

TABLE I.

Tube No.	Folic acid concentrate, specimen	Amt per cc, $\gamma$	% of max. growth
1	I	50.0	100
2	"	25.0	100
3	"	10.0	100
4	"	5.0	70
5	"	2.5	40
6	"	1.0	20
7	II	.025	100
8	"	.01	100
9	"	.005	100
10	"	.0025	100
11	"	.001	60
12	"	.0005	10
13	"	.00025	trace
14	III	.01	100
15	"	.005	100
16	"	.0025	100
17	"	.001	70
18	"	.0005	40
19	"	.00025	30
20	"	.0001	25
21	none		trace

It is evident that "folic acid" is essential to the growth of this strain of tetanus, and that its effect is exerted by extremely minute quantities.

## 13517

### Nature of Hydrosulphosol and Its Toxicity to Living Embryonic Tissue.

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Compounds containing the sulfhydryl ( $-SH$ ) radical have been shown to be of great practical value in promoting the healing of wounds. Glutathione is probably the best known compound of this type which is present in living tissues. It is of almost universal occurrence in animal tissues where the concentration has been found to roughly parallel the intensity of metabolic activity. The concentration is unusually high in regenerating or embryonic tissues. Voegtlin, Johnson and Thompson<sup>1</sup> reported that malignant tumor cells showed higher concentrations of glutathione than normal

<sup>1</sup> Voegtlin, C., Johnson, J. M., and Thompson, J. W., *Public Health Rep.*, U. S. Public Health Service, 1936, **51**, 1689.

tissue cells. Therefore, it appears that one of the important biological functions of glutathione, as well as other compounds of a similar nature, is related to growth.

Reimann<sup>2</sup> employed thiocresol in a concentration of 1:10,000 as a wet dressing on a number of stubborn wounds and reported a stimulation of cell division. On the basis of the results obtained from the use of thiocresol, and a number of other compounds containing the -SH group and their homologues containing other groups, he concluded that the sulfhydryl radical was the effective agent. Riley<sup>3</sup> used a lanolin ointment containing cysteine hydrochloride on skin reactions following deep X-ray or radium therapy and believed that the healing was hastened by the presence of the -SH radical.

Hydrosulphosol\* is an available and convenient source of -SH radicals. This fact, together with its value in ultraviolet burns, was discovered by Dr. Ralph R. Mellon (McBroom<sup>4</sup>) and his associates at the Western Pennsylvania Hospital's Institute of Pathology in Pittsburgh. As a result they suggested that it receive a thorough clinical trial in thermal burns. During the past year about 150 chemical and thermal burns have been treated by Dr. W. F. Pierce<sup>5</sup> and by Dr. A. E. Cruthirds.<sup>6</sup> About two-thirds of these have been serious, either in degree (second or third), or in extent, or both. The results have been so highly satisfactory as to justify the increasing clinical use that the solution is now receiving.

In addition, Hydrosulphosol contains the pentathionate ion in

TABLE I.  
Chemical Analysis of Hydrosulphosol

Specific gravity	1.032
pH of solution (glass electrode)	10.2
Solids remaining after evaporation of 100 g solution to constant weight	10.56
Constituent	%
Calcium from calcium oxide	0.549
Total sulfur (iodine titration)	1.46
Sulfur as sulfide ion	0.30
"    " thiosulfate ion	0.22
"    " from polysulfide	1.256
Sulfhydryl ion (methylene blue titration)	0.237

<sup>2</sup> Reimann, S. P., *J. A. M. A.*, 1930, **94**, 1369.

<sup>3</sup> Riley, J. F., *Brit. Med. J.*, 1940, **2**, 516.

\* Hydrosulphol is a liquid preparation supplied by the Earl Hill Wilson Foundation for Medical Research, Los Angeles, California.

<sup>4</sup> McBroom, J., *Med. Rec.*, 1940, **152**, 299.

<sup>5</sup> Pierce, W. F., *Am. J. Surg.*, 1941, **53**, 434.

<sup>6</sup> Cruthirds, A. E., personal communication.

compounds of polysulfides and thiosulfates in aqueous solution. The composition of the solution is given in Table I. Because of the nature of the solution it was not possible to obtain a more accurate analysis. On the addition of distilled water colloidal sulfur is rapidly precipitated indicating that the solution is unstable chemically. Available evidence points to the fact that the pH drops when the solution comes in contact with organic matter, as occurs in burns.

Methylene blue may be used as a test for compounds containing the  $-SH$  radical. The dye is rapidly decolorized in the presence of the sulfhydryl group and other reducing substances. The H of the sulfhydryl group readily combines with methylene blue, reducing it to the colorless compound. This is the only group present in Hydrosulphosol which was found to be capable of reducing methylene blue. The amount of methylene blue decolorized indicates that Hydrosulphosol contains about 0.237 g of the  $-SH$  ion per 100 cc. This is approximately 50 times the concentration of the ion present in human blood.

Another test for the presence of sulfhydryl-containing compounds is their ability to support growth of anaerobic organisms under aerobic conditions. Ten tubes of nutrient broth, each containing 10 cc, were heated in an Arnold sterilizer for 10 minutes to remove dissolved oxygen. To one series of 5 tubes were added 0.05 cc, 0.1 cc, 0.2 cc, 0.3 cc, and 0.4 cc Hydrosulphosol respectively. The other series served as a control. The tubes were each inoculated with one drop of a culture of *Clostridium sporogenes* and incubated at  $37^{\circ}C$  for 48 hours. A similar series was set up employing *Clostridium perfringens*. At the end of 48 hours the control tubes in both series showed no growth, whereas the tubes containing Hydrosulphosol showed luxuriant growth. An amount of Hydrosulphosol as small as 0.05 cc was sufficient to yield a heavy growth of the organisms in the presence of air. Smaller amounts were not tried.

These facts have stimulated investigations of Hydrosulphosol in the treatment of burns. Since it is being employed to an ever increasing extent it was considered of great interest and importance to see what effect the solution would have on living embryonic tissue fragments cultivated *in vitro*.

Accordingly, a series of dilutions of Hydrosulphosol were prepared in physiological salt solution. To 4 cc of each dilution was added 1 cc of a mixture of 3 parts of embryonic extract and 1 part of horse serum as a source of organic matter, in an endeavor to duplicate actual clinical conditions. The final dilutions of Hydro-

sulphosol were 1:20, 1:40, 1:60, 1:80, and 1:100. Five cc of each dilution were pipetted into sterile test tubes and placed in a 37°C water bath. Embryonic chick hearts were cut into fragments about 0.5 mm in diameter. About 100 fragments were pipetted into 5 cc of each dilution of Hydrosulphosol. At the end of 10 minutes the fragments were thoroughly washed and embedded in plasma contained in Carrel flasks. Slight growth occurred in the 1:20 dilution after an incubation period of 48 hours and normal growth occurred in a dilution of 1:80. It is believed that the toxicity of the compound to tissue was due to its high alkalinity. In the higher dilutions the alkalinity was sufficiently reduced to permit the growth of tissue. The original solution could not be adjusted to a lower pH owing to the fact that a rapid and heavy precipitate appeared on the addition of acid.

In another test a series of higher dilutions of Hydrosulphosol were prepared and allowed to remain in contact with the embryonic chick heart tissue fragments for 72 hours instead of only 10 minutes. The test was performed as follows: Tissue fragments were embedded in plasma contained in Carrel flasks. After the plasma had coagulated the surface was flooded with Hydrosulphosol dilution. One cc of Hydrosulphosol dilution was added to 2 cc of the coagulated plasma mixture (plasma-Tyrode solution-embryonic extract-tissue fragments). The dilutions ranged from 1:160 to 1:800. Although growth occurred in all dilutions only those from 1:400 and above showed proliferation as luxuriant as the controls. In the higher dilutions there was no evidence that stimulation beyond normal growth occurred. It must be remembered that chick heart tissue was used instead of skin. Tests are now under way using only embryonic skin for the tissue fragments.

*Conclusions.* Evidence has been presented to show that Hydrosulphosol is an available and convenient source of -SH radicals. The compound rapidly decolorizes methylene blue and supports the growth of the anaerobic organisms *Clostridium sporogenes* and *Clostridium perfringens* under aerobic conditions. The compound is relatively nontoxic to embryonic chick heart tissue fragments cultivated *in vitro*.