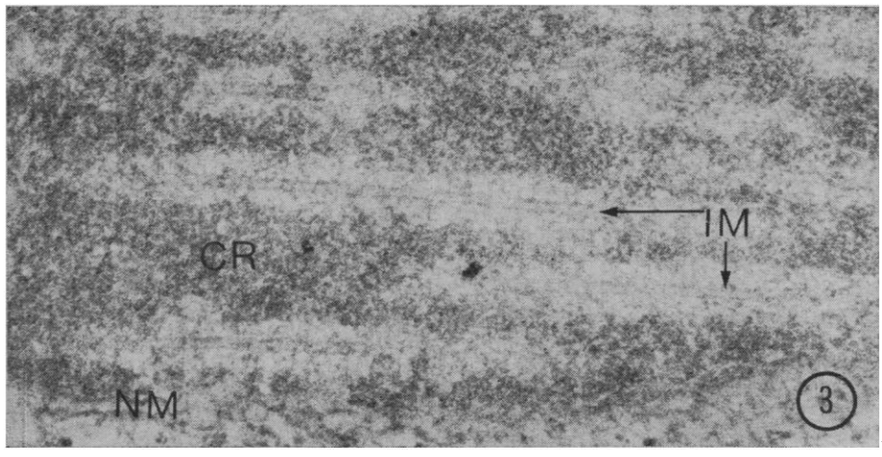

Fig. 3. Part of a telophase nucleus, showing intranuclear microtubules (*IM*), in longitudinal section, surrounded by less dense peritubular regions. *NM*, nuclear membrane. *CR*, chromatin (× 73,000).

The ultimate fate of the intranuclear microtubules is unknown. In late telophase they are not found, presumably because they have been depolymerized into morphologically unidentified subunits. Whether these hypothetical subunits really exist, and whether they remain in the nucleus, are open questions.

Thus, meiotic nuclei are added to the ever-increasing list of places where microtubules are found (*1, 3–9, 11*), and microtubules are added to the list of structures seen within nuclei (*5, 12*).

O. BEHNKE

Anatomy Department, Royal Dental College, Universitets Parken, Copenhagen, Denmark

ARTHUR FORER

The Carlsberg Foundation Biological Institute, Copenhagen, Denmark

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Nature of Seed Dormancy in Phacelia tanacetifolia

Abstract. *The inhibiting effect of light on germination of* Phacelia tanacetifolia *seed is overcome by removing the tip of the endosperm; however, immersion in solutions of high osmotic pressure reinstates the light sensitivity. Inhibition of germination by high temperature behaves similarly. Dormancy is ascribed to balance between mechanical constraint by the endosperm and "expansive force" of the embryo.*

Germination of the seed of *Phacelia tanacetifolia* (family Hydrophyllaceae) is strongly inhibited by light (*1, 2, 3*). However, if that part of the seed-covering structures (endosperm plus remnants of seedcoats) which directly cover the radicle is removed, full germination occurs in light. Removal of other parts of the seedcoat has no effect on normal germination, although a deep cut at the cotyledonary end allows the embryo to grow out abnormally from the cut surface (*4*). The isolated embryos grow just as well in light as in darkness. These facts have led us to conclude (*3*) that light does not prevent germination either by inhibiting growth of the embryo or by giving rise to an inhibiting substance in the seed (since such a substance should diffuse out through the cuts). Instead, control of germination rests in the balance between internal expansive forces developed by the embryo and mechanical restraint exerted by the tough endosperm, and this balance is modified by light. The same situation exists in light-requiring lettuce seed, in which full germination in darkness is made possible by appropriate cuts into the seed. Gibberellin induces germination of both types of seed, irrespective of illumination. In consequence, Ikuma and Thimann (*5*) deduced that the limiting factor in germination is not elongation of the radicle but the mechanical properties of the endosperm layer. A number of experiments with both species of seed have supported this explanation and even the effect of kinetin has been brought into line (*6*). Thus the earlier view that seed dormancy is due to inhibiting substances has been made increasingly untenable.

In general the mechanical restraint can be overcome by either or both of the following means: (i) softening of the endosperm, presumably by enzy-

matic hydrolysis or (ii) increase in the expansive force exerted by the embryo. Light would promote these mechanisms in lettuce, inhibit them in *Phacelia*. Injection of polysaccharidases into lettuce seed (*5*) was shown to imitate the effect of light, presumably by method (i).

Behavior of *Phacelia* seeds in the presence of an osmoticum (a medium to maintain high osmotic pressure) has now been found to support the importance of method (ii). The osmoticum is used to modify the embryo's expansive force. Seeds of *P. tanacetifolia* (*7*) were soaked in water for 90 minutes, and the endosperm at the extreme tip (radicular) end was cut off with a sharp razor blade; care was taken to avoid injury to the embryo. These seeds were sown in 10-cm petri dishes, covered with two discs of 9-cm Whatman No. 1 filter paper moistened with 5 ml of water or test solution, and incubated at 15°C until no further germination occurred. White light having energy of 15,000 ergs cm^{-2} sec^{-1} at seed level was used. Concentrations of osmotica and incubation temperatures, which are of critical importance, were determined in preliminary trials. In a representative experiment, the seeds, after removal of the tip, were subjected to four treatments: (i) 0.3*M* D-mannitol in light; (ii) 0.3*M* D-mannitol in darkness; (iii) 0.3*M* D-mannitol plus potassium gibberellate (160 mg/liter) in light, and (iv) water in light. There were 50 seeds in each group, and the cultures were scored for germination daily. Figure 1 shows that the seeds which had imbibed mannitol gave virtually complete germination, 96 percent, in darkness but only 20 percent in light. Thus osmotic treatment has reinstated the light sensitivity which removal of the tip had abolished. If gibberellin was added the germination in light was raised to 88 percent. The fourth group, soaked in water in light, germinated 82 percent. The reinstated light sensitivity is like the light sensitivity of intact seeds in that it is reversible by gibberellin. Similar results were obtained with another metabolically inert osmoregulator, carbowax 4000 (polyethylene glycol, average molecular weight, 4000). Germination of seeds without tips in 0.08*M* carbowax 4000 averaged 98 percent in darkness, 20 percent in light and 65 percent in light plus gibberelic acid.

Thermodormancy is a second type of dormant behavior in the seed of *P. tanacetifolia* (*1*). The optimum temperature for germination of this seed is approximately 15°C and as the temperature approaches 30°C germination falls almost to zero. If nongerminated seeds are later returned to the lower temperature, they germinate normally. The removal of the tip of these seeds, which overcomes light inhibition of germination, also releases thermodormancy, permitting complete germination at any temperature suitable for growth, even up to 30°C. We now find that exposure of these seeds to mannitol reinstates the sensitivity to temperature. Dry seeds, with tips removed as before, were allowed to imbibe 0.4*M* D-mannitol. When these seeds were incubated in darkness at 7°, 15°, and 25°C until no further germination occurred, the percentages of germination were 94, 78, and 40, respectively (Fig. 2). Suppression of germination due to higher temperatures had become evident again. (In the same experiment, seeds incubated at 16°C in light showed almost no germination. Control seeds, with their tips similarly removed, incubated in water showed 95 percent germination within 6 days at all the temperatures shown.)

We interpret these results as follows.

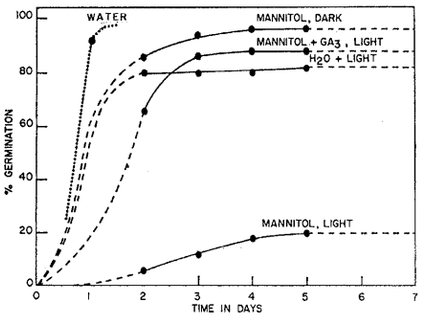

Fig. 1. Restoration of light sensitivity in seeds, after removal of the tip, by 0.3*M* mannitol and the effect of gibberellin (GA_3).

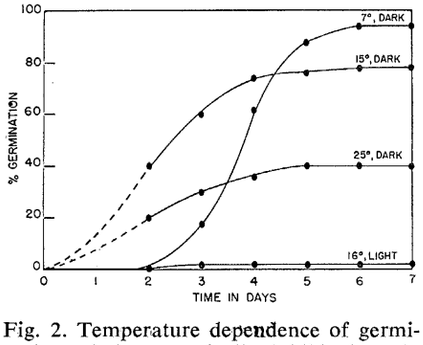

Fig. 2. Temperature dependence of germination of the osmotically inhibited seeds. The seeds were operated on at the radicle end and allowed to imbibe 0.4*M* D-mannitol.

Since the endosperm in front of the radicle has been removed, the seed coverings no longer constitute a block to extension of the radicle. The osmoticum simulates the effect of the endosperm by reducing the ability of the embryo to absorb water needed for cell enlargement. Since germination can now occur in the dark but not in the light (or in light in the presence of gibberellin), generation of "expansive force" in the embryo must be the limiting factor in germination, and it is evidently inhibited by light or high temperature. It is rendered insensitive to light by gibberellin.

A logical way in which the embryo could increase its expansive force would be to increase the content of osmotic materials in the cells by hydrolyzing stored macromolecules. Activities of several hydrolases in the seed were therefore studied. Amylases (largely α-amylase), protease, and lipase were all present in the dry seeds, but the total amount of each enzyme appeared to remain unchanged up to 36 hours of incubation, whether they were incubated in darkness or in light, in water or in gibberellic acid. Thus the sensitivity to light seems not to be directly related to synthesis of these enzymes; nor do gibberellins, in inducing germination, appear to activate amylases, proteases, or lipases. Nevertheless, evidence that synthesis of some enzyme is involved is given by the observation that actinomycin D, at a concentration of 80 mg/liter, inhibited the increase of germination caused by gibberellin in light. Control seeds (in light) germinated 38 percent, those in gibberellate 72 percent, and those in gibberellate plus actinomycin D 45 percent. Seeds in darkness germinated 88 percent, and this was not decreased by actinomycin D. In view of the known light-sensitivity of actinomycin D the inhibition is as strong as could be expected and shows that new synthesis of a protein is an essential reaction in germination. Cycloheximide (10 mg/liter) gave much more powerful inhibitions, preventing germination completely in darkness or in light plus gibberellate. The light sensitivity of enzymes in the isolated embryo is under study.

Recently Scheibe and Lang (*8*) gave evidence that irradiation with red light and treatment with gibberellins result in an increase in growth potential of the embryonic axis of light-sensitive (Grand Rapids) lettuce seed, the differential effect of red and far-red light on expansion being evident

in water but greatly magnified in 0.46*M* D-mannitol. These authors came to the same conclusion that we did, namely, that an increase in "growth potential" of the embryo is the essential factor in germination of dormant seeds. Since Grand Rapids lettuce is a light-promoted seed, the pattern appears to be a general one and to hold for photo-, scoto-, and thermodormancy.

SHEPLEY S. C. CHEN
MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing
KENNETH V. THIMANN
University of California, Santa Cruz

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Hemoglobins in Sheep: Multiple Differences in Amino Acid Sequences of Three Beta-Chains and Possible Origins

Abstract. *Among the three adult sheep hemoglobins (A, B, and C), two (A and B) are reportedly products of alleles. The β-chains of A and B differ by at least seven scattered amino acid residues whereas the β-sequence of C differs from A by at least 16 residues and from B by at least 21 residues. These changes suggest that the origin of C-β antedated the divergence of A and B. Five shared differences between A-β and C-β with respect to B-β can be interpreted as the result of selective advantage in favor of B. A complex of additional mechanisms has possibly been involved in maintaining the A-B-C polymorphism.*

The hemoglobins A and B of domestic sheep, described by Harris and Warren (*1*), form an electrophoretically distinguishable genetic polymorphism. Electrophoretic chromatographic patterns (fingerprints) of tryptic peptides (*2*), molecular dissociation-reassociation experiments (*3*), and amino acid composition of entire polypeptide chains (*4*) establish that relevant variation between the two hemoglobins is limited to the β-chains. Amino acid composition of entire chains indicates that the β-chains from hemoglobins A and B (A-β and B-β), although apparently allelic products (*1, 5*), possess multiple differences (*4*). To this already unusual situation van Vliet and Huisman (*6*) have recently added a third hemoglobin which they term hemoglobin C. An apparently identical component, hemoglobin N, has been described by Braend and Efremov (*7*). Various analyses by van Vliet and Huisman (*6*) and others (*8*) indicate that the characteristic differences between C and the other two hemoglobins are confined to the β-chains. Comparison of amino acid compositions of entire β-chains suggests that there are at least 17 differences between B and C and 12 between A and C (*4*). Two dimensional peptide patterns (fingerprints) (*9*) and partial analysis of tryptic peptides (*8, 10*) clearly indicate that such differences are scattered throughout the chains.

Two features (*11*) influencing the occurrence of hemoglobin C (Fig. 1), are the limitation of hemoglobin C to animals heterozygous or homozygous for the hemoglobin A gene (*6, 7, 8*), and the fact that although traces of hemoglobin C may be present in nonanemic sheep, this component becomes the sole form in animals severely anemic from either blood loss (*6*) or chemically induced hemolysis (Fig. 1). During recovery from anemia, hemoglobin C disappears while hemoglobin A reappears (*6*). These features suggest that the hemoglobin A-B-C system might provide a useful model for examining genetic and cellular factors concerned with regulation of protein synthesis in higher organisms. It seemed desirable first to delineate more exactly the differences between the three known sheep beta chains. The need for such additional information arises from the fact that protein structure and quantity synthesized may, in part, have a common genetic determinant (see *12*).

In this report we describe nearly complete sequences of the three sheep β-chains and discuss the nature and origin of the interchain differences.

Hemoglobins A, B, and C were obtained from a local flock of Dorset sheep. The α- and β-chains were separated by gradient elution from carboxymethylcellulose (*13*). Isolated chains were digested with trypsin, and peptides separated either by high-voltage filter-paper electrophoresis and solvent chromatography (*13*) or by column chromatography. The most successful method consisted of prior separation of peptides into groups by passage through Sephadex G-25 (Pharmacia) columns (1.8 by 240 to 440 cm) (*14*) and subsequent intragroup resolution through elution with a pyridine-acetate gradient from columns of Beckman Spinco 15A resin (*15*). This last combination of methods completely separated all but a few peptides (*16*) and gave higher yields than filter-paper methods.

The amino acid composition of individual peptides was determined (*17*), and the identity of individual tryptic peptides was established both by homology with known sequences of other hemoglobin chains (*18*) and by the radioactive-assembly technique of Dintzis (*13*). The latter method was used to identify B-β and C-β tryptic peptides. The A-β peptides were identified entirely by homology with those from B and C. Sequences of amino acids within peptides were derived by a combination of the fluorescent end-group technique (*19*) and modification of Edman's method of stepwise degradation (*20*). Selective hydrolysis of tryptic peptides by weak acids, chymotrypsin, leucine aminopeptidase, and carboxypeptidase A and B further aided sequence analysis.

Comparison of sheep β-chain sequences is made in Table 1. With one exception, the gross arrangement of tryptic peptides depends upon mutually confirmatory evidence from assembly experiments and from homology with the known sequences of horse and human hemoglobin chains (*18*). The outstanding example of the value of assembly analysis is the exceptional Pro-Asn-Lys (*21*) sequence of C-β-6 to -8, for which there is no obviously homologous peptide in man, horse, or sheep. This sequence is considered NH_2-terminal because its specific activity is the least of any on assembly with radioactive lysine and because we find that proline is the NH_2-terminal amino acid of the intact C-β chain (*22*). The precise placement of the first two resi-