dence on this point. Individual males of *Daphnia longispina* from three different lines which were known to be heterozygous for one or more mutant characters were mated with (usually) 8 to 16 sexual egg bearing females of a stock known not to carry these (dominant) mutant characters. These sexual eggs hatch poorly but from four such crosses (all of these crosses from which we have reared more than a single individual) we have had offspring of two classes—those with, and those without the mutant character. In two of these crosses, two dominant mutant characters were involved and segregation in the male occurred for both characters.

Since it is obvious that chromatic reduction and segregation are not to be expected in a haploid male, the demonstration of segregation in these males constitutes genetic evidence that they are diploid.

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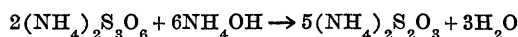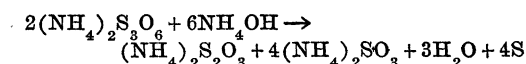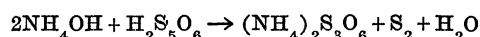## PENTATHIONIC ACID, THE FUNGICIDAL FACTOR OF SULPHUR

IN a previous paper (Young, '22)[1] it is pointed out that pentathionic acid is the fungicidal factor accompanying sulphur. It is further stated that this acid is a product of oxidized sulphur resulting only when oxygen and water are present. These earlier tests proved also that particulate sulphur is more readily oxidized and consequently more fungicidal than ordinary sulphur. This work was confirmed in the main by Tisdale ('25),[2] and its practical application by Lee and Martin ('27).[3] The conclusions have been questioned by some English workers ('25).[4]

During the present summer, the writers continued this investigation with the aim in view of ascertaining the definite chemical relationship of the toxic factor of sulphur to sulphur itself and to determine the effect of certain factors influencing this relationship. We assumed at the outset that the conclusion reached by Freundlich and Scholz ('22)[5] that pentathionic acid is a peptizing agent for sulphur made by the

reaction of H_2S and SO_2 and confirmed by Kruyt ('27),[6] the latter giving a simple diagram of the structure of the sulphur particle peptized by the pentathionic acid $(S)S_5O_6 = \begin{smallmatrix} H+ \\ H+ \end{smallmatrix}$, is correct. In all previous work it was generally supposed that pentathionic acid is peculiar to colloidal forms of sulphur. However, if a test for pentathionic acid (the ammoniacal silver nitrate test given in Mellar's Modern Inorganic Chemistry) is applied to sulphur the characteristic brown color develops and slowly changes to black. Hydrogen sulfide, or the sulfide ion, is the only sulphur compound that might give the same test. When flowers or flour of sulphur is treated with lead acetate, copper sulfate, or silver nitrate, no precipitate of the respective sulfides appears. Sulphur treated with ammoniacal copper sulfate gives no precipitate even on standing; likewise, no sulfide ion is obtained when sulphur is treated with ammonium hydroxide for several hours. Moreover, known solutions of sulfite, sulfate or thiosulfate ions do not respond to the above test for pentathionic acid. It can only be concluded that ordinary forms of sulphur have associated with them pentathionic acid. Our tests showed further that the acid is adsorbed quite completely by the sulphur particle, so much so that none can be washed off, as can be done in the case of hydrophilic colloidal sulphur using a simple ultra filter. The ordinary particle of sulphur flour is hydrophobic, partly because the pentathionic acid is too small in amount to effect hydration to an observable extent. However, when pentathionic acid is added to amorphous sulphur, it, along with other factors, causes complete hydration.

Strong ammonia destroys pentathionic acid if treated for some time, breaking it down to thiosulfate. Freundlich and Scholz used this treatment in determining the acid quantitatively by titrating the thiosulfate with iodine. They give the following reactions:

$$2NH_4OH + H_2S_5O_6 \rightarrow (NH_4)_2S_3O_6 + S_2 + H_2O$$

$$2(NH_4)_2S_3O_6 + 6NH_4OH \rightarrow \\ (NH_4)_2S_2O_3 + 4(NH_4)_2SO_3 + 3H_2O + 4S$$

$$2(NH_4)_2S_3O_6 + 6NH_4OH \rightarrow 5(NH_4)_2S_2O_3 + 3H_2O$$

On the other hand, strong ammonia does not completely destroy the $S_5O_6 =$ ion on the sulphur particle. In this case, we are not dealing with free pentathionic acid but with strongly adsorbed $S_5O_6 =$ which is not free. Consequently, when the pentathionic acid test is used on ammonia-treated sulphur, the nega-

[1] Young, H. C. The toxic property of sulphur. Ann. Mo. Bot. Gard. 9: 403–435, 1922.

[2] Tisdale, L. E. Colloidal sulphur: preparation and toxicity. Ann. Mo. Bot. Gard. 12: 381–418, 1925.

[3] Lee, H. Atherton, and J. P. Martin. The development of more effective dust fungicides by adding oxidizing agents to sulphur. SCIENCE 66: 178, 1927.
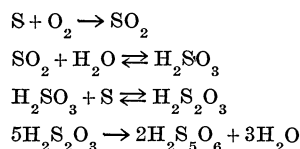
[4] Discussion on "The fungicidal action of sulphur." Ann. of Apl. Biol. 13: 308–318, 1925.

[5] Freundlich, H., and P. Scholz. Ueber hydrophobe und hydrophile Sole des Schwefels. Koll. Beih. 16: 234–266, 1922.

[6] Kruyt, H. R. "Colloids" translated by H. S. Van Klooster. John Wiley & Son (Inc.) Page 238, 1927.

tive $S_5O_6 =$ ion and the negative sulphur particle attract the positive $Ag(NH_3)_2 +$ ion which reacts with the $S_5O_6 =$ to form the characteristic compound which is in the end Ag_2S. A parallel case of the difference of reactions from normal under conditions of powerful adsorption is that a quantity of HCl sufficient to invert sugar will not do so when in a system adsorbed by charcoal.

Since pentathionic acid is present on most forms of sulphur and such forms of sulphur show a degree of toxicity, it is reasonable to assume that the acid is, or contains, the toxic factor. A complete proof of this was obtained by freeing sulphur of this acid and testing it for toxicity. At the outset it was found that when pure pentathionic acid was treated with strong ammonia, then brought back to pH6, or even more acid with HCl, the spores of *S. cinerea* and *V. inequalis* germinated as freely as in the checks. When sulphur was treated with strong ammonia and brought back to pH6, immediate germination tests were positive, but the suspension soon became toxic. Sulphur in the presence of oxygen and moisture is a continual source of pentathionic acid until equilibrium is reached. When the reaction was kept between pH7–7.5, the suspension was only slightly toxic. The oxidation of sulphur probably proceeds as follows:

$$S + O_2 \rightarrow SO_2$$
$$SO_2 + H_2O \rightleftarrows H_2SO_3$$
$$H_2SO_3 + S \rightleftarrows H_2S_2O_3$$
$$5H_2S_2O_3 \rightarrow 2H_2S_5O_6 + 3H_2O$$

In view of these reactions, which indicate the formation of pentathionic acid at the expense of sulphur, oxygen, and water, and those showing the alternate breakdown of this acid by ammonia into ammonium thiosulphate and sulphur, the difficulty Freundlich and Scholz experienced when trying to estimate pentathionic acid in the presence of sulphur can be explained. The reactions also account for the fact that although a solution of pentathionic acid with an added excess of ammonia fails to give a positive test for $S_5O_6 =$, the free sulphur thrown down as indicated in the step reactions develops sufficient $S_5O_6 =$ to give a positive test on standing. This entire procedure can account for the way ammonia gradually dissolves sulphur.

Sulphur prepared from H_2S and SO_2 is affected by strong ammonia in the same way except that the $S_5O_6 =$ ion is much more abundant, reacting more quickly to the pentathionic acid test and recurring more quickly after the sulphur has been treated with ammonia.

Pure pentathionic acid is not so completely destroyed with bases such as $Ca(OH)_2$, KOH, or NaOH. These react directly, each forming the respective pentathionate which gives a positive test. They were non-toxic to the organism used but regained their toxicity when brought to pH6, or below, with HCl. All the germination tests were made in hanging-drop cultures in Van Tieghen cells, and results are given in the table.

GERMINATION TESTS WITH VARIOUS TYPES OF SULPHUR

Treatment	Germination S. Cinerea	Germination V. inequalis
	%	%
1. Pentathionic acid, .062%......	0	0
2. " " .03 %......	2	trace
3. " " .0075.........	4	5
4. Pentathionic acid .03% + strong ammonia, then acidified to pH6 with HCl	80	......
5. Pentathionic acid .03% + CaOH then brought back to neutral pH7 with HCl..	72	......
6. Pentathionic acid .03% + CaOH then brought back to pH5 with HCl......	trace	......
7. Check-distilled water to pH7 with slight $Ca(OH)_2$...........	80	70
8. Ground flour of sulphur treated with strong ammonia then thoroughly washed with O_2 free water	27	......
9. Ground flour of sulphur washed with O_2 free water	1.6	......
10. Ground flour of sulphur treated with $Ca(OH)_2$ then brought to 6.4 with HCl	5	......
11. Ground flour of sulphur treated with gelatin to aid wetting	trace	......

It is quite evident from the above results that pentathionic acid is the toxic factor of sulphur and that this compound is quite sensitive to basic materials. They further show that the natural oxidation and dissolving of sulphur gives a continuous yield of pentathionic acid. Particulate sulphur oxidizes more readily as would be expected in such systems, and consequently is more toxic. The results explain why failures occur with so many commercial dusts and sprays which have been made up more from the standpoint of spreading and sticking than maintaining toxicity. Basic compounds aid in spreading and sticking but inhibit toxicity.

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