this direction is largely responsible for the great length of the book.

Besides the problems usually treated under the kinetic theory of gases there is included about fifty pages on specific heats and about sixty pages on electric and magnetic susceptibilities. These subjects are dealt with in a necessarily sketchy fashion, since they belong more properly in statistical mechanics. A most unusual feature of the book is the chapter of about one hundred pages on the subject of ionic mobilities on which the author is a distinguished authority.

Another novel feature of this book is the extensive use of the results of molecular beam experiments in discussing the basic questions of velocity distribution, low pressure and surface phenomena. There is unfortunately a serious slip on page 540, where it is stated that the magnetic deflection pattern has a maximum at a distance  $z_a$ . This maximum, as is well known, occurs at a distance more nearly equal to  $(1/3)z_a$ .

There are also extended changes in Chapter 5 on the "More Accurate Equation of State" and in Chapter 6 on "Transfer Phenomena" to include a treatment of molecular force fields. The changes have proved rather unfortunate, since they come at a time when the work of Massey and Mohr and of Uhlenbeck has shown that these considerations are quite inadequate and that the results of the quantum theory of collisions can not be neglected.

However, despite some defects this book remains one of the best to put into the hands of beginning students of kinetic theory.

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#### CYTOLOGY FOR STUDENTS

Introduction to Cytology. By L. W. SHARP. Pp. xiv+567. McGraw-Hill Book Co. 1934.

THE third edition of Sharp's "Introduction to Cytology" maintains the high standards of the previous editions, which have made this book the leading text in its field. The subject is treated from the standpoint of cell structure and morphology, with emphasis on chromosome behavior in relation to genetics. A general description of cells and tissues is followed by several chapters on various cell constituents. A description of chromosome structure, chromosome morphology, mitosis and meiosis serves as an introduction to six chapters on the more important aspects of the new hybrid science, "Cytogenetics." These are followed by chapters on chromosomes and sex, apomixis and cytoplasmic heredity. A historical sketch of the development of cytology is presented in the last chapter, followed by an extensive bibliography.

The transfer of most of the literature citations to footnotes makes the references available without breaking up the continuity of the text. An outstanding feature of the book is the impartial treatment of controversial subjects. A good balance is maintained between facts and theories which should be stimulating without misleading the student. The emphasis on cytogenetics is in keeping with the numerous important contributions on chromosome behavior in relation to genetics, taxonomy and evolution. To students in these fields of biology, as well as to students of general cytology, Sharp's book is indispensable.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## ON THE CULTIVATION OF SEVEN SPECIES OF TRYPANOSOMES IN VITRO<sup>1</sup>

DURING the last five years the writer has successfully cultured the following species of trypanosomes on N.N. media.<sup>2</sup> One or two strains representative of each species have been maintained *in vitro* for periods of from nine months to over three years.

With some experience one can distinguish the cultural forms of certain species from the others herein reported, on the basis of their morphology and rosette formation as they appear when cultivated *in vitro* under identical conditions. Thus the individual cell, as well as rosettes of Tr. duttoni, differ from Tr.

<sup>1</sup> From the Hygienic Laboratory, University of Michigan. americanum and Tr. avium. The Tr. americanum in culture differs from that of Tr. melophagium; the former has its peculiar groupings and movements and the individual cells are relatively much larger, while Tr. cruzi, by virtue of its broad and slender forms, its movement and rosette formation, can also, at times, be distinguished from species mentioned above.

All species studied formed circular colonies on the slant portion of blood agar tubes. Tr. americanum, Tr. duttoni and Tr. cruzi colonized much sooner and more readily on this medium than Tr. lewisi or Tr. rotatorium. The latter species formed colonies only after several months' cultivation; however, once they commence forming colonies, afterwards they colonize readily. The colonies of Tr. rotatorium at times reached about 8 mm in diameter and closely resemble colonies of B. megatherium, while the colonies of Tr.

<sup>&</sup>lt;sup>2</sup> F. G. Novy and W. J. MacNeal, "Contributions to Medical Research, Dedicated to Victor Clarence Vaughn," p. 549. George Wahr, Ann Arbor, Michigan, 1903.

TABLE I

The species of trypanosomes	The species of ani- mal from which the trypanosome was isolated	Cultures	
		No. of days main- tained in vitro	No. of sub- cultures made
Tr. americanum (Tr. theileri)	Cows (Near Ann Arbor, Mich.)	<b>1,01</b> 0	61
Tr. avium	Passer domesticus (English sparrow)	868	42
Tr. avium	Screech owl	883	46
Tr. cruzi	From guinea-pigs experimentally in- fected with intesti- nal contents of <i>Tri-</i> <i>atoma Geniculata</i>	619	34
Tr. duttoni	Mus Musculus (''Common house mice'')	231	14
<b>Tr.</b> lewisi	Rattus norwegicus (Laboratory white rats)	1,224	56
Tr. lewisi	Rattus norwegicus (Rats)	224	12
Tr. melophagium	Melophagus ovinus (Sheep keds)	375	28
Tr. rotatorium	Rana pipiens (American frogs)	834	44

americanum, Tr. cruzi and Tr. duttoni resemble more closely the B. typhosus colonies. Although the colonies resembled bacterial colonies, in all the microscopical examinations they revealed a solid mass of flagellates with healthy protoplasm. As a rule the individuals in the colonies were somewhat round and pear-shaped, but quite active, and those in the water of condensation were more active and usually had the morphology of crithidia or herpetomonas.

### INFECTIVITY AND SPONTANEOUS ATTENUATION OF TRYPANOSOMES IN VITRO

A strain of Trypanosoma lewisi (No. 201) was isolated on November 22, 1930, and was kept in vitro until the time of writing (March 31, 1934). Although this culture was infective for rats during its first few months in vitro, at the end of two years it had completely lost this power. Normal young rats were splenectomized and several cultures were inoculated in these animals, but no infection was produced. A recently isolated strain of Tr. lewisi cultivated in vitro for four months is still capable of producing an infection in rats. As a rule Tr. lewisi during its first year of subcultivation in vitro is infective for rats, but after about a year it gradually begins to lose this power. It is a definite fact that Tr. lewisi when kept in culture for two years on N.N. media is completely innocuous for rats.<sup>3, 4</sup>

The initial culture of  $Tr.\ cruzi$  was obtained on July 20, 1932. After maintaining it *in vitro* for 468 days, it was inoculated into *Mus musculus*, into hairless *P. m. gambelii* (American deer mice) and into a guinea-pig. All these animals contracted the infection, trypanosomes were demonstrable in scanty numbers in their peripheral circulation and were recultured. Thus this species of trypanosome is still infective after 586 days' cultivation *in vitro*.

Tr. duttoni was cultured in vitro on March 2, 1933; 135 and 229 days after initial cultivation it was still infective to *Mus musculus*.

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#### AN ARTIFICIAL SYMBIOSIS

In order to keep tissue cells in vitro alive and healthy it is the practise of tissue culturists to renew the medium at frequent intervals. Such a procedure is supposed to increase the supply of oxygen and decrease the concentration of harmful by-products of metabolism. The three most common methods are: (1) Cutting out a fragment of tissue from the old culture and transferring it to fresh medium (usually a mixture of embryonic or other tissue extract and plasma); or (2) bathing the clot containing the culture in physiological salt solution (Tyrode's solution) for part of an hour, adding a small quantity of new medium before resealing; or (3) continuous perfusion of the culture chamber with a renewable fluid. Now if oxygen and food materials could be delivered to the very doorway of the cells and the by-products of metabolism be carried away, much as is done by the capillary circulation in the intact animal, there should be an improvement in health of tissues in vitro. Like the capillary circulation, the interchange should be continuous and relatively rapid.

There are many animals (protozoans, coelenterates, flatworms, etc.) which can live without feeding, provided they harbor green algae. The animal lives in the light, dies in the dark. The animal obtains carbohydrates and oxygen from photosynthesis and has its carbon dioxide and nitrogenous wastes removed. The alga gets nitrogen compounds and carbon dioxide from animal respiration. The common, unicellular green alga, *Chlorella*, is frequently found thus living symbiotically. A sterile culture of *Chlorella* grows very well on an inorganic salt agar medium.

Cultures were made combining the green algae with embryonic chick connective tissue cells and macro-

<sup>&</sup>lt;sup>3</sup> F. G. Novy, W. B. Perkins and R. Chambers, Jour. Infect. Dis., 11: 411, 1912.

<sup>4</sup> A. C. Behrens, Jour. Infect. Dis., 15: 24, 1914.