

regularly came back immediately to the same zero on the micrometer after each change with no change in length, D_2O was now introduced in the same way. Observations were made, and when shrinkage and recovery were completed, D_2O was replaced with H_2O and observations again made. This was repeated for D_2O and usually also for H_2O . Fig. 1 shows the

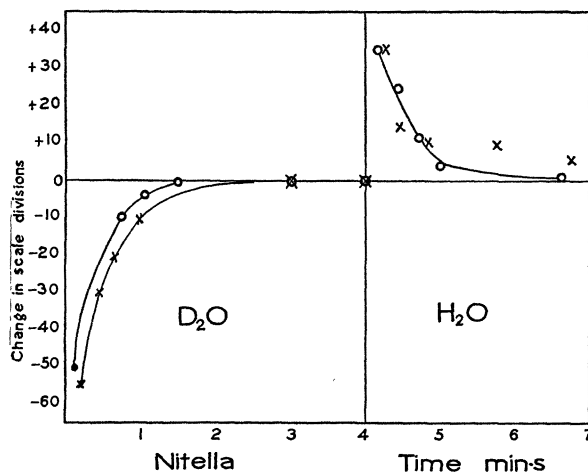


FIG. 1

changes in micrometer divisions, showing shrinkage after application of D_2O , followed by recovery (*i.e.*, swelling) to the original length, while on applying the H_2O , after D_2O , the filament first elongated and then returned to the original length. Altogether six tests for D_2O and five for H_2O were made. All experiments gave the same general behavior, but there was failure of the leaf to return to zero in some cases, either because of slipping of the attachment or the mechanical stimulation by bending against the sides of the glass cells. Observations in D_2O were made for 2 to 11 minutes, and in H_2O for 4 to 12 minutes.

The leaves of *Nitella* shrank 0.60–1.37 per cent. in D_2O , and elongated 0.40–0.70 per cent. in H_2O . These values were observed as nearly as possible at 5 or 10 seconds. The process of putting the water in the glass cell occupies 2 to 4 seconds. Even before the leaf appeared in the microscope field it had passed through a maximum, and by the time observation was made it was already returning toward zero. By this method the true maxima could not be observed, and since the whole recovery occurred in about 1 minute in D_2O , the time recorded in the earlier observations were actually only approximately measured. One notes that D_2O acts as a plasmolytic agent, although plasmolysis is concealed by the stretched elastic cell wall which follows the shrinkage. H_2O causes swelling of the cell, the counter change of that produced by D_2O .

It should be possible to make such cells as erythro-

⁴ D_2O was procured from the Stuart Oxygen Company. Its density was 1.1079 at 25°, and it was 99.9%+ pure.

cytes to swell on return to an H_2O solution from an equally concentrated D_2O solution, thus causing hemolysis. This is analogous to hemolysis caused by treating erythrocytes with hypotonic solutions, since H_2O is hypotonic to D_2O . We have tried using D_2O and H_2O solutions in 1.1 per cent. or 0.8 per cent. NaCl. Erythrocytes were obtained by centrifugation from 0.2 ml of defibrinated sheep blood. In the first experiment 1.1 per cent. NaCl solutions were used. In the second experiment 0.8 per cent. NaCl solutions were used, and the sediment was first washed in H_2O –NaCl solution by centrifugation and decantation. After that in both cases the erythrocytes were suspended in 1.0 ml of D_2O –NaCl, allowed to stand for 10 minutes, then centrifuged, and the D_2O –NaCl decanted, the decantate saved. The sediments were suspended in 5.0 ml of corresponding H_2O –NaCl solutions (1.1 and 0.8 per cent. respectively). Hemolysis was shown by the discolored supernatant fluids decanted after suspension and centrifugation. The remaining erythrocytes were hemolysed in 5.0 ml of pure H_2O . In the first experiment about 22 per cent. of the hemoglobin was in the H_2O –NaCl fluid, as compared with 78 per cent. in the fluid from the last sediment of cells. In the second experiment colorimetric measurements showed that the H_2O –NaCl solution contained 38.7 per cent. of the hemoglobin, 61.3 per cent. in the final sediment. The decanted D_2O was not perceptibly discolored.

The erythrocytes suspended in a D_2O –NaCl solution take up D_2O . These cells would have a vapor pressure much lower than that of the H_2O –NaCl, the H_2O in a few seconds enters the cells and this swelling brings hemolysis of the weaker part of the erythrocytes. Here again D_2O is hypertonic to H_2O and is an active osmotic agent.

The osmotic effects of D_2O as compared to H_2O produce some of the well-known phenomena associated with biological effects. The press, for example, mentioned the “burning sensation” experienced on drinking D_2O . Lewis⁵ describes a mouse which had access to D_2O only and “the more he drank of the heavy water the thirstier he became.” “The symptoms of distress that he showed seemed more pronounced after each dose, but not cumulative with succeeding doses.” Since D_2O violently dehydrates living cells it is quite understandable that D_2O causes thirst and other osmotic effects.

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DISULFIDE FROM AMMONIUM SULFATE IN THE PRESENCE OF MASHED ROOT-TIPS OF PHASEOLUS VULGARIS

A NATURAL result of the finding of nearly ten years ago that the sulphhydryl group and its partially oxi-

⁵ G. N. Lewis, *SCIENCE*, 79: 151–153, 1934.

dized derivatives play a significant part in the regulation of cell increase in number¹ has been stimulation of interest in the origin of the parent effective group.

The transformation of lifeless into living—the coming into expression in the chemical elements of those properties and combinations through which living substance is characterized is the becoming of growth.

Although there is an increasing literature dealing with the reactions of the biologically important sulfur groups *in vivo* and *in vitro*, and although there is much written of the ability of animals as well as plants to reduce the H_2S and even other sulfur compounds, not only elemental sulfur but also its highly oxidized derivatives such as sulfuric acid,^{2,3,4} the beginning formation of the growth significant groups has yet to be traced.

Since soil is the source and plants the presumed medium for transformation of inorganic to organic—and since in root-tips there is concentration of SH and S.S. in organic form,⁵ a first step in the search for the origin thereof would seem to be an inquiry into whether root-tips can make SH or S.S. from SO_4 .

To do this the common bean (*Phaseolus vulgaris*) was sprouted on wet filter paper over absorbent cotton soaked in well-water in large Petri dishes. From the roots so obtained when 1 to 2 cm in length there was cut the distal 1.0 to 1.5 mm of meristematic tissue with a safety-razor blade. The tissue is recognizable by its yellowish tinge as distinct from the dead white of the proximal length. Some hundreds were cut and kept from drying for each trial. These were mashed and ground with a glass rod in the bottom of a 10 cc test-tube with a pinch of fine beach sand previously treated with cleaning solution and vigorously washed with well-water for 50 shakings. The mash was mixed with 1 cc well-water and centrifuged. Two to four drops of the supernatant fluid were added to each of two 10 cc test-tubes, the one containing 1 cc of a 0.1 per cent. $(NH_4)_2SO_4$ solution and the other plain well-water for control. Other controls were set up of sulfate solution and well-water alone; *viz.*, with no extract, usually two sets of each. After 18 to 24 hours the mixtures saturated with solid $(NH_4)_2SO_4$ were tested on the spot-plate for SH and S.S. with ammoniacal sodium nitroprusside.

Free SH was never found in these experiments which were repeated many times. The disulfide—or

¹ F. S. Hammett, *Proc. Am. Philos. Soc.*, 68: 151–161, 1929.

² L. Rubentschik, *Centralbl. Bakt.*, 11: Abt. 73, 483–496, 1928.

³ A. Rippel and G. Behr, *Archiv. Mikrobiol.*, 7: 584–589, 1936.

⁴ B. E. Kline, E. B. Hart, J. G. Halpin and C. E. Holmes, *Wis. Agr. Expt. Sta. Bull.*, 430 (Ann. Rept., 1933–1934).

⁵ F. S. Hammett, *Protoplasma*, 5: 547–562, 1929.

S.S. form—however, was detected more often than not after cyanide reduction in the extract-sulfate mixture. It was never found in the sulfate solution alone or well-water alone—and only infrequently in the extract-well-water mixture and then in very much less concentration than in the corresponding extract-sulfate mixture. A positive reaction was never obtained when the extract-sulfate mixture was boiled before incubation. This indicates that boiling destroyed some agent responsible for the reduction of SO_4 to S.S. The reaction was not inhibited in the unboiled extract-sulfate mixture on incubation in the presence of thymol.

These results suggest the presence of an enzyme in the root-tips of *Phaseolus vulgaris* which is capable of reducing SO_4 to S.S.

Although the possibility that the reduction derived from bacterial action is not completely eliminated, yet the fact that thymol did not inhibit its expression and the fact that a negative test was repeatedly given in the boiled extract-sulfate mixture despite opportunity for contamination, is evidence consistent with the enzyme postulate. At any rate the findings justify extension of the inquiry which will now be along more quantitative and other lines of obvious interest.

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EXACT PROBABILITIES IN CERTAIN CARD-MATCHING PROBLEMS

WE consider three cases:

THE 3×3 CASE

With a deck of nine cards composed of 3 suits of 3 cards each, the number of possible “runs” is known to be 1,680. If each of these possible runs is “matched” against any fixed run (taken from a duplicate deck), the number of “hits” found may be called the “score” for that run. I have computed by direct elementary means the exact frequency with which each possible score will occur in a complete cycle of runs. This result is given in Table A:

TABLE A

Score	Freq.	Rel. freq.
0	56	.03333
1	216	.12857
2	378	.22500
3	435	.25893
4	324	.19286
5	189	.11250
6	54	.03214
7	27	.01607
8	0	.0
9	.1	.00060
	1,680	1.00000

The mean of the distribution is 3, and the standard deviation is 1.5.