have been studied (Plough, *5*), some of which were isolated by the methods of Tatum (*6*) and show the loss of certain enzyme systems normally carrying on specific essential syntheses, and so require one or more nutrilites in the medium. One of these mutant strains (#10–1)

TABLE 1

Medium	International units of penicillin						
	4	2	1	0.5	0.25	0.125	0.062
Original S. typhimurium #511							
Infusion broth	+++	+++	+++	+++	+++	+++	+++
S.D. + Casamino acid	+	+	++	++	++	++	– –
S.D. + cysteine	–	+	+	+	+	+	+
S.D. + tryptophane	–	+	+	+	+	++	++
S.D. alone	–	+	+	+	+	+	+
Mutant strain #10–1							
Infusion broth	–	–	++	+++	+++	+++	+++
S.D. + Casamino acid	–	–	+	+	++	++	++
S.D. + cysteine	–	–	–	–	+	+	+
S.D. – tryptophane	–	–	–	–	–	–	–
S.D. alone	–	+	+	·	+	+	+

isolated after ultraviolet radiation, requires the amino acid cysteine, which the parental strain can synthesize. Thus we have here exactly the opposite situation from that studied in *Staphylococcus* by Gale and Rodwell. The original *Staphylococcus* is Gram-positive, requires amino acids in the medium, and is penicillin-sensitive. The mutant strains can synthesize their own amino acids and are penicillin-resistant. Our original *Salmonella* is Gram-negative, can synthesize amino acids, and is penicillin-resistant. Our mutant cysteine-requiring *Salmonella* turns out to be much more penicillin-sensitive.

The tests were made by the tube method of assay (Plough, *4*), using a graded series of dilutions of penicillin in complete broth medium, and in synthetic medium containing dextrose and essential salts[2] plus particular nutrilites. Growth readings of visible turbidity were made at 24 hours. Results are shown in Table 1.

It is clear that the mutant strain #10–1 is more sensitive to penicillin than the parent strain, both when complete infusion broth medium is used and when the test is made in synthetic medium with complete nutrilites (S.D. + Casamino acid), or synthetic with the essential cysteine (S.D. + cysteine). When synthetic medium and tryptophane or synthetic medium alone is used, the essential cysteine is not present, and so there is no growth, regardless of penicillin.

This result brings *Salmonella* into line with the interpretation of Gale and Rodwell, though the wild type is the opposite of *Staphylococcus*. It further supports the conclusion that penicillin antagonizes the assimilation of one or more amino acids inside the bacterial cell. Study of the mechanism of the metabolic block and of other reactions of other mutant strains is being continued.

[2] The composition of the synthetic medium (noted in the table as S.D., salts and dextrose) was as follows, after MacLeod (*3*): NaCl 5.00 g; $(NH_4)_2SO_4$ 4.72 g; KH_2PO_4 2.72 g; dextrose 2.00 g; plus 1 ml of a solution containing 1 g each of $FeCl_2$, $MgCl_2$ and $CaCl_2$ in 600 ml. Distilled H_2O was added to make 1 l and pH was adjusted to 7.00 with N NaOH.

When Casamino acid is noted it was "Vitamin-free Casamino acid" Difco, a product hydrolyzed from casein.

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Inhibition of Salt Accumulation in Excised Wheat Roots by 2,4-Dichlorophenoxyacetic Acid

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Widespread interest in the growth effects of 2,4-dichlorophenoxyacetic acid (2,4-D) has led to a number of investigations designed to reveal its mechanism of action. Perhaps the most obvious metabolic effect observed is the striking decrease in the reserve carbohydrates of the treated tissues (*5*). More recently, changes in the respiratory activity of treated tissues have been reported. Brown (*1*) found significantly increased CO_2 evolution by bean seedlings 1 to 4 days after application of 2,4-D spray. Hsueh and Lou (*2*) report a stimulation of germination of rice and barley seedlings at concentrations of about 100 ppm of 2,4-D and, at higher concentrations (up to 1,000 ppm), inhibition of both germination and respiration. Increased CO_2 evolution by the roots of dandelion plants 5 to 15 days after the plants had been sprayed with 2,4-D mixture was observed by Rasmussen (*7*). Taylor (*10*) made detailed studies of O_2 uptake and CO_2 evolution of wheat and mustard seedlings (18 to 30 hrs old) treated with 2,4-D solutions of from 0.25 to 10 ppm. Manometric measurements over a period of 24 hours principally revealed decreases in both O_2 and CO_2 exchange in the two types of seedlings. Studies of Smith (*8*) on the stems of 2,4-D-treated bean seedlings show increases in the respiratory activity of treated tissue when calculated on a dry weight basis but decreased activity when calculated on protein-nitrogen basis. Control tissues were found to be significantly more sensitive to iodoacetate than 2,4-D-treated tissues under anaerobic conditions. Worth and McCabe (*12*) compared the effects of 2,4-D on the growth of several species of aerobic, facultatively anaerobic, and anaerobic bacteria. Growth of the aerobic organisms was greatly inhibited in 3 out of 4 organisms by concentrations of from 0.2% to 2% 2,4-D. Facultative anaerobes were not inhibited at any concentration and were stimulated at concentrations of from 0.002% to 0.2% in all cases. Growth of anaerobic

bacteria was inhibited over a wide range of concentrations of 2,4-D.

Preliminary results of studies concerned with the effect of 2,4-D on salt accumulation, which developed from a study of the effect of this compound on nitrate assimilation, are reported below. Excised roots of 4-day-old wheat seedlings were used as plant material. During the growing period the seedlings were supported on Fiberglas gauze stretched tightly over a 2-liter Pyrex crystallizing dish which contained 0.0001 M $CaSO_4$. The cultures were aerated and the vessels were kept in a dark room maintained at 22.0° ± 0.5° C. The plants were grown from White Federation 38 seeds harvested in the Sacramento valley of California in 1945. Plant material in each experimental flask consisted of 45 root segments, each 15 mm long, of which 20 were root tips and 25 were from more mature sections of the root. Because a less sensitive method for the chloride determination was used, it was necessary to employ samples of 35 root tips plus 100 of the mature sections in the experiments on chloride accumulation.

TABLE 1

Time (hrs)	Treatment*	NO_3^- absorbed (meq × 10^{-3})	Percentage of control
3	KNO_3	0.50	100
	KNO_3 + 2,4-D	0.20	40
6	KNO_3	2.30	100
	KNO_3 + 2,4-D	0.60	26
9	KNO_3	3.35	100
	KNO_3 + 2,4-D	0.90	27

* KNO_3, 0.001 M; 2,4-D, 10 ppm.

For the experiments, the root samples were placed in 5 ml of solution containing 0.001 M KNO_3 or 0.01 M KCl, with various additions, and aerated by shaking in a constant temperature bath at 25° C for 6 hrs. In the nitrate experiments, the culture solutions were decanted from the roots, and the roots washed 3 times at the end of the experiment. Nitrate analyses were made on the combined culture solutions and washings and separately on the roots. The phenoldisulfonic acid method was used following a modification of Burstrom's procedure described earlier (*6*). Although only culture solution analyses are reported in this paper, analyses on both roots and cultures were necessary to determine the effect of the treatments on the reduction of the nitrate. In the experiments reported below there was no significant nitrate reduction in any treatment. Chloride analyses were made according to Fajan's method (*11*) on an aliquot of the culture solution. The 2,4-D used was recrystallized twice from a commercial preparation and melted at 134 to 135° C (uncorrected).

Results of a time study (Table 1) show a marked inhibition of nitrate absorption by 10 ppm of 2,4-D within 3 hrs. This effect is clearly a primary 2,4-D inhibition of the accumulation mechanism and not a secondary effect following upon a general metabolic disturbance. In Table 2 it may be seen that concentrations as low as 0.1 ppm are inhibitory. Maximum inhibition was attained with 5 ppm. An attempt to determine the effect of pH on the toxicity of 2,4-D unexpectedly revealed that KH_2PO_4 at 0.001 M (used as a buffer) enhances the 2,4-D inhibition of nitrate accumulation very markedly (Table 3). In the presence of 0.001 M KH_2PO_4, 1 ppm of 2,4-D restricted the uptake of nitrate to form 4% of the control (pH 4.0) to 14% of the control (pH 6.0). These results should be compared to the effect of the same concentration of 2,4-D alone in 2 other experiments reported in Tables 2 and 4, where uptake of nitrate amounts to 58% and 62% of the controls, respectively. Although the inhibition is greater at the lower pH, the differences in absolute amounts are not large enough to indicate a pH relation conclusively. It will be noted that phosphate did not restrict nitrate uptake in the absence of 2,4-D.

TABLE 2

EFFECT OF CONCENTRATION ON INHIBITION OF NITRATE ABSORPTION BY 2,4-D

2,4-D (ppm)	NO_3^- absorbed* (meq × 10^{-3})	Percentage of control
0.0	1.90	100
0.1	1.65	87
1.0	1.10	58
5.0	0.65	33
10.0	0.70	37

* KNO_3 in all cases, 0.001 M.

TABLE 3

pH of buffer*	2,4-D (ppm)	NO_3^- absorbed† (meq × 10^{-3})	Percentage of control
6.0	0.0	2.60	100
4.0	1.0	0.10	4
5.0	1.0	0.30	12
6.0	1.0	0.35	14

* KH_2PO_4, 0.001 M adjusted to pH; indicated with KOH or H_2SO_4.

† KNO_3 in all cases, 0.001 M.

Attempts to reverse the effect of 2,4-D were made with certain dyes: methylene blue, quinone, and dichlorobenzenone indophenol, as well as iodine, cysteine, sodium sulfide, and a normal cellular substrate, citric acid. The dyes and iodine, all used at 10 ppm, enhanced the inhibitory effect of the 2,4-D to a small degree of questionable significance. Sodium sulfide (pH 6.0) at 1×10^{-3} M killed the roots; at 1×10^{-4} M its effect was not significant. Cysteine at 1×10^{-4} M had no significant influence. Citrate alone was able to reverse the 2,4-D inhibition. Its reversal activity was demonstrated in the presence of 1 ppm of 2,4-D and in a second experiment in which the roots were presoaked for 2 hrs in a solution of 25 ppm of 2,4-D, washed, then placed in KNO_3 with and

without added citrate. The citrate concentration was 0.05 M in both cases and adjusted to pH 5.0 with KOH.

TABLE 4

REVERSAL OF 2,4-D INHIBITION

Treatment*		NO_3^- absorbed (meq × 10^{-3})	Percentage of control
KNO_3		2.50	100
KNO_3 + 2,4-D		1.55	62
KNO_3 + 2,4-D + K citrate		2.45	96
Pre-soaked in 2.4-D‡	KNO_3	1.40	64†
	KNO_3 + K citrate	3.40	154†

* KNO_3, 0.001 M; 2.4-D, 1 ppm; K citrate, 0.05 M, pH 5.0.
† Based on average amount of KNO_3 accumulated by 45 root segments (controls) in 6 hrs, from 6 experiments.
‡ Roots aerated in a solution of 25 ppm of 2,4-D for 2 hrs.

Experiments with KCl, one of which is reported in Table 5, indicate that the 2,4-D inhibition of accumulation is not a specific nitrate effect.

It was felt that the 2,4-D inhibition of salt accumulation might be associated with a lower respiratory activity in the treated roots. Oxygen consumption by 45 root segments at 25° C was measured in a Warburg respirometer. The lower initial values in the third test (Table 6) are probably due to the fact that in this case, after they were cut, the roots stood 3 hrs in redistilled water before they were placed in the vessels. In the first 2 tests the roots were placed in the vessels as soon as they were cut.

TABLE 5

2,4-D INHIBITION OF CHLORIDE ABSORPTION

Treatment	Cl^- absorbed (meq × 10^{-2})	Percentage of control
KCl (0.01 M)	1.37	100
KCl (0.01 M) + 2,4-D (10 ppm)	0.70	48

Machlis (*4*) observed a similar effect with barley seedling roots. It may be seen that the oxygen consumption by roots after 5–7 hrs is significantly greater in KNO_3, KNO_3 plus 2,4-D, and 2,4-D alone than it is in distilled water. Differences in oxygen uptake between the roots in KNO_3, KNO_3 plus 2,4-D, and 2,4-D alone are probably not significant in these experiments though it is possible that more intensive studies might reveal such differences. It appears that the inhibition of salt accumulation by 2,4-D cannot be as conclusively related to diminished oxygen consumption as can its inhibition by various respiratory poisons as described by Machlis (*4*).

The results of the present investigation suggest that 2,4-D brings about a change in the relative amounts of energy released by the various possible respiratory pathways or components. Salt accumulation, for example, might be affected by the 2,4-D inhibition of a particular respiratory component. Lundegardh (*3*) has, indeed, presented evidence for the occurrence of two respiratory systems in plant tissues, a cyanide-insensitive system which he calls the "ground respiration" and a cyanide-sensitive system which he designates the "anion respiration." The latter component he associates with salt accumulation. As a consequence of the postulated inhibition of a particular respiratory pathway by 2,4-D and in order to account for the fact that oxygen consumption is not appreciably affected, it might be suggested further that as one component of the respiration becomes less active a second one may assume greater activity.

Another general mechanism for the inhibitory effect of 2,4-D on salt accumulation is suggested by recent work with azide. Spiegelman, Kamen, and Sussman (*9*) studied the inhibition of anaerobic synthesis in yeast by azide. These investigators suggest that azide uncouples anaerobic oxidation of carbohydrate from synthesis by a replacement reaction which prevents the formation of adenosine triphosphate. The suggestion that an uncoupling of oxidation and phosphorylation may likewise be involved in the 2,4-D inhibition of salt accumulation is supported by the observation that oxygen consumption of the roots is not diminished even though the accumulation is inhibited by 2,4-D.

TABLE 6

Test No.	Hour after experimental treatment	Oxygen consumption, mm^3/hr/45 root segments* KNO_3	KNO_3 + 2,4-D	2,4-D only	H_2O only
1	1st	64.5	64.5	65.8†	64.1†
	7th	54.7	53.1	59.7†	43.2†
	Change	− 10.8	− 11.4	− 6.1	− 20.9
2	1st	58.7	61.5	63.4†	53.9†
	5th	62.6	61.5	63.5†	39.4†
	Change	+ 3.9	0.0	+ 0.1	− 14.5
3	1st 3 hrs	49.9†	...	47.3	45.9
	5th	56.8†	...	52.2	44.7
	Change	+ 6.9	...	+ 4.9	− 1.2

* KNO_3, 0.0025 M; 2,4-D, Test 1, 5 ppm; Test 2 and 3, 10 ppm.
† Only one vessel in each experiment for these treatments; other rates are average values for two vessels.

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