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Antimalarial Activity of 1-Methyl-3-Nitro-1-Nitrosoguanidine*† (33417)

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1-Methyl-3-nitro-1-nitrosoguanidine (NMNG) has been shown to have anticancer activity in mice (1-3) and mutagenic activity in bacteria (4). This report presents evidence that NMNG is also an effective antimalarial agent *in vitro*.

Materials and Methods. Plasmodium berghei was cultured *in vivo* as previously described (5). The parasitemia in the infected mice is usually greater than 90% at day 7 after inoculation and death occurred within 10 days after inoculation. The mice were anesthetized with diethylether and the blood was removed from the infected mice aseptically by heart puncture. Usually 1.0 ml of blood was removed per mouse. The whole blood was suspended into an equal volume of modified acid-citrate-dextrose (ACD) solution, pH 5.0. Preincubation was performed as follows: 0.50 ml of the cell suspension (4×10^8 parasites/ml) was added to 0.95 ml of ACD solution containing the NMNG or the test substance. The preincubation control consisted only of the parasitized blood in the ACD solution. The mixture was allowed to stand at room temperature for 25 min. After the preincubation period, 0.1 ml aliquots were inoculated intraperitoneally into healthy white mice,[1] Walter Reed strain, weighing 25 g each (12 mice per group). To determine the extent of parasitemia, blood smears were prepared at intervals and stained with Giemsa staining solution. Parasitemia is the number of infected blood cells per 500 uninfected blood cells per mouse. The average parasitemia is expressed as the average of the sum of the parasitemias per group (12 mice per group) and was calculated just before the death of the first mouse in a group.

Results and Discussion. As observed in Table I, the infectivity of *Plasmodium berghei* is affected by NMNG. The ability of the plasmodia to infect healthy mice was destroyed when preincubated with 200, 400, or 500 μg of NMNG. Preincubation with 50 or 100 μg of NMNG showed a gradual increase in parasitemia in the mice after day 6 and 8 after inoculation respectively. Parasitemia was observed in the control group (no additions) as early as day 5 after inoculation.

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† The opinions and conclusions expressed herein do not necessarily reflect the views of the Department of the Army or of any other agency of the Federal Government and should not imply Department of the Army indorsement of or preference for the commercial products described.

[1] The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

TABLE I. The Effect of 1-Methyl-3-nitro-1-nitrosoguanidine on the Infectivity of *Plasmodium berghei*.[a,b]

Condition of preincubation	Average parasitemia (day)[b]						
	0	5	6	7	8	9	12
ACD control	0	50 (21–61)	244 (129–296)	793 (600–953)	1617 (1525–1875)	—	—
Plus 50 μg NMNG	0	np	<1	<5	105	484	1504 (1225–1775)
Plus 100 μg NMNG	0	np	np	np	<1	<5	1521 (1200–2050)
Plus 200 μg NMNG	0	np	np	np	np	np	np
Plus 400 μg NMNG	0	np	np	np	np	np	np
Plus 500 μg NMNG	0	np	np	np	np	np	np
Plus 1000 μg CQ	0	49	223 (107–357)	548 (345–685)	1041 (725–1580)	1429 (1010–1795)	—
Plus 0.02 ml EMS	0	37	155 (125–185)	921 (790–1050)	1535 (1085–2235)	— —	—

[a] See text for experimental conditions.

[b] The range of parasitemias per group is represented by the numbers within the parentheses; np represents no parasites observed in the slides; ACD, modified acid-citrate-dextrose solution, pH 5.0 (E. R. Squibb & Sons, New York); NMNG, 1-methyl-3-nitro-1-nitrosoguanidine (Aldrich Chemical Co., Inc., Wisconsin); CQ, chloroquine base (Winthrop Laboratories, New York); EMS, reagent grade ethylmethanesulfonate (Eastman Kodak, New York).

TABLE II. The Effect of Some Related Compounds on the Infectivity of *Plasmodium berghei*.[a]

Condition of preincubation	Average parasitemia (day)[b]					
	0	6	7	8	9	10
ACD control	0	159 (100–226)	404 (335–496)	1138 (693–1490)	1574 (1155–2069)	2357 (2050–2620)
Plus 200 μg NMNG	0	np	np	np	np	np
Plus 200 μg guanidine	0	202 (110–300)	478 (310–705)	930 (650–1200)	1521 (1350–1800)	—
Plus 200 μg nitroguanidine	0	155 (100–200)	386 (300–500)	1133 (630–1675)	1552 (1030–2048)	—
Plus 200 μg nitrosobarbiturate	0	<1	222 (157–275)	442 (220–744)	745 (525–1125)	1126 (805–1525)
Plus 200 μg *N*-methylnitrosourea	0	227 (120–278)	451 (355–595)	923 (710–1175)	1910 (1550–2310)	—
Plus 500 μg quinine sulfate	0	<1	176 (116–253)	417 (302–488)	598 (477–866)	898 (825–950)
Plus 1000 μg chloroquine base	0	<1	<5	452 (262–726)	1194 (855–1495)	3135 (2560–3375)
Plus 3000 μg chloroquine base	0	<1	<5	117 (107–135)	324 (225–486)	1941 (1575–2495)

[a] See text for experimental conditions.

[b] The range of parasitemias per group is represented by the numbers in parentheses.

TABLE III. The Effect of Some Anticancer Agents on the Infectivity of *Plasmodium berghei*.[a]

Condition of preincubation	Average parasitemia (day)[b]					
	0	7	8	9	10	11
ACD control	0	53 (21–88)	234 (180–300)	512 (454–635)	1370 (1210–1625)	—
Plus 200 μg NMNG	0	np	np	np	np	np
Plus 200 μg mitomycin C	0	<1	<5	101 (58–144)	324 (230–410)	589 (382–730)
Plus 200 μg nitromin	0	<1	337 (212–385)	876 (419–1125)	1681 (1525–1860)	1891 (1750–2050)
Plus 200 μg azaserine	0	<1	320 (255–400)	721 (630–900)	1516 (1050–2100)	1589 (1125–2250)
Plus 200 μg 6-DON	0	<1	294 (255–314)	732 (520–1150)	867 (515–1139)	1166 (850–1425)

[a] See text for experimental conditions.

[b] The range of parasitemias per group is represented by the numbers in parentheses.

Under these conditions, chloroquine and ethylmethanesulfonate had no effect on the infectivity of the malarial parasites. The average parasitemia in the preincubation experiments with chloroquine and ethylmethanesulfonate steadily increased along with the control. The mice that showed parasitemia died shortly after day 8 or 9 after inoculation. The mice that were inoculated with the plasmodia pretreated with 200, 400, or 500 μg of NMNG, however, remained healthy and showed no parasitemia for the duration of the experiment (30 days).

The results presented in Table II strongly suggest that the action of NMNG is rather specific. The data show that neither the guanidino-, the nitro-, nor the nitroso- group of NMNG is the active moiety. *N*-Methylnitrosourea was also found to be ineffective. Quinine sulfate and chloroquine were ineffective even at relatively higher doses.

Because of the reported anticancer effect of NMNG (1–3), other anticancer compounds were also tested and the results are presented in Table III. The mitomycin C, nitromin, 6-diazo-5-oxynorleucine (6-DON), and azaserine were kindly supplied by Dr. Harry B. Wood, Jr., of the Cancer Chemotherapy National Service Center at the National Cancer Institute. Under the conditions of the experiment, it appears that only mitomycin C may have some effect on the infectivity of *Plasmodium berghei*. The concentration used in the experiment, however, approximated the previously reported LD_{50} of 8.6 mg/kg body weight (6). The ineffectiveness of nitromin suggests that alkylation does not play a significant role in the mechanism of action of NMNG on the plasmodia.

The present data suggest that NMNG can penetrate the blood cells readily to destroy all forms of the malarial parasites in the mouse bloodstream. The mechanism of action of NMNG on the parasites cannot be established from the limited data presented in this paper. Because NMNG has been shown to be an effective mutagen in bacteria (4), it may be that NMNG alters the genetic mechanism of the plasmodia. It has been demonstrated, for example, that the infectivity of *Plasmodium gallinaceum* in chickens is greatly diminished after pretreatment with X-rays *in vitro* (7). The effect of mitomycin C also suggests an effect on the genetic mechanism of the plasmodia in that mitomycin C has been demonstrated to selectively inhibit the synthesis of DNA in *E. coli* (8). It should be noted, however, that a weak mutagen such as ethylmethanesulfonate does not affect the infectivity of the plasmodia (Table I). Further elucidation of the mechanism of action of NMNG and the *in*

vivo effectiveness of this and related compounds are being undertaken in our laboratory.

Summary. *In vitro* preincubation of 1-methyl-3-nitro-1-nitrosoguanidine with *Plasmodium berghei* destroys the infectivity of the plasmodia in mice. None of the structurally similar compounds tested had any effect. Of the anticancer compounds tested, only mitomycin C appears to have an effect. The high level of mitomycin C employed in the experiment (Table III) to obtain the small effect indicates that this compound may not be an effective antimalarial compound *in vivo*. The observations presented in this paper show that 1-methyl-3-nitro-1-nitrosoguanidine represents a promising novel type of antimalarial compound.

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Thyroglobulin-Like Substances in Human Thyroid Subcellular Particulates* (33418)

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In a study of thyroglobulin synthesis, Nunez *et al.* (1) have postulated that thyroglobulin originates from ribosomes of thyroid cells. It is believed that subsequent polymerization and iodination of the precursor protein take place in microsomes and mitochondria. It would be of interest to demonstrate such thyroglobulin precursors in subcellular particulates. This communication presents immunochemical evidence for the presence of thyroglobulin and thyroglobulin-like substance in thyroid mitochondrial and microsomal fractions.

Materials and Methods. Human thyroid glands obtained upon necropsy[1] were homogenized in buffered physiological saline (BPS, 0.16 *M* NaCl in 0.05 *M* phosphate buffer, pH 7.6). The mitochondria were isolated from the homogenate by centrifugation at 10,000 *g* for 10 min after removal of cell debris and nuclei at 1000 *g* for 15 min. The microsomal fraction was obtained by centrifugation for 1 hr at 105,000 *g* from the supernatant after the removal of mitochondria. Both the mitochondrial and microsomal fractions were washed twice with buffered physiological saline. By the treatment of the particulate fractions with sodium deoxycholate in a concentration of 0.5% at pH 7.8, deoxycholate-insoluble fractions were obtained. Cell sap fraction, then, represented the supernatant left over from the original thyroid homogenate after sequential removal of mitochondria and microsomes. Thyroglobulin was fractionated from cell sap by the method of Derrien *et al.* (2) and purified by starch-gel electrophoresis (3). All the fractions were stored in a frozen state until use.

To obtain antisera, thyroid mitochondrial

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[1] Unfortunately we were not able to control the postmortem period, but the thyroid tissue was frozen immediately upon removal from the body.