lein fades in alkali and can be regenerated by acidification but does not gel. The time of gelation after acidification is dependent upon the initial concentration of the dye (Table 1).

TABLE 1

Molar concentration of dye	Gelation time
1.0×10^{-2}	1 min.
4×10^{-3}	35 min.
1.7×10^{-3}	only viscous liquid

The fading process consists in a high dielectric solvent like water of the rate-determining addition of a univalent negative hydroxyl ion to the divalent negative ion of brom phenol blue forming a colorless carbinol. In solvents of dielectric constant less than 64.5, the kinetic process involves the univalent $(NaBr\Phi B)^-$ ion. On acidification the colorless carbinol reacts with hydrogen ion forming the yellow acid form of brom phenol blue, since the addition of alkali sufficient to neutralize the acid added regenerates the characteristic blue color of the dye in intensity dependent upon the length of time lapsing after acidification. The kinetics of this process is being investigated.

It is suggested that the gel formation is dependent upon hydrogen bond formation. The gel gives evidence of being thixotropic. It is stable for only a few hours, after which time brown crystals, presumably the acid form of the dye, appear and grow steadily throughout the gel.

A solution 10^{-2} M in dye and 0.2 N in NaOH was not completely faded in 27 days. When heated to 60–70° for 20 minutes to hasten the fading we observed that the blue color of the original solution was regenerated. After 4 hours at room temperature the color faded, approximating that of the original on the 27th day. Gelation occurred both before and after heating.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A PRACTICAL METHOD OF OBTAINING BACTERIA-FREE CULTURES OF TRICHOMONAS HOMINIS

Trichomonas hominis has been isolated in bacteria-free culture by using the following procedure: 6 mm Pyrex tubing 8 inches in length is plugged with cotton in each end, wrapped in paper and then sterilized by dry heat. The paper is removed from the sterile tube and, using a micro-burner, a capillary tube 16 inches long is drawn from one end of the tube. The distal tip is then broken off with a sterile forceps (great care must be exercised throughout the remainder of the procedure not to contaminate the terminal 4 inches of the capillary until it has been sealed). The tube is grasped in one hand and the proximal portion of the capillary tube in the other hand; then, by working on the edge of a very low flame, a series of loops or traps 3 or 4 mm in height are made as shown in the illustration.

Using a rubber tube (portion of an 18-F catheter) attached to a 10-cc syringe, the butt end is slid on the cotton-plugged end of the tube. Suction is then applied and the tube filled to within one inch of the top with sterile liquid medium. (Ringer 1 part, horse serum 8 parts.) Great care must be taken to see that no air bubbles are introduced into the capillary portion of the tube. The distal end of the capillary is sealed in a flame so that very little if any air is trapped in that portion of the tube. For stability and protection the capillary end of the tube is now slipped into a clear Cellophane envelope and the envelope fixed to the tube by adhesive tape. The tube is then incubated in a vertical position in a special rack (using pinch clothes pins for holders), for 48 hours and then observed for sterility. If no turbidity develops, for practical purposes it can be considered sterile. The liquid suspension of protozoa and bacteria is then carefully inoculated by layering into the top of the tube. The inoculated tubes are kept vertical and incubated at 37° C. From time to time the migration of the protozoa can be observed under the microscope by mounting the microscope on a platform so that the stage can be held in a 45 to 90° angle to the horizontal plane and using a mechanical stage prepared as illustrated. Trichomonas migrate slowly, and it usually takes 48

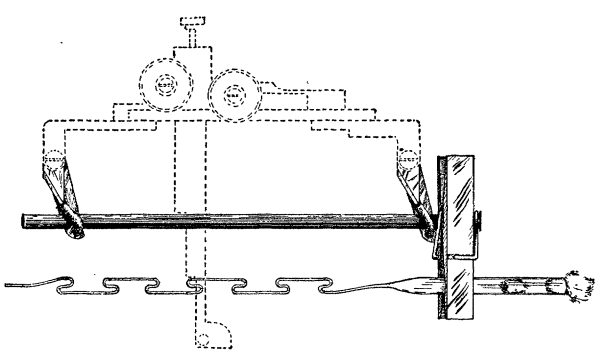

FIG. 1

hours or longer for the organisms to reach the bottom of the tube.

When the protozoa have reached the bottom of the tube the distal end of the capillary is cut off and sealed at the same time by applying a small flame to the area of the last trap. The severed sealed capillary is again observed for actively motile protozoa (usually many

present) and is then submerged in tr. iodine (7 per cent.) for one hour. At the end of this time the capillary is removed from the iodine by using a sterile forceps. The last sealed end is then carefully grasped by the fingers and the capillary held in a vertical position. The iodine drains toward the fingers, and when the outside of the tube is dry, segments from the distal end of the tube are broken off, with a sterile mosquito forceps, into selected culture media. *Trichomonas hominis* has been repeatedly isolated bacteria-free by using this technique.

Discussion

Our observations on the migration of motile protozoa seem to indicate that, with the exception of the free living phototactic organisms, their migration in liquid media is greatly influenced by the force of gravity. Very little progress is made against the force of gravity unless currents in the liquid support this movement. Currents in the liquid contents do not occur in capillary tubes, whose outside diameters do not exceed 0.8 mm. Migration of organisms in the liquid of the capillary apparently is influenced only by the motility of the organisms and the force of gravity, which result in gradual migration down the capillary. Motile bacterial organisms seem to be unable to make progress beyond the second trap and practically all of them are held back by the first trap.

The apparatus should be adaptable to the isolation of other actively motile flagellates and perhaps ciliates. However, we have not had the opportunity to test it using other organisms.

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THE USE OF THE HORSLEY-CLARKE INSTRUMENT ON THE RAT[1]

Since the reintroduction[2] of the Horsley-Clarke instrument[3] its use on the cat and monkey has become quite popular. A recent modification[4] has proved of considerable value. Lately it has been found possible to utilize the same instrument on albino rats by employing a few easily constructed special parts.

Ear plugs were dispensed with since the ear bars on the machine can be seated directly into the external auditory meatuses of the rat. Then, in order to center the animal, a c-shaped clamp is fastened to the ear bars. This makes it possible to loosen the screws holding the ear bars in position on the frame of the instrument and to move the animal back and forth, although the ear bars themselves remain rigidly seated in the rat's ears. A transverse bar and a light nose clamp were substituted for the usual mouth and eye clamps. After the ear bars are seated and the animal centered the nose-piece is adjusted so that the rat's long upper incisors rest just in front of the transverse bar. The nose clamp is then lowered. This centers and immobilizes the nose and holds the maxillae firmly against the transverse bar. No exact dimensions of these parts need be given, for they should be built so as to fit in the Horsley-Clarke machine with which they are to be used. Any machinist having the Horsley-Clarke instrument before him and with photographs of these parts which will be furnished on request should have no difficulty in making the special parts.

The operative procedures are quite simple. Under Evipal anesthesia (0.1 gm/kg. intraperitoneal) the hair is cut off the top of the head, the animal placed in the machine and an incision 1.5 to 2.0 cm long made through the skin in the midline. The electrode carrier is then set at the zero midsagittal plane and the electrode slowly lowered. If the tip of the electrode does not come to rest exactly upon the interparietal suture the animal is improperly placed in the machine and must be removed and replaced properly. The tip of the electrode is then raised and an opening in the skull made with the aid of a dental drill. Then the electrode is lowered to the desired point and the lesion made.

In working with rats it is very necessary that animals of uniform size and age be used. We have found it convenient to select animals weighing between 90 and 100 gms. If animals of more widely varying size are used little uniformity can be expected in the location of the lesions.

It would be desirable to produce chart sections of the rat's brain for the determination of coordinates of nuclei and fiber tracts, as has been done in the case of the cat and monkey. So far, however, such chart sections have not been made, since by the sacrifice of a few animals the location of any desired point in terms of the coordinates of the instrument can be determined by the method of trial and error.

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