dried for 4 h at 105°C and hydrolysed in sealed tubes for 6 h at 120°C with 6 *N* HCl. The hydrolysates were evaporated almost to dryness, taken up with 1 ml of distilled water, neutralized to pH 7.0 with crystalline *tris*(hydroxymethyl)aminomethane, and made up to 10 ml with 0.1 *M* Tris buffer pH 7.0. The estimations were made on 1 ml aliquots.

Whereas the histone hydrolysate gave only a very faint colour, the maximum absorption of which was at 460 nm, the albumin hydrolysates gave valid results which are presented in the Table.

It may be concluded from these results that histone apparently does not contain any detectable cystine, and that the molar ratio of cystine in bovine serum albumin is 18; this last value agrees with that given by CATSIMPOOLAS and WOOD[3], whereas EDSALL[4] quotes that of 17.5.

Estimation of cystine in bovine serum albumin

| Experiment no. | 1 | | 2 | | 3 |
|---|---|---|---|---|---|
| Dry weight of hydrolysed protein, mg | 10.4 | | 8.8 | | 9.2 |
| Hydrolysed protein in nmoles[a] | 157.6 | | 133.3 | | 139.4 |
| Total volume of neutralised hydrolysate, ml | 10 | | 10 | | 10 |
| Optical density at 504 nm | 0.550 | 0.550 | 0.462 | 0.465 | 0.484 |
| nmoles/ml | 285.2 | 285.2 | 239.5 | 241.1 | 251.0 |
| µg/ml | 68.4 | 68.4 | 57.5 | 57.9 | 60.2 |
| Molar ratio | 18.1 | 18.1 | 18.0 | 18.1 | 18.0 |
| Weight % | 6.58 | 6.58 | 6.53 | 6.58 | 6.55 |

[a] The molecular weight used for the calculations was 66,000, given by EDSALL[4].

*Résumé.* Nous présentons une micro-méthode colorimétrique qui permet de doser la cystine dans des hydrolysats de protéines, dans un domaine de concentration compris entre 20 et environ 400 nmoles/ml.

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*Service de Biochimie des Nucléoprotéides, Institut de Biologie physico-chimique, C.N.R.S., Paris (France), July 5, 1965.*

[3] N. CATSIMPOOLAS and J. L. WOOD, J. biol. Chem. *239*, 4132 (1964).
[4] J. T. EDSALL, Exposés a. Biochim. méd. *18*, 86 (Masson et Cie., Paris 1956).
[5] Permanent address: Eisai and Co. Research Laboratories, Bunkyo, Tokyo (Japan).

## A Differential Staining for Hypocotyl and Radicle

The radicle and the hypocotyl of seedlings may react very differently to environmental factors, such as temperature, illumination and growth regulators. In many developmental studies, it is essential to distinguish between these two organs.

Facing such a problem we were able to stain differentially the radicles and the hypocotyls of whole seedlings by *réactif genevois*. This reagent, which was developed by CHODAT, Genève (Switzerland), is a mixture of the dyes chrysoidin and Congo red (STRASBURGER[1], p. 813). CHODAT developed this reagent for differential staining of cell wall constituents. Cellulose is stained pink, lignin bright yellow and cutin and suberin yellowish brown. It was found that bathing the whole seedling in *réactif genevois* for 5 to 10 min followed by a rinse in water leaves only pink on the growing radicle. The hypocotyl may be stained somewhat yellowish-brown but the distinction between the two colours, and hence the two organs, is easily made (Figure 1). In all species tested the morphological boundary between the hypocotyl and the radicle, namely the place where the proximally (oldest) situated root hairs mark it, was identical with the boundary of the staining (Figure 2). This method was successfully tried in several species of different families of *Angiosperms*. There was no case in which this method did not work. The seedlings tested were of: *Lactuca sativa* (lettuce), *Pisum sativum* (pea), *Lycopersicum esculentum* (tomato), *Beta vulgaris* (beet), *Salsola inermis*, *S. volkensii* and *Atriplex dimorphostegia*.

It is suggested that the cuticular coating of the hypocotyl prevents the reaction of cellulose with *réactif genevois* from taking place. Since such a coating is absent from the epidermis of growing radicles, the cellulose of the epidermis reacts with the stain. Staining with $ZnCl_2$-KI-I

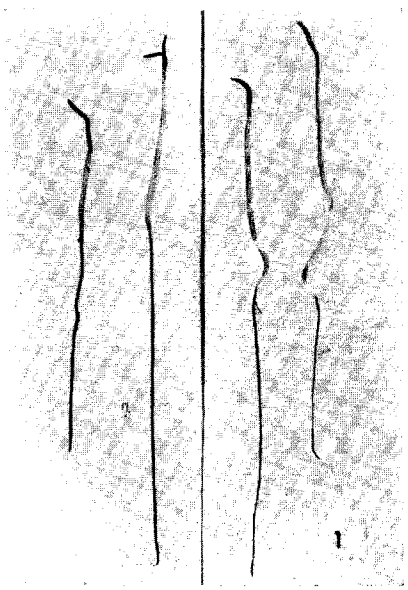

Fig. 1. Seedlings of *Atriplex dimorphostegia* stained with *réactif genevois* (2 on the right) and with carmine acetate (2 on the left). Note the sharp boundary between radicle (stained) and hypocotyl (unstained) with the former reagent.

[1] E. STRASBURGER, *Das botanische Praktikum*, 7th Ed. (Gustav Fischer, Jena 1923).

(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.

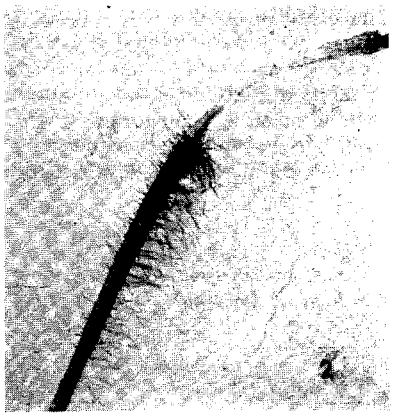

Fig. 2. The boundary between radicle and hypocotyl of a seedling of *Atriplex dimorphostegia* stained with *réactif genevois*. Note that the boundary of the staining corresponds with the boundary of root hairs.

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.

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## 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.

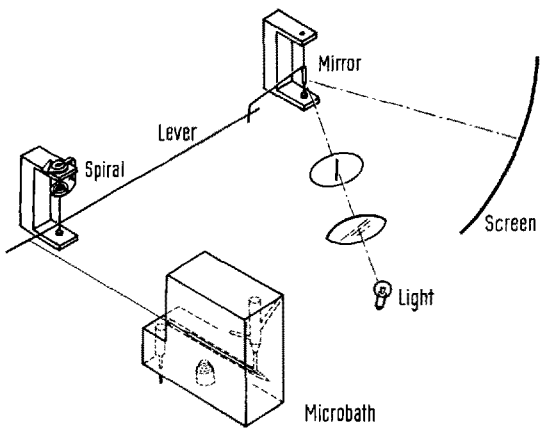


Fig. 1. Schematic drawing of the device used to amplify muscle contraction.

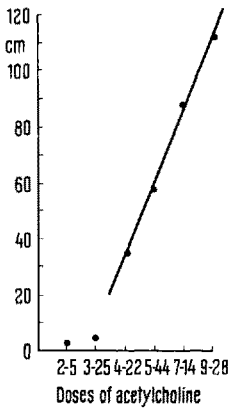


Fig. 2. Relationship between response of the rectum abdominal muscle of the toad and dose of acetylcholine (ng content in 0.05 ml).

[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).