RULES PROPOSALS 1993

AOSA Rules Committee
David F. Svik, Chairman

Seven proposals have been reviewed and approved by the Rules Committee for further consideration by the AOSA Members at the 1993 meeting. Approval does not mean that the Committee or its members endorse these proposals to the Rules.

These proposals are presented here as required by the Constitution so they may be evaluated at least 90 days in advance of the annual meeting. The name and address of the author(s) are noted if you wish to contact them for additional information or have comments for them.

Comments concerning any of these proposals may also be made in writing to the Rules Chairman prior to the June meeting. Additional comment time will also be available during the Open Rules meeting prior to the annual business meeting. **Extensive changes to these proposals are possible at the Open Rules meeting but are not encouraged by the Committee.**

Please note: Only a limited number of copies of these proposals will be available at the Open Rules meeting. We recommend that you bring this copy of the Newsletter with you to the AOSA meeting.

RULES PROPOSAL 1.

To have permissible temperature variation in laboratory germination of seed as ± 1°C. as stated in section 4.9c.

PRESENT RULE (If new rule, state "New Rule")

4.9c
Temperature. - Single numerals in the tables indicate constant temperatures. Two numerals separated by a dash indicate an alternation of temperature, the test to be held at the first temperature for approximately 16 hours and at the second temperature for approximately 8 hours per day. If the tests are not subjected to alternating temperatures over weekends and on holidays, they are to be held at the lower temperature during this time. In the case of species of ....

PROPOSED RULE (Exactly as it would appear in "Rules")

4.9c
Temperature. -Single numerals in the Tables indicate constant temperatures. Two numerals separated by a dash indicate an alternation of temperature, the test to be held at the first temperature for approximately 16 hours and at the second temperature for approximately 8 hours per day. If the tests are not subjected to alternating temperatures over weekends and on holidays, they are to be held at the lower temperature during this time. The permissible variation in the temperature specified in the rules for laboratory germination of seeds is ± 1°C. In the case of species of ....

SUPPORTING EVIDENCE (Research data, literature citations, published papers, or other appropriate information)

This rules proposal is submitted by the AOSA working group of the AOSA/ISTA Harmonization Committee. It addresses concerns on the part of EEC representatives about potential sources of variation in germination testing as a result of the absence of permitted variation in germination. USDA Handbook 30 indicates that the AOSA adopted the provision for ± 1°C. variation in 1949. This proposal, therefore brings the provision into the rules from Handbook 30.

SUBMITTED BY (Name, complete address, and phone number)

A.B. Ednie, Lab Services Division
Agriculture Canada
Ottawa, Ontario
Canada K1A OC6.
613-995-4907

DATE OF PROPOSAL 1/31/92

RULES PROPOSAL 2.

Proposal to delete the germination retesting rule which allows the 100-seed replicate with the lowest germination to be dropped and the remaining three averaged when replicates are out of tolerance (Rule 4.6) and to modify the rules for reporting percentage germination when more than one germination test has been conducted because of out-of-tolerance replicates (Rule 4.7).

PRESENT RULE

4.6 When to retest

a. Retest when the range of 100-seed replicates of a given test exceeds the maximum tolerated range in Table 2 (Column C, D or E). — To find the maximum tolerated range, compute the average percent of all replicates of a given test (drop a fraction less than 0.5, but increase to the next whole percent a fraction of 0.5 or more). The average is found in either Column A or B of Table 2 and the maximum tolerated range is found opposite in the appropriate Column (C, D, or E) ~~as outlined in paragraphs (1) and (2) that follow.~~

~~(1) When the range of percent germination of four 100-seed replicates exceeds the maximum tolerated range (Table 2, Column C), omit the lowest replicate. Compute the average of the three remaining replicates. Find the new average in Column A or B, and determine the allowable range from D. If the range of the three remainng replicates does not exceed the 3-replicate tolerated range, retesting is not necessary; the average of three replicates is the germination result. Retest if the range of the three replicates exceeeds the 3-replicate tolerated range.~~

~~(2) When only 200 seeds are germinated, retest if the range of the two replicates exceeds the 2-replicate tolerated range in Column E.~~

4.7 Calculation of percentage germination

b. When more than one test is made, the results of such tests or retests, which fall within one tolerance range, or tests by alternate methods, shall be averaged and reported as the percentage germination. When different official methods are employed, and the highest result is not within tolerance of the next lower, the higher percentage shall be reported. When retests are required and are satisfactory (section 4.6) but out of tolerance with the original test, ~~the higher percentage shall likewise be reported.~~

PROPOSED RULE

4.6 When to retest

a. Retest when the range of 100-seed replicates of a given test exceeds the maximum tolerated range in Table 2 (Column C, D or E). — To find the maximum tolerated range, compute the average percent of all replicates of a given test (drop a fraction less than 0.5, but increase to the next whole percent a fraction of 0.5 or more). The average is found in either Column A or B of Table 2 and the maximum tolerated range is found opposite in the appropriate Column (C, D or E). <u>See also section 4.7.b.</u>

4.7 Calculation of percentage germination

b. When more than one test is made, the results of such tests or retests, which fall within one tolerance range, or tests by alternate methods, shall be averaged and reported as the percentage germination. When different official methods are employed, and the highest result is not within tolerance of the next lower, the higher percentage shall be reported. When retests are required and are satisfactory (section 4.6) but out of tolerance with the original test, a third test must be conducted and the average of the tests which are within tolerance reported (if the result of the third tests falls between the first two, and is in tolerance with both, report the average of the three tests).

SUPPORTING EVIDENCE

The validity of the rule which allows dropping the low out-of-tolerance replicate has been one of the points under discussion by the AOSA/ISTA Harmonization Committee.

To assess the impact of dropping an out-of-tolerance replicate, data were analysed from 145 samples which had been retested because the replicates were out of tolerance (Ashton, 1992). Retest results for the samples were used as the best estimate of the "true" germination value of the sample. The data analysis showed that the procedure of dropping the low out-of-tolerance replicate gave a result on average 2.6% higher than the retest. In some cases the increase was as much as 8%. It was apparent from the data that dropping a replicate gave a biased result which did not reflect the true value of the sample. It was concluded that the reported percentage germination should not be based on the three highest replicate results but rather a retest should be conducted. The present AOSA Rule 4.7, by allowing a low out-of-tolerance test to be disregarded (when retesting was due to out-of-tolerance replicates), makes the assumption that a high result is "correct" or better than a lower result. The study with replicate results (Ashton, 1992) provides evidence that a low result (e.g. one 100-seed replicate) should not be arbitrarily discarded since it may, when combined with the higher results, be contributing to the best estimate of the sample quality. Since there is no valid method of choosing between two tests which are not in tolerance, the proposed rule would require that a third test be conducted.

Adoption of the proposal would harmonize the AOSA and ISTA Rules on this point.

Reference:

Ashton, D. B., 1992. *An evaluation of AOSA and ISTA germination test reporting procedures when replicate results are out of tolerance.* AOSA Newsletter, 66(2):21-29.

SUBMITTED BY: AOSA/ISTA Harmonization Committee,
Dr. L. Wiesner, Chairman (AOSA)

For clarification on this proposal, contact:
D. Ashton
Lab Services Bldg. 22
Central Experimental Farm
Ottawa, K1A 0C6, Canada
Phone: 613-995-4907 Fax: 613-992-5819

October 9, 1992

PROPOSAL 3.

Revises the wording of sections 2.5 a and 2.5 b to provide for reporting the percentage of a component found in very small amounts in a purity analysis as 0.01%, instead of 0.00%. The proposal also provides rules for rounding off of purity component percentages.

PRESENT RULE

2.5 a. Separation of component parts. - The working sample shall be weighed in grams to four significant figures and then shall be separated into four parts: (1) kind or cultivar to be considered pure seed; (2) other crop seed; (3) inert matter; and (4) weed seed. The four component parts shall be weighed in grams to the same number of decimal places as the working sample. The percentage of each part shall be determined to two decimal places.

Aids in the classification of crop seed, inert matter, and weed seed may include visual examination, use of transmitted light (diaphanoscope), or specific gravity (seed blowers). Specific instructions for classification as to pure seed, inert matter, and weed seed are given in sections 2.7 to 2.11, inclusive. Insofar as laws, and rules and regulations permit, classification as to weed or crop seed shall be according to Handbook No. 25: Uniform Classification of Weed and Crop Seeds.

b. Calculation of percent of component parts in the sample.

(1) Minimum working sample less than 25 grams: Percentages shall be based on the sum of the weights of the component parts and not on the original weight. However, the sum of the weights of the component parts shall be compared with the original weight of the working sample as a check against loss of material or other error.

(2) Minimum working sample of 25 grams or more: The other crop seed, inert matter, and weed seed shall be weighed and their percentages calculated on the basis of the original weight. The pure seed need not be weighed; its percentage may be determined by subtracting the sum of the percentages of the other three components from 100.

PROPOSED RULE

2.5 a. Separation of component parts. - The weight of the working sample shall be determined to the number of decimal places indicated below:

Weight of working sample in grams	Number of decimal places
Less than 1.0000	4
1.000 to 9.999	3
10.00 to 99.99	2
100.0 to 999.9	1
1000 or more	0

The working sample shall then be separated into the following parts: (1) kind or cultivar to be considered pure seed; (2) other crop seed; (3) inert matter; and (4) weed seed. The component parts shall be weighed in grams to the same number of decimal places as the working sample, except as provided for in 2.5 b. (2) where it is not necessary to weigh the pure seed component. The percentage of each part shall be determined to two decimal places.

Aids in the classification of crop seed, inert matter, and weed seed may include visual examination, use of transmitted light (diaphanoscope), or specific gravity (seed blowers). Specific instructions for classification as to pure seed, inert matter, and weed seed are given in sections 2.7 to 2.11, inclusive. Insofar as laws, and rules and regulations permit, classification as to weed or crop seed shall be according to Handbook No. 25: Uniform Classification of Weed and Crop Seeds.

b. Calculation of percent of component parts in the sample.

(1) Minimum working sample less than 25 grams: Percentages shall be based on the sum of the weights of the component parts and not on the original weight. However, the sum of the weights of the component parts shall be compared with the original weight of the working sample as a check against loss of material or other error.

(2) Minimum working sample of 25 grams or more: The other crop seed, inert matter, and weed seed shall be weighed and their percentages calculated on the basis of the original weight. The pure seed need not be weighed; its percentage may be determined by subtracting the sum of the percentages of the other three components from 100.

(3) When rounding off the calculated percentages of each component to the second decimal place, round down if the third decimal place is 4 or less and round up if the third decimal place is 5 or more, except that if any component

is determined to be present in any amount calculated to be less than 0.015%, then that component shall be reported as 0.01%. If any component is not found in the purity analysis, then that component shall be reported as 0.00%.

(4) The total percentage of all components shall be 100.00%. If the total does not equal 100.00% (e.g. 99.99% or 100.01%) then add to or subtract from the component with the largest value (normally the pure seed component).

SUPPORTING EVIDENCE

A survey conducted by the AOSA Uniformity Subcommittee (see AOSA Newsletter, September 1992) revealed a lack of uniformity in the interpretation of section 2.5 of the Rules for Testing Seeds. This proposal clarifies procedures for weighing and calculation of percentages of components in the purity analysis. The proposal provides for reporting of components found in very small amounts as 0.01%, rather than 0.00%. The survey indicated that most laboratories are using this practice now in reporting results. This change is proposed to recognize the need to provide meaningful information from the purity analysis and to promote uniform application of procedures in weighing and calculating results.

Examples of calculations:

Sample #1: A working sample of wheat, Triticum aestivum, weighs 100.7g. In the purity analysis, 32 fertile florets of barley, Hordeum vulgare, weighing 1.5g, 0.8g of inert material, and 10 seeds of pigweed, Amaranthus spp., weighing 0.0076 g were found.

crop seed = 1.5g/100.7g X 100 = 1.489%, which rounds off to 1.49%

inert = 0.8g/100.7g X 100 = 0.794%, which rounds off to 0.79%

weed seed = 0.0g/100.7g X 100 = 0.00%; however, since weed seeds were found, then the percentage shall be reported as 0.01%

pure seed = 100.00% - (1.49% + 0.79% + 0.01%) = 97.71%

Sample #2: A working sample of cabbage, Brassica oleracea var. capitata, weighs 10.35g. In the purity analysis 10.32g of pure seed, 4 seeds of onion, Allium cepa, weighing 0.01g, 0.003g of inert material, and no weed seeds were found.

The percentages are based upon the sum of weights of

the components, not upon the original working sample weight.

pure seed = 10.32g/10.33g X 100 = 99.903%, which rounds off to 99.90%

crop seed = 0.01g/10.33g X 100 = 0.097%, which rounds off to 0.10%

inert = 0.00g/10.33g X 100 = 0.00%; however, since inert material was found, then the percentage shall be reported as 0.01%

weed seed = 0.00g/10.33g X 100 = 0.00%, which is the percentage reported since no weed seeds were found

In this case, the sum of the percentages of the components, 99.90% + 0.10% + 0.01% + 0.00% = 100.01%. Since the total percentage of all components shall be 100.00%, 0.01% is subtracted from the component with the largest value, the pure seed, giving a percentage of 99.89%.

SUBMITTED BY

Uniformity Subcommittee
Rodney W. Young, Chair
Phone (301) 504-8605

RULES PROPOSAL 4.

Change temperature° C to 20° and change first count days to 4 for sunflower (Helianthus annuus) in Table 3

PRESENT RULE

Kind of seed	Substrata	Tempera-ture° C.	First count days	Final count days
Helianthus annuus sunflower	T, B	20-30	3	7

PROPOSED RULE

Kind of seed	Substrata	Tempera-ture° C.	First count days	Final count days
Helianthus annuus sunflower	T, B	20	4	7

SUPPORTING EVIDENCE

The Midwest Region II referee for 1991-1992, "Sunflower Germination of Diseased Seed, Comparing 20°-30°C to 20°" showed a significant increase in percent normal and a significant decrease in percent dead when tested at 20° vs. 20°-30°. The Plant Diagnostic Laboratory at NDSU determined that Rhizopus fungi was responsible for the seedling decay.

Test results were returned by 39 laboratories. Results from Samples 1 and 2 were combined to determine the effect of germination temperature on final results. Table 1 compares the results of all 39 labs.

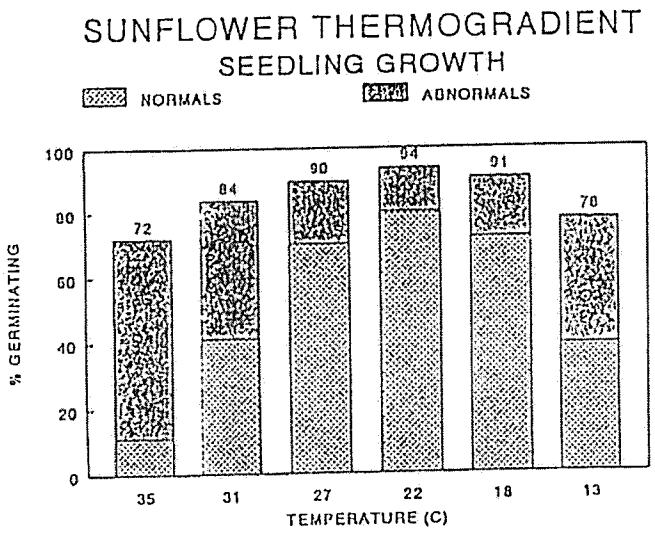
Table 1	Temp.	Normal	Abnormal	Dead
	20-30C	73.3	10.3	16.4
	20C	79.4	9.0	11.6
	Difference	6.1	1.3	4.8
	LSD .05	4.6	NS	4.7

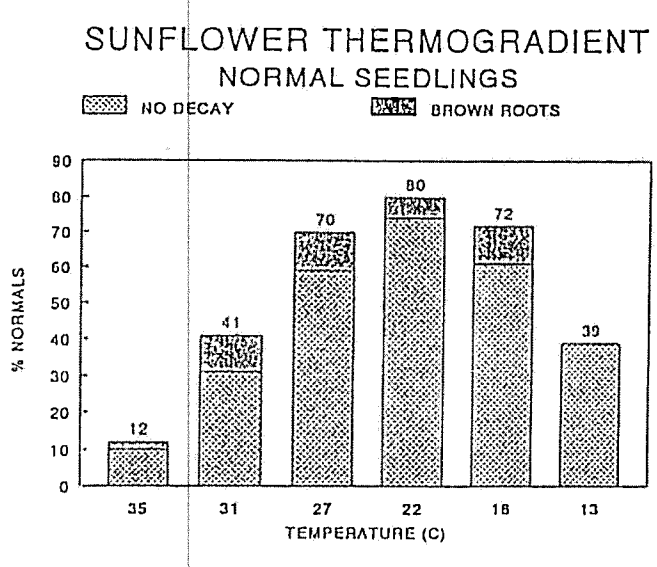
Results from samples 1 and 2 were combined and the 14 labs that were out of tolerance on either sample at either temperature were excluded entirely. Table 2 compares the results using the remaining 25 labs.

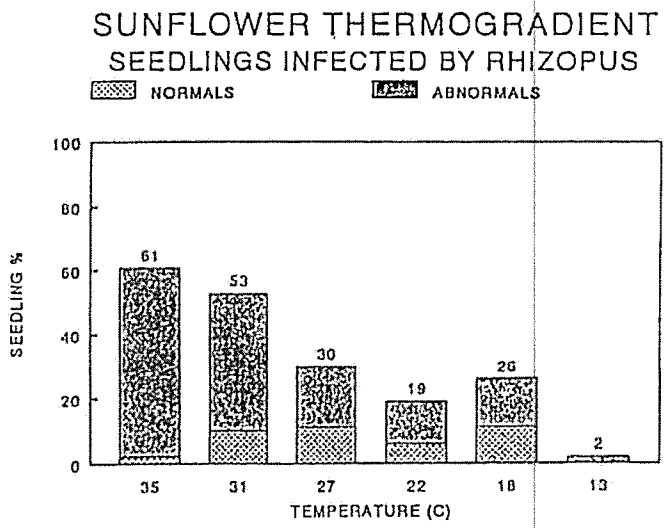
Table 2	Temp.	Normal	Abnormal	Dead
	20-30C	74.8	9.7	15.5
	20C	80.6	8.9	10.5
	Difference	5.8	0.8	5.0
	LSD .05	4.4	NS	3.4
	LSD .01	5.4	NS	4.5

A Thermogradient study was conducted by ISU on the same 2 seed lots as were in the Midwest Region II referee. The graphs represent the average across both seed lots with 6 replicates of 15 seeds/lot.

The graphs indicate that lower temperatures are helpful in slowing the growth of Rhizopus species and allowing the seedlings to develop normally.







A second referee comparing 20° C to 20°-30° C was conducted by 9 laboratories on 8 untreated sunflower lots (2 confection, 6 oil). The purpose of this referee was to determine if 20° would have any adverse effect on germination of good quality sunflower seed.

Each lab was instructed to germinate each sample at 20° C and at 20-30°C using 400 seeds for each temperature. This data represents 576 replicates of 100 seeds.

Table 3 compares 20°-30° C to 20° C for 8 samples averaged across 9 labs.

Table 3

Sample	Temperature 20°-30° C	Constant 20°C	Difference
1	91.5	91.9	+ 0.4
2	92.1	93.5	+ 1.4
3	84.7	85.3	+ 0.6
4	92.9	94.5	+ 1.6
5	88.8	91.2	+ 2.4
6	90.3	93.6	+ 3.3
7	91.1	93.4	+ 2.3
8	89.0	92.8	+ 3.8
Avg	90.1	92.3	+ 2.2

Table 4 shows the average across 9 labs and 8 samples compared at the 2 temperatures.

Table 4

Evaluation	20°-30° C	Constant 20° C	Difference
Normal	90.1	92.3	+ 2.2
Abnormal	6.2	4.7	- 1.5
Dead	3.7	3.0	- 0.7

Data from this referee shows that a 20° C germination temperature gives results that are at least as good as 20°-30° C on seed lots which were not expected to have seedling decay problems. In this referee 82% of the 20° C tests resulted in higher germinations than 20°-30° C. (See Appendix 1)

Laboratories participating in this referee were:

Central Seed Laboratory-Agriculture Canada-Ottowa Ontario Canada
Federal Seed Laboratory-Beltsville MD
Agrigenetics Corp-Prescott WI
Interstate/Payco Seed-West Fargo ND
Iowa State University Seed Science Center-Ames IA
Northrup King Co-New Deal TX
Minnesota Crop Improvement-St Paul MN
Wisconsin Crop Improvement-Madison WI
North Dakota State Seed Lab-Fargo ND

Submitted by: Quentin Schultz RST (715) 262-3223
Mark Ameinrud RST (701) 282-3373
Mark Hafdahl
North Dakota State Seed Laboratory
University Station
Fargo ND 58105
(701) 239-7210

October 12, 1992

APPENDIX 1. SUMMARY OF GERMINATION RESULTS COMPARING 20-30 VERSUS CONSTANT 20 USING 9 LABORATORIES AND 8 SUNFLOWER SAMPLES.

	LAB 1		LAB 2		LAB 3		LAB 4		LAB 5		LAB 6		LAB 7		LAB 8		LAB 9		COMBINED LABS	
SAMPLE	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C	20-30	20C
1	94.8	95.5	92.3	93.3	89.3	91.5	92.0	95.3	93.5	90.5	92.3	90.3	92.0	93.5	85.5	89.8	92.0	88.0	91.5	91.9
2	93.3	94.8	92.8	93.5	92.5	93.0	93.3	95.0	94.0	95.3	90.5	94.3	93.0	96.0	87.5	90.0	92.5	89.8	92.1	93.5
3	92.5	88.0	88.0	88.8	83.3	85.5	87.5	88.8	85.8	81.0	75.3	84.8	86.8	87.5	74.5	78.3	89.0	85.0	84.7	85.3
4	96.0	95.5	94.8	96.0	92.0	93.0	94.3	96.3	91.8	93.8	93.3	94.5	94.8	96.0	87.0	93.0	92.0	92.3	92.9	94.5
5	91.8	96.8	94.0	96.3	89.3	93.3	83.5	97.0	89.0	92.0	89.5	92.3	93.5	97.3	79.3	86.8	89.5	87.3	88.8	93.2
6	90.5	97.3	94.3	93.8	88.8	93.8	91.0	98.0	89.8	90.5	93.0	96.5	95.8	94.3	83.0	89.5	87.3	88.5	90.4	93.6
7	96.5	96.5	91.3	96.8	91.5	95.3	89.0	96.3	90.0	90.8	90.5	92.5	94.8	92.3	85.8	90.5	90.8	90.0	91.1	93.4
8	91.3	95.3	91.8	93.0	90.0	92.0	90.5	96.5	82.8	91.0	94.3	93.3	92.5	93.5	81.0	90.8	87.3	90.0	89.0	92.8
AVERAGE	93.3	94.9	92.4	93.9	89.6	92.2	90.1	95.4	89.6	90.6	89.8	92.3	92.9	93.8	82.9	88.6	90.0	88.8	90.1	92.3

LSD .05 = 4.16

Proposal 5.

RULES PROPOSAL: Acceptance of Handbook 25: Uniform classification of Weed and Crop Seeds. Third revision, 1993 by the AOSA membership.

PRESENT RULE: Uniform classification of Weed and Crop Seeds contribution No. 25 to Handbook on Seed Testing second revision 1977.

PROPOSED RULE: Uniform Classification of Weed and Crop Seeds. Contribution No. 25 to the Handbook on Seed Testing third revision 1993.

SUPPORTING EVIDENCE: The second revision contained references to about 650 species and each species was listed only as a weed or other crop when found as a contaminant in any species.

The third revision lists over 2000 species and many species can be listed as either a weed or other crop when found as a contaminant depending on which particular species they are contaminating.

SUBMITTED BY: Arnold L. Larsen
Colorado Seed Laboratory
E-10 Plant Science, CSU
Fort Collins, CO 80523

DATE OF PROPOSAL: October 20, 1992

Proposal 6.

RULES PROPOSAL: Editorial correction to make reference to Handbook No. 25 the same in 2.5a as it is in 2.8. Reference is to the statement "current edition."

PRESENT RULE: 2.5a.----according to Handbook 2.5----.

PROPOSED RULE: 2.5a. ---according to the current edition of Handbook 25:----.

SUPPORTING EVIDENCE: There is a need to alert users of the AOSA rules that Handbooks are updated frequently. We cannot conveniently refer to each specific change whenever they occur but some generic comment should bring it to the users attention so that they will check and confirm that they are using the latest edition.

SUBMITTED BY: Arnold L. Larsen
Colorado Seed Laboratory
E-10 Plant Science , CSU
Fort Collins, CO 80523

DATE OF PROPOSAL: October 20, 1992

RULES PROPOSAL 7.

PRESENT RULE

Definition of nucellus, AOSA Seedling Evaluation Handbook:

The tissue of the ovary wall from which the ovule develops; it may persist as nutritive tissue in some seeds (see perisperm).

PROPOSED RULE

Replace current definition of nucellus with the following:

Tissue of the inner part of an ovule, in which the embryo sac develops; it may persist as nutritive tissue in some seeds (see perisperm).

SUPPORTING EVIDENCE

The definition for nucellus currently found in the Seedling Evaluation Handbook best describes the term placenta.

> Placenta: Region in the ovary where ovules originate and remain attached at maturity. K. Esau, Anatomy of Seed Plants, 1977.

The term nucellus may be defined as follows:

> Inner part of an ovule in which the embryo sac develops. Commonly considered to be equivalent to the megasporangium. K. Esau, 1977, Anatomy of Seed Plants, John Wiley & Sons.

> The tissue of an ovule, in which the female gametophyte (embryo sac) develops; the megasporangium. (Dictionary of Botany, R.J. Little & C.E. Jones, 1980, Van Nostrand Reinhold Company.

> Tissue composing the chief part of the young ovule, in which the embryo sac develops; megasporangium. T.L. Rost, et al., 1984, Botany: A Brief Introduction to Plant Biology, John Wiley & Sons.

See attached references describing ovule development.

SUBMITTED BY
Deborah Meyer
California Department of Food & Agriculture, Seed Laboratory
1220 N Street, P. O. Box 942871
Sacramento, CA 94271-0001
(916) 654-1391

DATE OF PROPOSAL: November 9, 1992

K. Esau, 1977, Anatomy of Seed Plants, John Wiley & Sons.

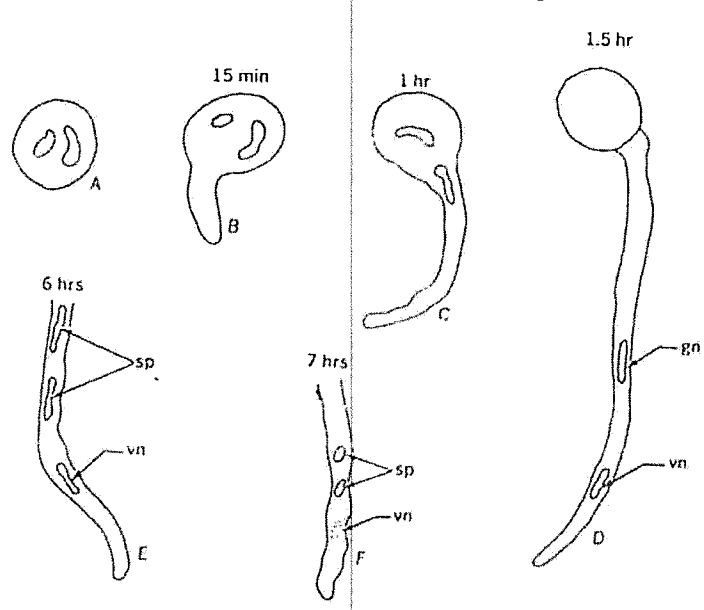


Figure 21.9 Germination of pollen grain of *Scilla* (monocotyledon) in vitro. Medium: 2% agar, 7% cane sugar, traces of sterile yeast. *A*, resting binucleate pollen grain, *B–E*, stages of germination at times after placement on medium indicated above the drawings. Only tips of the pollen tubes in *E* and *F*. The nuclei are compact at end of germination in *F*. Details: *gn*, generative nucleus; *sp*, sperm nucleus; *vn*, vegetative nucleus. (Adapted from R. A. Brink, *Amer. J. Bot.* 11:351–364, 1924.)

croscopy reveals that, upon germination of pollen, the intine becomes continuous with the pollen tube wall.[9,13]

The cytoplasm of the growing pollen tube commonly accumulates at the tip, with some differentiation between the apical and subapical regions. The vegetative nucleus, the sperms (or the generative nucleus), the cytoplasmic organelles, and small vesicles, all transferred from the pollen grain, occur in the subapical region (fig. 21.9,*D*,*E*). The apical end is devoid of organelles but contains numerous vesicles. The vesicles are derived from dictyosomes and probably also from endoplasmic reticulum and, at least some of them, are concerned with wall synthesis.[54] In many species, the older parts of the elongating pollen tube are successively sealed off by plugs of callose.

MEGASPOROGENESIS

Ovule

The ovule developing from the placenta of the ovary (chapter 20) is the site of formation of megaspores and development of the embryo sac (female gametophyte). The ovule commonly consists of the following principal parts (fig. 21.10): *nucellus*, the central body with vegetative cells enclosing the sporogenous cells; one or two *integuments* (thus, *unitegmic* and *bitegmic* ovules) enclosing the nucellus; *funiculus* (fig. 21.10,*C*), the stalk connecting the ovule with the placenta. The region where the nucellus, the integuments, and the funiculus merge is called the *chalaza*, a not clearly defined region.

as to how the embryo sac retains its shape and does not become crushed. A study relating the orientation of cell walls in the cotton ovule with the distribution of tension and compression stresses in a two-dimensional plastic model of the ovule suggests that the integuments serve to protect the developing embryo sac by insuring that all compressive forces circumvent it.[39,40]

During the development of integuments the ovule may remain upright (*atropous* or *orthotropous* ovule) or become inverted (*anatropous* ovule; fig. 21.10). Between these two basic types of ovule are many other variously curved and variously named forms of ovules.[10,42,50] The ovules also vary in the size of the nucellus. An ovule with a large nucellus is called *crassinucellate*, one with a small nucellus, *tenuinucellate*.

When the female gametophyte is ready for fertilization the ovule, like the ovary, is still relatively undifferentiated histologically. The vascular system, connected with that of the placenta through the funiculus, extends to the chalaza, usually as a single strand. But it may be more elaborate and may develop also in one or both integuments and become branched. The ground tissue is parenchymatic and the epidermis bears a cuticle. Because of the commonly epidermal origin of the integuments three cuticular layers may be distinguished: the outer, on the outside of the outer integument and the funiculus; the median, double in origin, between the two integuments; and the inner, also double, between the inner integument and the nucellus.

Parts of the ovule are disorganized during the development of the embryo sac and embryo. The vegetative tissue of the nucellus may be partly or entirely resorbed. In the latter instance, the embryo sac comes in contact with the inner epidermis of the adjacent integument. This epidermis commonly differentiates into the *integumentary tapetum*, or *endothelium*, a layer of deeply staining cells with an abundance of endoplasmic reticulum. A connection of this tapetum with the nutrition of the embryo is assumed because of the disintegration of the ovule tissue next to the tapetum and the persistence of the tapetum to seed maturity. This assumption is yet to be correlated with the characteristics of the walls of the tapetum and that of the embryo sac. No plasmodesmata have been seen between the embryo sac and the endothelium[12] and a double cuticle occurs between the two.[58] The wall of the embryo sac, however, may bear wall ingrowths indicating transfer activity.[45]

Megaspores

Megaspores result from the meiotic division of the megaspore mother cell, or *megasporocyte*. In crassinucellate condition, the archesporial cell undergoes a periclinal division and produces a parietal cell and the megasporocyte. In tenuinucellate ovules, the archesporial cell functions as the megasporocyte directly[10] (fig. 21.10,*A*,*B*). The common sequence of divisions of the latter (*Polygonum* type) is illustrated for *Solanum* in figure 21.11,*A*–*E*.[61] The megasporocyte may have rather dense cytoplasm or be vacuolated to various degrees. The cell undergoes two meiotic divisions resulting in the formation of a linear tetrad of haploid megaspores. The chalazal megaspore enlarges in preparation for the first mitotic division of the gametophyte. The other three megaspores degenerate. Fluorescence microscopy indicates that a temporary deposition of callose occurs in megasporogenesis, as well as in microsporogenesis.[46] Callose envelops the megaspore preparing for division and also appears in the walls separating the degenerating megaspores. All this callose soon disappears.

T. Rost, et al., 1984, Botany: A Brief Introduction to Plant Biology, John Wiley & Sons.

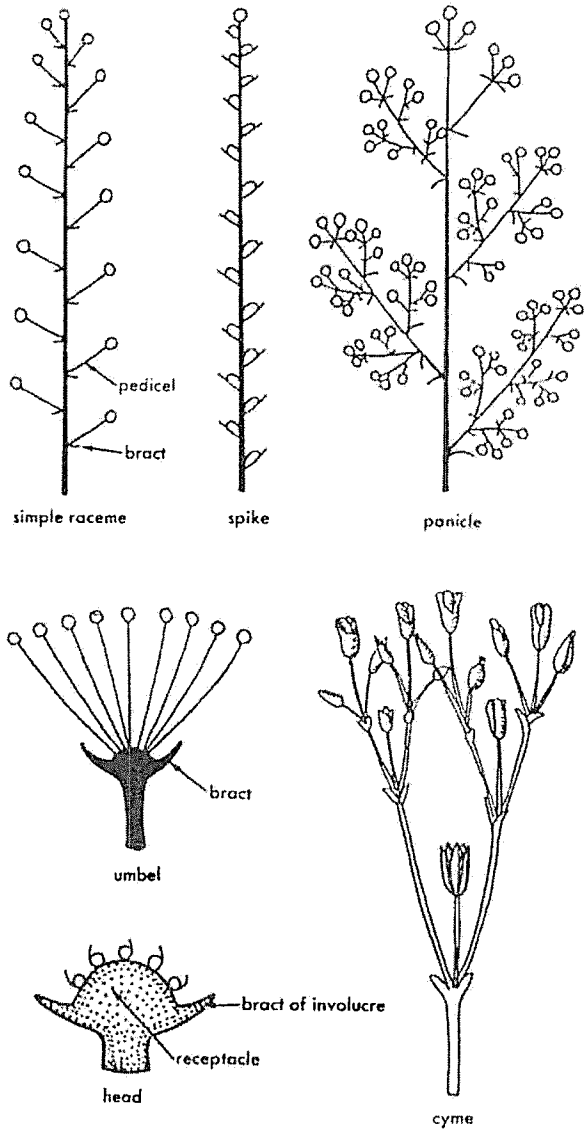


Figure 12.12. Diagram showing types of inflorescence

haploid, **male gametophyte** (gamete-producing plant) is to produce sperm which is necessary later for fertilization.

The pollen grain is surrounded by an elaborate, ornate cell wall that protects the gametophyte against dehydration. The pattern of the pollen wall is hereditary, and it varies widely among major groups of plants (Fig. 12.15). The walls contain a very hard material called sporopollenin that resists decay. As a result, pollen grains are among the most abundant fossils, and botanists have found them to be very useful in reasoning about the evolutionary history of plants.

After the pollen grains are mature, the anther wall splits open and the pollen are shed **(Fig. 12.14*F*).** In some manner (which will be discussed later), the pollen are transported to the stigma of adjacent or distant flowers. This process is called **pollination.** Once on the stigma, if the pollen and the stigma are genetically compatible, a pollen grain germinates to form an elongate **pollen tube** (Fig. 12.13*F*). The ornate pattern of the pollen grain wall serves as the means for receptive stigmas to recognize compatible pollen. The troughs and ridges of the pollen wall (Fig. 12.15) apparently contain proteins in specific patterns and [illegible] the patterns correlate with those of the stigma; metabolic events are triggered within the pollen grain to stimulate the pollen tube to grow into the pistil tissue. Some plants have developed different recognition systems that control pollen tube growth after pollen germination.

THE GYNOECIUM

The structure of the gynoecium depends on the number and arrangement of carpels comprising it. In the pea flower, for example, there is a single carpel [illegible] (Fig. 12.17) [illegible] **ovary,** an expanded basal portion, (2) the **style,** a slender stalk, and (3) a hairy irregular tip portion, the **stigma** (Fig. 12.17*B*). When the **pistil** is composed of one carpel, it is referred to as a simple pistil (Fig. 12.16*A*). In many other instances a compound pistil, consisting of [illegible] may occur (Fig. 12.16*B*).

The ovary is a hollow structure [illegible] several chambers, or **locules** (Fig. [illegible]). The carpels in a compound pistil is generally equal to the number of stigmas (Fig. 12.1), the number of locules, and sometimes, the number of faces of the ovary (Fig. 12.16*B*). The pea has a single stigma, and its ovary has one locule (Fig. 12.16*A*). There are three stigmas in the iris gynoecium; the ovary is three-sided (Fig. 12.16*B*), contains three locules, and is composed of three carpels.

Placentation

The tissues within the ovary to which the **ovules** are attached are called **placentae** (singular **placenta;** Fig. 12.18). The manner in which the placentae are distributed in the ovary is termed **placentation.** When the placentae are on the ovary wall, as in the pea (*Pisum*) and bleeding heart (*Dicentra;* Fig. 12.18*D*), the placentation is **parietal.** When they arise on the axis of an ovary that has several locules, as in lilies, *Fuchsia*, and tulip, the placentation is **axile** (Fig. 12.18*C*). Less frequently, the ovules are on the axis of a one-loculed ovary, in which event the placentation is **central,** as in the primrose family (Figs. 12.18*A* and 12.18*B*).

Style and Stigma

The style is a slender stalk that terminates in the stigma. It is through stylar tissue that the pollen tube grows. In some flowers, the style is very short (Fig. 12.17*B* and 12.17*C*) or entirely lacking; in others, it is long. In Zea mays, the corn silks are the styles (Fig. 12.7*C* and 12.7*D*). In general, the style withers after pollination, but in some plants (e.g., *Clematis*) it persists and becomes a [illegible] dispersal of the fruit. The stigmatic surface often has short cellular outgrowths that aid in holding the pollen grains; and sometimes it secretes a sugary and sticky solution, the **stigmatic fluid.** In many wind-pollinated plants, such as the grasses, the stigma is much branched or plumelike.

The Ovule

The ovule, the structure that will eventually become the seed, arises as a dome-shaped mass of cells on the surface of

A. Fahn, 1982, Plant Anatomy, Pergamon Press

ing pollen tube. There are investigators who suggest that a chemotactic attraction exists between the pollen tube and the tissues of the stigma, transmitting tissue and ovule (Rosen, 1964; Welk *et al.*, 1965, Tilton and Horner, 1980). According to other workers the very structure and arrangement of the transmitting tissue in the style direct the growth of the pollen tube (Schnarf, 1928; Renner and Preuss-Herzog, 1943; Jensen and Fisher, 1969).

In hollow styles the tubes of the germinating pollen grains grow between the papillae of the transmitting tissue, and, if they are absent, on the outer surfaces of the epidermal cells. In many plants the cuticle on the transmitting tissue disappears before pollination and the walls of the glandular tissue soften and swell. The pollen tubes may sometimes penetrate deeper into the transmitting tissue and grow between the cells. In solid styles the pollen tubes grow between the cells of the transmitting tissue. In grasses the pollen tube may even grow between the cells already in the stigma. On the stigma of grasses there are large multicellular hairs, consisting of several longitudinal rows of cells. The pollen tube penetrates between the inner cell rows of these hairs and from there to the transmitting tissue of the style. In the ovary the pollen tube penetrates via the transmitting tissue, which lines the ovary wall and the placenta, and eventually it reaches the ovule (Pope, 1946; Kiesselbach, 1949). Before the pollen tubes penetrate the transmitting tissue the walls of its cells swell, so that the tissue appears collenchymatous with mucilaginous walls and the connections between the cells weaken. As a result of these changes it is easy to macerate the transmitting tissue at this stage of development. The pollen tubes pass through the swollen, mucilaginous parts of the walls which they apparently digest (Schoch-Bodmer and Huber, 1947). Proofs have also been given that pollen tubes contain enzymes that are capable of disintegrating pectic substances (Paton, 1921). The protoplasts of the transmitting tissue may also be utilized by the developing pollen tube, but in many plants they may contract and die. As a result of this the style does not increase in width even when it contains many pollen tubes.

Apart from the transmitting tissue and vascular bundles, the style consists of thin-walled parenchyma and a typical cuticle-covered outer epidermis, in which stomata may sometimes be found.

THE OVULE

The ovule consists of the *nucellus* which is surrounded by one or two *integuments*, and it is attached to the placenta by a stalk i.e. the funiculus. At the free end of the ovule a small gap is left by the integuments; this opening is termed the *micropyle*. The region where the integuments fuse with the funiculus is termed the *chalaza*. A nucellar cell, usually one of those below the outermost layer at the micropylar end, differentiates into the *macro-* or *megaspore mother cell*. The nucellus is, therefore, considered to be the *megasporangium*.

Ovules may be of different form. The following two main types may be distinguished: (1) *orthotropous* or *atropous* in which the nucellar apex is in a straight line with the funiculus and is continuous with it; (2) *anatropous* ovule in which the apex of the nucellus is directed backwards towards the base of the funiculus (Fig. 251, nos. 6, 7). Between these two extreme forms there are different intermediate stages in which the ovule axis is variously bent (Fig. 251, nos. 8–10). A detailed terminology has been developed for all these forms, i.e. *hemianatropous*, *campylotropous*, and *amphitropous* (Schnarf, 1927; Maheshwari, 1950). In the Plumbaginaceae, *Opuntia*, and some other genera of the Cactaceae, the funiculus is very long and it surrounds the ovule; this type of ovule is termed *circinotropous* (Fig. 251, no. 11).

The ovules develop from the placentae of the ovary. The ovule primordium originates by periclinal division of cells below the surface layer of the placenta. At first the primordium appears as a conical projection with a rounded tip. The first sporogenous cell is already distinguishable in the primordial nucellus in that it is larger than the neighbouring cells, and it has a larger nucleus and denser cytoplasm. The inner integument begins to develop some distance from the nucellar apex. The initiation of this integument takes place by periclinal divisions in the protoderm (subdermal initiation of the inner integument occurs very

rarely). At first the integument appears as an annular ridge and later it grows towards the nucellar apex and so covers the nucellus except for the micropyle left at the free end of the ovule (Fig. 251, nos. 1–4). The initiation of the outer integument takes place by periclinal divisions of either the subdermal or dermal layer a little lower than the initiation of the inner integument. The development of the two integuments is similar. A subdermal initiation of the outer integument is rather common, while a dermal initiation is known, for example, from most families of the Centrospermae, Parietales, Capparidales, and Geraniales (Bouman and Calis, 1977). In many plants the outer integument does not reach the micropyle. In anatropous and bent ovules the growth of the integuments is asymmetric. In plants with sympetalous flowers the nucellus is usually enveloped by a single integument, while in more primitive dicotyledons and in many monocotyledons the ovule has two integuments. In some species the distal rim of one of the integuments is found to be lobed, e.g. the inner integument of *Hernandia peltata* (Heel, 1971).

There are two main possible ways in which the unitegmy (one integument) developed from the bitegmy (two integuments): (1) reduction of one of the integuments; (2) fusion of the integument primordia; (3) a process of integumentary shifting of one integument so it is formed over the other (Bouman and Calis, 1977; Bouman and Schier, 1979).

The nucellus is usually considered to be the megasporangium, but the homology of the integuments is still an unsolved problem. At the chalaza there is no differentiation between the tissues of the integuments and the funiculus.

In certain plants the structure of the ovules differs from that described above. There are ovules that lack integuments and those in which the number of integuments is greater than two. In certain plants, e.g. species of *Asphodelus*, a third integument develops from the base of the ovule; this structure is termed *aril* (Fig. 251, no. 12). The nucellus may be fused entirely to the integuments. In some ovules the integuments grow more than usual and may even close the micropyle, while in others the integuments do not reach the nucellar tip. (See also Chapter 21.)

The thickness of the nucellus in a mature ovule differs in various plants. It may be very thin - one to two cell layers surrounding the embryo sac—e.g. in *Quinchamalium chilense* of the Santalaceae (Johri and Agarwal, 1965), or it may consist of numerous cell layers, e.g. in *Pistacia* of the Anacardiaceae (Fig. 253, no. 1) and *Dysphania* of the Centrospermae (Eckardt, 1967). Also the integuments may vary in thickness, and the thinnest may consist of the two epidermal layers only. However, in such integuments the part closest to the micropyle may be somewhat thicker.

The entire surfaces of all the ovule parts are covered with cuticle. Thus it is possible to distinguish an *outer cuticle* which covers the funiculus and the outer integument externally, a *middle cuticle* which is double and is present between the two integuments, and an *inner cuticle* which is also double and is present between the inner integument and the nucellus.

During the development of the embryo sac the vegetative tissue of the nucellus is completely or partly destroyed, and its content is absorbed by the other parts of the ovule. In certain plants, e.g. the Centrospermae, the nucellus may, in the seed, produce a nutritious tissue which is termed *perisperm*. With the maturation of the ovule the histological structure of the integuments alters. In many plants the inner epidermis of the integument develops into a nutritious layer which is termed the *integumental tapetum*. This layer consists of tall, dark-staining cells. This feature is characteristic of those families in which the nucellus is destroyed early so that the integument is brought into contact with the embryo sac. It is a common feature in the Sympetalae.

Megasporogenesis

There are plants in which several megaspore mother cells appear in a single ovule, but usually only a single mother cell develops in each nucellus. Generally the sporogenous cell develops directly from a hypodermal nucellar cell (Fig. 252, no. 1). This cell is distinguishable from the neighbouring cells by its size, the size of its nucleus and the density of its cytoplasm. In certain plants indirect development of the sporogenous cell has

S. Blackmore & E. Tootill.(Eds.) 1984;.The Facts on File Dictionary of Botany, Market House Books Limited.

and infect the plant cortex. The cortex is stimulated to enlarge and form a nodule the centre of which is red due to the presence of haemoglobin (*see* leghaemoglobin). The bacterium fixes nitrogen by means of the enzyme *nitrogenase.
About 250 species of plants other than legumes also form symbiotic associations with nitrogen-fixing microorganisms. For example, bog myrtle (*Myrica gale*) and alder (*Alnus glutinosa*) have nitrogen-fixing root nodules that appear to contain fungi of the Plasmodiophorales. *Gunnera* and the water fern *Azolla* have blue-green algae in their roots. Free-living nitrogen-fixing microorganisms include the bacteria *Azotobacter*, *Klebsiella*, and *Clostridium* and all the blue-green algae and photosynthetic bacteria.

node A point on the stem from which one or more leaves arise. In the mature stem the nodes are usually well separated by internodes, which elongate through the action of *intercalary meristems. However in some plants, e.g. rosette plants and grasses, the nodes remain close together giving these species their characteristic growth form. The pattern of vascular connections between the stem and leaf at the node may be described as unilacunar, trilacunar, multilacunar, etc. depending on how many leaf gaps are left in the stele.

nomenclatural type *See* type.

nondisjunction The failure of paired (homologous) chromosomes to separate during anaphase I of meiosis. As a consequence, one daughter cell, and hence the two gametes formed from this, will receive both homologues. By corollary, the other daughter cell, and the gametes formed from this, will be deficient for the chromosome in question. Cells containing abnormal numbers of chromosomes are called aneuploids (*see* aneuploidy). *See also* monosomic, trisomic.

nonpersistent Describing pesticides that break down and become inactive relatively quickly. Such pesticides should be used if spraying soon before harvest. Examples are the natural insecticides derris and pyrethrum.

nonsense triplet A name given originally to triplets of bases that do not specify any amino acid and were thus once considered as having no function. There are three such triplets, UAG, UGA, and UGG, all of which have subsequently been found to act as 'stop-signals', i.e. they define the end of a polypeptide chain. Polypeptide initiation also requires a 'start signal', the signal being AUG or GUG, which also code for valine or methionine (or a methionine derivative) respectively.

normal curve (normal curve of errors) The bell-shaped curve obtained when a series of observations that shows a normal (Gaussian) distribution is plotted on a graph. The mean, median, and mode all occupy the same high middle point of the curve, which is perfectly symmetrical around this point.

normal deviate Symbol c. The ratio of the deviation, d, to the standard deviation, σ. It is used to assess experiments where the results do not fall into a limited number of classes but show a continuous range, for example the recording of crop yield in response to various fertilizer levels. To find whether any deviation is significant its probability is looked up in a table of normal deviates. *See also* t distribution.

northern coniferous forest *See* forest.

nu body *See* nucleosome.

nucellus A rounded or oval mass of parenchymatous tissue in an ovule, containing the embryo sac. Its size and shape may be used as a diagnostic character. It is almost totally surrounded by the integuments except for a small channel, the *micropyle, through which the pollen tube may grow prior to fertilization. At fertilization the nucellus may be

reabsorbed as the embryo develops or it may persist to form a nutritive *perisperm in some seeds.

nuclear membrane (nuclear envelope) A double membrane surrounding the nucleus. Each layer has a typical membrane structure (*see* plasma membrane) and is 4 nm to 6 nm in width. The outer membrane is studded with ribosomes on the cytoplasm side while the surface of the inner membrane next to the nucleus is smooth. Electron micrographs of many cells reveal connections between the outer membrane and the endoplasmic reticulum. Between the membranes there is a clear space, the *perinuclear cisterna*. At various points the two membranes fuse and pores are formed. The number of pores varies with the degree of nuclear activity. Extensions of the outer granular or fibrous region of the nucleus form cylindrical complexes within the pores. Their precise function is obscure but they appear to be involved in the transfer of information from nucleus to cytoplasm.

nucleic acid A complex organic acid comprising polymers of *nucleotides formed by condensation reactions that establish phosphodiester bonds between the component nucleotides. There are two types, *DNA and *RNA. The acidic properties of these compounds are due to phosphoric acid, a component of nucleotides. They are called nucleic acids because they were first associated only with the nucleus. However they have subsequently been found in chloroplasts and mitochondria.

nucleohistone A complex formed between the polynucleotides of DNA and basic proteins called histones, which only occur in the nuclei of eukaryotic organisms. Nucleohistone complexes are visible as *nucleosomes.

nucleolar organizer The region of chromosomal DNA that codes for ribosomal RNA. Such regions can be identified as secondary constrictions (the centromere being the primary constriction) often located towards the end of the chromosome. Like centromeres, nucleolar organizers are uncoiled regions of the chromosome and stain poorly. *Nucleoli in the interphase nucleus have been shown to be associated with these regions.

nucleolus A structure within the nucleus that stains densely with basic dyes and consists of proteins associated with RNA. The number and distribution of nucleoli varies but is usually characteristic for any one cell type. Electron micrographs show a central area of short fibres surrounded by a matrix of protein material with granules embedded in the peripheral region. Nucleoli are closely associated with the regions of chromosomal DNA that code for ribosomal RNA (*see* nucleolar organizer). The transcription of the code is dependent on a specific RNA polymerase found only in the nucleolus. A long precursor molecule is formed initially and is processed to produce two shorter molecules. The longer of these associates with proteins in the nucleolus to form the larger ribosomal subunits. The smaller molecules similarly associate with proteins to form the smaller subunits, possibly in the nucleolus or surrounding nucleoplasm. The formation of complete *ribosomes from the subunits only occurs when the latter reach the cytoplasm.

nucleoplasm *See* nucleus.

nucleoside A general term for the category of substances formed when a purine or pyrimidine base combines with carbon 1 of a pentose sugar. Such base–sugar complexes are much less common than base–sugar–phosphate complexes, which are known as *nucleotides.

nucleosome (nu body) A nodule, some 7–10 nm in diameter, consisting of *histones, around which is wrapped a strand of DNA about 150 base pairs long. One