experiment, 0.1 ml of a 1:50 dilution of an overnight growth of *S. aureus* FDA was inoculated into a tube of brain-heart medium containing 1.5 units of penicillin per ml. For the young culture, 0.1 ml of a 3-hour growth of the same organism was inoculated into another tube of the above medium containing penicillin (1.5 units per ml). These results are given in Table III.

It has been amply and quantitatively shown that, other conditions remaining as constant as possible, the rate at which penicillin kills susceptible organisms increases with the rate of growth of the organism. This quantitative data has previously not been extended beyond the temperature range favoring maximum growth. The representative data given in Table III are then of interest, for they indicate that at temperatures higher than 37°C the viable count in the presence of penicillin decreases at a faster rate, even though rate of growth in the control tubes is diminished. Thus, penicillin action either increases with temperature as such, or its action is a function of the rate of the metabolic processes of the organism.

An experiment similar to the previous one was carried out using rabbit serum as a medium. This was done to see whether the temperature-activity relationship would hold under these conditions. On the basis of the results of preliminary experiments, such as those given in Table IV, it appears that an increase in temperature does result in an increase in the killing action of penicillin, but the findings are not as definite as in the simpler media.

Summary. At elevated temperatures (50-60°C) penicillin (5 units per ml) hastens

TABLE IV.
Effect of Rate of Growth of *S. aureus* FDA and of Temperature on the Activity of Penicillin (1.5 units per ml) in Rabbit Serum.

Incubation of cultures at given temp. (in hrs)	Viable organisms per ml in thousands Temperature of Incubation					
	37°C		39°C		42°C	
	Control	Penicillin present	Control	Penicillin present	Control	Penicillin present
0	120	120	120	120	120	120
2	850	600	2700	550	800	120
4	11000	30	17000	10	46000	5.0

the death of *S. aureus* FDA and a strain of *S. agalactiæ*. At temperatures higher than that leading to maximum growth of the organism, penicillin kills *S. aureus* FDA faster than it does at the temperature of maximum growth. At the temperatures slightly higher than optimum, there still exists an interrelation between growth and the killing of bacteria by penicillin. It is believed that only at excessively high concentration, or very high temperature, does penicillin kill organisms while they are not undergoing growth.

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Use of Beta Beta' Dithiocyano Diethyl Ether (RID-O) to Control Mite Infestations in Mice.*

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When it was noticed that part of a colony of 3,000 mice used for cancer research were losing hair, they were examined for mites. The skin on the abdomen was scraped with a small sharp scalpel on which a drop of glycerine had been placed. The scrapings were then put in a drop of glycerine on a glass slide and examined with a microscope. Glycerine was used because mites remain active in this medium for many hours. The species of Acarina (mites) found was identified as *Myobia musculii*.

Since rotenone and derris root powder were not available, an attempt was made to kill the mites with DDT.† The results were disastrous because the DDT powder was much more toxic for mice than it was for mites. Some papers on the toxicity of DDT for mice, rats, cats, etc., appeared about this time and confirmed the observation that it is toxic for mice.[1,2,3] Animals that lick their fur and thus ingest agents applied externally, cannot be treated with DDT. In addition to the toxicity, DDT was of no value in this case because it did not appear to kill the mites. This lack of lethal effect of DDT on mites may be related to the fact that mites are not insects, but arachnids.

The next insecticide tried was a liquid

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† A small sample of DDT was very kindly supplied by the Research Department of the School of Pharmacy of the University of Maryland.

1 Lillie, R. D., and Smith, M. I., *Public Health Rep.*, Washington, D.C., 1944, **59**, 979.

2 Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. G., Smith, R. B., Jr., and Calvery, H. O., *Ibid.*, 1944, **59**, 1009.

3 Woodard, G., Nelson, A. A., and Calvery, H. O., *J. Pharm. and Exp. Therap.*, 1944, **82**, 152.