Efficacy of combinations of difluoromethylornithine and bleomycin in a mouse model of central nervous system African trypanosomiasis

(polyamines/rational chemotherapy/sleeping sickness/ornithine decarboxylase)

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ABSTRACT   DL-α-Difluoromethylornithine, a polyamine biosynthesis inhibitor, and bleomycin, a currently used antineoplastic agent, have each previously been shown to be curative for acute short-term infections of mice with *Trypanosoma brucei brucei*, an African trypanosome closely related to those that cause the human disease African sleeping sickness. These agents were tested singly and in combination in a previously described mouse model of sleeping sickness with demonstrable brain involvement. The original model is extended by using two additional strains of outbred mice and by demonstrating that melarsoprol, an arsenical and currently the only drug used for human African trypanosomiasis involving the brain, was also curative for these brain infections. Neither difluoromethylornithine nor bleomycin alone was curative for the brain infections; however, many combinations of the two drugs were found to be 100% curative with no evidence of immediate toxicity.

Human African trypanosomiasis, or sleeping sickness, is caused by either of two subspecies of *Trypanosoma brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*. Although this tsetse fly-transmitted disease has been controlled in many parts of Africa, outbreaks continue to occur. A recent example is the epidemic occurring in Uganda (1). Drugs available to treat this disease are generally quite toxic and not always curative; no new drugs have been introduced in the last 30 years. Melarsoprol, a toxic organic arsenical, is the only available drug that is effective in patients with advanced sleeping sickness—i.e., with central nervous system (CNS) involvement (2, 3).

A major obstacle in the development of new drugs over the years has been the lack of a convenient and reliable laboratory model of the CNS infection. Recent studies of Jennings and colleagues (4–6) have demonstrated that a strain (TREU 667) of *T. brucei brucei*, a veterinary parasite, establishes CNS infections in mice and may thus provide a model of CNS trypanosomiasis. These semichronic infections are resistant to Berenil (diminazene aceturate), a widely used veterinary drug, if treatment is delayed for 3 weeks, even though such treatment initially causes the mice to become aparasitemic. Relapse occurs because Berenil apparently does not reach parasites sequestered in the CNS (4–6). This is a situation similar to that in the human disease, for which suramin and pentamidine are used in the early stages of trypanosomiasis but, because neither crosses the blood–brain barrier, they cannot be used to treat advanced sleeping sickness (6).

We have reported previously that two agents, DL-α-difluo-

romethylornithine (DFMO) and the antitumor agent Blenoxane, will each individually cure rodents with acute infections of African trypanosomes (7–11). Moreover, these two agents act synergistically in that cures of mice with acute infections can be obtained with considerable reductions (>75%) from the single-drug cure doses (9, 12). Both drugs were studied by us because of their effects on trypanosome polyamine metabolism (13). The polyamines putrescine and spermidine occur universally in cells and are required cofactors for cell proliferation and differentiation and for synthesis of macromolecules (14). DFMO is an enzyme-activated irreversible inhibitor of the key enzyme of polyamine biosynthesis, ornithine decarboxylase (EC 4.1.1.17), and is being developed as a potential antitumor agent (15, 16). Blenoxane (bleomycin sulfate) is a commercial mixture of glycopeptide antibiotics, which consist of bleomycinic acids with various amine moieties, including spermidine and agmatine. Proof of the interaction of both drugs with polyamine metabolism was shown by the ability of exogenously supplied polyamines to negate their curative effects—DFMO by putrescine, spermidine, or spermine (17) and bleomycin, alone or in combination with DFMO by spermidine or spermine (10, 12). In the present study, these drugs were examined singly and in combination for efficacy against the mouse model of CNS trypanosomiasis.

MATERIALS AND METHODS

Three sources and two strains of animals were used: female CF-1 mice from Charles River Farms (Wilmington, MA), male and female Swiss/Webster (S/W) mice (caged separately) from Royal Hart Animal Laboratories (New Hampton, NY), and female S/W mice from Taconic Farms (Germantown, NY). The TREU 667 strain of *T. brucei brucei* was obtained as a frozen stabilate from F. W. Jennings of the University of Glasgow. From that stabilate additional frozen stabilates were made (18) after three to six passages in mice and rats. For each experiment one to three mice were infected from a stabilate and their blood was collected 5–7 days after inoculation. This blood was used to infect experimental animals with 10^5 parasites. Before beginning drug treatment, mice were examined for parasitemia, and those with parasites seen in blood films were randomly assigned to treatment groups.

Individual treatment schedules as well as the strain of mouse

used in various experiments are indicated in *Results*. DFMO (Merrell Dow, Cincinnati, OH; MDL 71,782) was always administered as a solution in the drinking water at the percentage indicated in *Results*. On the basis of the observation that a 25-g mouse takes 5 ml of water per day (7), 1% DFMO equates to a dose of 2 g/kg per day. Bleomycin (outdated vials of Blenoxane were used, the gift of Bristol Laboratories, Syracuse, NY) was weighed and dissolved in 0.85% NaCl to yield a solution of 0.7 mg/ml (this yielded 1.5 units of activity per ml according to the manufacturer's assay), which was administered by intraperitoneal injection at the doses given in *Results*. Diminazene aceturate (Hoechst, Federal Republic of Germany; the active principal of Berenil, which is 45% diminazene aceturate) was dissolved in 0.85% NaCl and administered intraperitoneally at the dose schedule given in *Results*. Melarsoprol [Mel B (Arsobal), a gift from Specia, Paris] was supplied as a 36 mg/ml solution in propylene glycol. This was diluted to 0.36 mg/ml with propylene glycol and administered intravenously at the dose schedule given in *Results*. The polyamines spermidine and spermine were purchased from Sigma and were dissolved in 0.85% NaCl for intraperitoneal injection.

After a specific therapeutic regimen was completed, weekly wet-mount blood examinations were made to detect parasitemia. Animals with parasitemia were then sacrificed. Animals negative for parasitemia through day 200 after inoculation were considered cured (this was 165–179 days after treatment was completed). Nonrecrudescent animals from some experimental groups were followed only to day 140 (see *Results*) but were tested further for latent parasitemia by subinoculation of blood and brain tissue intraperitoneally into uninfected irradiated animals. Blood from apparently cured mice was collected in the presence of 0.05 ml of a 30 mg/ml heparin solution to prevent coagulation; afterwards these animals were sacrificed and dissected, and their brains were homogenized in a phosphate buffer (100 mM sodium phosphate/73 mM NaCl/1.5% glucose, pH 8.0) by using a motor-driven Potter–Elvehjem homogenizer with a Teflon pestle. The final volume of the brain homogenate was brought to 2.0 ml with phosphate buffer. Aliquots (0.50 ml) of blood or brain homogenate were injected intraperitoneally into recipient mice (two per tissue sample) that had received 600 rads of gamma radiation 24 hr previously. Daily wet-mount blood preparations were made from recipient mice beginning on day 3 and continuing through day 7. Those without parasitemias at day 7 were considered as not having been injected with viable parasites, thus confirming the cure of the experimental mice from which the tissue samples had been collected.

RESULTS

In earlier studies with this murine model of African trypanosomiasis (19), we found that in control animals there are two or three parasitological crises followed by a plateau of high parasitemia, and the mice survive 60–80 days if untreated. As shown in Table 1, line A, mice were cured by a single dose of 10 mg of diminazene aceturate (Berenil) per kg when the drug was given on day 4 of infection. Because these mice had parasitemia at the time of treatment, the results indicated that there was no inherent resistance to Berenil in this model. When animals were treated with Berenil on day 21 after inoculation, however, only 3 of 67 were cured (Table 1, line B). Melarsoprol, the established drug for CNS therapy, was 100% curative when given in the standard human multidose regimen (3–7 days starting on day 21; Table 1, lines C–E). The dosage of 3.6 mg/kg is the same as that used to treat late-state human sleeping sickness. We further extended the model by using two previously untried outbred mouse strains, CF-1 and S/W (see footnotes c, e, and f of Table 1).

Because these data indicated the suitability of this model for study of chemotherapy of late-stage trypanosomiasis, we used it to evaluate the effectiveness of DFMO and bleomycin in various combinations and schedules. In these experiments treatment was always begun 21 days after inoculation. As shown in Table 2, neither DFMO nor bleomycin was curative alone. The higher doses of both drugs did, however, produce aparasitemic periods. One regimen of DFMO (Table 2, line G) produced occasional cures similar to the cure observed with Berenil (Table 1, line B). None of the combinations tried with less than 1% DFMO produced cures (Table 3). However, evenly distributing a total bleomycin dose of 1.75 mg/kg over the 7 days of simultaneous administration of 0.25% DFMO (Table 3, line A) produced longer aparasitemic periods than higher total doses (2 mg/kg bleomycin + 0.50% DFMO for 14 days) over a shorter period as shown in Table 3, line D.

A 100% cure rate was eventually achieved with DFMO and bleomycin together, as for example when 1% DFMO in the drinking water for 14 days was combined with bleomycin given a total of 6 times at 3.5 mg/kg per day (Table 3, line L). A 50% reduction in the total dose of either compound greatly reduced the cure rate (Table 3, lines G, H, and I), and reducing both by 50% eliminated any cures (Table 3, lines E and F). Though the numbers are small, the data suggest that distribution of the bleomycin over 6 days (Table 3, line L) might be more effective than the same total dose given over 3 days (Table 3, lines J and K).

Table 1. Responses of mice infected with *T. brucei brucei* (TREU 667) to Berenil and Mel-B

	Drug	Daily dose, mg/kg[a]	Days after inoculation when treatment was given	No. of mice relapsing[b] Day 35			Day 56			Day 77		Day 98	Day 119	Total no. mice treated	% cured
A.	Berenil	10	4											10[c]	100[d]
B.	Berenil	10	21	15	11	12	10	8	3	2	2	1		67[e,f]	4[d,g,h]
C.	Mel-B	3.6	21–23											15[c]	100[d]
D.	Mel-B	3.6	21–23											9[e]	100[g]
E.	Mel-B	5	21–27											29[f]	100[d]

[a] Doses of Berenil are in terms of diminazene aceturate, the active principal.
[b] Mice were checked weekly for parasitemia beginning on day 35. Numbers below the day represent the number of animals relapsing at that time.
[c] S/W mice from Taconic Farms.
[d] Mice considered cured remained negative through day 200.
[e] CF-1 mice.
[f] S/W mice from Royal Hart Animal Laboratories.
[g] Mice considered cured remained negative through day 140, after which blood and brain tissues were subinoculated into irradiated mice. The recipient mice did not develop parasitemias unless noted otherwise.
[h] Subinoculation from one animal did result in a parasitemia in the recipient.

Table 2. Response of CNS trypanosomiasis model to DFMO and bleomycin singly

	Drug	Daily dose mg/kg	% in water	Days after inoculation when treatment was given	Day 35	Day 56	Day 77	Day 98	Day 119	Total mice treated	% cured
A.	Bleomycin	0.25		21–27	8 2					10[a]	0
B.	Bleomycin	2.00		21	10					10[b]	0
C.	Bleomycin	7.00		21–23	3 3 3	1				10[b]	0
D.	Bleomycin	7.00		21–27	3 2	9 2 10 1	1 2			30[a]	0
E.	DFMO		0.25	21–28	7 3					10[a]	0
F.	DFMO		0.50	21–35	7 3					10[b]	0
G.	DFMO		2.00	21–28	4 2 2	6	5 3	3 2	1	30[a]	7[c]
H.	DFMO		2.00	21–35	1	2 3		2 1	1	10[b]	0

[a] S/W mice from Royal Hart Animal Laboratories.
[b] CF-1 mice.
[c] Mice considered cured remained negative through day 200.

Various other combinations of DFMO and bleomycin were also curative. The total dose of DFMO shown in Table 3, lines N and O, is the same as that in Table 3, lines J, K, and L, but DFMO is given at twice the rate for half the time. Under these conditions, bleomycin given over 3 days is effective. However, administration of both compounds within a week still does not allow 100% cure if the dose of either compound is reduced by 50% (Table 3, lines G and M). A 50% reduction in the total bleomycin dose is possible if the DFMO dose is doubled to 2% in the drinking water for 2 weeks with the bleomycin given during the second week; this is the lowest dose of bleomycin (3.5 mg/kg per day for 3 days) that produced a 100% cure rate (Table 3, line Q). The higher total doses of bleomycin with 2% DFMO, shown in Table 3, lines R–T, provided 100% cures as would have been expected. Unexpectedly, 1 of 30 animals in Table 3, line P, did relapse even though the dose of bleomycin was higher than that shown in Table 3, lines N and O, and the DFMO dose was the same.

We also tested the ability of the polyamines spermidine and spermine to block the curative effects of DFMO and bleomycin. Two groups of 10 animals were infected with T. brucei brucei and treated with 2% DFMO in their drinking water for days 21–28 after inoculation and with bleomycin at 7 mg/kg on days 26, 27, and 28. This regimen (Table 3, line N) produces a 100% cure rate. When spermidine at 100 mg/kg was also administered intraperitoneally on days 26, 27, and 28, the cure rate was reduced to 70%. Spermine administered at 50 mg/kg on the same days had no effect; i.e., all these animals were cured.

DISCUSSION

The sensitivity of the TREU 667 mouse model to Berenil treatment at an early (day 4) stage, its subsequent refractoriness to the same drug at day 21, and our finding of a complete curative effect of melarsoprol validate and extend the CNS model developed by Jennings et al. (4, 5). A situation similar to that with Berenil was found with the two polyamine-related drugs DFMO and bleomycin. Both have been shown to be highly effective in treating acute T. brucei brucei infections (7, 10) but neither was curative when used alone in the CNS model employed here. Schedules of DFMO alone provided a small percentage of cures (7%) at the maximal dose given; this was similar to the cures achieved with Berenil (4%). These sporadic cures may represent biological variations in the course of infection in these outbred mice—e.g., lack of establishment of CNS infection in some mice or variation in the integrity of the blood–brain barrier. We cannot rule out efficacy of DFMO alone in this late-stage model at very high doses or for longer periods. However,

the pattern of relapse after treatment with 2% DFMO for 14 days indicates no greater degree of efficacy than with 2% DFMO for only 7 days. Bleomycin given alone produced no cures of this late-stage model even with a total dose approximately 5 times the minimal dose that produced 100% cures when combined with DFMO.

Several different combinations and regimens of DFMO and bleomycin were found to be curative. We have demonstrated eight dose combinations which reliably produced 100% cures, in a total of 72 mice, without obvious untoward effects. Synergism between these two drugs is indicated by their curative effects in combination, with a general failure of either drug to cure as a single agent. In addition, an increase in dose of one agent allows a decrease in the other. For example, three doses of 3.5 mg of bleomycin per kg are 100% curative when combined with 2% DFMO for 2 weeks but not with a lower dose of DFMO. Similarly, 1% DFMO for 2 weeks is 100% curative with six doses of bleomycin at 3.5 mg/kg but not with only three doses. Also, in general, a better therapeutic response is achieved when both agents are given over approximately the same period, as is shown in Table 3.

Polyamine depletion is apparently critical to the activity of the combination because coadministration of polyamines previously negated combination therapy (9, 12), as did spermidine in this present study. The molecular basis for synergism seems related to the blockage in parasite cell division induced by polyamine depletion (20) and the ability of bleomycin to selectively cleave DNA of nondividing cells (21). This can be compared to the potentiation of 1,3-bis-(S-chloromethyl)-1-nitrosourea-mediated chromosomal damage by DFMO-induced polyamine deficiency (22).

One consideration in the potential clinical use of DFMO and bleomycin together would be the toxicity of such a combination. It should be noted, however, that bleomycin itself is the drug of choice in human testicular cancer (23, 24) and has been used in treatment of advanced Hodgkin disease (25) and brain tumors (26). Extensive preclinical and clinical evidence demonstrated that the major toxic effect of bleomycin chemotherapy is pulmonary fibrosis (27). Toxicity is cumulative and delayed, occurring clinically within several weeks or longer after initiation of therapy (28), and is heightened by prior exposure to other pulmonary toxins and procedures such as radiotherapy (23). DFMO has been generally well tolerated by experimental animals even in high single doses or prolonged multiple dosage (29). Although human clinical experience is limited (16), all the data obtained so far clearly indicate that DFMO is exceptionally well tolerated (15, 16). With regard to effects of the combination of the two, recent work with hamsters indicates that some

Table 3. Response of CNS trypanosomiasis model to DFMO + bleomycin

Drug	Daily dose mg/kg	Daily dose % in water	Days after inoculation when treatment was given	Day 35			Day 56			Day 77			Day 98	Day 119	Total mice treated	% cured
A. DFMO		0.25	21–28												10[a]	0
Bleomycin	0.25		21–27				1	1		3	1	1	1	2		
B. DFMO		0.50	21–35	9											9[b]	0
Bleomycin	0.50		21													
C. DFMO		0.50	21–35	7	3										10[b]	0
Bleomycin	1.00		21													
D. DFMO		0.50	21–35	4	5										9[b]	0
Bleomycin	2.00		21													
E. DFMO		1.00	21–28												10[b]	0
DFMO		0.50	29–31		1	3	6									
Bleomycin	3.50		29–31													
F. DFMO		1.00	21–28	5		3	1	1							10[b]	0
Bleomycin	3.50		26–28													
G. DFMO		1.00	21–28				1	1	1			1			10[b]	60[c]
Bleomycin	7.00		26–28													
H. DFMO		1.00	21–35		1		4	1	1		1				10[b]	10[c,d]
Bleomycin	3.50		28–30													
I. DFMO		1.00	21–35				2	2							10[b]	60[c]
Bleomycin	3.50		33–35													
J. DFMO		1.00	21–35										1		10[b]	90[c]
Bleomycin	7.00		28–30													
K. DFMO		1.00	21–35										1	1	10[b]	80[c]
Bleomycin	7.00		33–35													
L. DFMO		1.00	21–35												8[e]	100[f]
Bleomycin	3.50		26–28 33–35													
M. DFMO		2.00	21–28	2			2	1			1				10[b]	40[c]
Bleomycin	3.50		26–28													
N. DFMO		2.00	21–28												18[b,e]	100[c,f]
Bleomycin	7.00		26–28													
O. DFMO		2.00	21–28												10[b]	100[c]
Bleomycin	7.00		29–31													
P. DFMO		2.00	21–28									1			30[a]	97[f]
Bleomycin	7.00		21–27													
Q. DFMO		2.00	21–35												9[e]	100[f]
Bleomycin	3.50		33–35													
R. DFMO		2.00	21–35												10[e]	100[f]
Bleomycin	3.50		26–28 33–35													
S. DFMO		2.00	21–35												8[b]	100[c]
Bleomycin	7.00		21–23													
T. DFMO		2.00	21–35												9[b]	100[c]
Bleomycin	7.00		28–30													
U. DFMO		2.00	21–35												9[b]	100[f]
Bleomycin	7.00		33–35													

[a] S/W mice from Royal Hart Animal Laboratories.
[b] CF-1 mice.
[c] Mice considered cured remained negative through day 140, after which blood and brain tissues were subinoculated into irradiated mice. The recipient mice did not develop parasitemias unless noted otherwise.
[d] Subinoculation from one animal did result in a parasitemia in the recipient.
[e] S/W mice from Taconic Farms.
[f] Mice considered cured remained negative through day 200.

aspects of bleomycin lung toxicity may be increased by concomitant DFMO administration but not remarkably so (30). We did not examine the treated mice for lung pathology, but we noticed no overt signs of toxicity despite the long observation period. Thus, study of combination therapy in humans would appear feasible and without prohibitive risk.

The general development of a rational approach to chemotherapy of parasites has been urged for some time (31–33). The success of the DFMO/bleomycin combination against a CNS model of *T. brucei brucei* represents the culmination of a progressive approach to chemotherapy of African trypanosomiasis. The drug combination can be further tested in another CNS model in the rodent *Microtus montanus* infected with the human pathogen *T. brucei gambiense*. DFMO alone has already been shown to be effective against short-term infections of *M. montanus* by *T. brucei gambiense* but, again, to be essentially ineffective against late stage infections (9). Regardless, our overall experience indicates that singly both bleomycin and particularly DFMO have significant activity against numerous types of African trypanosomes and now clearly indicates that the com-

bination of DFMO and bleomycin has potential in treating human African trypanosomiasis involving the CNS.

1. Gashumba, J. (1981) *New Sci.* 89, 164.
2. Williamson, J. (1976) *Trop. Dis. Bull.* 73, 531–542.
3. Meshnick, S. R. (1983) in *Parasitic Diseases: The Chemotherapy,* ed. Mansfield, J. (Dekker, New York), Vol. 2, pp. 165–199.
4. Jennings, F. W., Whitelaw, D. D. & Urquhart, G. M. (1977) *Parasitology* 75, 143–153.
5. Jennings, F. W., Whitelaw, D. D., Holmes, P. H., Chizyuka, H. G. B. & Urquhart, G. M. (1979) *Int. J. Parasitol.* 9, 381–384.
6. Murray, P. K. & Jennings, F. W. (1983) in *Animal Models for Experimental Bacterial and Parasitic Infection,* eds. Keusch, G. & Wadstrom, T. (Elsevier/North-Holland, New York), pp. 343–354.
7. Bacchi, C. J., Nathan, H. C., Hutner, S. H., McCann, P. P. & Sjoerdsma, A. (1980) *Science* 210, 332–334.
8. McCann, P. P., Bacchi, C. J., Hanson, W. L., Cain, G. D., Nathan, H. C., Hutner, S. H. & Sjoerdsma, A. (1981) *Adv. Polyamine Res.* 3, 97–110.
9. McCann, P. P., Bacchi, C. J., Clarkson, A. B., Jr., Seed, J. R., Nathan, H. C., Amole, B. O., Hutner, S. H. & Sjoerdsma, A. (1981) *Med. Biol.* 59, 434–440.
10. Nathan, H. C., Bacchi, C. J., Sakai, T. T., Rescigno, D., Stumpf, D. & Hutner, S. H. (1981) *Trans. R. Soc. Trop. Med. Hyg.* 75, 394–398.
11. Karbe, E., Bottger, M., McCann, P. P., Sjoerdsma, A. & Freitas, E. K. (1982) *Tropenmed. Parasitol.* 33, 161–162.
12. Bacchi, C. J., Nathan, H. C., Hutner, S. H., McCann, P. P. & Sjoerdsma, A. (1982) *Biochem. Pharmacol.* 31, 2833–2836.
13. Bacchi, C. J. (1981) *J. Protozool.* 28, 20–27.
14. Pegg, A. E. & McCann, P. P. (1982) *Am. J. Physiol.* 243, C212–C221.
15. Sjoerdsma, A. (1981) *Clin. Pharmacol. Ther.* 30, 3–22.
16. Janne, J., Alhonen-Hongisto, L., Seppanen, P. & Siimes, M. (1981) *Med. Biol.* 59, 448–457.

17. Nathan, H. C., Bacchi, C. J., Hutner, S. H., Rescigno, D., McCann, P. P. & Sjoerdsma, A. (1981) *Biochem. Pharmacol.* 30, 3010–3013.
18. Brohn, F. H. & Clarkson, A. B., Jr. (1978) *Acta Tropica* 35, 23–33.
19. Amole, B. O., Clarkson, A. B., Jr., & Shear, H. L. (1982) *Infect. Immun.* 36, 1060–1068.
20. Bacchi, C. J., Garofalo, J., Mockenhaupt, D., McCann, P. P., Diekema, K. A., Pegg, A. E., Nathan, H. C., Mullaney, E. A., Chunosoff, L., Sjoerdsma, A. & Hutner, S. H. (1983) *Mol. Biochem. Parasitol.* 7, 209–225.
21. Barranco, S. C. (1978) in *Bleomycin: Current Status and New Developments,* eds. Carter, S. K., Crooke, S. T. & Umezawa, H. (Academic, New York), pp. 81–90.
22. Tofilon, P. J., Oredsson, S. M., Deen, D. F. & Marton, L. J. (1982) *Science* 217, 1044–1046.
23. Crooke, S. T. (1981) in *Cancer and Chemotherapy,* eds. Crooke, S. T. & Prestayko, A. W. (Academic, New York), Vol. 3, pp. 97–109.
24. Vurgrin, D., Cvitkovic, E., Whitmore, W. F., Jr., Cheng, E. & Golbey, R. B. (1981) *Cancer* 47, 833–839.
25. Case, D. C., Young, C. W., Nisce, L., Lee, B. J., III, & Clarkson, B. D. (1976) *Cancer Treat. Rep.* 60, 1217–1223.
26. Takeuchi, K. (1975) *Int. J. Clin. Pharmacol.* 12, 419–426.
27. Meares, C. F., DeRiemer, L. H. & Goodwin, D. A. (1979) in *Bleomycin: Chemical, Biochemical and Biological Aspects,* ed. Hecht, S. M. (Springer, New York), pp. 309–323.
28. Comis, R. L. (1978) in *Bleomycin: Current Status and New Developments,* eds. Carter, S. K., Crooke, S. T. & Umezawa, H. (Academic, New York), pp. 279–292.
29. Koch-Weser, J., Schechter, P. J., Bey, P., Danzin, C., Fozard, J. R., Jung, M. J., Mamont, P. S., Seiler, N., Prakash, N. J. & Sjoerdsma, A. (1981) in *Polyamines in Biology and Medicine,* eds. Morris, D. R. & Marton, L. J. (Dekker, New York), pp. 437–453.
30. Giri, S. N., Hyde, D. M., Schwartz, L. W. & Younker, W. R. (1982) *Am. J. Pathol.* 109, 115–122.
31. Cohen, S. S. (1979) *Science* 205, 964–971.
32. Cohen, S. S. (1982) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* 41, 3061–3064.
33. Wang, C. C. (1982) *Trends Biochem. Sci.* 7, 354–356.