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# VERNALIZATION

OR

## Lyssenko's Method for the Pre-treatment of Seed

BY

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(Bulletin No. 9 of the Imperial Bureau of Plant Genetics, Herbage Plants and Occasional publication of the Imperial Bureau of Plant Genetics, for plants other than herbage.)

Published by the
IMPERIAL BUREAUX OF PLANT GENETICS,
Aberystwyth and Cambridge,
Great Britain.

*Price* 2/6. *March*, 1933.

TITLE PAGE OF WHYTE AND HUDSON'S ACCOUNT OF LYSENKO'S VERNALIZATION METHOD (1933)

# HISTORY OF RESEARCH IN VERNALIZATION

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Introduction:— It requires to be stressed at the outset that the treatment and responses associated with the term "vernalization" represent only one aspect of the work on the developmental physiology of plants, which is receiving the attention of many research workers at the present time. Research on developmental physiology may be said to have begun with the experiments of KLEBS in the years up to about 1918. Earlier experiments had been made, but KLEBS may be regarded as the initiator of the modern extension of this branch of plant physiology, the main thesis of which is that it should be possible to control and direct the processes of growth and development of a plant by exposure under artificial experimental conditions to the particular factors of environment to which it is exposed in the cultivated field or greenhouse or in nature.

In this research on developmental physiology, reviewed by the author elsewhere (WHYTE, 1946), it appears that the decisive factors of the environment which control growth and development are temperature and light (its presence or absence), and that, once these factors have had an opportunity of operating to the required degree, a further set of conditions, including such factors as the relationship between carbohydrate and nitrogen, water relations, general nutrition and so on, then operate and determine whether a plant shall ultimately exhibit vegetative growth or reproduction (development).

A plant displays the capacity to respond to the temperature of its environment in certain instances remarkably soon after fertilization, within 5 or 6 days in one case studied. This capacity is progressively lost with approaching dormancy, and is reacquired when dormancy of the ripe seed is broken and germination begins. As soon as chlorophyll is formed in the leaves of the young seedling, the light régime of the environment can begin to operate, and from then on the behaviour of a plant appears to depend upon the interrelationship of these two factors, temperature and light. Plants such as winter cereals proceed most rapidly to reproduction if they are first exposed to a certain quota of low temperature, followed by an exposure to a sufficient number of days of the appropriate length, together with an optimal temperature.

The terms of reference of this article limit consideration to the acquisition by germinating seeds or young seedlings, either in their natural environment or under experimental conditions, of that particular dose of low temperature under the influence of which they can proceed most rapidly and efficiently to reproduction, provided the environment to which they are subsequently exposed is optimal for the reproductive processes of that variety. It is therefore necessary to ignore the wider questions relating to the effect of temperatures at all stages of a plant's life on its reproductive behaviour. The effect of temperature on the photoperiodic reaction of plants is being discussed by MURNEEK (pages 39 et seq.). From investigations of plant growth and development such as those being conducted by WENT at the California Institute of Technology, Pasadena (1943, 1944a, 1944b), a much fuller understanding of the significance of temperature will be forthcoming; using air-conditioned greenhouses, it is now possible to maintain the total environment under control throughout the life of a plant, from fertilization to seed formation.

Taking again the example of winter cereals in the latitudes in which they are cultivated, it has long been the practice to arrange their sowing dates in such a way that they are exposed while in the field to the low temperatures desirable, but apparently not obligatory, for progress to reproduction. If these varieties are sown in spring alongside the spring varieties, differentiation of flower primordia may occur after a considerable period of time, but earing does not occur normally. It was the aim of the chillers of seed to give such grain their specific quota of low temperature in the laboratory or the barn and to sow them in the spring in the hope that they would come into ear in the same season, that is, they would behave like spring varieties, although not, as some have erroneously stated, to the extent of being "converted" into spring varieties.

GASSNER (1918) was one of the early chillers of grain, giving cereals in the laboratory the dose of low temperature they would normally receive in the field after being sown in the autumn in Germany. His work is discussed later. In subsequent years others chilled various types of seeds, but the interest of agricultural physiologists became transferred to the relations between light and development and particularly to the work of GARNER and ALLARD and their successors on the photoperiodic reactions and categories of plants.

The interest in temperature was, however, revived and the attention given to developmental physiology as a whole stimulated by the elaboration by LYSENKO and his associates at the Institute of Plant Breeding and Genetics, Odessa, of the technique which has come to be known throughout the English-speaking world as vernalization. This technique is considered by the Russians to differ from that of GASSNER in recognizing that only a minimum of growth is essential during low temperature exposure so that development can take place.

The theoretical principles which were elaborated from the experimental data quickly exerted a revolutionary effect on the whole trend of Soviet research in plant biology, expressed in a spate of scientific and agronomic articles dealing with theory and practical applications.

Largely through the medium of the publications of the Imperial Agricultural Bureaux (1933, 1935), this technique and the theoretical principles were made available to English-speaking readers, and this was followed by many experiments on the vernalization of a wide range of crops under a wide range of environments. The technique was regarded as the solution of the problem of bringing crops to maturity in the short Northern summer and of producing crops in drought-affected regions, of bringing high cash value crops such as market garden or greenhouse plants to maturity in time to catch early markets, or of producing seed in one year from biennial or perennial crops not normally fruiting in their first year. It must be admitted that this was a period of dogmatic assertions and exaggerated or ill-founded claims, of uncertain techniques and an incomplete understanding of the fundamental biological principles. That period is now past and it is therefore appropriate to review its consequences, and to examine the changes in scientific knowledge and agricultural or horticultural practice which have been achieved.

It will be convenient to deal with this by using a combination of the historical and geographical approaches, thus indicating the international nature of the problems and how investigators in many different countries have been tackling them. In the U. S. S. R., where the technique of vernalization had its origin, the emphasis throughout has naturally been on the applied and practical results to be obtained from its use. After the "fashion" created by the I.A.B. publications had died down, the nature of the interest in countries outside the U.S.S.R. has varied considerably. In Germany it was primarily practical, concerned with its use in making possible the cultivation of such only partially adapted crops as the soybean. Interest in India is both academic and practical, the latter to the extent that centres for vernalization of crop seeds have been suggested, in order to supply cultivators with properly treated seed which, when sown, would produce plants which would reach maturity more rapidly than those from untreated seed and thus avoid a period of adverse environment such as excessive heat, drought or floods.

In Great Britain, on the other hand, research has been concerned primarily with the fundamental biological processes underlying the technique of vernalization. The work of F. G. GREGORY, O. N. PURVIS and others of the Research Institute of Plant Physiology of the Imperial College of Science and Technology, South Kensington has provided an important analysis of the causal factors in this reaction to temperature treatment. The historical trend of these experiments is reviewed later.

**Chilling of Seed:**— Many reviewers have suggested that there is nothing new in the vernalization technique. MCKINNEY (1940) has brought into prominence a little of this historical background, stating that "it seems only fair to point out that the basic concepts involved in these studies (on vernalization and developmental phases) have been known within certain circles for many years. They simply have not been recognized in all circles of plant science until recently." After stating that the older horticultural and agricultural journals and the older scientific journals and text books demonstrate that some growers and some botanists had already recognized part or all of these concepts, MCKINNEY refers to an early report in 1857 by KLIPPART of Ohio, and even earlier records (20 years before KLIPPART) of the production of a crop of grain from spring-sown winter-wheat seed which had been subjected to low temperatures before sowing. KLIPPART's statement is so much to the point that it is again quoted here from MCKINNEY.

> "To convert winter into spring wheat, nothing more is necessary than that the winter wheat should be allowed to germinate slightly in the fall or winter, but kept from vegetation by a low temperature or freezing, until it can be sown in the spring. This is usually done by soaking and sprouting the seed, and freezing it while in this state and keeping it frozen until the season for spring sowing has arrived. Only two things seem requisite, germination and freezing. It is probable that winter wheat sown in the fall, so late as only to germinate in the earth, without coming up, would produce a grain which would be a spring wheat, if sown in April instead of September. The experiment of converting winter wheat into spring wheat has met with great success. It retains many of its primitive winter wheat qualities, and produces at the rate of 28 bushels per acre."

From these and similar examples, one can bring the discussion into modern times by reference to the classic experiments of KLEBS and GASSNER which, as already noted, provided the starting point of the modern branch of biological research concerned with developmental physiology.

The work of KLEBS was concerned with the control of growth and development by appropriate adjustments of the decisive factors of the environment, temperature and light. Three developmental phases were recognized, not all of the same nature. Ripeness-to-flower is a qualitative phase not recognizable morphologically, and is dependent on the temperature effect in relation to assimilation and dissimilation. The initiation of flower primordia and the formation of inflorescences and flowers are quantitative phases, recognizable morphologically. This research by KLEBS and the postulation of a form of phasic development contain at least a reference to most of the problems at issue in the study of the developmental processes in plants, and of the reasons for the transformation from a vegetative to a reproductive state. He recognized the effect of the decisive factors, temperature, light and/or darkness, alone or in combination, upon a series of phases or conditions, each one of which must be established before the next can begin; a hint of an after-effect of light treatment; evidence of reversal of development; the internal metabolic changes associated with vegetative growth or reproductive development.

KLEBS was, however, primarily concerned with the effect of light. GASSNER (1918) was primarily interested in temperature, and temperature only in the early stages of plant growth, just after germination. As GASSNER considered that the shooting and flowering of winter cereals depends to a marked degree on their passing through a period of low temperature, experiments were conducted with the following objects:— to determine (*1*) the principles underlying the effect of low temperatures on flower production, and (*2*) further details regarding (*a*) co-operation of the temperature of germination, and temperature and other conditions during the course of vegetation, and (*b*) the significance of the vegetative period in understanding the relative peculiarities of summer and winter cereals.

GASSNER sowed the seed of cereals in sand at different dates between January 10th and July 3rd, and subjected them to the following temperatures during germination: 1 to 2°, 5 to 6°, 12° and 24°C. Selected seedlings were subsequently placed in pots and then planted out. Tables and graphs in his paper show (*a*) date of sowing, (*b*) temperature during germination, (*c*) "appearance above ground," when the young leaf had reached a length of 20 to 25 mm., and (*d*) shooting. The crop plants used were Petkus winter rye and Petkus spring rye. The results may be summarized as follows (GASSNER, 1918):

The temperature during germination had no influence on shooting of spring rye; plants germinated at the temperatures quoted above, all shooting at regular and uniform intervals. In winter rye, only the plants germinated at 1 to 2°C. exhibited shooting regularly throughout the whole vegetative period. Plants germinated at 5 to 6°C. only shot regularly if their "appearance above ground" had occurred before the end of April, and those germinated at 12° and 24°C. only if they had appeared above ground before the middle of April and the end of March respectively.

Some plants did not shoot at all, but of those which did, the plants that had appeared above ground simultaneously attained shooting all the sooner, the lower the temperature at which they had been germinated. Thus, plants which appeared above ground in the middle of March and which had been germinated at 1 to 2°C. shot about 9 days, 21 days and 41 days earlier than those that had been germinated at 5 to 6°C., 12°C., and 24°C. respectively. The illustrations in GASSNER'S article indicate the further difference that plants germinated at a low temperature not only reached the shooting stage more rapidly but also more regularly than those germinated at a higher temperature.

GASSNER'S conclusions regarding the physiological difference between spring and winter rye were as follows: Spring rye is practically independent of any need to pass through a cold period before it can achieve shooting (Blütenauslösung). The flowering (Blütenbildung) of winter rye depends on its passing through a cold period either during germination or at some stage subsequent to germination.

GASSNER made some observations regarding the correlation between winterhardiness, sugar content and flowering. Without going into any great detail with regard to the problem of death of the plant cell through cold, he stated that the following may be quoted as definite facts:

(1) The growing of winter plants at a low temperature induces increased winter-hardiness;

(2) low temperature is a condition for the "release of flower formation;"

(3) cultivation at low temperature increases sugar content; it may be assumed that winterhardiness and sugar content are in a causal relationship, while the "release of flower formation" depends on sugar content;

(4) winterhardiness and the cold-requirement, so important for flowering, are correlatively connected.

It can thus be seen that, in the period before the technique of vernalization was evolved and received such publicity, research workers in several countries were concerned with this type of study and had proceeded a certain distance in the direction of controlling growth and development by the manipulation of the environment. Progress was being made in the Soviet Union as well, where MAXIMOV continued the experiments of GASSNER and treated seedlings with low temperature, thereby influencing the whole of their further development. Winter cereals which, when sown in spring, did not normally reach the heading stage, headed and flowered normally when the seedlings had been exposed to a short period of chilling. It was found possible to direct or determine the further course of development by treating them during the early stages of growth with appropriate temperatures and a given periodicity of light. Although these experiments provided a basis for understanding the behaviour of winter varieties in the field when sown in autumn in the normal way, the technique was still, however, applicable chiefly to laboratory trials and crop cultivation in greenhouses, owing to the fact that the treatment was applied to seedlings, which could not be handled on a bulk basis like seeds.

TOLMAEV (1929) came even nearer to the development of a technique which could be used in agronomic practice. His aim was to keep the seeds in the form of seeds for as long a period as possible, and yet at the same time to break their dormancy and permit growth to start very slowly. This was presumably done because it was considered that the temperature during or just after germination is the effective factor in inducing subsequent flowering, and at the same time to provide treated grains, not seedlings, which might still be sown in the normal manner. Working on winter wheat, for example, TOLMAEV applied partial soaking to retard growth during germination; by keeping such seed for 2 months at 0°C., he obtained plants which produced ears in the first year when sown in spring, even after the latest date of sowing. Similar results are claimed with sugar beet, and conversely, it is stated that a sugar beet plant can be maintained in a vegetative condition for at least 4 years by withholding that factor of th environment (temperature) appropriate for development.

To explain these results, practically identical with the results of vernalization described below, TOLMAEV produced what MAXIMOV has described as a "peculiar theoretical interpretation." According to TOLMAEV, winter cereals will not ear nor will sugar beet flower until a definite active phase has been completed in their "stem plasm,' a definite work must be done and a sufficient amount of the products of disintegration accumulated. In spring plants, the stem plasm is ready for its function at the time of seed maturity. Those conditions, for instance, low temperature, which stimulate the accumulation and preservation of the products of distintegration, also stimulate fruit bearing, and on the contrary the absence of the accumulation of the products of disintegration during intensive growth under the influence of light and high temperatures depresses stalk formation.

**U.S.S.R.:**— Working at the Experiment Station at Ganja in Azerbaidzan, LYSENKO observed the influence of the thermal factor on the duration of the developmental phases in cereals and cotton (1928). It was

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им. тов. Орджоникидзе в Гандже.

Выпуск 3.

Т. Д. ЛЫСЕНКО.

# Влияние термического фактора на продолжительность фаз развития растений.

## ОПЫТ СО ЗЛАКАМИ И ХЛОПЧАТНИКОМ

Баку — 1928.

Title page of LYSENKO's first article on "Effect of the Thermal Factor on Length of Phases in Development of Plants," published in the Trudy of the Azerbaidžan Experiment Station, No. 3 (1928), *cf.* p. 38.

found that the time required for the completion of some of the recognizable stages in the growth of cereals such as seedling emergence, ear emergence, full flowering, wax ripeness, etc., are completed more rapidly at higher temperatures than at lower. It was noted, however, that in spring and more especially in winter forms the phase of shooting (ear formation and elongation of internodes) does not always show this relation. With an increase in the temperature at which the crops are grown, this phase is in some cases initiated earlier, and in others takes place later or not at all. When absent, the plants remain at the tillering or rosette stage. LYSENKO called this retention of the winter habit "hibernalism," that is, the property of plants to remain for an indefinitely long period at the phase of tillering without flowering stems.

After moving from Azerbaidzan to the Institute of Plant Breeding and Genetics, Odessa, LYSENKO continued to study, in collaboration with DOLGUSIN, this property of hibernalism in cereals and the nature of the difference between spring and winter forms. The results of the experiments carried out for this purpose were presented at the All-Union Congress of Genetics, Selection, Seed Production and Animal Production in 1929 (DOLGUSIN and LYSENKO, 1929), and may be indicated from the following quotation of their conclusions:

(a) Experiments on time of sowing showed that there are no definite dates which can be used to distinguish winter forms from spring forms. The later the date of sowing, the larger is the number of varieties which retain the winter habit; when varieties are sown in the second half of April, or even later under the conditions of Azerbaidzan, not only all the winter and alternative forms but also the majority of the spring forms remained at the tillering stage.

(b) One of the main factors preventing shooting is the high temperature of the period subsequent to sowing. However, after a pre-sowing chilling of slightly sprouted or imbibed grain at 2.5 to 3.5' C. for a definite period varying according to variety (Kooperatorka wheat = 38 days; winter barley = 28 days), winter cereals will proceed to the shooting stage no matter when sown. After such a pre-sowing treatment, the rate of shooting increases according as the temperature of the period following sowing itself increases. Since a high temperature after sowing retards shooting in plants raised from unchilled grains, it is argued that in cereals the period from seed swelling to ear emergence covers at least two biologically consecutive phases. The first of these does not produce any morphological changes in the plant and is directly related to the thermal factor, high temperatures prolonging its duration. (Compare with the condition of ripeness-to-flower postulated by KLEBS, 1918). The duration of the second phase (shooting) is, like all other phases, inversely related to temperature, progressing most rapidly at the higher temperatures.

(c) Thus, the retardation of shooting observed in cereals sown without pre-treatment and subjected to a high temperature after sowing is due to the prolongation of the unrecognizable phase; if the temperature of the period after sowing reaches a maximum at and beyond which this phase for a given variety cannot be passed, then this variety will behave as a winter form, and will not ear.

(d) Since the temperature of the environment rises with the approach of summer, it is observed in sowings of a mixture of varieties that the later the sowing, the greater is the number of varieties which change from a spring to a winter habit

Having worked out a thermal constant for a number of varieties, LYSENKO and his associates at Odessa proceeded to apply this particular method of chilling to slightly sprouted grain of cereals and seeds of other crops. In 1931 and 1932, LYSENKO published instructions for the treatment of seed by this method (jarovizacija) which came to he known in countries other than the Soviet Union as vernalization (Imperial Agricultural Bureaux, 1933). The technique had as its theoretical basis the principle of phasic development. Although it is not possible to discuss this controversial hypothesis here, it is necessary to give the principles postulated by LYSENKO in order to appreciate the Russian interpretation of the results obtained in this work.

In the 4 or 5 years up to and including 1934, LYSENKO formulated his theoretical conceptions as follows:

(*a*) growth and development are not identical phenomena;
(*b*) the entire process of the development of an annual seed plant consists of individual *étapes* or stages;
(*c*) the stages or phases always occur in a strict sequence, and a subsequent stage cannot commence until the preceding stage has been completed;
(*d*) different stages of development of the same plant or crop require different environmental conditions for their completion.

These postulates are related to the technique of vernalization in the following way. If growth and development are not identical phenomena, it should be possible to reduce the rate of progress of growth to a minimum while allowing for rapid development. LYSENKO expressly confines his distinction in terms of the rate of growth and development, the former meaning the rate of accumulation of dry matter or increase in size of a plant, the latter meaning the rate of progress through the stages of development as postulated by him. The technique of vernalization is based on a very reduced rate of growth, in order to prevent formation of seedlings, combined with a rapid rate of development under the influence of the optimal environment, that is, low temperature.

Discussion of postulates (*b*) (*c*) and (*d*) is outside the scope of this review, but it should be noted that LYSENKO assumed that the low temperature used in the vernalization technique is an obligatory requirement for a certain phase of development; once that requirement has been met, the seed, seedling or plant can proceed to the next phase. This strict sequence of phases is strongly criticized by physiologists in other countries; a number have expressed the view that low temperature is not obligatory for ultimate reproduction, and that vernalization by short day is possible. If it is supposed to be the light period of a short day which has the vernalizing effect, obviously this could not operate on soaked grain kept in complete darkness.

According to the Russian literature, vernalization rapidly became a regular agronomic practice throughout Russia. It was used to ensure reliable crops in regions in which the growing period was short owing to frost or drought, or to ensure the production of two crops per season on the same land (for example, a grain crop followed by a catch crop for green manure or forage). Courses for farmers were held at the Institute at Odessa and elsewhere. In most parts of Russia the low winter temperatures made it possible for farmers to vernalize their grains and seeds in the barns, once the elements of the technique had been acquired.

From data collected by M. A. OLJHOVIKOV, the following acreages in the U.S.S.R. were sown with vernalized seeds in the years 1932-7 inclusive:

| | |
|---|---|
| 1932 | 43,000 |
| 1933 | 200,000 |

| | |
|---|---|
| 1934 | 600,000 |
| 1935 | 2,100,000 |
| 1936 | 7,000,000 |
| 1937 | 10,000,000 |

More recent data have not been seen; it is not known to what extent, if at all, Russian farmers are now using the method, or whether any centres for treatment and distribution of vernalized seeds have been operating.

From an academic point of view, the technique of vernalization, and more particularly the theoretical principles which were evolved on the basis thereof, received a great deal of attention among Russian physiologists. Writing at the request of the author, MAXIMOV (1934) stated that the doubts and contradictions that had arisen would suffice to show that the theory was far from being completely formulated. "A single investigator or group of investigators around him are, of course, unable to solve all the most difficult problems of the physiology of development," problems which now open to physiologists and others an extensive and fertile field of research.

One of the early interests of Russian investigators in connexion with vernalization was in the possibility Of diagnosing whether seeds had had their total treatment required. Methods based upon a staining technique or on the change in iso-electric point were tried, but no satisfactory conclusion seems to have been reached. Other investigators wrote on subjects such as the relation of enzymes and hormones to vernalization, the physiological and biochemical characteristics of vernalized as compared with untreated seeds, and of the plants produced from these seeds. The outlook on the breeding of crop plants changed, the emphasis then being placed on the need for preliminary analyses of environmental requirements followed by breeding based upon these specific and varietal characteristics. All these aspects are discussed more fully elsewhere (WHYTE, 1946); it is at present desirable to refer to two particular aspects of this work in the U.S.S.R., namely, the hormonal interpretation of development with particular reference to the vernalized grain (CHOLODNY, 1939, see also CAILAHJAN, 1937), and TUMANOV'S work on the relation between developmental phases and winterhardiness (1940).

A considerable amount of literature has accumulated on the evidence for and against the existence of a hormone which causes flowering in plants. It appears that some such substance does operate, but it has not yet been possible to isolate it, still less to apply it to plants by injection or other methods in order to induce flowering. This hormone is, however, generally associated with the action of light upon leaves, and is assumed to be responsible for transmitting a response taken up by a leaf to the growing point under its control, causing it to exhibit the characteristics either of growth or development. CHOLODNY has, however, produced an interpretation of the low temperature effect associated with vernalization based on the existence and operation of a hormone.

The investigations of SCHANDER (1934), CHOLODNY (1935), LAIBACH and MEYER (1935) and others have shown that seeds contain reserves of various hormones received from the mother plant and concentrated chiefly in the endosperm or cotyledons. This reserve is believed to come into action during germination, in the early stages of which the phytohormones are transmitted to the organs of the embryo which are beginning their growth and development. SCHANDER showed that the activating substances contained in cereal grains at the very beginning of germination pass over to the embryo through the special elongated cells of the aleurone layer, and regarded these substances as being required for further development and growth of the embryo. CHOLODNY (1935) claimed that auxin or a related compound which is accumulated in the endosperm during germination of cereal grains is also transmitted into the embryo. This auxin has been identified as indole-3-acetic acid (HAAGEN-SMIT, LEECH and BERGREN, 1942) ; it is rapidly liberated from its storage form in the endosperm by simple moistening or alkali hydrolysis, differing in this respect from the auxin in leaves and stems (THIMANN and SKOOG, 1940).

In 1936 CHOLODNY formulated the nature of the internal factors which act upon the growing points during vernalization. According to the hypothesis which he developed, the embryo is stimulated to activity by the pretreatment technique but lacks the capacity for normal growth, because of the insufficient moisture and low temperature accompanying the vernalization treatment; the embryo absorbs from the endosperm the growth hormones which are stored there in great quantity. As these hormones are generally used primarily for growth, and as under the conditions of the vernalization treatment there is almost no growth, the concentration of the hormone in the cells of the embryo is assumed to have risen considerably above the normal for that stage of the plant's life. It is further assumed that this increase of the intracellular concentration of the hormone in the growing points causes an acceleration in the progress of the meristematic cells of the young plants through the first phases of development. Thus the interval of time which separates these first phases of development from the later stages associated with preparation for fruiting is shortened. The full result is assumed to be that the whole cycle of plant development is completed sooner than it would be under normal conditions (CHOLODNY, 1939).

Reference will be made in a later section to the experiments of GREGORY and PURVIS on the vernalization of excised embryos, on which they base their own criticism of CHOLODNY'S hypothesis. CHOLODNY felt the need to introduce a correction into his hypothesis because of the rapid advances being made in the knowledge of phytohormones. "It is hardly possible at the present time to doubt that the qualitative aspect of the complex of phytohormones acting upon the embryo is not also without its own effect upon the development of the embryo. The composition of that complex and the chemical nature of the active substances found in the tissues of the embryo and of the plant developing from it undoubtedly also change under the influence of the external and internal conditions in which development is being maintained."

CHOLODNY considers that his working hypothesis is confirmed by some of his own experiments on the pre-treatment of seed with various solutions of indole-acetic acid, and blastanin or embryo extract (CHOLODNY, 1936), as well as by some later experiments by THIMANN and LANE (1938) and by CAILAHJAN and ZDANOVA (1938). These investigators found that a short treatment of growing seeds with increased concentrations of phytohormones affected the subsequent development of plants, what might be called "vernalization by hormone." In some cases it was claimed that the growth of the vegetative organs was either inhibited or accelerated; in others fructification was intensified and occasionally a conspicuous curtailment of the vegetative period was recorded. The whole question has however been experimentally investigated by HATCHER (1943) and HATCHER and GREGORY (1941) whose work is reviewed later.

This work has completely negated the theory of CHOLODNY. As far as the hormonal treatment of seeds is concerned, the balance of evidence is at the moment against any after-effect of the above nature (TEMPLEMAN, 1939; STEWART and HAMNER, 1942), although HAMNER (1938) has referred to a U. S. Patent being taken out by one WENDT for the application of acetylene to pineapple plants. HAMNER states: "The prompt differentiation of flower primordia and fruit development which ensue in treated plants as compared with non-treated plants which differentiate flower primordia many weeks and even months later is worthy of critical experimentation."

In his book entitled "The physiological bases of winterhardiness in cultivated plants," TUMANOV (1940) deals with the literature on a number of aspects directly or indirectly related to developmental physiology, including the relation between winterhardiness and phasic development as understood in Russia. In the research of VASILJEV (1934), KUPERMAN (1936), KUPERMAN and ZADONCEV (1936), SALTYKOVSKII and SAPRYGINA (1935) and others, it has been claimed that frost resistance is connected with phasic development. These experiments were made chiefly with winter cereals; frost resistance was found to be generally lower, and at times conspicuously so, in plants from vernalized seeds, that is, from seeds with embryos which had, as far as could be ascertained, completed the thermo-phase, to use the terminology of phasic development. The following percentage survival of wheat plants observed by TUMANOV (1935) is quoted as an example:

| | VERNALIZED | UNVERNALIZED |
|---|---|---|
| Ukrainka: early sowing | 16 | 92 |
| Ukrainka: late sowing | 12 | 94 |
| Moskov. 02411: early sowing | 7 | 93 |
| Moskov. 02411: late sowing | 0 | 96 |

TUMANOV and IVANOVA found that both early and late sowings of vernalized grain of Lutescens wheat became depleted at higher temperatures (-11 to -12°C.) than sowings from unvernalized grain, and that generally the frost resistance was higher with the shorter periods of presowing vernalization. After 20 days of vernalization of winter wheat and 15 days in winter rye, the frost resistance of the resulting plants was conspicuously reduced.

Special investigations conducted in the Leningrad region indicate the risks to which autumn sowings were exposed when the early autumn temperatures favour the natural vernalization of seeds after sowing. When Lutescens 0329, Moskov 02411 and Minhardy had been sown at the end of August, they were found to have completed their tIiermo-phase on Jan. 13

in 1934-5, on Jan. 1 in 1935-6, and almost entirely by Nov. 19 in the 1936-7 season.

Such a variation in time of completion of the thermo-phase cannot be attributed to variation in climatic conditions alone. A partial or complete vernalization as far as the thermo-phase may occur while the developing grain are still attached to the mother plant, and is also regarded as possible during prolonged drying on the field in a cold and wet season, when the seeds may be sufficiently imbibed to begin the slow growth necessary before development through the thermo-phase can begin.

TUMANOV recognizes two possibilities resulting from the after-sowing vernalization in the open: (*1*) the seeds may be completely vernalized before the beginning of winter, (*2*) the vernalization of the thermo-phase may be completed either by the beginning of winter or during the winter. In the first case, especially when the plants have had an opportunity to grow for a considerable period, they would lose their ability to harden, and their resistance to frost would thus be much reduced. Some investigators believe, however, that frost resistance begins to fall in vernalized plants with the beginning of the photo-phase. Some investigations have indeed shown that the capacity to resist frost falls rapidly when the plants are grown after completion of the thermo-phase under conditions which favour the progress of the photo-phase, namely, high temperatures and long day. On the other hand, TUMANOV states that a fall in frost resistance may also be noted when plants are grown under conditions not favouring the photo-phase, as may be the case with late autumn sowings of vernalized seeds.

TUMANOV refers to his own investigations in 1936/7. When the plants were tested on Nov. 2, and the thermo-phase was not completed until Nov. 19, frost resistance was found to be reasonably high; the percentage of survival in Lutescens 0329 at -12 to -18°C. varied from 98 to 84. When the plants were tested on Dec. 14, that is, 25 days after the end of the thermo-phase, frost resistance had fallen, percentage survival varying from 85 to 27 at temperatures of -13 to -22°C. Before the tests were made, all plants had been kept under conditions which would ensure their hardening. In these investigations, the loss of the capacity to resist frost was observed only when plants were grown for some time after the completion of the thermo-phase; this was the case with winter rye, Vjatka, the percentage survival being 78 to 44 in the first test, and only 25 at -13°C. in the second test, while no plant survived temperatures lower than -15°C.

The situation is different when the thermo-phase is completed so late that no growth is possible before the plants are frozen. In TUMANOV'S investigations in 1935/6, when vernalization was completed by Jan. 1 in winter wheats and by Dec. 1 in winter rye, the percentage survival at -14 to -18°C. varied from 96 to 74 in Lutescens and from 81 to 70 in Vjatka. Similar results were obtained in another experiment where the percentage survival was much higher after later sowings.

It may be noted at this point that OLEINIKOVA has shown that the thermo-phase can be completed in the open during the winter under the snow cover in the winter conditions at Leningrad. Winter wheats were sown on Nov. 17, Jan. 1, Feb. 1, and March 1 and 31 in the open; from the beginning of April, the various series were transferred to greenhouses where the temperatures were such as to prevent any further progress through the thermo-phase by those plants which had failed to complete this phase in the open. Winter rye failed to ear only after the last date of sowing, and winter wheats after the last two dates. It is therefore concluded that, even in strongly "winter" plants, the thermo-phase may be fully or partially passed during the winter.

TUMANOV considers that the completion of the thermo-phase alone does not yet necessarily mean that frost resistance is markedly reduced, provided the conditions for subsequent growth are not present. Different conditions arise, however, under a deep snow cover or during a temporary thaw, when plants may resume active growth very readily and would thus have a reduced resistance to frost. TUMANOV considers it possible that, after the thermo-phase, the growing point is capable during resumption of growth of changing the state of the protoplasm in all other tissues in existence at that time, and suggests a hormonal mechanism as the possible explanation.

KUPERMAN (1936) produced evidence that vernalized plants accumulated much smaller quantities of sugars than unvernalized plants, but TUMANOV and FEDEROVA found that vernalized plants have sufficient amounts of sugars and that a sugar deficiency cannot be regarded as the reason for their lower frost resistance. The amount of sugars in vernalized plants first rose very slowly and later, in April, more or less rapidly, reaching 26 per cent on May 7, or 10 per cent lower than in unvernalized plants, and similar to unvernalized plants before wintering.

TUMANOV and IVANOVA found that the exposure of vernalized plants to the conditions requisite for the second phase of hardening had no effect, although it is during this phase (of hardening) that plants should show a considerable increase in their frost resistance. TUMANOV therefore assumes that those changes that originate during vernalization cause the protoplasm to be readjusted in such a way that it loses the ability to undergo the appropriate changes during the second phase of hardening, regardless of the presence of protective substances, dehydration and low temperatures.

Since enzymatic processes are very active during the period of germination, it was natural that studies should be made of their behaviour in relation to vernalization. Whether any change which may be noted in their activity is governed ultimately by the concentration of hormones present depends upon the confirmation of CHOLODNY'S hormonal interpretation of vernalization. The studies on enzymes in relation to vernalization made by RICHTER and his associates and DEMKOVSKII, and on the iso-electric point by RICHTER, GAVRILOVA and others, are described in the early reviews of vernalization (I.A.B. 1935).

OVECKIN and others (1936) studied the biochemical changes in winter wheat grains during vernalization. Grains were vernalized at 0 to 1°C. under normal air conditions, and also in a 0.003 per cent concentration of chloroform or ethylene chloride. Samples were taken on every sixth day and records made of respiration rate, sugar content, catalase activity, content of mono-amino-acids and reduced glutathione, and of the percentage of fully vernalized grains that grew when planted out of doors. The presence of chloroform and ethylene chloride reduced the percentage of fully vernalized grains; the respiration rate, catalase activity, and content of reduced glutathione were all lower in grains vernalized in ethylene chloride. The oxidation-reduction processes are intensified during vernalization, but there is no relation between content of sugars and mono-amino-acids and vernalization.

SAPOZNIKOVA (1935) analysed vernalized seed of *Lupinus angustifolius*. The content of reducing sugars in seeds treated at 6 to 7°C. increases with the progress of vernalization, a fact regarded as suggesting an increasing activity of the enzymes acting on carbohydrates; however the content of reducing sugars gradually falls in seeds vernalized at 4 to 5°C. The amount of active enzymes rises with vernalization at 6 to 7°C. to a maximum on the last day of treatment; enzymes increase less rapidly with vernalization at 4 to 5°C. during the first 12 days and then fall. The control of active enzymes is measured by their activity at 35°C. The protease content in seeds vernalized at 6 to 7°C. was found to rise to a climax on the day of sowing, while in those vernalized at 4 to 5°C. its changes were indefinite. The activity of catalases, peroxidases and respiration varied in the different series. Marked activity of catalases and peroxidases is a feature of vernalized lupine seeds that give rise to plants with a reduced vegetative period, and this character is stated to be useful for distinguishing vernalized from unvernalized seeds.

From determinations of nitrogenous substances (total N, insoluble and soluble N, amino-N, amide-N, and ammoniacal N) in seeds and plants during vernalization and in those under conditions which prevent vernalization, KONOVALOV (1938) found their behaviour to vary considerably. When vernalization was prevented, the disintegration of the proteins extended to the end products, whereas during vernalization the proteins retained their form, but became more readily soluble. This worker concludes that nitrogenous substances appear to be re-synthesized during vernalization, and regards this transformation as a distinctive feature of the vernalization process. PASEVIC (1940) found that vernalization induces changes in the protein substances of the wheat germ affecting both their colloidal state and their amino-acid content.

In some early studies on the effect of vernalization on the rate of accumulation of dry matter, KONOVALOV (1936) found that vernalized plants of wheat and lentils accumulated more dry matter per unit of time than the unvernalized control, and the yield of organic matter was consequently increased. More recently, KONOVALOV and POPOVA (1941) found that the synthesizing capacity of vernalized plants is higher than in the unvernalized controls. By the time of earing, vernalized plants contained 26 per cent more organic matter than those from soaked and germinated seeds and 52 per cent more than plants from seeds sown dry.

KONOVALOV (1944) has continued his work at the Timirjazev Institute of Plant Physiology, Moscow, on the effects which vernalization of seed exerts on the growth and physiological processes in the leaf relative to the yield of grain or seed ultimately produced. The chief concern in these experiments has been productiveness of a plant as governed by the intensity of photosynthesis, the extent of its leaf area, and the duration of activity by the leaves.

The intensity of photosynthesis was not materially affected by vernalization, but the interval between emergence of the leaf and its death was shortened. The factor most closely connected with yield was leaf area of a plant, and it was this which was markedly affected by vernalization. The successive emergence of leaves was more rapid with than without vernalization, and each leaf reached its maximal size sooner. A growing leaf uses much of the products of metabolism for its growth; vernalization has the advantage of hastening the growth of leaves and ensuring its early completion, after which the products are released for the benefit of the embryonic ear. It was noticed that the content of nitrogenous substances in the lowest leaves was diminished after the leaves had ceased to increase in size.

The synthetic activity of the leaves reached the maximum sooner in vernalized than unvernalized plants, and, after remaining stationary for a while, gradually decreased. The accumulation of dry-matter was likewise more abundant in vernalized plants. Consequently there was more material available for translocation to the developing ear, thus accounting for the well-being of vernalized plants which is to be especially observed during a dry season.

The yield of late-maturing wheat in KONOVALOV'S experiments was increased by vernalization because, although tillers were reduced in number, their ears bore better and more numerous spikelets, as well as more numerous and heavier grain. In the early-maturing varieties the good development of the ears could not compensate for the reduced number of tillers, and vernalization did not therefore increase the yield.

A similar result is reported by BUZOVIR (1936), who made experiments over two seasons with varieties of winter and spring wheat and a variety of millet; at the beginning of vegetative growth in winter wheat and throughout the vegetative period in the other plants, the rate of elaboration and accumulation of dry matter was greater in vernalized plants. The accumulation of carbohydrates was also greater in vernalized plants, particularly during the period from jointing and stem elongation to milk ripeness.

In experiments made by ZAICEVA (1939) with spring and winter wheats sown with vernalized and unvernalized seeds, it was found that the chlorophyll content increased as the plants advanced towards sexual maturity, reaching a maximum of over 6 mg. of crystalline chlorophyll per grm. Of leaf weight in both varieties, by the time of heading or thereabouts and falling rapidly thereafter. In rapidity of development, the spring wheat was somewhat ahead of the vernalized winter wheat. No such regularity was noted in unvernalized winter wheat plants that remained at the tillering phase, the chlorophyll content varying from the beginning of tillering between 3 and 4 mg. of crystalline chlorophyll per grm. of leaf weight, the highest figures being obtained in the leaves nearest to the spikes. Advance in development is considered to be the chief factor causing this conspicuous variation.

An important aspect of Soviet research is the work of KOSTJUCENKO and ZARUBAILO on the vernalization of grain during the period preceding dormancy while the grain is ripening on the mother plant. This is a question which has very considerable practical implications, and affects all matters connected with plant introduction and the cultivation of plants in an environment differing widely from that in which the seeds were produced.

The experiments of KOSTJUCENKO and ZARUBAILO are discussed along with those of GREGORY and PURVIS on the same aspect in a later section of this review.

Another small point may be mentioned before leaving the Russian research on vernalization. It has been found that not all plants can be vernalized in the form of seeds or grains, and that the low temperature applied does not begin to have an effect on certain types until a seedling has been formed. This applies more particularly to certain species of Brassica such as rape and turnip.

**Germany:**— The name of Professor W. RUDORF of the Kaiser Wilhelm-Inst. f. Züchtungsforschung, Müncheberg, Mark, is chiefly associated with work on and reviews of vernalization as applied to German conditions and crops. A review of the literature on vernalization was published in 1936 (RUDORF and HARTISCH, 1936), and a summary of experiments made at Müncheberg, Giessen, Göttingen, Berlin, Berlin-Dahlem, Heidelberg and Leipzig was published in 1938 (RUDORF, 1938a). RUDORF himself studied the relation between vernalization and photoperiods and resistance to cold.

Using winter, dual-purpose and summer forms of wheat and barley, RUDORF (1938b) applied different temperatures and different photoperiodic treatments with a view to discovering a method of producing material for studying the physiological causes of cold resistance. Resistance to cold under periods of chilling ranging from 20 to 60 days at 3 to 5°C. was determined, and it was found that such resistance is largely governed by the condition of the plants in the dormant period before the thermo-phase has been completed, that is, before vernalization is complete. The more this internal condition is degraded by vernalization the less is the resistance to cold. Varietal differences are noted; in certain varieties a reversal in the reaction can be seen after 60 days' chilling. It remains a question whether such a turning point can also be determined in definite winter forms chilled for more than 60 days. When the thermo-phase has been partially completed, the capacity to harden is annulled to a degree which is in proportion to the duration of the treatment. The further work which was then stated to be in progress was the discovery of varieties with as long a thermo-phase as possible (maximum requirement of low temperature) and the maximum cold resistance at that phase, and the discovery of varieties which would still exhibit great hardening capacity even after completing the thermo-phase.

In 1936 HARDER and STÖRMER (1936) showed that white mustard (*Sinapis alba*) responded to temperature of germination best in short-day conditions. An exhaustive comparative study of vernalized mustard and oats made by VON DENFFER (1939) under a series of daylengths artificially maintained showed that this different behaviour of these two plant types occurred in the time between initiation of flower primordia and flowering. The relative shortening of the purely vegetative phase from germination to the visual appearance of flower buds in mustard, and shooting in oats, was on the other hand approximately the same for all daylengths (from continuous light down to 12 hour day) as compared with control plants which had not been vernalized.

The increase in duration of vernalization led in both mustard and oats to a gradual shortening of the vegetative phase, which asymptotically approached a limiting value. This occurred in winter oats (var. Eckendorfer) after 80 days treatment, in mustard after 50 days vernalization. The limiting duration of the vegetative phase after optimal duration of vernalization was dependent upon the daylength under which the plant was grown; being minimal in continuous light, maximal in short days. The extension of daylength leads in both plant types to a gradual shortening of the vegetative phase, and again approaches a limit value. The limiting duration of the vegetative phase under optimal photoperiod is dependent upon the duration of vernalization.

The leaf number on the main shoot of oats is directly related to the development, as McKinney and Sando showed for wheat, and Purvis and Gregory for Petkus winter rye; the same direct relation between leaf number and time of flower formation was established also in *Sinapis alba*. The results can be interpreted without difficulty along the lines of the flower hormone hypothesis of Purvis and Gregory.

Rudorf, Stelzner and Hartisch (1937) described a technique for vernalizing seed at high temperatures, presumably for use with crops such as soybeans. The aim in this work was to prevent fungal and bacterial growth and to avoid excessive elongation of the seedling. It is necessary to keep the seed in motion and at the same time to maintain the temperature and moisture in the seed containers within the desired limits; a certain amount of aeration must be practicable and it must be possible to darken the whole apparatus. A portable apparatus is described, movement being secured by the use of small disinfection drums.

Germany is a borderline environment as far as the cultivation of soybeans is concerned, yet the wide range of products that can be obtained from this crop made it desirable to grow it wherever possible. Experiments have therefore been made on the manipulation of development of this crop. Rosenbaum (1937*a*, 1937*b*) attempted to vernalize soybeans; although agreeing that the conditions of treatment laid down by Lysenko are practically correct, it is considered that similar effects may be attained with other temperatures, and that, by suitable adjustment of the conditions of growth and development, the vernalization treatment may be dispensed with entirely. The environmental conditions following sowing are stated to govern the course and rate of development more than does previous vernalization, for which reason it is considered to be difficult to make reliable comparisons of the results of vernalizing different varieties. A reduction in time taken to flower may be more readily obtained by means of the photoperiodic after-effect, although this is said to entail a reduction in yield. Rosenbaum does not recommend either vernalization or photoperiodic induction of soybeans for the use of the practical farmer or the plant breeder in Germany.

Another German worker who does not consider that a thermo-phase is obligatory in winter wheat is Voss (1938). By using short day under conditions of a constant high temperature of about 20°C., it was found possible to induce shooting in German winter wheats. The manner in which a variety reacts to treatment may differ within the variety under (*a*) combined cold and short-day treatment, and (*b*) short-day treatment alone, at high temperatures. Given a very long growth period, winter wheat may be brought to flowering even under continuous illumination and high temperatures. Having found that winter wheat can pass from the vegetative to the reproductive state most readily under the influence of different external factors, in particular low temperature and short day operating alone or in combination, VOSS suggests a new definition for "winter wheat" and "summer wheat." Wheat varieties which, under a constant high temperature (approximately 20°C.), constant illumination (by natural or artificial light not exceeding 1500 lux), and otherwise favourable conditions in a greenhouse, do not begin to shoot within 4 to 6 weeks are described as winter wheats.

**Great Britain:**— The chief work on the vernalization of cereals in England began about 1931 at the Research Institute of Plant Physiology, Imperial College of Science and Technology, London and 1934-35 at the School of Agriculture, Cambridge. BELL (1935, 1936) worked at Cambridge with wheat, barley and oats, but the experiments were not continued and BELL has since investigated the developmental physiology of sugar beet (see BELL and BAUER, 1942, and WHYTE, 1946). In his work on cereals, an acceleration in earing was observed in the winter varieties of barley and wheat but none in the oat varieties. Many of the varieties giving the greatest response in the early stages (more rapid germination) showed no acceleration in caring, and conversely the winter varieties in which vernalization produced the maximum difference in earing date showed little or no early response. The type of response is a varietal character.

Following upon these experiments, a series of sowings was made with vernalized and untreated seed of winter and spring varieties of barley, wheat and oats, one in October and six successive ones in the following spring between Mar. 8 and April 5. The treatment consisted of exposure to 3°C. for 14 days. All but one of the varieties showed a slight acceleration in germination. The acceleration in earing was slight or nil in the autumn sowings, but in the spring sowings there was a progressive increase in acceleration with delay in sowing in the winter varieties and, conversely, a progressive decrease in the spring varieties. This difference in behaviour is attributed to the rise in temperature with progressive lateness of sowing; greenhouse sowings under higher temperature came into ear sooner than field sowings in spring varieties and later in most winter varieties, showing that low temperatures in the open had effected a certain amount of vernalization. Examination of growing points was also made in these trials and revealed great differences in varietal behaviour.

The long series of experiments made by GREGORY, PURVIS and other workers of the Research Institute of Plant Physiology, Imperial College of Science and Technology, South Kensington, has provided valuable data on the fundamental biological processes concerned in the response of growing seed to vernalization. The work on effects of temperature during germination was embodied in a publication (PURVIS 1934) on the analysis of the influence of temperature during germination on the subsequent development of certain winter cereals and its relation to the effect of length of day.

Winter rye (var. Petkus) was the experimental material used. One series was grown in normal long day at high and low levels of nitrogen supply and subjected to temperatures of 1°C. and 18°C. respectively during germination; a second series was grown in short day of 10 hours with similar temperature treatments. In the long-day series, cold germination reduced the time required for earing and flowering, not so in the short-day series. Low temperature in the long-day treatment also reduced the rate of tillering, not in the short-day plants. The effects of temperature were found to be independent of nitrogen manuring.

Under long days and after germination at 1°C., flowering occurred 10 weeks after planting out; after germination at 18°C. it was delayed until autumn when a few ears appeared. Under short days ear emergence was indefinitely delayed after both temperature treatments. Examination of the growing points revealed, however, that under both temperature treatments and daylengths ear initials are laid down. *After germination* at 18°C. flower initials are laid down *earlier in short days than in long days* and the number of initials is considerably increased, and with it the potential grain-yielding capacity Such ears, however, die within the leaf sheaths, and the stems fail to elongate. After germination at 1°C. the characteristic temperature after-effect, namely, early flower differentiation, was manifest only under long days while under short days the effect was slight. When plants which had received some weeks of short-day treatment were subjected to long days, ears were rapidly formed after germination at 1°C. and more slowly after germination at 18°C. Even with plants germinated at high temperature, flower differentiation may be induced by short-day treatment alone, showing that cold germination is not essential for reproduction. Stem elongation and ear emergence, however, fail unless long days are given subsequent to ear differentiation. These results illustrate a point stressed from the start in the work from this Institute, namely, the great importance of determining the time of formation of the flower primordia rather than the time of emergence of the inflorescence in studies of this kind.

The concept of minimal leaf number has also been a characteristic of the work of GREGORY and PURVIS. Observations made by PURVIS (1934) on the spike primordia indicate that a minimal number of leaves must be formed before the differentiation of flower initials can occur. Under short-day conditions this is always about twenty-two, irrespective of variety and temperature, while under long-day conditions the minimal number is less for plants germinated at low than at high temperatures, namely, 12 for the former (in this particular experiment) and 22 to 25 for the latter. This reduction in leaf number combined with the more rapid elongation of the spike leads to acceleration in the onset of floral differentiation and thus induces a behaviour more closely resembling that of spring varieties in which the minimal number is only seven. The effect of the greater minimal number of leaves required before "ripeness to flower" is attained in short days is partially counterbalanced by the higher rate of differentiation of the ear in these plants, particularly in those germinated at low temperatures. The increased rate of floral differentiation in short days also affords an explanation of the photoperiodic after-effect observed by RASUMOV in short-day plants like millet. However, in long-day plants like oats or barley, the floral differentiation is almost independent of daylength, only the later stages being hastened by long day. All spring cereals are also of this type. On the other hand, winter rye behaves as a long-day plant only when germinated at low temperatures. When germinated at 18°C. the floral differentiation shows the typical short-day behaviour, being promoted by short day, though stem elongation and further stages of floral development still retain the long-day reaction. Thus the effect of chilling in winter cereals sown in the spring is to reduce the minimal number of leaves required for flower initiation and to enable the plant to attain the condition of "ripeness to flower" at an earlier date while long days still prevail. It is in this way that PURVIS interprets the phenomena connected with vernalization.

After a review of vernalization as a method of hastening flowering (PURVIS, 1936), PURVIS and GREGORY proceeded to a comparative study of vernalization of winter rye by low temperature and short days, the first of a series of papers published (1937) under the general title of "Studies in Vernalization of Cereals," aiming at a closer analysis of the proximal cause of the effect of the low temperature applied during germination. A preliminary report appeared in Nature (GREGORY and PURVIS, 1936a).

The treatments with low temperature or preliminary short days both resulted in acceleration of flowering. With the low temperature treatment, 4 days' exposure produces a definite response; the magnitude of the response increases with the duration of treatment up to a limit, of 14 weeks, after which the flowering behaviour of winter rye is indistinguishable from that of spring rye. With increasing exposure to low temperature the leaf number in winter rye is reduced from approximately twenty-five to seven.

With the exposure of seedlings of winter rye to preliminary short days (10 hours) the magnitude of the effect on flowering increases with length of treatment up to 6 weeks, but longer exposures retard flowering. Reduction of leaf number to a minimum of sixteen occurs after 6 weeks' treatment with short days, but longer treatment again leads to increase.
In both spring and winter rye, PURVIS and GREGORY state that there is a minimal leaf number, seven, which cannot be further reduced, and a maximum, about twenty-five, which cannot be further increased. The primordia between the eighth and twenty-fifth are indeterminate and can produce either leaf or spikelet; each primordium is double, consisting of a flowering branch (spikelet) with a subtending bract, either part of which may he inhibited according to the length of day or temperature of germination.

To explain the mechanism of these reactions of a germinating seed or a seedling to low temperature or short day, PURVIS and GREGORY (1937) postulated the existence of a hypothetical "flower-forming" substance, the effect of which on the labile primordia varies according to the factors to which the plant is exposed. Although stressing that the whole situation is not yet clear, they state that the following facts are established:

(*a*) The hypothetical substance is not preformed in the endosperm and transferred to the embryo, as the embryo isolated from the seed behaves similarly (see experiments on excised embryos described below).

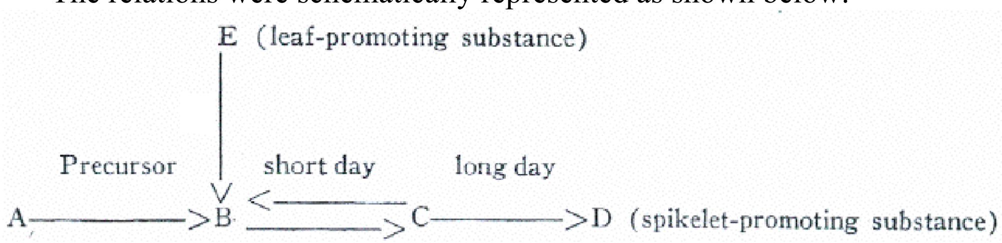
(*b*) The change can be reversed by high temperature (again see below). It appears possible that during vernalization some precursor of a "flower-forming" substance accumulates in the embryo. This substance may be supposed to be translocated and accumulated in all growing points of the shoot, and when a critical concentration is reached induces flower initiation. The increasing rate of formation of primordia indicates that the substance increases autocatalytically. In spring rye about 2 weeks at normal summer temperatures after germination suffice to reach the critical level, while in unvernalized winter rye somewhat less than 6 weeks are required.

(c) An oxidative reaction is involved in the formation of the substance, as anaerobic conditions completely inhibit its production during vernalization of winter rye and partially so in spring rye.

During vernalization the "flower-forming" substance or its precursor increases in concentration so that the critical level is reached earlier, and thus spikelet initiation sets in earlier in the sequence of labile primordia, and a progressive reduction in leaf number occurs, finally reaching the minimum of seven characteristic of spring rye. The progressive effect of longer duration of low-temperature vernalization can formally be accounted for on these lines.

The relations to day-length are more difficult to formulate. The outstanding problem here is related to the fact that in both spring and winter rye in continuous short days a maximum leaf number is found, which is approximately constant and independent of temperature of germination. In spring rye the "flower-forming" substance is already present in high concentration, but only in long days do the early members of the series of labile primordia form spikelets. It appears, therefore, that two stages are involved in the process. A precursor depends for its formation on a genetic factor in spring rye and on low temperature of germination in winter rye. This precursor accumulates in all the growing-points, increasing autocatalytically. A second stage in the process involves reactions depending on day-length.

The relations were schematically represented as shown below:




The next experiments reported by GREGORY and PURVIS (1938a) continued the process of narrowing down the problem of the localization of the vernalization effect. It was concluded that the process of vernalization is localized in the embryo itself and is entirely independent of changes in the endosperm or aleurone layer which may take place during germination. It was therefore concluded that the growing embryo is able to synthesize hormones from a simple substratum containing glucose and inorganic salts (including nitrates), and that CHOLODNY'S interpretation based upon the existence of large quantities of blastanin in the endosperm and the increased transference and concentration of this substance in the developing embryo is inadequate.

The phenomenon of reversal of vernalization (devernalization) by high temperature had already been reported by GREGORY and PURVIS (1936c);

in Studies 2 (GREGORY and PURVIS, 1938*a*), a loss of vernalization by drying down the vernalized grain for periods longer than 6 weeks is demonstrated by experiment. Although the effect of flowering vanishes, an after-effect is shown by the high tiller production; this is attributed to a reversal in the hormone synthesis noted (as indicated in the diagram above). This line of approach was continued in Studies 3 (GREGORY and PURVIS, 1938*b*) when anaerobic conditions were utilized to inhibit growth by means other than low temperature. This made possible a successful experimental demonstration of a quantitative reversal of the vernalizing effect of low temperatures by higher temperatures, as well as the complete inhibition of the low temperature effect in the absence of oxygen; and definitely proved that low temperature and not mere inhibition of growth is the essential factor in vernalization.

Continuing the study of the part played by the various tissues of the grain in the process of vernalization, GREGORY and DE ROPP (1938) found that in the absence of a carbohydrate supply the isolated embryo cannot be vernalized by low temperature. Also it should be noted that previous to the excision of the embryo in the work of GREGORY and PURVIS the grain had already been soaked in a sterilizing solution for a period of 5 hours. DE ROPP (1939) therefore studied more closely the conditions obtaining in the grain during the first few hours of germination, and found that, during the preliminary soaking, a "regulator" controlling its auxin production and distribution enters the embryo from the endosperm and aleurone layer.

The fifth Study in this series from the Research Institute of Plant Physiology concerns an investigation by PURVIS (1939) on the inheritance of the spring and winter habit in hybrids between Petkus winter rye and Petkus spring rye. This is thus outside the strict terms of reference of the present review. The cross gave an F1 generation in which the spring habit was completely dominant, while in the F2 germination the spring and winter plants were in an approximate 3: 1 ratio. In the F2 generation, however, there is within the "spring" and "winter" classes a dispersion of flowering dates, suggesting a less simple inheritance. PURVIS explains this on the basis of independent segregation of factors for early and late ripening as distinct from the spring and winter habit. The Russian interpretation of results such as these is discussed elsewhere by WHYTE (1946).

Returning again to the question of the nature of the biological processes occurring within a vernalized grain, NUTMAN (1939) investigated by cytological technique the processes occurring in the embryo-sac of rye subsequent to fertilization; an aspect of direct interest in connexion with the fact that vernalization can be carried out on a developing embryo (see later section of this review). It was hoped to obtain some indication of the functions of the various tissues of the developing fruit and to throw some light on the possible role of hormones in development. This study has led to the discovery of new facts hitherto overlooked, more particularly the part played by the nucellar tissue in the organization of the mature grain and a sequence of degenerative changes associated with each phase of development. NUTMAN considers the possible role of nekrohormone (or wound hormone) in the development of the rye grain, comparing his observations with those of LA RUE and AVERY (1938) on immature excised embryos of *Zizania aquatica* in artificial culture.

NUTMAN'S next investigation (1941) amplified the earlier observation made by GREGORY and PURVIS (1938*a*) that ears harvested as early as 5 days after pollination produced viable grain, and the earlier observations made on the development of immature barley kernels removed from the plant by HARLAN and POPE (1922, 1926). Although the smallest viable grain found by NUTMAN was only one-sixteenth the weight of normal grain, it was found that all plants whatever their original grain weight reached approximately the same size at the end of their period of growth. Over the greater part of the experiment the plants grown from dwarf grain exceeded those grown from normal grain in (*a*) relative growth rate (on a fresh weight basis), (*b*) rate of tiller production, and (*c*) rate of leaf formation. These three results are attributed by NUTMAN to a single effect, namely, to the rate of development of the meristem of plants grown from dwarf grains being higher.

PURVIS has returned in the eighth paper (1944) in this series to the vernalization of excised embryos. It had already been shown (GREGORY and PURVIS 1938b) that the excised embryo of rye when supplied with nutrient salts and glucose responded to vernalization in the same manner as the whole grain and to a comparable degree. Similar results had previously been reported by GREGORY and PURVIS (1936*a*), by KONOVALOV (1937) and by BASSARSKAJA and GROSSMAN (1941), and confirm LYSENKO'S statement that the locus of vernalization lies within the embryo. Further, PURVIS (1940) demonstrated "that the isolated stem apex of the embryo, if supplied with sugar and subjected to low temperature, regenerates the whole plant, which shows itself to have been vernalized." MCKINNEY and SANDO (1935) noted a similar result when they mutilated embryos before chilling, but maintained the contact with the endosperm. GREGORY and DE ROPP (1938) had further shown that if sugar is omitted from the medium in which these excised embryos are grown, they remain unvernalized. The general conclusions drawn by these British workers from their experimental results is that the large accumulation of blastanin found by CHOLODNY (1936) in the endosperm of maize does not play any essential part in the vernalization process; the embryo is capable of synthesizing any hormones that may be necessary for vernalization from external sources of carbohydrate in the presence of inorganic salts, with possibly the aid of additional substances already present in the embryo before imbibition. Embryos excised from unsoaked seed can still be vernalized, thus showing that the objection to the excised embryo technique raised by CHOLODNY (1939) and quoted by WHYTE (1939) is not valid.

PURVIS enumerates the factors which can possibly participate in the vernalization process as follows:

*a*) the embryo and its constituents,
*b*) the source of organic carbon,
*c*) the source of nitrogen and the mineral salts,
*d*) the effect of low temperature.

Preliminary experiments conducted in 1939 comprised the subjection of excised embryos to a low temperature for 6 weeks on agar media containing varying proportions of carbohydrates and nitrogen. A difficulty was experienced in that even on media containing little or no sucrose some degree of vernalization was apparent, and this applied only to a few individual embryos. It appeared that quite small amounts of sucrose were adequate for vernaliztion and that a source of this sugar may have been available within the individual embryo, either as stored sugars or starch in the tissues or as starch grains adhering to the scutellum. A technique was therefore developed involving periods of starvation for the embryos at high temperature to remove available sugar by respiration and this successfully reduced the variability of the low sugar series, although it raised a further set of problems requiring further investigation. The scope of the eighth study is, however, confined to a consideration of the effect on vernalization of varying the carbohydrate and nitrogen supply, the effectiveness of various carbohydrates in growth and vernalization, and to a discussion of the results obtained "within the limits imposed by the unforeseen difficulties of the experiment."

The whole question of the auxin production in the developing and ripe ear has been systematically studied and its relation to vernalization has been investigated by HATCHER (1945). This work has experimentally tested the hormone hypothesis of CHOLODNY and has shown this theory to be completely untenable. Preliminary reports have appeared in Nature (HATCHER and GREGORY, 1941; HATCHER, 1943). The auxin content was determined by the standard *Avena* coleoptile test of WENT (1928). The following salient points have been established. Mature cereal grains of different species vary considerably in total auxin content and concentration, giving a descending series: maize, oat, rye, wheat, barley. The auxin of the rye grain is located in the endosperm and chiefly in the aleurone: none could be definitely detected in the embryo itself. The auxin contents of spring and winter rye varieties are not different. The auxin is detectable in the embryo either during development or during germination either at normal or vernalizing temperatures. Auxin, far from being accumulated in the embryo of grain undergoing vernalization as CHOLODNY supposed, is not concerned in the process. The other findings in this work are not of immediate concern and are therefore very briefly mentioned.

Free auxin appears in the rye grain in the third week after fertilization, accumulates during the next month to a peak value and disappears almost completely with ripening. A "precursor" is present similar to that described in maize by AVERY, BERGER, and SHALUCHA (1941) and isolated by BERGER and AVERY (1944). This precursor releases indole-3-acetic acid on alkaline hydrolysis (optimal conditions N/50 NaOH). The precursor and free auxin appear at the same time and both accumulate to a peak value, up to which time they are present in a constant ratio of 2 : 1. During ripening the free auxin disappears at a greater rate, so that in the ripe grain of rye the ratio reaches a value of 80 : 1 precursor to free auxin. Spring and winter rye are quite similar in the matter of auxin production.

When prematurely harvested the same cycle of auxin accumulation and disappearance occurs, but the peak value is the lower the earlier the grain is removed from the plant. No auxin is present in the ear prior to emergence, and is then first formed in the anthers, where a cycle of accumulation and disappearance occurs similar to that in the carpel.

The shortening of the first leaf after low temperature treatment noted by THIMANN and LANE (1938) referred to above, and held by them to support CHOLODNY'S hypothesis, has been shown to be quite independent of the vernalization process, for it occurs equally in spring and winter rye and is a different effect of low temperature.

It has not been possible to deal in any detail with the differences of opinion between various workers regarding the question of reversibility of vernalization. It has already been noted that plants have been successfully "devernalized" by drying, or by exposure to high temperature or to anaerobic conditions. The latest and confirmatory experiments on devernalization by high temperature are reported by PURVIS and GREGORY (1945). Petkus winter rye was vernalized for 42 days at 1°C. and the seeds were then subjected to a range of temperatures for varying periods of time (temperatures, 25°, 30°, 35° and 40°C.; durations, 1, 2, 3, 4, 5, 6 and 10 days). Petkus spring rye was subjected to similar heat treatments without previous low temperature treatment. Results obtained at 35°C. are given in table where 'scores' give a positive measure of the degree of vernalization; other results will be later published in full (the method of scoring is described by GREGORY and PURVIS, 1938a).

| Duration of heat treatment | Spring rye (unvernalized) | | Winter rye (vernalized) | |
|---|---|---|---|---|
| | 'Score' | No. of replicates | 'Score' | No. of replicates |
| 0 (Controls) | 123 ± 0.12 | (52) | 100 ± 3.0 | (47) |
| 8 hours | 120 ± 0.77 | (48) | 95 ± 4.6 | (44) |
| 16 hours | 121 ± 0.93 | (53) | 90 ± 4.9 | (35) |
| 1 day | 120 ± 0.65 | (62) | 75 ± 6.5 | (35) |
| 2 days | 121 ± 0.68 | (47) | 80 ± 5.9 | (39) |
| 3 days | 124 ± 0.40 | (55) | 72 ± 9.5 | (16) |
| 4 days | 123 ± 0.46 | (49) | 72 ± 6.4 | (33) |
| 5 days | 120 ± 0.64 | (42) | 63 ± 6.6 | (35) |

PURVIS and GREGORY drew the following conclusion from these data:

(*a*) Heat treatment of the seed is without effect on the flowering behaviour of spring rye, thus showing that there is no question of a lethal action. Spring rye heated at 40°C. for 4 days scored 120, substantially the same as at 35°C. The reduction in score in winter rye is therefore not due to any injury effect, as is claimed by investigators who believe that vernalization is not reversible.

(*b*) A progressive and significant reduction in the score accompanies prolonged heat treatment of winter rye. The delay in flowering is then due presumably to the reversal of the vernalization effect. Complete devernalization did not occur. After treatment at 40°C. for 2 days the score was 79 ± 8.0 (four times that of unheated and unvernalized winter rye) whereas a further day at 40°C. killed all the seed.

In the same experiment, after the preliminary vernalization for 42 days at 1°C. and the subsequent heat treatment as above, the seeds were then revernalized by exposure to low temperature for a further 6 weeks. The effect on ear development is shown in the following table.

| DURATION OF HEAT TREATMENT | 'SCORE' | NO. OF REPLICATES |
|---|---|---|
| 0 (Controls vernalized 12 continuous weeks at 1°C.) | 112 ± 1.18 | (17) |
| 8 hours heating followed by six weeks at 1°C. | 113 ± 0.95 | (48) |
| 16 hours | 117 ± 0.82 | (41) |
| 1 day | 117 ± 0.45 | (42) |
| 2 days | 113 ± 0.93 | (39) |
| 3 days | 118 ± 0.65 | (54) |
| 4 days | 117 ± 0.63 | (54) |
| 5 days | 116 ± 0.65 | (55) |

The scores are slightly higher than those of the control series vernalized for 12 weeks continuously, thus proving reversibility of the vernalization process in both directions. The efficacy of the high temperature devernalization in these experiments depends upon the duration of the previous low-temperature period and thus upon the "intensity" of vernalization; this aspect is now being investigated.

*Field experiment at East Malling Research Station, 1942.*— Soviet research on vernalization had indicated that increases in yield could be obtained with spring cereals, and techniques for the treatment of spring cereals were included by LYSENKO (see I.A.B. 1935) and DOLGUSIN (ibid.) in their recommendations to agronomists. The requisite temperatures varied with the type of cereal used: thus 0-2°C. was suggested for winter wheat and barley; 2-5° for hard late wheats, spring barley, and oats; and 10-12°C. for soft spring wheat and hard early wheat. The duration of vernalization recommended varied from 35-40 days for winter wheat, to 5-7 days for early spring wheat.

In the field experiment under discussion (GREGORY, 1945), two each of the cereals wheat, barley, and oats were used. Vernalization treatments were used covering the range of temperatures recommended by the Soviet agronomists, and at each temperature two durations, one long and one short, were employed.

The varieties used and the vernalization treatments given are stated below:

| | VARIETY: |
|---|---|
| *Wheat* | Little Joss, April Bearded. |
| *Barley* | Plumage Archer, Spratt Archer. |
| *Oats* | Victory, Resistance |

| TEMPERATURE: | DURATION OF VERNALIZATION IN WEEKS: |
|---|---|
| 37°F. (2.8°C.) | 4 and 2 |
| 40°F. (4.4°C.) | 3 and 2 |
| 45°F. (7.2°C.) | 3 and 2 |
| 50°F. (l0°C.) | 2 and 1 |

Sowing date (1) April 6-11; (2) May 4-9.

There were thus, in all, six varieties with four vernalization temperatures each with two durations of treatment and in addition two controls. The whole experiment was carried out with two sowing occasions. Each treatment was replicated eight times giving in all (6 x 10 x 2 x 8) 960 plots. A factorial design was used for the experiment which covered an area of approximately 0.6 acres. As the results are shortly to be published in full, only a brief summary is given here.

The variety, Little Joss wheat, was found to behave like a winter wheat and showed a very large response to vernalization as seen from the table.

*Yield of Grain and Straw for Little Joss Wheat in cwt, per acre:*

| Temperature of vernalization | Duration of treatment | | Duration of treatment | |
|---|---|---|---|---|
| | Long | Short | Long | Short |
| | Grain | | Straw | |
| 37°F. | 17.5 | 10.7 | 33.2 | 28.2 |
| 40°F. | 17.0 | 11.6 | 35.1 | 28.0 |
| 45°F. | 13.1 | 12.0 | 30.2 | 27.0 |
| 50°F. | 6.9 | 6.1 | 22.9 | 22.9 |
| Control (unvernalized) 3.5 | | | 22.7 | |

The effect in the true spring varieties of the vernalization treatments on grain yields are given in the table below.

*Effect of Treatment on Grain Yield in cwt. per acre in True Spring Cereals: —*

| Variety | Vernalized | Control | Difference |
|---|---|---|---|
| April Bearded | 21.5 | 21.1 | + 0.4 ± 0.73 |
| Plumage Archer | 28.2 | 25.8 | + 2.4 ± 0.90 |
| Spratt Archer | 24.8 | 22.3 | + 2.5 ± 1.19 |
| Victory | 19.9 | 18.9 | + 1.0 ± 0.86 |
| Resistance | 23.6 | 23.1 | + 0.5 ± 0.97 |
| Mean of all varieties | 23.6 | 22.2 | + 1.4 ± 0.42 |

With each variety vernalization has led to an increase in grain yield, but only in the case of the two barley varieties are these increases statistically significant. The mean effect of vernalization on all varieties combined is about 5 per cent and is significant. The mean effects of temperature of vernalization on grain yield combining all varieties used were:

37°F. 23.5 cwt.; 40°F. 24.0; 45°F. 23.3; 60°F. 23.6.

There is no suggestion of an optimal temperature of vernalization, and consequently no support can be found in this experiment for the view that spring cereals require higher temperature than winter cereals during vernalization, as LYSENKO recommended. Nor is there any evidence that shorter durations are more effective; the mean grain yield for long duration being 23.2 cwt. and short duration 23.8 cwt.

Observations were made during the course of the experiment on ear emergence in the true spring cereals; only a small effect of vernalization was recorded, ranging from less than 1 day above the controls in Plumage Archer barley to 4 days in Resistance oats. This acceleration in ear emergence although statistically significant is too small to be of practical importance in the climate of Great Britain. A small effect of vernalization was noted on the total number of ears produced, but this was not consistent among the varieties used, some giving an increase, others a decrease, so that the mean overall effect was not significant.

This experiment, in which a high order of accuracy was attained (S.E. of mean yield per acre less than 2 per cent of the mean), showed an effect of vernalization on grain yield not exceeding 10 per cent in the barley varieties, 5 per cent in the oats, and no effect on spring wheat, and of these results only those with spring barley attained statistical significance.

**United States of America:**— Reference has already been made to the review on vernalization published by McKINNEY (1940). The author noted that the majority of investigations outside Russia fail to recognize any great commercial gain to be derived from the application of vernalization to the small grains, rice, maize, sorghum, forage crops and cotton in the regions where these crops are adapted. McKINNEY believes the general opinion to be that the crop problems can best be solved through developing more adapted genotypes. Some commercial value is attached to the chilling method when used to force flowering in daffodils, Dutch and Spanish iris, and Easter lily. Apart from possible application to seed production in biennials, or in speeding up seed production in genetical and general crop improvement studies, McKINNEY considers that the relation between temperature, photoperiod, intensity and quality of light must be fully understood before satisfactory results can be obtained from the initial chilling.

McKINNEY and SANDO (1933) themselves conducted experiments on earliness and seasonal growth habit in wheat, finding that sexual reproduction can be greatly accelerated by first subjecting the slightly germinated seeds of winter wheat to low temperatures in the dark for 50 to 65 days before sowing. By growing plants from treated seed subsequently at higher growing temperatures in a long day, it is possible to obtain two or more crops of winter wheat in a year, according to the variety.

Seasonal growth habit in wheat is considered as an aspect of earliness and lateness. So far as earliness is concerned, McKINNEY and SANDO state that the winter varieties have low temperature and short-day optima during the initial growth phase, whereas spring varieties have optima at the higher temperatures and the longer photoperiods. "Earliness and lateness of sexual reproduction appear to depend on the interrelation of several characters such as the number of internodes per culm, the growth rate of the internodes, the duration of the elongation of the internodes, and environmental-response characters which influence the expression of these characters." In relating environmental response to number of internodes, McKINNEY and SANDO forecast the number-of-leaves interpretation put forward by GREGORY and PURVIS.

LOJKIN (1936) succeeded in vernalizing Turkey Red and Leaf's Prolific wheat by exposure to temperatures of 1 to 3°C., but low temperature did not shorten the vegetative period of the spring cereals, Blue Stem wheat and Clydesdale oats. Drying the vernalized seeds and exposing them to warm temperatures decreased or nullified the vernalization already produced.

The location of the American sugar beet seed industry in areas where thermal induction, that is, vernalization in the field under the natural environment, is guaranteed, is an interesting example of the practical application of this type of research. Details are given in Chapter 18 of Crop Production and Environment (WHYTE, 1946).

**India:**— Agricultural physiologists in India have shown much interest in the developmental physiology of the crops grown in that country, and a clear appreciation of the problems at issue in the study of the relations between growth and development. For example, the degree of interest is indicated by the fact that a discussion on vernalization was held by the Imperial Council of Agricultural Research in December, 1939, at which it was emphasized by some of the attending physiologists that (*a*) experimental data must be obtained to discover whether particular strains of a selected crop will or will not respond to vernalization; (*b*) to obtain this information experiments with different strains should be undertaken in different regions; and (*c*) a study of the effect of the prevailing after-sowing environmental factors of given regions on the life cycle of both vernalized and untreated seeds is essential in order to evaluate the practical possibilities of the method for Indian agriculture. In discussing the question of practical application, SEN (1940) reviews some technical aspects which will require to be considered before the method can be expected to become part of the routine agronomic practice of India. It is not anticipated that Indian cultivators will master vernalization on their own, and SEN suggests that a central station for vernalization should be opened, as most seeds can be dried, stored and distributed after vernalization without suffering damage.

It is a well-known part of the vernalization technique that a certain minimum amount of germination (growth) is essential before vernalization can be effective. When vernalizing mustard, one of the Indian crops on which experiments have been made, this period has to be watched very carefully, as only *unsplit* vernalized seeds can have any practical value (SEN and CHAKRAVARTI, 1942). Sprouted chilled seeds have to be sown very carefully as drying is fatal for them; unsplit seeds can be dried and stored without losing their subsequent germinating capacity. Having found that vernalized seeds of mustard produce plants which flower significantly earlier (SEN and CHAKRAVARTI, 1938), these workers in the United Provinces of India made subsequent experiments for 4 years to discover (*a*) the optimal conditions and period of chilling necessary to induce maximum vernalization in unsplit chilled seeds, (*b*) response of different strains, (*c*) effect on progeny, (*d*) period for which unsplit chilled seeds can be dried and stored without reversal of vernalization, and (*e*) the effect of the temperature and daylength subsequent to sowing on the developmental physiology of plants from vernalized and control seeds.

The technique of vernalization used by Russian and other workers has been fully described elsewhere (I. A. B. 1933, 1935); it may he of interest to describe the technique used under Indian conditions by SEN and CHAKRA-

VARTI (1942). The following quotation applies to large samples, that for small samples having already been described earlier (1938).

For these larger samples modifications were introduced, particularly in regard to the containers of seeds and provision for absorption of $CO_2$ from the respiring seeds. The seeds to be chilled are first soaked under excess of water to induce them to absorb about 60 per cent of their weight of water, a procedure which generally requires 6 to 8 hours, according to the room temperature. After removal of excess water by spreading the seeds over several layers of absorbent cloth, they are placed in muslin bags or unglazed porcelain pots of suitable sizes and then placed inside the moist chamber of the chilling cabinet.

Any watertight box of required dimensions with removable lid can be used for a moist-chamber. When boxes of thin wood are used, they should be thoroughly asphalted inside and out. The inside of the box is lined with blotting paper and sufficient water is placed at the bottom of the box to maintain the absorbent lining moist throughout the period of chilling. For absorption of $CO_2$, a concentrated solution of potassium hydroxide is kept at the bottom of the box in a large, flat porcelain dish, the rim of which is previously paraffined to prevent creeping of KOH solution. A removable thick wire-net frame is placed over the KOH dish to protect the seeds against any accidental contact with the solution. Seeds in bags are suspended from hooks screwed on to the removable lid of the box, care being taken to see that the suspended bags do not touch the wire-net guard, or the moist blotting-paper lining of the box. When unglazed porcelain pots are used as seed containers, they are placed on the wire-net guard above the potash solution. From daily readings of the maximum-minimum thermometer, the temperature range to which the seeds are subjected is recorded. Obviously, from these readings no definite idea is obtainable about the duration of the recorded temperatures each day.

SEN and CHAKRAVARTI consider that an electrically operated cabinet of the Frigidaire type with an automatic device for maintaining a constant low temperature is undoubtedly the most suitable appliance for chilling seeds, but having no available electrical supply were themselves obliged to use a kerosene-operated Electrolux. An ordinary ice-box can be used, or even a wide-mouthed thermos-flask when the temperature required is not below 5°C. and only small samples of seeds are to be chilled. The thermos-flask is half-filled with freezing mixture and the soaked seeds are hung in a muslin bag from a hook screwed on the underside of the cork stopper of the flask. The process of daily renewal of the freezing mixture ensures the necessary removal of CO, and a supply of fresh air. Additional moisture when required can be given to the seeds by dipping the bags in ice-cold water; the excess water automatically drips down into the flask.

After the required periods of chilling, any mustard seeds which may have sprouted are discarded and the unsplit seeds are washed and dried at room temperature until they attain a constant weight. This period varies in the United Provinces from 3 to 5 days, according to the season. The seeds are then packed in a sealed container and stored inside an Electrolux.

All five strains of mustard vernalized by SEN and CHAKRAVARTI responded to the treatment, the degree of response varying with variety.

Seeds which sprout as well as those which remain unsplit during the period of chilling are vernalized, but, for the same dose of chilling, plants from sprouted chilled seeds are earlier. The vernalization effect is not transmitted to the progeny. When growth of the embryo is successfully confined within the elastic limits of the seed coat, the unsplit chilled seeds can be dried and stored for long periods (at least 863 days) without any resultant devernalization.

It was found that the mustard Type 27 used in these experiments has no obligatory low-temperature requirement for the thermo-phase, plants from untreated seeds flowering even when the minimum night temperature is 20°C. or more. Partial natural vernalization can, however, be induced in Type 27 when the embryo develops under a low temperature, and artificial vernalization has its maximal effect in 6 weeks. Once the thermo-phase has been completed, mustard appears to develop towards maturity best in a photoperiod of 13 hours, with an optimal associated temperature of 30°C.

Following these experiments, other Indian workers at the Botany Dept., Presidency College, Calcutta (SEN GUPTA and SEN, 1944) made observations on the effects of time of sowing, photoperiods and vernalization on the growth and development of the two varieties of mustard, Tori No. 7 and Rai No. 5. Finding no response to vernalization, it was stated that their results did not confirm those of SEN and CHAKRAVARTI. The latters' reply (1944) was to the effect that their own results applied only to the five strains tested. They considered that no definite conclusions by SEN GUPTA and SEN are warranted because the maximum period of chilling they used was only 30 days. The fact that unsprouted soaked seeds were chilled at 2 to 4°C. for different periods does not in itself ensure that they were properly vernalized; the technique or the conditions following chilling have not been described, and the conclusions are based on data obtained from a single sowing of Rai No. 5 and Tori No. 7.

Other crops dealt with by physiologists in India include gram (*Cicer arietinum*), rice and wheat. Low temperature applied to gram at the time of germination accelerates subsequent inception of the reproductive phase, while high temperature definitely retards it. The degree of response in both cases is a varietal character (PAL and MURTY, 1941). PILLAY (1944) has reported a preliminary experiment to determine whether pre-sowing treatment with low temperature would induce earlier flowering and high yield in gram under the conditions of Orissa. Seeds of uniform size of the variety, Sabour Type 4, were soaked in water for 22 hours at room temperature. The smaller seeds were then placed in a Kelvinator at 0 to 2°C., one lot for 7 days and another for 14 days. The latter seeds produced plants which flowered only 3 days earlier than the controls, and there was no significant difference in dry weight of pods or of stems.

Much work has been done in the United States of America, Japan, India and other countries on the developmental physiology of rice, more particularly. with regard to photoperiods. In addition, Indian and Russian workers write about the vernalization of rice by short day or temperature. Using the former treatment in Bengal, SIRCAR and PARIJA (1945) obtained results of agricultural importance with a winter variety of rice, "Rupsail." The time to flowering of this variety has been reduced from 133 to 47 days by "vernalization by short days." This is thought to be the shortest period yet reported within which winter varieties of rice will flower. ALAM (1940/41) had already concluded that all varieties require a minimum period of 30 days for vegetative growth and a subsequent period of about 30 days of exposure to short days for flowering. This acceleration claimed by SIRCAR and PARIJA is applicable to the cultivation of rice in Bengal. A variety of fine rice could be grown in a much shorter time; by inducing early maturity it could escape flood; early harvesting would leave sufficient time for preparation of fields for subsequent crops in the rotation.

Two experiments on vernalization of rice by temperature may be noted. PARTHASARATHY (1940) vernalized sterilized seeds in darkness or continuous light at 10 to 20°C. for three weeks. Those vernalized in darkness flowered 4 to 5 days earlier than the control. SKRIPCINSKII (1940) obtained a distinct response from one variety only, British India 1220, in tests made for 3 years at the Rice Testing Station, Krasnodar. This variety eared 10 days earlier after vernalization at 6 to 8°C. and 8 days earlier after PARIJA vernalization at 15 to 17°C. than the control plants grown in short day.

In addition to experiments on the relation between light and temperature and the course of development, Indian workers have also adopted a special pre-sowing treatment for inducing or increasing drought resistance. CHINOY (1942) developed the technique for wheat, and PARIJA (1943) has applied it to rice; water requirements for the treated plants were significantly less than for the control plants and the yield of grain significantly greater when the interval of watering is 8 days. Similar experiments have been made with jute, the effects of alternate moistening and drying and after-sowing light conditions being observed on drought resistance and earliness of flowering (KAR, 1944). There was an increased resistance to drought after treatment, but the earliness observed was due to the photoperiods following sowing.

PAL and MURTY (1941) state that, although low temperature at the time of germination may be indispensable for the normal development of English winter wheat, Indian wheats come into ear (without chilling) even at high summer temperatures, indicating that low temperature is not indispensable. A further indication that Indian varieties have little if any requirement of low temperature, but that response to light can be readily obtained is also seen in the experiments of KAR (1940).

SEN and CHAKRAVARTI (1945) have been testing the response of wheats to vernalization since 1938. The non-Indian varieties, Holdfast, Little Joss, Yeoman, Juliana and Yorkwin have been used, and significant responses have been obtained, that is, earlier emergence of the inflorescence in plants from pre-chilled seeds. Results of attempts to vernalize Indian varieties of wheat in different parts of India have led to the general acceptance of the fact that the cultivated strains of Indian wheat will not respond to vernalization because of their shorter life cycles. SEN and CHAKRAVARTI have, however, found that a very good response was obtainable in the first, second and third generation hybrids made by them between T.P. 4 (Indian wheat) and Yeoman (Cambridge winter wheat). Further trials have shown that a response can be obtained in a number of pure strains of cultivated Indian wheats and have now led to a systematic study in collaboration with Dr. B. P. PAL, Imperial Economic Botanist, of the vernalization response of all the available strains of Indian wheats in his collection. A report will shortly appear in a joint paper with B. P. PAL of the results of vernalizing 150 strains of Indian wheats. Though an earliness of agricultural significance in ear emergence of certain cultivated Indian wheats can be obtained by the use of vernalized seeds, it is found that the number of tillers, the factor positively correlated with yield, is smaller. Trials on closer spacing in sowings are therefore being made.

**Response to Temperature Before Seed Ripening:**— The discovery reported independently by KOSTJUCENKO and ZARUBAILO in Russia and GREGORY and PURVIS in England that the low temperature effect associated with vernalization can be obtained on ripening seeds while they are still attached to the mother plant has made it desirable to give careful consideration to the environment, and particularly to the temperatures experienced before harvest.

KOSTJUCENKO and ZARUBAILO (1935, 1936, 1937) laid down a trial of winter wheat varieties at the Polar Experimental Station of the All-Union Institute of Plant Industry (VIR) at Hibiny. The grain used had been grown in two widely differing environments, namely, Hibiny itself (N. Lat. 67°44') and Kirovobad (N. Lat. 40°41'). In both sowings vernalized and unvernalized grains were used. In September of the sowing year it was seen that the plants grown from these grains of different origin were distinct in their behaviour. Plants from Kirovobad grain artificially vernalized were then at the milk-ripe stage, while those from unvernalized Kirovobad grain were at the tillering stage. On the other hand, plants from Hibiny grain also artificially vernalized were almost at the stage of wax ripeness, while plants from the same grain not artificially vernalized exhibited partial or complete flowering; some of the latter had set seed in the ears of the main stem.

Thus, these Russian varieties of wheat, generally regarded as winter forms, behaved as spring forms when grown from grain which had ripened at Hibiny, but retained their winter habit when grown from Kirovobad grain. KOSTJUCENKO and ZARUBAILO conclude that the Hibiny grains had been able to pass their "stage of vernalization" or thermo-phase under the natural conditions of these northern latitudes, while the Kirovobad grains had not done so and still required artificial vernalization before they could reach maturity in one season after being sown in spring. It was assumed that the embryos of the Hibiny grain were vernalized by low temperature while still attached to the mother plant. An embryo which has not entered the dormant state may be sensitive to vernalization in the same degree as one which has been brought from the resting non-reactive condition by soaking. The details of these and other experiments by these Russian investigators are discussed more fully elsewhere (WHYTE, 1946)

After their experiments on the vernalization of excised embryos, GREGORY and PURVIS (1936*b*, 1938*a*) proceeded independently to study the effect of vernalization on developing embryos and immature ears. Having shown that the vernalization process occurred in the embryo apart from the endosperm, it appeared possible to apply the low-temperature treatment after anthesis, while the embryo is developing, and before the onset of dormancy.

A preliminary experiment was performed in 1935, when the ripening ears were chilled by two methods:

(*1*) The ears together with several nodes of the stem were cut off and kept in water in a refrigerator for 5 weeks at 1°C. Control ears similarly treated were kept in a dark room at normal temperature until the grain ripened off. After the low-temperature exposure the treated ears were allowed to complete ripening at room temperature.

(*2*) The second method consisted in treating ears attached to the plant. The selected ears after anthesis were inserted into wide glass test-tubes, plugged with cotton wool. In one set these tubes were placed in the necks of vacuum flasks containing crushed ice. The ears were thus kept at low temperature but did not come into contact with free water. Control ears were similarly treated and placed in vacuum flasks but without ice. After 24 days the ears were removed and allowed to ripen normally in the open air.

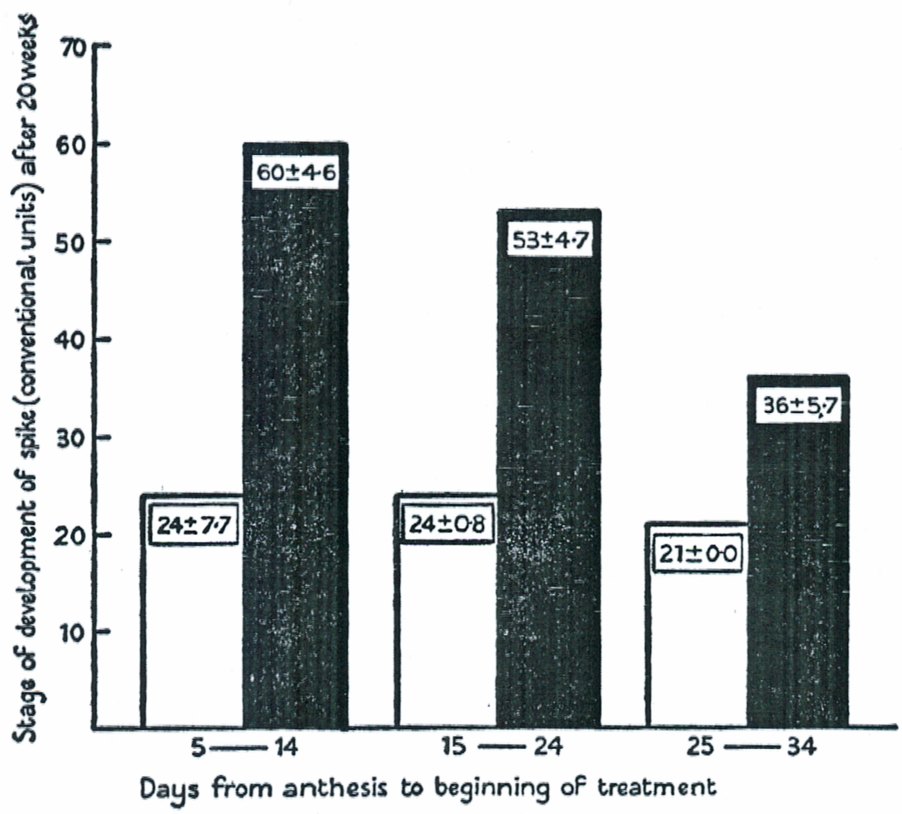


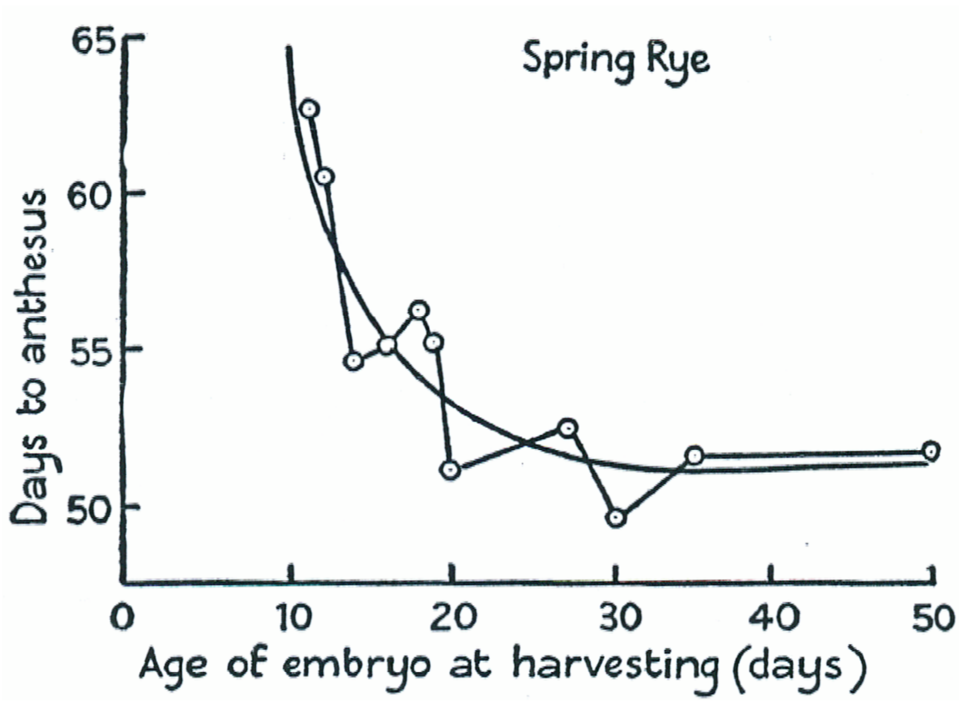

Illustration from GREGORY and PURVIS' classic 'Studies in the Vernalization of Cereals' (II, The Vernalization of Excised Mature Embryos, and of Developing Ears, in Ann. Bot., N. S. 2, 5, 1938) showing the relation between age of embryo at beginning of vernalization in the ear, and effectiveness of the treatment (black vernalized; white = unvernalized controls).

The ripe grains obtained from plants thus treated in 1935 were sown on March 17, 1936, in pots of sand, without further low-temperature treatment. Flowering was irregular, especially in those plants resulting from grain which had been treated in vacuum flasks, where the duration of the treatment was less than that known to be optimal for vernalization, but flowering was appreciably hastened by both methods of chilling and results were sufficiently encouraging to warrant repetition of the experiment in the following year, when longer periods of treatment were used.

*Effect of Vernalizing Developing Ears of Petkus Winter Rye 1935:—*

| METHOD OF TREATMENT | TIME OF TREATMENT | DAYS FROM PLANTING TO ANTHESIS IN RESULTING PLANTS. | |
|---|---|---|---|
| | | RIPENED AT 1°C | RIPENED AT NORMAL AIR TEMPERATURE |
| Cut ears in water | 5 weeks | 102 | 146 |
| Attached ears in vacuum flasks | 24 days | 110 | 164 |

When a larger number of ears was treated in 1936, the ears were placed in a refrigerator while still attached to the stem, and in some cases whole plants were treated in this way. The period of chilling was increased to 45 days, and final ripening was carried out at medium temperatures. The ears were air-dried and the grain sown on May 31, 1937, without further treatment with low temperature.




**TEXT-FIG. 2. Spring rye. Relation between age of embryo at harvesting and time to anthesis in resulting plants.**

Another figure from GREGORY and PURVIS' article referred to on opposite page.

*Vernalization of Growing Embryos in the Developing Ear of Petkus Winter Rye, 1936:—*

| TREATMENT | NO. OF PLANTS | STAGE OF FLOWER INITIATION | CONDITION OF PLANTS 15 WEEKS AFTER PLANTING | |
|---|---|---|---|---|
| | | | STAGE OF FULLY FORMED EARS | MATURITY IN CONVENTIONAL UNITS |
| Vernalized | 156 | 65 per cent | 38 per cent | 53 |
| Control | 49 | 49 per cent | 0 percent | 24 |

Reference should be made to the original article for the method of measuring maturity in conventional units. It is clear from these results that the low temperature during the previous season had a marked effect, although GREGORY and PURVIS note that not all the treated grains had attained the vernalized condition. Only 38 per cent of the treated grains produced plants with fully emerged ears, although others were approaching that stage. These data led to the same conclusion as that reached by KOSTJUCENKO and ZARUBAILO with regard to the sensitivity of an active embryo and the insensitivity of a dormant one.

GREGORY and PURVIS (1938*a* and *b*) dated the ears at anthesis and thus determined the age of the treated embryos. The results of the experiment showed that they could be grouped into 10-day classes covering the period from 5 days to 35 days after anthesis. The treatment is effective from the earliest stage of the development of the embryo, decreasing in intensity as beginning of treatment is delayed. Low temperature is therefore effective only while the embryo is actively growing, and ceases to be operative when the embryo becomes dormant. GREGORY and PURVIS conclude that the effect is quantitative, depending on the duration of the exposure to low temperature as had been shown to be the case in normal vernalization (PURVIS and GREGORY 1937).

An important result which probably indicates how early a developing embryo is fully sensitive to environmental conditions is that obtained by GREGORY and PURVIS (1938*a* and *b*), who showed that seed obtained from ears removed from the parent plant as early as 5 days after anthesis germinated after being sown in the following spring. Completely normal plants were produced, although the individual immature grains were very small (4 x 1 mm.) and had apparently no reserves.

When winter rye was used, the plants grown from seeds having a range of maturity of 5 to 50 days showed no difference in the stage of development reached after 17 weeks. As the plants had not been vernalized, no ears emerged. In spring rye, on the other hand, a variation was found in the days from germination to anthesis varying from $51.8 \pm 1.17$ days (mean of ten plants) in completely matured seeds, to $62.7 \pm 0.91$ days (mean of three plants) in grain from ears removed 11 days after anthesis, the earliest removed ears to give viable grain. A decrease in the time taken to flower is correlated with maturity of the ripening grain. GREGORY and PURVIS suggest the possibility of partial devernalization of the very immature grains of spring rye.

Later studies made at the Research Institute of Plant Physiology, London, have been concerned with a more detailed investigation of the processes occurring in the embryo-sac of a cereal subsequent to fertilization, and of the conditions of formation and the subsequent growth of dwarf embryos of rye. NUTMAN'S account of the former investigation (1939) is concerned with the anatomical and cytological evidence for the formation of growth-promoting substances in the developing grain of rye.

In a later paper NUTMAN (1941) describes the formation of the dwarf grain of rye in ears harvested at an immature stage, and compares the morphology and anatomy of dwarf and normal grains and embryos. He confirms observations made by GREGORY and PURVIS with rye, and earlier by

HARLAN and POPE with barley (1922, 1926) that ears removed from the plants as early as 5 days after fertilization produce viable grain.

**Conclusion:**— It has been seen that research on the biological processes concerned in vernalization has almost reached its limit in the meantime, particularly in the very detailed studies of GREGORY and PURVIS. Until their hypothetical precursor has been isolated and its nature defined, striking advances in this direction are not to be expected.

Neither does it appear probable that the method of vernalization will itself become widely used, except possibly in those districts in countries such as the U.S.S.R. or India where conditions of drought or flood make a difference of a few days in maturity a desirable objective. In countries not experiencing extreme conditions, the low-temperature treatment will not be used when superior genotypes can be found, and would therefore be confined to genetical or similar research, or in the production of market garden crops if a few days' earliness means increased economic return.

The greatest economic significance of this research appears to be, however, in the two incidental discoveries which have been made, namely, the possibility of vernalization before seed ripening and of devernalization and revernalization according as conditions favour one process or the other. vernalization of developing embryos while still attached to the mother plant is an important factor in the selection of areas for seed production, and in plant introduction and the moving of seeds from one environment or latitude to another. Early strains may become late, and vice versa.

Devernalization also affects a number of agronomic questions, as in many environments a temporary period of high temperature may partially or completely annul any degree of vernalization already achieved naturally in the field. Waterlogging of a field in a wet winter might also be expected to produce the anaerobic conditions found to favour devernalization; seeds in a heavy clay soil may possibly be more liable to devernalization through anaerobic conditions than those in light well-aerated soils.

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