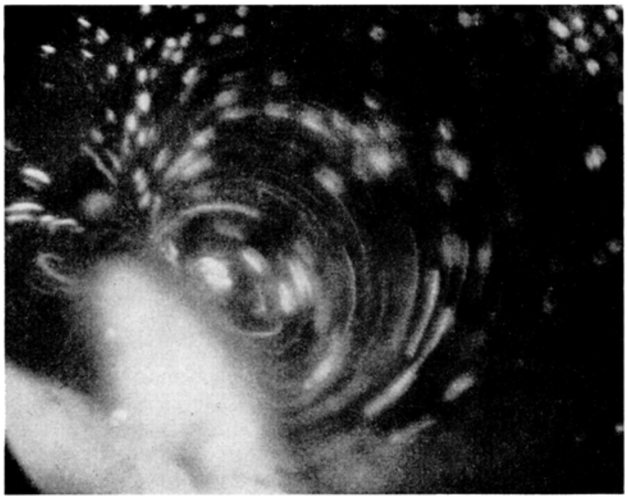

Fig. 1

Fig. 2

Fig. 3

les mouvements d'eau entourant la partie antérieure de l'animal qui entre dans le champ d'observation, ou les courants laissés en arrière par une paramécie qui est en train de sortir du cadre.

Les premières observations indiquent que la méthode d'enregistrement microphotographique est également utilisable dans le cas d'autres êtres unicellulaires munies de cils ou de flagelles. En comparaison avec la méthode classique du dessin, elle élimine l'influence d'une impression subjective du chercheur, elle permet d'analyser toute l'image compliquée simultanément dans tous ses détails, et enfin elle semble remplacer une illustration par des documents qu'on peut obtenir en nombre voulu.

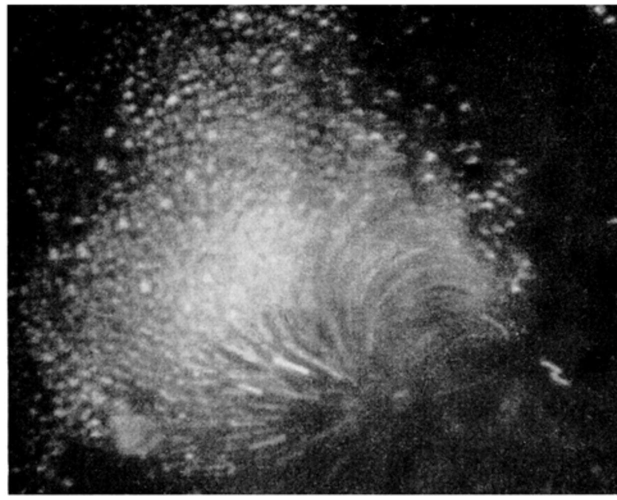

Fig. 4

Summary. The movement of a liquid medium around a protozoan cell evoked by cilia was registered microphotographically. Bright illuminated particles of emulsion or suspension, photographed in the dark field during a long exposure time, mark a course of the water microstreams.

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PRO EXPERIMENTIS

Further Simplification of a Supernatant Flow System for Tissue Culture

This paper concerns a further simplification of flow systems for automatic uniform provision of small volumes of supernatant to tissues in culture previously described from this laboratory[1,2]. The system is illustrated in the Figure.

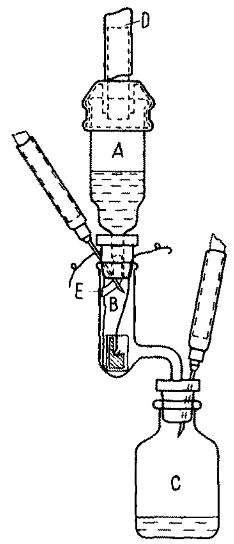
The supernatant flows from a 15 mlcapacity Buchner funnel with medium porosityfritted disc into the culture chamber B and thence to the collection bottle C. The rate of supernatant flow is regulated automatically by the unaided passage of air through the dialysing membrane D. The piece of dialysing membrane is held in an airtight fashion over the end of a length of Pyrex Nr. 234100 glass tubing by a segment of latex tubing ($^1/_4$" I.D., $^1/_{16}$" wall). (The rate of flow can be altered by using glass tubing of different diameters or membranes of different porosity). The open end of the glass tubing is inserted into the upper end of the Buchner funnel through a red rubber, sleevetype, serum stopper. The fritted disc prevents the regurgitation of air into the reservoir from the culture chamber and thus helps smooth the flow of supernatant. When the flow system and chamber is first put into the incubator, the expansion of air in the Buchner funnel forces 3 or 4 ml of supernatant through instead of the usual daily 1–2 ml.

[1] A. W. B. CUNNINGHAM and B. D. ESTBORN, *Chamber and Flow System for Quantitative Tissue Culture*, Laboratory Investigation, March-April (1958), p. 156.

[2] A.W.B. CUNNINGHAM and W.E. HERBST, *A Simplified Quantitative Tissue Culture Flow System*, Laboratory Investigation, May-June (1960), p. 384.

[3] This research was supported by a P.H.S. Grant H-2249 from the National Heart Institute, U.S. Public Health Service.

This has the beneficial effect of helping to wash away blood or breakdown products resulting from the explantation. The needle air-vents with cotton bacterial filters in the culture chambe B and receiving bottle C allow free exit for gases to the external air and promote free flow from B to C. The piece of cellulose sponge E inserted into the lower end of the Buchner funnel and touching the side of the culture chamber serves to conduct the supernatant to the side of the culture tube, smooth out the flow and prevent traumatisation of the culture by drops of supernatant. A piece of chemically clean braided fiberglass sleeving in the outflow arm of the culture tube facilitates the exit of supernatant from the culture tube and thus helps to prevent changes in the level of supernatant in the culture chamber. Bacteria are filtered out of the air during its passage through the dialysing membrane thus preservin gthe sterility of the supernatant.



Schematic diagram of apparatus

Zusammenfassung. In der Arbeit wird eine Einrichtung für kontinuierliche Flüssigkeitszufuhr beschrieben, wie sie für Gewebskulturen wertvoll sein kann. Durch eine feinporöse Membran kann die Menge der Flüssigkeit reguliert werden.

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COGITATIONES

Spontaneous Miniature Activity and Gradation of Transmission at the Neuromuscular Junction

The discovery of the randomized spontaneous miniature activity at the neuromuscular junction by Fatt and Katz[1,2] opened new perspectives for the interpretation of facilitation of synaptic transmission[3–5]. Specifically, it was shown in frog- and mammalian nerve-muscle preparations that the neurally evoked endplate potential (epp) results from a statistical coincidence of quantal units of identical magnitude (i.e. the min epp's)[6–8]; that a conditioning nerve impulse enhances the probability of occurrence of spontaneous miniature activity as well as increases the quantum content of response to a subsequent nerve volley[9,10]; and that the quantum content of a neurally evoked response increases in the course of tetanic stimulation[9,10], while marked augmentation of spontaneous miniature activity persists for several minutes in the post-tetanic period[8,11].

However, del Castillo and Katz ([12], see there Table I) also pointed out that rate of spontaneous miniature discharges and quantum content of evoked epp are not necessarily covariant: e.g. Mg ions effect a decrease of quantum content of epp, while leaving the frequency of spontaneous miniature activity unaltered. Furthermore, a decrease of quantum content of epp after prolonged tetanic stimulation is accompanied by increase of min epp-frequency[9]. Weak anodic polarization of terminal motor nerve branches augments the evoked epp, but the spontaneous discharge rate remains unaltered; cathodic polarization, on the other hand, depresses transmission and augments spontaneous discharge rate[12,13]. An additional instance of dissociation between spontaneous and evoked activity is provided by the fact that spontaneous discharges continue in the absence of Na-ions and in preparations depolarized by K_2SO_4[14], wherefrom it is apparent that spontaneous miniature activity 'does not depend upon the occurrence of electrical activity of the action potential type in any structural unit within the nerve terminal'[5].

To these instances of dissociation between direction of change of spontaneous and evoked junctional activity may now be added the finding of Kraatz and Trautwein[15] that 2, 4-dinitrophenol, while greatly augmenting spontaneous random discharge rate, diminishes the epp-amplitude. Hydrazinium-ions[16], and tetrodotoxin in the presence of NH_4^+-ions[17] block transmission without affecting the discharge rate of min epp's. Conversely, guanidine apparently augments the evoked epp[18], while spontaneous miniature activity does not change[17]; however, spontaneous giant potentials appear with an amplitude smaller than that of evoked epp's, but larger than that of min epp's. Botulinus toxin, on the other hand, depresses frequency of spontaneous discharges and reduces the amplitude of the evoked epp as well[11].

In summary, then, there is ample evidence that the mechanism for spontaneous quantal discharges can operate in autonomy from nervous control; and that the frequency of spontaneous activity does not uniquely define the potential of the presynaptic apparatus to transmit incoming nerve volleys to the postsynaptic site. The differences between spontaneous and evoked junctional activity, to which del Castillo and Katz[3] directed attention, can, however, be ascribed to the responsiveness of the motor nerve endings to invasion by a nerve impulse (see: factor 'N' in [12]). This, then, would constitute an additional limiting step in the chain of events of transmission, and would attribute to spontaneous miniature activity a permissive, rather than a determining role in junctional transmission and facilitation of transmission.

It then becomes pertinent to inquire of what nature this limiting neural event is, and whether it is subject to gradation by physiologic and pharmacologic parameters.

In the spinal cord, posttetanic potentiation of the monosynaptic reflex is paralleled by augmentation of the pre-

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