TABLE II

EFFECT OF THYROIDECTOMY ON MALE RATS (3½ MOS. OLD) OPERATED ON AT THE AGE OF 1 MONTH

No. rats	Body wt. (gm)	Weight in mg				Maternal behavior
		Ant. Hyp.	2 Adr.	2 Thyr.	2 Testes	
20	215	13	36	.....	2,965	Yes
20*	290	6	37	41	2,989	No

* Controls.

for long periods on a pile of newborn rats in a can placed on a table; (4) picking up the newborn rats and licking them in a maternal way; (5) desire to doze and snuggle in corners or up against objects, if given the freedom of a large shelf in the laboratory; (6) tendency to huddle in small groups, at intervals licking each other affectionately, when allowed to roam at will on the floor.

In both groups, the pituitaries were considerably enlarged, compared with those of the male controls. No mammary development was observed in the thyroidectomized males.

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EGG QUANTITY AND THE RESPIRATORY RATES OF SEVERAL MARINE EGGS

IN previous publications[1] the rates of oxygen consumption by the eggs of *Fucus, Cumingia, Nereis, Chaetopterus* and *Arbacia* were expressed in mm^3O_2 per hour per 10 mm^3 eggs. The quantity of eggs was determined in volume units by centrifuging to approximate equilibrium packing of the eggs in calibrated vaccine tubes having diameters of the order of 2 mm. As the eggs distort to pack tightly under strong centrifugal force, the measured volumes were regarded as only slightly too great, due to interstitial space among the eggs. The centrifugal force used was not reported.

More recently, in the case of *Arbacia,* Gerard and Rubinstein[2] have compared volume determination by centrifuging, and by haemocytometer counts and dilution counts with measurements of egg size. They have found centrifuge determinations to average 80 per cent. or more too great. This was with relatively low centrifugal force, 400–750 × gravity. Shapiro[3] has compared the equilibrium centrifuge volume of *Arbacia* eggs at various centrifugal forces with the volume determined by haemocytometer and dilution counts. He finds that at 2700 × g centrifuge volumes agree with determinations by haemocytometer counts to within an average of approximately ± 10 per cent. Fifteen determinations showed an average greater volume by centrifuging of 12 per cent., while fourteen showed an average lesser volume of 7.7 per cent.

Since the magnitude of the error of volume determination by centrifuging depends on the centrifugal force (being greater at low force), the particular centrifugal forces used in deriving the respiratory rates referred to above should be reported. When conversion factors have been established for the several eggs the rates may then be converted to absolute volume units. Late in the summer of 1934, with the kind assistance of Dr. Samuel Pond, the same (unaltered) centrifuge previously used at the Marine Biological Laboratory for the eggs referred to (except *Fucus*) was accurately calibrated under conditions previously used. The centrifugal force was 2850 × g, or if an allowance of 10 per cent. speed retardation during calibration is made, it may have been as high as 3400 × g. The duration of the original centrifuging in the cases of *Cumingia, Nereis* and *Chaetopterus* was 15 minutes. *Arbacia* eggs were centrifuged in some cases 15, in some 10 and in some 18 minutes. *Fucus* eggs were centrifuged 15 minutes or longer at lower centrifugal force, probably of the order of 1500 × g.

This does not affect relative rates previously given for the same eggs measured before and after fertilization. Comparisons[4] of absolute rates of different species of eggs (or of the same species when volumes are measured by different methods) are untenable,[2] except upon the assumption that the errors of volume determination are small (or are similar[5]).

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

STROBOSCOPIC OBSERVATION OF CILIARY MOVEMENT IN THE PROTOZOA

STROBOSCOPIC observation is carried out by means of light interrupted into consecutive flashes of known frequency and duration. In the study of normally beating cilia under a microscope supplied with stroboscopic light it is possible by varying the flash frequency to obtain the effect of slowing the cilia to any

1 *Jour. Gen. Physiol.,* 15: 167–200, 1931; and 16: 475, 1933.
2 *Ibid.,* 17: 375, 1934.
3 *Biol. Bull.,* in press.
4 *Jour. Gen. Physiol.,* 16: 497, 1933.
5 J. Runnström, *Protoplasma,* 20: 1, 1933.

speed desired or of arriving at complete standstill. If the number of ciliary beats per second be represented by x, then the effect of motionless cilia will be obtained at a flash frequency of x or multiples of x, and also at $\frac{1}{2}\ x$, $\frac{1}{4}\ x$, and on down to the limits of visual perception. The individual cilium will be seen at more than one position at "harmonics" above x, thus making it possible by varying the frequency to determine the true speed of ciliary beat. After x has been roughly determined the higher harmonics may be used to reduce eyestrain and cut down the probable error. Consistent practice will overcome the subjective difficulties involved.[1]

Although the stroboscopic method gives far more accurate results than narcosis or other common techniques for studying ciliary movement it has been rarely used and then usually through the medium of the motion picture camera. The cost of film makes the photographic method impracticable for extended observations.

Very satisfactory stroboscopic effects were obtained by the writer by interrupting the focused rays of a projection lamp at their point of convergence by means of a rotating 9″ bakelite disk bearing two $\frac{3}{8}$″ holes one inch from the periphery and at opposite sides of the disk. This arrangement gives two flashes per revolution at a time relation of 5 per cent. light to 95 per cent. darkness. At a ciliary frequency of, say, 20 cycles per second the duration of each flash is but .0025 seconds, which amply satisfies the optical requirements concerned. The disk is mounted on a shaft of a small electric motor controlled by a variable resistance. The speed is ascertained by a polygraph mechanically connected to an eccentric on the shaft. Either dark-ground or conventional condenser may be used and the usual magnifications employed.

Preliminary studies have been made on *Vorticella microstoma, Paramecium caudatum, Epistylis* sp., and *Stentor coeruleus*. Temperature was not under strict control in this introductory work, varying with the room from 20° to 24° C.

The *Vorticella* studied showed ciliary frequencies of from 6 to 8 cycles per second.

P. caudatum was studied in groups accumulated in debris in an old culture. Three zones of cilia were found: (1) the "body" cilia; (2) the longer cilia of the oral groove, which were seen to begin at a point just aboral to the anterior tip of the animal, coming up around the tip and progressing posteriorly in a narrow triangular area with its base at the cytostome; (3) the cilia of the gullet. The zones were differentiated by their differences in speed and nature of beat. Because of the movements of the animals it has been possible to date to measure accurately the frequency of the cilia in group 2 only, a value of 8 cycles per second being found. Studies of these cilia in six *Paramecium* in one group revealed a variation of less than 0.1 cycles per second.

The average of several individuals in two colonies of *Epistylis* gave: colony A, 12 and colony B, 11 cycles per second. A pair of animals in colony *A*, which from their position on the stalk were daughters of a recent division, showed consistent frequencies of 48 for y and 50 for z at the $4x$ harmonic. This is an absolute frequency of 12 and 12.5, respectively.

The "body" cilia of *Stentor* were found to be more or less irregular in action, so the membranelle series was used for this study. Individuals from a flourishing culture showed a range of frequencies of 32 to 42 cycles per second; those from an old run-down culture were very consistent at 26 per second. Preparations sealed against evaporation dropped off as much as half in speed in twelve hours. The pH and oxygen supply are under investigation. The metachronal wave covers seven membranelles, five of which are in recovery, two in effective stroke. The effective stroke ends in a vertical position, while in recovery the membranelles do not bend back in the same plane, but out and down over the edge of the animal until at the end of recovery they are twisted nearly 180° away from their position at the beginning. This results in the effective stroke in a lash directed upward and inward. The twisting reaction in *Stentor* resulting in the detachment of the foot is due to a lengthening of the effective stroke of the membranelles to about 25° past the vertical, the frequency remaining unchanged. Once the animal is freed and swimming ahead the character of the beat returns to normal, the frequency still unchanged.

Interesting fields of investigation are suggested by these results obtained by the stroboscope. The forms studied from run-down cultures showed the least variation in frequency of ciliary beat. The variations found in forms from flourishing cultures may be correlated in some way with division, thus suggesting a new approach to the problem of the relation of division to vitality. Stroboscopic observations are important in the question of coordination in ciliates, *e.g.*, none of the so-called coordinating fibrillar systems so far described in *Paramecium* will explain the three different zones of ciliary activity in that animal. Further, the determination of the frequency of beat and the length of the metachronal wave makes it possible for the first time to calculate the speed of the coordinating impulse in Protozoa. In *Stentor*, the only form in which accurate measurements of the metachronal wave have been made to date, the value of 30 microns was obtained. This, multiplied by the highest frequency obtained, gives a maximum value of only 1.2 mm per

[1] Gray, *Proc. Roy. Soc. London*, B 107: 313, 1930.

second. The other ciliates studied obviously have a much lower speed of coordinating impulse. These values are so far below the velocities of true neural impulses that it is not possible to consider a "neuromotor" factor in the coordination of these Protozoa.

Further investigations are in progress along the lines indicated here and will be reported in detail.

The writer is indebted to Dr. W. M. Barrows for suggesting the use of the stroboscope in the study of the Protozoa.

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DARKFIELD MICROMANIPULATION WITH AN ULTROPAQUE ILLUMINATOR

RECENTLY I had been studying the surface precipitation reaction[1] in the ameba. The experimental work consisted in tearing *Amoeba proteus* and *A. dubia* with a microneedle in various salts solutions. This technique is essentially similar to that used by Chambers and Reznikoff.[2] If an ameba is carefully torn in a solution containing an appropriate concentration of Ca- or Sr-ions, a new plasmalemma will be formed at the zone of tearing. Before the new plasmalemma is formed, some of the protoplasm will exude into the surrounding medium in the form of granules, crystals and unorganized matter. This more or less rapid disintegration of protoplasm will continue until the new plasmalemma is formed. The time at which this new plasmalemma is produced may be more easily determined if the ameba is studied under darkfield illumination.

Although special darkfield condensers are available for micrurgical technique, their use is somewhat limited. A specially designed moist chamber is needed. The regular substage condenser must be removed before the darkfield condenser can be installed. Brightfield illumination as furnished by the substage condenser is indispensable for making preliminary adjustments of the microneedles. In order to use both brightfield and darkfield illumination it is necessary to interchange these condensers. This procedure complicates the micrurgical technique and frequently the original adjustments of the microneedles are disturbed.

An ideal arrangement should furnish either brightfield or darkfield illumination without involving an interchange of condensers. Also this arrangement should permit the use of any style or height of moist chamber. The Ultropaque illuminator[3] adequately fulfils these requirements.

This instrument is a vertical illuminator permitting the use of a large series of objectives. The light source, consisting of a low voltage Mignon lamp, is an integral part. Color, neutral or heat-resisting filters may be conveniently used. The entire unit is mounted on the microscope in place of the revolving nosepiece. The substage condenser need not be removed. Each objective is furnished with a ring condenser. A slight rotation of this condenser will produce varying degrees of illumination ranging from brightfield to darkfield without disturbing those adjustments previously made either with the microscope or with the micromanipulator.

I use the following procedure. The amebas are mounted in a shallow hanging drop on a coverslip which is then placed on the moist chamber. The ring condenser is adjusted to give darkfield illumination. The preliminary adjustments of the microneedles are made in a brightfield furnished by the substage lamp and condenser. Two toggle switches conveniently placed near the micromanipulator are used to control the two sources of light. At any time, during or after the ameba is torn, the substage lamp may be quickly turned off and the Ultropaque turned on. Thus the effect produced by the microneedles on the protoplasm may be immediately studied with an excellent darkfield illumination.

Some of the intermediate degrees of illumination are exceedingly useful. In a semi-darkfield the *plasmalemma/medium* interface may be seen very distinctly. A semi-brightfield is useful in studying the nucleus; while the minute granules within the ectoplasm may be made visible if extreme darkfield is used. In transmitted light these ectoplasmic granules are not evident. Thus any change in the size, aggregation or number of granules can be readily detected. In a darkfield the tips of the microneedles when in focus appear as very bright luminous points.

The use of darkfield illumination makes possible a closer check on the condition of the cytoplasm. The importance of being able to detect various changes in the cytoplasm (Brownian movement, streaming, etc.) while micrurgical experiments are being conducted has also been pointed out by Plowe.[4]

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[1] L. V. Heilbrunn, *Arch. f. exp. Zellf.*, 4: 246, 1927.
[2] *Jour. Gen. Physiol.*, 8: 369, 1926.
[3] Made by E. Leitz.
[4] *Protoplasma*, 12: 196, 1931.

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