amount of material having an absorption band at 240 mμ wavelength was found in some 2% ethanol fractions. This absorption band is characteristic of steroids containing the α–ß unsaturated ketone structure. Definitely separated from this was a much larger amount of material giving this absorption which appeared in the late 4% and early 6% ethanol fractions. On oxidation with periodic acid this material yielded formaldehyde (*2*).

The last two fractions mentioned were accumulated from several dogs. The crude material was acetylated and sublimed in high vacuum at 180° C. Most of the material sublimed. The sublimate was chromatographed on aluminum oxide. Material giving the 240 mμ absorption appeared in the benzene-ether fractions. On crystallization from ether pentane, the mp of the compound was 212°–220° C (corrected).[3] A sample of compound F acetate obtained through the courtesy of Dr. Harold Mason of the Mayo Clinic gave 214°–220.5° C mp (corrected). A mixture of the two compounds gave the same value, 214°–220° C. On the other hand, a mixture of the unknown with a known sample of cortisone acetate (mp 227°–236° C) gave a significant depression (mp 197°–209° C).

Chloroform solutions of both the unknown acetate and the sample from Dr. Mason gave infrared spectra indicating an α–ß unsaturated ketone, a keto group on carbons 11 and/or 20, an alcohol group, and an acetoxy group. The absorption of chloroform in the fingerprint region was so great, however, that positive identification from this region could not be made. The compound was too insoluble in carbon disulfide to give a spectrum. Attempts to obtain a satisfactory dry film with the small amount of material which remained were unsuccessful and must await accumulation of more substance.

When treated with chromic acid the acetate underwent oxidation and a compound was isolated which became opaque at 70°–100° C and melted at 220°–229° C (corrected). The small amount of material did not permit further purification. A known sample of compound E acetate showed opacity in the same range and melted at 227°–236° C (corrected).

The solubilities and region of elution from both magnesium silicate and aluminum hydroxide indicate a highly oxygenated polar steroid. The ultraviolet absorption spectrum and the formation of formaldehyde on oxidation with periodic acid place the compound among those having an α–ß unsaturated ketonic group in ring A, and a ketol side chain similar to the active adrenal steroids. The infrared spectrum confirms the presence of these groups and indicates at least two alcohol groups, one of which was easily acetylated. The mp of the acetate is similar to that of 17-hydroxycorticosterone acetate, is not depressed when the acetate is mixed with a known sample of this compound, and rises on oxidation with chromic acid toward that corresponding to cortisone acetate. The compound isolated from the blood flowing from the adrenal veins of dogs, therefore, appears to be 17-hydroxycorticosterone.

[3] Melting points were determined on the Kofler micro hot stage and measured from first softening to complete liquefaction.

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The Effect of 2,4-Dichlorophenoxy Acetic Acid and Various Other Substances upon the Respiration of Blue Lupine Seedling Roots

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The outstanding capacity of 2,4-D, the synthetic plant hormone, to control certain phases of plant growth has been known for a number of years (*8*). Concurrent with growth and exerting some regulating influence upon it is the process of respiration. In this study, the process of respiration as affected by a synthetic hormone, 2,4-D, is considered from the over-all viewpoint of oxygen uptake and carbon dioxide release, as shown in the respiration of excised seedling roots.

Materials and methods. Selected seeds of *Lupinus angustifolius* L., first treated with a wetting agent (Teel), then sterilized in a 0.25% formaldehyde solution, were germinated in sterile Petri dishes on moist filter paper at a temperature of 22° C. About three days were required for germination of roots of desirable size, namely, 3 cm, giving a dry weight of approximately 5 mg. Two to three were used per flask, suspended in 0.1 M KH_2PO_4. The experimental procedure was that of the conventional Direct Method of Warburg.

Experimental results. The effect of 2,4-D upon the respiratory rates of blue lupine. In setting up this experiment, it was assumed that 2,4-D exerts a specific inhibitory effect upon plant cells. The concentration of 2,4-D necessary to bring about an inhibition of oxygen uptake was determined over a range from 0.05 to 0.00001 M. A concentration of 0.05 M is inhibitory at both pH 4.5 and 5.0 (29% and 27% respectively), whereas at 0.01 M the inhibition is slow and gradual, although obvious (20% and 17%). Below 0.001 M (15%) significant inhibitory effect was absent. Auxin (IAA) at a concentration of 0.0016 M showed neither an inhibition nor an acceleration at pH 5.0.

The effect of substrates at a concentration of 0.01 M upon the respiration of 2,4-D-treated and untreated roots. Various compounds and substrates associated with plant metabolism were used in an attempt to overcome inhibition by 2,4-D (0.05 M) and thus give some clue as to wherein the mechanism might be located. These sub-

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TABLE 1

COMPARISON OF THE REACTION IN OXYGEN OF ASCORBIC ACID AT 0.001 M *in Vitro* AND *in Vivo*

Treatment	O_2 uptake in mm³* during period of:					
	10 min	20 min	30 min	40 min	50 min	60 min
(1) Ascorbic acid (*in vitro*)	1(1)	1(0)	1(0)†	29(28)	35(6)	38(3)
(2) Ascorbic acid in N_2 (*in vitro*)	1(1)†	0(−1)	3(3)	3(0)	.....	.....
(3) Ascorbic acid + roots (*in vivo*)	(a)14(14)	26(12)	37(11)†	65(28)	80(15)	92(12)
	(b)19(19)	39(20)	59(20)†	95(36)	122(27)	.....
(4) 2,4-D‡ + ascorbic acid (*in vitro*)	1(1)	2(1)	2(0)†	21(19)	32(11)	38(6)
(5) 2,4-D‡ + ascorbic acid + roots (*in vivo*)	5(5)	6(1)	9(3)†	17(8)	21(4)	26(5)
(6) 2,4-D‡ + roots	5(5)	9(4)	12(3)	16(4)	18(2)	20(2)
(7) Ascorbic acid§ + glutathionine‡ (*in vitro*)	1(1)†	2(1)	3(1)	3(0)	.....	.....
(8) Ascorbic acid§ + glutathione‡ in N_2 (*in vitro*)	0(0)†	1(1)	1(0)	0(−1)	.....	.....

* Figures in parentheses are the increase of O_2 uptake for a 10-min interval.
† Substance was added to flask from side arm at the end of this interval.
‡ Substance added at the beginning of the experiment. § Ascorbic acid added at end of 10-min interval.

stances included glucose; sucrose; glycine; glycollic,[3] succinic, fumaric, lactic, citric, and pyruvic acids; glutathione; ascorbic acid; and indoleacetic acid (0.0016 M). Essentially only one compound showed any indication of overcoming the inhibition of 2,4-D. This was in the case of ascorbic acid, where an increase was pronounced. But since it is generally believed that ascorbic acid is easily oxidized in an oxygen atmosphere, runs with ascorbic acid were made *in vitro* as well as *in vivo* (i.e., in the presence of roots) to determine whether the accelerated uptake was due entirely or partly to processes in the root, or to the spontaneous reaction of ascorbic acid in oxygen.[4] Experiments were carried out with ascorbic acid alone and in the presence of 2,4-D or glutathione. (Previous experiments revealed that glutathione in the presence of roots was noninhibitory.) Experiments *in vitro* and *in vivo* were conducted simultaneously, using similar reaction mixtures. The results may be found in Table 1.

These data show that when ascorbic acid alone (*in vitro*) is present (after tipping) in an atmosphere of pure oxygen, oxidation seemingly takes place completely and irreversibly over a period of 20 to 30 min. When the same conditions prevail in the presence of roots, a somewhat similar response is obtained. However, from these two experiments (Table 1, No. 3) one can, upon close scrutiny of the data, observe that in the presence of roots ascorbic acid either (1) causes a depression in the normal respiration, or (2) is itself utilized only partly (50%–70%), apparently being inhibited by some substance or mechanism in the roots. This statement is based on the fact that these normal roots, which respire linearly over a 60-min period, do show a dropping off in rate after addition of ascorbic acid.[5]

A second striking fact is noted when one compares the effects of (1) 2,4-D on roots alone, (2) 2,4-D in the presence of ascorbic acid alone, and (3) 2,4-D in the presence of ascorbic acid and roots. The 2,4-D has no apparent effect on ascorbic acid alone (No. 4), but in the presence of roots and 2,4-D (No. 5) oxidation of ascorbic acid is altered. This is more revealing in light of the response of roots in the presence of 2,4-D alone (No. 6). Assuming noninhibition of the roots by ascorbic acid, the indication is that there is only about 25% oxidation of ascorbic acid, or about half as much as utilized in the presence of roots alone (No. 3).

In an attempt to relate these phenomena with the second explanation—the partial utilization of ascorbic acid—experiments were performed with glutathione (GSH) (0.01 M) and in a nitrogen atmosphere. Both sets of experiments produced expected responses and agreed with statements from the literature.[6] The effect of a complete nitrogen atmosphere (No. 2) is similar to the suppressive effect of glutathione on ascorbic acid in an oxygen atmosphere. Therefore, the reaction *in vitro* of ascorbic acid in these experiments is an oxidative one.

[3] Glycollic acid apparently gave better response than the basic medium (control), but this had no effect in overcoming the inhibition of 2,4-D.

[4] According to Weissberger and LuValle (7), auto-oxidation of L-ascorbic acid at pH 4.7 to pH 9.2 proceeds at a rapid rate even in the absence of the cupric ion.

[5] For example, case 3a at 30 min (before addition of ascorbic acid) shows 37 mm³ O_2 uptake. At 60-min normal respiration value should be 74. Since ascorbic acid *in vitro* takes up 38 mm³ O_2, (Table 1, 1 and 4) the two combined should equal 112. The value is actually 92. Assuming 74 mm³ is due to the normal respiration, then only 18 mm³ remaining (92–74) can be attributable to ascorbic acid. This, out of a potential of 38 mm³, is roughly 50% utilization. Case b is similar if calculated for the 50-min period, and shows roughly 70% utilization of ascorbic acid. These values are not to be taken as absolute, but merely as exemplary to the case in point.

[6] Harrow (3) states that the oxidation of ascorbic acid by oxygen and ascorbic acid oxidase is prevented by the addition of glutathione. Borsook *et al.* (1) indicate that if glutathione is present, it will reverse a portion of the oxidation product (dehydro AA) formed from the oxidation of ascorbic acid.

Conclusions. The results obtained seem to indicate that none of the intermediates or substrates used, with the possible exception of ascorbic acid, is concerned with an enzyme system assumed to be inhibited by 2,4-D. There appears to be abundant evidence (*3*, p. 400) that ascorbic acid is active in a wide variety of tissues, and that it is easily oxidized by both an ascorbic acid oxidase and by atmospheric oxygen. Ascorbic acid oxidase (AAO) also is apparently rather widely distributed in nature (*3*, p. 176). It might be assumed that this oxidase is present in lupine roots and acts in its normal role (as stated). The optimum pH for the reaction is 5.6 (*5*). The reaction is specific for ascorbic acid and oxygen with the formation of water. It might be further assumed that some concentration of glutathione is present in the roots also (based on experiment No. 3). If now, when 2,4-D is added, there is greater reduction in O_2 uptake, one explanation may be that 2,4-D inhibits AAO and thus the oxidation of the greater part of ascorbic acid. If there is an established ratio of GSH to AAO in the root, that normally controls the oxidation of ascorbic acid,[7] any inhibition of one would enhance the reaction of the other. In this case the inhibition would appear to be of the substance normally accelerating O_2 uptake, namely AAO. Mitchell *et al.* (*6*) have demonstrated that *p*-chlorophenoxyacetic acid preserves a relatively high vitamin C content in bean pods when applied just before harvesting, in contrast to samples not so treated (up to 76% more in treated than in untreated). Fults *et al.* (*2*) have reported that Red McClure potatoes treated with 2,4-D retain significantly more ascorbic acid (and red color) both at harvest and after 60 days' storage than the untreated samples. One can assume that oxygen alone will oxidize ascorbic acid to a lesser extent than AAO. The remaining O_2 uptake might easily be that mediated by other oxidations and respiratory systems present in the roots.

This explantion may best be described schematically in the following stepwise manner:

1. AA (reduced) $\xrightarrow[in\ vitro]{O_2}$ AA (oxidized $\longrightarrow$
   (L-AA) (dehydro AA)
   irreversible "X" products

2. AA (reduced) $\xrightarrow[GSH]{O_2}$ no reaction (*in vitro*)

3. AA (reduced) $\xrightarrow[\text{AA oxidase (}-2H\text{)}]{O_2}$ AA (oxidized) + H_2O
   $\uparrow$ GSH $\downarrow$
   (*in vivo*) (possible assumption—see footnote 7)

4. AA (reduced) $\xrightarrow[\text{AA oxidase + 2,4-D}]{O_2}$ no reaction (*in vivo*)

According to experiments on growth with both higher plants (*4*) and microorganisms (*8*), it was concluded that oxygen was rendered unavailable to the organism by the action of 2,4-D. It was further concluded that the growth in the presence of 2,4-D was similar to growth of anaerobic organisms. This evidence indicates that 2,4-D inhibits an oxidase and that the balance of the respiration is carried on by some "extra-2,4-D" system not involving this particular oxidase. It is also believed that an oxidase system is similarly involved in the respiratory inhibition of blue lupine roots by 2,4-D.

Further experimentation is necessary to elucidate these contentions in relation to the specific oxidase and possibly to a dehydrogenase system (or systems) involved.[8] From indirect evidence presented, involving several intermediates generally related to dehydrogenase stems (malic and succinic), one might surmise that the normal scheme of Krebs, if indeed it is present at all, is not the one involved in the response of blue lupine roots to 2,4-D.

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[7] The following scheme for interaction of L-AA and glutathione in oxidation-reduction reactions in biological systems is a rather widely accepted one:

$$\text{L-AA} \xrightarrow[\text{AAO}]{\text{(}-2H\text{)}} \text{dehydro AA.} \qquad \text{GSH (reductase)} \xleftarrow{\text{(}+2H\text{)}}$$

[8] In two subsequent experiments, malonate (0.01 M) and iodoacetate (0.001 and 0.0001 M) produced no inhibitory response whatsoever. Iodoacetate and 2,4-D, both at 0.01 M, gave somewhat similar inhibitory responses. Further screening experiments involving adenosine triphosphate, hexosediphosphate, coenzyme 1, cytochrome C, and onion juice revealed no apparent correlative evidence.