normal serum cross reacts with *H. capsulatum* cells or that a low titer of histoplasma antibodies is present and not detectable in the complement fixation test.

Summary. Antibodies in sera from patients with systemic mycoses were demonstrated by the indirect method of staining with fluorescent antibody. In spite of a general autofluorescence of pathogenic fungi, addition of human antiserum and a fluorescein-labeled rabbit antihuman gamma globulin resulted in characteristic fluorescing reactions. In the case of cryptococcosis antibodies were demonstrated by the fluorescent technic but not by other serologic methods.

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Comparison of Opacimetric and Hematocrit Methods in Measurement of Mitochondrial Swelling.* (23966)

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Study of the osmotic properties of isolated mitochondria is undeveloped in comparison with the maturity of similar studies of the red blood cell. The opacimetric method has been most widely used in mitochondrial studies for it is simple and lends itself readily to kinetic studies of volume changes. For the erythrocyte, the results obtained with the opacimetric method are generally in good agreement with the results of a variety of other methods which depend on different properties of the red cell system(1). For mitochondria, comparisons of the opacimetric method with others have been reported for only 2 alternative technics. Tedeschi and Harris(2) have shown that the optical density of mitochondrial suspensions is inversely proportional to the mitochondrial volume measured by photomicrography. The per cent dry weight of mitochondrial pellets

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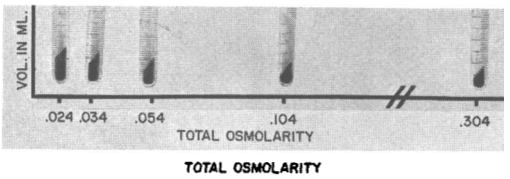

FIG. 1. Effect of osmolarity of medium on mitochondrial volume. Equal amounts of mitochondria sedimented in Shevky-Stafford tubes. Smallest division on tubes is .01 ml.

has been shown to decrease with decrease in optical density of the suspensions from which they are spun down(3). The latter correlation has been confirmed in this laboratory.

In the course of a study of mitochondrial swelling it was observed that mitochondrial pellets from suspensions of low optical density are macroscopically larger than those from more opaque suspensions of equal quantities of mitochondria. In this report quantitative data are presented which show that the volume of a mitochondrial pellet, *i.e.*, the mitocrit,‡ is inversely related to optical density of the suspension before centrifugation. These data compare the opacimetric and hematocrit methods for measuring relative mitochondrial volume.

Materials and methods. Male rats (Holtzman Co., Madison, Wis.) were fed *ad libitum*. Mitochondria were isolated(5) from a 13.5% homogenate of four livers in 0.30 M sucrose which was 0.002 M with respect to ethylenediamine tetraacetic acid (EDTA) at pH 7.4, by centrifugation for 12 minutes at 7100 x gravity (American Instrument Co., high speed refrigerated centrifuge, tube angle of 32°). They were washed twice in the same medium. The resultant stock suspension contained mitochondria from 1.5 g wet weight of liver per ml of sucrose-EDTA solution. Swelling was produced by diluting 2.0 ml aliquots of the stock suspension to 30 ml with a series of hypotonic sucrose solutions. Final concentrations in the series of mitochondria-sucrose systems were: 10% mitochondria, 0.002 M tris (hydroxymethyl) aminomethane (Tris) buffer at pH 7.4, and sucrose at various molarities. For direct measurement of mitochondrial pellet volumes, 6.5 ml aliquots from members of this series were delivered to Shevky-Stafford graduated centrifuge tubes and centrifuged for 30 minutes at 5700 x gravity. The mean of the upper and lower limits of the top of the mitochondrial pellet was taken as its volume. For optical density measurements, 1.0 ml aliquots from the mitochondria-sucrose systems, at various sucrose molarities, were periodically diluted to 4.0 ml with sucrose solutions of a corresponding molarity, also buffered with 0.002 M Tris at pH 7.4. Optical density readings at 520 mμ were obtained with the Coleman Junior Spectrophotometer, Model 6A. After about 10 minutes at room temperature, slow secondary changes of the initial optical density values are observed. These secondary degenerative changes, also observed by Tedeschi and Harris(2), are believed to be similar to those occurring in CCl_4- or inorganic phosphate-induced isotonic swelling(5). It was found that during the time necessary for the mitocrit determinations (45-60 min), the optical densities of the corresponding mitochondrial suspensions could be maintained to within 4% of their initial values by keeping the mitochondria at 0-1°C, except during the short interval necessary for optical density readings which were carried out at 3-8°C.

Results. Fig. 1 is a photograph of mitochondria sedimented in Shevky-Stafford tubes. Each tube contained the same amount of mitochondria. It is evident that as the osmolarity of the medium decreases, the volume of the mitochondrial pellet increases.

The results from a repetition of this experi-

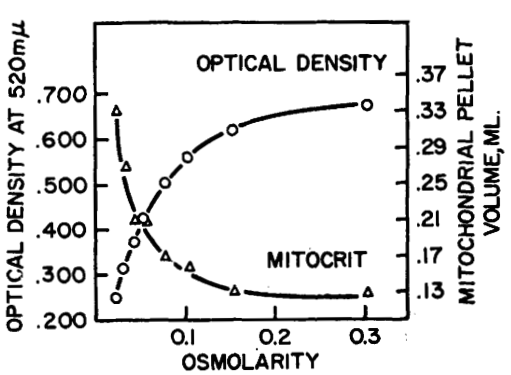

FIG. 2. Volume and optical density of mitochondria as functions of osmolarity of medium.

‡ The hematocrit, etymologically, refers to a blood measurement. Jackson and Pace(4) introduced the term mitocrit for measurement of mitochondrial pellet volume.

ment are shown in Fig. 2. Here, the mitochondrial pellet volumes and the corresponding optical densities have been plotted as functions of the final total osmolarity of the medium. The data clearly show that mitochondrial pellet volume increases continuously with progressive decrease in the osmolarity of the mitochondrial medium, and that associated with the volume increase there is a corresponding decrease in optical density of the mitochondrial suspension. This experiment has been performed, with minor variations, 5 times, always with the same result: mitochondrial volume increases as optical density decreases. The same result was also obtained in an experiment in which mitochondria in 0.30 M sucrose were induced to swell by the presence of inorganic phosphate.

For comparison of the rate of change of optical density with that of mitochondrial pellet volume, in Fig. 2 the units of the ordinates for the 2 parameters have been adjusted to make the total change in optical density equal to that in volume. The 2 curves are nearly symmetrical. However, as osmolarity decreases, the increase in mitochondrial volume at low osmolarities is somewhat greater than the corresponding decrease in optical density. This is probably due to incomplete packing of the grossly swollen mitochondria concomitant with their decreased specific gravity.

Discussion. A practical and a theoretical conclusion may be drawn from the results here presented. As to the former, measurements of mitochondrial pellet volumes confirm the validity of the optical density method of measuring relative mitochondrial volume. These data may be added to the evidence from photomicrographic measurements(2) and from measurements of mitochondrial water content(3). Thus, there is ample justification for choosing the simple opacimetric method for routine work.

Theoretically, the data are pertinent to the recently disputed view that mitochondria behave as osmometers. It is widely held that isolated mitochondria are impermeable to sucrose and similar non-electrolytes, and that they respond to osmotic changes in their environment according to classical osmotic pressure theory. Evidence for this view includes the stability of mitochondrial volume in sucrose at or near 0.3 M(2,5,6), mitochondrial retention of a variety of cofactors, substrates, and ions through the isolation procedure(7), similarity in the rules governing transfer of water and non-electrolytes across mitochondrial membranes to those which apply to plant cell membranes and to the red blood cell(2), relative impermeability of mitochondria to sucrose shown by Jackson and Pace(4), and the studies of "colloid-osmotic swelling" in this laboratory(5) which indicate that mitochondrial membranes are impermeable to sucrose.

On the other hand, Werkheiser and Bartley (8) have presented data in support of the view that mitochondrial membranes are partially permeable to sucrose and possibly also to molecules as large as inulin. In addition, the view that mitochondrial volume changes are in accord with classical osmotic pressure theory has been challenged indirectly by Fonnesu and Davies(9), on the grounds that small concentrations of adenosine monophosphate, with Mn^{++} or Mg^{++}, could largely protect the mitochondria from swelling in hypotonic sucrose solutions as low as 0.04 M. Possible alternative interpretations of these experiments are presented elsewhere(5). The results here reported on the behavior of mitochondria in hypotonic sucrose solutions clearly follow classical osmotic pressure theory. The progressive increase in mitocrit values as the external osmotic pressure is progressively decreased, is the expected behavior if the mitochondria are osmotic systems impermeable to sucrose. It may be pointed out that the permeability characteristics of the mitochondrial membrane can only be inferred indirectly from measurement of mitochondrial volume changes, regardless of the method used. Nevertheless, if future work confirms the recently expressed view that the mitochondrial membrane is permeable to sucrose (8), or that the mitochondria under certain circumstances do not adjust osmotically to external 0.04 M sucrose(9), then it would appear to us that a wholly new theoretical approach, other than classical osmotic pressure theory, will have to be taken to explain the experimental results presented here and in the

work of Tedeschi and Harris(2) and others, cited above.

Summary. The opacimetric and hematocrit methods for measuring relative mitochondrial volume are compared. Data from mitochondria swollen by exposure to hypotonic sucrose solutions demonstrate the validity of the simple opacimetric method, since, as osmolarity decreases, rate of increase in mitochondrial pellet volume approximates rate of decrease in optical density of the mitochondrial suspension. The results are discussed in terms of a current dispute regarding permeability of the mitochondrial membrane to sucrose.

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Action of Colicines on *Shigella boydii* and on their Rough and Mucoid Variants. (23967)

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Certain antibiotic substances produced by strains of *Escherichia* and *Shigella* have been defined as colicines. A recent review of colicines(1) has been published by Fredericq, a pioneer in this particular field. Halbert and Gravatt(2) have reported on the relation of *Shigella* type specificity and susceptibility to antibiotic producing strains of *Escherichia coli.* As extension and partial confirmation of their report, 170 *Shigella boydii* cultures† consisting of serotypes 1 through 11 and the 5 provisional serotypes were tested for possible characteristic patterns of sensitivity to 28 colicines. Some correlation between serological type and sensitivity to colicines has been revealed by this study.

The present report deals with an observation made during the testing of the aforementioned *Shigella* cultures. It was noted that R (rough) and M (mucoid) colony variants seemed somewhat more susceptible to colicines than S (smooth) colonies of the same original culture.

Procedures. Further experiments were performed to establish the validity of these sensitivity differences. A group of colicine producing strains of *Escherichia* and *Shigella*‡ were tested against randomly selected *Sh. boydii* strains utilizing the chloroform-killed plate assay technic of Gratia and Fredericq(3). R variants were obtained from an S colony of *Sh. boydii* 1, Strain No. 1701, by the methods indicated in Table I, and simultaneously tested with the S parent organism against colicines. The results shown in Table I and Fig. 1 indicate the marked sensitivity of R variants to some colicines employed in contrast to the relative resistance of the S parent culture. Sensitivity differences between R or M variants and their corresponding S parent cultures were also demonstrated in 2 other

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