NUCLEIC ACID CHANGES IN AFTER-RIPENING SOUR CHERRY SEEDS

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Margaret Susan Sutherland

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ABSTRACT

Sutherland, Margaret Susan, M.Sc.

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NUCLEIC ACID CHANGES IN AFTER-RIPENING

SOUR CHERRY SEEDS

Major Professor: L. J. LaCroix

Changes in the nucleic acid fractions of sour cherry seeds were observed during a 16 week after-ripening treatment under cool moist conditions. Embryos were fed uridine-U-^{14}C after which the extracted nucleic acids were fractionated by chromatography on methylated albumin-kieselguhr columns. The amount of nucleic acid material in each fraction was determined as a per cent of the total amount extracted and the incorporation of uridine into each fraction was determined as a per cent of the total incorporation.

The relative amount of ribosomal RNA increased with after-ripening time while the relative amounts of messenger RNA and DNA + DNA-RNA decreased. No change occurred in the relative amount of soluble RNA with after-ripening.

The ability of sour cherry embryos to synthesize ribosomal RNA increased with after-ripening time as indicated by increased relative incorporation or uridine-U-^{14}C into this fraction. Relative incorporation of label into messenger RNA decreased with after-ripening while incorporation into the DNA + DNA-RNA fraction increased up to 8 weeks of after-ripening and then decreased. No change occurred in the relative incorporation of uridine-U-^{14}C into soluble RNA with after-ripening.

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ABBREVIATIONS

ADP	Adenosine 5'-diphosphate
AMP	Adenosine 5'-phosphate
DNA	Deoxyribonucleic acid
2,4-DNP	2,4-Dinitrophenol
EMP pathway	Embden-Meyerhof-Parnas pathway
GA_3	Gibberellic acid
GMP	Guanosine 5'-phosphate
IAA	Indoleacetic acid
MAK	Methylated albumin coated kieselguhr
RNA	Ribonucleic acid
m-RNA	Messenger-ribonucleic acid
r-RNA	Ribosomal-ribonucleic acid
s-RNA	Soluble-ribonucleic acid
R.Q.	Respiratory quotient
TCA cycle	Tricarboxylic acid cycle
Tris	Tris(hydroxymethyl)aminomethane

INTRODUCTION

Seed dormancy in plants is a remarkable phenomenon which incorporates the cessation of growth and the maintenance of viability under adverse conditions. Because of these features it is most desirable that an understanding of the biochemical mechanisms involved be sought. To appreciate the importance of such an endeavour one should first become aware of the significance of seed dormancy to the survival of the plant and to man's control of the plant world. For the plant, seed dormancy is a highly useful adaptation enabling the reproductive structure to withstand environmental conditions which are unfavourable to the commencement or continuation of growth. This ensures continuation of the species and time for seed dissemination. For man, delayed germination makes possible the harvest and storage of cereal grains but creates the problems of propagating certain horticulturally significant plants and controlling wild plants whose seeds can lie dormant in the soil for many years.

A viable mature seed which fails to germinate under ideal conditions of temperature, moisture and oxygen is said to be in a dormant state. The type of dormancy found in stone fruit can be called embryo dormancy and is difficult to overcome. Naked embryos from such fruits either fail to germinate or produce abnormal stunted seedlings. The only effective method of overcoming embryo dormancy is by after-ripening the seeds, that is, by subjecting them to moist conditions at low temperatures for a specific length of time. This treatment is often called stratification while the metabolic changes leading to germination which occur in the seed as a result of the treatment are referred to as after-ripening.

During after-ripening, factors such as seed coat impermeability to water and gaseous exchange, the presence of inhibitors and promoters of germination in the seeds or covering structures, and the temperature regime all regulate the rate at which the metabolic blocks to germination are overcome. The mechanisms by which these factors influence the metabolism of the seed are mostly unknown. They might involve the induction or repression of an enzyme system which in turn might relieve a deficiency in some necessary substrate or coenzyme. Furthermore the actual metabolic changes which occur during after-ripening have not been fully elucidated as yet.

Since the metabolism of a cell is ultimately controlled by nucleic acids, a study of these compounds might shed some light on the mechanisms which control the metabolic changes. With this in mind the present work was initiated to determine the effects of after-ripening on the nucleic acid metabolism of sour cherry seeds (Prunus cerasus L.). These seeds were chosen because they have a definite rest period, are readily available and other studies have shown interesting metabolic changes during after-ripening.

LITERATURE REVIEW

Man has been aware of the problem of seed dormancy since his early attempts to cultivate plants but it has not been until the turn of the century that concerted efforts have been made to understand and deal more effectively with the phenomenon. The causes of seed dormancy are varied but in general fall into five major classes (72), namely, 1. rudimentary embryos, 2. physiologically immature embryos, 3. mechanically resistant seed coats, 4. impermeable seed coats and 5. the presence of germination inhibitors. More than one type of seed dormancy may be present in a given seed. In each case either physical or biochemical factors or both serve as blocks to germination. Whether dormancy is imposed by physical or chemical restrictions, germination can be brought about by special environmental factors which initiate and influence the metabolic changes required for germination. Under natural conditions, blocks to germination are eliminated by time, temperature changes, leaching, decay of surrounding fruit tissue, light and other factors. Much work has been done to determine what physical and chemical factors are involved in dormancy in many species and how germination can be brought about. However, little knowledge has been gained in understanding the mechanisms by which the chemical environment within the seed coverings and/or embryos can sustain dormancy and by which the chemical changes leading to germination are initiated and expressed.

As early as 1906 Crocker (20) was aware of most of the ways in which seed coats are currently understood to affect germination. These are chiefly impermeability of the coats to water and gaseous exchange, mechanical resistance to the expansion of the embryo and the presence of

growth inhibitors. Seed coat impermeability to water is exhibited in a number of plant families, e.g., the waterlily, mallow and legume families and provides one of the most complete blocks to germination (59). When white clover seeds are ripened in hot dry weather they form so called hard seeds. Hyde (32) observed that the hard seeds of several species of legumes have a fissure in the impermeable seed coat along the groove of the hilum which functions as a hydroscopic valve. This valve opens in dry air permitting water to escape but closes rapidly in moist air. Thus seeds can dry further through outward diffusion of water while re-entry of water is prevented. Corns and Schraa (19) reported that removal of the endocarp from silverberry seeds resulted in almost complete germination of the seed which otherwise required stratification. They proposed that dormancy is caused by seed coat impermeability since treatment of unstratified fruits with germination stimulators did not promote germination and the germination inhibitor extracted from the endocarp was unable to inhibit germination of naked seeds.

Seed coverings can also prevent germination by mechanically restricting embryo expansion. In the olive, the seed is surrounded by a thick bony indehiscent endocarp which is mechanically hard and water impermeable (29). Wellington (79) observed that the covering layers of wheat grains, if not disturbed during after-ripening, exert sufficient mechanical resistance to prevent expansion of the embryo until the water absorbing capacity of the seed has been sufficiently increased by transfer of reserves from the endosperm. Chen and Thimann (12) ascribe dormancy in Phacelia tanacetifolia Benth. to a balance between mechanical restraint

by the endosperm and the expansive force of the embryo. The inhibitory effect of light and high temperature on germination of the intact seed can be overcome by removal of the tip of the endosperm. However, light and temperature sensitivity are reinstated by immersion of the seeds in solutions of high osmotic pressure which simulate the effect of the endosperm by reducing the ability of the embryo to absorb water needed for cell enlargement. Crocker et al (21) obtained quantitative data that correlated germination with the changes in coat resistance and embryo pressure in black walnut and hickory seeds. Moist storage caused the calculated shell resistance to decrease and the softening was attributed to the activity of microorganisms since walnuts stored under sterile conditions did not soften.

Studies with isolated embryos, fully and partially coated seeds and atmospheres enriched with oxygen have provided inferential evidence that seed coats can control germination by preventing gaseous exchange. Toole et al (72) cite numerous examples in which removal of the covering structures from grass species has improved germination. This effect has been attributed to restriction of the entry of oxygen by the coats. One of the most extensively studied examples of the effects of seed coats in relation to oxygen requirements is the cocklebur (Xanthium). Using controlled gas mixtures of oxygen, carbon dioxide, nitrogen and hydrogen, Thornton (69) showed that secondary dormancy was induced in moist seeds only when oxygen was absent from the atmosphere. Dormancy in the upper seed was attributed to a differential ability of the inner seed coat to restrict the movement of gases. More recent work by Wareing and Foda (78) has demonstrated the presence of inhibitors within the embryo which

are destroyed by increased oxygen tension. Cell free extracts from the radicles of germinating seedlings were shown to contain a heat labile compound capable of bringing about the in vitro breakdown of the inhibitory material in the presence of oxygen. Thus the role of the seed coat appears to be the prevention of the loss of the inhibitor by oxidative breakdown or leaching. Early work on seed dormancy in wild oats by Atwood (1) indicated that dormancy was caused by restricted gaseous exchange by the seed coats since high oxygen tension promoted germination of dormant seeds. However, Black (5) has since demonstrated that the effect of oxygen is to reduce the levels of inhibitors present in the seed. Black also showed that, in the induction of secondary dormancy in imbibed seeds by anaerobic conditions, there is a marked increase in the inhibitor level in the caryopses. Increased germination of Betula pubescens Ehrh. in oxygen has been attributed to oxidative inactivation of the inhibitor present in the pericarp (4). In the case of dormant buds, Vegis (76) and Pollock (57) suggest that the restricted supply of oxygen limits the oxidative breakdown of pyruvate or acetyl-CoA via the TCA cycle. The pyruvate which accumulates is then used to form such compounds as ethyl alcohol and acetaldehyde which act as growth inhibitors.

A number of compounds have been shown to stimulate or inhibit germination when applied to seeds. Of these, the gibberellins and kinins have been extensively studied. Gibberellic acid has been shown to stimulate germination of seeds which have low temperature after-ripening requirements such as the apricot and peach (11), Lambert cherry (24), wild oats (53) and hazel and beech (25). Black and Naylor (6) were able

to prevent the onset of seed dormancy in a very dormant line of wild oats by allowing stems cut when seeds were in the milk stage to take up potassium gibberellate from solution. In a study on the germination of Polygonum convolvulus L. Timson (70) found that GA_3 was effective in bringing about germination of non-after-ripened seeds only when the apex of the nut was removed. Presumably the pericarp was acting as a barrier to the entry of GA_3. He found also that thiourea, uracil, thymine, cytosine, guanine and adenine all stimulated germination whereas neither KNO_3 nor glucose acted as dormancy breakers. The effects of various inorganic ions on dormancy in rice seed was studied by Roberts (61). Germination of dormant seeds was stimulated by nitrate, nitrite and hydroxylamine, nitrite being more active than nitrate. The possibility that nitrate reductase might be involved is suggested by the fact that greater germination was obtained in the presence of nitrate plus molybdate, the latter being a stimulator of this enzyme. The activity of nitrate reductase was studied in connection with nitrate stimulation of germination of after-ripening seeds of Agrostemma githago L. (7). Dormant embryos showed no nitrate reductase activity even in the presence of KNO_3 while after-ripened embryos contained substantial activity without induction and still greater activity in the presence of KNO_3. Roberts observed that steeping rice seeds in solutions of low pH stimulated germination while pH's between 3.0 and 11.0 had no effect and pH's higher than 12.0 retarded the breaking of dormancy. By application of both nitrate and high concentrations of H^+ a higher percentage of germination was obtained than expected for either ion alone. Na, K, Mg, Mn, Fe, Co, Ni and Zn ions had no effect on germination of

dormant rice seeds while Cu, Hg and Ag retarded the breaking of dormancy at concentrations which did not affect the percentage germination of non-dormant seeds. In further studies Roberts observed the effects of some organic growth substances and organic nutrients on dormancy in rice seeds (62). GA_3 and to a lesser extent kinetin were found to stimulate germination while IAA and thiourea had very slight stimulatory effects. Unlike wild oats, placing flowering tillers in solutions of GA_3 did not overcome dormancy in the developing seeds. Of the other substances tested on rice seeds, coumarin and the salts of some organic acids slightly delayed the breaking of dormancy while ethylene, L-alanine, β-alanine, thiamine hydrochloride, pyridoxine hydrochloride and nicotinic acid had no effect. It was recently demonstrated (68) that the growth regulator abscisin II (dormin) inhibited germination of seeds which have a chilling requirement for germination (Fraxinus ornus L. and F. americana L.). GA_3 and GA_1 were able to reverse the abscisin II-induced inhibition of root development.

The following naturally occurring germination inhibitors have been isolated from a variety of plant parts: ammonia from beet seed, hydrogen cyanide from the seed constituent amygdalin, ethylene from ripe fruit, essential oils including both aldehydes and mustard oils, alkaloids such as cocaine, caffein, physostigmin and nicotine, unsaturated lactones such as coumarin and parascorbic acid and unsaturated organic acids. As mentioned previously germination inhibitors have been extracted from Xanthium (78). No difference was found in inhibitor content between the upper (dormant) and lower (non-dormant) seeds of Xanthium or between dormant and after-ripened upper seeds. Thus the change from the dormant to

non-dormant state was not due to changes in inhibitor content but more likely involved activation of enzyme systems for the breakdown of the inhibitor. These workers showed that inhibitors with the same characteristics as those in Xanthium could be extracted from lettuce seed. Black (5) in his studies on inhibitors in the hulls of Avena fatua L. was also unable to show any change in inhibitor content during natural after-ripening. Knowles and Zalik (43) demonstrated the presence of an inhibitor in the endocarp of seeds of Viburnum trilobum Marsh. and Hilton et al (31) obtained evidence for the presence of inhibitors in resting seeds of Amelanchier laevis Weig. and two Sorbus spp. Miyamoto et al (51) showed that post-harvest dormancy in wheat is caused by inhibitors which are located in the seed coat. In this case loss of dormancy during storage was correlated with natural inactivation of the inhibitors. GA_3 was shown to reverse the inhibitory effect of one of these substances in wheat embryos. Lipe and Crane (47) have isolated an inhibitor of seed germination from the outer and inner integuments of Lovell peach seeds. Its action was expressed at the micropylar end of the seed where it inhibited radicle elongation. Experimental evidence indicates that it is identical with abscisin II. Termination of rest in peach seeds by stratification is correlated with the disappearance of the inhibitor. Removal of the integuments from unstratified seeds brings about germination but seedlings are often dwarfed. Evidence suggests that such dwarfing is caused by concentrations of the inhibitor which are not high enough to prevent germination. The effects of the inhibitor were found to be antagonistic to GA_3 and IAA. The former can stimulate germination of peach seeds (11).

There are a number of cases in which naturally occurring gibberellins have been found in seeds. Frankland and Wareing (25) demonstrated the presence of gibberellins in hazel and beech seeds. During moist low temperature after-ripening of hazel seeds there was an increase in the level of gibberellins, while after-ripening of beech seeds resulted in a qualitative change in the chromatographic pattern of the gibberellins. Also, there was no significant change in concentration of auxins and inhibitors during after-ripening (26). Since exogenous GA_3 as well as kinetin and thiourea will stimulate germination of dormant hazel and beech seeds, it is likely that the effect of the endogenous gibberellins is to counteract the natural inhibitor. Dormant and non-dormant peanut seeds contain growth promoters with the same chromatographic properties as IAA and GA_3 (28). Inhibitors were found only in dormant seeds. The interaction of growth inhibiting and stimulating substances in Fraxinus excelsior L. was studied by Villiers and Wareing (77). When Fraxinus fruit is shed, the embryo is small but morphologically complete and contains a growth promoting substance tentatively identified as indoleacetonitrile. As the embryo grows to full size, this substance gradually disappears and fully grown seeds will not germinate unless after-ripened in moist storage at low temperatures. Dry seeds contain no growth inhibitors but on imbibition of water there appears a growth inhibitor which prevents radicle growth and the concentration of this inhibitor increases on further imbibition. There is no reduction in activity of the inhibitor in either the endosperm or embryos during or after chilling. However, fully chilled embryos contain a germination stimulator. This evidence suggests that dormancy in F. excelsior is imposed by the presence of a growth

inhibitor in both endosperm and embryo. During chilling, dormancy is overcome by the production of a germination stimulating substance in the embryo. The literature on germination inhibitors has been reviewed by Evenari (23) and Toole et al (72).

It is well known that visible light can inhibit or promote germination of certain dormant seeds. The subject has been reviewed by Toole et al (72), Toole (71) and Koller et al (45). Borthwick et al (8) studied the action of light on lettuce seed germination and found that it was influenced in a reversible manner by radiation in the red and far-red regions. The main mechanism involved is the now well established low energy, reversible red/far-red mechanism (72) in which the pigment phytochrome participates. The light stimulus received by the phytochrome system must then in some way be transmitted to the metabolic sites within the seeds which are responsible for the chemical changes that lead to germination. GA_3 has been shown to promote dark germination of light-requiring lettuce seed (41). The site of action of red light and GA_3 in germination of photosensitive seed appears to be the same (33). However, the red light effect is different from the GA_3 effect in that far-red light reverses the effect of red light but not the effect of GA_3. GA_3-induced dark germination of photosensitive lettuce seeds was found to be inhibited by 6-azauracil and 2-thiouracil and this inhibition was reversed by uracil but not by thymine, deoxycytidine and orotic acid (40). This suggests that GA_3-induced dark germination is dependent on RNA synthesis and not on DNA synthesis. The same effect was observed for light-induced germination of these seeds but to a lesser extent. This provides further evidence that the mechanism of control of GA_3-induced dark germination

and light-induced germination are not identical. The failure of light-sensitive lettuce seeds to germinate in the dark is dependent on the presence of intact endosperm and pericarp (34). Smith and Frankland (66) found that inhibition of germination of photosensitive lettuce seeds by the uracil derivatives 2-thiouracil, 5-fluorouracil and 5-bromouracil required higher concentrations for half seeds than for intact seeds. Other analogues of nucleic acid precursors, inhibitors of protein synthesis and analogues of amino acids showed no difference in concentrations required for inhibition of intact or half seeds. These results suggest that uracil derivatives inhibit a facet of nucleic acid metabolism specifically related to the ability of the embryo to overcome seed coat restrictions, i.e., the mechanism catalysed by phytochrome. Germination of light-sensitive lettuce seed can be inhibited by coumarin and xanthatin as well as far-red light (42). The effect of this inhibition can be reversed by kinetin plus red light but not by either alone. Khan (39) showed that dormancy in the upper seed of Xanthium can be broken by kinetin which antagonized the endogenous inhibitor present in the embryo. In this case the effect of kinetin is dependent on the reversible phytochrome system as well as DNA-dependent RNA synthesis.

Seed dormancy may also be due to metabolic blocks to embryo growth which may originate in the embryo itself or in the covering structures. The factors which bring about germination of dormant seeds whether they are after-ripening at low temperatures, light quality, or loss or inactivation of a germination inhibitor, in some way influence the metabolism of the seed in favour of reactions which lead to germination.

In one of the earlier studies on chemical changes during after-ripening of dormant seeds, Eckerson (22) showed that there was an increase in titratable acidity in the initial stages of after-ripening of seeds of Crataegus mollis Schule. Correlated with this was an increase in water holding capacity of the seed and a rise in the activity of catalase and peroxidase. Treatment of the seed with dilute hydrochloric, butyric or acetic acid was found to shorten the after-ripening time.

In his study of the after-ripening of juniper seeds Pack (56) found that lipids decreased while amino acid nitrogen, nitrogen of amides, peptides and nucleic acid derivatives and carbohydrates and organic acids increased. The level of phosphatides almost doubled.

Lasheen and Blackhurst (46) found that embryo dormancy in blackberry seed can be broken by low temperature after-ripening. During after-ripening at room temperature starch decreased but sucrose and reducing sugars increased. These carbohydrate transformations were greatly accelerated at low temperatures. As after-ripening progressed, catalase, peroxidase and lipase activities increased rapidly, reaching a maximum at five months. These workers demonstrated the presence of growth inhibitory substances in the endosperm, testa and embryo of freshly harvested seeds. They were unable to correlate inhibitor content with failure of the naked embryo to germinate and thus suggested that a growth promoting substance might also be involved.

In a study of enzyme activities during an eight week stratification of Adonis vernalis L. Berenegovskaya and Bubenchikov (3) showed

that the activity of catalase gradually increased with a subsequent sharp decrease before germination. Polyphenoloxidase activity was not observed during stratification but was demonstrated before germination. Peroxidase was active during stratification but its activity ceased abruptly. The respiration of seeds during stratification was mainly at the expense of enzymes sensitive to cyanide.

Popov (60) studied changes in the activity and localization of respiratory enzymes in apple seeds during stratification. He found that peroxidase and succinic dehydrogenase activities increased, the most intense activation occurring during the second half of the stratification period when cytochrome oxidase appeared. No increase in enzyme activity was observed during storage of dry seeds. The highest enzyme activity was found in the axial organs of the germ, in the conducting system and in the cell layers of the cotyledon and endosperm near the radicle and shoot.

Hatano (30) showed that low temperature stratification of pine seeds elevated oxygen uptake by the seeds and isolated embryos at the initial stage of germination. Addition of 2,4-DNP, sodium azide and pentachlorophenol, which are uncouplers of oxidative phosphorylation, and coumarin enhanced oxygen uptake in stratified seeds but inhibited it in unstratified seeds. Addition of 2,4-DNP also had a strong effect on the R.Q. values.

Ballard and Lipp (2) found that 2,4-DNP and other uncouplers or inhibitors of oxidative phosphorylation did not disturb the carbon dioxide induced breaking of dormancy in Trifolium subterraneum L. On the contrary they promoted germination in a substantial proportion of the seeds.

Roberts in his study on dormancy in rice seed (63) observed that inhibitors of terminal oxidases such as CO, cyanide and azide stimulated germination of dormant seeds while other respiratory inhibitors including 2,4-DNP had no effect. His evidence favoured cytochrome oxidase as the enzyme involved. He also noted that violet light in the presence of CO increased percentage germination but had no effect on its own. Since growth following germination in CO in the dark was extremely stunted while light by itself brought about resumption of normal growth it seemed that growth following germination is dependent upon respiration involving cytochrome oxidase whereas germination itself is stimulated by blocking the enzyme. Histochemical studies indicated that in addition to the embryo there were two layers of potentially high activity of redox enzymes - the testa and aleurone layer.

Naylor and Simpson (53) showed that exogenously applied sucrose stimulated germination of dormant seeds of A. fatua and that GA_3 could substitute for the sugar requirement. They suggested that natural inhibition of germination in wild oats involved restriction of both sugar accumulation and utilization of sugar in growth. Since evidence was obtained for the presence of a natural gibberellin, they proposed that control of dormancy is probably by a gibberellin-inhibitor antagonism. In further studies (65) they found that hydrolysis of starch is blocked in dormant seeds in spite of the fact that dormant and non-dormant seeds contain similar amounts of α- and β-amylases. Examination of the maltase content of imbibed dormant and non-dormant seeds showed a marked increase in maltase in non-dormant seeds during imbibition while this did not occur in dormant seeds unless treated with GA_3. These observations led to the

conclusion that gibberellin is necessary for the synthesis or release of maltase. The critical role of maltase is associated with the initial disruption of intact starch grains, an action which also requires amylases. It appeared that maltose acted as an end product inhibitor of α-amylase activity on raw starch and that the function of maltase in vivo was to remove the block by hydrolyzing maltose. It was noted that the importance of oxygen or aerobic conditions in breaking dormancy may be linked directly with maltase activity as the enzyme is sensitive to alcohol, a product of anaerobiosis. Simpson (64) found that another feature of embryo dormancy in wild oats was a restriction in the activity of 3'-nucleotidase which could be overcome by GA_3. Studies by Naylor (52) on the response of aleurone cells of A. fatua to GA_3 indicate that another important factor in dormancy in wild oats is the inability of dormant aleurone cells to mobilize amino acids without the aid of gibberellin.

Nucleotide synthesis during after-ripening of hazel seeds has been studied by Bradbeer and Floyd (10). They found that during the first eight days of chilling there was a marked increase in the ability of cotyledon slices to synthesize adenosine and AMP. The ability to accumulate ADP increased slightly during this time and continued to rise over the following 26 days. In embryonic axes there was a fall in adenosine synthesis and a rise in nucleotide synthesis during the first eight days. Since these changes were completed before after-ripening ceased, it was suggested that they play an early part in the process of after-ripening.

Wood and Bradbeer (80) found that cotyledon slices from previously unimbibed non-chilled hazel seeds did not incorporate detectable amounts

of ^{32}P-phosphate into any nucleic acid fraction eluted from MAK columns. The r-RNA did not show division into heavy and light components. Chilling and imbibition brought about changes in the s-RNA fraction and the appearance of light and heavy r-RNA components. Chilling beyond ten days resulted in considerable nucleic acid synthesis particularly in the r-RNA fraction. A study of the metabolism of acetate-2-^{14}C in hazel seeds (9) showed that both cotyledon slices and embryonic axes of dormant seeds actively metabolized acetate. The operation of the following enzymes and enzyme systems was inferred: TCA cycle, glutamate dehydrogenase, aspartate aminotransferase and those enzymes involved in the synthesis of lipids, proteins and sucrose. Since no evidence was found for the appearance of further metabolic pathways during chilling, it was suggested that the block to germination in dormant seeds might be the partial block of a metabolic pathway. Quantitative changes in the distribution of acetate-2-^{14}C label during chilling indicated a fall in lipid synthesizing ability of the cotyledons and a rise in aspartate, glutamate and protein synthesizing ability of the embryonic axes. The embryonic axes also developed increased TCA cycle activity and showed increased sucrose labelling.

Pollock and Olney (58) found that after-ripening of sour cherry seeds at 5°C brought about a linear increase in the capacity for growth after a lag of four weeks. This capacity for growth was paralleled in the embryonic axes by an increase in dry weight indicating translocation of material from the cotyledons. Also it was preceded by cell divisions and a sharp increase in respiratory rate. The increase in respiration

was paralleled by an increase in respiratory capacity as measured by the effect of 2,4-DNP. In seeds after-ripened at 25°C, growth capacity did not develop, little translocation to the potentially growing cells of the embryo occurred, respiratory rate remained approximately constant but respiratory capacity rose within the first four weeks and thereafter declined to its original level. In a further study (55) these workers found that total nitrogen and phosphorous increased during low temperature after-ripening. They demonstrated that the rate of nitrogen translocation from the cotyledons to the embryonic axes was equal to the rate of cell division so that the nitrogen concentration per cell remained constant. The rate of phosphorous translocation was in excess of cell division and the concentration of phosphorous in the cell increased. In seeds kept at 25°C phosphorous tended to shift from normal compounds such as nucleic acids and nucleotides and accumulate as inorganic phosphate. They interpret their results as suggesting that one cause of breaking the rest period may be the increased availability of phosphate acceptors. They point out that it is not clear whether this is a primary reaction responsible for breaking dormancy or whether it is just one of many secondary reactions following activation of the cells.

In his study of seed dormancy in sour cherries Jaswal (36) observed that during the first six weeks of moist low temperature after-ripening, lipids, sugars, dry weight and respiratory rate in the embryonic axes remained unchanged while the predominant pathway for glucose oxidation was via the EMP pathway. About the seventh week of after-ripening there was an abrupt change in metabolic activity and the pentose phosphate

pathway became the major pathway for glucose breakdown as indicated by studies of C_6/C_1 ratios, 2,4-DNP effect on respiration and the contribution of C_6 and C_1 of glucose to the amino acid fraction. At the same time the respiration rate rose rapidly, the lipid level decreased and sugars and dry weight increased. No change in C_6/C_1 ratios was observed in the cotyledons but a similar though less marked increase in respiration occurred. Indirect evidence suggested that lipids were being oxidized via the glyoxylate cycle and translocated in some form to supply the increased respiratory requirements of the embryonic axes.

Until the present work no study had been made of nucleic acid metabolism in after-ripening cherry seeds. However, Oknina (54) studied the nucleic acids in seeds of growing apples and cherries. During the undifferentiated stage of the seed bud, content of nucleic acids, particularly RNA, was high. During the early stages of cotyledon development the content of DNA was high. As development and formation of the seed pod progressed the content of nucleic acid persisted at a high level in all parts of vital development. Nucleic acids dropped to lower levels at complete ripening of the seed and maintained the same level during the period of quiescence.

MATERIALS AND METHODS

The seeds used in this study were those of the sour cherry, Prunus cerasus L. var. Montmorency. Seeds were recovered at intervals from an after-ripening treatment, and fed uridine-^{14}C. The nucleic acids were then extracted and chromatographed on methylated albumin-kieselguhr (MAK) columns. The OD and radioactivity of the column effluent were determined to elucidate the changes in nucleic acid metabolism occurring during after-ripening.

AFTER-RIPENING TREATMENT

The sour cherry seeds were obtained from the Canada Department of Agriculture Research Station, St. Catharines, Ontario. All experiments were carried out with the 1965 crop. The material was dried and stored at 5°C.

A single layer of seeds was incubated between 3-4 cm layers of a moist sand-peat mixture (1:1). Flats containing the seeds were soaked with water, placed in polyethylene bags, and stored in a cold room at 1°C for 16 weeks. A control flat was kept at room temperature.

CONTAMINATION STUDIES

Two sterilizing agents, Domiphen Bromide (Mann Research Laboratories Inc.) and Wescodyne (West Chemical Products Ltd.) were tested as to their effectiveness in destroying bacterial and mould contamination on the seeds. Endocarps were removed from dormant seeds, which were then soaked in sterile distilled water. The sterilizing agents were used in the following concentrations: Domiphen Bromide - 1% aqueous solution

and Wescodyne - 75 ppm aqueous solution. The treatment times were 5 or 10 minutes with the seed coats on followed by 3 or 5 minutes with seed coats removed, for both chemicals. Following each treatment, the seeds were rinsed 5 times with sterile distilled water. Seeds for the controls were rinsed in the same manner but received no chemical treatment. Ten embryos were placed in sterilized 25 ml Erlenmeyer flasks containing 4 ml of 0.1 M sodium acetate buffer, pH 5.4 and 0.10 mg of cold uridine to simulate the radioactive feeding medium. The flasks were incubated for 48 hours at room temperature in the dark and then frozen. Samples were thawed, the feeding medium poured off, and a serial dilution of it was plated on potato dextrose agar. After 4 days at room temperature, the number of colonies on each plate was recorded.

A dilution study of Domiphen Bromide was made to determine the lowest effective concentration. Concentrations of 1%, 0.1%, 0.05% and 0.01% were tested in the same manner as above using the 5 minute - 3 minute sequence of timing.

The effect of Domiphen Bromide on the respiration of the seeds was studied. The seeds were treated in the following ways: (a) with a 1% solution for 5 and 3 minutes, with and without seed coats respectively; (b) with a 0.05% solution for the same time sequence; and (c) with 0.1% for 5 minutes with seed coats and 0.05% for 3 minutes without seed coats. O_2 uptake at 25°C was used as a measure of respiration and was determined using a Warburg respirometer. Ten embryos were incubated in a Warburg flask containing 3 ml of 0.1 M sodium acetate buffer, pH 5.4. Control flasks contained embryos prepared in the same manner but not sterilized.

CO_2 was absorbed in 0.2 ml of 2 N NaOH in the center well. The details of O_2 uptake calculations were taken from Umbreit et al (74).

Next, the effect of Domiphen Bromide on the incorporation of uridine into nucleic acids was studied. Seeds were sterilized as described in (c) above. One hundred embryos were then transferred into the radioactive feeding medium, the nucleic acids extracted and the specific activity of the total nucleic acid sample compared to that obtained when seeds were sterilized with calcium hypochlorite as described below.

FEEDING EXPERIMENTS

The seeds were harvested from the after-ripening medium at intervals of 4, 8, 12 and 16 weeks. After removal of the endocarp, seeds were sterilized by soaking them in 4.0% (w/v) calcium hypochlorite solution for 5 minutes. This was followed by 5 rinses with sterile distilled water. The seed coat and endosperm were removed and the embryos placed in 0.1% calcium hypochlorite solution for 3 minutes after which they were rinsed 5 times with sterile distilled water. One hundred embryos were transferred to each of two sterilized 50 ml Erlenmeyer flasks, containing 9 ml of 0.1 M sodium acetate buffer, pH 5.4 and 5 μc of uridine-U-^{14}C (specific activity 290 mc/mM) in 1 ml of water. CO_2 was trapped in a center well containing 2 ml of ethanolamine. The flasks were sealed with serum caps and placed in a constant temperature bath at 25°C for 48 hours after which the samples were frozen until extracted.

Seeds and embryos from the same sample were transferred to petri dishes containing moist filter paper and allowed to germinate at room

temperature. Those seeds whose radicle penetrated the seed coat in 1 - 2 weeks were considered to have germinated. In the case of embryos, extension of the hypocotyl was the criterion for germination.

EXTRACTION OF NUCLEIC ACIDS

The procedure used for the extraction of nucleic acids was essentially that of Cherry (13). Before extraction the sample was thawed, the center well removed and the remaining substrate poured off. Microbial contamination was determined from an aliquot of the substrate. The embryos were rinsed 5 times with cold water and homogenized for 7 minutes in a Lourdes tissue grinder. The homogenizing medium contained 20 ml of 0.01 M Tris-HCl, pH 7.6, 0.06 M KCl and 0.01 M $MgCl_2$; 3 ml of 5.5% sodium lauryl sulfate (dupanol); 40 mg of bentonite; and 34 ml of water saturated phenol. The sample was further homogenized using a Potter-Elvehjem tissue grinder until all cells were broken and the homogenate contained no visible particles. The homogenate was then centrifuged at 15,900 x g and the aqueous layer removed. The residue was stirred with an additional 20 ml of homogenizing medium, centrifuged and the aqueous layer again removed. The combined aqueous layers were twice treated with equal volumes of water saturated phenol. The nucleic acids were precipitated from the final aqueous fraction by the addition of 2 volumes of cold ethanol. In the case of the 4, 8 and 12 week samples from flat III, 0.2 M potassium acetate was added to the ethanol. After sitting in the cold for 1/2 hour the precipitate was collected by centrifugation and dissolved in 30-40 ml of 0.05 M sodium phosphate buffer, pH 6.7. The extract was dialysed against

this buffer for 48 hours in the cold and then made up to 50 ml. Forty ml of sample were used for column chromatography.

PREPARATION OF MAK COLUMNS

The MAK columns were made according to the procedure of Mandell and Hershey (49) with slight modifications.

The methylated albumin was prepared as follows: Five g of bovine serum albumin, fraction V, were suspended in 500 ml of absolute methanol to which 4.2 ml of 12 N HCl were added. The mixture was allowed to stand in the dark for 5 days with occasional shaking. After this time the suspension was adjusted to pH 6.0 with NaOH and the precipitate was collected by centrifugation. The precipitate was then washed 3 times with methanol and 3 times with ether. The ether was evaporated and the powdered precipitate was dried and stored in vacuo over KOH.

Material referred to as washed protein-coated kieselguhr or washed MAK for the second layer of the column was prepared as follows: Ten g of kieselguhr (John Manville Co.) were suspended in 50 ml of 0.1 M NaCl in 0.05 M sodium phosphate buffer, final pH 6.7, and boiled to remove trapped air. To the cooled suspension was added 2.5 ml of a 1% aqueous solution of methylated albumin followed by an additional 10 ml of buffered saline. The suspension was transferred to a glass column 2 cm in diameter which contained a small piece of glass wool covered by a 2 cm layer of glass beads. The column was packed by adding 5-10 ml portions of the suspension to the tube and forcing out the excess liquid under an air pressure of 3 psi. The column was then washed with 150 ml of 0.4 M NaCl in 0.05 M sodium phosphate buffer, pH 6.7, after which the

entire contents of the column was suspended in 63 ml of 0.4 M NaCl and stored in the refrigerator. New washed MAK was prepared each month.

To prepare the column for fractionating nucleic acids the following suspensions of kieselguhr were boiled and cooled: (a) 8 g in 40 ml of 0.1 M NaCl; (b) 6 g in 40 ml of 0.4 M NaCl; and (c) 1 g in 10 ml of 0.4 M NaCl. Two ml of 1% methylated albumin and 15 ml of additional saline solution were stirred into the first suspension. The first layer of the column was formed on top of the glass beads with this as described above. Any material adhering to the walls of the tube above the first layer was washed into the column with 0.1 M NaCl to avoid contaminating the material in the next layer. Ten ml of washed MAK were added to the second suspension and the second layer was formed with it in the same manner as the first. A third layer was formed with the third suspension and this was covered by a 2 mm layer of glass beads. The column was washed immediately with 150 ml of 0.3 M NaCl in 0.05 M sodium phosphate buffer, pH 6.7, the starting concentration of the elution gradient.

CHROMATOGRAPHY OF NUCLEIC ACIDS

Forty ml of the nucleic acid sample were adjusted to 0.3 M NaCl by addition of the salt and loaded on the column under a pressure of 3 psi. The nucleic acid was eluted from the column with a linear gradient of NaCl from 0.3 M to 1.5 M or 0.3 M to 1.2 M in 0.05 M sodium phosphate buffer, pH 6.7. Fractions containing 5 ml each were collected. The initial flow rate of the column was kept between 6.5 and 7.5 minutes per fraction by adjusting the level of the gradient apparatus above the column. The OD at 260 mμ and the radioactivity of each fraction were determined.

REGENERATION OF MAK COLUMNS

When the gradient elution was completed, 150 ml of 2.0 M buffered NaCl were passed through the column under 3 psi. The column was then equilibrated again with 150 ml of 0.3 M buffered saline in preparation for the next sample.

DETERMINATION OF RADIOACTIVITY

Sample radioactivity was determined with a Nuclear Chicago liquid scintillation counter using the channel ratio method. Radioactivity in the remaining substrate, the CO_2 fraction (made to 10 ml with methyl cellosolve), and the total nucleic acid sample was determined by counting aliquots of each in dioxane scintillator containing 50 g of naphthalene, 4 g of PPO (2,5-diphenyloxazole), 100 mg of POPOP (1,4-bis[2-(5-phenyloxazolyl)]-benzene), 833 ml of dioxane, and 167 ml of ethyl cellosolve per liter of solution.

The total radioactivity in each fraction of the column effluent was determined by a similar procedure to that of Chroboczek and Cherry (17). Two ml aliquots of each fraction were mixed with 0.5 mg of carrier DNA and the nucleic acid precipitated in 5% trichloroacetic acid. The precipitate was collected on a Millipore filter (0.30 μ pore size) and washed with water. The dried filter was placed in a vial containing toluene scintillator (4 g PPO, 100 mg POPOP in 1 liter of toluene) and counted. The vials were reused after removal of the filter as all radioactivity remained on the filter and the counting efficiency of the scintillator did not deteriorate.

VIRUS ASSAY

A personal communication from the seed supplier stated that there was a possibility that the seeds may be infected with necrotic ring spot or sour cherry yellows viruses. To assay for these viruses, the procedure of George (27) was adopted. Several seeds were macerated in a few ml of water with a mortar and pestle. The resulting juice was applied with a pipe cleaner to the cotyledons of 5 cucumber seedlings (Chicago pickling variety) previously dusted with carborundum. Seedlings were inoculated just as the primary leaf was unfolding and were kept either in the greenhouse or in a growth chamber at 76°F under continuous light. Aliquots of several of the nucleic acid samples were assayed in a similar manner after dialysis.

RESULTS

Seed dormancy in Prunus cerasus, which can be classified as embryo dormancy, can be overcome by a 16 week after-ripening period. It has been observed (36) that at the seventh week of after-ripening there is a shift in the pathway of glucose oxidation in the embryonic axes from the EMP to the pentose phosphate pathway. Other metabolic changes such as a decrease in lipid level, an increase in sugars and dry weight (36) and translocation of nitrogen and phosphorous from the cotyledons to the embryonic axes have also been observed. Control of the metabolism of a cell lies in its nucleic acids. Thus one should observe changes in the nucleic acids of after-ripening cherry seeds which would parallel the metabolic changes. To this end, nucleic acids were extracted from cherry seeds at different stages during after-ripening. The nucleic acids were fractionated on MAK columns and the amount of newly synthesized RNA in the ribosomal and messenger regions was examined.

CONTAMINATION STUDIES

Since it is desirable to remove microbial contamination from tissue which is to be fed radioactive material for an extended period of time, several chemicals were tested as to their effectiveness in destroying contamination on the seeds. One per cent and 0.1% solutions of Domiphen Bromide reduced colony counts to less than 30 per ml of feeding medium, when seeds were treated for 10 or 5 minutes with seed coats on followed by 5 or 3 minutes with seed coats removed. Wescodyne at a concentration of 75 ppm reduced contamination from one to four million for the controls to 41,000 colonies per ml.

Of these two chemicals Domiphen Bromide appeared the most effective. To measure the toxic effect of this chemical at the concentration used, respiration as measured by oxygen uptake, and incorporation of uridine-2-^{14}C into the nucleic acid fraction of cherry seeds were studied. The results of the oxygen uptake experiments are found in Table 1. Using 0.1% or 0.05% with and without seed coats reduced oxygen uptake to 37% and 45% of that of the control. A mixed treatment of 0.1% with seed coats and 0.05% without seed coats reduced oxygen uptake to 56% of that of the control. It should be noted that there was no way of eliminating the respiration of microbial contamination from measurements made on the control. Thus the value obtained for the control includes two components, the respiration of the seeds and that of the contamination. In the experiments on uridine-2-^{14}C incorporation, Domiphen Bromide treatment decreased the specific activity of the total nucleic acid sample from 343 dpm/OD as obtained with calcium hypochlorite sterilization to 260 dpm/OD for non-after-ripened seeds. In view of the drop in respiration and the decrease in uridine incorporation brought about by Domiphen Bromide treatment, it was decided that this chemical was too toxic relative to calcium hypochlorite. The latter was used in all further experiments.

VIRUS STUDIES

Some of the trees from which the seed supply was obtained showed symptoms of necrotic ring spot or sour cherry yellows viruses. These viruses are transmitted in pollen and seeds. Nothing is known about the effect of storage or after-ripening treatment on the viability of the

TABLE 1.

THE EFFECT OF DOMIPHEN BROMIDE STERILIZATION ON THE OXYGEN UPTAKE OF DORMANT PRUNUS CERASUS SEEDS

Treatment of Seeds *		Oxygen uptake	
Concentration of Domiphen Bromide (%)		µl/hr/sample	% of control
with seed coats	without seed coats		
0.05	0.05	29.8	45
0.10	0.05	37.1	56
0.10	0.10	24.8	37
0.00	0.00	66.1	100

* The treatment sequence involved incubation with Domiphen Bromide for 5 minutes with seed coats on followed by 3 minutes with seed coats off. The control seeds were treated in the same manner, but in water.

viruses in the seeds. If viruses were present in the seeds in considerable numbers and if they were actively reproducing during after-ripening or feeding of the seeds, their contribution to the nucleic acids extracted from the seeds might lead to incorrect interpretation of changes in nucleic acid during after-ripening.

Attempts to detect the presence of virus in the seeds or infectivity in the nucleic acid extracts by mechanical transmission to cucumber seedlings failed. It was concluded that the percentage infection was too low to detect, prolonged storage brought about inactivation of the virus, or the technique failed to demonstrate the presence of virus.

EFFECT OF AFTER-RIPENING TREATMENT ON GERMINATION

The effect of a moist cold temperature treatment on the germination of sour cherries can be seen in Figure 1. A full 16 weeks after-ripening brought about 95% germination of the embryos. The presence of the seed coat restricted germination at all stages of after-ripening with the germination percentage reaching only 18% at 16 weeks. At 25° the percentage germination of embryos at 4 weeks was high (44%) but further treatment at this temperature did not improve germination. The seed coat was effective in keeping germination almost at zero even after 16 weeks treatment at 25°.

COLUMN CHROMATOGRAPHY

In preliminary experiments with MAK columns it was found that after short storage times the methylated albumin prepared according to the procedure of Mandell and Hershey (49) lost its ability to fractionate

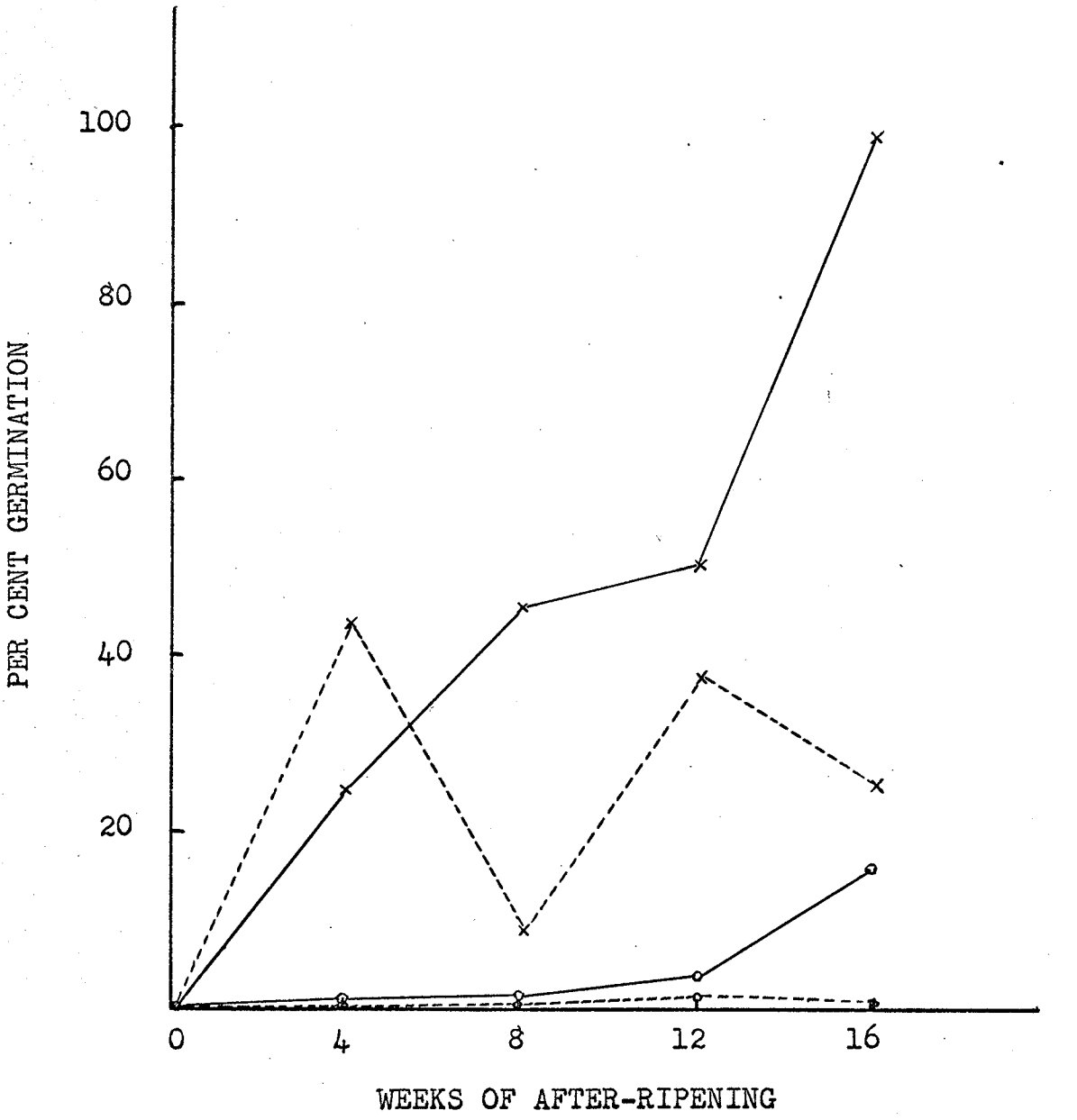

× Without seed coat
o With seed coat
—— After-ripened at 1°C
---- After-ripened at 25°C
PER CENT GERMINATION
100
80
60
40
20
0
4
8
12
16
WEEKS OF AFTER-RIPENING

Fig. 1. The Effect of After-ripening on Germination of *Prunus cerasus* Seeds.

nucleic acid preparations. Any hydrochloric acid not removed from the methylated albumin during preparation would reduce the basicity of the protein on storage. Thus it was decided to adjust the pH of the methylated albumin suspension to 6.0 immediately after methylation. This step improved the removal of residual hydrochloric acid from the protein and was successful in prolonging the storage life of the methylated albumin by several months.

It was desirable during this work to use the MAK columns more than once. To determine if this was possible one sample of nucleic acid was divided in two and the first half run as usual. When the gradient elution was completed 150 ml of 2.0 M buffered NaCl was passed through the column and the OD of these fractions read to determine if any additional nucleic acid was removed from the column. The column was then equilibrated again with 0.3 M buffered saline. The second half of the sample was loaded on the column and eluted with the gradient. The results from this treatment were compared to those from a sample divided between two fresh columns.

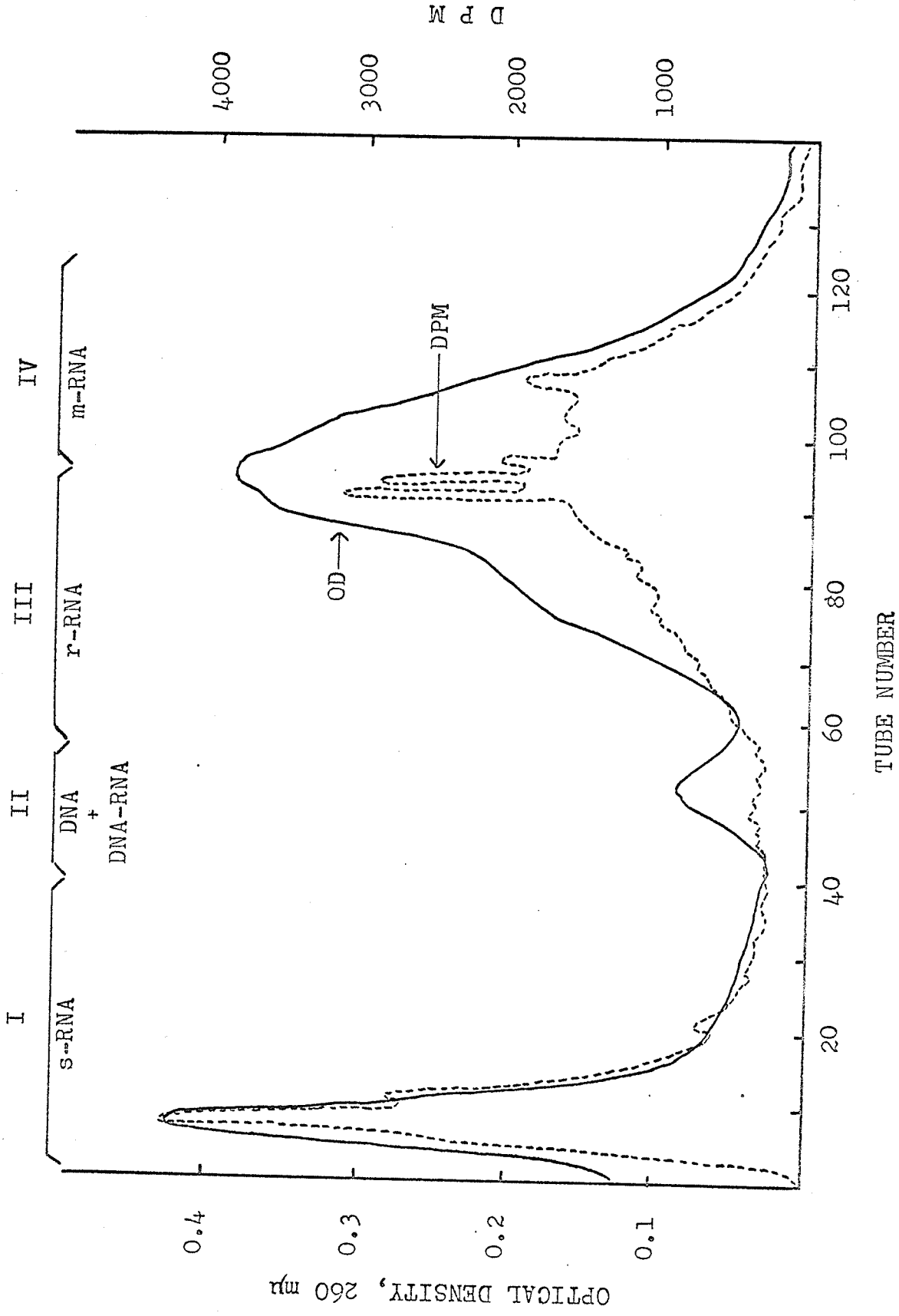
To further test the reliability of the regeneration procedure the ribosomal-messenger RNA peak from one column effluent was collected, dialysed overnight against 0.05 M sodium phosphate buffer pH 6.7, made to 0.3 M NaCl and rechromatographed on the regenerated column. In the first experiment differences in the two halves of the sample chromatographed on one column were no greater than differences in the two halves of the sample chromatographed on two fresh columns. In the second experiment no difference in the elution profile of the peak could be detected between the original and the rechromatographed sample.

CHROMATOGRAPHY OF NUCLEIC ACIDS

The nucleic acids from sour cherry seeds were separated into four fractions by chromatography on MAK columns as shown in Figures 2 and 3. The elution profiles are similar to those reported for peanut cotyledons (14), Xanthium buds (16), pea epicotyls (15) and hazel seed cotyledons (80) to name a few of the many reports in the literature. According to the current practise the four regions are referred to as I - soluble RNA, II - DNA + DNA-RNA, III - ribosomal RNA, IV - messenger RNA. The experimental evidence for this characterization will be discussed later. As seen in the figures, the s-RNA region eluted in one main fraction although a shoulder was often observed as is more evident in Figure 2. Two peaks of radioactivity were found in this region and the whole of area I appeared heterogenous with respect to radioactivity. Two fractions were observed in the ribosomal regions which have been referred to as light r-RNA and heavy r-RNA in the literature (14). A definite shoulder was always found on the side of the ribosomal region. This shoulder will be referred to as m-RNA. In regions III and IV three definite areas of radioactivity were observed, each showing two peaks.

Sedimentation experiments were carried out on the total nucleic acid sample before chromatography and on the nucleic acid material eluted from the MAK columns. An aliquot of eluent from the top of each peak was centrifuged at 167,540 x g in a Spinco Model E analytical ultracentrifuge. Movement of the boundary was followed with UV optics. Two definite boundaries were observed in the total sample, one with a sedimentation constant of 2.52 S and the other 11.19 S. Because of

Fig. 2. Separation of Nucleic Acids from 4-week After-ripened Prunus cerasus Seeds by MAK Column Chromatography.


I
II
III
IV
s-RNA
DNA
+
DNA-RNA
r-RNA
m-RNA
OD
DPM
OPTICAL DENSITY, 260 mμ
0.4
0.3
0.2
0.1
D P M
4000
3000
2000
1000
20
40
60
80
100
120
TUBE NUMBER

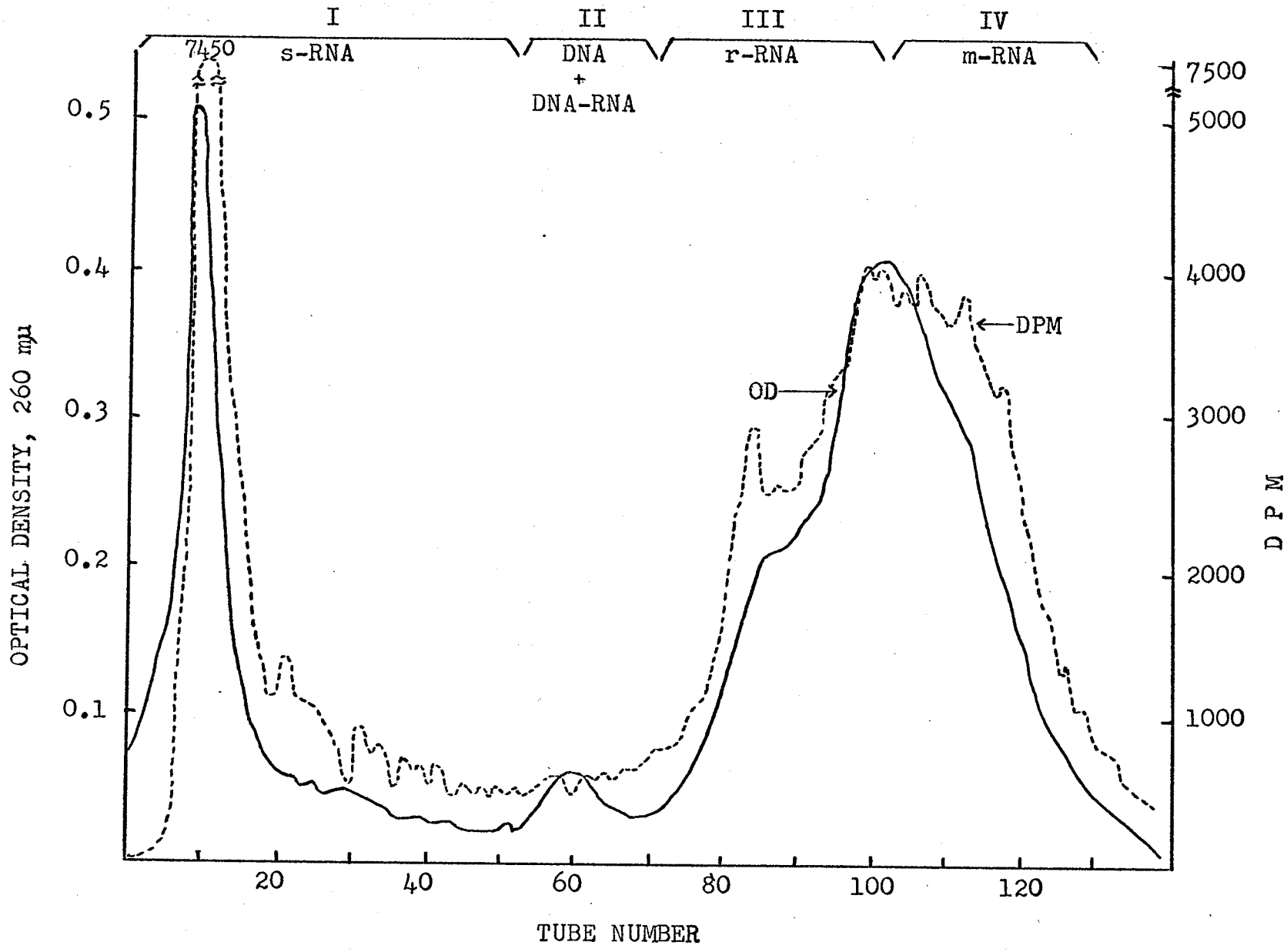

I
II
III
IV
7450
s-RNA
DNA
+
DNA-RNA
r-RNA
m-RNA
OD
DPM
OPTICAL DENSITY, 260 mμ
D P M
TUBE NUMBER
0.5
0.4
0.3
0.2
0.1
7500
5000
4000
3000
2000
1000
20
40
60
80
100
120

Fig. 3. Separation of Nucleic Acids from 8-week After-ripened Prunus cerasus Seeds by MAK Column Chromatography.

instrumentation difficulties in obtaining tracings of the boundaries from the photographs taken during the sedimentation runs, the error in these values may be as high as ±2 S. Sedimentation constant values obtained for the column effluent were 3.09 S for the s-RNA fraction, 13.41 S for the light r-RNA and 13.91 S for the heavy r-RNA. The values for the s-RNA fraction are in agreement within the estimated error with that obtained for wheat germ s-RNA (37). On the other hand the values obtained for the r-RNA fractions are lower than expected and furthermore they do not indicate the presence of two different RNA types as has been reported (35).

CHANGES IN THE NUCLEIC ACID FRACTIONS DURING AFTER-RIPENING

The various kinds of nucleic acids present in a cell have different metabolic roles. Changes in the total amount or in the rate of synthesis of any type during after-ripening would give information as to possible metabolic blocks within the embryo. The total amount of nucleic acid material in each fraction eluted from the column was determined by summing the OD's of all the tubes in each peak. Similarly the total incorporation of uridine-^{14}C into each fraction was determined by summation over each peak of the amount of radioactivity in all the tubes.

The amount of nucleic acid material in each fraction is given in Table 2. It can be seen that there was very little if any change in the amount of s-RNA during after-ripening when expressed as a per cent of the total nucleic acid extracted. In region II there was a drop in the relative amount of nucleic acid material during after-ripening. Table 2

TABLE 2. AMOUNT OF NUCLEIC ACID FROM AFTER-RIPENING *PRUNUS CERASUS* SEEDS IN EACH FRACTION ELUTED FROM THE MAK COLUMN

Fraction	After-ripening temperature	After-ripening time									
		0 Weeks		4 Weeks		8 Weeks		12 Weeks		16 Weeks	
		OD	% of total	OD	% of total	OD	% of total	OD	% of total	OD	% of total
I	1°	4.214	21.9	4.137	20.2	3.704	27.4	4.464	22.8	4.423	21.3
				4.056	24.8	3.806	20.1	3.097	20.6	4.223	20.1
				4.357	23.2	3.831	21.6			5.650	23.2
	25°			3.789	21.1	3.921	24.5				
II	1°	2.248	11.7	2.381	11.7	1.453	10.7	1.110	5.7	1.280	6.2
				1.524	9.3	1.260	6.7	0.714	4.8	1.773	8.4
				1.767	9.5	1.467	8.3			1.768	7.3
	25°			1.639	9.1	1.128	7.1				
III	1°	5.732	29.8	8.155	39.9	4.268	31.6	9.632	49.1	8.913	42.8
				4.870	29.8	8.349	44.2	6.389	42.4	9.383	44.7
				6.427	34.2	6.870	38.7			10.252	42.1
	25°			6.577	36.6	7.246	45.3				
IV	1°	7.070	36.7	5.771	28.2	4.106	30.3	4.400	22.4	6.185	29.7
				5.910	36.1	5.493	29.0	4.846	32.2	5.652	26.8
				6.216	33.1	5.593	31.4			6.707	27.5
	25°			5.977	33.2	3.711	23.2				
Total	1°	19.264		20.442		13.531		19.606		20.801	
				16.360		18.908		15.046		21.031	
				18.767		17.761				24.377	
	25°			17.982		16.006					

also shows a trend towards an increase in the percentage of r-RNA and a decrease in the percentage of m-RNA with after-ripening time. Similar changes occurred at both treatment temperatures.

The incorporation of ^{14}C into each fraction eluted from the MAK column is given in Table 3. It was observed that the total dpm in each region at a given after-ripening time varied greatly from sample to sample. However when the total dpm were expressed as a per cent of the total incorporation, results were more consistant. No definite change was observed in the percentage incorporation into the s-RNA fraction. In fraction II during after-ripening at $1^{\circ}C$ an increase in the percentage incorporation was observed between 4 and 8 weeks followed by a drop at 12 and 16 weeks. A definite increase in the percentage incorporation into the r-RNA fraction was observed while the percentage incorporation into the m-RNA fraction decreased with after-ripening time. Similar changes occurred at both temperatures except in region II where the percentage incorporation at $25^{\circ}C$ at 8 weeks after-ripening time decreased.

The specific activity of each fraction of nucleic acids eluted from the MAK columns was expressed as dpm/OD x 5 and is given in Table 4. Again the values in each fraction at a given after-ripening time varied greatly from sample to sample. This is a reflection of the large variation in the total dpm in each fraction.

The most interesting changes observed during after-ripening were in the ribosomal and messenger regions. The amount of nucleic acid in each fraction is given in Table 5 as a per cent of the total amount in the combined regions. By expressing the results in this fashion an idea of the changes in the two fractions relative to each other can be obtained.

TABLE 3. RADIOACTIVITY IN EACH FRACTION OF NUCLEIC ACIDS FROM AFTER-RIPENING *PRUNUS CERASUS* SEEDS

Fraction	After-ripening temperature	After-ripening time									
		0 Weeks		4 Weeks		8 Weeks		12 Weeks		16 Weeks	
		dpm	% of total	dpm	% of total	dpm	% of total	dpm	% of total	dpm	% of total
I	1°	2,090	11.4	9,473	30.8	18,734	49.5	4,783	-	100,590	35.0
				29,700	43.3	15,498	33.0	58,994	28.8	59,437	33.2
				37,748	30.3	49,170	21.7			31,948	21.9
	25°			8,833	17.5	11,073	12.6				
II	1°	2,148	11.7	3,018	9.8	4,945	13.1	-	-	17,109	6.0
				5,619	8.2	3,643	7.8	18,920	9.2	14,003	7.8
				11,961	9.6	25,860	11.4			9,361	6.4
	25°			6,387	12.6	7,859	8.9				
III	1°	5,648	30.7	8,446	27.4	8,153	21.6	4,809	-	96,863	33.7
				14,737	21.5	15,199	32.4	71,399	34.8	63,729	35.6
				37,058	29.8	80,204	35.4			60,208	41.3
	25°			18,119	35.9	42,236	48.1				
IV	1°	8,474	46.2	9,805	32.0	6,010	15.9	3,990	-	72,744	25.3
				18,463	26.9	12,572	26.8	55,805	27.2	41,787	23.4
				37,719	30.3	71,853	31.6			44,373	30.4
	25°			17,192	34.0	26,724	30.4				
Total	1°	18,340		30,742		37,841		8,800		287,306	
				68,517		46,911		205,118		178,956	
				124,485		227,086				145,890	
	25°			50,530		87,932					

TABLE 4.

SPECIFIC ACTIVITY OF EACH FRACTION OF NUCLEIC ACID FROM AFTER-RIPENING *PRUNUS CERASUS* SEEDS

Fraction	After-ripening temperature	Specific Activity (dpm/ODx5)				
		After-ripening time				
		0 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
I	1°	99	458	1012	214	4548
			1464	814	3810	1131
			1733	2567		1131
	25°		466	565		
II	1°	191	253	681	-	2673
			737	578	5299	1580
			1354	3525		1060
	25°		779	1393		
III	1°	196	207	382	100	2173
			605	364	2235	1360
			1153	2335		1172
	25°		551	1160		
IV	1°	240	340	293	181	2352
			625	458	2303	1479
			1214	2569		1322
	25°		574	1442		

TABLE 5.

AMOUNT OF RIBOSOMAL AND MESSENGER RNA EXTRACTED FROM AFTER-RIPENING *PRUNUS CERASUS* SEEDS

Fraction	After-ripening temperature	Amount of nucleic acid per fraction as per cent of ribosomal and messenger RNA				
		After-ripening time				
		0 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
r-RNA	1°C	44.8	58.6	51.0	68.5	62.4
			45.2	60.3	56.8	59.0
			50.7	55.2		60.4
mean			51.5	55.5	62.7	60.6
	25°		52.4	66.2		
m-RNA	1°	55.2	41.4	49.0	31.4	37.6
			54.8	39.7	43.1	41.0
			49.2	44.8		39.6
mean			48.5	44.5	37.3	39.4
	25°		47.6	33.8		

It was observed that after-ripening at 1° brought about a continued increase in the r-RNA fraction relative to the m-RNA fraction up to 12 weeks followed by a slight drop which may not be significant. For treatment of the seeds at 25°C only values for 4 and 8 weeks are available and these indicate a large increase in r-RNA relative to m-RNA.

Incorporation of radioactive uridine into the r-RNA and m-RNA fractions is expressed as a per cent of the total incorporation for the combined regions in Table 6. At 1°C, after-ripening brought about a steady increase in incorporation into r-RNA relative to that in m-RNA. Similar but larger changes occurred between 4 and 8 weeks during treatment of the seeds at 25°C.

Table 7 gives the relative amounts of ribosomal and messenger RNA, the relative incorporation of uridine into the two fractions and the relative specific activity of the two fractions. With no after-ripening there was a greater amount of m-RNA than r-RNA. By 4 weeks of after-ripening the amount of r-RNA surpassed that of m-RNA and continued to increase with respect to it up to 12 weeks. At 16 weeks the amount of r-RNA was still greater than that of m-RNA but to a slightly lesser degree. During treatment at 25°, the amount of r-RNA exceeded that of m-RNA at 4 weeks whereas at 8 weeks the reverse occurred. Incorporation of uridine into m-RNA with no after-ripening and after 4 weeks of after-ripening at 1° was greater than that for r-RNA. Further after-ripening resulted in increased incorporation into the r-RNA fraction such that it became greater than that in the m-RNA fraction. However, at 25° incorporation into r-RNA at 4 weeks was greater than for

TABLE 6.

INCORPORATION OF URIDINE INTO RIBOSOMAL AND MESSENGER RNA OF AFTER-RIPENING *PRUNUS CERASUS* SEEDS

Fraction	After-ripening Temperature	^{14}C incorporation as per cent of total radioactivity in ribosomal and messenger RNA				
		After-ripening time				
		0 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
r-RNA	1°C	39.9	46.3	57.6	54.7	57.1
			44.4	54.7	56.1	57.5
			49.6	52.7		60.5
mean			46.8	55.0	55.4	58.4
	25°		51.3	61.2		
m-RNA	1°	60.1	53.7	42.4	45.3	42.9
			55.6	45.3	43.9	42.5
			50.4	47.3		39.5
mean			53.2	45.0	44.6	41.6
	25°		48.7	38.8		

TABLE 7.

RELATIVE INCORPORATION, AMOUNT AND SPECIFIC ACTIVITY
OF RIBOSOMAL AND MESSENGER RNA
FROM AFTER-RIPENING *PRUNUS CERASUS* SEEDS

	After-ripening temperature	Relative values expressed as the ratio of $\frac{\text{m-RNA}}{\text{r-RNA}}$				
		After-ripening time				
		0 Weeks	4 Weeks	8 Weeks	12 Weeks	16 Weeks
OD	1°C	1.230	0.708	0.962	0.457	0.694
			1.210	0.659	0.760	0.603
			0.966	0.814		0.654
mean			0.961	0.812	0.608	0.650
	25°		0.909	2.760		
dpm	1°	1.505	1.161	0.737	0.830	0.751
			1.253	0.827	0.782	0.666
			1.018	0.896		0.738
mean			1.144	0.820	0.806	0.718
	25°		0.949	0.635		
specific activity (dpm/OD)	1°	1.224	1.642	0.767	1.810	1.082
			1.033	1.258	1.030	1.088
			1.053	1.100		1.128
mean			1.243	1.042	1.420	1.099
	25°		1.042	1.242		

m-RNA and at 8 weeks the relative incorporation into r-RNA was greater still. There did not seem to be a definite relation between the relative specific activites of messenger and ribosomal RNA with after-ripening time. However, it was noted that the specific activity of m-RNA was greater than that of r-RNA in all cases except one.

DISCUSSION

Dormancy in Prunus cerasus seeds is primarily due to embryo dormancy but a secondary seed coat effect is also observed. Treatment of the seeds for four months under moist conditions at temperatures just above freezing is required to bring about normal germination. Change from the dormant to the non-dormant state brought about by the after-ripening treatment could be due to the synthesis or activation of an enzyme system or the production or release of necessary substrates and coenzymes which are required for reactions leading to germination. Changes in the nucleic acid metabolism of the embryos should parallel these changes and give some idea of the mechanism involved in overcoming the blocks to germination.

Since there were no reports of a method of extracting nucleic acids from sour cherry seeds, the method used by Cherry (13) with a few modifications was adapted to this system. Nucleic acids were extracted from the seeds at various stages during after-ripening and separated into four fractions by MAK column chromatography. Changes in the amounts of nucleic acid material in each fraction and in the incorporation of uridine-^{14}C during a 48 hour feeding period at 25° were taken as measuring the changing capacity of the embryos to synthesize the different fractions of nucleic acids at different after-ripening times. It was observed that with longer after-ripening times the relative amount of r-RNA increased (Table 2). The percentage incorporation of uridine-^{14}C into the r-RNA fraction also increased with after-ripening time indicating an increased capacity of the embryo to synthesize r-RNA (Table 3). A definite increase in the amount of r-RNA relative to the amount of m-RNA was observed (Table 5) with after-ripening time. Except for dormant embryos the amount of r-RNA

present was always greater than the amount of m-RNA (Table 7). The incorporation of uridine-^{14}C into r-RNA increased with respect to m-RNA with after-ripening time (Table 6). At 0 and 4 weeks after-ripening incorporation into m-RNA exceeded incorporation into r-RNA while further after-ripening brought about greater incorporation into r-RNA than into m-RNA (Table 7).

A number of difficulties were encountered in obtaining a uniform extraction of the nucleic acid material. A major difficulty was the fact that the phenol method of extraction was not quantitative. This no doubt accounts for a good deal of the variability observed and made it difficult to compare actual OD values from sample to sample. Large differences were observed in the incorporation of label into the s-RNA fraction in duplicate samples from the same flat of after-ripening seeds and in samples from different flats at the same after-ripening time. As a result no reliable correlation could be obtained between the after-ripening time and the amount of nucleic acid material in or the incorporation of label into this fraction. Also it was not always possible to obtain a clear-cut peak in region II of the chromatogram. This could not be attributed to the failure of the column to fractionate the sample as in some cases the following sample fractionated as expected. Furthermore, it did not seem to be due to incomplete homogenization of the sample as microscopic observation of the tissue residue indicated that very few if any cells or nuclei remained intact. The ability to recover a clear-cut peak in this region was improved by adding potassium acetate to the ethanolic precipitation of the nucleic acids. Regions III and IV of the chromatograms were the most reliably reproduced. As a result most of the attention has

been focused on these fractions in the interpretation of the results.

It is interesting to note that on a few occasions a small peak of labelled nucleic acid material eluted from the column a short time after the end of the m-RNA peak. The nature of the peak is unknown. It has been recently reported (38) that a rather large amount of newly synthesized RNA is tenaciously bound to MAK columns. It can be eluted with sodium lauryl sulfate or dilute NH_4OH. As initially eluted this RNA is contaminated with RNA which elutes on rerun in the regions of ribosomal and messenger RNA. The nature of this tightly bound RNA has not been determined as yet. No attempts were made to recover this RNA fraction in the nucleic acid studies reported here. Since no further nucleic acid material was removed from the column during the regeneration procedure when a very strong saline solution was used, it was decided that either this fraction was absent from nucleic acid extracts of cherry embryos or that it remained bound to the column throughout the entire use of the column. It is possible that the small peak occasionally recovered after the m-RNA peak is part of this tenaciously bound RNA fraction but there is no evidence to confirm this.

One of the main difficulties encountered in this study was the wide variation in incorporation of uridine-^{14}C into the nucleic acid fractions of samples recovered at the same after-ripening time from different flats. The most likely explanation for this is the fact that it was difficult to reproduce exactly from flat to flat the same conditions in the after-ripening medium particularly with reference to the water content. The moisture level of the medium may influence the rate of

chemical changes in the seeds during after-ripening. It was observed that, for the samples at a given after-ripening time, those which had higher incorporation levels also had somewhat higher germination percentages indicating that they had either progressed faster towards the non-dormant state during after-ripening or that the seeds were losing their dormancy during prolonged dry storage. In spite of this the percentage incorporation into each fraction of nucleic acids at a given after-ripening time was fairly constant. In view of this problem it appears that a more uniform after-ripening treatment should be sought for further studies of metabolic changes during after-ripening.

Another major difficulty encountered in this work was the high microbial contamination levels on the seeds. Several different chemical agents were tested as to their ability to destroy this contamination. The only chemical which was found to be very effective in removing contamination was Domiphen Bromide. However, it also proved to be quite toxic to the seeds. Antibiotics were not tested since many of them directly affect various aspects of nucleic acid metabolism and in doing so would lead to a false picture of nucleic acid changes during after-ripening. Calcium hypochlorite, the sterilizing agent used throughout the nucleic acid studies, held the contamination levels at several million bacteria per ml of feeding medium. Lonberg-Holm (48) and Hock (44) have reported that the presence of bacterial contamination greatly influenced the amount of ^{32}P-phosphate incorporation into the ribosomal and messenger RNA regions and the profile of radioactivity in the MAK column chromatograms. Sobota, Leaver and Key (67) on the other hand report that levels of

contamination of 3 - 4 x 10^4 bacteria per g fresh weight did not appreciably affect radioisotope incorporation into nucleic acids of plant tissue. In the studies reported here no correlation was found between the observed variability in incorporation and the level of bacterial contamination. Nevertheless it is felt that a better method of sterilization of the seeds is desirable and in any case the level of contamination should always be checked.

A number of studies have been carried out to characterize the nucleic acids in the various fractions obtained on MAK column chromatography. In a study on nucleic acids extracted from soybean hypocotyl Ingle, Key and Holm (35) identified the soluble, and light and heavy ribosomal RNA regions by comparison of material from sucrose gradient centrifugation and MAK column eluent. The DNA + DNA-RNA region was shown to be about 95% DNA by the diphenylamine reaction. The OD and radioactivity in this region did not match well while the radioactivity was associated with the DNA. The composition of this DNA was found to be different from that of the total DNA of soybean hypocotyl and the RNA associated with this fraction had the same GMP/AMP ratio as the DNA. The m-RNA or D-RNA region was found to be contaminated with some heavy r-RNA. This fraction was more rapidly labelled than the others and had a composition similar to that of bean DNA. Chroboczek and Cherry (18) found that most of the r-RNA of peanut cotyledons was contained in the microsomal pellet which sedimented at 105,000 x g while the s-RNA was isolated from the supernatant fraction. Cherry (13) observed that the DNA peak (fraction II) of peanut cotyledons contained at least 3

components, RNA, rapidly metabolized DNA and non-metabolic DNA. No attempt was made to characterize the nucleic acid fractions obtained from cherry embryos and it was assumed in all considerations of the results that they were comparable to those reported in the literature. That this is most likely the case is indicated by the sedimentation experiments. These studies showed that the sedimentation constant for peak I was similar to that reported for s-RNA in higher plant tissue while that of the r-RNA region indicated the presence of only one type of r-RNA. Since there was much difficulty in measuring the movement of the boundaries the values obtained for the sedimentation constants should be considered as approximations only.

The most significant change observed in the nucleic acid fractions with after-ripening was the increase in r-RNA with respect to the total nucleic acid extracted and with respect to the m-RNA fraction. This was also found to be the case in hazel seed cotyledons during after-ripening (80). These observations most likely reflect a greater demand for protein synthesis as after-ripening progresses. It has been observed that there is an increase in the respiration rate in after-ripening cherry embryos (36) particularly in the embryonic axes. This indicates that the metabolic activity of the embryos increases with after-ripening time which would require increased amounts of the enzymes involved. A change in the pathway of glucose breakdown from the EMP pathway to the pentose phosphate pathway has also been observed to take place during after-ripening (36). The initial stages of the changeover might involve activation of enzymes already present in the embryo but as the new pathway became more significant there would be a greater demand for synthesis

of new enzymes. As the metabolic activity of the embryos increases with after-ripening there would be a greater demand for enzymes to break down and mobilize reserve materials in the cotyledons to provide energy and cofactors for reactions leading to germination. Thus one of the metabolic blocks to germination in dormant embryos might be insufficient r-RNA to meet the demand for synthesis of proteins required for germination. One of the effects of the after-ripening treatment might then be to increase the capacity of the embryos to produce the necessary r-RNA and/or form complete and active ribosomes.

Marcus and Feeley report that unimbibed peanut seeds showed a low level of incorporation of amino acids into proteins while imbibed seeds showed a greatly increased activity (50). Their evidence indicated that the soluble enzymes required for protein synthesis were active before imbibition and that it was the ribosomal fraction that was activated on imbibition. The increase in activity was associated with polysome formation. They explained their observations on the basis that the ribosomes were functional in the dry seed and hydration brought about the combination of them with either a previously present m-RNA or one that was synthesized as a result of the liberation of an inhibitor. They point out, however, that the ribosomes from unimbibed seeds would not readily support protein synthesis when m-RNA fractions from imbibed peanut cotyledons or turnip yellow mosaic virus RNA were used instead of polyuridylic acid. This might indicate that the ribosomes from unimbibed seeds are deficient in some way. Since the present study indicated that increased amounts of r-RNA were formed on after-ripening it is possible that one of the blocks to germination in the embryo might be a deficiency

in the ribosomes such that they are unable to support adequate protein synthesis for germination. After-ripening treatment in some way then would relieve this deficiency. Further work to determine the protein synthesizing ability of the ribosomes at various stages during after-ripening would provide interesting information as to the validity of this speculation.

The observed decrease in the relative amount of m-RNA synthesis with after-ripening of cherry embryos indicated that lack of m-RNA was not likely a block to germination in these embryos. It is possible however that in dormant embryos the m-RNA is in some way prevented from interacting with the ribosomes for protein synthesis. In this case after-ripening would be responsible for removing this barrier. It would be interesting to determine the composition of the m-RNA fraction at different after-ripening times to see if different species of m-RNA were formed at different times. This might be expected in view of the observed shift in the pathway of glucose oxidation. Also it has been observed by Chroboczek and Cherry (17) that the composition of m-RNA from peanut cotyledons changed with different stages of germination. Thus it is not unreasonable to expect that during after-ripening which prepares seeds for germination, similar changes might occur.

It was noted that similar changes in nucleic acid fractions occurred as a result of treatment of the seeds at 1°C and 25°C. However, only after-ripening of the seeds at 1°C was fully effective in bringing about increased ability of the seeds to germinate. Consequently it might be questionable to consider the changes that were observed to be the result of low temperature after-ripening of the seeds. However, it should be

noted that at 25°C, germination of intact seeds failed to rise above zero even after 16 weeks treatment while germination of the embryos was relatively high (Figure 1). This indicated that the seed coat exerts a strong inhibitory influence on the embryo which is overcome only at lower after-ripening temperatures. For the feeding experiments, seed coats were removed from the embryos to facilitate entry of the labelled substrate. From the germination data it would seem that this step also removed a considerable amount of inhibitory influence from the embryos. It may be that the changes observed here can take place at both temperatures and are only a part of the overall chemical changes necessary for germination. Further changes which might occur at the lower temperature only, such as the derepression of an enzyme system, might only be detected as a change in the composition of the m-RNA fraction and would thus have not been observed in this study.

Although there are no reports of the presence of germination stimulators or inhibitors in sour cherry seeds there is much evidence in the literature to suggest that this is probably the case. Tuan and Bonner (73) showed that chromatin of buds of dormant potato tubers was incapable of supporting DNA-dependent RNA synthesis. The chromatin of non-dormant buds of potato tubers in which dormancy had been overcome by treatment with ethylene chlorohydrin was highly effective in supporting DNA-dependent RNA synthesis. They concluded that the genetic material of buds of dormant potato tubers is largely in a repressed state and that hormone stimulated breaking of dormancy is accompanied by derepression of genetic material. In this connection it is interesting to note that abscisin II is an inhibitor of nucleic acid synthesis in duckweed cultures,

suppression of the DNA fraction preceding that of the RNA fractions (75). This chemical has also been found in peach seed endocarps and has been shown to inhibit germination and cause dwarfing of seedlings from non-after-ripened seeds. Thus it seems likely that abscisin II acts as a germination inhibitor by suppressing nucleic acid synthesis. The effect of the seed coat on germination of cherry seeds observed in this study suggests the possibility of the presence of an inhibitor which might diffuse into the embryo and thereby cause embryo dormancy. Such an inhibitor might act in a similar manner as abscisin II may do in peach seeds by inhibiting nucleic acid synthesis. Its inhibition could be specific for r-RNA synthesis. This could result in the formation of deficient ribosomes and thus prevent enzyme synthesis necessary to bring about the changes in metabolism that are required for germination. If low temperature after-ripening brought about inactivation of this hypothetical inhibitor one would expect to see an increased ability of the embryos to synthesize the necessary r-RNA and this has been observed. A study of the presence of germination inhibitors in sour cherry seeds and of their effect on nucleic acid metabolism would be enlightening in view of these speculations.

It can be concluded from the results presented here that one of the metabolic changes observed during the after-ripening of sour cherry seeds is the increased capacity of the embryos to synthesize r-RNA with after-ripening time. The block to r-RNA synthesis in dormant embryos may be due to the action of some as yet undiscovered inhibitor that low temperature after-ripening might inactivate. The other metabolic changes which occur during after-ripening likely require and/or are supported by

protein synthesis. Blocks to r-RNA synthesis would result in formation of deficient ribosomes or insufficient numbers of them and protein synthesis necessary for germination would be inhibited. After-ripening overcomes this block to germination by in some way increasing the capacity of the embryos to synthesize r-RNA.

SUMMARY

Seed dormancy in sour cherries is primarily due to embryo dormancy. After-ripening of the seeds under cool moist conditions brings about almost total germination of the embryos after 16 weeks. The seed coat was observed to restrict germination at all stages of after-ripening and this inhibition was only partly overcome after 16 weeks at 1°C.

The nucleic acids extracted from sour cherry seeds were separated into four fractions by chromatography on MAK columns. These fractions are referred to as soluble RNA, DNA + DNA-RNA, ribosomal RNA and messenger RNA. The sedimentation constant of the s-RNA fraction eluted from the column was found to be 3.09 S while those of the light and heavy ribosomal RNA's were 13.41 S and 13.91 S respectively.

No change occurred in the relative amount of s-RNA or in the percentage incorporation of uridine-^{14}C into this fraction with after-ripening time. The relative amount of nucleic acid in the DNA + DNA-RNA peak decreased during after-ripening while the percentage incorporation of uridine-^{14}C increased up to 8 weeks and then decreased. During after-ripening the relative amount of r-RNA increased and the percentage incorporation of uridine-^{14}C into this fraction also increased. The relative amount of m-RNA and the percentage incorporation of uridine-^{14}C into the m-RNA fraction decreased on after-ripening.

Dormant sour cherry seeds contained a greater amount of m-RNA than r-RNA but by 4 weeks of after-ripening the amount of r-RNA surpassed that of m-RNA and continued to increase with respect to it during further after-ripening. Incorporation of uridine-^{14}C into the m-RNA fraction of

dormant and 4 week after-ripened seeds was greater than that for r-RNA but further after-ripening resulted in increased incorporation into the r-RNA fraction such that it became greater than in the m-RNA fraction. The specific activity of m-RNA was always greater than that of r-RNA.

It is suggested that the increase in r-RNA with after-ripening reflects the increased ability of the embryos to synthesize r-RNA to meet increasing demands for protein synthesis as the seeds pass from the dormant to non-dormant state.

BIBLIOGRAPHY

1. Atwood, W.M. 1914. A physiological study of the germination of Avena fatua. Botan. Gaz. 57: 387-414.

2. Ballard, L.A.T. and A.E. Grant Lipp. 1967. Seed dormancy: breaking by uncouplers and inhibitors of oxidative phosphorylation. Science 156: 398-399.

3. Bereznegovskaya, L.N. and A.A. Bubenchikov. 1967. Biochemical changes in seeds of Adonis vernalis during pregermination. Nauch. Dokl. Vyssk. Shk. Biol. Nauki 4: 97-100. Chem. Abst. 67: 41029.1967.

4. Black, M. 1956. Interrelationships of germination inhibitors and oxygen in the dormancy of seeds of Betula. Nature 178: 924-925.

5. Black, M. 1959. Dormancy studies in seed of Avena fatua. I. The possible role of germination inhibitors. Can. J. Botany 37: 393-402.

6. Black, M. and J.M. Naylor. 1959. Prevention of onset of seed dormancy by gibberellic acid. Nature 184: 468-469.

7. Borriss, H. and J. Schulze. 1966. The activity of nitrate reductase in the embryos of dormant and after-ripened Agrostemma seeds. Z. Pflanzenphysiol. 55: 449-457. Chem. Abst. 66: 73271.1966.

8. Borthwick, H.A., S.B. Hendricks, E.H. Toole, and V.K. Toole. 1954. Actions of light on lettuce seed germination. Botan. Gaz. 115: 205-225.

9. Bradbeer, J.W. and B. Colman. 1967. Studies in seed dormancy. I. The metabolism of [2-^{14}C] acetate by chilled seeds of Corylus avellana L. New Phytol. 66: 5-15.

10. Bradbeer, J.W. and V.M. Floyd. 1964. Nucleotide synthesis in hazel seeds during after-ripening. Nature 201: 99-100.

11. Chao, L. and D.R. Walker. 1966. The effect of temperature, chemicals and seed coat on apricot and peach seed germination and growth. Proc. Am. Soc. Hort. Sci. 88: 232-238.

12. Chen, S.S.C. and K.V. Thimann. 1966. Nature of seed dormancy in Phacelia tanacetifolia. Science 153: 1537-1539.

13. Cherry, J.H. 1964. Association of rapidly metabolized DNA and RNA. Science 146: 1066-1069.

14. Cherry, J.H., H. Chroboczek, W.J.G. Carpenter, and A. Richmond. 1963. Nucleic acid metabolism in peanut cotyledons. Plant Physiol. 40: 582-587.

15. Cherry, J.H. and K.J. Lessman. 1967. Comparison of nucleic acids in maize shoots and pea epicotyls. Am. J. Botany 54: 181-188.

16. Cherry, J.H. and R.B. van Huystee. 1965. Comparison of messenger RNA in photoperiodically induced and noninduced Xanthium buds. Science 150: 1450-1453.

17. Chroboczek, H. and J.H. Cherry. 1965. Production of messenger RNA during seed germination. Biochem. Biophys. Res. Commun. 20: 774-779.

18. Chroboczek, H. and J.H. Cherry. 1966. Characterization of nucleic acids in peanut cotyledons. J. Mol. Biol. 19: 28-37.

19. Corns, W.G. and R.J. Schraa. 1962. Dormancy and germination of seeds of silverberry (Elaeagnus commutata Benh.). Can. J. Botany 40: 1051-1055.

20. Crocker, W. 1906. Role of seed coats in delayed germination. Botan. Gaz. 42: 265-291.

21. Crocker, W., N.C. Thornton, and E.M. Shroeder. 1946. Internal pressure necessary to break shells of nuts and the role of shells in delayed germination. Contrib. Boyce Thompson Inst. 14: 173-201.

22. Eckerson, S. 1913. A physiological and chemical study of after-ripening. Botan. Gaz. 55: 286-299.

23. Evenari, M. 1949. Germination inhibitors. Botan. Rev. 15: 153-194.

24. Fogle, H.W. and C.S. McCroy. 1960. Effects of cracking, after-ripening and gibberellin on germination of Lambert cherry seeds. Proc. Am. Soc. Hort. Sci. 76: 134-138.

25. Frankland, B. and P.F. Wareing. 1962. Changes in endogenous gibberellins in relation to chilling of dormant seeds. Nature 194: 313-314.

26. Frankland, B. and P.F. Wareing. 1966. Hormonal regulation of seed dormancy in hazel (Corylus avellana L.) and beech (Fagus sylvatica L.). J. Exptl. Botany 17: 596-611.

27. George, J.A. 1962. A technique for detecting virus-infected Montmorency cherry seeds. Can. J. Plant Sci. 42: 198-203.

28. Gordin-Sharir, A. and P.F. Wareing. 1964. Auxin and gibberellin-like substances in peanut (Arachis hypogaea). Nature 204: 1308-1309.

29. Hartmann, H.T. and D.E. Kester. 1959. Plant Propagation: Principles and Practises. Prentice-Hall Inc., Englewood Cliffs,N.J.

30. Hatano, Ken-ichi. 1966. Changes in respiratory pattern in stratified pine seeds. J. Jap. Forest. Soc. 48: 274-279. Biol. Abst. 48: 40332.1967.

31. Hilton, J., A.S. Jaswal, B.J.E. Teskey, and B. Barabas. 1965. Rest period studies on seeds of Amelanchier, Prunus, and Sorbus. Can. J. Plant Sci. 45: 79-85.

32. Hyde, E.D.C. 1954. The function of the hilum in some Papilionaceae in relation to the ripening of the seed and the permeability of the testa. Ann. Botan. 18: 241-256.

33. Ikuma, H. and K.V. Thimann. 1960. Action of gibberellic acid on lettuce seed germination. Plant Physiol. 35: 557-566.

34. Ikuma, H. and K.V. Thimann. 1963. The role of the seed coats in germination of photosensitive lettuce seeds. Plant Cell Physiol. 4: 169-185.

35. Ingle, J., J.L. Key, and R.E. Holm. 1965. Demonstration and characterization of a DNA-like RNA in excised plant tissue. J. Mol. Biol. 11: 730-746.

36. Jaswal, A.S. 1965. Metabolic changes during after-ripening in Prunus cerasus seeds. Ph.D. Thesis, University of Manitoba, Winnipeg, Manitoba.

37. Kay, C.M. and K. Oikawa. 1966. Hydrodynamic and optical rotatory dispersion studies on wheat germ soluble ribonucleic acid. Biochemistry 5: 213-223.

38. Key, J.L. 1967. Aspects of RNA metabolism in plant tissue. Plant Physiol. 42: S-39.

39. Khan, A.A. 1966. Breaking of dormancy in Xanthium seeds by kinetin mediated by light and DNA-dependent RNA synthesis. Physiol. Plantarum 19: 869-874.

40. Khan, A.A. 1967. Dependence of gibberellic acid-induced dark germination of lettuce seed on RNA synthesis. Planta 72: 284-288.

41. Khan, A.A., J.A. Goss, and D.E. Smith. 1957. Effect of gibberellin on germination of lettuce seed. Science 125: 645-646.

42. Khan, A.A. and N.E. Tolbert. 1965. Reversal of inhibitors of seed germination by red light plus kinetin. Physiol. Plantarum 18: 41-43.

43. Knowles, R.H. and S. Zalik. 1958. Effects of temperature treatment and of a native inhibitor on seed dormancy and of cotyledon removal on epicotyl growth in Viburnum trilobum Marsh. Can. J. Botany 36: 561-566.

44. Kock, B. 1967. Nature of a rapidly labelled RNA fraction described in higher plant systems. Plant Physiol. 42: 1149-1152.

45. Koller, D., A.M. Mayer, A. Poljakoff-Mayber, and S. Klein. 1962. Seed germination. Ann. Rev. Plant Physiol. 13: 437-464.

46. Lasheen, A.M. and H.T. Blackhurst. 1956. Biochemical changes associated with dormancy and after-ripening of blackberry seeds. Proc. Am. Soc. Hort. Sci. 67: 331-340.

47. Lipe, W.N. and J.C. Crane. 1966. Dormancy regulation in peach seeds. Science 153: 541-542.

48. Lonberg-Holm, K.K. 1967. Nucleic acid synthesis in seedlings. Nature 213: 454-457.

49. Mandell, J.P. and A.D. Hershey. 1960. A fractionating column for analysis of nucleic acids. Anal. Biochem. 1: 66-77.

50. Marcus, A. and J. Feeley. 1965. Protein synthesis in imbibed seeds. II. Polysome formation during imbibition. J. Biol. Chem. 240: 1675-1680.

51. Miyamoto, T., N.E. Tolbert, and E.H. Everson. 1961. Germination inhibitors related to dormancy in wheat seeds. Plant Physiol. 36: 739-746.

52. Naylor, J.M. 1966. Dormancy studies in seed of Avena fatua. 5. On the response of aleurone cells to gibberellic acid. Can. J. Botany 44: 19-32.

53. Naylor, J.M. and G.M. Simpson. 1961. Dormancy studies in seed of Avena fatua. 2. A gibberellin sensitive inhibitory mechanism in the embryo. Can. J. Botany 39: 281-295.

54. Oknina, E.Z. 1959. Nucleic acids in apple and cherry seeds. Biol. Nuklein. Obnema u Rastenu, Ufa, Sbornik. 1959: 63-69. Chem. Abst. 55: 3738.1961.

55. Olney, H.O. and B.M. Pollock. 1960. Studies of the rest period. II. Nitrogen and phosphorous changes in embryonic organs of after-ripening cherry seeds. Plant Physiol. 35: 970-975.

56. Pack, D.A. 1921. Chemistry of after-ripening, germination and seedling development of juniper seeds. Botan. Gaz. 72: 139-150.

57. Pollock, B.M. 1953. The respiration of Acer buds in relation to the inception and termination of winter rest. Physiol. Plantarum 6: 47-77.

58. Pollock, B.M. and H.O. Olney. 1959. Studies of the rest period. I. Growth, translocation, and respiratory changes in the embryonic organs of after-ripening cherry seeds. Plant Physiol. 34: 131-142.

59. Pollock, B.M. and V.K. Toole. 1961. After-ripening, rest period and dormancy. Yearbook Agr. U.S. Dept. Agri. 1961: 106-112.

60. Popov, Yu. G. 1965. Changes in activity and location of respiratory enzymes in apple seeds in stratification. Sb. Rabot Molodykh Uch. Nauch.-Issled. Zon. Inst. Sadovod. Nechernozem. Polosky, Moscow 1965: 239-242. Chem. Abst. 66: 62647.1966.

61. Roberts, E.H. 1963. The effect of inorganic ions on dormancy in rice seed. Physiol. Plantarum 16: 732-744.

62. Roberts, E.H. 1963. The effects of some organic growth substances and organic nutrients on dormancy in rice seed. Physiol. Plantarum 16: 745-755.

63. Roberts, E.H. 1964. The distribution of oxidation-reduction enzymes and the effects of respiratory inhibitors and oxidizing agents on dormancy in rice seed. Physiol. Plantarum 17: 14-29.

64. Simpson, G.M. 1965. Dormancy studies in seed of Avena fatua. 4. The role of gibberellin in embryo dormancy. Can. J. Botany 43: 793-816.

65. Simpson, G.M. and J.M. Naylor. 1962. Dormancy studies in seed of Avena fatua. 3. A relationship between maltase, amylases and gibberellins. Can. J. Botany 40: 1659-1673.

66. Smith, H. and B. Frankland. 1966. Specific inhibition by uracil derivatives of the mechanism of dormancy release in light sensitive lettuce seeds. Nature 211: 1323-1324.

67. Sobota, A.E., C.J. Leaver, and J.L. Key. 1967. Incorporation of ^{32}P-orthophosphate into nucleic acid by excised plant tissue - the possible influence of bacteria. Plant Physiol. 42: S-39.

68. Sondheimer, E. and E.C. Galson. 1966. Effects of abscisin II and other plant growth substances on germination of seeds with stratification requirements. Plant Physiol. 41: 1397-1398.

69. Thornton, N.C. 1935. Factors influencing germination and development of dormancy in cocklebur seeds. Contrib. Boyce Thompson Inst. 7: 477-496.

70. Timson, J. 1967. The germination of Polygonum convolvulus L. New Phytol. 65: 423-428.

71. Toole, E.H. 1959. Effects of light on germination of seed. Photoperiodism and Related Phenomena in Plants and Animals pp 89-99. American Association for the Advancement of Science, Washington, D.C.

72. Toole, E.H., S.B. Hendricks, H.A. Borthwick, and V.K. Toole. 1956. Physiology of seed germination. Ann. Rev. Plant Physiol. 7: 299-324.

73. Tuan, D.Y.H. and J. Bonner. 1964. Dormancy associated with repression of genetic activity. Plant Physiol. 39: 768-772.

74. Umbreit, W.W., R.H. Burris, and J.F. Stauffer. 1957. Manometric Technique. Burgess Publishing Co. Minneapolis 15, Minnesota, U.S.A.

75. Van Overbeek, J., J.E. Loeffler, and M.I.R. Mason. 1967. Dormin (abscisin II), inhibitor of plant DNA synthesis? Science 156: 1497-1499.

76. Vegis, A. 1964. Dormancy in higher plants. Ann. Rev. Plant Physiol. 15: 185-224.

77. Villiers, T.A. and P.F. Wareing. 1960. Interaction of growth inhibitor and a natural germination stimulator in the dormancy of Fraxinus excelsior L. Nature 185: 112-114.

78. Wareing, P.F. and H.A. Foda. 1956. Possible role of growth inhibitors in dormancy of seeds of Xanthium and lettuce. Nature 178: 908-910.

79. Wellington, P.S. and V.M. Durham. 1961. The effect of covering layers on the uptake of water by the embryo of the wheat grain. Ann. Botan. 25: 185-196.

80. Wood, A. and J.W. Bradbeer. 1967. Studies in seed dormancy. II. The nucleic acid metabolism of the cotyledons of Corylus avellana L. seeds. New Phytol. 66: 17-26.