tive adenylate cyclase activity in rat caudate nucleus during halothane anesthesia in oxygen. The present halothane response and that of Nahrwold et al.[5] was obtained using air as the halothane carrier. Nevertheless, the measurement of cAMP is known to be dependent on tissue fixation[12] and subsequent handling, and consequently some other variable of the experimental procedures that is not readily apparent may yet account for the different results observed above.

cGMP: Rats administered halothane in air for 1 h exhibited a highly significant, dose-related increase in cGMP values above the control value (0.14 p moles/mg protein). This result for whole brain appears consistent with and confirms the results of Nahrwold et al.[5], who observed a several-fold increase in mouse cortex cGMP after administration of halothane in concentrations of 1% or more. The smaller response observed here for whole brain is consistent with relative weight contributions of different parts of the brain, and the marked decreases in cGMP observed[5] in cerebellum. The absolute values of cGMP are comparable to those of Dinnendahl[14], but somewhat lower here than those of Nahrwold et al.[5]. Differences in cGMP values may in part result from regional differences in samples or from post-decapitation anoxia[4,13]; however, constant levels of ATP and PC in these same samples (see below) would appear to rule out any major effect of anoxia. These metabolites decrease in response to anoxia[5,12].

Energy metabolites. Whole brain ATP and PC remained essentially unchanged (the slight increases are not statistically significant) from control values of 22 and 28 n moles/mg protein, respectively, following halothane administration. These control values and the halothane responses are comparable to results obtained by others[4,5,15].

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2 To whom reprint requests should be addressed at the Department of Neurobiology and Anatomy.
3 V.M. Wiegant, Brain Res. Bull. *3*, 611 (1978).
4 D.F. Dedrick, Y.D. Scherer and J.F. Biebuyck, Anesthesiology *42*, 651 (1975).
5 M.L. Nahrwold, W.D. Lust and J.V. Passonneau, Anesthesiology *47*, 423 (1977).
6 P. Divakaran, F. Joiner, B.M. Rigor and R.C. Wiggins, J. Neurochem. in press (1980).
7 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1979).
8 F. Murad, V. Manganiello and M. Vaughan, Proc. natl. Acad. Sci. USA *68*, 736 (1971).
9 A.L. Steiner, A.S. Pagliara, L.R. Chase and D.M. Kipnis, J. biol. Chem. *247*, 1114 (1972).
10 O.H. Lowry and J.V. Passonneau, in: A Flexible System of Enzymatic Analysis, p. 151. Academic Press, New York 1972.
11 S.Y. Woo, M. Verosky, Y. Wulliemoz and L. Triner, Anesthesiology *51*, 27 (1979).
12 W.D. Lust, J.V. Passonneau and R.L. Veech, Science *181*, 280 (1973).
13 A.L. Steiner, J.A. Ferrendelli and B.M. Kipnis, J. biol. Chem. *247*, 1121 (1972).
14 V. Dinnendahl, Naunyn-Schmiedebergs Arch. Pharmak. *284*, 55 (1974).
15 B.K. Siesjo, in: Brain Energy Metabolism, p. 233. Ed. B.K. Siesjo. John Wiley and Sons, Chichester 1978.

Anthocyanins in iris flowers

N. Ishikura[1]

Department of Biology, Faculty of Science, Kumamoto University, Kurokami, Kumamoto 860 (Japan), 1 September 1979

Summary. 6 kinds of anthocyanin have been found in the flowers of 5 iris species. They were identified as the 3-p-coumaroyl-rutinosido-5-glucosides and the 3-rutinosido-5-glucosides of malvidin, petunidin and delphinidin. The distribution pattern of the iris-flower pigments is discussed; it shows that *Iris* species belonging to the section Apogon differ from the plants of the sections Xiphium and Eupogon owing to the occurrence of malvidin glycosides in addition to delphinidin glycosides.

In Japan, there are 7 *Iris* species including 5 varieties and 2 forms[2], and hundreds of *I. ensata* var. *hortensis* garden varieties have been produced by Japanese horticulturists. They have been mainly bred in 3 distinct regions: Tokyo (old district name: Edo), Kumamoto (Higo) and Mie (Ise). In the preceding paper[3], the author showed that 90 *Iris* varieties of the so-called 'Higo' type which were examined could be divided into 3 types; A (79 varieties), B (10) and C (1) on the basis of the distribution pattern of the glycosides consisting of 11 kinds of anthocyanins. In order to provide a comparison with those experimental results, the author has carried out the present study on the flower anthocyanins of 4 wild species and also *I. tectorum* which was originally distributed in China.

Flower anthocyanins in 1% methanolic HCl extract were separated into several bands by paper chromatography in n-BuOH-HCl-H_2O (7:2:5). 6 pigments were identified: the 3-p-coumaroylrutinosido-5-glucoside of malvidin (MP), petunidin (PP) and delphinidin (DP), and the 3-rutinosido-5-glucosides of malvidin (M), petunidin (P) and delphinidin (D). 3 of these, MP, PP and DP, have hitherto been found as ensatin[3], petanin[4] and delphanin (= violanin)[5], respectively. R_f values were 0.45 (MP), 0.48 (PP), 0.37 (DP), 0.17 (M), 0.15 (P) and 0.10 (D). Each band was further purified by paper chromatography in HOAc-HCl-H_2O (3:1:8) (R_f values were 0.78, 0.73, 0.68, 0.80, 0.74 and 0.53, respectively). The ratio of individual anthocyanins in the flower extracts were estimated by a Tôyô Digital Densitorol DMU-33C (500 nm filter) using the paper chromatograms. The identification of the separated pigments was carried out as described earlier[6] and also by spectral and chromatographic comparison with authentic specimens. The anthocyanin distribution in the plants was as follows; Apogon section - *I. ensata* var. *spontanea:* MP (5)[7], PP (3), M (1), P (0.5), delphinidin glycoside (÷D) (0.5); *I. laevigata:* MP (3.5), PP (4.5), M (0.5), P (1), (÷D) (0.5); *I. sanguinea:* DP (6), PP (3), D (0.9), P (0.1), malvidin glycoside (trace). Evansia section - *I. japonica:* DP (5.5), PP (4), D (0.4), P (0.1), malvidin glycoside (trace); *I. tectorum:* DP (10), D (trace). Anthocyanins of both *I. ensata* var. *spontanea* and *I. laevigata* were very similar to those of A type of the 'Higo' varieties containing ensatin as the major pigment, but *I. sanguinea* and *I. japonica* were different from any of the 3 'Higo' types, because they contained only a trace amount of malvidin glycoside. According to Ueno et al.[8], *I. setosa* contains ensatin and a trace amount of delphinidin

hexoside. Thus, it should be noted that anthocyanins of Japanese wild iris consist of the glycosides of malvidin, petunidin and delphinidin. Of the plants in the Apogon section, *I. chrysographes* and *I. delavayi* have also been shown to contain a malvidin derivative in addition to delphanin[9]. Differing from those plants, *Iris* species belonging to the section Xiphium, e.g. *I. hollandica* cv. 'Wedgwood'[10] and *I. tingitana* cv. 'Prof. Blaauw'[11], and to the section Eupogon, e.g. tall bearded garden iris cv. 'Floridor' (Cayeux 1929)[12], contain only delphinidin glycoside.

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2 K. Tomino, Bull. Lib. Arts Dept., Mie Univ. *28*, 1 (1963).
3 K. Hayashi, Proc. imp. Acad. Tokyo *16*, 478 (1940).
4 N. Saito, R. Hotta, K. Imai and K. Hayashi, Proc. Japan Acad. *41*, 593 (1965).
5 K. Takeda, Y. Abe and K. Hayashi, Proc. Japan Acad. *39*, 225 (1963).
6 N. Ishikura and E. Yamamoto, Kumamoto J. Sci., Biol. *14*, 9 (1978).
7 Figures in parentheses give approx ratio (out of 10).
8 N. Ueno, E. Takemura and K. Hayashi, Bot. Mag., Tokyo *82*, 155 (1969).
9 J.B. Harborne, Phytochemistry *3*, 151 (1964).
10 N. Ishikura, Pl. Cell Physiol., Tokyo *19*, 887 (1978).
11 S. Asen, R.N. Stewart, K.H. Norris and D.R. Massie, Phytochemistry *9*, 619 (1970).
12 P. Werckmeister, K. Hayashi and Y. Yazaki, Züchter *36*, 233 (1966).

Some aspects of ovular development during megasporogenesis in *Pisum sativum* L.[1]

F.J. Medina and M.C. Risueño

Morfogénesis Celular, Instituto de Biología Celular, C.S.I.C. Velázquez 144, Madrid-6 (Spain), 30 July 1979

Summary. This work reports some findings which will aid us in our understanding of the female phase of *Pisum sativum* embryology. We have observed that the central planes between one ovule and the next form an angle of 60°. We suggest that this ovule arrangement may make it easier for the pollen tube to reach the ovular micropyle in the fertilization process. On the other hand, ovary and ovule growth do not depend on the megasporogenesis stage but have individual characteristics for each plant, every one depending on its own phenotype.

The main aspects of ovule and ovary structure in *Pisum sativum* have been understood for a long time. Cooper[2] reported the existence of a single ovary and the pattern of the ovular growth, the early arching of the ovules and the existence of only 1 megaspore mother cell (MMC) in each ovule. Recently, Rembert[3] has described the ovule and the ovary on *Trifolium repens*, a species belonging to the same family as *Pisum* (Papilionaceae) by means of scanning electron microscopy; his results confirm the earlier studies of Cooper and add some details about ovular development. Nevertheless, nothing has been said either about the relative disposition of the ovules within the ovary or whether the ovule and ovary growth are related to the megasporogenesis progress or not.

Material and methods. Floral buds of *Pisum sativum* L. were collected from plants growing in a greenhouse. Ovaries were dissected from the floral buds and fixed in 3% glutaraldehyde in 0.025 M cacodylate buffer for 2 h, then postfixed in 1% osmium tetroxide in the same buffer for 1 h, and after dehydrated in alcohol series and embedded in Epon 812. Semithin sections of 2 µm were made with a LKB pyramitome and observed under the light microscope, either directly by phase-contrast or stained with toluidine blue.

Results. Pisum sativum L. ovules are clearly orientated to the stylar pole of the ovary from the time that differentiation of the MMC begins. Their relative position is not random, because once a central, well-oriented section of an ovule has been obtained, it is possible to get another section, also well-orientated, of the contiguous ovule, by turning the section plane 60° approximately (figures 1, 2

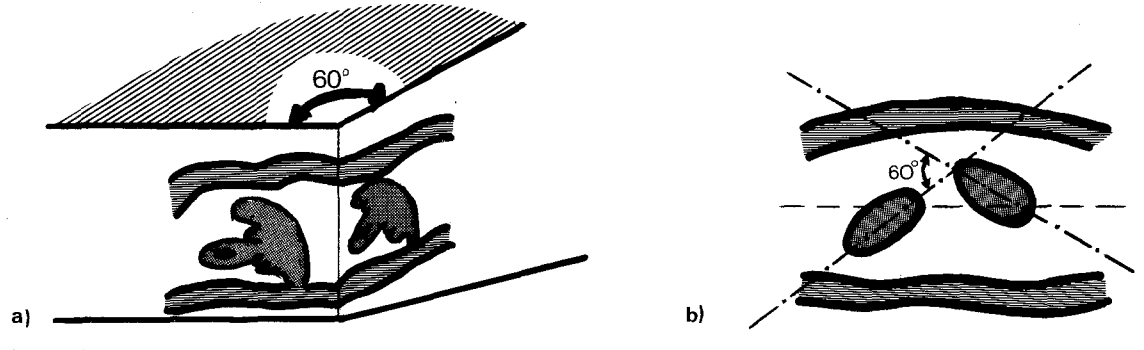

Fig. 1. Schematic drawings of the relative arrangement of the ovules within the ovary. *a* Front view of the Epon block showing 2 well-orientated sections of 2 contiguous ovules. We can get these sections by turning the section plane an angle of 60°. *b* Top view of 2 contiguous ovules; ---, central suture of the ovary, .-.-., micropyle - MMC - chalaza plane.