the venous blood sugar of man. This response is variable in quantity and is not increased by increased dosage. The production of anesthesia enhances the increase in venous blood sugar produced by the hyperglycemic factor. This effect of anesthesia is attributed to an increase in peripheral blood flow rather than to an augmented hyperglycemic effect.

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Experimental Histoplasmosis in the White Rat.* (18132)

J. G. MIDDLETON, D. L. MCVICKAR, AND J. C. PETERSON.†
(Introduced by J. W. Ward.)

From the Departments of Pediatrics and Pathology, Vanderbilt University School of Medicine, Nashville, Tenn.

The true effectiveness of any substance of potential promise in the therapy of an infectious disease can be evaluated only in terms of its capacity to inhibit or kill the pathogenic microorganism in the tissues of an infected host. It is perhaps not unreasonable to suggest that lack of a standardized laboratory procedure for testing the *in vivo* activity of chemotherapeutic agents has been responsible for the delay in the development of an effective method of treatment of the systemic mycotic infections. With increasing recognition of the prevalence of these diseases has come an awareness of the necessity for some specific form of therapy, since the systemic mycoses, though not of common occurrence, have a high mortality rate. Extensive experience with histoplasmosis at the Vanderbilt University Hospital led us, a little more than a year ago, to undertake the development of a standardized technic for infecting animals with *Histoplasma capsulatum*. The widespread interest in histoplasmosis is reflected in the recent appearance of two articles on the experimental production of this fungous disease in animals. Campbell and Saslaw(1), and Howell, Kipkie and Bruyers(2), have been able to demonstrate consistently successful infection of mice with *H. capsulatum*. The present paper is a report and discussion of the procedures we have developed for inducing generalized histoplasmosis in the white rat.

Methods and experiments. Animals. Since it was anticipated that certain drugs to be tested might have to be administered by gavage, mice were not used because of their small size. Guinea pigs had been shown (unpublished data) to be unsuitable because of a too great lack of uniformity in reaction to infection with *H. capsulatum*. White rats, of the Sherman strain, were tried and found to be reasonably satisfactory; healthy, white females, weighing 125 ± 8 g were used throughout the experiments.

Inoculum. Preliminary experiments, over a period of a few months, with small groups of animals inoculated with equal numbers of organisms in each experiment, showed a great variation in mortality rate. The hypothesis was advanced, then proven correct, that even though the absolute numbers of organisms injected into the rats were identical, the number of *viable* organisms in the inoculum differed greatly. Accordingly, a rigid technic and schedule of transfer was instituted: under these conditions, transfer of cultures at three-day intervals produced a growth which, at the time of transfer, yielded a relatively constant percentage of viable organisms. After virulence tests on several strains of *H. capsulatum* obtained from different sources, V. U. strain No. 621,† which appeared more viru-

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1. Campbell, C. C., and Saslaw, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 469.

2. Howell, A., Jr., Kipkie, G. F., and Bruyers, P. T., *U. S. Public Health Rep.*, 1950, v65, 722.

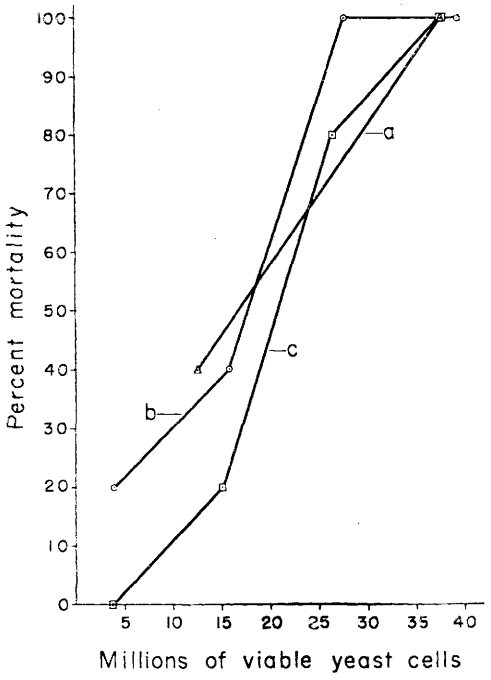

FIG. 1.
Relationship between number of viable yeast cells of *Histoplasma capsulatum* injected intravenously into rats and the percentage mortality obtained. Curves "a", "b", and "c" represent three different experiments carried out at several months intervals.

lent than the others, was chosen and used throughout the studies reported here.

Yeast cells from 3-day-old cultures were suspended in physiological saline, and adjusted photometrically to a standard density containing a known number of organisms. On the basis of the previously established probable percentage of viable organisms, suspensions of yeast cells were made up to contain the desired numbers of viable organisms per ml. (In each experiment, determination was made of the percentage of viable organisms in the standard suspension, and any deviation from the expected percentage taken into account in the final evaluation of dosage-mortality relationships.)

Procedures. Preliminary runs indicated that a 0% to 100% mortality prevailed when the number of viable organisms injected per animal was varied between 4 to 40 millions. Five rats were used for each of 4 different dosage levels of organisms; the animals were inoculated by tail vein with 1 ml of a suspension containing the calculated probable number of viable yeast cells. In view of the large number of organisms found necessary to effect a high mortality, it was felt desirable to inject a small group of animals with killed organisms. For this purpose 5 rats were injected intravenously with 1 ml containing the maximal number of viable organisms, which had been heat-killed. In another experiment, 5 rats were injected intraperitoneally, with the maximal number of organisms. All animals were observed carefully until death ensued, or until after four weeks had elapsed, after which interval of time the animals rarely succumbed.

Results and discussion. The results of 3 typical runs, carried out several months apart, are shown in Fig. 1. Curve "a", which has only 2 points since the 2 higher dosage levels also yielded 100% mortality, is given here because it represents the results obtained in one of the earliest runs, and shows good correspondence with curves "b" and "c", which represent experiments carried out at considerably later dates. The LD_{50} for these 3 runs ranges between 17 and 21 million viable yeast cells per rat.

No effects, other than a transitory weight loss, were shown by the rats inoculated intravenously with maximum doses of heat-killed organisms, or intraperitoneally with the same doses of viable organisms.

All animals which succumbed to the infection showed a steady weight loss, amounting to about 30% of their original weight, by the time of death. Such animals also manifested an increasing lethargy, with markedly decreased intake of food and water. The interval between the time of inoculation and the time of death showed no constancy, either within or between groups, and averaged about 15 ± 6 days.

These results, which demonstrate that fatal experimental histoplasmosis may be regularly produced in rats by intravenous injection of the yeast cells of *H. capsulatum*, have one

† V.U. strain No. 621 was isolated from a 5-month-old infant who died with disseminated histoplasmosis in 1949.

or two points of interest which deserve comment.

Perhaps the most striking aspect of the experiments is the enormous number of viable organisms found necessary to establish a fatal infection. That the reaction was a true infection seems established by tissue sections from dead animals, which showed characteristic, developing, granulomatous areas containing numerous giant cells congested with the yeast cells of *H. capsulatum*. Furthermore, the injection of heat-killed organisms in maximal number was without significant pathological effect. Our results are in accord with Campbell and Saslaw(1), who used an inoculum which was about equivalent to ours, in terms of the number of organisms per gram of body weight necessary to produce death in the infected animal. (It may be noted, however, that Campbell and Saslaw used dosage levels based on the absolute numbers of yeast cells, stating that "it (is) impracticable to attempt to determine the actual number of viable cells.") Howell *et al.*(2) used intracerebral inoculation, and took into consideration the number of viable organisms in the inoculum. Interestingly enough, it may be calculated from their data that the intracerebral route of administration requires only 1/200 the number of viable yeast cells of *H. capsulatum* as does the intravenous route. It seems unlikely that a difference of this magnitude could be attributable to a difference in virulence of the strains employed; probably it is a reflection of the difference in the capacity of the host's defense mechanisms to cope with invasion by these different routes. This point is further illustrated by the ineffectiveness of intraperitoneal injection in our tests, and in those of Campbell and Saslaw, who found it necessary to add gastric mucin in 5% concentration to the yeast cell suspension in order to obtain infection by the intraperitoneal route.

From the standpoint of using the infected rat for *in vivo* studies of fungicidal agents, it is perhaps unfortunate that a change from 0% to 100% mortality should occur with only a ten-fold increase in the number of viable organisms injected. This fact, together with the inevitable uncertainty regarding the percentage of viable organisms in an inoculum, makes it essential to employ more than one dosage level of viable organisms, in order to achieve good approximation to any particular desired percentage mortality in the control animals.

Summary. The yeast cell phase of *Histoplasma capsulatum*, when injected intravenously in suitable numbers, has been shown to yield a consistently reproducible fatal infection in the rat. The necessity for standardizing the inoculum on the basis of the number of viable organisms, rather than the absolute number of organisms, is emphasized.

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Experimental Intraocular Infection of Guinea Pigs with Mumps Virus.*† (18133)

VERN S. BOLIN,‡ JOHN A. ANDERSON,§ AND GLEN R. LEYMASTER.
From the Lockhart Memorial Pediatric Research Laboratory, Department of Pediatrics, and the Department of Public Health and Preventive Medicine, School of Medicine, University of Utah, Salt Lake City.

The virus of mumps is known to infect

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‡ Present Address: Department of Public Health and Preventive Medicine, School of Medicine, University of Utah, Salt Lake City.

§ Present Address: Department of Pediatrics; Stanford University School of Medicine, San Francisco, Calif.

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