streptomycin-resistant strains point to the possibility of utilizing grisein for the control of infections caused by bacteria resistant to other antibiotics. However, its narrow antibiotic spectrum would limit its practical application.

Summary. *Streptomyces griseus* is widely distributed in soils, peats and in composts. Only very few strains of this organism are capable of producing streptomycin. Most of the strains produce no antibiotic at all, whereas certain strains produce antibiotics that are distinctly different from streptomycin. One such new antibiotic was isolated and designated as grisein.

Grisein is active against certain Gram-positive and Gram-negative bacteria. Its antibacterial spectrum is much more limited than that of either streptomycin or streptothricin. Cultures of bacteria that are made resistant, by serial passage, to streptomycin still remain sensitive to grisein. Cultures of bacteria originally sensitive to grisein give rise easily to strains resistant to this antibiotic.

Grisein shows a rather low toxicity to experimental animals and is rapidly excreted in the urine. It was found capable of protecting experimental animals against infections with *S. schottmülleri* and *S. aureus*.‡

‡ The authors are indebted to Mr. Otto Graessle, of the Merck Institute, for testing the animal toxicity of grisein, and to Dr. H. Boyd Woodruff, of the Merck Laboratories, for checking the sensitivity of streptomycin-resistant strains of different bacteria.

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Determination of Oxygen Consumption in the Albino Rat.*

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Technical aspects of the measurement of gaseous metabolism in the albino rat would appear, from a cursory survey of the literature, to be almost a closed chapter in biologic technology. Yet the determinations, as executed, have proved inaccurate, inconsistent, and time-consuming. The method to be described below appears to offer several distinct advances over those currently in use.

The machine devised for these studies is of the closed-circuit type, measuring only oxygen consumption. Its major structural features are illustrated in Fig. 1.

Animal chamber, spirometer box, and water bath are all constructed of heavy sheet brass, which is adequate for the rapid equalization of temperature throughout the system. The temperature is maintained within narrow limits by means of a tubular model heating element controlled through a suitable relay by a thermoregulator immersed in the water bath. The water is kept circulating by a noiseless electric stirrer. Sensitivity of the thermoregulator is assayed by a blank test, measuring the variations in volume of the inclosed oxygen that are induced by the automatic activation and deactivation of the heating element; such variations have not exceeded 0.5 cc. The temperature within the animal chamber is maintained at 28°C, stated by Benedict and MacLeod[1] to be the temperature of thermic neutrality for the albino rat.

Dimensions of the animal chamber are 9″ x 6″ x 5½″. Its lid is provided with a large window, composed of 2 panes of glass separated by an air space to limit condensa-

* Paper No. 831, Research Series, the University of Arkansas.

1 Benedict, F. G., and MacLeod, G., *J. Nutrition*, 1929, **1**, 367.

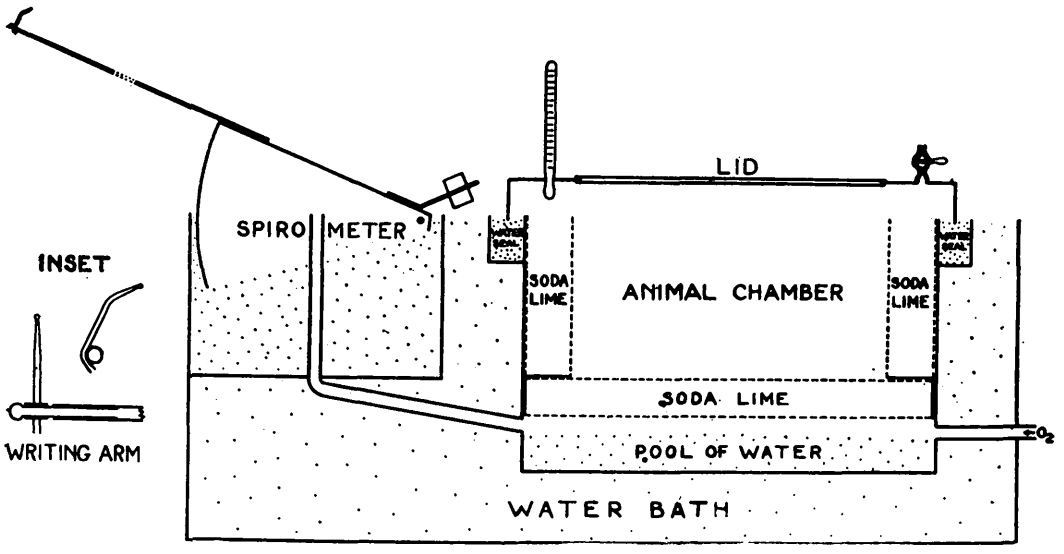

FIG. 1.
Basal Metabolism Machine. Longitudinal section. About ¼ scale. Heating element, thermoregulator, and water stirrer not shown. Inset: writing arm, side view as inserted into writing lever, end view of writing arm and collar without the pin which inserts into writing lever.

tion of water. The thermometer† and a stopcock, to allow free movement of air into the system whenever the apparatus is opened, are fitted into the lid. The chamber is rendered airtight by a water-filled moat into which the overhanging rim of the lid dips.

The rat is placed into a wire mesh cage, somewhat smaller than the space available, and this is introduced in turn into the animal chamber, where it is flanked fore and aft by soda lime boxes made of copper window screening. The inner cage rests on a false-bottom floor, formed by a third soda lime container, which is itself elevated about an inch above the true floor of the animal chamber in order to provide space for a pool of water below the CO_2 absorbent.

Koehler[2] points out the advisability of maintaining a relatively high humidity within the chamber, partly to keep the soda lime at maximum absorptive efficiency and partly to induce the animal to relax. He considers, however, that a humidity above 75% causes restlessness. The present author, on the other hand, observed that the soporific influence varies directly with the vapor tension and that the 99% humidity provided at 28°C by a pool of water is most effective, at least in the rat. Under that regime, the animal almost immediately composes itself for sleep, sometimes on the first trial, usually after 3 or 4.

The spirometer bell is made of thin-walled brush copper, which is far less vulnerable than aluminum to attack by soda lime. It is a Krogh type spirometer, pivoting on jewel bearings and thus providing freedom of movement without the lateral shifting encountered in the ordinary cylindrical spirometer. The bell is counterweighted in such manner that, with the system open to the outside, it descends slowly and without acceleration. Its dimensions are 5″ x 2½″ x ½-3½″, giving a volume of about 300 cc, which is well above the requirement of even a hyperthyroid rat. Customarily, however, the spirometer is refilled during the rest period about 10 minutes before the test run; this is done from an ordinary vitalometer in order to avoid disturbance of the rat by the

† The thermometer manufactured by the Warren E. Collins Co., Boston, for use in the Benedict Roth Metabolism Machine has a brass jacket which can be screwed and cemented in place.

[2] Koehler, A. E., *J. Biol. Chem.*, 1932, **95**, 67.

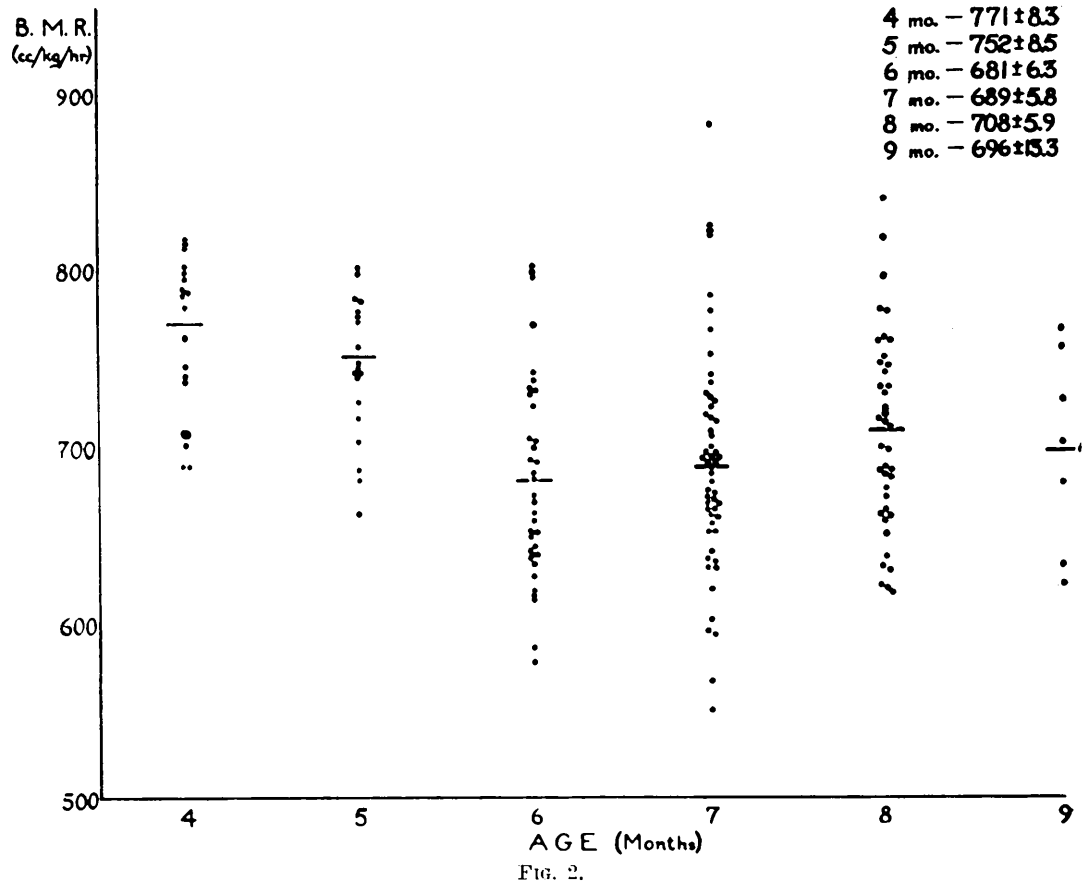

FIG. 2.
Relation of Oxygen Consumption to Age in Male Rats. Mean and probable error for each age level shown at upper right corner.

hiss of gas as it comes from a compressed oxygen tank.

At the tip of the tubular stainless steel writing lever is attached a free-swinging writing arm made of capillary glass tubing (see inset, Fig. 1). Such a writing point exerts a constant light pressure, determined solely by its own weight, against the kymograph drum. Fire-polishing the tip converts it into a smooth ball of low friction.

Use of the spirometer and writing point described above leads to a high degree of sensitivity, so much so that the slightest movement of the rat communicates itself as a gross hump to the otherwise smooth kymogram. Such precision affords an integral record of activity on the oxygen line; it also makes possible the measurement of oxygen consumption over so short an interval as 5 minutes. Such short periods were found to give lower and more consistent readings than the much more prolonged runs usually considered necessary.

Inscription of the tracing in this manner, however, introduces complications in measurement, since it brings about an interplay between 3 arcs, described by the drum's periphery, the spirometer bell, and the free-swinging writing arm. This requires that steps be taken to insure a constant, exactly reproducible spatial relationship between the kymograph and the basal metabolism machine, and that a measuring rule be made for the individual machine. The former control is easily made by fastening the machine and the kymograph to wooden bases so shaped that they fit together in only one position. The measuring rule is constructed

in convenient form by transfer onto a plate of window glass of the record made by withdrawal of successive 5 cc volumes of air from the chamber into an accurate burette. The rulings are scratched on this glass plate with a diamond point and are then filled with colored wax; the transparent rule thus produced can be placed on each tracing and used to read off the oxygen consumption values accurately and quickly. Simultaneous ordinates for the signal magnet time record are transcribed onto the oxygen line of each tracing by manual operation of the drum and writing lever, after the test run.

In practice, then, the fasted rat is placed into the small wire mesh cage referred to above, and this is inserted into the animal chamber of the basal metabolism machine. Oxygen is admitted and allowed to flow out of the open stopcock and to bubble out of the spirometer until all air in the system is replaced by oxygen. Then the chamber is closed and at least 30 minutes are allowed for the animal to fall asleep and for the system to reach equilibrium. A 30-minute tracing is made and the best 5-minute period noted, along with an estimate of activity as determined from the smoothness of the tracing.

When the oxygen line indicates by an absence of any waves that the rat has been sleeping soundly throughout the recording period, all of the 5-minute readings give practically identical rates of oxygen consumption. Any movement which does occur elevates the succeeding readings in direct proportion to the intensity and duration of the activity; even a mere shift in position by a visibly sleeping animal increases the oxygen consumption for at least the next 5-minute period.

The graph of Fig. 2 shows the distribution of values, expressed as cc/kg/hr, according to age, in 172 determinations on 42 adult male rats. Only those observations made on sleeping animals are included, and all rats were housed in a constant temperature room at 26°C. Averages for the individual age groups show, with other observers, a progressive decline with age, reaching what appears to be a plateau at about 6 months. The mean oxygen consumption of the 133 determinations made in the 6-9-month age range is 692 cc/kg/hr $\pm$ 3.0 (P. E.), which may be taken as the basal metabolic rate obtained in "metabolic maturity." This figure is equivalent to 635 Cal/m^2/24 hr, using the formula, S. A. $=$ 9.1 $\times$ W2/3, for calculation of surface area.

Summary. 1. A method is described for determination of oxygen consumption in the rat. Its major advances are: (a) Humidity control, inducing sleep. (b) Sensitive recording method, which shows body movements on the oxygen line and makes feasible readings covering 5-minute periods.

2. Values obtained in 172 determinations on 42 male rats show a decline from 771 cc/kg/hr at age 4 months and 752 cc/kg/hr at 5 months to a plateau at 6 months and maintained to at least 9 months. The average for 133 determinations on rats of 6-9 months is 692 cc/kg/hr $\pm$ 3.0 (P. E.), or 635 Cal/m^2/24 hr.

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The Susceptibility to Furacin of Bacterial Strains Resistant to Sulfonamides or Antibiotics.*

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Furacin or 5-nitro-2-furaldehyde semicarbazone has been found to be both bactericidal and bacteriostatic for a large group of Gram-positive and Gram-negative bac-

* This work has been aided by a grant from the Eaton Laboratories, Inc., Norwich, N.Y.