to be within ± 1° of the ambient air temperature and the air movement to be turbulent but continuous at a rate of about 2 m.p.h.

TABLE 1

| Species | No. of animals | Average weight (gm) | Local frostbite noted | Survival time (hrs) |
|---|---|---|---|---|
| Mouse, Carworth CF1 strain | 10 | 19 | Feet & tails | 0.4 |
| Canary | 2 | 20 | | 0.6 |
| Rat, Wistar | 7 | 242 | Feet & | .75–1.6 |
| albino | 5 | 227 | tails | 2.0 |
| Rabbit, New | 3 | 1,760 | Ears | 3.5–5.0 |
| Zealand white | 6 | 1,774 | | 5.0–6.5 |
| Chicken, white | 8 | 1,642 | Combs | 3.3–16.0 |
| Leghorn | 3 | 1,537 | & feet | 16.0–29.5 |
| Pigeon, Army | 5 | 395 | Feet | 22–24 |
| carrier | 4 | 397 | | 24–48 |
| | 2 | 408 | | 48–78 |

The animals were confined, one at a time, in metal cages with cardboard bottoms. The rectal temperatures of rats and rabbits and the cloacal temperatures of pigeons and chickens were measured either by thermometer or by copper-Constantan thermocouples read with a potentiometer. In a few cases the animals were

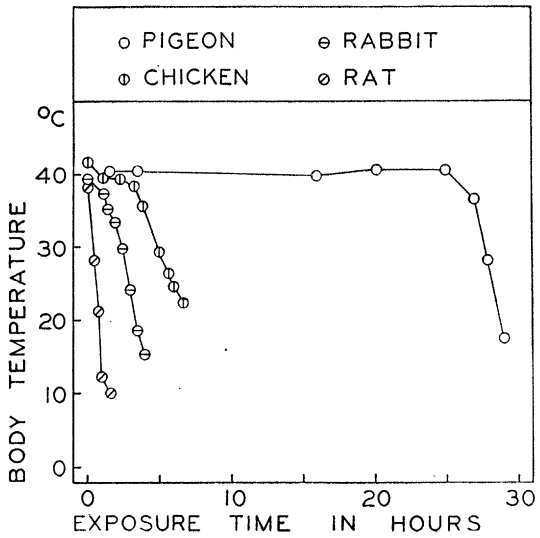


FIG. 1

removed from the cold room into a cool room for insertion and reading of the rectal thermometer. Although the animals had room for movement in their cages, they sat quietly in one place and exposed a minimum of surface. They were not fed during the experiment.

A summary of the results is given in Table 1. Body temperature for representative individuals is plotted against time in the cold in Fig. 1. The last temperatures recorded were obtained on moribund animals. The data indicate that the adaptability to low environmental temperatures varies considerably among species as well as within a species. The pigeon appeared to be especially well adapted to cold, one pigeon surviving without food for 78 hrs in an ambient temperature of − 35° C. Local frostbite was noticed on the ears of rabbits, on the combs of chickens, on the feet of chickens, pigeons, mice, and rats, and on the tails of rats and mice.

Our observations are in accord with the experiments of Giaja (*2*), who found the lowest temperature at which the animal can maintain its body temperature for one hour to be: for the pigeon, − 85° C; for the chicken, − 50° C; for the rabbit, − 45° C; and for the white rat, − 25° C. Giaja's experiments in which environmental temperature was the variable and our experiments in which time was the variable placed species in the same order in regard to the degree of their resistance to cold.

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## The Respiration of *Streptomyces griseus*


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Since the presence of air is necessary both for the growth of *Streptomyces griseus* and for the production of streptomycin, an investigation of the respiration of this organism was pertinent. The experiments were designed to determine (1) oxygen consumption during the growth of the culture, (2) its relationship to antibiotic production, and (3) whether a correlation exists between the abilities of different strains to produce the antibiotic and their different rates of respiration.

Strain A2, which was most generally used in these studies, was derived as a single colony isolate from *S. griseus* H9 received from S. A. Waksman. Other strains such as 4c5 and R.M. 1067 were obtained by irradiating A2 with ultraviolet light. All cultures were grown in 500-ml Erlenmeyer flasks containing 125 ml of a modified streptothricin assay medium and were agitated on a reciprocal shaker. Assays for streptomycin were made according to the method described by Loo, *et al.* (*2*), and the growth of the organism was based upon the dry weight of washed and centrifuged mycelium. Studies of oxygen consumption were made in a Warburg apparatus using the techniques described by Umbreit, Burris, and Stauffer (*3*). Fifteen-ml aliquots of the culture were used for determinations of mycelial weight and 2 ml for respiration studies. When dextrose was added to the medium during measurements of oxygen consumption,

this was done from the side arm, and a similar amount of water was added to the control flasks. All analyses were made on samples taken from triplicate flasks at various periods during the growth and decline of the culture.

TABLE 1

GROWTH, RESPIRATION, AND PRODUCTION OF STREPTOMYCIN OF *S. griseus* A2

| Age of culture (hrs) | Wt. of mycelium (mg) | $O_2$ consumed (µl) | Mycin produced (units/ml) |
|---|---|---|---|
| 0 | 0.7 | 32 | 0 |
| 12 | 4.8 | 344 | 0 |
| 24 | 11.2 | 281 | 50 |
| 36 | 12.2 | 127 | 188 |
| 48 | 10.2 | 63 | 227 |
| 60 | 8.1 | 40 | 223 |
| 72 | 6.9 | 30 | 211 |
| 84 | 6.6 | 30 | 201 |
| 108 | 6.6 | 30 | ... |

The growth of *S. griseus* followed the general pattern which has previously been described (*2*): the weight of the mycelium increased until about 36 hrs after the flasks were inoculated, then steadily declined (Table 1). Respiration followed a different pattern. During the early period of growth, respiration was at its highest level; thus, in one experiment this occurred when only 39% of the maximum growth had been obtained (Table 1).

TABLE 2

RESPIRATION OF *S. griseus*

| Age of culture (hrs) | $QO_2$ | $QO_2$ when dextrose added | Age of culture (hrs) | $QO_2$ | $QO_2$ when dextrose added | Age of culture (hrs) | $QO_2$ | $QO_2$ when dextrose added |
|---|---|---|---|---|---|---|---|---|
| 0 | 32 | ... | 0 | 29.5 | ... | 0 | 46.0 | ... |
| 19 | 40.5 | 45.2 | 15 | 41.4 | 45.1 | 12 | 71.8 | ... |
| 45 | 12.6 | 22.2 | 39 | 10.7 | 14.8 | 24 | 25.3 | ... |
| 69 | 7.5 | 19.0 | | | | 36 | 10.3 | ... |
| 93 | 3.8 | 0 | | | | 48 | 6.2 | ... |
| | | | | | | 60 | 5.5 | 13.0 |
| | | | | | | 72 | 4.3 | 9.1 |
| | | | | | | 84 | 4.6 | 11.7 |
| | | | | | | 108 | 4.0 | 7.9 |

Respiration was then gradually reduced during the remainder of the growth and decline of the culture. This can best be noticed when the $QO_2$ is compared at various periods of growth (Table 2). Both total oxygen consumption and the oxygen consumption per unit weight of mycelium decreased during the period in which the total amount of the *S. griseus* present in the culture was increasing. This diminution of oxygen demand continued into the decline phase, when the total weight of the fungus was steadily reduced. Streptomycin was not produced in the culture until about 12–24 hrs of growth and reached a maximum somewhere between 48 and 60 hrs. Greatest antibiotic production therefore occurred during the period of low oxygen consumption and was not detected during the early period when respiration was at its highest level.

The decrease in oxygen consumption during the growth of *S. griseus* could be due to two factors: (1) a depletion of sugar in the medium with a concomitant reduction in metabolism, and (2) an inherent change in respiration of the cells with age. The addition of dextrose to the medium should then result in an increased respiration, if depletion of the media was the main factor in decreased respiration. Though some increase in respiration occurred when the dextrose concentration was increased, the increase never reached the levels of respiration of the very young culture (Table 2). It is apparent, then, that two phenomena were involved in the change in oxygen consumption of *S. griseus*. One of these factors is the very high rate of respiration of the young mycelium. Synthetic metabolism was at its highest level during this period. In the first 12 hrs the mass of mycelium increased 6.5 times, whereas in the next two consecutive like periods the increase was only 2.3 and 0.09 times, respectively. Oxygen consumption was related to synthetic ability during these periods, since the changes in the $QO_2$ of the mycelium follow the changes in growth rate in various stages of the culture's development. During the period of decrease in the weight of mycelium present in the culture, the reduced respiration was expected, though some oxygen might have been utilized in some of the enzymatic processes of autolysis. The other factor responsible for reduced respiration was the amount of dextrose removed from the solution by the growing culture. This process has been shown to be very rapid in young cultures (*1*). Though increases in oxygen consumption occurred on the addition of dextrose, the fact that it never reached the level of the young cultures indicates that both the age of the culture and the dextrose concentration are involved in the respiration fluctuations which occur in shake culture.

The ability of different strains of *S. griseus* to produce streptomycin could not be correlated with their rates of respiration. Strain R.M. 1067, which produces no detectable amounts of streptomycin, had a $QO_2$ of 51.9, whereas A2, which produced 240 units/ml had a $QO_2$ of 23.3. Another strain, 4c5, which produced even more streptomycin (350 units/ml) had a $QO_2$ of 61.0. No discernible relationship therefore exists between oxygen consumption and streptomycin production either at various stages in the culture of the fungus or between strains of different synthetic capacities.

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