freezing under vacuum, proved tedious until we were able to devise methods which gave reproducible results. The details of these methods will be published later.

The results appearing in Table 1 and graphically in Figs. 1–3 show that freezing treated eggs under vacuum decreases considerably their expansion during freezing and reverses the rate of freezing in the various parts of the egg content when freezing takes place at about 20° F. In the fresh, untreated eggs the white freezes first and creates a solid wall around the yolk, so that when the latter freezes there is no room for expansion and the egg cracks. When the eggs are treated and frozen under vacuum, the yolk freezes first and pushes the white toward the shell so that all the room available in the air space is used during the expansion of the white. This, of course, allows better utilization of the space available within the egg. Removal of the gas from the yolk, on the one hand, and lowering of the freezing point of the white by the treatment, on the other, seem the probable explanation of this surprising result. A contributing factor also is the small decrease in volume of the thick white during the freezing of the treated eggs through a partial breaking down of its mucin content, due probably to the change of pH under the reduced pressure of CO_2 (*1*), a fact which is associated with a change in volume. In one particular case, where the change of thick white to liquid was about 50%, the change in volume was about 3%. All these factors working together help to secure a better economy of space within the egg during freezing and reduce the necessary treatment to a minimum. Under these conditions the treatment does not affect the quality of the eggs at all; on the contrary, the whipping quality of the white is somewhat improved. The loss of weight during storage at 20° F and 85% relative humidity was, in our experiments, 0.15% per month, in comparison with a 0.23% loss obtained during the storage at about 32° F. This loss would be still less if the humidity of the room were kept higher. Eggs kept in an undercooled condition at 23° F for 7 months lost 1.8–2.9% (*10*), and if the theoretical formula developed by Greenlee (*2*) is valid, the calculated loss at storage temperatures below 28° F is zero.

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Reversal to Penicillin Sensitivity in a Cysteine-requiring Mutant of *Salmonella*[1]

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The action of penicillin in producing bacteriostatic and bactericidal effects is believed to be due to chemical interactions with essential SH and perhaps NH_2 groups in the medium (Bailey and Cavalitto, *1*), and so a metabolic block is produced which may be irreversible. Additional evidence for the point of view that penicillin is a metabolic antagonist has recently been given by Wilson and du Vigneaud (*7*), who showed that L-penicillamine—but not D-penicillamine—inhibits the growth of young rats when added to a diet which otherwise permits good growth. When aminoethanol and methylated derivatives were added to the L-penicillamine-containing diet the inhibiting effect of the latter was counteracted. More direct evidence of specific metabolic antagonism is given by Gale and Rodwell (*2*), whose experiments with *Staphylococcus* are interpreted as showing that penicillin acts to impair the ability to assimilate glutamic acid. These investigators have studied the nutritional requirements of penicillin-sensitive *Staphylococcus*, and of resistant strains derived from the original culture by training or mutation. It appears that the parental strains cannot synthesize most of the essential nutrilites, but can concentrate within the cell free amino acids, especially glutamic acid, provided they are available in the surrounding medium. The mutant resistant strains have lost the ability to concentrate free glutamic or other amino acids, but have concurrently developed the power to synthesize all these essential nutrilites within the cell from their inorganic constituents. Since the mutant strains are thus not dependent for growth on the assimilatory processes they should be independent of the antagonistic effects of penicillin, as they are.

These studies still leave many questions unanswered. One of the most obvious concerns the status of the Gram-positive organisms like *Bacillus subtilis*, which are heterotrophic, i.e., synthesize all their nutrilites except glucose, yet are still penicillin-sensitive. It is of considerable interest, therefore, to test the penicillin sensitivity of nutritional mutants of other bacteria, particularly those that are originally penicillin-resistant.

We wish to record one such series of tests in a Gram-negative organism, the results of which give excellent correlation with the metabolic antagonism theory of the action of penicillin. We are studying radiation-induced mutations in *Salmonella typhimurium*, a food poisoning pathogen which is heterotrophic and highly resistant to penicillin. A number of different kinds of mutations

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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