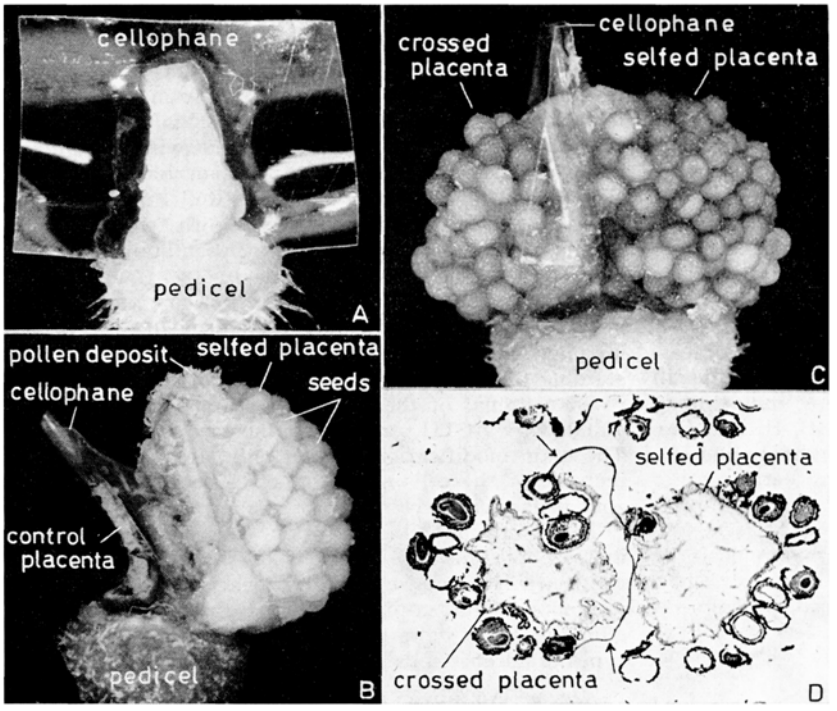


Figure A–D. Differential treatments of placentae in vitro in *Petunia axillaris*. A) Explant after insertion of cellophane as seen in face view of one of the placentae. ×7,8. B) Culture as seen 14 days after differential treatment (control vs selfed). Control placenta (left) has shrivelled and on the selfed placenta (right) several seeds are obvious. ×12,8. C) Culture 21 days after differential treatment (selfed vs crossed). Both placentae bear numerous seeds. ×13,3. D) Transection through middle region of the culture shown in C. Cellophane between the placentae is seen cut as a black coil (arrow-marked); seeds cut in favourable plane show the embryo. ×10,7.

tion, many cultures showed beginnings of seed development on both placentae, and mature seeds were formed in 21 days after pollination (Figure C, Table). Irrespective of their affiliation either to the selfed or to the crossed placenta, the majority of seeds contained normal embryo and endosperm (Figure D). There was a high degree of positive correlation between the seed set obtained on selfed placenta and that on the crossed placenta; the t value also showed no significant difference. The seeds (crossed as well as selfed) readily germinated and gave rise to normal seedlings. Squash preparations of root tips of seedlings obtained from selfed placentae showed the diploid chromosome number 14.

Thus, the technique of introducing a mechanical barrier such as cellophane between the placentae has made differential treatments of the 2 placentae of the *same ovary* possible. Further, this technique reduces the sample variation between the treatments to a minimum and facilitates a direct deposition of pollen grains on *definitive loci* of a placenta; also it offers a wide scope for studies of pollination with mixed pollen, irradiated pollen, and labelled pollen.

Zusammenfassung. Es wird eine Methode zur Vornahme verschiedener Bestäubungsversuche an ein und derselben Plazenta beschrieben.

K. R. Shivanna[6]

Department of Botany, University of Delhi, Delhi 7 (India), 10 August 1970.

[1] N. S. Rangaswamy and K. R. Shivanna, Nature, Lond. *216*, 937 (1967).
[2] K. R. Shivanna and N. S. Rangaswamy, Phytomorphology *19*, 372 (1969).
[3] K. Pearson and A. Lee, Biometrica *2*, 357 (1903).
[4] V. G. Panse and P. V. Sukhatme, *Methods for Agricultural Workers* (Indian Council of Agricultural Research, New Delhi 1967).
[5] The r value, the t value and f values for the 2 treatments were +0.557, 0.636 and 144 respectively.
[6] Acknowledgments. I am grateful to Dr. N. S. Rangaswamy for guidance, to Prof. B. M. Johri for facilities and to Dr. K. M. M. Dakshini for his help in statistical analyses.

Demonstration by a New Staining Method of Different Types of Cells in Adrenal Glands

In recent years much has been learned about hormone biosynthesis of adrenal glands but no substantial progress was made about their light microscopical appearence. However, some evident changes in physiological conditions in the classical zones of the adrenal cortex suggest that a better knowledge of the adrenal morphology is essential. In spite of this, the information required for adrenal morphology up to the present is obtained from sections stained with haematoxylin and eosin or with a few other histological and histochemical routine methods.

A new staining method recently proposed by Novelli[1] for collagen and reticulum gives interesting and sometimes surprising results if it is applied to the study of the histophysiology of adrenal glands, provided that a suitable fixation with dichromate has been used; several different types of cells in the various cortical zones and medulla can be demonstrated.

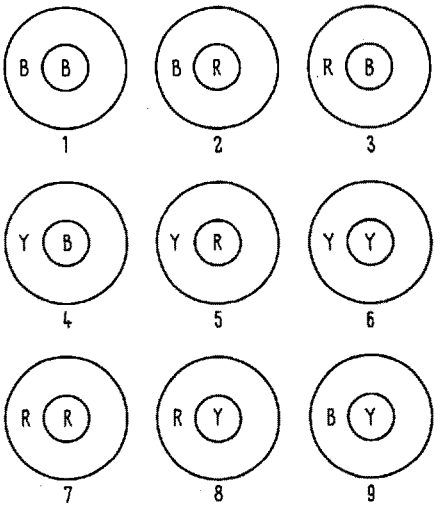
The following procedure is suggested: Fixation for 48 h at room temperature (22 °C) in Mueller fluid with 10% formalin; pH between 5–6, eventually adjusted with a 5% aqueous solution of potassium chromate. Paraffin sections of 5 µm. 1. Bring sections to water and then into 0.04% Evans blue solution in picric acid (aqueous saturated) for 20 min. 2. Rinse in water and place in 1% aqueous fucsin acid solution for 10 min. 3. Rinse in water, dehydrate, clear and mount.

Results. Adrenal cells assume different color combinations of the 3 acid stains employed. These combinations

(Figure) are basically the following: 1. cytoplasm blue (or violet blue) and nucleus blue (BB); 2. cytoplasm blue, nucleus red (BR); 3. cytoplasm red, nucleus blue (RB); 4. cytoplasm yellow, nucleus blue (YB); 5. cytoplasm yellow, nucleus red (YR); 6. cytoplasm yellow, nucleus yellow (YY); 7. cytoplasm red, nucleus red (RR); 8. cytoplasm red, nucleus yellow (RY); 9. cytoplasm blue, nucleus yellow (BY).

The distribution in the classical zones of the adrenal cortex and in the medulla are summarized as follows: Glomerulosa: BB, BR, RB; Fasciculata: BB, BR, RB, YB, YR, YY, RR, RY and BY; Reticularis: BB, BR, RB, YR, YY, RR; Medulla: RB, YR, YY, RR and RY.

The predominant types of cells are in normal guinea-pig: into glomerulosa: BB, BR; in the surface of fasciculata: BB, BR and YB, YR; in the inner zone (interfacies): RB and RR; in the reticularis: BB, BR. In the medulla: RY. The RB and RR types correspond to cells with a considerable amount of basic proteins associated to RNA. The BB and BR are cells with a certain grade of affinity for Alcian blue (pH 2.5), low amount of RNA and BERENBAUM[2] reaction for masqued lipids negative. The YY and YR are cells with cytoplasm stainable with BERENBAUM method but with an appreciable amount of RNA and are Alcian blue negatives. Pheochromocytes of the medulla (RY) are cells PAS positives and strongly stainable with pyronin. The medulla granula of epinephrine appear stained brown, granula of nor-epinephrine are black.



Different color combinations of the 3 acid stains (see text).

Another feature of adrenal sections stained by this new method is the presence into sinusoids of the reticularis and medulla of a homogeneous colloid-like material, staining blue. This blue material is particularly evident in the adrenal of the rats. The stimulation of adrenal glands with ACTH produces an increase of this material. The main modifications of the cells after ACTH are an increase of the cells of RB and RR types in the inner zone of fasciculata.

The cortisone administration seems to produce an increase of RR and RB cells of the glomerulosa and reticularis. Kidney ischemia by renal artery compression produces in guinea-pigs an increase of RB cells in the glomerulosa. A more deepened identification of the new types of adrenal cells requires supplementary researches.

Résumé. Au moyen d'un nouveau procédé de coloration proposé par l'A., on peut faire ressortir dans les glandes surrénales de Cobaye et d'autres animaux de laboratoire, differentes catégories de cellules caractérisées par diverses colorations cytoplasmatiques ou nucléaires. On propose une classification provisoire de ces catégories cellulaires.

A. NOVELLI

Istituto di Patologia Generale, II^a Cattedra, Università di Genova, I-16132 Genova (Italy), 3 December 1970.

[1] A. NOVELLI, Anat. Anz., in press (1970).
[2] M. C. BERENBAUM, Q. J. microsc. Sci. *99*, 231 (1958).

Dépouillement automatique des enregistrements polygraphiques de sommeil

L'analyse automatique des enregistrements polygraphiques de longue durée, en particulier du sommeil chez l'homme, peut rendre de très grands services. Elle permet de traiter avec une meilleure fiabilité de nombreux enregistrements, d'obtenir rapidement et sans peine l'essentiel des informations concernant la succession des cycles du sommeil, ainsi que des résultats chiffrés pour les autres variables enregistrées en polygraphie, telles que les mouvements oculaires, les activités musculaire, cardiaque et respiratoire. Diverses méthodes, tant analogiques que digitales, ont été proposées (BERGER et MEIER[1], ESTRIN[2], ITIL et al.[3], MACGILLIVRAY[4], SMITH et al.[5], FROST[6]). Certaines d'entre elles ne sont cependant pas adaptées à la pratique courante d'un laboratoire. Nous avons cherché à réaliser un système d'analyse à la fois précis et d'utilisation suffisamment simple pour un travail de routine chez de nombreux patients.

Les enregistrements de sommeil chez l'homme sont réalisés sur un appareil EEG classique (Schwarzer à 12 canaux) et sur bande magnétique (Philips Analog 7). On enregistre trois dérivations EEG antéro-postérieures, l'électrooculogramme (EOG), l'électromyogramme (EMG), l'électrocardiogramme (ECG) et l'électrospirogramme (ESG). Le dépouillement des bandes magnétiques se fait en temps différé à une vitesse 16 fois plus élevée que celle de l'enregistrement.

Le système d'analyse (Figure) effectue une première réduction des données par voie analogique, à l'aide de 18 analyseurs distincts; les données réduites sont alors directement traitées par voie digitale pour aboutir au résultat. La partie analogique comprend 12 filtres passe-bande, dont les fréquences de résonance sont réparties dans les différentes bandes du spectre (du delta lent au bêta rapide), un discriminateur de pente et un discriminateur d'amplitude et de temps pour l'analyse de l'EEG. Il y a en outre un discriminateur d'amplitude pour les mouvements oculaires lents, un discriminateur de pente et d'amplitude pour les mouvements oculaires rapides, un intégrateur pour l'activité musculaire, un dispositif à seuil à réglage automatique pour la fréquence cardiaque et, enfin, un discriminateur d'amplitude pour la fréquence respiratoire. Cette partie analogique est construite en système modulaire «Camac»[7] et reliée à la partie digitale par une interface constituée par des modules «Camac» standards. La partie digitale est un ordinateur Nova de 4 kmots de mémoire.