rhythm in driven ventricular strips. This action appeared to be independent of change in resting excitability and to result from enhancement of intrinsic rhythmicity. Vagal action on the auricle depressed the pacemaker, decreased contraction amplitude and shortened refractory period. Contractility and refractory period changes were independent of rate change. In the atropinized auricle high concentrations shortened refractory period by direct muscle action. During the stage of shortened refractory period there was a critical interval when a single extra shock might elicit multiple responses. In the turtle ventricle, which has no vagal endings, high concentrations shortened refractory period, decreased conduction velocity and raised threshold for electrical stimulation.

1. Cushny, A. R., *Heart,* 1909, vI, 1.
2. Cash, J. T., and Dunstan, W. R., *Phil. Trans. Roy. Soc. London,* 1898, v190, 239.
3. Wedd, A. M., and Blair, H. A., PROC. SOC. EXP. BIOL. AND MED., 1945, v60, 64.
4. Tenney, S. M., and Wedd, A. M., *Circulation Research,* in press.
5. Wedd, A. M., Blair, H. A., and Dwyer, G. K., *J. Pharm. Exp. Therap.,* 1941, v72, 394.
6. Hermanns, L., *Z. Biol.,* 1912, v58, 261.

Received July 8, 1953. P.S.E.B.M., 1953, v84.

Effects of N-Methylformamide and Related Compounds in Mouse Sarcoma 180.* (20590)

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Recently a series of formamides was evaluated as possible solvents for parenteral administration of organic chemicals in the sarcoma 180 screening program of this Institute(1). The compounds proved too toxic for this purpose. However, 2 of the agents, formamide and N-methylformamide (Fig. 1), were found to inhibit the growth of the assay tumor. A third, N,N-diethylformamide, had questionable inhibitory activity whereas other members of the series were without effect. The present report is a study of the inhibitory actions of the formamides in sarcoma 180 (S-180). Special emphasis is given to the N-methyl derivative since it appears to be the most effective of the series. The inhibitory action of urethane against S-180, which has been mentioned previously(1), is also reviewed since the chemical configuration of this agent resembles that of the formamides (Fig. 1) and since it has been found effective against a variety of experimental and clinical neoplasms(2-5).

Tumor inhibition test. Female mice of the Swiss-Webster (CFW) strain weighing 18-22 g were employed. Small, uniform cubes (*ca*

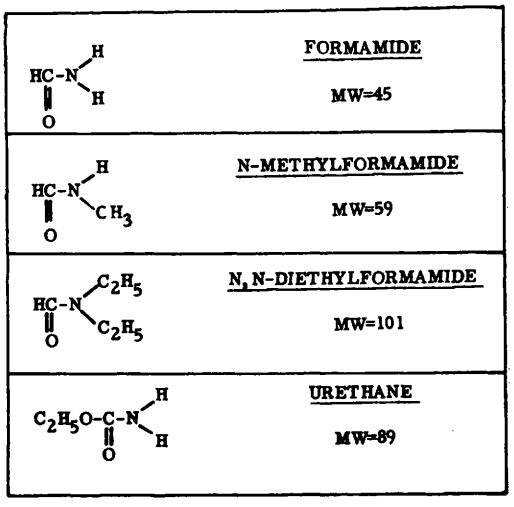

FIG. 1.

* This study was supported in part by an institutional grant from the American Cancer Society and in part by funds from the National Cancer Institute, U. S. Public Health Service.

TABLE I. Inhibition of Growth of Mouse Sarcoma 180 by Formamides. Treatment initiated 24 hours after implantation.

Compound	Mean diameter of tumors, mm ± SD	Toxicity: Avg wt change, g‡	Toxicity: Mortality
I. Observations at end of therapy			
N-Methylformamide*	3.1 ± 1.0	—4.5	1/30
Controls	11.9 ± 1.7	— .5	0/30
Formamide*	6.4 ± 1.6	—4.0	0/30
Controls	11.7 ± 1.2	—1.0	0/30
Diethylformamide*	10.3 ± 1.5	+ .5	0/30
Controls	11.2 ± 1.3	+ .5	0/30
Urethane†	6.7 ± 1.1	—4.0	3/30
Controls	11.4 ± 1.5	— .5	0/30
II. Observations 1 wk after end of therapy			
N-Methylformamide	10.2 ± 1.9	—2.0	9/30
Controls	14.4 ± 2.0	—5.5	7/30
Formamide	11.3 ± 1.0	—4.4	11/30
Controls	13.8 ± 1.1	—5.5	6/30
Diethylformamide	14.5 ± 2.5	—4.0	2/30
Controls	14.0 ± 2.9	—3.5	3/30
Urethane	10.6 ± 1.8	—6.5	18/30
Controls	15.3 ± 2.0	—5.0	4/30

* Given intraperitoneally, 500 mg/kg/day.
† " " , 1000 " .
‡ Cumulative from day of implantation.

2 x 2 x 2 mm) of mouse S-180 were implanted by trocar subcutaneously in the right axillary region of recipient mice. Treatment was initiated either 24 or 96 hours later. The agents† were dissolved in isotonic NaCl and administered either by intraperitoneal injection or by oral intubation in the volume of 0.5 ml/20 g mouse once daily on each of 7 successive days. Control animals were simultaneously injected with a similar volume of isotonic NaCl. One day after completion of therapy tumor diameters in two perpendicular axes were measured through the skin by means of vernier calipers; the averages of the two diameters were used to obtain the mean diameter of each experimental group.

Results and discussion. Treatment initiated 24 hours after implantation. Part I of Table I illustrates that significant inhibition of tumor growth was obtained by the end of therapy in those animals treated with approximately equimolar doses of N-methylformamide, formamide, and urethane. Since the weight of S-180 varies directly as the cube of its mean diameters(6), it was possible to calculate that the ratio of tumor mass in animals given N-methylformamide to that in controls was 1:57; in those given formamide and urethane the proportions were 1:6.1 and 1:4.9, respectively. On the other hand, the effect of N,N-diethylformamide was relatively negligible; the ratio of mass in treated and control tumors was 1:1.3. Moreover, N,N-diethylformamide failed to cause significant inhibition of S-180 when given in the dose of 1120 mg/kg/day which is equimolar to the dose of formamide employed in Table I. It thus appears that the N-methyl group enhances the activity of the formamide moiety while other substitution suppresses inhibitory effects.‡

The results in Table I also indicate that the inhibitory effects noted were not persistent. Part II of the table describes the status of tumors in the animals which survived for as long as one week after the end of therapy. By this time the treated tumors had resumed rapid growth and the inhibitions noted one week earlier were now less evident. A further analysis of the recovery of S-180 from inhibition by N-methylformamide will be presented below. The fatalities, recorded in Part II, Table I, in groups treated with the 3 active substances were presumably due to intoxication by the agents, for the majority of these deaths occurred during the week of treatment or within the first 48 hours thereafter. Moreover, the size of tumors in these animals at time of death was significantly less than that found in untreated, tumor-bearing mice(6). On the other hand, in the control groups and in mice given N,N-diethylformamide, fatalities occurred in animals bearing tumors of a size usually associated with death due to intoxication by S-180(6). The dose of urethane employed was sufficiently depressant to mice to result in loss of righting re-

† The authors are indebted to the Rohm and Haas Co., Philadelphia, for generous supplies of purified N-methylformamide.

‡ Preliminary observations with more than 60 different formamides have failed to uncover any which are inhibitory to S-180 other than formamide and its N-methyl derivative. Of particular interest has been the finding that acetamide, N,N-dimethylformamide, N-ethylformamide, and thioformamide were among the ineffective formamides.

TABLE II. N-Methylformamide Inhibition of S-180 Growth in Mice. Intraperitoneal *vs.* oral routes. Treatment initiated 24 hours after implantation.

Dose, mg/kg/day	Intraperitoneal route: No. of mice surviving	Intraperitoneal route: Mean diameter of tumors, mm ± SD	Oral route: No. of mice surviving*	Oral route: Mean diameter of tumors, mm ± SD
500	10/10	2.6 ± .5	10/10	2.5 ± .5
400	"	2.6 ± .6	"	2.9 ± 1.4
300	"	3.1 ± 1.2	"	4.1 ± 1.4
200	"	6.1 ± .9	9/10	6.1 ± 1.2
100	"	8.3 ± 1.7	8/10	8.1 ± 1.4
Controls	20/20	10.8 ± 1.2	11/20	10.5 ± 1.2

* Deaths were all accidental, incident to intubation.

flexes and to cause complete immobilization for several hours after each injection. It is likely that the high incidence of mortality among urethane-treated animals was, in part, attributable to central depression. On the other hand, no depressant actions were observed after administration of either formamide or its two derivatives. It will be noted in Table I that the active agents caused marked weight loss by the end of therapy. This debilitation cannot be considered the sole cause of tumor inhibition for the growth of S-180 is only moderately affected in mice which have been even more severely debilitated by dietary restriction. As an example, mice have been placed on a regimen of 0.5 g of diet§/individual/day with water *ad lib* at 24 hours after tumor implantation. They lost an average of 6 g in body weight over a period of 8 days while their tumors grew to a mean diameter of 8.0 mm. Control mice receiving both food and water *ad lib* during this period gained an average of 0.5 g in body weight and the mean diameter of their tumors was 10.9 mm(6). Furthermore, numerous examples have recently been reported of compounds which cause marked weight loss without significant inhibition of S-180(7). The response of the growth of S-180 to varying doses of N-methylformamide is shown in Table II. The table also indicates that the agent is equally effective whether given by intraperitoneal injection or by oral intubation.

TABLE III. N-Methylformamide Inhibition of S-180 Growth in Mice. Treatment initiated 96 hours after implantation.

Treatment	No. of mice	Mean diam. of tumors (mm ± SD) at Start of therapy	Mean diam. of tumors (mm ± SD) at End of therapy
N-Methylformamide*	15	5.4 ± .7	4.1 ± .7
Controls	15	5.0 ± .9	14.1 ± 1.9

* Intraperitoneal injections, 500 mg/kg daily for 7 days.

§ Purina Laboratory Chow.

Treatment initiated 96 hours after implantation. As in the case of 24-hour implants, the growth of 96-hour tumors was inhibited by N-methylformamide (Table III). This finding is to be contrasted with the effects of other agents previously shown to be active against S-180. For example, 2,4,6-triethylenimino-*s*-triazine(8) and certain of the folic acid antagonists(9) produce marked inhibition when therapy is initiated in mice with 24-hour implants; however, these agents are relatively ineffective when therapy is delayed until 96 hours. This difference between N-methylformamide and the last mentioned agents cannot be explained at present. It may, however, be related to changes in metabolism of S-180 between 24 and 96 hours after implantation, for implants require at least 48 to 72 hours before they become vascularized and attain maximal rates of growth(6). The transient nature of the inhibition caused by N-methylformamide has been noted above in the results shown in Table I. As in the previous experiment, the treated tumors of Table III resumed growth during the week following the end of therapy. In 3 other experiments mice with 96-hour implants were given a single injection of N-methylformamide which caused transient inhibition, as illustrated in Fig. 2, without inducing significant loss of body weight.

Microscopic alterations in treated tumors. The microscopic effects of N-methylforma-

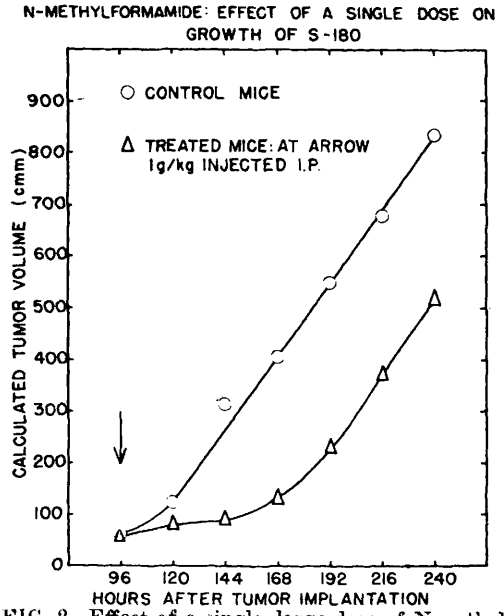

FIG. 2. Effect of a single, large dose of N-methylformamide on growth of S-180. Curves are avg tumor volumes of 15 controls and 15 treated mice; volume of each tumor calculated from its mean diameter.

mide were studied in tumors from mice given successive daily injections of 500 mg/kg/day (treatment initiated with 24-hour implants). Groups of 3 animals were sacrificed at 24-hour intervals from 1 to 8 days after initiation of therapy. Earliest evidence of microscopic alteration was found in tumors removed at 3 days; thereafter, cellular changes became progressively more pronounced. Briefly, the aberrations included the presence of enlarged cells with swollen, frequently vacuolated cytoplasm and with abnormal nuclei exhibiting either bizarre distributions of chromatin or pyknosis. Although such alterations represent significant changes in tumor cells, their non-specific nature deserves emphasis, for similar disturbances have been found in S-180 treated with certain other inhibitory agents (*e.g.*, folic acid antagonists(10), 2,4,6-triethylenimino-*s*-triazine(11) and N,N′,N″-triethylenephosphoramide(12)).

Mode of action. Formamide and N-methylformamide, like amides in general, are relatively stable substances. Therefore the possibility of direct chemical combinations with vital cellular constituents seems unlikely unless such reactions were to involve unknown enzymatic processes. In regard to the latter it should be kept in mind that the products of the hydrolysis of the agents, namely, formic acid, ammonia, and methylamine, are biologically active substances. It is also conceivable that formamide and its N-methyl congener could act as metabolite antagonists for certain simple substances which closely resemble the agents in molecular configuration. Specifically, it is tempting to propose an antagonism of single carbon units, for the role of one-carbon intermediates in vital biosynthetic mechanisms is well-established(13-15). In a preliminary test of this hypothesis the administration of formate, acetate, and glycine failed to block the inhibitory effects of the formamides in S-180 or to reduce their toxicity.‖ Since antagonists of folic acid are known to interfere with incorporation of formate into polynucleotide adenine, guanine, and thymine(16, 17) and since such antifolics are known to be potent inhibitors of S-180(9), attempts were made to prevent formamide inhibition with either folic acid or synthetic citrovorum factor. These two metabolites also failed to block tumor inhibition or toxicity.‖

Another possible mechanism was suggested by the observation of Skipper, *et al.*, that both urethane and formamide inhibit the growth of *Escherichia coli*. Inhibition of growth by either agent was prevented by the presence of small amounts of 2,6-diaminopurine (DAP) or of its riboside(18,19). DAP was tested as a possible antagonist of the formamides in S-180 but in the manner employed failed to influence the tumor inhibition

‖ All blocking experiments were started 24 hours after S-180 implantation. In separate experiments, formamide and N-methylformamide were injected in doses of 500 mg/kg/day for 7 consecutive days. The dose of the candidate blocking agent was injected just prior to the dose of the inhibitor. The following daily doses of these candidates were employed (mg/kg): sodium formate, 500; sodium acetate, 500; glycine, 500; folic acid, 25; synthetic citrovorum factor (leucovorin), 10; 2, 6-diaminopurine, 35. The authors are grateful to Dr. J. M. Ruegsegger of the Lederle Laboratories Division of American Cyanamid Co., Pearl River, N. Y., for supplies of leucovorin and to Dr. G. H. Hitchings, The Wellcome Research Laboratories, Tuckahoe, N. Y., for supplies of 2,6-diaminopurine.

or toxicity caused by the latter substances.‖

Finally another possible mode of action of the formamides is suggested by their hepatotoxicity, a property which will be the subject of a future publication. The toxic actions of both formamides in mice, rats, and dogs are referable to hepatic dysfunction and, following large doses, parenchymal necrosis. Moreover, preliminary studies have revealed that mice, in which the growth of S-180 has been inhibited, also evidence a simultaneous hepatic insufficiency. Whether or not tumor inhibition in mice reflects indirectly a primary hepatic damage is at present under investigation. It is of some interest to note that urethane itself has been found hepatotoxic(20, 21) although a possible relationship between this action and its capacity to affect experimental and clinical tumors has not previously been considered(2-5).

Summary and conclusions. 1. Formamide and its more potent N-methyl derivative have been described as transient inhibitors of the growth of mouse sarcoma 180. This effect is not a property of formamides in general since other compounds containing the formamide moiety have failed to affect the growth of the tumor. 2. N-methylformamide has been shown to exert its effects when therapy is started either 24 or 96 hours after implantation of the tumor or when the agent is given in a single, large dose. Further, the compound has been found equally effective whether given by the oral or intraperitoneal route. 3. Histologic changes of a non-specific nature have been described in tumors from animals treated with N-methylformamide. 4. In view of the structural resemblance between formamide and urethane, the actions of both agents have been compared. Urethane, like formamide, inhibits the growth of S-180; however, treatment with the former substance causes toxic manifestations not encountered in mice given formamide. 5. Several conceivable mechanisms have been proposed to account for the inhibitory effects of the formamides. Further studies of these suggested modes of action may provide useful information concerning biochemical mechanisms involved in the growth of tumors. 6. In view of the hepatotoxicity of formamide and its N-methyl derivative it is not expected that these agents will prove therapeutically useful.

1. Stock, C. C., *Am. J. Med.*, 1950, v8, 658.

2. Haddow, A., and Sexton, W. A., *Nature*, 1946, v157, 500.

3. Paterson, E., Ap Thomas, I., Haddow, A., and Watkinson, J. M., *Lancet*, 1946, v1, 677.

4. Karnofsky, D. A., *New England J. Med.*, 1948, v239, 226, 260, 299.

5. Gellhorn, A., *Cancer Res.*, 1953, v13, 205.

6. Stock, C. C., Clarke, D. A., Philips, F. S., Buckley, S. M., and Moore, A. E., in preparation.

7. Stock, C. C., Philips, F. S., Moore, A. E., Buckley, S. M., Clarke, D. A., Barclay, R. K., and Sugiura, K., *Cancer Res.*, 1953, Suppl. 1, 91.

8. Unpublished observations.

9. Moore, A. E., Stock, C. C., Sugiura, K., and Rhoads, C. P., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 396.

10. Stock, C. C., Biesele, J. J., Burchenal, J. H., Karnofsky, D. A., Moore, A. E., and Sugiura, K., *Ann. N. Y. Acad. Sci.*, 1950, v52, 1360.

11. Buckley, S. M., Stock, C. C., Crossley, M. L., and Rhoads, C. P., *Cancer*, 1952, v5, 144.

12. Buckley, S. M., Stock, C. C., Parker, R. P., Crossley, M. L., Kuh, E., and Seeger, D. R., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 299.

13. Shive, W., *Fed. Proc.*, 1953, v12, 639.

14. Buchanan, J. M., and Wilson, D. W., *Fed. Proc.*, 1953, v12, 646.

15. Welch, A. D., and Nichol, C. A., *Ann. Rev. Biochem.*, 1952, v21, 633.

16. Skipper, H. E., Mitchell, J. H., Jr., and Bennett, L. L., Jr., *Cancer Res.*, 1950, v10, 510.

17. Goldthwait, D. A., and Bendich, A., *J. Biol. Chem.*, 1952, v 196, 841.

18. Skipper, H. E., Schabel, F. M., Jr., Thompson, J. R., and Wheeler, G. P., Personal communication.

19. Skipper, H. E., and Schabel, F. M., Jr., *Arch. Biochem. and Biophys.*, 1952, v40, 476.

20. Doljanski, L., and Rosin, A., *Am. J. Path.*, 1944, v20, 945.

21. Meacham, G. C., Tillotson, F. W., and Heinle, R. W., *Am. J. Clin. Path.*, 1952, v22, 22.

Received July 27, 1953. P.S.E.B.M., 1953, v84.