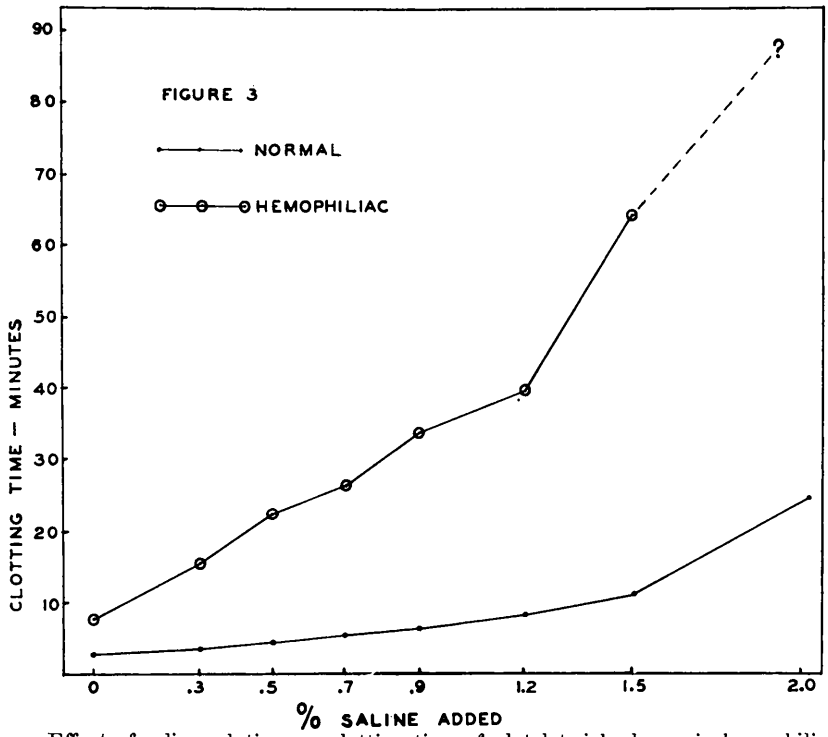

Effect of saline solutions on clotting time of platelet-rich plasma in hemophilia as compared with normal; reactions taking place in solutions of varying tonicity.

however, for it is entirely possible that the increased lysis of platelets by supplying an excess of thromboplastin serves to counteract some other deficiency or excess in hemophilia.

As suggested by Muhrer, one of the chief drawbacks in the testing of substances active in reducing clotting time in hemophilia is the lack of suitable experimental animals. These findings with human blood are sufficiently close to those obtained with the blood of hemophilic swine to suggest the possibility that study of the latter may throw light on the human disease.

I am indebted to Dr. Philip Wagley of the Johns Hopkins Hospital and to Dr. Harry Eagle of the United States Public Health Service for technical aid and advice in carrying out this study.

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Effect of 2-Methyl-1,4-Naphthoquinone on Glycolysis of *Schistosoma mansoni*.*

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Earlier observations indicated that oxidative metabolism is not essential for the survival of *Schistosoma mansoni*.[1] Since schistosomes have a high rate of both anaerobic

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[1] Peters, L., Welch, A. D., and Bueding, E., unpublished data.

TABLE I.
Effect of Quinones on Oxygen Uptake and Glycolysis of Schistosomes.

Compound	Molar concn.	Medium	O_2 uptake		Glucose removal		Lactic acid production	
			µl*	% change	µg*	% change	µg*	% change
—	—	Salt	1.21		33.5		30.4	
2-Methyl-1,4-naphthoquinone	1x10-4	"	0.59	—51	0	—100	7.2	—76
" "	2x10-5	"	1.25	+ 3	21.6	— 36	23.2	—24
1,4-Naphthoquinone	2x10-5	"	0.94	— 21	13.6	— 58	18.1	—40
1,2- "	2x10-4	"	1.15	— 5	28.9	— 13	28.0	— 7
1,4-Quinone	2x10-5	"	1.26	+ 4	24.8	— 26	27.1	— 11
"	2x10-4	"	0.94	—22	7.0	— 79	16.1	—48
1,4-Hydroquinone	2x10-5	"	1.18	— 6	28.2	— 16	28.3	— 7
"	2x10-4	"	0.87	— 28	16.7	— 50	20.9	—32
3-Methoxy-2-methyl-1,4-naphthoquinone	1x10-3	"	0.51	— 58	16.7	— 50	13.6	—55
3-Hydroxy-1,4-naphthoquinone†	1x10-3	"	0.58	— 52	25.3	— 25	22.3	—27
3-Hydroxy-2-piperidino-methyl-1,4-naphthoquinone†	1x10-3	"	1.19	— 2	29.4	— 12	27.4	—10
3-Hydroxy-2-amyl-amino-methyl-1,4-naphthoquinone†	1x10-3	"	0.80	—34	21.9	— 35	22.3	—27
—		Buffered serum	1.40		37.6		44.6	
2-Methyl-1,4-naphthoquinone	5x10-4	" "	1.06	—24	4.9	— 87	13.7	—69
" "	1x10-4	" "	1.42	+ 2	23.6	— 37	31.3	—30
1,4-Naphthoquinone	1x10-4	" "	1.18	—16	28.3	— 25	36.5	—18
Quinone	2x10-3	" "	—	—	12.0	— 68	20.2	—55
Hydroquinone	2x10-3	" "	—	—	21.4	— 46	34.9	—22

* Per hour and mg wet wt.
† Kindly supplied by Abbott Laboratories, Chicago.

and aerobic glycolysis, studies have been made of the glycolytic processes and of their inhibition by compounds of low toxicity for mammalian species.

Paired adult schistosomes were removed from the mesenteric or portal veins, or from the larger hepatic vessels of mice infested with *S. mansoni*. The organisms were placed in a glucose-containing salt medium (0.137 *M* NaCl, 0.0027 *M* KCl, 0.0003 *M* $CaCl_2$, 0.001 *M* $MgCl_2$, 0.066 *M* phosphate; pH, 7.4) or in a mixture of 40 volumes of human serum (previously degassed and adjusted to pH 7.4) and 3 volumes of 0.5 *M* phosphate buffer (pH 7.4). A high phosphate concentration is essential for the optimal respiration and glycolysis of schistosomes *in vitro*. Incubation was then carried out in small vessels (vol. 4 to 5 ml) in 0.8 ml of medium at 38.6°C in an atmosphere of air; the consumption of oxygen was measured manometrically. The concentrations of glucose[2] and of lactic acid[3] in the medium were determined before and after the incubation period.

The rate of aerobic glycolysis of schistosomes was inhibited by 2-methyl-1,4-naphthoquinone (representative results are recorded in Table I). Oxygen uptake was inhibited to a much smaller extent than was the removal of glucose or the formation of lactic acid. The concentration of 2-methyl-1,4-naphthoquinone required to produce a 50% inhibition of glycolysis was about 5 times greater in human serum than in a protein-free salt medium. If schistosomes were incubated for 30-60 minutes at 38°C in the serum-medium, containing the naphthoquinone in a concentration of 1×10^{-4} *M*, and were then transferred into fresh medium without the quinone, inhibition of glycolysis was maintained for a period of at least 4 hours. As recorded in Table I, a related compound, 1,4-naphthoquinone, was somewhat more effective than the 2-methyl derivative in inhibiting glycolysis of schistosomes in a salt medium, but it was less effective in serum. 1,2-Naphthoquinone was a much weaker inhibitor of glycolysis of schistosomes than was 1,4-naphthoquinone or 2-methyl-1,4-naphthoquinone. Furthermore, 3-methoxy-2-methyl-1,4-naphthoquinone† and substituted 3-OH-naphthoquinones were much less potent inhibitors of glycolysis of *S. mansoni* than was 1,4-naphthoquinone or 2-methyl-1,4-naphthoquinone. It appears, therefore, that introduction of a hydroxy- or a methoxy-group in position-3 decreases markedly the activity of a naphthoquinone against schistosomes *in vitro*.

When mice infected with schistosomes were fed a diet containing 1 to 1.5% 2-methyl-1,4-naphthoquinone for one week and, during this period, were injected intraperitoneally with subcurative doses of sodium antimony III-biscatechol-2,4 disulfonate ("Fuadin") (7.5 mg/kg every 8 hours for 5 days), the schistosomes removed from the livers of these mice showed a markedly decreased rate of glycolysis, as indicated by 60 to 90% reduction of glucose removal and of lactic acid formation. Furthermore, the combined administration of the antimonial compound and the quinone resulted in the complete disappearance of the schistosomes from the mesenteric veins and in many cases from the portal vein of the host. The administration of 2-methyl-1,4-naphthoquinone alone, or of "Fuadin" alone, resulted in a much smaller decrease in the glycolytic activity (10-20%) of the parasites and did not lead to the exodus of the worms from the mesenteric veins.

Conclusions. 2-Methyl-1,4-naphthoquinone markedly inhibits aerobic glycolysis of *Schistosoma mansoni in vitro*. Since glycolysis, rather than oxidative metabolism, appears to be essential for the survival of these organisms, and since the toxicity of 2-methyl-1,4-naphthoquinone for mammalian species is low, the effect of this compound in experimental schistosomiasis has been studied. Results in mice suggest that the compound may act synergistically with subcurative doses of antimonials ("Fuadin").

[2] Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 61, 69.

[3] Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535.

† Kindly supplied by Dr. C. Colwell of The Velsicol Corporation, Chicago.