QUANTITATIVE AND QUALITATIVE CHANGES IN SHOOT APICAL MERISTEM OF SAFFRON *CROCUS* DURING THE PERIOD OF GROWTH AND DEVELOPMENT

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Abstract

Cytological, dimensional and mitotic activity changes in shoot apical meristem of saffron *Crocus* (*Crocus sativus* L.) during the period of bud growth and development are described. After the establishment of new apical meristem in November, slow activity of apex during the winter and spring produces a series of covering scales. Apical meristem of *Crocus sativus* is structurally simple and has a 2-3 layered tunica and a small unlayered corpus. No appreciable growth occurs in the shoot apex between mid-May till mid-July; this period is the phase of corm dormancy. From the middle of July onwards, the shoot apex begins to form leaf primordia which continue to increase in number till the beginning of August. In the period between the beginning and middle of August, the prefloral phase, rapid changes occur in the topography of the shoot apex leading to flower initiation and differentiation, the latter being of a protandrous type. But growth and development in saffron *Crocus* take place in seven stages: establishment of new apical meristem; formation of scales; leaf formation; prefloral phase; flower initiation and differentiation, growth of flower stalk; and growth of flower bud. Mitotic activity is highest in the prefloral phase. At this stage, with decrement in the length and increment in the breadth of the apex, the ratio of length to breadth is decreased.

Introduction

Crocus sativus L. is a perennial and herbaceous plant of the family Iridaceae. The red stigmatic lobes of the species are valuable for their odoriferous, colouring and medicinal properties and are widely employed in the food, drug and textile industries [6]. The plant is sexually sterile [5] and has been propagated vegetatively through corms since at least the 4th century B.C. [6].

Keywords: Flowering; Mitotic activity; Ontogenesis; Saffron

At the present time, establishing the connection between the transpiring processes in stem meristems and subsequent formative processes is one of the important tasks in the study of plant ontogenesis. Exchange of developmental programs occurs in apical stem meristems under the influence of hormonal substances during certain periods of ontogenesis. In ler to clarify the mechanisms governing the lization of various morphogenetic processes, it is cessary to establish what kind of changes occur in cal stem meristems during the period of program change. Development and differentiation in the ot apex of certain bulbous plants such as tulip [12], *ium candidum* [10] and *Iris* [11] has been reviewed. formation relating to seasonal periodicity in developmental events, particularly differentiation in the ot apical meristem, is lacking as far as saffron is ncerned [1,2,3].

The purpose of the present work is to clarify basic riods in the ontogenesis of saffron *Crocus* and anges occurring in the shoot apex specially with a w to studying flower initiation and differentiation.

Materials and Methods

Corms of saffron *Crocus* were obtained from the lage of Natanz. They were harvested at monthly invals between November (1992) and May (1993), at rtnightly intervals from May to mid-July, at weekly ervals between mid-July and early August, and later at intervals of three days till late August. At each ge corms with nearly uniform diameter (3-4 cm) re harvested.

Apical buds were excised from the corms together th a portion of surrounding tissue and were immedily fixed in FAA, Carnoy and Navashin solutions [3,]. After fixation, the buds were washed under runng water and transferred to 70% alcohol. Infiltration d embedding in paraffin wax was carried out by ssing the material through the usual alcohol-toluole ries [13]. The dehydrated material was gradually intrated with, and finally embedded in, paraffin wax 3]. Longitudinal sections, 7 µm thick, were cut. The ctions were stained with hematoxylin according to arris [3] and Schiff's reagent according to Feulgen 3].

Hydrolysis of DNA was conducted in 1 N HCl at -64°C for a period of 15 min. Observations were ade on five median longitudinal sections. The length and breadth of the shoot apex were directly measured by an ocular micrometer, the youngest leaf primordium being generally taken as the base line. The ratio of length to breadth was computed at each stage. The average of mitotic index in the meristematic region of shoot apex ($\frac{\text{Number of dividing nuclei} \times 100}{\text{Total nuclei}}$) was calculated on five median longitudinal sections. Changes in these parameters at different stages of bud growth and development were graphically drawn.

Results

Phenology of *Crocus sativus*

After the completion of flowering (late August), a daughter cormlet with one or two apical buds was formed near the base of the flower stalk on the maternal corms. The next three months (December to February) were marked by intensive growth of the leaves. The root system expanded greatly. The daughter corm also intensively grew during this period, whereas the maternal corm wrinkled and dried up.

With the advent of drying in April, the leaves and roots gradually dried out. This concealed underground, period of the saffron *Crocus* corm' life lasts from May to July. In August, root primordia appeared at the base of the corm and generative primordia took form in the apical bud. With a decrement of heat and increment of moisture in September, the apical bud grew intensively. Rapid growth of the flower stalk started at the beginning of October and flowering set in again in November (Fig. 1).

Properties of Vegetative Meristem and Its Mode of Action

After the completion of flowering, a new apical meristem forms near the base of the flower stalk on the maternal corm. At the early stage of formation, the apical meristem is small in size and slightly convex in form. Thereafter, the apex increases rapidly in size and produces the covering scales of apical bud (Fig.

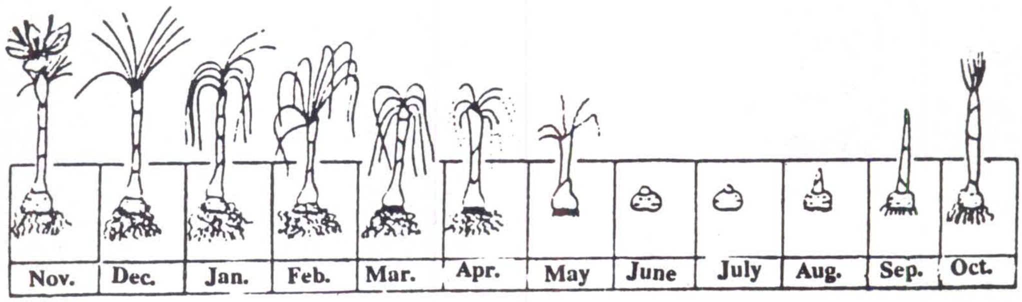

Figure 1. Ontogenesis of *Crocus sativus* L.

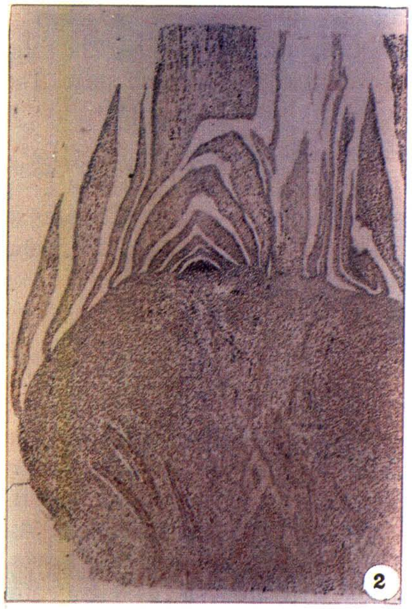

Figure 2. New apical meristem producing covering scales of bud (x 15)

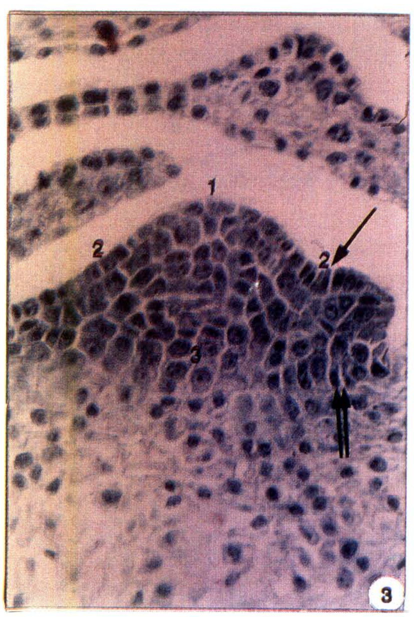

Figure 3. Vegetative meristem with 2-3 layered tunica and small corpus. Apical axial zone (1), lateral zone (2) and rib meristem (3) are distinguished. Periclinal divisions of subtunica layers (double arrow) and anticlinal divisions of tunica layers (single arrow) in lateral zones of meristem produce a scale primordium (x 300).

2).

The apical meristem is structurally simple with a two- or three-layered tunica and an unlayered corpus at its core (Fig. 3). During the winter and spring, the apical meristem periodically produces covering scales. The scale primordia arise as lateral protuberances initiated from the tunica. The initiation is marked by periclinal divisions in tunica layers. A lateral protuberance is thus formed and continued periclinal and anticlinal divisions in the subtunica and tunica layers contribute to the gradual enlargement of the scale primordium (Fig. 3).

No appreciable growth occurs in the apex between mid-May and the first week of July (period of dormancy). The first signs of growth in the shoot apex are manifest sometimes between the first and second week of July. The apex is shaped like a dome and some incompletely differentiated léaf primordia are observed laying well below the tunica layers on either side, lateral to the axis. The mode of action of apical meristem in leaf formation is similar to the formation of scales. Thus, from the middle of July up to the beginning of August the apex continues to produce a series of leaf primordia which undergo transition from primordial to young leaf stages of development (Fig. 4).

Properties of Prefloral Stage

After the completion of leaf formation, the shoot apex is broadly domed, high and bilaterally symmetrical, structurally simple with a two-layered tunica and a homogenous corpus composed of large nucleated cells with dense cytoplasm devoid of vacuoles. The tunica cells are elongated at right angles to the surface of the apex (Fig. 5). Thus, between the first and second week

Figure 4. Vegetative meristem at the early stages of leaf formation (arrow) (x 150).

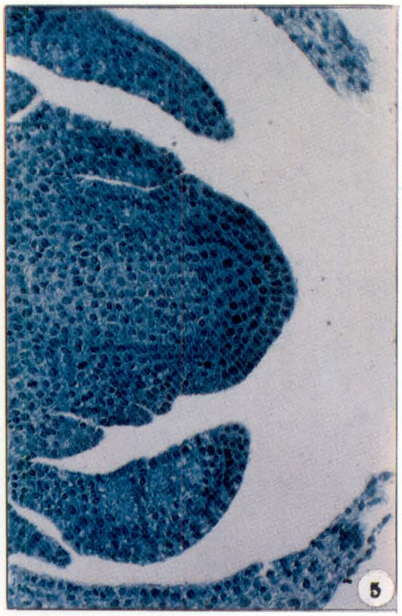

Figure 5. High and dome form meristem with activated tunica and corpus at the beginning of the prefloral phase (x 150)

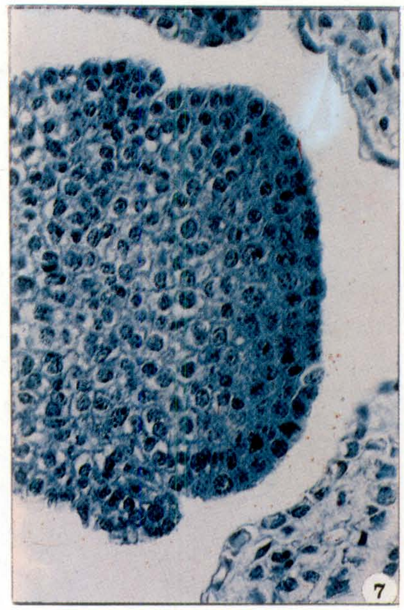

Figure 7. Fully expanded prefloral meristem with homogeneous cytological state (x 240).

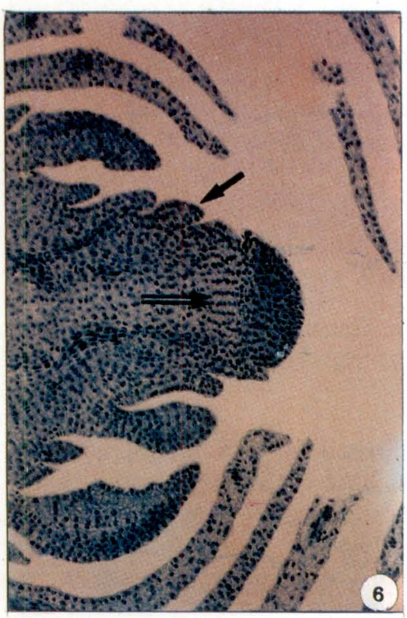

Figure 6. Prefloral meristem with activated tunica and expanded corpus. Periclinal divisions in rib meristem and elongation of resulting cells are obvious (double arrow). Covering sheets of leaves and flowers are formed by low activity of lateral zones (x 99).

of August, the apex reaches the transition or prefloral stage. After the high dome stage is reached, the apex completely flattens out (Figs. 6,7) and differentiates into the terminal flower during the second week of August. In the prefloral phase, despite the period of leaf formation, cells of the apical axial zone of meristem are activated. Due to periclinal divisions in corpus, anticlinal divisions in tunica layers and enlargement of upper cells of ribmeristem shoot apex is completely flattened (Figs. 6,7). At the end of the prefloral phase, a central zone with vacuolated cells is covered by a very active meristemic zone that originates from tunica and corpus (Fig. 7). In the prefloral phase, the lateral zones of meristem are somewhat inactivated and their limited activities produce covering sheets of flower (Fig. 6).

Differentiation of Floral Organs

In the second week of August, floral differentiation occurs in the flattened floral apex. Perianth initiation is expressed when the flattened floral apex depresses into a concavity (Fig. 8). The lobes are initiated by periclinal divisions in the subtunica layers and anticlinal divisions in tunica layers. Both outer and inner perianth primordia are formed in succession (Fig. 9). The perianth primordium swells into protuberances as a result of continued division. Stamen initiation is accompanied by further depression of the floral apex.

Figure 8. Mitotic divisions occurring in the tunica of floral apex which result in central depression (single arrow) (x 300).

Figure 10. Lobes of staminal primordia (S.P.) have formed near the perianth primordia (P.P.) (x 120).

Figure 9. Lobes of perianth primordia (P.P.) have formed. Anticlinal (single arrow) and periclinal divisions (double arrow) are obvious (x 150).

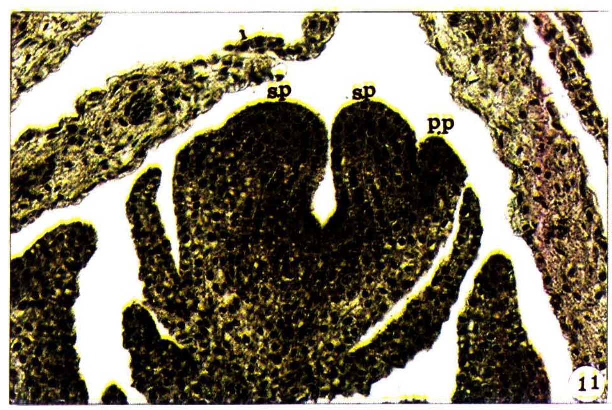

Figure 11. Dipping of central depression and formation of staminal primordia (S.P.) (x 150).

Stamens are initiated by periclinal divisions in subtunica and anticlinal divisions in tunica layers (Figs. 10, 11). The concavity becomes fully depressed forming a notch enclosed between well differentiated perianth and stamens. The tunica and subtunica layers, which displayed localized meristematic activity, now undergo active cell division at the base of the notch indicating the initiation of gynocium by forming small protuberances on both sides (Fig. 12).

During the formation of carpels, differentiation of stamens is completed and wall layers, tapetum and

Figure 12. Longitudinal section of a flower bud. Stamen st, carpel c, perianth P, sheet sh, flower stalk fs and leaves le are obvious (x 60).

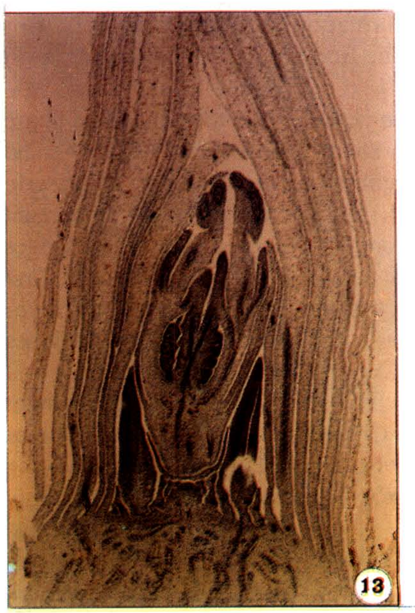

Figure 13. Longitudinal section of a differentiated flower bud with ovule primordia which are newly formed (x 19.8).

sporogenous cells are gradually differentiated. When these layers are completely differentiated in juvenile anther, ovular primordia are formed (Fig. 13). Protandrous type of flower formation in *Crocus sativus* L. occurs in a centripetal manner.

Frequency of Mitotic Figures

During the period of dormancy, the frequency of mitotic divisions as observed in the longitudinal sections is relatively low both in the tunica and the corpus regions. Thereafter, it continues to increase progressively with time till early August. In the second week of August, however, a decline in the trend is observed. The highest frequency of mitotic figures is observed during the first week of August (prefloral phase) (Diagram 1).

Length and Breadth of the Shoot Apex

A small but gradual increase in the length of the apical meristem occurs from the middle of May till early August, after which the length of the meristematic region shows a marked decline (Diagram 2). A continued increase in the breadth of the apical meristem is observed from the middle of May up to the middle of July; after which it shows a sudden decrease. Subsequently, the breadth again continues to increase till early August (Diagram 3). There is gradually a progressive increase in the ratio of length to breadth in the apical meristem till late July, after which the ratio begins to decrease; the decrease being

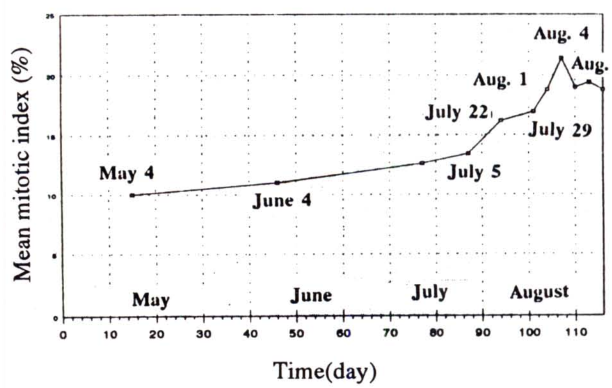

Diagram 1. Changes in mean mitotic activity

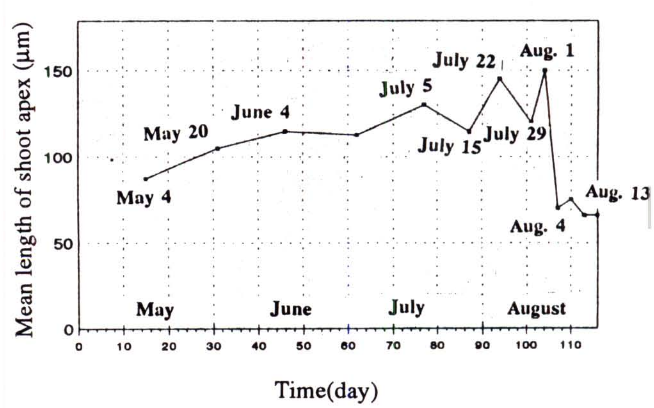

Diagram 2. Mean length of apical meristem during the period of leaf and flower formation

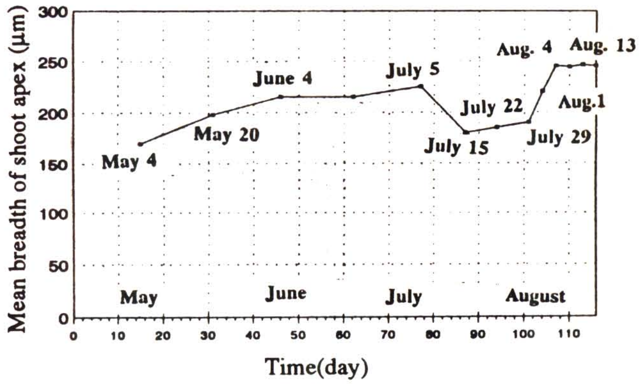

Diagram 3. Changes of mean breadth

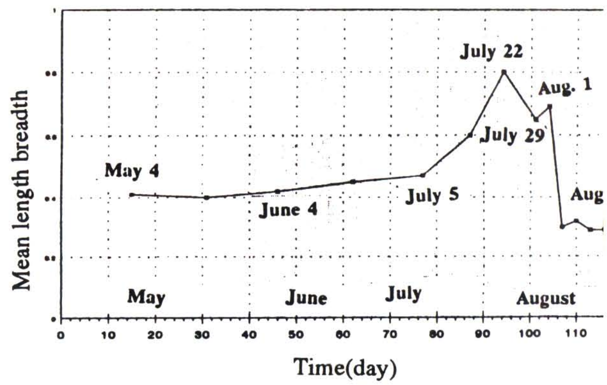

Diagram 4. Ratio of length to breadth in apical meristem during the period of leaf and flower formation

more pronounced during the first week of August (Diagram 4).

Discussion

Study of the growth and differentiation in apical meristem of *Crocus sativus* L. shows seven different stages in this period: 1-The period of establishment of apices during the time of flowering of the plant (November). 2-The period of covering scales formation during the winter and spring; at the end of this period, with the cessation of activity above the ground and the death of the foliage and roots, corms are considered to be in a state of dormancy which lasts till early July. 3-The period of active formation of leaf primordia in apical bud after the ending of dormancy (from the first week of July up to the beginning of August). 4-Prefloral phase in the first week of August. 5-The period of generative organs differentiation which falls at a time when the corm is underground and vegetative organs are absent (from the second week of August till late August). 6-The period of flower stalk growth (October). And 7-The period of flowering (November).

The present study on the growth and differentiation in buds of the saffron plant during the period between May to the middle of July suggests that this period is a phase of arrested growth and developmental activity or dormancy in the corms. With the resumption of growth, a steady increase in cell division and elongation occurs. At first, leaf primordia form and after transition of the prefloral phase followed by rapid changes in the topography of shoot apex, generative primordia initiate in the apical bud, whereas during these stages corms are apparently dormant and not only roots but also vegetative organs are not observed above the soil. Thus, in spite of the findings of Azizbekova and Milyaeva [1] and Ebrahimzadeh [2], there is not a six-month interval between the prefloral phase and flower formation in *Crocus sativus* L. This result agrees with the findings of Koul and Faroog [4] and Rees [7-9].

Transition of the vegetative to the generative phase is accompanied by sharp changes in dimensions and structure of plant apices, as well as by an increase in mitotic activity [1]. The present study, in agreement with Koul and Faroog [4], showed that such changes in the apices of *Crocus sativus* L. occurred in the first week of August so that apical meristem had the highest mitotic activity at this time. This result disagrees with the report of Azizbekova and Milyaeva [1] and Ebrahimzadeh [2]. The structure of vegetative shoot apex in the saffron *Crocus* compares well with that of *Iris* as described by Rodrigues Pereira [11], both being members of the same Iridaceae family. In each case, there is a two-three layered tunica enclosing a corpus of larger unlayered cells and the rate of cell division is initially very low. In *Iris*, the first sign of transition from vegetative to flowering phase is an increase in cell division in the rib meristem. This, however, is not true for the saffron plant. The difference may be due to the fact that flowers in *Iris* are borne on an elongated scape [11].

Acknowledgements

We are grateful to the National Research Center for Genetic Engineering and Biotechnology for the financial support given during the course of this research.

References

1. Azizbekova, N.Sh. and Milyaeva E.L. *Ontogenez*, **9**, (3), 309-14, (1978).
2. Ebrahimzadeh, M. H. (1978). *Pazhoohanded*, Final

report of basic science researches, N. 21, basic Science
2.
Johansen, D.A. *Plant microtechnique*, pp. 523. McGraw-Hill Book Company, New York, London, (1940)
Koul, K.K. and Faroog, S. *J. Indian Bot. Soc.*, **63**, 153-160, (1982).
Matthew, B. *The Crocus: A revision of the genus Crocus (Iridaceae)*. B.T. Bastford Ltd., London, (1982).
Negbi, M., Dagan, B., Dror, A. and Basker, D. *Israel J. Bot.*, **38**, 95-113, (1989).
Rees, A.R. *The growth of bulbs*. Academic Press, London and New York, (1972).
8. Rees, A.R. *Ann. Appl. Biol.*, **98**, 544-48, (1981).
9. Rees, A.R. In *Handbook of flowering*, Vol. 1, (ed. A.H. Halevy), pp. 259-67. CRC Press, Boca Raton, Florida, (1985).
10. Riviere, S. C.P. Sean. Hebd. Acad. Sci., Paris, 257. Group II, 1-3, (1963).
11. Rodriques Pereira, A.S. *Acta. Bot. Neerl.*, **11**, 77-138, (1962).
12. Sass, J.E. *Iowa St. Coll. F. Sci.*, **18**, 447-56, (1944).
13. Schneider, H. *Botanical sciences in staining procedures*, (ed. G. Claris), pp. 315-67. Williams and Wilkins, (1981).