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Title: PHYSIOLOGICAL EFFECTS, DISTRIBUTION AND METABOLISM OF DIMETHYL SULFOXIDE IN DMSO-TREATED SEED POTATO TUBERS (SOLANUM TUBEROSUM L.)

Abstract approved: Ralph Garren, Jr.

The effect obtained from DMSO on tuber sprouting of 'Norgold Russet' and 'Netted Gem' was found to be related to physiological age when whole and cut apical-halves were routinely immersed 30 minutes in 4, 10 or 25% (v/v) DMSO. Treatment did not induce sprouting during the rest period. Tubers possessing a high degree of apical dominance also were not affected, but treatments applied at the stage characterized by multiple sprouting induced statistically significant increases in sprout number. Apical-half tubers responded more consistently than whole tubers. Sprout numbers increased with increasing concentration of DMSO. This relationship was observed primarily with 'Norgold Russet'.

Sprout morphology was modified by DMSO and ethephon (2-chloroethyl-phosphonic acid). Tubers at the branched-multiple

sprouting stage developed some spindly sprouts following treatment with 25% DMSO. At the 10 and 25% concentration, DMSO had a slight effect in preventing "little tuber" disorder. Ethephon at 5000 ppm completely suppressed this morphological symptom and promoted branched-multiple sprouting. It is postulated that the effect of DMSO may result from a modification of gibberellic acid gradients or their distribution.

At physiological ages characterized by multiple and branched-multiple sprouting, DMSO treatment of apical-half tubers did not affect the level of ascorbic acid. Glutathione level generally increased, but statistically significant differences were associated mainly with the 25% DMSO concentration. Respiration rates of whole and apical-half tubers at these same physiological ages increased following treatment with 25% DMSO. The response may have been due to phytotoxicity.

Liquid scintillation detection revealed that $DM^{35}SO$ absorption was over five times greater by cut halves than by whole tubers. Autoradiographic studies showed that intact periderm acted as a partial barrier whereas buds, cut surfaces and adjacent tissues were readily permeated. Within a short time following treatment, $DM^{35}SO$ penetrated the xylem ring only in cut tubers. Where clear definitive interpretation was possible, the ^{35}S label was not detected in phloem tissue, but it was clearly present in all other tissues of tubers autoradiographed after ten-days' sprouting in the dark.

The compounds derived from metabolism of DMSO were qualitated and quantitated by gas chromatography, flame photometric detection, mass spectrometry and gravimetric methods. DMSO was reduced to dimethyl sulfide and oxidized to dimethyl sulfone in cut and whole 'Norgold Russet' tubers sprouted in the dark. Accumulation of metabolites was proportional to the amount of DMSO absorbed. Complete suppression of sprouting with CIPC [isopropyl N-(3-chlorophenyl) carbamate] was associated with decreased metabolism of DMSO. Ratio of dimethyl sulfide to dimethyl sulfone was high, which is inverse to data reported for DMSO-treated mammals. Much more dimethyl sulfone was recovered from sprouts than from periderm or internal tissues. Most of the absorbed DMSO, however, remained unchanged even after six-weeks' sprouting and was recovered from sprouts as well as from tuber tissues. No other metabolites were identified and evidence for de-methylation was not found. So far as is known, this is the first elucidation of DMSO metabolism in plant tissue.

Physiological Effects, Distribution and Metabolism of Dimethyl Sulfoxide in DMSO Treated Seed Potato Tubers (*Solanum tuberosum* L.)

by

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INTRODUCTION

Continued interest in the practical application of dimethyl sulfoxide (DMSO) in agriculture has resulted from the increasing amount of information obtained concerning its effects on membrane permeability, which enhances absorption and translocation of chemicals. This information, which has been the subject of several reviews, indicates that DMSO should be placed in a priority category in crop research, especially in relation to plant physiological and pathological problems (Klarner, 1968; Leake, 1967; Smale, 1969).

DMSO treatment has increased the yield of certain crops, including flower bulbs, peas, potatoes and soybeans (Smale, 1969). Davidson (1967) reported more wound periderm formation and increased sprouting after immersing cut potato tubers in 4% DMSO. Increased sprouting was related to greater tuber numbers and a larger harvest (Estes, 1969; Henninger, 1971). In potato production, greater plant density usually means more but smaller tubers; consequently, DMSO could aid in controlling growth in varieties which require tuber size control. This would be an asset to fresh marketing, processing and seed tuber growing.

Although considerable information is available on the effect of DMSO on plant functions, studies concerning its absorption and distribution in crop plant tissue have been limited to fruit trees (Garren, Jr., 1967; Keil, Smale and Wilson, 1969; Smale, 1969). Investigations on DMSO metabolism have been confined to mammalian and bacterial organisms (Gerhards and Gibian, 1968; Wood and Jacob, 1968). From a review of the literature, however, the potential of plant tissue to metabolize sulfur compounds similar to DMSO is clearly indicated.

Statement of the Objectives

There is need for more intensive study of the effect of DMSO on the growth and physiology of sprouting in seed potato tubers. Potato tubers also represent an excellent substrate for uptake and metabolic studies. The present investigation was undertaken (1) to determine if DMSO has a differential effect on sprouting of seed potato tubers ranging in physiological age (PA) from recently harvested dormant tubers to aged tubers held a full storage season; (2) to determine if ascorbic acid (AA) and glutathione (GSH) content or the respiration rate of sprouting seed potatoes, at a PA responding to DMSO treatment by enhanced sprouting, were changed due to treatment; (3) to elucidate the routes of penetration and amount of DMSO absorbed by cut and whole seed potato tubers during an immersion treatment which has

been shown to enhance sprouting; (4) to ascertain qualitatively and quantitatively the primary metabolic fate of DMSO absorbed into seed potato tubers which were either allowed to sprout after treatment or were chemically treated to suppress sprouting.

The entire study was conducted in the laboratory. The results are intended to establish fundamental knowledge for future toxicological studies on metabolism and action of DMSO in plant tissue.

REVIEW OF LITERATURE

DMSO Effects on Growth and Development of Storage Organ Crops

Beneficial and often striking physiological responses have been attributed to the action of DMSO when applied to flower bulbs, potatoes and sweetpotatoes (Davidson, 1967; Estes, 1969; Henninger, 1971; Smale, 1969; Whatley, Thompson, and Mayes, 1968). The range of influence extends from simply improving sprouting to increasing yield of potato and bulbs of daffodil, Dutch iris and tulip.

All of the published accounts of DMSO as a seed potato treatment indicate that the main effect on sprouting is an increase in the number of stems in the field. Visibly or statistically distinct benefits to potato plant vigor or accelerated growth in the field have not been shown. In contrast, Whatley, Thompson and Mayes (1968) found DMSO markedly accelerated the growth of sprouts in three sweet potato varieties when their roots were immersed from 5 to 15 minutes in 12% DMSO.

When potato yields were related to stem number, an increase was reflected by greater tuber numbers and often a larger total harvest weight following DMSO pre-plant treatment (Estes and Henninger). Davidson (1967), working with cut 'Norgold Russet' and 'Russet Burbank' ('Netted Gem') seed pieces, found that the increase was related to DMSO concentration, even though phytotoxicity occurred at the

wound area after a three minute dip at levels as high as 16% or 32% DMSO. Concentrations as high as 50% to 100% injured whole 'Kennebec' tubers soaked for 30 minutes (Estes, 1969). Optimal effects with 'Kennebec' on stem number were found at 5% and 10% DMSO concentrations. Field trials by Henninger (1971) with 4% DMSO showed significance in stem increases for both 'Norgold Russet' and 'Russet' Burbank' cut tubers. Even though Davidson (1967) did increase suberization of cut pieces with DMSO, he and Henninger (1971) found the advantage in sprouting was offset by decay when DMSO was used without a fungicide. Whatley (1969) found, however, that increase in sprouting of sweetpotato was obtained independently of fungicide application.

Physiological Aging of Seed Potato Tubers

The physiological age (PA) of a tuber provides a useful relationship to important aspects of its life cycle. When no special measures are taken, the normal course of advancing PA occurs in cold storage (Burton, 1966; Lindblom, 1968). Potato PA may be related to chronological age from date of initiation of the tuber on its parent plant, through the entire storage period, terminating with senescence and lack of ability to sprout. Chronological age does not always indicate the physiological state because environmental conditions during storage exert a profound influence (Goodwin et al., 1969; Toosey,

1963). High storage temperatures especially advance PA (Toosey, 1963). Nevertheless, chronological age in storage under optimal conditions has served as an index of PA (Kawakami, 1962, 1963; Krijthe, 1962).

Morphological expression of PA is characterized by sprouting tendency. The first PA is the rest period which immediately follows harvest (Burton, 1966, 1968). No sprouting occurs during this time because of the presence of internal inhibitors defined by Hemberg (1958) as the mixture of acid and neutral components, designated as the inhibitor β complex.

Subsequent to the termination of rest, there occurs a hormone-mediated advance in sprouting tendency (Emilsson and Lindblom, 1963; Krijthe, 1962; Lindblom, 1968). Initially, a single sprout stage of apical dominance ensues, whereby lateral buds are prevented from sprouting by the presence and basipolar movement of indoleacetic acid (IAA) from the apical sprout. This basic concept was first proposed by Thimann and Skoog (1933) and later emphasized for tuber sprouting by Michener (1942). Several auxins including IAA, indolebutyric acid (IBA) and indolepyruvic acid (IPA) have been isolated from potato tissue (Montuelle and Cornette, 1964; Lindblom, 1966). An apical dominance stage generally does not occur if tubers have been held after harvest for short periods at conventional storage temperatures of 1-5°C (Burton, 1966; Goodwin, 1963; Morris, 1966).

If they are placed at 15-20°C following rest, single sprouting and dominance become established. After a certain sprout length is achieved, Toosey (1959, 1962) contends that the tubers may again be placed in cool storage and the attendant PA maintained for a considerable period.

The apical dominance stage is followed by a period during which unbranched sprouting occurs. After approximately six months' storage, depending on variety, PA advances toward a condition whereby nearly all of the buds will produce numerously branched sprouts. Bushnell (1928, 1929), Ivins (1963), Krijthe (1962) and Werner (1954) state that by de-sprouting or sectioning single-sprouted tubers dominance is upset and multiple sprouting induced, thereby advancing PA.

Some varieties become predisposed to "little tuber" disorder when chronologically aged or when exposed to conditions which accelerate the PA, including high storage temperature, desiccation, repeated sprout removal and cool soil temperature at planting (Krijthe, 1962; Langille, 1969; Toosey, 1963). In this condition, the potential for sprout growth is comparatively feeble, but that for young tuber formation is vigorous. Often abundant immature tubers are formed on the old seed piece without actual plant development.

Tuber PA is considered as an expression of modulating internal hormone quotients. Lindblom (1968) has formulated a theory of

sprouting behavior for potato, which states that the potential sprouting ability is dependent on the IAA/GA (gibberellic acid) ratio. High as well as low ratios give rise to many sprouts. Accordingly, this follows a second degree relationship, which is determined by the relative amounts of the hormones and not by the absolute ones. He maintains that inhibitors do not prevent sprouting, but function by forming a complex with growth promoting substances. These concepts are not yet fully substantiated. Nonetheless, Lindblom (1966, 1968) cites his analyses and those of others to show that GA is very low at the end of rest. Afterwards it increases and parallels the change from single to multiple sprouting. IAA, at its highest concentration, was correlated with the smallest number of sprouts. Recently, Goodwin (1967b) proposed the existence of a specific correlative inhibitor, which is not IAA, but is induced by the presence of IAA in tuber and mature stem tissue, and is active in preventing very short sprouts from continuing growth.

Processes of stolon elongation and tuber initiation in relation to "little tuber" formation are also undoubtedly under hormonal control. Madec (1963), Palmer and Smith (1969), Slater (1963) and Smith and Rappaport (1969) concluded the interplay of gibberellins and endogenous growth inhibitors to be a requisite in regulation of tuber initiation. Abscisic acid (ABA) was shown to induce tuber formation by El-Antably, Wareing and Hillman (1967) but not by Smith and Rappaport

(1969). These latter authors, however, did find an uncharacterized GA inhibitor to be higher in the young tuber than in the stolons. Furthermore, Okazawa (1959) and Timm et al. (1960) noted that GA stimulated elongation of stems and stolons but inhibited tuber formation. Aged tubers had a low GA content which increased when storage conditions were modified to rejuvenate sprouting vigor.

Ascorbic Acid and Glutathione in Potato Tuber Physiology

AA occurs naturally as L-ascorbic acid and is found in potato tubers in both the reduced and oxidized (dehydroascorbic acid) forms. Values for AA differ widely, depending upon variety, growing conditions, length of time and temperature in storage, portion of tuber analyzed, and degree of sprout formation in conjunction with the sprouting conditions.

Decreasing AA levels are proportional to the length of time in storage at 3-4°C. The decrease is significantly slower at temperatures above 10°C (Burton, 1966; Mayfield et al., 1937; Torfason, 1965). Rudra (1936), Smith and Gillies (1940) and Szalai (1959a) reported AA to be higher in the apical and peripheral regions, but Mudambi and Hanning (1961) found the central zone to be higher than the cortex.

The influence of AA on sprouting of potatoes and changes in AA content accompanying sprouting of tubers of different PA have not been

resolved (Burton, 1966; Emilsson and Lindblom, 1963). During sprouting AA apparently increases in the sprouts and decreases in the tuber (Lampitt, Baker and Parkinson, 1945; Smith and Paterson, 1937). Pett (1936) reported a very rapid rise in AA after two day's sprouting when cut pieces were planted in wet sand. However, Guthrie (1937) showed the increase to be a factor of cutting and not sprouting. Bud forcing chemicals and chemicals applied to suppress sprouting have little effect on AA (Driver, 1957; Emilsson and Lindblom, 1963; Guthrie, 1937; Torfason, 1965).

The generally accepted formula for GSH is γ-glutamylcysteinylglycine. Guthrie (1932) was first to report its occurrence in plants (potato tubers). Two forms, reduced (GSH) and oxidized (GSSG), exist in all plants. In stored potato tubers, a high level of GSH is related to breaking of the rest period (Emilsson, 1949; Tagawa and Nishiyama, 1956). Values decrease 2-5 times thereafter, but tubers held for extended periods do not necessarily show low GSH content.

Szalai et al. (1958) observed GSH to rise with progressive sprouting, the intensity of the rise being correlated with sprouting vigor. Pett (1936) also noticed an increase in GSH at sprout initiation, but a drastic decline ensued after a few days. Even though Emilsson (1949) detected more GSH in the sprouts than in tuber tissue, he could not relate this to more vigorous sprouting. Many chemical treatments, especially ethylene chlorohydrin, enhance sulfhydryl content

in resting tubers (Guthrie, 1933; Szalai, 1959b). Gibberellic acid and thiourea induce an increase in sprouts (Palladina and Pervova, 1966).

AA and GSH are abundant in tissues of high metabolic activity and have been implicated as significant redox factors acting as coupled electron carriers in respiration (Mapson, 1959; Mapson and Moustafa, 1956). Both compounds protect cell membrane integrity from chemical challenges (Archer et al., 1967; Kosower and Kosower, 1969). Furthermore, GSH is a cofactor for several enzymes (Mapson, 1959). The balance between activities of different classes of enzymes can be controlled in part by the redox state of the sulphydryl group in equilibrium with GSH (Spragg, Lievesley and Wilson, 1962). These authors also stressed its importance in mitotic division.

Potato Tuber Respiration

Respiratory activity is different for whole and cut tubers. Sound intact potatoes have a basal respiration rate which may be stimulated by a number of treatments including chemical vapors, bruising, heat and cold storage (Smith, 1968). Cut tubers are subject to wound respiration (Johnstone, 1925). Respiratory rate of halved tubers during the first week ranged from two to four times that of whole tubers. With freshly cut seed, Mulder (1956) and Tombesi and Tarantola (1952) found the peak of CO_2 evolution at 24 hours; whereas, Timm

and Schweers (1965) recorded a maximum at five days. All authors report the rate to decline after a week often approaching that of whole tubers.

Tubers taken from cold storage and warmed have a higher rate of respiration for several days before subsiding to a constant level. The lower the storage temperature the higher the initial rise in respiration following removal of tubers to warm temperatures. Barker (1936) relates this to the concentration of sucrose formed during cold storage. According to James (1953), the breakdown of sucrose is the rate-controlling reaction in respiration, and a respiration increase develops from a temperature induced shift in the starch-sugar equilibrium.

Respiration intensity of sprouting potatoes partly depends upon rate of sprouting and may be correlated with weight of sprouts. Nevertheless, temperature is the primary factor influencing potato tuber respiration. In the range of 5 to 25°C the rate increases about two-fold for every 10°C rise in the temperature, as long as no change in respiratory substrate occurs (Burton, 1966).

Respiratory processes in potato tubers involve the Embden-Meyerhof pathway, the citric acid cycle and possibly the pentose phosphate shunt (Norton, 1963). The final stages appear to be shared by cytochrome oxidase and o-diphenol oxidase, the relative importance depending on the maturity of the tuber (Mondy, Klein and Smith, 1960).

Mapson and Burton (1962) found 70% of the terminal respiration of intact tubers passes over the cytochrome system. Other authors have found that cytochrome oxidase activity accounts for the whole of the respiration of tubers (Goddard and Holden, 1950; Thimann, Yocum and Hackett, 1954).

Emilsson (1949) summarized earlier literature concerning chemical treatment and potato tuber respiration. Bud forcing chemicals increase the rate many fold. Respiration curves show increases soon after the beginning of treatment. Maximum rate occurs within 50 to 60 hours, which is followed by a slow decrease to values within the range of untreated tubers. This rise was found to precede bud growth, and the rate returned to rates of untreated tubers before growth began. Many sulfur compounds (thiourea, thiocyanates, dimethyl disulfide and ethyl mercaptan) caused higher respiration rates. Several low molecular weight alcohols decreased respiration while effectively shortening the rest period.

Chemical sprout enhancement has been related to increased respiration. Thus, Smith and Rappaport (1965) found that gibberellin, applied before sprouting, stimulated respiration for 20 days or longer. Timm and Schweers (1965) likewise observed that GA stimulated respiration of unsprouted but not sprouted tubers.

Potato Tuber Anatomy

Artschwager (1918) and Reeve, Hautala and Weaver (1969) have differentiated the potato tuber into periderm (phellem, phellogen and phelloderm), cortex, vascular ring, perimedullary zone and pith. The periderm is six to ten cell layers in thickness, being broken only by small lenticel-like structures. Woolley (1962) did not observe intercellular spaces between the orderly rows of periderm cells, but there were many areas in which these cells were replaced by irregular cells with air-filled intercellular spaces extending to the corky external part of the periderm. These regions occupied about 3 to 10 percent of the tuber surface of 'Russet Burbank'. Individual cork cells of the periderm are brick-shaped with thin suberized walls. At maturity they are devoid of contents. Wound periderm resembles normal periderm in cell structure (Artschwager, 1924).

The eyes (buds) are differentiated in the axils of small scale leaves, which occur in the same phyllotaxy as the foliage leaves. There may be several axillary bud primordia in each eye. The vascular ring approaches the periphery in the region opposite the eyes, branching out in the buds and bud scales. In the main portion of the vascular ring, the water conducting tissues are weakly developed. Porous vessels of secondary xylem can be found only in separated individual groups (Hayward, 1938).

Parenchyma cells are smaller and consequently denser in the cortex than in the central pith. Artschwager (1918) and Edelman, Jefford and Singh (1969) confirmed the presence of phloem scattered throughout the potato tuber. Differentiation of this tissue, in contrast to that in other dicotyledonous plants, takes place internally as well as externally to the xylem.

The pith forms a small central core from which radiate arms of medullary parenchyma, the region appearing irregularly stellate in transection. These arms extend into the basal regions of the eyes and there branch further into the axes of the buds (Reeve, Hautala and Weaver, 1969).

A continuous arrangement of intercellular spaces exists within most internal tissues of potato. Woolley (1962) found the arrangement in 'Russet Burbank' to consist of a lattice of stellate chambers having approximate diameters of 10 to 15μ interconnected by long, narrow passages, about 3μ in diameter. The total volume of intercellular space has been estimated to be 0.2 to 1.0% by Woolley and 0.62 to 1.34% by Burton (1950). Differences may be related to variety.

Potato periderm presents an effective barrier to passage of gases or solvents (Burton, 1950; Burton and Hannan, 1957). Movement of solutions into cut potato (Laties, 1962; Woolley, 1962) also meets with resistance. Sprouts and buds, however, are more readily penetrated (Burton, 1955). Crafts and Yamaguchi (1964) traced the

penetration of labeled herbicides and growth regulators into potato tuber cylinders by autoradiography. In the apoplast, Monuron moved most rapidly, Simazine was intermediate, while 2,4-D and 2,4,5-T were slowest. IAA, or a metabolite, followed a symplastic route. Their data show distance of penetration to be limited for most compounds tested.

Penetrant Properties of DMSO

DMSO has been shown to be rapidly absorbed and translocated in animal and plant tissue, even though it lacks surfactant properties. Use of the compound as a cosolvent in pesticide or nutrient formulations has met with wide success in solvating and distributing molecules on the surface as well as inside of plants. No theory yet proposed completely explains the mode of penetration, although the plasma membrane may be a primary site of action (DeBruijne and Van Steveninck, 1970b; Elford, 1970; Leake, 1967; Smale, 1969; Spilker, 1970). Some suggestions which take into account differences in observed influx and efflux kinetics include: readily reversible altered configuration of protein molecules in membranes or tissues, solvation of hydrophobic diffusion barriers, direct membrane poisoning action and nonselective increase in tissue permeability (Franz and Van Bruggen, 1967; Rammler and Zaffaroni, 1967; Schmid, 1968).

Evidence for DMSO modification of cellular diffusion is available. Callus cell permeability of Nicotiana tabacum is increased by DMSO (Delmer and Mills, 1969). Much of the evidence in plants points to a differential effect of DMSO on element transport. ^{32}P (sodium phosphate) moved to a greater extent into roots of strawberry plants in the presence of 0.055% DMSO than in its absence (Garren, Jr., 1967), but Chamel and Simiand (1970) could not show increased ^{32}P transport (potassium or ammonium phosphate) when corn or bean leaves were treated with 0.5% DMSO. Beans grown in nutrient solution containing DMSO did not show increased uptake of Zn or Mn (Estes, 1969), but uptake of Zn by excised barley roots was enhanced by 1 to 10% solutions of DMSO (Schmid, 1968). An adverse influence on metabolic energy producing sites may well be one mode of action for DMSO, because significantly inhibited uptake of labeled zinc, methionine and uracil in bean tissue treated with 10% DMSO could not be fully related to permeability changes (Bajaj et al., 1970).

In animal tissues and branches of trees, DMSO may be completely distributed in one to two hours following administration (Elford, 1970; McDermot, Finkbeiner and Zanette, 1967; Smale, 1969). This suggests rapid movement in extracellular fluid. Experiments with live animals have shown that topically applied DMSO penetrates the skin readily. In the dog, 80% of the applied dose had penetrated the skin barrier after four hours and 90% after 24 hours (Kolb et al., 1967).

Distribution was confined to the skin and muscle underneath treated areas. Absorption through rat skin is much more rapid and the distribution greater than in the dog (McDermot, Finkbeiner and Zanette, 1967). About one-third of the applied DMSO dose was absorbed in 15 minutes; after one hour uptake was virtually complete. All organs were labeled and the highest concentrations could be found in most cases at one to two hours. Disposition of $DM^{35}SO$ administered dermally to rabbits was discribed by Hucker, Ahmad and Miller (1966). Concentrations of radioactivity were appreciable after 30 minutes in all tissues except the eye lens. After four hours values were 3 to 60 times higher and accumulation was greater in the heart, plasma, aqueous humor and vitreous humor than in most other tissues. Fat tended to accumulate the least label. In man, dermal application results in urinary excretion of DMSO shortly after administration and serum levels become maximal after about four hours.

Several accounts of DMSO absorption and translocation in plants have been published. Garren, Jr. (1967) recorded movement of $DM^{35}SO$ through the bark of a young pear tree and observed that it became systemic in the foliage. Peach leaves and fruit sprayed with labeled compound accumulated a portion of the ^{35}S (Keil, Smale and Wilson, 1969). Only 1 to 2% of the label deposited on treated plant parts was passed to unexposed fruit or leaves. $DM^{35}SO$ injected into the sap stream of peach trees moved much farther. In a treated

branch, label was detected 80 cm from the point of treatment within one hour (Smale, 1969). Basipetal movement, which could be detected only after the third day, was restricted to stem tissue not more than 10 cm below the area of introduction. An account of $DM^{35}SO$ localization in Lupinus albus seedlings by Caujolle et al. (1968) demonstrated complete distribution within five days following germination of seed on treated media. Accumulation of ^{35}S occurred around cellular membranes and later in the nucleus.

Work reported by Smale (1969) points to the advantage of simultaneous use of a surfactant with DMSO. If Triton X-100 was used with DMSO, 40 times more was absorbed and translocated in 24 hours to peach seedling leaves above the site of treatment.

Metabolism of DMSO in Mammals and Microorganisms

Gerhards and Gibian (1968) and Wood and Jacob (1968) have reviewed most of the fate and excretion studies performed with man and animals. Simple oxidation and reduction seem to be the preferred routes of elimination other than excretion of the unchanged DMSO molecule. DiStefano and Borgstedt (1964) first reported DMS as a metabolite of DMSO in animals. Their later quantitative study with cats showed that DMS appears in the breath within seconds after DMSO treatment (Borgstedt and DiStefano, 1967). A total respiratory excretion of 3% of the administered amount was detected by gas

chromatography. DMS respiratory excretion values from DMSO treated rats and man approximate those of the cat (Hucker, Ahmad and Miller, 1966; Kolb et al., 1965; Kolb et al., 1967).

Dimethyl sulfone was simultaneously reported as a metabolite of DMSO administered to humans and rats (Gerhards, Gibian and Raspe, 1965) and rabbits (Williams et al., 1965). Quantitative investigations by Hucker, Ahmad and Miller (1966) demonstrated 10 to 15% and 25% conversion of DMSO to $DMSO_2$ in rats and rabbits, respectively. In man, dermal application leads to 5 to 10% $DMSO_2$ excretion in urine; if DMSO is orally administered, 21 to 23% of it is oxidized (Hucker et al., 1967). All of the mammalian fate studies conclude that the largest percentage of a treatment dose is eliminated as unchanged DMSO.

When rabbits were given separate injections of DMS, DMSO, and $DMSO_2$, both DMS and DMSO led to the excretion of the sulfide, sulfoxide and sulfone (Williams, Burstein and Layne, 1966). Dimethyl sulfone, however, was not metabolized. No other metabolites of significance have been detected in DMSO treated animals. There is reason to doubt that demethylation occurs to any extent because oxidation to labeled sulfate in vivo represented only a trace of the $DM^{35}SO$ dose given to rats (Gerhards and Gibian, 1967). In the related area of simple sulfide metabolism, Maw (1953b) found no increase in urinary sulfate in rats treated with DMS. Canellakis and Tarver (1953),

however, reported methane thiol to be metabolized to sulfate and CO_2 in rats.

A more complete degradation of DMSO occurs in microorganisms. Originally, Ando et al. (1957) described DMSO conversion to DMS by E. coli. Subsequently, Rammler and Zaffaroni (1967) demonstrated Aerobacter aerogenes fed ^{14}C-DMSO evolved 80% of the label as carbon dioxide. DMS accounted for an additional 6% of the radioactivity. A small proportion of the remaining ^{14}C was retained by the cells. Their data suggest incorporation of the label as formate to purines in nucleic acids or as methyl groups in RNA. These data support oxidation to be the preferred route of DMSO catabolism in lower organisms.

Related Organic Sulfur Chemistry in Plants

Most of the organic sulfur compounds of wide occurrence in plants can be grouped according to the following basic structures: R-S-H (thiols), R-S-R' (sulfides), R-S-S-R' (disulfides), $R\text{-}S_x\text{-}R'$ (polysulfides), $R\text{-}\overset{O}{\overset{\|}{S}}\text{-}R'$ (sulfoxides), $R\text{-}\overset{O}{\overset{\|}{\underset{O}{\underset{\|}{S}}}}\text{-}R'$ (sulfones) and $(CH_3)_2\overset{+}{S}\text{-}R$ (methyl sulfonium compounds) (Robinson, 1963). Methane thiol is evolved from cooked (Gumbmann and Burr, 1964) but apparently not from fresh potato tubers (Self, Rolley and Joyce, 1963). Potato tissue or juice, however, has been shown to reduce methyl or ethyl disulfides to the corresponding thiols, probably in a manner

similar to reduction of the disulfide form of glutathione (GSSG) to its respective thiol (GSH) (Miller, 1933).

That methane thiol can be evolved in minute amounts from plant parts where ethylene biosynthesis takes place is indicated. Yang (1968) proposed the operation of a system whereby the unoxidized form of methionine can be converted to carbon dioxide, formic acid, dimethyl disulfide, methane thiol and ammonia. The production of ethylene by potato tubers in storage was first suggested by the data of Burton (1952).

A pathway in yeast accounting for the synthesis of S-methyl-L-cysteine from a reaction between serine and methane thiol has been elucidated (Wolf, Black and Downey, 1956). The synthesized amino acid, a lower homolog of methionine, was tentatively identified in 'Katahdin' potato tubers (Talley, Fitzpatrick and Porter, 1964). Several plant lyases have been described which cleave S-alkyl cysteines or S-alkyl cysteine sulfoxides to yield alkyl mercaptans or alkyl alkane thiolsulfinates. These enzymes have been isolated from garlic, onion, broccoli and Albizzia, but there are no reports confirming their existence in potato tissue (Fowden, 1964).

The simple volatile sulfide, DMS, can arise from methionine and derivatives, especially the methylated oxidation product dimethyl-β-propiothetin or methyl methionine sulfonium salts. Cantoni and Anderson (1956) and Challenger et al. (1957) presented evidence of

enzymatic DMS evolution from the thetins found in algae. More pertinent is the enzymatic or heat induced DMS liberation from methyl methionine sulfonium salts. These salts, which decompose on heating to yield homoserine and DMS, have been isolated from several plants including potato tubers (Keenan and Lindsay, 1967; Werner, Hossli and Neukom, 1969). Challenger et al. (1957) infer enzymatic liberation of DMS from the amino salt in plants, but volatile sulfide production from potato tubers occurred only when heated (Gumbmann and Burr, 1964; Werner, Hossli and Neukom, 1969). Furthermore, Virtanen (1965) reported that plant systems enzymatically cleave S-methyl-L-cysteine sulfoxide, giving rise to simple sulfides as secondary products.

Evidence for the presence of simple sulfoxides in potatoes is questionable and none have been reported in other plant tissues (Child and Fothergill, 1967). Dimethyl sulfone, however, has been extracted from certain Equisetum spp. by Karrer and Eugster (1949) and Karrer et al. (1949), and from Cladonia deformis (Bruun and Sörensen, 1954).

More complex sulfoxides and sulfones exist in potatoes as amino acids. Le Tourneau (1956) recovered methionine sulfoxide and sulfone from tubers and etiolated sprouts of 'Russet Burbank'. Mulder and Bakema (1956) also identified methionine sulfoxide from tubers by two dimensional paper chromatography. Some evidence, however, places methionine sulfoxide as an oxidation artifact of methionine,

which occurs during sample preparation (Talley, Fitzpatrick and Porter, 1964). Later work with an amino acid analyzer verified that fresh 'Kennebec' tuber extracts yielded 0.57μ mole methionine sulfoxide/gram dry wt and 5.86μ mole methionine/gram dry wt (Fitzpatrick and Porter, 1966). As mentioned previously, Talley, Fitzpatrick and Porter were able to tentatively identify S-methyl-L-cysteine sulfone and/or sulfoxide in extracts from 'Katahdin' tubers. Doney and Thompson (1966) found that methyl cysteine or methionine participated readily in enzymatic oxidation — reduction conversions in plant tissue.

It seems clear from the literature reviewed that plant tissues are capable of reducing and further oxidizing sulfoxides. Plants have a great capacity to degrade almost every foreign organic compound (Casida and Lykken, 1969). Although processes in animals are often more rapid than in plants, pathways appear to be no more sophisticated.

I. INFLUENCE OF DMSO ON SPROUTING AND SPROUT MORPHOLOGY OF SEED POTATO TUBERS OF DIFFERENT PHYSIOLOGICAL AGES

Materials and Methods

Plant Material

Oregon Foundation Grade 'Norgold Russet' and 'Netted Gem' seed potato tubers were obtained in 100 lb lots from the Barlow Farms, Inc., Corvallis, Oregon. The tubers were obtained shortly after harvest and were held in cold storage at 3°C and 80% RH. Preliminary trials with both varieties from September, 1968 through August, 1969 provided an estimate of optimum time in storage required to obtain the desired physiological ages for experimentation during 1969 and 1970.

Physiological age classes were selected according to groupings previously defined (Burton, 1966; Kawakami, 1962, 1963; Krijthe, 1962; Lindbloom, 1968; Toosey, 1963). The classification system used in the present study is outlined below.

Physiological Age Classification	Physiological and Morphological Characteristics
PA-1	Rest period stage - time following harvest during which tubers show a lack of consistent bud extension growth under optimal sprouting conditions.

PA-2E	Early apically dominant single sprout stage - period immediately following PA-1 when only one or two sprouts are formed because of the influence of a high IAA to GA ratio.
PA-2L	Late apically dominant single sprout stage - period immediately preceding definite correlative inhibition.
PA-3E	Early correlative inhibition stage - manifested by multiple unbranched sprouts, varying in growth rate, as influenced by a decreasing dominance of the apical growing points of the tuber.
PA-3L	Late correlative inhibition stage - characterized by increased multiple sprouting over PA-3E. Inactive or damaged shoot apices are evident and the ensuing dominance imbalance results in numerously branched sprouts. Low relative humidity and higher sprouting temperatures in addition to advanced chronological age influence expression of this stage.
PA-3LM	Metabolism study late correlative inhibition stage - the physiologic age observed during investigation of the metabolism of DMSO in potato tubers as reported in Section IV. This stage closely resembles PA-3L except for the presence of fine, fibrous roots promoted by high humidity during the experimental period.
PA-4	Senescent, aged tuber stage - at this stage potatoes display "little tuber" symptoms soon after sprouting.

Photographs of 'Norgold Russet' apical-half tubers exemplifying sprout morphology characteristic of each of the physiological age classes except PA-1 are shown in Figures 1-7. The morphology depicted at a given physiological age is representative of that observed for both whole and apical-half tubers of either 'Norgold Russet' or 'Netted Gem'.

Treatment Methodology

Experiments with age classes PA-1 through PA-3L were performed with both varieties during 1969 and 1970. Age classes PA-3LM and PA-4 were utilized only in 1970 with 'Norgold Russet'. Whole and cut seed tubers (apical-halves) were compared in all trials except PA-4 in which half tubers were used. Removal of tubers from storage and application of treatments took place at certain arbitrary dates which were predicted to coincide with the desired physiological age required for an experiment.

DMSO treatments consisted of immersing soil free, surface dry tubers for 30 minutes in 0, 4, 10, or 25% (v/v) aqueous DMSO (Crown Zellerbach Corp., 99% minimum purity) containing 0.01% Triton X-100 surfactant (Rohm and Haas Corp.). All tubers were held at 20°C for 24 hours prior to treatment. Concentrations were selected on the basis of previous reports or recommendations for use of DMSO in field trials (Estes, 1969; Herschler, 1968). Rindite, an effective

sprouting stimulant (7 parts ethylene chlorohydrin, 3 parts ethylene dichloride and 1 part carbon tetrachloride by volume) was used according to the method of Keller and Berces (1966), and served as a standard during PA-1 experiments to compare efficacy of DMSO in shortening the rest period.

For the PA-4 study, ethephon (2-chloroethylphosphonic acid, Amchem Products, Inc.), 5000 ppm aqueous solution containing 0.01% Triton X-100, five minute immersion, served as the standard for uniform reversion of "little tuber" symptoms. This method was chosen on the basis of preliminary success after screening several chemicals, including gibberellic acid for their influence in rejuvenation of aged seed potatoes.

Each experiment except series PA-3LM, was arranged as a completely randomized design on wire screen frames in a ventilated room in the dark. Biological and physical conditions of post-treatment storage and some parameters of methodology are presented in Table 1.

In experiments with PA-3LM tubers, qualitative and quantitative studies of the metabolic fate of DMSO in potato tissue were carried out simultaneously with observations on sprouting and sprout morphology. These biochemical studies are reported in Section IV. The tubers, after immersion in DMSO, were held for 24 hrs at 20°C and ambient humidity. Afterwards, the cut surfaces of all apical-half tubers were protected with a fungicidal dust application of 80%

Table 1. Biological and physical parameters of methodology used to evaluate the influence of DMSO on sprouting and sprout morphology of seed potato tubers of different physiological ages.

Physiologic Age	Tuber Harvest		Post harvest storage		No. days[a] @ 3°C		Treatment date		Sample size		Post treatment storage		Date of sprout counts	
	'Norgold Russet'	'Netted Gem'	Temp.	% R. H.	'Norgold Russet'	'Netted Gem'	'Norgold Russet'	'Netted Gem'	Whole	Half	Temp.	% R. H.	'Norgold Russet'	'Netted Gem'
PA-1	9-4-69	9-4-69	20°C	80	0	0	9-6-69	9-6-69	25	25	20°C	80	see table 2 for No. of days to 80% and 100% sprouting.	
PA-1	9-12-70	9-12-70	20°C	80	0	0	9-16-70	9-16-70	25	25	20°C	80		
PA-2E	9-4-69	9-4-69	20°C	80	0	0	10-20-69	12-4-69	25	25	20°C	80	12-15-70	1-29-70
PA-2E	9-12-70	9-12-70	20°C	80	0	0	10-28-70	11-30-70	25	25	20°C	80	12-23-70	1-25-71
PA-2L	9-4-69	9-4-69	3°C	75	10	10	9-26-69	9-26-69	25	25	20°C	80	11-21-69	11-21-69
PA-2L	9-12-70	9-12-70	3°C	75	10	10	10-4-70	10-4-70	25	25	20°C	80	11-29-70	11-29-70
PA-3E	10-17-68	10-17-68	3°C	75	122	123	2-28-69	2-28-69	25	25	20°C	80	3-28-69	3-28-69
PA-3E	10-4-69	10-4-69	3°C	85	138	154	3-2-70	3-18-70	25	25	20°C	80	3-30-70	4-15-70
PA-3L	10-17-68	10-17-68	3°C	75	231	233	6-16-69	6-18-69	25	25	20°C	40	7-14-69	7-16-69
PA-3L	10-4-69	10-4-69	3°C	85	294	262	8-4-70	7-4-70	25	25	20°C	40	9-1-70	8-1-70
PA-3LM	10-4-69	--	3°C	85	180	--	4-13-70	--	95	--	20°C	90	5-25-70	
PA-3LM	10-4-69	--	3°C	85	210	--	5-13-70	--	--	120	20°C	90	6-24-70	
PA-4	10-4-69	--	3°C	85	316	--	8-27-70	--	--	50	20°C	80	10-22-70	

(a) Experiments from PA-1 through PA-2E were with tubers held at 20°C from date of harvest to treatment.

Experiments from PA-3E through PA-4 were with tubers receiving an initial ten-day conditioning from 10°C to 3°C in four successive temperature reductions, immediately following harvest. In addition, they received a 24-hour conditioning at 20°C before treatment. These periods are not included in the figures for storage at 3°C.

polyethylene carbamate (Niagara Chemical Co.). Whole tubers received no fungicide treatment. Finally, each treatment group was placed in a stoppered five-gallon glass container aerated at the rate of 100 ml/min for six weeks. Periodic CO_2 and O_2 measurements were made with an Orsatt apparatus.

Tuber weight classes for experiments covering PA-2E through PA-4 are given in Tables 3 and 4. Weights for PA-1 were 75 g each for half tubers and as close as possible to 100 g each for whole tubers. In order to minimize bias and sampling error except where uniform weights were used, tuber weight classes were randomly selected within a close range for each treatment group.

Evaluation Procedure

End of the rest period, PA-1, was determined according to Krijthe (1962) when at least 80% of the tubers held under the conditions of temperature and humidity specified in Table 1 developed sprouts at least 3 mm long. Number of days was also recorded at 100% sprouting.

Observations of experiments covering PA-2E through PA-3LM included sprout counts and morphological changes in sprouting character due to treatment. A sprout is defined as an individual shoot arising directly from one of the axillary buds in each potato eye. Counts were made by detaching each shoot at the base. Minimum

sprout lengths and the range considered for the count were recorded. Numbers were recorded after four to eight weeks sprouting, depending on the experiment (Table 1). Sprout count data were analyzed statistically as a completely randomized design according to Steel and Torrie (1960). Only sprouting morphology and influence of "little tuber" expression were considered in evaluation of DMSO or ethephon treatments with aged tubers, PA-4.

Results and Discussion

Influence of the Rest Period

With the exception of one experiment, treatment of tubers with DMSO had no effect on the rest period (Table 2). Thus in 1969, 25% DMSO reduced the rest requirement of 'Norgold Russet' whole and apical-half tubers about 20%, but in 1970 no effect was obtained. Other concentrations were equally ineffective in both years. The rest requirement of 'Netted Gem' tubers also was not appreciably changed by DMSO. Rindite, however, induced germination in one-sixth to one-third the time required by the control.

Smith and Rappaport (1961) have demonstrated a rise in gibberellin activity towards the end of the rest period but induction of early sprouting with Rindite does not necessarily stimulate the production of GA (Wareing and Saunders, 1971). Hemberg (1958) hypothesized that a specific complex of acid and neutral inhibitors plays an essential role in bud dormancy. The acid component, isolated from potato and eventually identified as abscisic acid (ABA) acts as a GA antagonist (van Es and Hartmans, 1969). An hypothesis receiving widespread support is that bud dormancy may be controlled by a balance between endogenous inhibitors, such as ABA, and the growth-promoting hormones including cytokinins and especially gibberellins (Wareing and Saunders, 1971). The data obtained suggest that DMSO does not affect this balance in favor of earlier sprouting.

Table 2. Influence of DMSO and Rindite on the rest period (PA-1) of 'Norgold Russet' and 'Netted Gem' seed potato tubers.

	1969		1969		1970		1970	
	'Norgold Russet'		'Netted Gem'		'Norgold Russet'		'Netted Gem'	
Treatment	whole	half	whole	half	whole	half	whole	half
			Days to 80 Percent Sprouted					
Rindite	15	12	14	14	10	9	12	10
0% DMSO	46	46	56	56	53	53	58	58
4% DMSO	46	46	56	56	53	53	58	58
10% DMSO	43	43	56	56	50	50	56	58
25% DMSO	39	37	58	56	51	50	54	53
			Days to 100 Percent Sprouted					
Rindite	18	16	16	16	14	14	14	11
0% DMSO	51	50	63	63	58	58	62	62
4% DMSO	52	50	61	60	58	59	62	62
10% DMSO	48	48	60	60	55	54	60	60
25% DMSO	41	40	65	62	57	56	58	56

Effect on Sprout Number and Sprout Morphology

A resume of the effect of four levels of DMSO on two varieties at five physiological ages on increasing sprouting and influencing sprout morphology is presented in Table 3. The data show clearly that the effect obtained from DMSO on tuber sprouting is directly related to PA. Thus, at the early apically-dominant single sprout stage, PA-2E, sprouting was not significantly affected by DMSO (Figure 1). This stage is characterized by either high IAA content or a low GA/IAA ratio (Burton, 1966; Lindblom, 1966). After storage for about a week at 3°C, however, tubers had entered PA-2L (Figure 2) and responded to DMSO treatment. Increased sprouting of whole tubers was observed only with 25% DMSO treatment. Half tubers generally responded to both 10% and 25% DMSO, possibly because cut tubers absorb more DMSO (Section III). From a practical standpoint, 10% DMSO is to be preferred to enhance sprouting of cut tubers because of the higher incidence of field decay when more concentrated solution is used.

Tubers held in storage about three months had entered PA-3E (Figure 3). Response of whole tubers of each variety at this stage was erratic. 'Netted Gem' apical-half tubers also did not benefit from treatment but 'Norgold Russet' showed nearly a linear response with treatment concentration. Davidson (1967) found 'Russet Burbank'

Table 3. Influence of DMSO on sprout numbers and sprout morphology of 'Norgold Russet' and 'Netted Gem' whole and apical-half seed potato tubers of different physiological ages. Sprout count values represent the mean of 25 tubers (PA-2E --- PA-3L) and 95 tubers (PA-3LM).

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	sprout morphology	Sig. Incr. in Sprouting (P=.05) % of Control	Mean no. sprouts per tuber	LSD .05	LSD .01
PA-2E	whole	'Norgold	1969	0	89.60-110.70	104.50	10-20	no change		0.96		
"	"	Russet'	"	4	96.50-115.00	103.20	"	with treat-		1.12		
"	"	"	"	10	88.70-111.30	108.50	"	ment.		1.28		
"	"	"	"	25	89.10-106.40	102.20	"	"		1.28	NS	NS
"	"	"	1970	0	84.30-107.20	99.20	10-22	"		1.16		
"	"	"	"	4	89.20-118.30	102.50	"			1.32		
"	"	"	"	10	89.40-109.20	97.70	"			1.04		
"	"	"	"	25	86.40-115.10	100.30	"			1.20	NS	NS
"	"	'Netted	1969	0	73.20-128.60	81.40	10-16	"		1.08		
"	"	Gem'	"	4	68.90-119.80	86.20	"			1.00		
"	"	"	"	10	72.60-125.00	80.70	"			1.08		
"	"	"	"	25	70.10-125.80	82.50	"			1.36	NS	NS
"	"	"	1970	0	79.20-120.50	108.50	10-20	"		1.40		
"	"	"	"	4	69.50-118.10	110.80	"			2.08		
"	"	"	"	10	73.50-122.70	102.50	"			1.72		
"	"	"	"	25	75.00-116.90	102.10	"			1.24		
"	half	'Norgold	1969	0	58.43-90.10	72.40	"	"		1.36		
"	"	Russet'	"	4	60.10-90.50	68.80	"			1.36		
"	"	"	"	10	61.50-100.90	69.70	"			1.36		
"	"	"	"	25	59.00-100.00	73.10	"			1.24	NS	NS

Table 3. (Cont.)

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	Sprout morphology	Sig. Incr. in Sprouting (P=.05) % of Control	Mean No. sprouts per tuber	LSD .05	LSD .01
PA-2E	half	'Norgold	1970	0	60.50-80.70	58.20	10-23	no change		1.24		
"	"	Russet'	"	4	55.00-81.60	63.10	"	with treat-		1.40		
"	"	"	"	10	50.60-74.30	56.30	"	ment.		1.44		
"	"	"	"	25	50.00-77.10	57.10	"			1.44	NS	NS
"	"	'Netted	1969	0	38.30-60.10	50.30	10-16			1.56		
"	"	Gem'	"	4	35.00-66.00	48.60	"			1.56		
"	"	"	"	10	44.70-70.00	55.80	"			1.64		
"	"	"	"	25	47.20-66.60	54.30	"	"		1.36	NS	NS
"	"	"	1970	0	68.30-100.50	75.50	10-20			1.36		
"	"	"	"	4	65.50-106.20	72.40	"			1.32		
"	"	"	"	10	69.80-99.50	73.20	"			1.36		
"	"	"	"	25	66.00-98.30	78.30	"	"		1.20	NS	NS
PA-2L	whole	'Norgold	1969	0	97.50-119.50	108.50	20-40			1.40		
"	"	Russet'	"	4	90.30-109.00	99.50	"			1.68		
"	"	"	"	10	92.50-115.00	103.40	"			1.84		
"	"	"	"	25	91.10-116.00	100.20	"	"	57	2.20	.45	.60
"	"	"	1970	0	82.10-107.50	93.50	20-45			1.32		
"	"	"	"	4	89.00-111.90	99.00	"			1.68		
"	"	"	"	10	86.30-114.20	94.70	"			1.72		
"	"	"	"	25	82.00-106.90	97.50	"	"	42	1.88	.40	NS
"	"	'Netted	1969	0	96.00-126.70	108.40	20-50			1.40		
"	"	Gem'	"	4	92.50-133.00	108.30	"			1.28		
"	"	"	"	10	86.10-117.90	102.00	"			1.44		
"	"	"	"	25	88.00-122.80	104.80	"	"	40	1.96	.36	.48

Table 3. (Cont.)

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	Sprout morphology	Sig. Incr. in Sprouting (P=.05) % of Control	Mean No. sprouts per tuber	LSD .05	LSD .01
PA-2L	whole	'Netted	1970	0	72.40-101.40	89.90	20-40	no change		1.44		
"	"	Gem'	"	4	88.00-106.30	92.40	"	with treat-		1.72		
"	"	"	"	10	81.40-105.40	90.10	"	ment.		1.84		
"	"	"	"	25	70.10-109.20	88.60	"		67	2.40	.47	.62
"	"	'Norgold	1969	0	52.30-70.50	60.90	20-40			1.68		
"	"	Russet'	"	4	48.30-72.50	62.50	"			1.72		
"	"	"	"	10	58.90-78.00	68.00	"		36	2.28		
"	"	"	"	25	58.80-70.90	62.00	"		76	2.96	.50	.67
"	"	"	1970	0	48.50-66.20	55.30	20-45			1.64		
"	"	"	"	4	39.00-70.60	58.00	"			1.80		
"	"	"	"	10	44.00-63.50	51.70	"	"	68	2.76		
"	"	"	"	25	42.30-70.40	58.20	"		61	2.64	.49	.65
"	half	'Netted	1969	0	63.50-80.00	72.00	20-50			1.48		
"	"	Gem'	"	4	64.70-86.50	74.10	"			1.68		
"	"	"	"	10	68.60-92.50	76.10	"	"	108	3.08		
"	"	"	"	25	66.00-86.50	73.30	"		124	3.32	.50	.67
"	"	"	1970	0	58.40-81.30	70.00	20-40			1.44		
"	"	"	"	4	56.00-93.40	76.00	"			1.88		
"	"	"	"	10	61.50-84.70	74.80	"	"		2.00		
"	"	"	"	25	55.00-90.90	74.20	"			2.28	NS	NS
PA-3E	whole	'Norgold	1969	0	68.50-103.20	88.20	25-60			3.36		
"	"	Russet'	"	4	66.90-100.40	89.90	"			3.28		
"	"	"	"	10	72.50-111.30	86.40	"	"	37	4.60		
"	"	"	"	25	62.00-99.70	88.30	"		59	5.36	.69	.91

Table 3. (Cont.)

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	Sprout morphology	Sig. Incr. in Sprouting (P=.05) % of Control	Mean No. sprouts per tuber	LSD .05	LSD .01
PA-3E	whole	'Norgold	1970	0	83.50-119.20	101.40	20-65	no change		4.00		
"	"	Russet'	"	4	77.50-120.40	98.60	"	with treat-		3.88		
"	"	"	"	10	89.80-116.00	99.20	"	ment.		5.00		
"	"	"	"	25	88.40-111.20	103.20	"			4.60	NS	NS
"	"	'Netted	1969	0	89.90-112.60	104.50	20-60			3.68		
"	"	Gem'	"	4	96.50-109.30	101.60	"			3.80		
"	"	"	"	10	88.50-119.60	99.90	"			4.28		
"	"	"	"	25	95.10-109.70	104.10	"	"		4.52	NS	NS
"	"	"	1970	0	116.40-130.00	122.30	"			4.32		
"	"	"	"	4	118.50-132.40	125.00	"			4.92		
"	"	"	"	10	114.00-136.00	127.90	"			4.48		
"	"	"	"	25	116.00-138.40	124.10	"	"	84	7.96	1.26	1.67
"	half	'Norgold	1969	0	48.20-70.20	63.50	25-60			3.68		
"	"	Russet'	"	4	50.30-72.16	65.10	"		26	4.64		
"	"	"	"	10	46.66-70.50	62.20	"		48	6.55		
"	"	"	"	25	44.25-71.38	62.60	"	"	100	7.36	.72	.96
"	"	"	1970	0	53.50-86.50	70.00	20-65			3.32		
"	"	"	"	4	55.00-84.10	74.20	"		73	5.76		
"	"	"	"	10	54.60-82.50	73.70	"		76	5.84		
"	"	"	"	25	53.80-82.30	70.10	"	"	136	7.84	1.36	1.80
"	"	'Netted	1969	0	51.80-89.50	70.50	20-60			3.80		
"	"	Gem'	"	4	52.20-94.70	70.80	"			3.88		
"	"	"	"	10	54.50-89.00	68.60	"			4.08		
"	"	"	"	25	60.50-86.90	69.50	"	"	70	6.48	.89	1.18

Table 3. (Cont.)

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	Sprout morphology	Sig. Incr. in Sprouting (P=.05) % of Control	Mean No. sprouts per tuber	LSD .05	LSD .01
PA-3E	half	'Netted	1970	0	55.00-80.10	70.00	20-60	no change		2.84		
"	"	Gem'	"	4	56.10-90.30	69.50	"	with treat-		4.08		
"	"	"	"	10	49.60-83.20	73.00	"	ment.		3.84		
"	"	"	"	25	52.10-80.70	74.10	"			3.88	NS	NS
PA-3L	whole	'Norgold	1969	0	78.00-95.50	86.20	20-45			9.08		
"	"	Russet'	"	4	79.80-98.50	89.50	"			8.24		
"	"	"	"	10	77.60-93.20	90.00	"			9.32		
"	"	"	"	25	76.40-95.60	89.70	"	"		10.00	NS	NS
"	"	"	1970	0	90.20-120.50	106.50	20-35	some fine,		9.88		
"	"	"	"	4	88.60-116.30	110.20	"	weak sprouts		10.36		
"	"	"	"	10	82.50-120.10	109.10	"	at 25%		10.20		
"	"	"	"	25	86.30-114.20	108.90	"	DMSO	28	12.68	1.73	2.30
"	"	'Netted	1969	0	74.00-89.20	80.40	20-40	no change		4.92		
"	"	Gem'	"	4	69.10-90.50	82.10	"	with treat-		5.28		
"	"	"	"	10	73.50-87.80	81.80	"	ment.	28	6.28		
"	"	"	"	25	77.30-91.50	80.70	"		89	9.32	1.34	1.77
"	"	"	1970	0	96.40-140.50	129.50	20-35			5.40		
"	"	"	"	4	100.10-151.60	132.30	"			6.32		
"	"	"	"	10	94.30-147.20	128.90	"		49	8.04		
"	"	"	"	25	99.50-146.30	129.10	"		59	8.60	1.31	1.73
"	half	'Norgold	1969	0	108.60-150.30	131.50	20-45	some fine,		7.64		
"	"	Russet'	"	4	110.00-149.20	132.50	"	weak sprouts	25	9.56		
"	"	"	"	10	106.70-154.20	132.20	"	at 25%		8.72		
"	"	"	"	25	118.40-147.50	130.40	"	DMSO	31	10.04	1.48	NS

Table 3. (Cont.)

Physiological Age	Tuber	Variety	Year	DMSO %	Weight Range g	Mean Weight g	Range sprout length mm	Sprout morphology	Sig. Incr. in sprouting (P=.05) % of Control	Mean No. sprouts per tuber	LSD .05	LSD .01
PA-3L	half	'Norgold	1970	0	100.00-115.10	109.40	20-40	numerous fine,		16.76		
"	"	Russet'	"	4	93.40-116.10	110.70	"	weak sprouts	27	21.24		
"	"	"	"	10	103.20-109.50	106.30	"	at 25%	34	22.40		
"	"	"	"	25	101.90-116.30	103.50	"	DMSO	60	26.80	3.39	4.49
"	"	'Netted	1969	0	93.20-118.30	108.40	20-40	some fine,		6.28		
"	"	Gem'	"	4	94.50-109.80	103.50	"	weak sprouts	82	11.44		
"	"	"	"	10	99.30-111.50	106.10	"	at 25%	61	10.12		
"	"	"	"	25	99.50-113.00	104.90	"	DMSO	92	12.04	1.26	1.67
"	"	"	1970	0	100.00-129.40	116.50	20-35			7.12		
"	"	"	"	4	92.00-131.10	118.20	"		54	10.96		
"	"	"	"	10	89.80-128.20	116.70	"		58	11.28		
"	"	"	"	25	88.60-130.40	117.40	"	"	51	10.72	1.49	1.97
PA-3LM	whole	'Norgold	"	0	96.70-110.30	103.36	10-45	development of		7.14		
"	"	Russet'	"	4	97.00-116.40	102.21	"	numerous fine		7.93		
"	"	"	"	10	98.50-109.50	104.60	"	roots at all	20	8.60		
"	"	"	"	25	96.30-109.80	104.50	"	DMSO concentrations	17	8.33	.73	.96
"	half	"	"	0	75.00-75.00	75.00	10-40			6.29		
"	"	"	"	4	" "	"	"			6.85		
"	"	"	"	10	" "	"	"		33	8.37		
"	"	"	"	25	" "	"	10-25	"	88	11.84	.75	.99

('Netted Gem') responded best to treatment at time of removal from cold storage, whereas 'Norgold Russet' produced more sprouts after a pre-warming period. In the present study all tubers were pre-warmed 24 hours prior to immersion in DMSO.

After a seven-month storage period, sprouting morphology was characteristic of PA-3L following treatment (Figure 4). DMSO increased sprout numbers of whole tubers at 10% and 25% concentrations but the results were not consistent from year to year, especially with 'Norgold Russet'. Apical-half tubers of both varieties, however, showed increased sprouting at all DMSO levels. Tubers sprouted under the treatment conditions of the metabolic study, PA-3LM (Figure 5) were identical in sprout morphology to PA-3L tubers except for the presence of numerous roots which developed at the high humidity in the glass containers. Measurement of CO_2 at weekly intervals showed an average concentration range of 3 to 6%. Burton (1968) reported that CO_2 is often regarded as a growth suppressant, but at concentrations up to about 7% it can cause marked stimulation of sprout growth, the optimum being in the range 3 to 5%.

Changes in morphology due to DMSO treatment occurred only in a few cases. These were limited to an increase in weak sprouts developing in response to treatment with 10% or 25% DMSO. Only tubers at a more advanced PA produced these symptoms (Table 3). Palladina and Pervova (1966) similarly found the formation of fine, rapidly

'Norgold Russet' apical-half seed potato tubers showing the effect of DMSO or ethephon treatment at different physiological ages (PA)

Figure 1. PA-2E, early apically dominant single sprout stage. No effect from DMSO treatment.

Figure 2. PA-2L, late apically dominant single sprout stage. Tubers treated with 25% DMSO. Earliest PA at which DMSO has an effect.

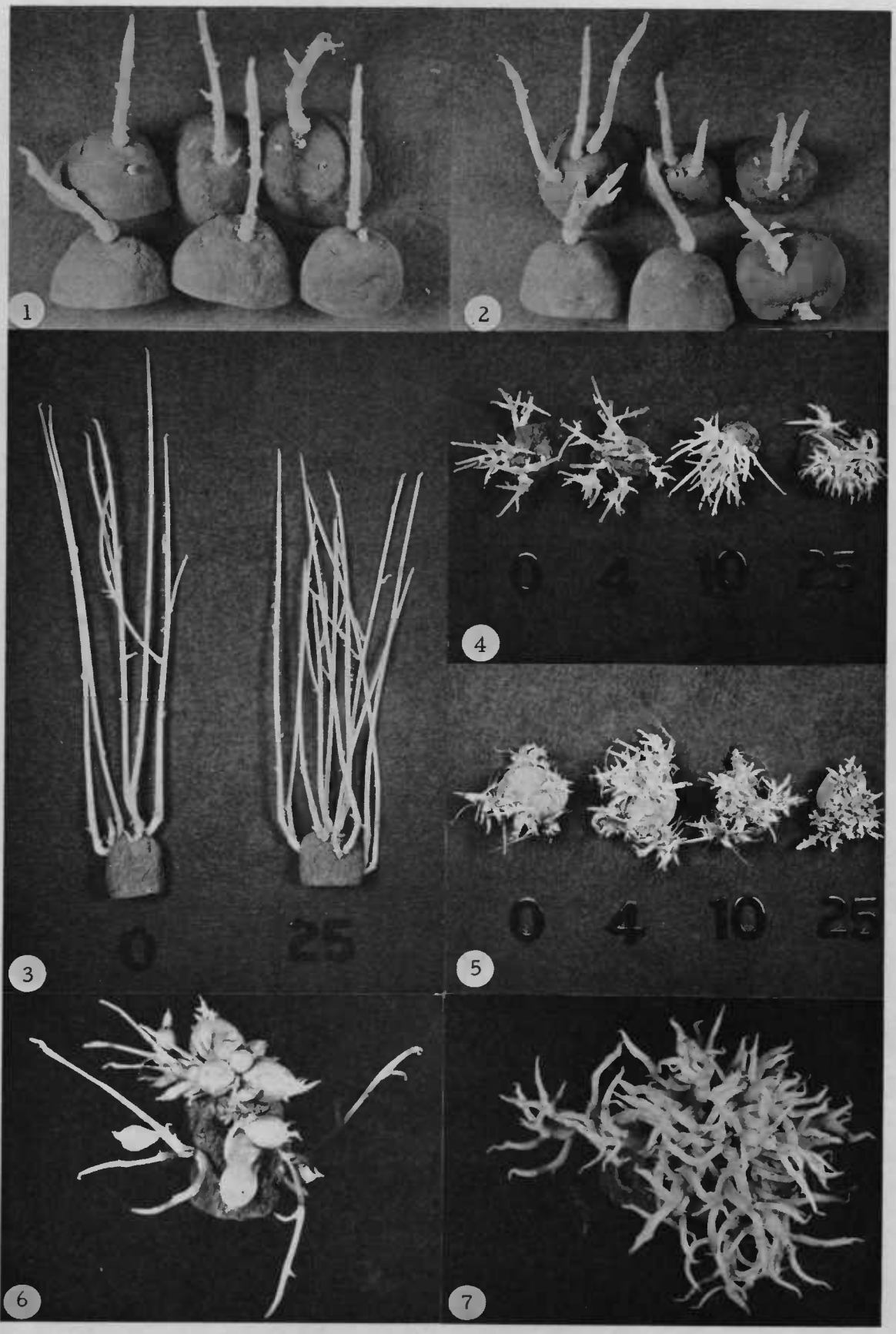
Figure 3. PA-3E, early correlative inhibition stage. Multiple unbranched sprouting showing the effect of 25% DMSO on increasing sprout numbers.

Figure 4. PA-3L, late correlative inhibition stage. Branched-multiple sprouting showing the effects of four concentrations of DMSO on sprout numbers.

Figure 5. PA-3LM, PA observed during investigation of the metabolism of DMSO in potato tubers. This stage was almost identical to PA-3L.

Figure 6. PA-4, senescent aged tuber stage showing "little tuber" symptoms.

Figure 7. PA-4, senescent aged tuber stage. Tuber treated with 5000 ppm ethephon 24 hours after removal from cold storage. Complete reversion of the "little tuber" condition.


1
2
3
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25
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6
7

growing sprouts followed immersion of tubers in 0.01-0.03% GA for 30 minutes. Rate of sprout growth was not affected by DMSO except that 25% DMSO delayed the onset of sprouting by one to two days. Estes (1969) also found DMSO immersion treatment had no effect on the growth rate of potato stems in the field.

The mechanism by which DMSO enhances sprouting is not understood. Evidence from several studies indicates that the compound may overcome apical dominance (Smale, 1969). Estes (1969) proposed the effect was exerted through an auxin interaction such as transport or degradation within the potato tuber. In previous investigations, however, PA had not been considered in relation to the response obtained. Thus, Estes used tubers which probably had been in storage from fall until treatment in June, while Davidson (1967) reported on tubers held eight to ten months prior to treatment. Furthermore, tubers used for seedpieces in Henninger's (1971) work had been stored at least six months. As a consequence the results of these authors in showing increased sprouting due to DMSO treatment are based on tubers which already had reached a PA characterized by multiple- or branched-multiple sprouting.

Lindblom (1968) maintains that a high ratio of GA to IAA is responsible for multiple sprouting. Single sprouting tubers can be advanced to a multiple sprouting stage by treatment with GA (Bishop and Timm, 1968). Another hypothesis proposes a continuous

production of a specific correlative inhibitor induced by basipolar movement of IAA (Goodwin, 1967b), the inhibitor being continuously destroyed as it moves up into the sprouts. In the terminal portion of growing shoots, there is a sensitive region in which the inhibitor is proposed to act. In shoots longer than a certain critical length, the inhibitor is inactivated before it reaches this sensitive zone. In short sprouts, the inhibitor prevents stem elongation. Multiple sprouting tubers apparently must have a mechanism offsetting the effects of the correlative inhibitor.

In the present study DMSO had no effect on PA-2E tubers which display strong apical dominance and reportedly contain a high IAA/GA ratio. Although the results were not entirely consistent the most pronounced DMSO increases in sprout numbers were associated with PA-2L, PA-3E and PA-3L apical-half tubers. This indicates that DMSO exerts its influence when GA is high in relation to IAA and that larger quantities of DMSO absorbed by cut tubers may have an influence on the level or distribution of the growth promoter. An effect on IAA or the inferred correlative inhibitor, nevertheless, cannot be discounted. Finally, it is interesting to speculate on the role of dimethyl sulfide evolution from DMSO-treated tuber tissue (Section IV) in affecting endogenous hormonal balances.

Rejuvenation of Aged Tubers

Results of the study with senescent tubers are summarized in Table 4. 'Norgold Russet' tubers held in cold storage nearly a year developed uniform "little tuber" symptoms within three weeks following cutting into apical-halves (Figure 6). Ethephon, which has been shown by Warner and Leopold (1969) to release ethylene in treated plant tissue, prevented development of symptoms and induced numerous branched sprouts (Figure 7). Langille (1969) observed an alteration of "little tuber" in 'Katahdin' when GA and ethephon were applied simultaneously, while ethephon applied alone had no effect. He speculated that ethylene production stimulated growth of axillary buds and these elongated in response to GA. In the present study ethephon alone prevented rhizome formation. Since the tubers were not planted, no conclusion can be drawn regarding the subsequent plant development.

Reversion of "little tuber" and induction of normal multiple-branched sprouting (Figure 4) developed in 28% of the tubers which had been treated with 10% DMSO and in 16% of those treated with 25% DMSO. The higher DMSO concentration had a pronounced lethal effect on aged tubers and 32% failed to sprout. Okazawa (1959) found that GA stimulated elongation of stems and stolons and also inhibited tuber formation. Smith and Rappaport (1969) associated tuberization

Table 4. Effect of DMSO and ethephon on "little tuber" (PA-4) expression in 'Norgold Russet' apical-half seed potato tubers. Values represent number of treated tubers rated in each of four sprout morphology classes.

Treatment	Weight range g	Mean weight g	Sprout Morphology			
			Little tuber	Branched multiple sprouting	Weak sprouting	dead
Ethephon 5000 ppm	47.20-100.30	75.40	0	50	0	0
0% DMSO	39.70-118.50	68.50	50	0	0	0
4% DMSO	38.50-116.70	71.40	48	1	1	0
10% DMSO	42.00-125.20	73.10	31	14	4	1
25% DMSO	36.00-120.40	74.50	21	8	5	16

with a decline in GA concentration. They also isolated a GA antagonist which was higher in young tubers than in stolons and which could not be related to ABA. However, ABA has been considered a necessary growth substance in formation of tubers (El-Antably, Wareing and Hillman, 1967). The influence of DMSO in counteracting "little tuber" development may be mediated through an effect on GA rather than on interfering in the action of endogenous inhibitors.

II. EFFECT OF DMSO ON ASCORBIC ACID, GLUTATHIONE AND RESPIRATION OF SPROUTING SEED POTATO TUBERS

Materials and Methods

Plant Material and Treatments

'Norgold Russet' and 'Netted Gem' seed potato tubers were obtained and stored as described previously. DMSO-treated 100 g, PA-3E and PA-3L apical-half tubers of both varieties, were used for AA and GSH estimations in 1969. In 1970, 'Norgold Russet', PA-3L, apical-half tubers were treated with a sprout suppressant. 'Norgold Russet' whole and apical-half tubers were used for the respiration studies. PA-3E tubers were used in 1969 and PA-3L tubers in 1970.

For AA and GSH analyses, and the respiration study, date and method of PA selection and treatment methods were the same as those reported previously (Section I). Treatments for the sprout suppressed series consisted of the control, 25% DMSO, and 25% DMSO followed 72 hours later with a five second immersion in 0.5% CIPC [isopropyl N-(3-chlorophenyl) carbamate; Columbia-Southern Co.] containing 0.01% Triton X-100. CIPC treated tubers were kept in a separate room at 20°C, 40% RH, to avoid volatile contamination of other treatments.

Estimation of Ascorbic Acid and Glutathione

Treated and control apical-half tubers were assayed for AA and GSH at 7, 21 and 35 day intervals during the course of sprouting in the dark at 20°C. An additional dormant untreated lot was assayed within a few hours after removal from cold storage. Three tubers from each treatment were assayed individually and the means tested for significance as a completely randomized design according to Steel and Torrie (1960).

The assayed tissue from the 7 and 21 day samples consisted of 20-g sections from tuber apexes, including dormant buds, growing sprouts, periderm and cortical-medullary tissues. The 35 day samples included all of these tissues except the sprouts. The sprout-suppressed series included treated and untreated controls with sprouts removed prior to every assay.

GSH was estimated according to the basic iodometric titration method of Woodward and Fry (1932) as fully detailed in Patterson and Lazarow (1955) with additional refinements suggested by Thomson and Martin (1959). All chemicals used in the assays for GSH and AA were reagent grade (GSH and AA standards, Sigma Chemical Co.; all other reagents, Allied Chemical Co.). Standardizations were made prior to each experiment. Over 90% recovery was obtained when GSH and AA were added to control homogenates. Titration end points were not affected by addition of DMSO.

The extraction procedure was modified slightly for potato samples in the present study. Twenty g tissue sections were diced into 20 ml of 4% (w/v) sulfosalicylic acid (SSA) in a 200 ml flask, 50 ml of 2% SSA and 1 ml of 1% KCN were added, and the tissue was homogenized for five minutes in an ice bath with a Virtis homogenizer (Virtis Research Equipment, Type 30) at 40,000 rpm. The homogenate was transferred to a beaker, allowed to stand 30 minutes with periodic stirring, then filtered under vacuum through Whatman No. 1 paper upon addition of 1 g analytical grade Celite (Johns-Manville). After adjustment of the filtrate volume to 100 ml with 2% SSA, titrations were made in triplicate, each in less than two minutes.

The assay for ascorbic acid was carried out as part of the Patterson and Lazarow procedure for GSH. Following GSH titrations, additional filtrate aliquots were titrated, also in triplicate, with 2,6-dichlorophenolindophenol to a faint pink end point persisting for 15 seconds. The basis of the reaction is reduction of the dye by AA in acid solution. Under the conditions employed GSH did not interfere in the reaction. Although some other reducing substances such as ferrous, stannous or cuprous ions may give values in excess of the actual AA content (A. O. A. C., 1965) these were not considered to be a problem with potato extracts.

Respiration Measurements

Standard DMSO immersion procedure and concentrations were used for all respiration tuber samples. Duplicate five-tuber samples were placed, 24 hr after treatment, in one-gallon glass containers sealed with rubber stoppers containing inlet and outlet tubes. Aeration was maintained at 200 ml per minute with fresh air drawn from outdoors. The experiments were conducted in a dark room at 20°C.

Respiration rate was determined with an infrared CO_2 analyzer (Beckman Instruments, Model 215 A) at two-day intervals during a 20 day period. Respiration values were recorded as a mean of the duplicate five-tuber samples representing each treatment.

Results and Discussion

Influence on Ascorbic Acid

Treatment of tubers with DMSO did not significantly affect ascorbic acid content in either variety (Table 5). Levels were not significantly different between tubers prevented from sprouting with CIPC and those mechanically de-sprouted before assay. An inverse relationship existed between AA content and tuber PA. Also, levels were much lower in samples analyzed without sprouts 35 days following treatment. This is interpreted to mean that the sprouts contained more AA than tuber tissue or that the level dropped rapidly after the

Table 5. Effect of DMSO on ascorbic acid levels in sprouting and sprout-suppressed 'Norgold Russet' and sprouting 'Netted Gem' apical-half seed potato tubers at two physiological ages. Values represent the mean of three analyzed tubers.

Variety	Seed Piece Physiological Age	Treatment	Ascorbic Acid mg/100g Sprouting Period in Days 0	7	21	35
'Norgold	Early correlative	Dormant tuber	10.99			
Russet'	inhibition (PA-3E)	0% DMSO		14.85	15.09	9.77
		4% DMSO		14.53	15.66	8.89
		10% DMSO		16.38	15.56	8.14
		25% DMSO		15.35	14.02	9.61
		LSD 0.05		NS	NS	NS
"	Late correlative	Dormant tuber	6.24			
	inhibition (PA-3L)	0% DMSO		10.40	12.65	6.63
		4% DMSO		9.71	11.99	7.20
		10% DMSO		9.81	9.62	7.79
		25% DMSO		9.31	12.20	8.13
		LSD 0.05		NS	NS	NS
"	Late correlative	Dormant tuber	10.25			
	inhibition (PA-3L)	0% DMSO				
	Sprout suppression	sprouts removed		10.94	8.44	8.53
		25% DMSO sprouts removed		10.78	8.75	8.63
		0.5% CIPC/ 25% DMSO		12.81	11.22	11.37
		LSD 0.05		NS	NS	NS
'Netted	Early correlative	Dormant tuber	10.87			
Gem'	inhibition (PA-3E)	0% DMSO		13.87	13.88	9.03
		4% DMSO		11.39	12.22	7.87
		10% DMSO		13.02	12.61	8.30
		25% DMSO		11.87	13.09	9.13
		LSD 0.05		NS	NS	NS
"	Late correlative	Dormant tuber	7.84			
	inhibition (PA-3L)	0% DMSO		9.37	14.35	8.17
		4% DMSO		9.35	12.20	8.93
		10% DMSO		10.94	14.23	8.95
		25% DMSO		10.45	12.69	8.77
		LSD 0.05		NS	NS	NS

third week. Most likely both of these factors interact to cause a reduction of AA in tuber tissue. Lampitt, Baker and Parkinson (1945) and Smith and Paterson (1937) found AA increased in sprouts and decreased in the tuber during sprouting. Emilsson (1949) observed a gradual but slow decline of AA during sprouting. In contrast, Szalai (1959a) reported AA content decreased during the first eight days, then increased up to the 22nd and declined thereafter. This pattern was followed in his experiments by all tuber portions, with the apexes showing the most deviation.

Sprout stimulants have been shown to have a small effect on AA. Ethylene chlorohydrin slowly increased the AA content in old tubers and maintained it longer in cut tubers (Guthrie, 1937). Treatment with Rindite has been shown to lengthen the duration of a natural increase in AA associated with sprouting (Szalai, 1959a). Information about the effect of other bud-forcing chemicals on AA is lacking. Torfason (1965) found CIPC-treated 'Netted Gem' potatoes contained slightly less AA than untreated. Certain other sprout suppressants had no effect (Driver, 1957).

Enhancement of Glutathione

'Norgold Russet' (PA-3E) apical-half tubers showed a significant increase in GSH 21 days following treatment with 4, 10, or 25% DMSO (Table 6). The increase ranged from 24 to 31% depending on

Table 6.. Effect of DMSO on glutathione levels in sprouting and sprout suppressed 'Norgold Russet' and sprouting 'Netted Gem' apical-half seed potato tubers at two physiological ages. Values represent the mean of three analyzed tubers.

Variety	Seed Piece Physiological Age	Treatment	Glutathione : mg/100g Sprouting Period in Days 0	7	21	35
'Norgold Russet'	Early correlative inhibition (PA-3E)	Dormant tuber	11.07			
		0% DMSO		11.39	10.88	9.79
		4% DMSO		12.55	13.53	11.03
		10% DMSO		12.95	14.25	10.53
		25% DMSO		14.98	14.60	12.50
		LSD 0.05		NS	2.55	NS
"	Late correlative inhibition (PA-3L)	Dormant tuber	10.39			
		0% DMSO		8.82	9.96	7.68
		4% DMSO		9.35	9.73	7.96
		10% DMSO		10.82	13.18	10.48
		25% DMSO		15.46	16.23	12.88
		LSD 0.05		2.39	3.49	2.32
		0.01		3.48	5.09	3.38
"	Late correlative inhibition (PA-3L) Sprout suppression	Dormant tuber	9.84			
		0% DMSO sprouts removed		11.37	8.79	7.97
		25% DMSO sprouts removed		12.37	10.74	8.70
		0.5% CIPC/ 25% DMSO		11.46	11.08	8.91
		LSD 0.05		NS	NS	NS
'Netted Gem'	Early correlative inhibition (PA-3E)	Dormant tuber	10.65			
		0% DMSO		8.51	7.71	6.02
		4% DMSO		10.14	10.76	7.40
		10% DMSO		10.52	9.89	6.46
		25% DMSO		12.71	14.68	9.46
		LSD 0.05		1.80	3.57	1.93
		0.01		2.61	NS	NS
"	Late correlative inhibition (PA-3L)	Dormant tuber	7.56			
		0% DMSO		8.85	7.92	6.83
		4% DMSO		10.71	10.14	7.80
		10% DMSO		11.01	10.23	8.28
		25% DMSO		15.56	11.44	9.36
		LSD 0.05		4.80	3.19	NS

the treatment. Differences in the GSH levels were not significant at 7 or 35 days. Since assays made at 35 days did not include sprouts this indicated that the increase found at 21 days may have been associated with the sprouts.

'Norgold Russet' tubers treated at a more advanced PA (PA-3L), with 25% DMSO, showed a significant ($P=0.01$) increase in GSH occurred at all dates. No other concentrations had a significant effect with the exception of the 10% treatment at 35 days. Enhancement of GSH by 25% DMSO averaged 68% greater than the control.

Although the GSH increases associated with both physiological ages at all DMSO concentrations and at each date were not statistically significant, there was a tendency toward linearity with treatment as shown in Table 6. Furthermore, GSH concentration generally increased slightly from the first to third weeks of sprouting. There were no significant differences among PA-3L control and 25% DMSO-treated tubers which were de-sprouted before analysis, and 25% DMSO-treated tubers prevented from sprouting with CIPC. That de-sprouted 25% DMSO-treated tubers did not have a higher GSH content over the controls in the sprout-suppressed experiment after 35 days does not agree with results from the other experiment with PA-3L tubers (Table 6).

Response of 'Netted Gem' to DMSO treatment exceeded that of 'Norgold Russet'. Generally, significantly increased GSH levels were

associated only with 25% DMSO treatment. Increases in GSH ranged from 24 to 90% in PA-3E tubers and 44 to 75% in PA-3L tubers. Glutathione means which were not significantly different at the 5% level were, nevertheless, higher than control means. They did not follow a linear relationship with treatment concentration as had been found with 'Norgold Russet'.

The present study did not show variety, number of sprouts and vigor of sprouting to be related to GSH content. Also, GSH level of dormant tubers was not significantly above or below that of sprouted tubers. Some investigations have shown GSH content to be higher in sprouts, but this was not associated with a concomitant depletion in the tuber (Emilsson, 1949; Pett, 1936). Values for GSH in untreated sprouting potato tubers as reported in the literature agree with those found in the present study.

Although many bud-forcing chemicals significantly increase GSH, the effectiveness of a chemical in terminating rest and increasing GSH are not related (Emilsson, 1949; Guthrie, 1933, 1940). Effectiveness of chemicals which enhance sprouting following natural termination of the rest period and their relationship to GSH have not been studied in detail. However, gibberellic acid and thiourea, which alter apical dominance and induce more sprouts at several different physiological ages, have been shown to increase GSH in sprouts (Palladina and Pervova, 1966).

Evidence regarding the relation between AA, GSH and sprouting is conflicting (Emilsson and Lindblom, 1963). Increased levels have not been directly associated with greater sprout numbers or more vigorous growth rate either in this study or in those reported in the literature. DMSO-induced stimulation of GSH in sprouting tubers is important since the sulphydryl and AA constitute a potential coupled redox source for cellular metabolism. Interestingly, Wood et al. (1967) observed GSH was reduced in concentration in DMSO-treated rabbit eye lenses. Borgstedt and DiStefano (1967) and Sommer and Tauberger (1964) have implied DMSO can be directly metabolized to DMS in the presence of sulphydryl. If reduction of DMSO to DMS proceeds via GSH, then differences in rate of reduction between sprouting and CIPC sprout-suppressed tubers might be related to GSH content (see Section IV). CIPC application did not affect GSH level in tuber tissue, but higher GSH concentrations were associated with sprout tissue. Glutathione may function in activating glycolytic enzymes dependent on free SH groups and therefore ultimately influence the level of cellular NADPH. The pyridine nucleotide can act as a co-factor for both oxidation and reduction of DMSO as suggested by Gerhards and Gibian (1967) and Wood and Jacob (1968).

DMSO damage of rat cell endoplasmic reticula has been shown to be prevented by AA (Archer et al., 1967). Both GSH and AA protect cell membrane integrity from chemical challenges, not unlike the

DMSO challenge to membrane protein configuration, which could occur in treated potato tissue (Archer et al., 1967; Kosower and Kosower, 1969; Mayer and Avi-Dor, 1970; Rammler, 1967; Rammler and Zaffaroni, 1967). While GSH protects cell membranes from damage by free radicals; DMSO offsets formation of these radicals by its capacity to be oxidized (Kosower and Kosower, 1969; Rammler, 1967).

Even though the order of biological toxicity of DMSO is considered to be low, Schmid (1968) contends it behaves as a poisoning agent in plants, attacking some aspect of metabolism. Bajaj et al. (1970) also found a severe inhibitory effect on respiration, protein and RNA metabolism of Phaseolus vulgaris tissue. In this regard, sustained levels of sulphydryl could act as a primary de-toxifying agent by acting through a GSH-conjugating system with DMSO (Boyland and Chasseaud, 1967; Wood et al., 1967).

Effects on Respiration

Respiration rates for all treatments were initially high and decreased with time (Tables 7 and 8). Tubers (whole and apical-half) at the multiple unbranched sprouting stage, PA-3E, were not consistent in their respiratory response to DMSO. Aside from small increases over the control in the first week, DMSO generally reduced respiration slightly.

Tubers at the branched multiple sprout stage PA-3L (Table 8) showed a lesser initial respiratory rate than PA-3E tubers but followed the same general decline with time. The respiratory rate of the 25%

Table 7. Effect of 30-minute DMSO immersion treatments on respiration rate of 'Norgold Russet' whole and apical-half seed potato tubers (PA-3E).

		Milligrams CO_2 per Kg per Hour									
		Days Following Treatment									
Treatment	Tuber	2	4	6	8	10	12	14	16	18	20
0% DMSO	whole	21.45	21.86	16.20	15.35	15.68	15.68	14.55	13.86	14.25	14.25
	apical-half	45.67	31.17	18.19	18.19	17.95	16.50	17.68	18.30	17.90	17.68
4% DMSO	whole	22.46	20.50	17.23	15.01	14.68	14.68	14.20	14.20	13.66	13.25
	apical-half	43.82	30.90	17.33	17.17	16.89	17.17	16.30	16.59	17.35	17.20
10% DMSO	whole	20.35	20.55	17.18	15.03	15.50	14.62	13.49	13.78	14.22	14.22
	apical-half	48.52	33.07	18.79	16.30	15.95	15.40	15.40	17.25	16.31	15.71
25% DMSO	whole	23.75	22.05	18.13	16.00	15.25	15.61	14.01	14.66	14.71	15.02
	apical-half	50.25	35.21	17.95	17.33	17.35	16.29	16.29	16.26	17.05	16.80

Table 8. Effect of 30-minute DMSO immersion treatments on respiration rate of 'Norgold Russet' whole and apical-half seed potato tubers (PA-3L).

		Milligrams CO_2 per Kg per Hour									
		Days Following Treatment									
Treatment	Tuber	2	4	6	8	10	12	14	16	18	20
0% DMSO	whole	25. 31	20. 55	20. 83	16. 50	16. 25	16. 50	17. 31	17. 01	16. 85	16. 73
	apical-half	31. 17	27. 09	18. 36	16. 89	19. 20	20. 80	21. 20	21. 20	22. 43	22. 68
4% DMSO	whole	26. 24	21. 37	21. 68	15. 61	15. 97	16. 25	15. 97	15. 24	15. 24	15. 61
	apical-half	32. 41	26. 50	20. 02	18. 40	18. 40	19. 31	19. 60	22. 60	21. 83	23. 40
10% DMSO	whole	25. 40	21. 30	20. 80	17. 40	16. 38	16. 84	17. 50	17. 17	17. 25	16. 90
	apical-half	30. 35	26. 04	21. 32	20. 82	19. 30	18. 90	18. 21	20. 07	21. 32	22. 48
25% DMSO	whole	27. 50	23. 81	22. 67	20. 88	18. 23	18. 46	19. 37	19. 25	18. 04	17. 18
	apical-half	34. 36	28. 24	25. 05	26. 03	25. 78	25. 14	24. 07	24. 50	25. 20	25. 93

DMSO treated tubers (whole and apical-half) was consistently higher than the control and all other treatments.

Sprouting of tubers was slower with 25% DMSO during the first week than with other treatments. Cut PA-3E tubers respired at twice the rate of whole tubers during the first few days. The difference was not as great with PA-3L tubers. Temporary increase in potato respiration due to wounding has been reported (Johnstone, 1925; Mulder, 1956).

Some investigations have related chemical sprout enhancement to increased respiration. Smith and Rappaport (1965) found GA stimulated respiration before sprouting, and the stimulation was maintained for 20 days or longer. At the same time GA induced more sprouts. In similar studies Timm and Schweers (1965) observed no detectable respiratory response to GA of previously sprouted tubers although treated unsprouted tubers showed a marked rise in respiration. Many sulfur compounds including thiourea, thiocyanates, dimethyl disulfide and ethyl mercaptan caused higher respiration rates of dormant tubers (Miller, 1933). In Miller's study, dimethyl sulfide did not affect respiration. This compound was found to be a metabolite of DMSO in treated potato tubers (Section IV).

DMSO effects on plant respiration have been examined in only a few cases. A 69% reduction in O_2 utilization was observed by Schmid (1968) when excised barley roots were treated with 25% DMSO. Bean tissues incubated in 10% DMSO showed severe metabolic inhibition of

respiration (Bajaj et al., 1970). The relatively small amount of DMSO absorbed by treated tubers (0.1-3.5 mg/g fresh wt, Section III, Table 9) probably would not adversely affect metabolism except in the area of greatest localization. In fact, Fine and Stahl (1970) were able to stabilize labile cytochrome oxidase with 1 to 2% DMSO in phosphate buffer. A large part, if not all, of the respiration of potato tubers proceeds through this enzyme (Goddard and Holden, 1950; Mapson and Burton, 1962; Mondy, Klein and Smith, 1960). Injury to the cut surface or to the buds of tubers treated with 25% DMSO could account for the observed rise in respiration in the present study.

III. ABSORPTION AND DISTRIBUTION OF DMSO IN TREATED 'NORGOLD RUSSET' SEED POTATO TUBERS

Materials and Methods

Determination of Absorption of $DM^{35}SO$ by Whole and Cut Tubers

Plant Material and Treatment

Oregon Foundation Grade 'Norgold Russet' seed potato tubers, free from damage and disease, were washed and separated into approximate size categories prior to storage at 3°C. After a storage period of 200 days, 12 PA-3L tubers were transferred to 20°C for 24 hours, then nine whole tubers and three apical-half tubers were weighed prior to $DM^{35}SO$ treatment. The 12 tubers were then divided into three lots, each containing one from each weight group. Twenty-five ml of $DM^{35}SO$ (radiolabeled DMSO obtained from the Radiochemical Centre, Amersham, England) having a total specific activity of 3.37×10^9 cpm was diluted to 100 ml with distilled water to make a 25% (v/v) stock solution. Triton X-100 (0.01%) was added as a surfactant. The weaker solutions, 4 and 10%, were obtained by dilution. Treatment consisted of 30-minute immersion followed by one hour drying prior to radioactivity assay. Tuber size category, weight in grams and $DM^{35}SO$ treatment scheme are shown on the following page.

$DM^{35}SO$ Treatment Scheme

Tuber Category	4% $DM^{35}SO$	10% $DM^{35}SO$	25% $DM^{35}SO$
200 g whole	214.79 g	207.30 g	196.95 g
100 g whole	116.07	104.87	115.11
75 g half	74.50	76.55	73.88
50 g whole	41.61	40.30	46.42

Preparation of Samples and Assay of Radioactivity

Following treatment, each tuber was placed in the tared grinding flask of a Virtis homogenizer and ground five minutes in an ice bath. The grinding medium consisted of 100 ml of 2% (w/v) sulfosalicylic acid for each 100 grams of tissue. A small amount of Dow-Corning Antifoam A was sprayed into the medium to aid in uniform homogenization and elimination of air bubbles. The total weight of the material in the tared flask was then determined. While being stirred magnetically, five aliquots were transferred by a large bore automatic pipet into tared 20 ml Pyrex vials. After weighing, 2 ml of concentrated nitric acid were added to each vial which was covered with a 20 ml beaker, and the contents wet ashed for four hours at 80°C. After cooling, the pH was adjusted to 7.0 with NaOH.

Dilutions and calculations were based on the weight of the wet ashed digest, which then could be related to the fresh weight of the $DM^{35}SO$ treated tuber. To test the method for recovery efficiency,

several control tuber homogenates were fortified with 1 ml of DM^{35}SO having a total specific activity of 2.20 x 10^7 cpm/ml.

Radioactivity was determined with a Packard Tri-Carb Model 3310 Automatic Liquid Scintillation Spectrometer. The ^{35}S was counted at a gain setting of 8.5 and discriminator setting of 50-1000. Bray's scintillation fluid was used (Bray, 1960), and 125 mg of radioactive digest was added to 20 ml of the solution in each counting vial. Quenching and counting efficiency were determined by internal standardization. The efficiency of the system was approximately 60%. All counting data were calculated back to the hour of treatment b y using the decay factors listed by Wang and Willis (1965). Mean counts from five wet ashed samples per tuber were used to calculate the total ^{35}S activity in the homogenate and accordingly the quantity of DM^{35}SO absorbed by each tuber.

Determination of DM^{35}SO Distribution in Whole and Cut Tubers

Plant Material and Treatment

Uniform 'Norgold Russet' tubers, each weighing approximately 100 g, and apical-half tubers weighing 75 g each were used in this study. Source of the tubers and method of selection for condition were the same as specified above. A 25% (v/v) solution of DM^{35}SO containing 0.01% Triton X-100 and having a total specific activity of 5.85 x 10^9 cpm was used for all immersion treatments. Each treatment tuber represented a replicate. Tuber type, number of replicates, length

of immersion and post-immersion time lapse prior to autoradiography are shown:

Tuber	Replicates	Immersion	Time to Autoradiography
whole	5	1 min	1 hour
whole	5	30 min	1 hour
half	5	30 min	1 hour
whole	5	30 min	10 days
half	5	30 min	10 days
whole	5	30 min	1 hour (periderm removed before autoradiography)

Tubers immersed in 25% DMSO required one hour to dry, which was considered zero time of autoradiography. To determine immediate localization patterns occurring during 30 minutes immersion, the tubers were autoradiographed as soon as dry. To determine if DMSO diffused throughout the tuber or became differentially localized over an extended time, some tubers were also autoradiographed after ten days.

Since ^{35}S may have been extruded into internal tissues from the heavily labeled periderm while under compression in a botany press, the following experiment was conducted. Prior to autoradiography in the press, the periderm was removed from whole tubers following DM^{35}SO treatment. These results were compared to sections of tubers with intact periderm.

Autoradiographic Method

Longitudinal and transverse 600μ serial sections were sliced with a sliding microtome. Four longitudinal and four transverse sections were taken from the middle of both whole and cut tubers. Slicing for the longitudinal sections was begun at the distal end of the tuber. To include apical buds, four transverse sections were removed, beginning 2 to 3 mm from the tuber apex.

To insure that ^{35}S localized in the periderm or cortical tissues would not be drawn across the face of the cut tuber during slicing with the microtome blade, tissue sections were lifted free of the blade with flexible forceps before cutting had advanced more than 2 mm. For additional assurance, some sections received a distilled water rinse. All sections, whether or not rinsed, were blotted between filter paper to take up excess moisture before autoradiography.

Autoradiography and Photographic Reproduction

For autoradiographing, four serial sections from treated tissue and two sections from an unlabeled DMSO treated tuber (chemographic control) were apposed to 5 x 7 inch Kodak No-Screen (Estar base) medical X-ray film. Sections and emulsion were separated by 0.5 mil Saran wrap. Aluminum foil was placed over the sections and the assembly was inserted into a lead backed, rigid, Kodak X-ray exposure holder. Loaded holders were positioned in a botany press maintained at -15°C.

After four hours the press was tightened under 20 lbs pressure. Length of exposure was determined empirically (Comar, 1955). Kodak Panatomic-X was used for making negatives and prints were obtained with Kodabromide paper.

Results and Discussion

Absorption of $DM^{35}SO$

Absorption of $DM^{35}SO$ from 4, 10 and 25% (v/v) solutions by whole and apical-half tubers one hour following 30 minutes immersion was approximately linear with treatment concentration (Table 9). One out-of-line value (200 g category whole tuber/25% $DM^{35}SO$) occurred. A difference in the anatomy of the periderm may have been responsible for the deviation. According to radioactive counting, the samples were in close agreement, suggesting no experimental error. More than 98% of the activity in the fortification experiments was recovered.

Absorption by whole tubers was related to tuber weight, small tubers showing a greater percentage uptake (Table 9). This was to be expected since the proportion of exposed tissue to weight of tuber is inversely proportional to tuber weight. Sharrock (1968) verified this relationship for water absorption by whole tubers. He studied uptake by removing cores of tissue from 144 tubers in the size range of 50-500 g. These samples were immersed for 24 hrs in distilled water,

dried, reweighed and the percentage increase in weight calculated. Figure 8 shows a visually fitted curve of the results when the sample was plotted with percentage water uptake by weight against initial weight of the tubers. Sharrock suggested that in any absorption study, the variable shape of tubers must be taken into consideration because this would also affect the relation of proportion of exposed tissue to initial weight.

Apical-half tubers absorbed over five times more $DM^{35}SO$ than did whole tubers (Table 9). The following autoradiographic study presents evidence for an explanation of this difference.

Penetration and Distribution of $DM^{35}SO$

The previous absorption experiment demonstrated that amounts of $DM^{35}SO$ taken up by whole and apical-half tubers were markedly different. Within the two tuber types, however, uptake was approximately linear with treatment concentration. This suggested that although tissue quantity differed, $DM^{35}SO$ localization probably did not vary with the strength of the immersion solution in the range 4 to 25%. Consequently, a single concentration could be used to study penetration and distribution without biasing the results. In order to maximize specific activity and shorten autoradiographic exposure time, the concentration chosen was 25% (v/v) $DM^{35}SO$.

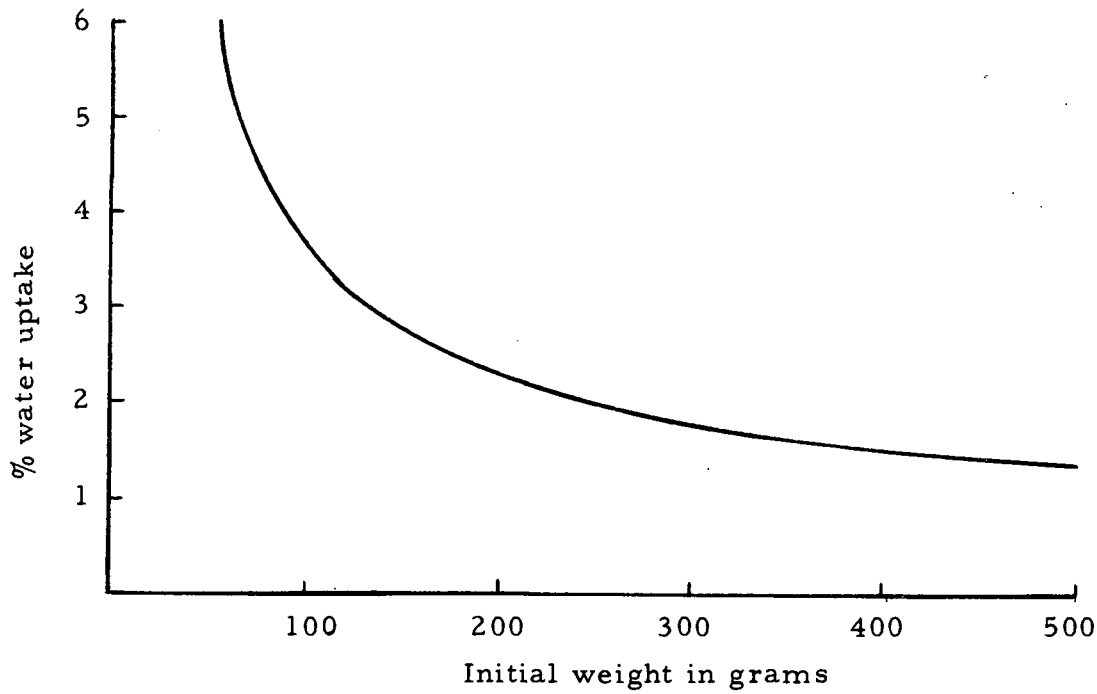


Figure 8. Relation between water uptake and weight of tuber for a single 144-tuber sample (after Sharrock, 1968).

Table 9. Absorption in mg/g fresh weight of $DM^{35}SO$ by dormant 'Norgold Russet' (PA-3L) whole and apical-half tubers immersed 30 minutes in three concentrations (v/v) of labeled DMSO. Values in parenthesis represent mean amount absorbed when corrected for concentration and averaged over the three original values. These means are used in Section IV to calculate estimated DMSO absorption (Tables 10-12).

Tuber Category	4% $DM^{35}SO$	10% $DM^{35}SO$	25% $DM^{35}SO$
200 g whole	0.0849	0.1930	0.2038
100 g whole	0.0930	0.2810	0.5304
	(0.0968)	(0.2419)	(0.6047)
75 g apical-half	0.5200	1.4700	3.4800
	(0.5549)	(1.3873)	(3.4683)
50 g whole	0.1087	0.3434	0.6843

Distribution of radiolabel was assumed to represent that of intact $DM^{35}SO$ in tubers sectioned within one hour following treatment. Label distribution in sections made ten days following treatment could represent that of intact $DM^{35}SO$ and its metabolic products, including $DM^{35}S$ and $DM^{35}SO_2$. After six-weeks' sprouting, however, most of the DMSO absorbed by potato tubers was found as unaltered compound in sprouts, periderm and internal tissues (see Section IV). Undoubtedly, label distribution in the 10-day sections represents primarily $DM^{35}SO$.

Autoradiographs selected for photographic reproduction and presented in Figures 9-24 were typical of those from serial sections of all replicates. No artifacts were produced by the chemographic controls.

Whole Tubers

When tubers were immersed less than one minute, intact periderm was not readily penetrated by $DM^{35}SO$ after one hour (Figure 9). Tubers immersed 30 minutes showed $DM^{35}SO$ localization in the periderm and in the first few millimeters of cortex after one hour (Figures 10 and 14). Resistance of bud tissue to penetration was many times less than that of intact periderm and $DM^{35}SO$ moved into the surrounding cortex rapidly (Figures 12-14). A single needle puncture greatly reduced resistance to penetration, as depicted by the heavily

labeled spot in Figure 11.

In Figures 11 and 12 are shown sections from which the periderm, an eye and approximately 5 mm of underlying cortical tissue were removed one hour after immersion for 30 minutes in $DM^{35}SO$, before the sections were exposed to X-ray film. These autoradiographs verify that penetration into cortical tissue was not an artifact of compression during exposure of frozen sections in a botany press under 20 lbs pressure. They depict a greater depth of $DM^{35}SO$ penetration than that shown in Figures 10 and 14. The peeled sections, however, were exposed for 1000 hours, whereas, those shown in Figures 10 and 14 were exposed 200 hours and 500 hours, respectively. Apparently, trace amounts of $DM^{35}SO$, which were not detected with less exposure time, reached the midcortical region in one hour following treatment.

Mechanical resistance to diffusion is an inherent feature of the compact, suberized tissue of intact potato periderm; consequently, it is comparatively impermeable to water loss and uptake (Burton and Hannan, 1957). Burton (1955) found the rate of evaporation from mature tubers stored a month at 10°C following harvest was only 0.01 to 0.015 $mg/cm^2/hr/mm$ Hg VPD. If tubers were peeled and subject to free air circulation the rate increased to nearly 5 $mg/cm^2/hr/mm$ Hg VPD. Wound periderm is as resistant as normal periderm to movement of water. Tuber sprouts were intermediate in water retention,

the loss being about 1.0 to 1.4 mg/cm^2 sprout surface/hr/mm Hg VPD. Smith and Rappaport (1965) contended that the primary avenue of entry for aqueous hormone solutions such as gibberellic acid was through tuber buds and not periderm. In the present study, penetration of DM^{35}SO into whole tubers was much greater through buds than through periderm.

Movement of DM^{35}SO through the bark of trees and through leaves and fruit has been demonstrated (Garren, Jr., 1967; Keil, Smale and Wilson, 1969; Smale, 1969). Smale (1969) emphasized the importance of a surfactant in enhancing DMSO penetration through stem tissue. He used 0.1% Triton X-100 and obtained 40 times greater uptake. Although 0.01% Triton X-100 was used with DM^{35}SO in the present study a higher concentration may have influenced greater penetration through potato periderm.

Artschwager (1924) and Goodwin (1967a) demonstrated that vascular tissues within buds are joined to the dispersed xylem ring and phloem groups of the tuber itself. It is interesting to speculate why DM^{35}SO, which rapidly entered buds and adjacent cortical tissue, was never detected in the xylem ring of treated whole tubers. The compound enters xylem vessels readily when cut tubers are immersed in aqueous DM^{35}SO (Figures 17, 18 and 22). A possible explanation is suggested from the data of Goodwin (1967a). He found that xylem vessels at the base of buds on dormant tubers were blocked with

tannin-like substances. Generally, 10 to 50μ of each vessel appeared to be completely filled with this deposit and an additional length of 40-150μ was incompletely filled. Deposits were not found in the vessel elements within the bud itself or elsewhere in the tuber. When sprout growth commenced, new vessels with clear lumina were differentiated from procambial tissues, but the old vessels remained filled with the tannin-like material.

Goodwin (1967a) observed obstruction of xylem vessels in apical buds which had been growing until tubers were harvested and in lateral buds which passed directly from dormancy into correlative inhibition. In the present study diffusion of rapidly moving $DM^{35}SO$ through buds could be confined to slower permeation of adjacent cortical parenchyma under identical cytological limitations. Once the original influx had been absorbed by the cells, it was unlikely that rapid movement into water conducting vessels would ensue, even though DMSO is preferentially attracted to aqueous routes in biological tissues (Elford, 1970).

After ten days, distribution of $DM^{35}SO$ was detected throughout whole tubers including the growing sprouts (Figures 15 and 16). Label was not found in the dispersed phloem (white flecked areas) which was found throughout the tuber, both internal and external to the xylem ring. These unlabeled areas were verified to be authentic phloem by comparing the autoradiograph with the phloem pattern of the original

tissue section. No attempt was made to determine if ^{35}S had entered the intercellular spaces.

The ability of DMSO to easily penetrate animal and plant membranes is well documented in the literature. Based on their work with yeast cells, De Bruijne and Van Steveninck (1970b) proposed DMSO penetration follows simple first-order kinetics, active transport not being involved. Yeast cells treated with up to 20% DMSO formed a steady state equilibrium at an intercellular concentration of 70% of the medium concentration (De Bruijne and Van Steveninck, 1970a). The DMSO was present in these cells in an osmotically active form, and no substantial adsorption inside the cell took place. Whether the physics of uptake is similar with potato tuber cells remains to be shown.

Detailed studies were not made of the intracellular localization of $DM^{35}SO$ in potato parenchyma. According to the known behavior of DMSO and from the gross autoradiographic data, however, the compound apparently was localized within individual cells, the concentration depending on proximity to the source of influx as well as the length of subsequent incubation. Parenchyma cells contain water-filled vacuoles whereas the protoplast of phloem sieve cells and sieve-tube elements is much more viscous. These latter cells contain callose at the sieve area as well as sucrose and related oligosaccharides at concentrations exceeding 20%. Variable amounts of

Autoradiographs of 'Norgold Russet' whole seed potato tubers immersed in 25% (v/v) $DM^{35}SO$ for 30 minutes, except Figure 9 (<1 min).

Figure 9. Longitudinal mid-tuber section cut one hour following treatment. Section is free of buds and shows label localization in periderm without further penetration.

Figure 10. Longitudinal mid-tuber section cut one hour following treatment. Dense label is localized in periderm and beneath bud of an eye. Cortical tissue adjacent to periderm is labeled and demonstrates $DM^{35}SO$ penetrates the periderm during a 30 minute immersion treatment. Compare with Figure 9.

Figure 11. Longitudinal mid-tuber section cut one hour following treatment. The tuber was injured with a single puncture from a large bore needle prior to treatment. Following treatment the periderm was removed and the section then exposed to film. Autoradiograph shows dense ^{35}S localization at point of periderm injury. Penetration of $DM^{35}SO$ into cortex is verified as not being a compression artifact occurring during exposure.

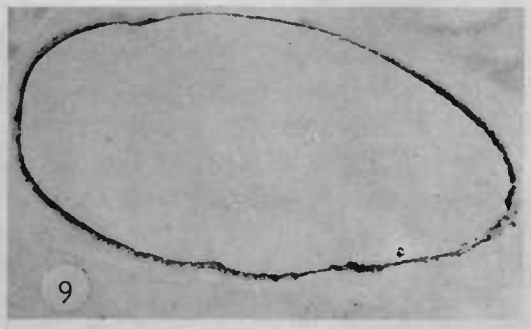
Figure 12. Section similar to Figure 11. Autoradiograph illustrates rapid $DM^{35}SO$ penetration through a bud into surrounding tissue. The bud was removed before exposure to film.

Figure 13. Transverse section of a whole tuber apex cut one hour following treatment showing dense label localization in the apical bud area, periderm and adjacent cortex.

Figure 14. Transverse mid-tuber section cut one hour following treatment showing labeling in periderm and adjacent cortex, especially beneath a bud. Compare with Figure 10.

Figure 15. Transverse section cut ten days following treatment from the apical region of a tuber. Radiolabel is distributed in all tissues except the dispersed external and internal phloem.

Figure 16. Transverse mid-tuber section cut ten days following treatment. Radiolabel is distributed as in Figure 15 and shows localization in growing sprout.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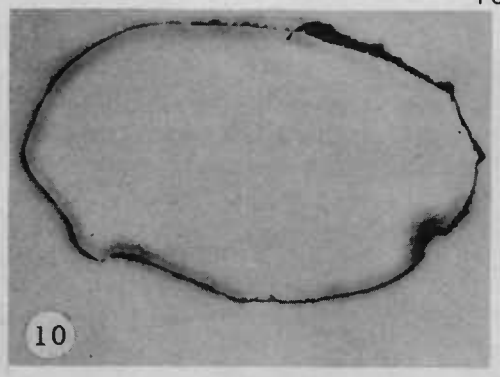
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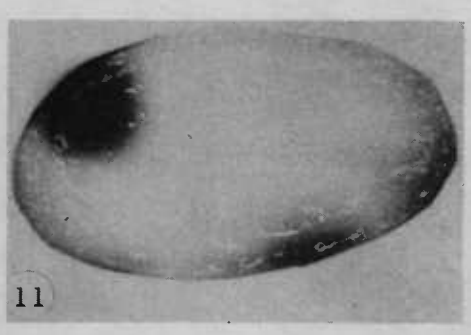
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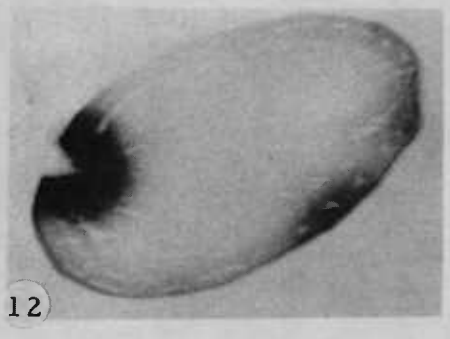
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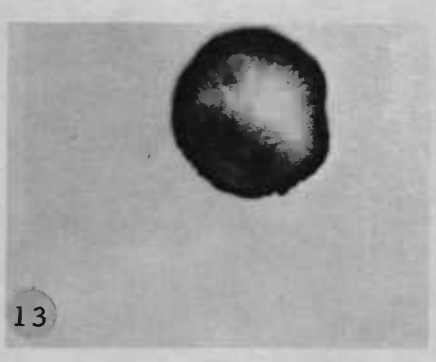
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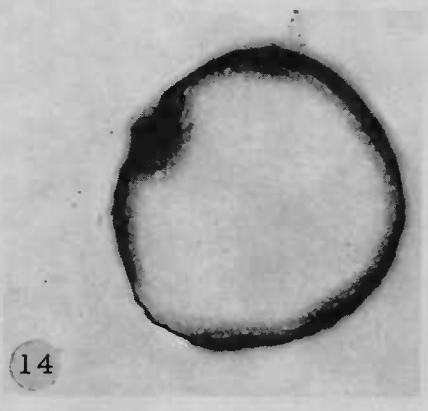
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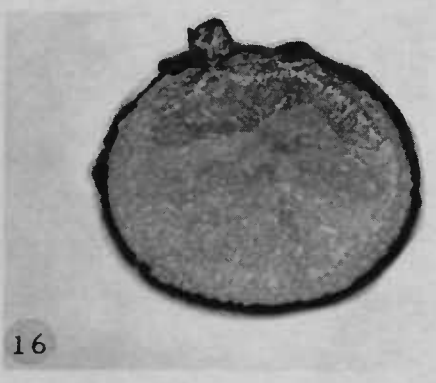
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proteinaceous slime dispersed in the vacuolar sap are also present (Esau, 1965). A portion of the phloem may be nonconducting because the sieve elements have ceased to function. The non-aqueous nature of this tissue may explain why $DM^{35}SO$ was not observed to penetrate potato tuber phloem in whole tubers, even after ten days incubation.

Two metabolites of $DM^{35}SO$ which have been identified from treated seed potato tuber tissue (Section IV) may account for a small percentage of the labeling of ten-day sections. The hydrophilic product $DM^{35}SO_2$ probably follows the distribution of $DM^{35}SO$, in which it is soluble. The metabolite $DM^{35}S$, while considered hydrophobic, will dissolve in water to the extent of 2% (w/v) at 25°C (Crown Zellerbach Corporation, 1969). Most of the tissue-associated $DM^{35}S$ may have been lost through volatilization (B.P. 37.3°C) during preparation of sections prior to autoradiography consequently contribute little to the localization pattern of ^{35}S in ten-day sections.

Apical-half Tubers

After one hour following treatment, the cut surface of apical-half tubers was penetrated 5 mm by $DM^{35}SO$ (Figures 17 and 18). The xylem ring, an accessible aqueous route, was distinctly labeled. Vascular groups of the xylem have been reported to be discontinuous in mature potato tubers (Artschwager, 1924). This is especially evident from the vascular labeling shown in longitudinal (Figure 18) and

transverse (Figure 22) sections. Penetration of apical buds and the subsequent distribution of $DM^{35}SO$ in one-hour sections was identical in apical-half and whole tubers (Figures 21 and 13).

In preliminary studies, apical-half tubers immersed for 30 minutes in 25% (v/v) DMSO lost approximately 32% more water after one hour's treatment than did tubers immersed in 0% DMSO. Cowie and Toporowski (1961) proposed that a 2 to 1 association hydrate, accompanied by the evolution of considerable heat, is formed by DMSO with water. This hydrate contains about 67 volume percent DMSO. They contend that the hydrogen bonds which exist between water and DMSO are stronger than those existing between water molecules. Since the most abundant constituent of the cell is water, and because proteins, polysaccharides and nucleic acids are sheathed in an ordered arrangement of bound water, it has been rationalized that DMSO could alter the configuration of each of these substances.

Gerhards and Gibian (1967) found that the water in animal skin or cornea can be replaced by DMSO up to 100 molar percent. Within one hour after immersion of Guinea-pig taenia coli muscle in Kreb's solution containing 20% DMSO, Elford (1970) showed the solvent to be distributed uniformly throughout a volume equivalent to the total initial water content. He proposed that there was simultaneous permeation into cells and radial diffusion in extracellular fluid. These data may explain why $DM^{35}SO$ readily permeated tissue beneath the cut surface

of apical-half tubers.

Label density adjacent to the cut in both one-hour (Figures 17 and 18) and ten-day sections (Figures 19 and 20) indicates $DM^{35}SO$ localization in the intercellular spaces. Woolley (1962) and Laties (1957, 1962) found water or aqueous solutions passed through potato tuber tissue only with great difficulty. Laties was unable to force water through tissue on a Seitz filter. Woolley found a pressure differential of four bars necessary to force about 0.1 ml water or sucrose solution per hour through each square centimeter of cross section of a cylinder 1.5 cm in length. Under these conditions air was displaced and the intercellular spaces became filled with water. Tissue merely submerged in water did not form an accumulation in the spaces. Diluted india ink penetrated only about 3 mm, the carbon particles flocculating into aggregates which could not pass through the spaces, regardless of whether the pressure was applied for 1 or 24 hours. Apparently, $DM^{35}SO$ is a unique solvent capable of displacing air or reacting with water vapor in the intercellular spaces. Additional work is needed to determine if the present autoradiographs represent a true account of the intra- and extracellular localization of $DM^{35}SO$.

Ten days after treatment, the dense $DM^{35}SO$ front observed in one-hour sections had moved 3 to 4 times the original distance (Figures 19 and 20). Concentration of $DM^{35}SO$ and/or its metabolites

was much less in the remaining tuber. The presence of ^{35}S was observed in sprouts (Figures 20, 23, and 24) but the concentration appeared to be no greater than that in the surrounding cortical and medullary tissues. Another less densely labeled front extending basipetally from the tuber apex is shown in Figure 19. The origin of the label was probably the cortical sink formed by an influx of $DM^{35}SO$ through apical buds. Distribution of $DM^{35}SO$ and its metabolites after ten-days metabolism was identical in mid-tuber transections from both apical-half tubers (Figures 23 and 24) and whole tubers (Figures 15 and 16).

Phloem tissue appeared to be labeled only in the area of greatest ^{35}S concentration near the cut surface of apical-half tubers (Figures 17-20). Elsewhere the phloem existed as an unlabeled semi-anastomosing system dispersed throughout the tuber. Edelman, Jefford and Singh (1969) were not able to confirm whether the phloem forms a true anastomosing system. They found some evidence for a specific translocatory pathway such as symplast connected to the growing sprout. These authors implied that elucidation of the degree of interconnection within the phloem of potato tubers would be a valuable contribution.

Autoradiographs of 'Norgold Russet' apical-half seed potato tubers immersed 30 minutes in 25% (v/v) $DM^{35}SO$.

Figure 17. Longitudinal mid-tuber section cut one hour following treatment, showing dense label localization in periderm and an advancing front inward from the cut tuber surface. Rapid label penetration is evident in both vascular ring and immediately adjacent tissues, excluding phloem strands.

Figure 18. Section similar to Figure 17 except $DM^{35}SO$ localization is discontinuous in the characteristically segmented vascular ring of a potato tuber.

Figure 19. Longitudinal mid-tuber section cut ten days following treatment. Dense radiolabeled front has advanced 3X over sections cut at one hour (Figures 17-18). Distribution of label appears throughout all tissues except the dispersed external and internal phloem. Heavier labeling appears in the apical region resulting from rapid $DM^{35}SO$ penetration through apical buds, as illustrated in Figure 21.

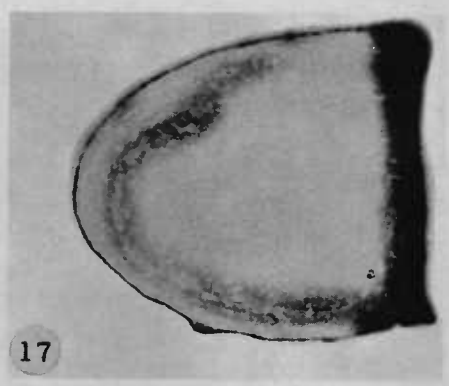
Figure 20. Section similar to Figure 19 except ^{35}S is evident in the growing sprout.

Figure 21. Transverse section of a tuber apex cut one hour following treatment showing dense label localization in the apical bud area and periderm.

Figure 22. Transverse mid-tuber section cut one hour following treatment, showing labeling in periderm, cortical tissue and the segmented vascular ring area. Compare with Figure 18.

Figure 23. Transverse section of a tuber apex cut ten days following treatment. Radiolabel is distributed throughout all tissues except the dispersed external and internal phloem. Compare with Figure 15.

Figure 24. Transverse mid-tuber section cut ten days following treatment, showing ^{35}S distribution in all tissues except phloem.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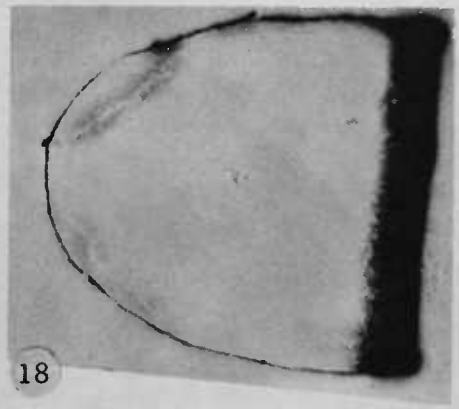
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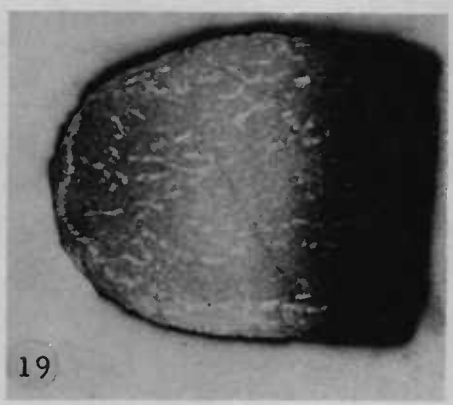
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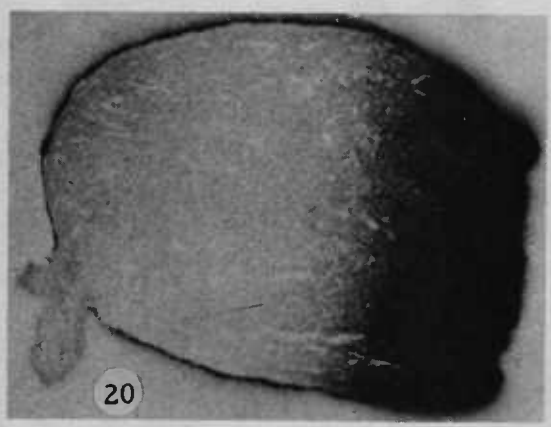
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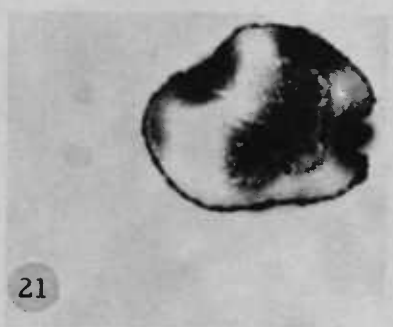
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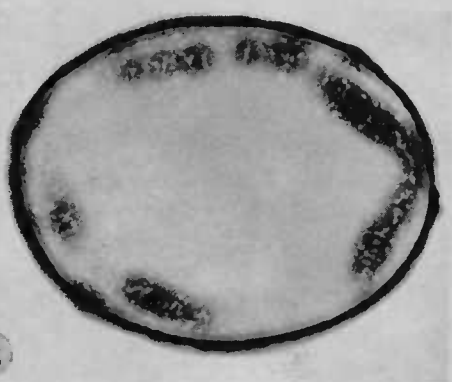
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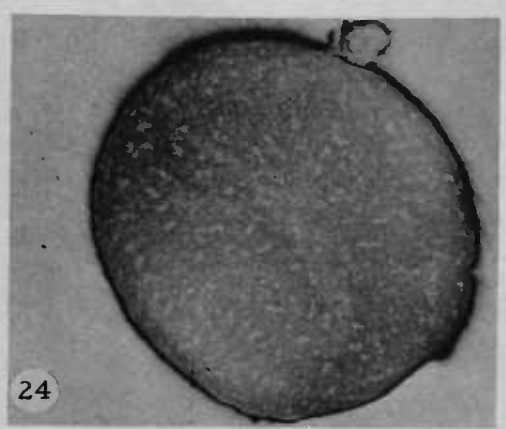
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IV. METABOLISM OF DMSO IN TREATED 'NORGOLD RUSSET' SEED POTATO TUBERS

Materials and Methods

Volatile Organic Sulfur Metabolism Following Treatment

Preparation of Standards

Mercuric chloride forms coordination complexes with sulfides, insoluble mercaptides with thiols and a decomposition complex with disulfides (Robinson, 1963). The complex formed with bis-methyl-thiomethane has not been reported. The original compounds can be regenerated by treating the mercuric chloride precipitates with acid, except in the case of disulfides (Robinson, 1963). Disulfides are split by mercuric salts so they cannot be quantitatively isolated in their original form. Nevertheless, Gumbmann and Burr (1964) were able to utilize the acid regeneration method to advantage in a gas chromatographic analysis of disulfides from cooking potatoes.

Four standard reference compounds: methane thiol (CH_3-SH), dimethyl sulfide (CH_3-S-CH_3), dimethyl disulfide (CH_3-S-S-CH_3) and bis-methyl thio methane (CH_3-S-CH_2-S-CH_3) (Crown Zellerbach Corp.) were either bubbled through 5% mercuric chloride ($HgCl_2$) or added directly to it. In all cases white precipitates formed. These were filtered, washed with distilled water, dried and then stored in

separate vials in a desiccator. Formulas for three of the above sulfur compounds precipitated with $HgCl_2$ have been elucidated:

Methane thiol:	$(CH_3\text{-}S)_2$ Hg and CH_3-S HgCl (Blackburn and Challenger, 1938)
Dimethyl sulfide:	$2(CH_3)_2\text{-}S \cdot 3HgCl_2$ (Faragher, Morrell and Comay, 1929; Phillips, 1901)
Dimethyl disulfide:	CH_3-S HgCl, $xHgCl_2$ and CH_3SO_2H (Blackburn and Challenger, 1938)

Regeneration of Volatile Sulfur Standards from their Mercuric Chloride Complexes

Ten mg of the dried precipitate from each mercuric chloride complex were placed in separate 25 ml Erlenmeyer flasks and stoppered with previously boiled rubber serum caps. Another flask contained a mixture of all four precipitate species. Hydrochloric acid purified by passing through activated charcoal was diluted to 6N and 0.5 ml was injected through the serum cap. The flasks were warmed a few minutes at 30°C. Samples were then removed for gas chromatographic analysis.

Gas Chromatography of Regenerated Volatile Sulfur Standards

One ml aliquots of the regenerated volatiles were taken up with a Hamilton gas tight syringe and chromatographed under the following conditions:

GLC Instrument	F & M Model 810 (hydrogen flame ionization detector)
Column	6 foot x 1/8-inch OD stainless steel. Packed with 80-100 mesh Porapak Q
Column temperature	200°C.
Injector temperature	175°C
Detector temperature	250°C
Carrier gas flow rate	50 ml/min N_2
Recorder	1 mV series 8000 Barber-Coleman

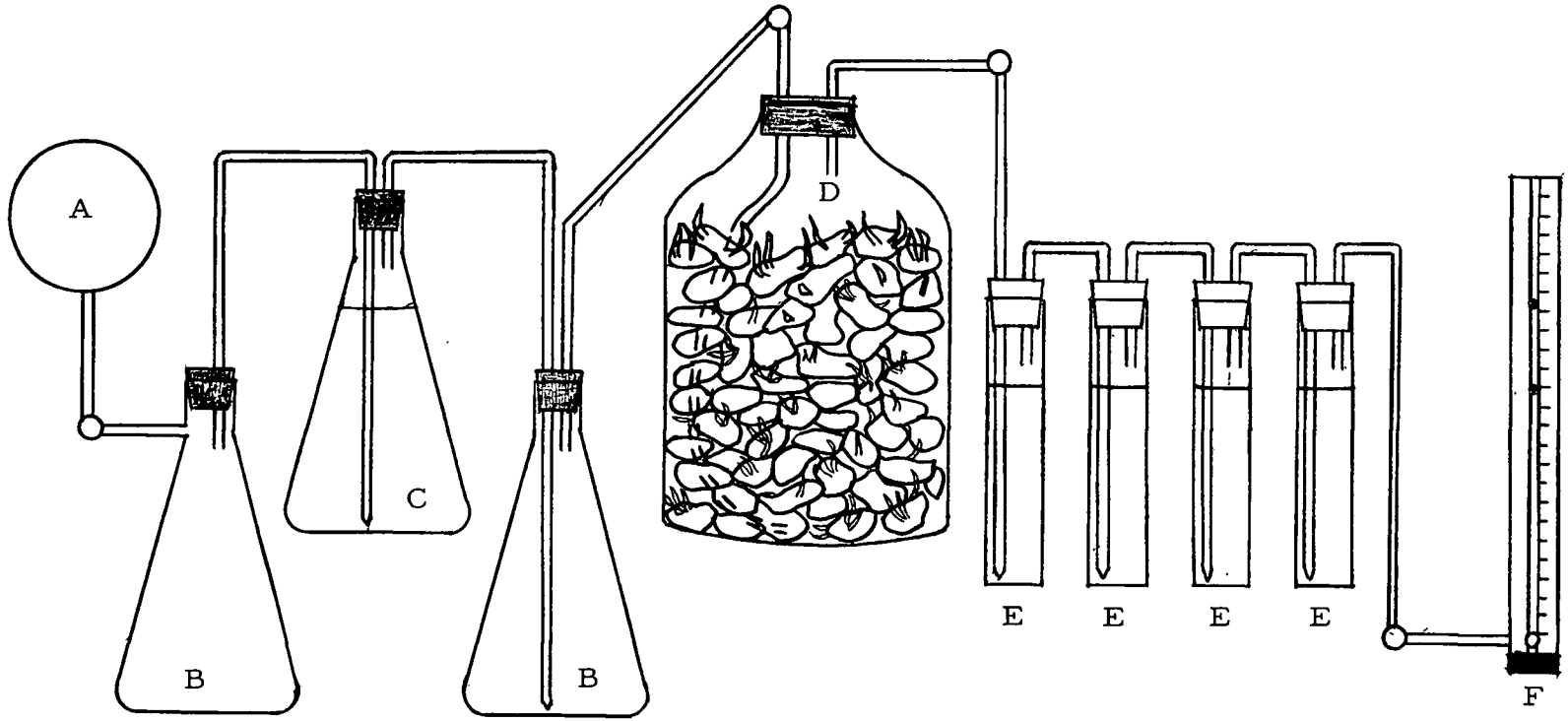
Collection of Volatile Organic Sulfur Metabolites from Treated Samples

Preliminary Qualitative Experiment (1969). A preliminary experiment was performed to determine if DMS (the only reported volatile sulfur metabolite of DMSO) or other sulfur compounds could be recovered from DMSO treated seed potatoes allowed to sprout six weeks in glass vessels which were held in the dark at 20°C. Whole Oregon Foundation Grade 'Norgold Russet' seed potato tubers (PA-3LM) were obtained and selected for PA in a manner similar to that described in the Sprouting and Sprout Morphology Section. Three groups of 95 tubers weighing 9575 g, 9610 g and 9590 g fresh weight were treated (standard immersion method), respectively, with 0, 10, and 25% (v/v) aqueous DMSO. The three groups were separately placed in stoppered five-gallon glass vessels containing inlet and

outlet tubes. The inlet tube was connected to a 5% potassium permangante one-liter trap to remove sulfur contaminants from a fresh air stream which was drawn from outdoors at a rate of 100 ml/min. The outlet tube was joined to a series of eight traps connected in tandem. Each of the first four traps contained 200 ml of 4% mercuric cyanide and the remaining four traps contained the same quantity of 3% mercuric chloride. Mercuric cyanide selectively removes thiols from gas streams containing thiols and sulfides (Robinson, 1963). Precipitates forming in the absorption train were filtered weekly, washed with water, dried, regenerated and gas chromatographed with the standards.

Qualitative and Quantitative Estimations (1970). On the basis of results from the preliminary experiment an extensive study was made with the same variety and PA class. The following modification was made. Only the 3% mercuric chloride traps (four in tandem) were used for scavenging sulfur volatiles. A diagram of the absorption apparatus is shown in Figure 25.

Whole and apical-half tubers immersed for 30 min in 0, 4, 10 and 25% aqueous DMSO comprised one part of the experiment. Two other treatments were also included: a group of 10% DMSO treated apical-half tubers and a similar group of 25% DMSO treated whole tubers were immersed, 72 hrs later, for five seconds in 0.5%



(A) vacuum pump
(B) safety trap
(C) 5% potassium permanganate trap
(D) potato tuber sample
(E) 3% mercuric chloride trap
(F) flowmeter

Figure 25. Diagram of absorption apparatus for collection of volatile organic sulfur compounds.

CIPC [isopropyl N-(3-chlorophenyl) carbamate, Columbia-Southern Co.] containing 0.01% Triton X-100. These treatments compared the metabolism of DMSO in the presence and absence of sprouting.

The sample size, including fresh and estimated dry weight for all treatment groups, is presented as part of Tables 11 and 12. The experiment continued for six weeks at 20°C in the dark. At seven-day intervals, precipitates accumulating in the traps were filtered, washed with distilled water, dried over silica gel and weighed on an analytical balance. These weights provided quantitative estimations of the volatile organic sulfur metabolism of DMSO in potato tissue, as fully described in Results and Discussion. Ten mg samples from each seven-day precipitate collection were regenerated and gas chromatographed with the standards for qualitative analyses.

Extraction and Qualitative and Quantitative Assay of Other Organic Sulfur Compounds Following Treatment

Extraction Method

Upon termination of both the preliminary and final volatile recovery experiments after six-weeks' sprouting, each treatment group was separated into sprouts, periderm and internal tissues. Tissue fractions were ground with five volumes of 80% reagent grade acetone in a stainless steel Waring blender until thoroughly homogenized. The homogenate was kept in stoppered flasks for 48 hours with occasional

agitation. The residue was then filtered through S & S No. 589 filter paper, resuspended in five volumes of 95% acetone and refluxed at 100°C for one hour.

Following filtration of the refluxed material, the original and final filtrates were combined and reduced in vacuo at 35°C to 100 ml. This amount of filtrate consisted of a water-acetone azeotrope in which were dissolved the unknown sulfur components. Certain suspended lipids and fatty materials were removed either by aspiration or centrifugation. The clarified solution was finally reduced in vacuo to 20 ml, brought to 60 ml with absolute methanol, and stored in serum-stoppered Wheaton bottles at 0°C until gas chromatographed.

Gas Chromatography of Extracted Compounds

DMSO and $DMSO_2$ (supplied by the Crown Zellerbach Corp. as either pre-purified or 99% minimum purity standards) were used as reference compounds. This was considered advisable because of the likelihood of these being present in DMSO-treated tissue as indicated in the review of literature. Standard solutions were prepared by initially obtaining extracts of untreated whole sprouted 'Norgold Russet' tubers grown in the dark in a room isolated from DMSO-treated plant material. To the extracts were added known quantities of standards to yield solutions of 1, 5 or 10 μg/μl. This method of preparing reference solutions more closely approached the conditions of the test

samples because previous experiments showed standards in methanol or water produced larger GLC peaks.

Extracts from DMSO-treated and untreated tubers were gas chromatographed under the operating conditions shown below. An on-column injection system was used because $DMSO_2$ was found to have a tendency to 'hang-up' in the injection port. Following each injection of sample, the column was washed with four 1µl volumes of distilled water. Under these conditions, good peak area replication was achieved.

GLC Instrument	F & M Model 810 (hydrogen flame ionization detector)
Column	8 foot x 1/8-inch OD stainless steel. Packed with 10% Carbowax 20M on 80-100 Diatoport S (Hewlett-Packard Corp.)
Column temperature	150°C
Injector temperature	225°C
Detector temperature	300°C
Carrier gas flow rate	50 ml/min N_2
Recorder	1 mV series 8000 Barber-Coleman

GLC recovery (zero time) verifications of DMSO absorbed by samples of whole and apical-half tubers were performed. After the surface of the tubers had dried following a standard 30-minute immersion in 25% (v/v) DMSO, they were extracted with acetone as previously

described. Data from this recovery experiment were compared for agreement to that obtained by the radiometric method of estimating DMSO absorption at zero time (see page 68). Dimethyl sulfone recoveries were verified by fortification of control homogenates and subjecting these to extraction and GLC.

Compounds which could be resolved into separate peaks were identified routinely by co-chromatography and agreement of retention time with the standards. Quantification was achieved by triangulating peak areas according to the method of McNair and Bonelli (1969).

Supplemental and Confirmatory Identification of Organic Sulfur Compounds Isolated by GLC Following Treatment

Flame Emission Detection

Gas chromatographic peaks of regenerated sulfur volatiles and peaks identified from extracts of DMSO-treated tissue were further substantiated by verifying the presence of sulfur with a Melpar Flame Photometric Detector (Melpar, Inc.) attached to a Model 200 Aerograph gas chromatograph. The respective columns and other operating parameters for GLC separation of the various sulfur components were the same as those stated earlier.

Under the conditions of flame photometric detection (FPD), a photomultiplier tube monitors the chemiluminescent emission above a hydrogen-rich, hydrogen-air flame. Insertion of a narrow bandwidth

filter (394 mμ) between the flame emission detector on the GLC and the photomultiplier makes the response selective for detection of sulfur components only. FPD is relatively insensitive to hydrocarbons. Selectivity of over 50, 000 to 1 has been reported for sulfur compounds as opposed to organic compounds devoid of this element. Consequently, for most trace analyses a confirmed FPD response represents sulfur (Grice, Yates and David, 1970).

Mass Spectrometry

Mass spectral data were obtained to confirm identity of the volatile and other sulfur compounds isolated by GLC. Spectra were obtained from three samples showing typical GLC peaks representative of all samples taken during the entire experimental period. These were:

1. A regenerated volatile organic sulfur metabolite sample from whole tubers treated with 25% DMSO.
2. An extracted tissue sample from whole tubers treated with 0% DMSO.
3. An extracted tissue sample from whole tubers treated with 25% DMSO.

An Atlas CH-4, Nier-type (9-inch, 60 degree sector) single-focusing, dual ion source mass spectrometer equipped with an EC-1 intake valve was used in conjunction with an F & M Model 810 gas

chromatograph. Compounds were separated by GLC and, as the peaks emerged, approximately ten percent of the GLC column effluent was allowed to enter the ionization chamber of the mass spectrometer. The remaining effluent was vented into the air through a heated tube.

Spectra were taken at peak apexes and on the leading and trailing edges to insure adequate coverage of both resolved and incompletely resolved components. Some spectra were also recorded at arbitrary intervals before and following peak emergence. These served as backgrounds to account for column bleed or sample retention.

The instrument has two ion sources (20 eV and 70 eV), the 70 eV source being used to provide the fragmentation patterns. These were recorded as mass spectra using a Honeywell Model 1508 Visicorder. Operating conditions for the GLC-MS analyses were:

GLC (volatile sample)

Column	Porapak Q (previously described)
Column temperature	175°C
Carrier gas	Helium, 30 psi

MS (volatile sample)

Scan speed	2.8 sec to cover m/e 14 - 200
Filament current	20μA
Electron voltage	70 eV
Accelerating voltage	3 KV

Analyzer pressure	1×10^{-6} Torr
Multipler voltage	1.60 KV

GLC (extracted samples)

Column	10% Carbowax 20 M (previously described)
Column temperature	165°C
Carrier gas	Helium, 25 psi

MS (extracted samples)

Scan speed	2.8 sec to cover m/e 14 - 200
Filament current	40 μA
Electron voltage	70 eV
Accelerating voltage	3 KV
Analyzer pressure	1×10^{-6} Torr
Multiplier voltage	1.60 KV

Results and Discussion

Gas Chromatography of Reference Sulfur Compounds

The four compounds selected as references for detection of volatile metabolites from DMSO treated potato tubers were easily separated on Porapak Q by GLC following acid regeneration from their mercuric chloride complexes. The order of elution was according to molecular weight as shown in Figure 26. Part of the dimethyl disulfide may be accounted for as methane thiol because of the decomposition which

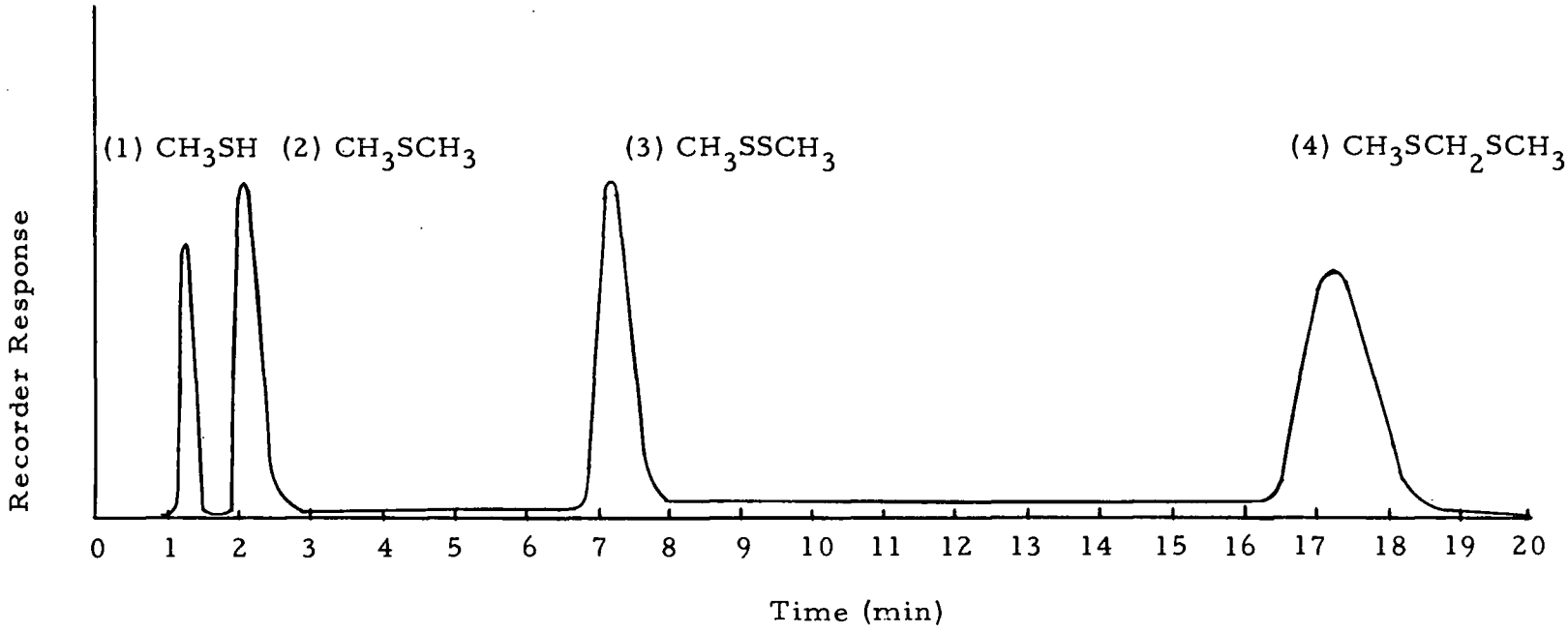


Figure 26. Gas chromatographic separation of volatile sulfur reference compounds [(1) methane thiol, (2) dimethyl sulfide, (3) dimethyl disulfide, (4) bis-methylthiomethane] acid regenerated from their mercuric chloride complexes. Six-foot by 1/8-inch stainless steel column; Porapak Q 80-100 mesh.

occurs upon reaction with mercuric chloride (Gumbmann and Burr, 1964). No report was found concerning the nature of the regenerated product from the complex with bis-methylthiomethane.

Dimethyl sulfoxide and $DMSO_2$ standards were well separated on Carbowax 20 M. A typical chromatogram is presented in Figure 27. Retention times closely agreed with those reported by Hucker et al. (1967). Plots of area under the peaks vs. concentration were linear for $DMSO_2$ and nearly linear for DMSO.

Recovery of Absorbed DMSO and Fortification of $DMSO_2$ at Zero Time

Recovery of absorbed DMSO by extraction and GLC following a standard 30-minute immersion treatment was compared for agreement with the radiometric assay reported in Section III. The results are shown in Table 10. Agreement of values from radiometric assay with GLC were approximately 80% with whole tubers and 75% with apical-half tubers. Radiometric assay is generally accepted as more sensitive than other methods. GLC extraction methods for removal of DMSO from plant tissue may have been subject to certain limitations. Tomita and Terajima (1970) report DMSO strongly adsorbs to starch in solution. Gerhards and Gibian (1967) and Rammler and Zaffaroni (1967) have shown some DMSO is irreversibly bound to biological tissues, but the amount is small. That DMSO was not lost during

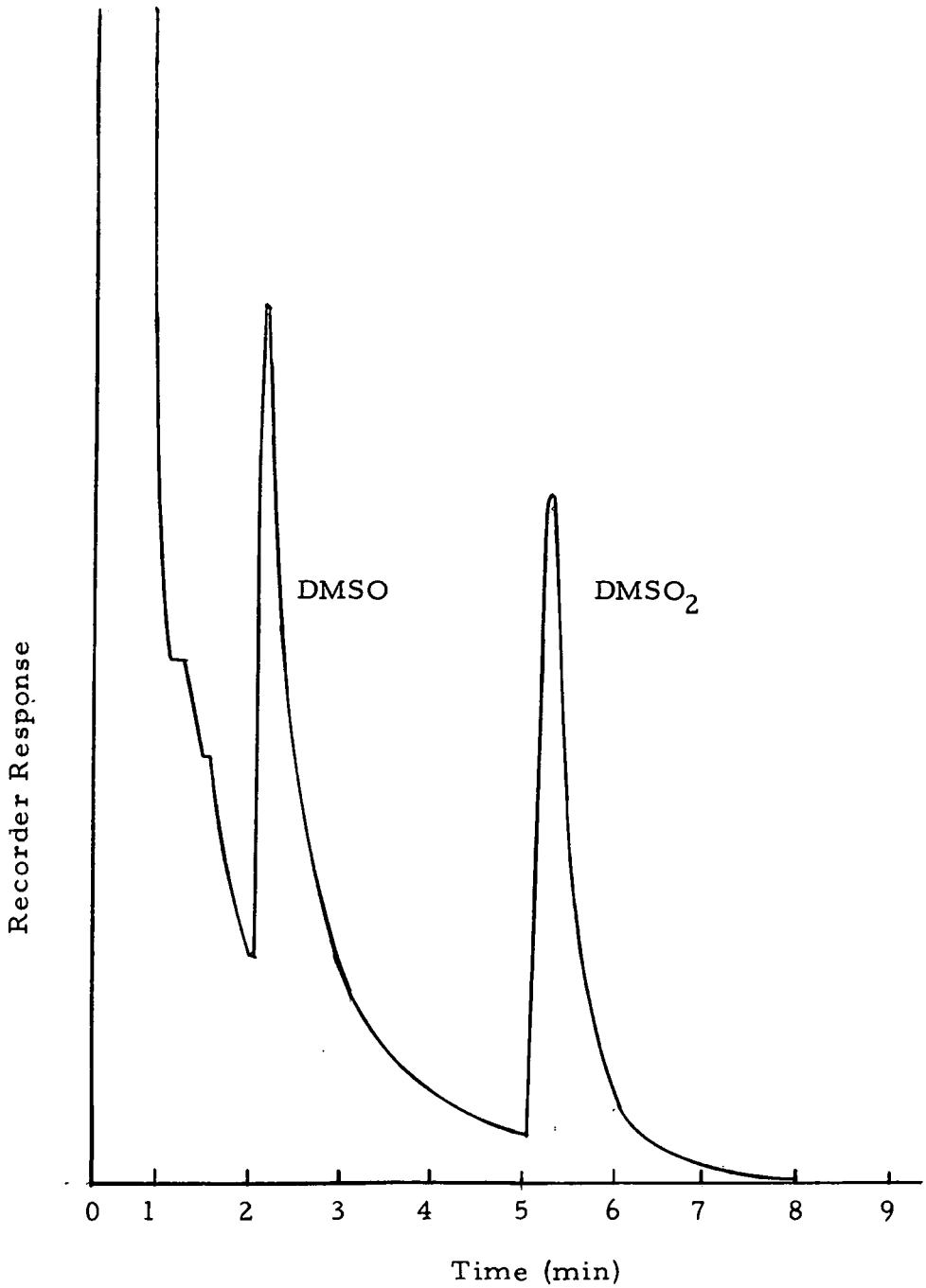


Figure 27. Gas chromatogram of 5 μg each of DMSO and $DMSO_2$ in an acetone extract of untreated whole, sprouted 'Norgold Russet' tubers. Separation on 8-foot by 1/8-inch stainless steel column; 10% Carbowax 20 M on 80-100 Diatoport S.

Table 10. Gas chromatographic analysis of DMSO absorption as compared to radiometric assay. Tubers immersed in 25% (v/v) DMSO 30 minutes and prepared for extraction and assay within one hour of treatment.

Seed Tubers	Fresh Weight g	Dry Weight g	- Gas Chromatography - Recovered DMSO grams/100g Fresh Weight				- Radiometry - Estimated DMSO Absorption grams/100g fresh weight	GLC percent recovery
			I	II	III	Mean		
10 whole	1060.70	212.99	0.0479					
10 whole	1093.40	222.72		0.0493				
10 whole	1171.20	249.46			0.0471	0.0481	0.0605	79.50
10 apical halves	750.00	160.35	0.2607					
10 apical halves	750.00	155.02		0.2552				
10 apical halves	750.00	157.95			0.2651	0.2603	0.3468	75.06

reduction of extracts in vacuo was verified by chromatographing the evaporate. Over 90% of the $DMSO_2$ added to control homogenates was recovered.

Although the results indicate discrepancy between radiometry and GLC, no correction factors were used. In all subsequent assays, DMSO absorption was calculated from radiometric values and DMSO residues or metabolites from GLC data. This may explain the variance between the values for estimated absorbed DMSO and those for total recovery of residual DMSO and metabolites after six-weeks' sprouting as reported in Tables 11 and 12. Otherwise, the data suggest increased DMSO adsorption with time or incorporation into other fractions of the sulfur pool of potato tubers.

Metabolism of DMSO by Seed Potato Tubers

Preliminary Qualitative Experiments (1969)

Approximately 10 mg of black ppt accumulated in the mercuric cyanide traps from all treatment combinations after six-weeks' sprouting. After 48 hours white ppt began forming in the $HgCl_2$ traps from both 10% and 25% DMSO treatments but not from the 0% DMSO control. No volatile sulfur compounds were recovered upon regeneration of the black mercuric cyanide ppt, suggesting absence of thiols in the effluent from DMSO-treated tubers. Regeneration of the ppt from the $HgCl_2$

traps led to recovery of a compound agreeing in retention time and co-chromatography with authentic DMS. No other GLC peaks were obtained, even at the most sensitive instrument range.

Two peaks were resolved on Carbowax 20 M from extracts of sprouts, periderm and internal tissues prepared at the end of the six-week experimental period. These peaks agreed in retention time and co-chromatography with authentic DMSO and $DMSO_2$. The preliminary experiment suggested DMSO was reduced to DMS and oxidized to $DMSO_2$ by potato tuber tissue.

Final Qualitative and Quantitative Experiments (1970)

Volatile Metabolism. Identification of evolved organic sulfur compounds by GLC and FPD from each of the eight DMSO-seed potato tuber treatment combinations confirmed the presence of DMS and the absence of other sulfur volatiles. No sulfur compounds were trapped in $HgCl_2$ from the 0% DMSO controls. A definitive mass spectral fragmentation pattern agreeing with the published spectrum confirmed identification of DMS (standard reference: American Society for Testing and Materials, 1969). The characteristic ion fragment at m/e 35 was well defined, as were the base peak (m/e 47) and the molecular ion (m/e 62). Ions at m/e 45, 46, and 51 completed the identity key.

Quantitative evolution of DMS was estimated during the six-week experimental period from the weight of the $HgCl_2$ complex obtained at

seven-day intervals.

Complex: $2\ C_2H_6S \cdot 3\ HgCl_2$

M. W. 938.84

% DMS Composition: 13.23

Collection of the evolved thioether was considered quantitative because 95% of the complex accumulated in the first trap and a negligible amount in the fourth trap. Following each filtration of complex ppt, fresh 3% (w/v) $HgCl_2$ was replaced in the first trap. In other experiments 0.1% DMSO added to 3% $HgCl_2$ did not result in formation of any detectable ppt; consequently, it was unlikely that evaporating DMSO from treated tissue would cause an artifact in the quantitative procedure. Several reports substantiate DMSO does not volatilize to any extent from dermal application to man and animals (Hucker et al., 1967; Mc Dermot, Finkbeiner and Zanette, 1967). The same condition may apply to potato tubers; however, no attempt was made to account for volatile DMSO loss in the present investigation.

Non-volatile Metabolism. The presence of DMSO and the oxidation product $DMSO_2$ was detected in sprouts, periderm and cortex-pith tissue fractions from all treatment combinations except the 0% DMSO controls.[1] Identification was verified by excellent agreement of

[1] Trace amounts of sulfur were detected by FPD in extracts from 0% DMSO whole and apical-half tuber sprout fractions. These peaks agreed in retention time with $DMSO_2$ but the amounts were too small to quantitate.

samples with standards in coincidence of retention time and co-chromatography by GLC and FPD.

The DMSO peak produced a mass spectral fragmentation pattern with the following characteristics: m/e 63 (base peak), m/e 78 (molecular ion) and further ions at m/e 15, 29, 45 and 61. The GLC peak corresponding to $DMSO_2$ yielded a mass spectrum with the base peak at m/e 15, molecular ion at m/e 79 and formation of characteristic ions at m/e 29, 33, 45 and 94. Percent relative intensity of the ion fragments from both compounds was in general agreement to mass spectral data for DMSO and $DMSO_2$ published by the American Society for Testing and Materials (1969).

Cumulative Metabolism. The course of DMS evolution during the experimental period is shown in Figure 28. DMS appeared within 24 hours from all DMSO treatment combinations and was produced for more than six weeks except in the case of whole tubers immersed in 4% DMSO. The curves are similar in shape, but the highest DMSO treatments show the most variability. Peak DMS evolution generally occurred two to three weeks following treatment and coincided with the period of most rapid sprout growth.

Cumulative levels of DMS, DMSO and $DMSO_2$ recovered from DMSO-treated whole and apical-half tubers after six-weeks' sprouting are reported in Tables 11 and 12, respectively. The tables include

(A) 4% DMSO whole tuber
(B) 10% DMSO whole tuber
(C) 25% DMSO whole tuber
(D) 25% DMSO/0.5% CIPC whole tuber
(E) 4% DMSO apical-half tuber
(F) 10% DMSO apical-half tuber
(G) 10% DMSO/0.5% CIPC apical-half tuber
(H) 25% DMSO apical-half tuber

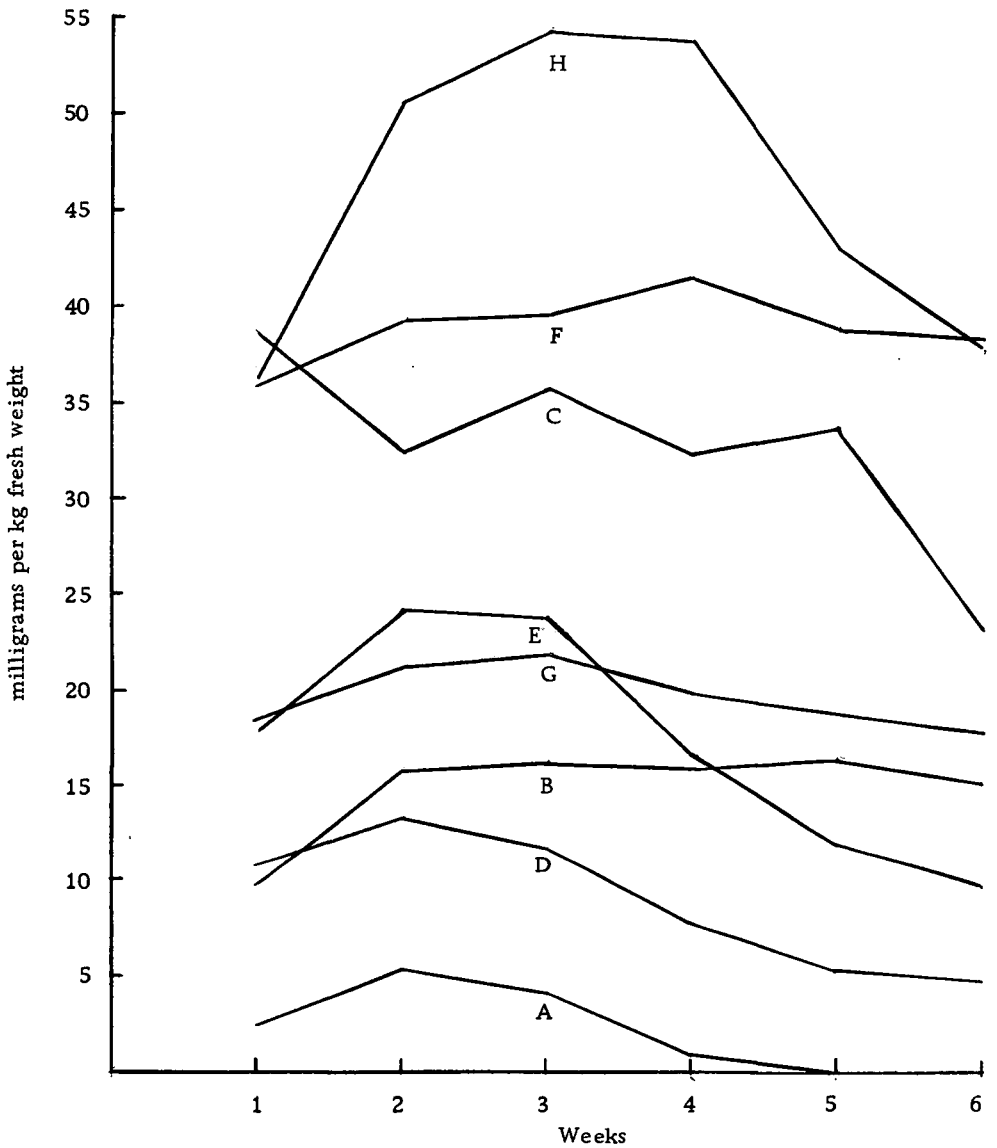


Figure 28. Course of DMS evolution from DMSO-treated 'Norgold Russet' whole and apical-half seed potato tubers during six-weeks' sprouting in the dark.

Table 11. Cumulative levels of DMS, DMSO and $DMSO_2$ in dimethyl sulfoxide treated 'Norgold Russet' whole seed potato tubers after six-weeks' sprouting in the dark.

	Immersion Treatments				
	0% DMSO	4% DMSO	10% DMSO	25% DMSO	0.5% CIPC/ 25% DMSO
Fresh Weight 95 Tubers (g)	9820	9710	9935	9925	10144
Estimated Percent Dry Weight	20.83	20.83	20.83	20.83	19.98
Estimated Initial Dry Weight (g)	2045	2022	2069	2067	2026
Final Dry Weight, Six Weeks' Sprouting (g)					
Sprouts	98	97	109	113	sprouts suppressed
Periderm	165	169	171	178	219
Cortex-Pith	1610	1551	1602	1564	1689
Total	1873	1817	1882	1855	1908
Percent Estimated Dry Weight	91.59	89.86	90.96	89.74	94.17
Estimated DMSO Absorption (g)	--	0.94	2.40	6.00	6.00
Metabolite Recovery (ppm final dry weight)					
DMS Total	0	67	472	1050	284
DMSO Sprouts	0	323	751	1330	--
DMSO Periderm	0	864	1292	3479	5723
DMSO Cortex-Pith	0	182	323	851	1554
DMSO Total Whole Tuber	0	253	436	1133	2033
$DMSO_2$ Sprouts	Trace	150	349	783	--
$DMSO_2$ Periderm	0	Trace	71	100	27
$DMSO_2$ Cortex-Pith	0	6	21	36	11
$DMSO_2$ Total Whole Tuber	Trace	13	45	88	13
Total Recovery (g)	--.	0.60	1.79	4.22	4.45
Percent Recovery	--	63.83	74.58	70.33	74.17

Table 12. Cumulative levels of DMS, DMSO and $DMSO_2$ in dimethyl sulfoxide treated 'Norgold Russet' apical-half seed potato tubers after six-weeks' sprouting in the dark.

	Immersion Treatments				
	0% DMSO	4% DMSO	10% DMSO	25% DMSO	0.5% CIPC/ 10% DMSO
Fresh Weight 120 Half Tubers (g)	9000	9000	9000	9000	9000
Estimated Percent Dry Weight	19.98	19.98	19.98	19.98	19.98
Estimated Initial Dry Weight (g)	1798	1798	1798	1798	1798
Final Dry Weight, Six-Weeks' Sprouting (g)					
Sprouts	209	243	258	249	sprouts suppressed
Periderm	142	137	154	148	208
Cortex-Pith	1264	1237	1208	1206	1494
Total	1615	1617	1620	1603	1702
Percent Estimated Dry Weight	89.82	89.93	90.10	89.15	94.66
Estimated DMSO Absorption (g)	--	4.99	12.49	31.21	12.49
Metabolite Recovery (ppm final dry weight)					
DMS Total	0	581	1295	1550	622
DMSO Sprouts	0	1780	3019	15504	--
DMSO Periderm	0	1081	3415	8755	4797
DMSO Cortex-Pith	0	1302	4147	10999	4837
DMSO Total Half Tuber	0	1355	3898	11492	4832
$DMSO_2$ Sprouts	Trace	409	492	1029	--
$DMSO_2$ Periderm	0	120	249	301	75
$DMSO_2$ Cortex-Pith	0	75	86	220	61
$DMSO_2$ Total Half Tuber	Trace	129	166	353	62
Total Recovery (g)	--	3.34	8.69	21.49	9.39
Percent Recovery	--	66.93	69.57	68.86	75.18

data on fresh weight, estimated dry weight at the beginning of the experiment and final dry weight following extraction of tissue for analysis at the end of the experiment. Estimated initial dry weight and final dry weight were within 90% agreement. The difference may be accounted for by loss of substrate (CO_2) during sprout growth and by loss of lipid and minor constituents during extraction. Burton (1966) estimated the loss in dry matter to be about 3% per month when potatoes were sprouted at 20°C.

Estimated DMSO absorption was calculated on a fresh weight basis from the radiometric absorption data reported in Table 9, Section III. Recovery of metabolites is expressed in ppm final dry weight.

Sprouting whole tubers metabolized DMSO to DMS roughly proportional to the concentration of the immersion treatment and to the amount of DMSO absorbed in the range 0.1-0.6 mg/g fresh weight. Total DMS evolution from sprouting apical-half tubers was approximately linear with treatment concentration up to 10% DMSO and to the amount of DMSO absorbed in the range 0.55-1.4 mg/g fresh weight. This indicates that the mechanism responsible for reduction was probably saturated in apical-half tubers when the absorbed DMSO concentration exceeded 1.4 mg/g fresh weight.

Metabolism of DMSO to DMS was reduced 73% in whole tubers and 50% in apical-half tubers when sprouting was completely suppressed with 0.5% CIPC. A larger percentage of the initially absorbed

DMSO (autoradiographic study) and of the residual DMSO after six-weeks' sprouting (GLC analysis) was localized in the periderm of whole tubers. These data and the larger percentage of DMSO expressed as DMS in whole vs. apical-half tubers (Table 13) suggest most of the reduction activity may be associated with sprouts and periderm.

At the end of the experiments, most of the absorbed DMSO was recovered as the unchanged molecule, ranging from 60 to 90% in whole tubers and 77 to 90% in apical-half tubers, depending on the level of treatment. Residual DMSO was recovered from all tissue fractions. In apical-half tubers, levels ranged on the order periderm < cortex-pith < sprouts, and in whole tubers on the order cortex-pith < sprouts < periderm. Apparently, DMSO moves readily in the sap stream and/or the transpiration stream connecting growing sprouts with ground tissues of the tuber.

Dimethyl sulfone accumulated in all tissues analyzed and ranged in concentration on the order cortex-pith ≤ periderm < sprouts. Accumulation of $DMSO_2$ was more proportional to level of DMSO treatment in apical-half tubers than in whole tubers. Sprout tissue may be the primary locus of DMSO oxidation, because $DMSO_2$ levels in sprouts were many fold greater in whole tubers and several fold greater in apical-half tubers than in other tuber tissues. Furthermore, oxidation of DMSO to $DMSO_2$ was reduced 85% in whole tubers

and 63% in apical-half tubers when sprouting was completely suppressed with 0.5% CIPC.

Growth of micro-organisms was negligible during both preliminary and final metabolic studies. Bacterial contamination was never observed under careful inspection. The growth of fungi appeared after the fourth week in all treatments, but colonization was confined to small scattered areas occupying less than 0.1% of the total surface area of the tubers. Sprouting characteristics during the final metabolic study have been described earlier in Section I.

General Considerations of the Metabolism of DMSO in Biological Systems

Rammler and Zaffaroni (1967) proposed a model scheme for the total oxidation of DMSO (Figure 29). The indicated pathways suggest oxidation, reduction and methyl group transfer might occur in biological organisms treated with DMSO.

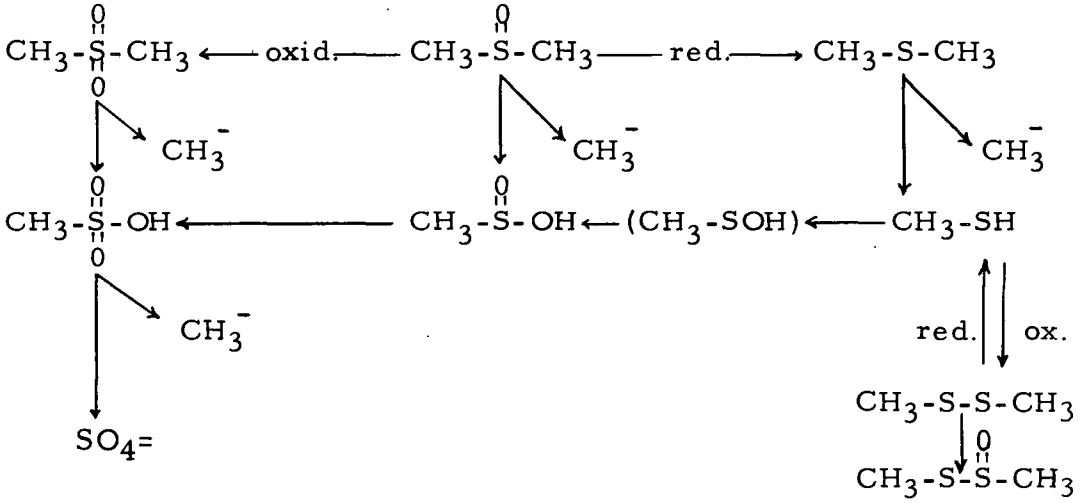


Figure 29. Proposed hypothetical scheme for oxidative metabolism of DMSO (after Rammler and Zaffaroni, 1967).

In mammals DMS and $DMSO_2$ and in bacterial organisms DMS only have been identified as metabolites of DMSO, although in the latter there occurs a more complete degradation resulting in evolution of CO_2 (Gerhards and Gibian, 1968; Wood and Jacob, 1968). The present study substantiates conversion of DMSO to DMS and $DMSO_2$ in plant tissue. Ratio of DMS to $DMSO_2$ was high in DMSO treated potato tubers (Table 13) whereas an inverse relationship in DMSO metabolism was found with mammals (Table 14).

Table 13. Percentage of estimated absorbed dimethyl sulfoxide expressed as metabolites in 'Norgold Russet' after six-weeks' sprouting in the dark.

	Immersion Treatments			
	4% DMSO	10% DMSO	25% DMSO	0.5% CIPC/ 25% DMSO
Whole tuber				
DMS	13	37	32	9
$DMSO_2$	2.5	3.5	2.7	0.4
				0.5% CIPC/ 10% DMSO
Half tuber				
DMS	19	17	8	8.5
$DMSO_2$	4.2	2.2	1.8	0.9

Table 14. Percentage of administered dimethyl sulfoxide excreted as metabolites in man and animals as reported in the literature.

	Man	Animals
DMS	1.5 to 3.0 dermal[e]	3.5 rats [e]
	3.5 intravenous [d]	3.0 cats [a]
$DMSO_2$	17.8 dermal [c]	10 to 15 rats [b]
	21.0 to 23.0 oral [c]	9.5 rabbits [f]
		28.0 rabbits [b]

(a) Borgstedt and DiStefano (1967)
(b) Hucker, Ahmad and Miller (1966)
(c) Hucker et al. (1967)
(d) Kolb et al. (1965)
(e) Kolb et al. (1967)
(f) Williams, Burstein and Layne (1966)

Demethylation of DMSO probably does not occur in potato tuber tissue, although the possibility cannot be excluded. No measurement was made of a change in the level of sulfate with DMSO treatment. Demethylated products such as methane thiol and dimethyl disulfide were not detected in the effluent from treated tubers. Furthermore, chromatographed tissue extracts produced no spurious sulfur peaks when subjected to FPD; therefore, evidence for the presence of methyl sulfonic acid (CH_3SO_3H) or methyl sulfinic acid (CH_3SO_2H) was lacking.

In rats given $DM^{35}SO$, no appreciable label was detected in the sulfate fraction of urine (Gerhards and Gibian, 1967). DMS fed to rats also did not lead to an increase in urinary sulfate (Maw, 1953b). In

addition, methyl sulfonic acid and $DMSO_2$ given to animals were excreted primarily as the unchanged molecules (Maw, 1953a; Williams, Burstein and Layne, 1966). These data do not support demethylation as a primary mode of DMSO degradation in higher organisms.

Reduction of DMSO could proceed by two mechanisms. Wood and Jacob (1968) reviewed the biological literature and reported sulfoxide amino acids are reduced to the corresponding thioethers in the presence of thiols. DMSO has also been shown to be directly reduced to DMS in vitro upon addition of GSH or cysteine (Borgstedt and DiStefano, 1967; Sommer and Tauberger, 1964). Application of DMSO to seed potato tubers was shown to stimulate an increase in GSH; consequently, the ensuing pathway may account for part of the DMS evolved from treated tubers.

$$R\text{-}\overset{\overset{O}{\|}}{S}\text{-}R + RSH \longrightarrow R\text{-}S\text{-}R + RSOH$$

(GSH, cysteine)

$$RSOH + RSH \longrightarrow R\text{-}S\text{-}S\text{-}R$$

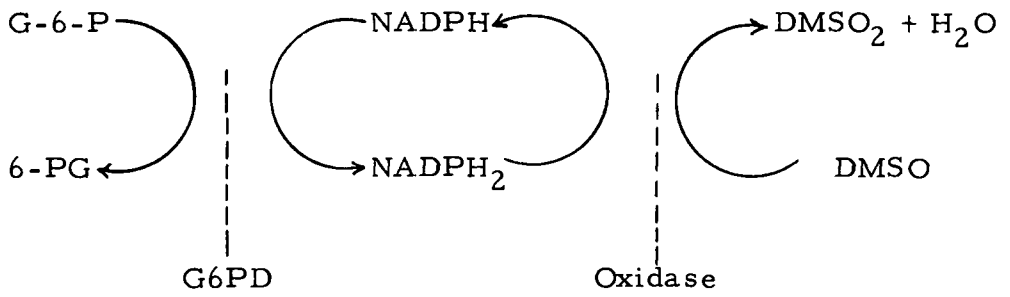
(GSH, cysteine) (GSSG, cystine)

Another mechanism shown by Black et al. (1960) to account for conversion of methionine sulfoxide to methionine in yeast cells requires specific enzymes and a pyridine nucleotide redox partner.

$$R\text{-}\overset{\overset{O}{\|}}{S}\text{-}R' \xrightarrow[\text{enzymes}]{NADPH_2} R\text{-}S\text{-}R'$$

An analogous pathway could account for most of the DMSO reduction in potato tubers.

Oxidation of DMSO by animal microsomes proceeds as a coupled reaction with the glucose-6-phosphate dehydrogenase system. The following scheme has been proposed by Gerhards and Gibian (1967).



Rammler (1967) found catalase and peroxidase were capable of oxidizing DMSO to $DMSO_2$. These enzymes are abundant in potato tuber tissue (Smith, 1968).

DMSO does not affect the activity of glucose-6-phosphate dehydrogenase, even at concentrations up to 30%. (Gerhards and Gibian, 1967). Rammler (1967) reported 1% DMSO stimulated catalase and peroxidase but higher concentrations greatly reduced activity. The low concentration (0.1-3.5 mg/g fresh weight) of DMSO absorbed into potato tubers may enhance the activity of these enzymes.

The natural occurrence of simple sulfides, sulfoxides and sulfones in sprouting potato tubers has not been reported. More complex homologs, however, are found in tubers as amino acids (Fitzpatrick

and Porter, 1966; Le Tourneau, 1956; Talley, Fitzpatrick and Porter, 1964). It has been suggested that sulfur amino acids readily participate in redox conversions in plant tissue (Doney and Thompson, 1966). Simple oxidation and reduction of DMSO in potato tuber tissue undoubtedly proceeds without induction of new enzyme systems.

SUMMARY AND CONCLUSIONS

The solvent properties of DMSO in relation to the growth and physiology of plants has been the subject of many agricultural investigations, but little information is available on its mode of action. Few studies have been undertaken on the influence of DMSO in plant biochemistry. Metabolic research has been confined to animals and micro-organisms. Considerable interest has developed concerning the effects on plant growth and yields. Increased sprouting of seed potato tubers, following DMSO pre-plant immersion treatment, has been reported to induce greater tuber numbers and a larger marketable crop.

The present investigation on DMSO-treated seed potato tubers was conducted along the following lines: (1) influence of DMSO on sprouting and sprout morphology at different physiological ages (PA); (2) effect of DMSO on ascorbic acid, glutathione and respiration; (3) absorption and distribution of DMSO following treatment; and (4) metabolism of DMSO following absorption. For these studies, whole and cut apical-half tubers were routinely immersed 30 minutes in 4, 10 or 25% (v/v) DMSO.

The effect obtained from DMSO on tuber sprouting of 'Norgold Russet' and 'Netted Gem' was directly related to PA. Treatment did not induce sprouting during the rest period. Single-sprouted tubers

showing strong apical dominance were not influenced by treatment; however, sprout numbers were significantly increased when tubers were treated within the PA ranges characterized by multiple sprouting. Apical-half tubers, which absorb more DMSO than whole tubers, responded most consistently.

Sprout morphology of tubers at the branched-multiple sprouting PA, was altered by 25% DMSO with the development of more fine, weak sprouts than the control. Senescent 'Norgold Russet' tubers, treated with 10 or 25% DMSO, developed less "little tuber" disorder than the control, but the higher concentration was considerably phytotoxic. Ethephon at 5000 ppm completely suppressed this morphological symptom and promoted branched-multiple sprouting. These data suggest DMSO may exert its influence by stimulating the production of gibberellic acid or by altering its distribution.

Ascorbic acid content was not affected when apical-half tubers of both cultivars were treated at the multiple and branched-multiple sprouting stage. Glutathione level generally increased, but statistically significant differences were associated mainly with the 25% DMSO concentration. Respiration rates of whole and apical-half tubers at these same physiological ages increased following treatment with 25% DMSO. Injury to the buds or cut tissues may have caused the increased respiration.

Absorption of $DM^{35}SO$ was related to treatment concentration and over five times more was absorbed by cut apical-halves than by whole tubers of 'Norgold Russet'. Intact periderm acted as a partial barrier to penetration; but buds, cut surfaces and adjacent tissues were readily permeated. $DM^{35}SO$ penetrated the xylem ring shortly after immersion only in cut tubers. Where clear definitive interpretation was possible, the ^{35}S label was not detected in phloem tissue, but it was clearly present in all other tissues of tubers autoradiographed after ten-days' sprouting in the dark.

DMSO was reduced to DMS and oxidized to $DMSO_2$ in cut and whole 'Norgold Russet' tubers sprouted in the dark. Accumulation of metabolites was proportional to the amount of DMSO absorbed. Complete suppression of sprouting with CIPC was associated with markedly decreased metabolism of DMSO. Ratio of DMS to $DMSO_2$ was high. In DMSO-treated mammals the inverse has been reported. Much more $DMSO_2$ was recovered from sprouts than from periderm or internal tissues. Most of the absorbed DMSO, however, remained unchanged even after six-weeks' sprouting and was recovered from sprouts as well as tuber tissues. No other metabolites were identified and evidence for de-methylation was not found. So far as is known, this is the first elucidation of DMSO metabolism in plant tissue.

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