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Folic Acid in the Growth of *Cl. tetani.*

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Recent work in this laboratory[1] has demonstrated the requirement by a strain of *Cl. tetani* for a component of liver extract originally described in another connection by Snell and Peterson[2] and subsequently studied by Stokstad.[3] This material, together with thiamine and riboflavine, fully accounted for the activity of a fraction of liver extract which had been found essential to the growth of our tetanus organism. The active component of Snell and Peterson's fraction has been further investigated by Mitchell, Snell and Williams,[4] who have carried the purification considerably further, and have named the agent "folic acid" since they find green leaves to be a convenient source. Its chemical constitution has not yet been determined.

Through the courtesy of Professor Williams it has been possible to test his purified material as to its growth-promoting effect on the tetanus bacillus. Three specimens, representing varying degrees of concentration have been made available. The amounts required to produce half maximum growth of *L. helveticus* were given as follows:

Specimen	1	6.0 gamma
"	2	0.005 "
"	3	0.0016 "

These preparations were used in varying quantities to replace the Snell and Peterson or Stokstad factor in an otherwise complete medium.[1] After seeding with our strain of *Cl. tetani* and anaerobic incubation for 24 hours, relative growths were determined in a photoelectric nephelometer. The results, expressing degree of growth approximately as percentage of maximum are shown in Table I.

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1 Mueller, J. H., and Miller, P. A., *J. Bact.*, in press.

2 Snell, E. E., and Peterson, W. H., *J. Bact.*, 1940, **39**, 273.

3 Stokstad, E. L. R., *J. Biol. Chem.*, 1941, **139**, 475.

4 Mitchell, H. K., Snell, E. E., and Williams, R. J., *J. Am. Chem. Soc.*, 1941, **63**, 2284.

TABLE I.

Tube No.	Folic acid concentrate, specimen	Amt per cc, γ	% 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.

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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[1] reported that malignant tumor cells showed higher concentrations of glutathione than normal

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