(STRASBURGER[1], p. 731) gave strength to this hypothesis. $ZnCl_2$-KI-I stained the radicles of seedlings of *A. dimorphostegia* blue, and their hypocotyls yellow. These are the typical reactions of the dye to cellulose in the former organ and to cutin in the latter.

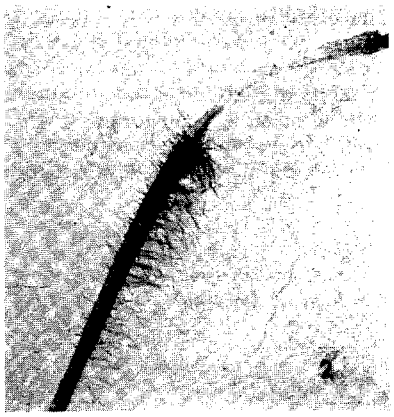

Fig. 2. The boundary between radicle and hypocotyl of a seedling of *Atriplex dimorphostegia* stained with *réactif genevois*. Note that the boundary of the staining corresponds with the boundary of root hairs.

Staining with 2% carmin in acetic acid followed by a rinse in water left a red colour on the radicles of *A. dimorphostegia*, while the hypocotyls remained unstained. With this stain, the boundary between the organs was not as clearly defined as with *réactif genevois* (Figure 1).

$ZnCl_2$-KI-I is effective only when highly concentrated, and slight dilution, even by the water within or adhering to the seedling, will render this reagent ineffective. Carmin-acetate did not clearly define the boundary between the two organs. Therefore *réactif genevois* appears to be most suitable in distinguishing between the radicle and hypocotyl in whole seedlings.

Résumé. Dans les études où la distinction entre l'hypocotyle et le radicule est nécessaire, le *réactif genevois* s'est montré le colorant le plus approprié.

M. NEGBI

Department of Botany, The Hebrew University, Jerusalem (Israel), May 12, 1965.

A Modification of the Technique of the Gaddum and Stephenson Microbath

GADDUM and STEPHENSON[1] have described a method for the evaluation of substances contained in small volumes, which has been employed for the determination of substance 'P' (GADDUM and SZERB[2]) and acetylcholine (SZERB[3]). In this method, muscular contraction sets in motion a small mirror which reflects a beam of light to a photocell. The graphic inscription is taken after adequate amplification of the cell's potential. The final amplification reaches up to 600 times the muscular contraction.

In this work a very simple and economic modification of the amplifying system is described. The microbath used is the same as originally described, its capacity is 0.06 ml, which is reduced by the muscle inserted. The amplifying system consists of two levers which, rotating simultaneously, set in motion a small mirror which reflects a beam of light onto a screen. The levers rotate horizontally around two axles similar to those used in watches, thus putting no weight on the small muscle. The levers are made of glass capillaries with an external diameter inferior to 0.5 mm. The first lever, whose arm relation is 1:10 is joined to the muscle at one end and at the other to the second lever by means of a drop of liquid vaseline or light oil. A small mirror attached to the axle of the second lever reflects a beam of light.

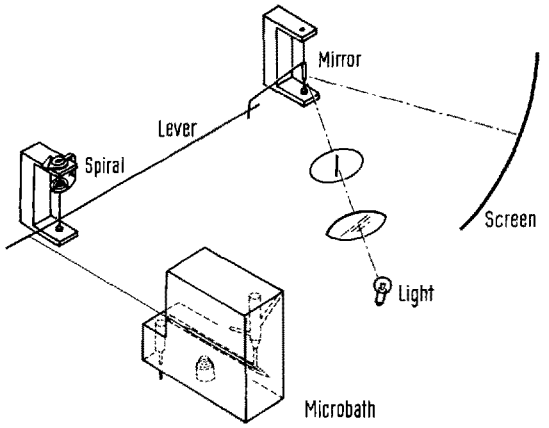

Fig. 1. Schematic drawing of the device used to amplify muscle contraction.

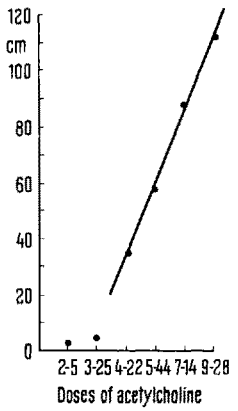

Fig. 2. Relationship between response of the rectum abdominal muscle of the toad and dose of acetylcholine (ng content in 0.05 ml).

[1] J. H. GADDUM and R. P. STEPHENSON, Brit. J. Pharmacol. *13*, 493 (1958).
[2] J. H. GADDUM and J. C. SZERB, Brit. J. Pharmacol. *17*, 451 (1961).
[3] J. C. SZERB, J. Physiol. *158*, 8P (1961).

By means of a watch spiral placed on the first axle a convenient tension is applied to the muscle (maximum 250 mg). The beam of light is reflected by the mirror onto a centimeter-graduated semi-circular screen, placed at a distance of 1 m and equidistant at every point from the mirror. Under these conditions, the system provides an amplification of about 1000 times. The responses are measured by reading directly on the screen, in cm, the course of the beam of light. Doses of acetylcholine were generally used which produced readings of about 100 cm.

For the dosage of acetylcholine, a small longitudinal strip of the rectum abdominal muscle of the toad (*Bufo arenarum* Hensel) was employed. This was obtained from the zone above the abdominal vein. In most cases this zone is no more than 2–3 mm wide, and is easily distinguished from the rest. Relaxation is rapid, allowing for a dose to be tried every 5–10 min. In this muscle the response was proportional to the logarithm of the dose, and the effects were clearly different when tried in two points with doses whose relation was 3:4 or 4:5. The responses were reproducible and in 10 experiments the index of precision gave a mean value for λ of 0.047 $\pm$ 0.007. The useful doses for this muscle oscillated between 8–15 ng in 0.05 ml.

Résumé. Une modification à la technique du microbain pour le dosage de matériel biologique est décrite. La contraction musculaire, amplifiée mécaniquement, est mesurée par le trajet d'un rayon de lumière sur un écran.

H. BUZZO

Instituto de Investigación Médica 'Mercedes y Martín Ferreyra', Córdoba (Argentina), July 16, 1965.

STUDIORUM PROGRESSUS

Arousal Threshold in the Cat as a Function of Sleep Phase and Stimulus Significance

Recently, a number of investigators, notably DEMENT and KLEITMAN[1] and JOUVET et al.[2], have differentiated in both man and animals between two types of naturally occurring sleep. The predominant phase of sleep is referred to as *high voltage sleep*; the other, which is associated with dreaming in humans, is variously called *paradoxical sleep, activated sleep, rhombencephalic sleep*, and *REM sleep*. In addition to its low voltage EEG characteristics, this latter stage of sleep has a number of other distinguishing features. The one which is most relevant to this paper is that arousal thresholds are reported to be higher in paradoxical sleep than in high voltage sleep[2–4]. However, assessment of arousal thresholds was based primarily on electrical stimulation of the brain stem reticular formation[2,3] and on responses to neutral auditory stimuli[4]. Those studies which have presented meaningful stimuli to subjects during high voltage and paradoxical sleep were primarily concerned with investigating learning and discrimination during the sleeping state as evidenced by differential responsiveness while still asleep[5]. In addition, these investigators established stimulus significance by employing an aversive situation.

The experiment reported here directly investigated differences of arousal to significant and non-significant stimuli during high voltage and paradoxical sleep and used an appetitive situation to establish stimulus significance (the delivery of milk to food and liquid deprived cats).

Two adult cats were intensively investigated. Each animal was prepared with permanent recording electrodes on the cerebral cortex, in the dorsal hippocampus, and in the dorsal neck muscles. After recovery from surgery, each animal was adapted to a training compartment equipped with a loudspeaker, an observation window, and a dish connected to an automatic milk delivery device. Stimulus significance was manipulated by a four-stage program of training with awake subjects which consisted of: (a) adaptation to trains of clicks of various durations and intensities; (b) conditioning to associate the termination of a train of clicks with delivery of milk (the click intensity was 81 db SPL for one subject and 85 db SPL for the other); (c) extinction training which consisted of presentation of the clicks alone; and (d) reconditioning which was effected in the same manner as the original conditioning.

During all conditioning and testing, the subjects were maintained on an 18 to 20 h food and liquid deprivation schedule. A short duration conditioning stimulus was used initially, and then gradually lengthened to 100 sec (the situation was that of a Pavlovian delayed conditioning paradigm). Test trials, which were presented only during sleep, consisted of a 50 sec train of clicks alone. As soon as clicks presented during sleep resulted in behavioral arousal, the clicks were terminated by the experimenter. Cortical and hippocampal EEG and dorsal neck EMG were monitored on a Grass polygraph in order to distinguish between high voltage and paradoxical sleep.

Figure 1 represents the percentage of awakenings for each cat from high voltage sleep to clicks during each of the four stages of the experiment: (A) before conditioning, (B) after conditioning, (C) after extinction, and (D) after reconditioning. Statistical analysis indicates a significant difference in the percentage of arousals between each successive pair of experimental conditions, i.e. between (A) and (B), (B) and (C), and between (C) and (D). The results of test trials after extinction were essentially the same as the results of test trials before conditioning, indicating that the clicks during these two stages of the experiment had approximately the same arousal effects. It should be

[1] W. DEMENT and N. KLEITMAN, EEG clin. Neurophysiol. *9*, 673 (1957).
[2] M. JOUVET, F. MICHEL, and J. COURJON, C. r. Soc. Biol., Paris *153*, 101 (1959).
[3] O. BENOIT and H. BLOCH, J. Physiol., Paris *52*, 17 (1960).
[4] W. DEMENT, EEG clin. Neurophysiol. *10*, 291 (1958).
[5] N. BUENDIA, M. GOODE, G. SIERRA, and J. P. SEGUNDO, Exper. *19*, 208 (1963).