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The relation  $a/b = 3/\sqrt{2}$  may perhaps be derived by starting with a result of Sitte and Glaser. These authors<sup>2</sup> derived the following relation:

$$h/(Z m_p c) = R/(Z' \sqrt{Z})$$

where Z' is the total number of particles in the universe, Z the total number of "heavy" particles (whose mass is large in comparison with the mass of the electron), and R the equilibrium radius of the universe. If we distinguish between protons and neutrons (a distinction not made by Sitte and Glaser), we must put

$$Z'=2P+N, \ Z=P+N$$

where P is the total number of protons or electrons, and N the total number of neutrons, respectively.

We thus find

$$\frac{h}{(P+N)m_pc} = \frac{R}{(2P+N)\sqrt{P+N}}$$

or

$$b = R \sqrt{P + N}/(2P + N).$$
  
Now, according to Eddington<sup>3</sup> and the author<sup>4</sup>  
 $R^2 = P a^2,$ 

or the surface of a sphere which would include the total equilibrium volume of the universe, is equal to the sum of the spheres of action of all electrons. Hence

$$a/b = (2P+N)/\sqrt{(P+N)P}.$$

If we make the very simple assumption that in the state of equilibrium the number of protons equals the number of neutrons, we thus actually find

$$a/b = 3/\sqrt{2}$$
.

The occurrence of the integers three and two in this formula finds its explanation in the fact that we distinguish three essential types of primordial particles, two of which are "heavy."

Conversely, we may conclude from the observational value of the ratio between the electronic radius and the Compton wave-length of the proton that in the equilibrium state of the universe one third of its primordial particles are protons, one third electrons and one third neutrons.

UNIVERSITY OF NOTRE DAME

ARTHUR E. HAAS

# SCIENTIFIC APPARATUS AND LABORATORY METHODS

# A GROWING YEAST MEDIUM FOR THE CUL-TIVATION OF AN HEMOPHILIC BACILLI AND OF AN ORGANISM CAUSING A BRONCHITIS IN CHICKENS<sup>1</sup>

THE writers obtained much better growth of Hemo-philus gallinarum and Hemophilus influenzae<sup>2</sup> when cultivated with growing yeast<sup>3</sup> than could be obtained with the use of a chicken blood medium.

The growing yeast supplies all the growth requirements for both organisms, and they have been successfully cultivated for several months in such a blood-free medium (by weekly transfers).

The yeast and the hemophilic bacilli were cultivated on a medium of the following composition: Difco dehydrated nutrient agar, 23 grams; Difco phenol red maltose, 10 grams; salt, 8 grams; added to 1,000 cc of the broth in which 400 grams of raw potato had been cooked. The sterilized tubed medium was used in the form of slants, at the base of which a small amount of sodium chloride solution was used to prevent drying of the surface. The growing yeast on this medium results in a change of the pH from acid to alkaline in

<sup>2</sup> K. Sitte and W. Glaser, Zeitschr. f. Physik, 88: 103, 1934.

<sup>3</sup> A. S. Eddington, Proc. Roy. Soc. London (A), 133: 605, 1931.

<sup>1</sup>Published by permission of the Director of Research as Contribution No. 527 of the Rhode Island Agricultural Experiment Station.

<sup>2</sup> Cultures obtained through the courtesy of Dr. John H. Dingle, Harvard Medical School.

<sup>3</sup> Pure yeast obtained from Fleischmann's Stock and Poultry Yeast.

reaction within 24 hours at  $37^{\circ}$  C. incubation. The change in pH is possibly responsible for the success of the medium. Difco phenol red dextrose and sucrose have been substituted and used instead of the maltose, with similar results. A medium prepared by using plain agar, Bacto beef extract, maltose, phenol red and salt, in the same proportions as the medium already described, would indicate the change from acid to alkaline results from the reaction of the yeast on the beef extract, because, when Bacto peptone is substituted for the beef extract, the medium remains acid.

Since the growing yeast fulfilled the growth requirements of the hemophilic organisms better than blood, the question arose as to whether it would be of value in studying other respiratory diseases of poultry.

An infectious bronchitis of chickens of a clinically similar type<sup>4</sup> as that which our studies had indicated was of a filterable virus nature was studied in this respect.

Cultures were carefully obtained from the edematous fluids of the lungs of infected birds and used along with pure cultures of yeast to inoculate the medium. Previous investigations had indicated that the lungs were frequently free of bacteria as found by the use of chicken blood media.

Growth other than yeast was obtained from one chicken out of five in this manner. Stained preparations indicated two different types of organisms in

<sup>4</sup> A. Haas, Anz. Akad. Wiss. Vienna, 67: 161, 1930 and 69: 91, 1932.

<sup>4</sup> J. P. Delaplane, L. E. Erwin and H. O. Stuart, *Jour. Agr. Res.*, 52: 5, 382, 1936. addition to the yeast. The first seven transfers of this culture induced symptoms of bronchitis in chickens when inoculated intra-tracheally. One type of organism overgrew the other, and it was no longer infective after the seventh transfer. The cultures in which no growth but yeast occurred failed to induce the infection in chickens, indicating the causative agent had grown in the one in which other organisms had been noted.

A culture obtained from the trachea near its bifureation with the lungs was found to show, upon microscopic examination of stained smears of what appeared as pure yeast colonies, a moderate growth of an apparently pure culture of an organism which had been one of the types present in the first successful cultures. Chicken blood at the base of nutrient agar slants, inoculated with the same material, remained sterile.

The organisms in question are comparatively large, very irregular in shape, appear singly, double or in clumps, and are decolonized with Gram's stain after 24 hours' incubation. The shape may vary from irregular circular to pear or rod shapes.

The typical symptoms of the bronchitis have been successfully reproduced during twelve transfers of the cultures over a three-week period from the time they were first isolated. The same organisms have been successfully reisolated from the infected chickens, and in turn found to be capable of inciting the disease.

The organisms have repeatedly failed to grow in or on chicken blood and other types of media, so that further studies will be required before any identification is possible.

The use of such a medium has resulted in the isolation of an organism which may have otherwise escaped detection.

> J. P. DELAPLANE H. O. STUART

RHODE ISLAND STATE COLLEGE

### PRESERVATION OF ANATOMICAL SPECIMENS

A SIMPLE method to preserve anatomical specimens for museum and teaching purposes may be utilized by applying the following solution; one part of formalin, three parts of 95 per cent. alcohol and two parts of ordinary white shellac. The cadavers from which the specimen is obtained have been preserved for varying periods of time from several months to a year or longer, with equal parts of 95 per cent. alcohol, phenol and glycerin, to which a small quantity of formalin has been added, approximately one pint to five gallons of the above solution.

Groups of muscles, leaving the origin and insertion upon the bones with nerves and blood vessels if desired, hearts and other organs have now been kept in this laboratory for the past five years with this method. After dissection, if necessary, the material is allowed to dry from 8 to 24 hours and then with a sprayer two or three applications of the solution are used, allowing each to dry before another coating is given. Respraying the specimens every year or two helps to keep them in good condition.

JOHN R. PATE

THE GEORGE WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

# CELLOPHANE USED FOR PROJECTION DRAWINGS

In studying the vascular pattern of the thymus, a quick, inexpensive method for showing the relationships of vessels was found by projecting the sections on sheet Cellophane such as is used for wrapping parcels, etc. Different parts were represented by colored ink, and mistakes were easily rectified by washing them out. Composite line-drawings were made from a number of single sheets and these in turn put together, until the completed drawing was made. The Cellophane wrinkles somewhat when removed from the roll, but this has not been found to be a hindrance as the pieces were kept flattened out on cardboard by thumb tacks. This method has proved very useful when time and expense were limited.

CHRISTIANNA SMITH

MOUNT HOLYOKE COLLEGE

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