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IMMUNOSUPPRESSIVE, ANTIVIRAL AND ANTITUMOR ACTIVITIES OF CYTARABINE DERIVATIVES

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Abstract—Although cytarabine (cytosine arabinoside, *ara*-cytidine, Cytosar) is a potent immunosuppressant, antiviral and antitumor agent in animals and man, maximum inhibitory effects require the use of complex injection schedules. Previous reports have shown that good immunosuppressive and antitumor activities were attained with simple injection schedules using the 5′-adamantoate derivative. The current results show that a variety of 5′-acylates were equally as active as the 5′-adamantoate in suppressing immune responses in rodents (hemagglutinin formation in mice and hamsters, skin graft rejection in rats), and as antitumor agents in mice (L1210 leukemia). Similar results were attained in protecting mice from the lethal effects of intracranial herpes simplex infection, and in inhibiting DNA synthesis in phytohemagglutinin-stimulated human lymphocytes. The hypothesis for the enhanced potency of these newer derivatives was as follows: After injection of these insoluble derivatives, there is a finite time required for dispersion and solubilization. The freely circulating derivatives are resistant to deamination (and inactivation). After enzymatic hydrolysis to the free acid and cytarabine, the latter is then free to exert its inhibitory activities. The net effect is the maintenance of relatively low levels of cytarabine for long periods of time.

CYTARABINE* has been shown to possess potent immunosuppressive,[1–11] antiviral,[12,13] and antitumor[14–17] activities in animals. In man, one detailed report of its immunosuppressive activities has been presented,[18] its antiviral activity has been described,[19–22] and it is routinely employed as an antitumor agent.[23–25] Although cytarabine is a potent agent in the above-mentioned situations, the use of this compound for maximum immunosuppressive and antitumor effects requires the use of very complex and precise dose schedules.[5,6,15–17] For this reason, a synthetic derivative program was begun in these laboratories with the goal of obtaining a more easily usable derivative. The first compound which had clearly superior activities compared to the parent compound was adamantoyl cytarabine.[26,29] Since that time, a variety of cytarabine derivatives have been synthesized (the chemical characteristics have been described in a separate communication[30]). This report describes the immunosuppressive, antiviral and antitumor activities of some 5′-acylates and 5′-sulfonates of cytarabine.

METHODS

Hemagglutinin responses of mice and hamsters

Male, 25 g ICR mice (Upjohn stock colony) and male, 50–60 g golden hamsters (Olsen Laboratories, Madison, Wis.) were injected intraperitoneally with 10^9

* Cytosar, 1-β-D-arabinofuranosylcytosine, *ara*-cytidine, cytosine arabinoside.

sheep erythrocytes. A standard hemagglutinin assay was performed on doubling-dilutions of sera obtained 7 days later from groups of five to six animals. The hemagglutinin titers were expressed as the reciprocal of $\log_2$ of the highest serum dilution displaying hemagglutinating activity. Since the data in Fig. 1 represents many separate experiments, the titers were "normalized" such that the controls in the separate experiments had titers of exactly 10 (which was the usual average anyway) so that the composite figure could be drawn. The compounds were either dissolved or suspended in 0·25 per cent aqueous methylcellulose (Vehicle 122, Upjohn) and injected intraperitoneally in 0·2-ml volumes.

Antitumor activity (L1210 leukemia) in mice

Male, 20 g BDF_1 mice (Jackson Laboratories, Bar Harbor, Me.) were inoculated intraperitoneally with 10^6 L1210 cells (eight mice/group). The results were expressed as the per cent increase in life span (% ILS) calculated from median survivals of experimental and control groups. Compounds were either dissolved or suspended in Vehicle 122 and injected intraperitoneally in 0·2-ml volumes.

Determination of water solubilities

The derivatives were added to water at room temperature and stirred for 60 min. The optical densities of the saturated solutions obtained after Millipore filtration (0·45 μ) were measured at their absorption maxima (ca. 270 mμ). The molar extinction coefficients in water ranged from $7{\cdot}9 \times 10^3$ for the adamantoate to $9{\cdot}7 \times 10^3$ for the benzoate, with the rest falling between 9·2 to $9{\cdot}5 \times 10^3$ (cytarabine was $9{\cdot}45 \times 10^3$). A general molar extinction coefficient of 1×10^4 was then used to calculate the concentration of each derivative and their solubilities were expressed in micrograms per milliliter. Any compound soluble in excess of 2000 μg/ml was considered soluble.

Skin graft rejection in rats

LBN skin grafts were fitted onto the backs of Lewis rats as described previously.[20] Rejection was considered complete when the graft became thickened and hard. All compounds were injected intraperitoneally, 66 mg/kg/day, beginning 1 day before transplant through day 20 into groups of at least 16 Lewis rats. No gross toxicity was observed with any compound under these conditions.

Anti-herpes simplex virus activity in mice

Male, 18 g ICR mice (Upjohn stock colony) were infected with the HFRK-9B strain of herpes simplex virus (HSV). Virus stocks were stored and diluted in Hanks balanced salt solutions (HBSS) containing 0·5 per cent lactalbumin hydrolysate. Test animals were injected intracranially at zero time with 0·03 ml virus containing a 100 LD_{50} dose of HSV. The test compounds were prepared in a water-based vehicle containing, per milliliter, 10 mg carboxymethylcellulose, 4 mg polysorbate 80, and 0·42 mg propylparaben, and were injected intracranially in 0·05-ml volumes. Each group was observed twice daily and deaths were recorded up to 7 days post-infection. Per cent survival was calculated on the basis of the number of animals alive at 7 days. Any deaths before 2 days were not included, since they were attributed to the trauma of intracranial injection.

Thymidine uptake by phytohemagglutinin-stimulated human lymphocytes

The effect of cytarabine and selected derivatives on DNA synthesis in phytohemagglutinin (PHA)-stimulated human lymphocytes was assessed by measuring tritiated thymidine incorporation. Human venous blood was defibrinated with glass beads and red cells were removed by settling for 30–45 min at 37°. Total white cell counts were made in a 2 per cent acetic acid–water solution; viable leukocyte counts were made with trypan blue stain; differential white cell counts were made using Giemsa stain. Cells and autologous serum were added to minimal essential medium, Eagle's (MEM), supplemented with antibiotics and L-glutamine, to give final concentrations of $0{\cdot}5 \times 10^6$ viable lymphocytes per ml and 17–18% (v/v) serum. Sterile PHA-P solution (Difco) was added at a ratio of 0·1 ml per 64 ml of suspension. One-ml aliquots of the suspension, with and without PHA, were distributed to screw-capped tubes. The adamantoate and palmitate derivatives were suspended first in absolute ethanol at a concentration of about 20 mg/ml and then were diluted to 2 mg/ml with 10^{-5} M acetic acid. These stock solutions were further diluted just before use with MEM (cytarabine was dissolved in MEM directly). The amounts of ethanol and acetic acid present in the assay tubes were well below inhibitory concentrations.

In an attempt to differentiate between long-duration and short-duration inhibitory activities, 0·1-ml aliquots of test compounds were added either at the beginning of the incubation or at 8 hr before termination of the incubation. Correspondingly, when the compounds were present throughout the incubation, 0·1-ml aliquots of C^3H_3-thymidine (8 μc, 100 mμmoles) were added 16 hr before termination. When the compounds were added 8 hr before termination, 0·1-ml aliquots of C^3H_3-thymidine (8 μc, 28 mμmoles) were added 4 hr before termination. The incubation was conducted at 37° for 64 hr. At the end of the experiment, the tube contents were washed serially with 10-ml aliquots of saline, 10 per cent trichloroacetic acid (TCA) and 5 per cent TCA. The precipitates were heated with 1·0-ml aliquots of 5 per cent TCA for 15 min at 88–90°. After centrifugation, the radioactivity in 0·25-ml aliquots of the supernatant was determined in a scintillation counter. The studies were conducted with lymphocytes from four individuals. The range of incorporation was from 180–310 dis./min in nonstimulated controls to 18,000–27,000 dis./min in the PHA-stimulated controls.

RESULTS

Immunosuppressive and antitumor effects in mice

The results of injecting the derivatives into mice 1 day after the injection of sheep erythrocytes or L1210 cells are displayed together in Fig. 1 to show the remarkable similarities obtained in these two test systems. Whenever inhibition of hemagglutinin formation was observed, antitumor effects were also observed. Under these conditions, the most active compounds were the stearate, palmitate, 4-methoxybenzoate, benzoate, and adamantoate esters of cytarabine. Administration of these active compounds generally tripled the lifespan of the leukemic mice with occasional cures (defined as 45-day survivors) and generally obliterated the hemagglutinin response to sheep erythrocytes.

At first glance, it might appear that one common characteristic that the active derivatives possessed was low water solubility. While this may be true, close examina-

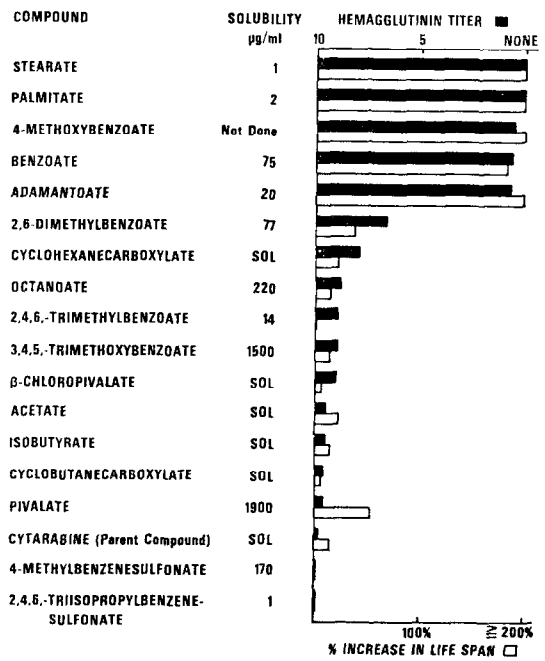

FIG. 1. Immunosuppressive and anti-L1210 effects of cytarabine derivatives in mice. Groups of 5 ICR mice were injected with 10^9 sheep erythrocytes and the serum hemagglutinin titers were measured 7 days later. The titers are expressed as the reciprocal of the $\log_2$ of the highest dilution demonstrating hemagglutination and were "normalized" so that the control titers were exactly 10, as described under Methods. Groups of 8 BDF_1 mice were inoculated with 10^6 L1210 cells/mouse and the number of deaths was recorded. The per cent increase in life span (open bars) was calculated from the mean survival time of the controls (usually 6·5 days). The solubilities in water were measured as described in Methods and are expressed in micrograms per milliliter. Compounds were considered soluble (SOL) at a solubility in excess of 2000 μg/ml. All compounds were injected once, intraperitoneally, 200 mg/kg (except the palmitate which was injected at 150 mg/kg), 1 day after sheep erythrocyte injection or L1210 cell inoculation.

tion of the solubilities displayed in Fig. 1 shows that low solubility, although apparently necessary, was not in itself sufficient for activity. For example, the 2,4,6-triisopropylbenzenesulfonate and the 2,4,6-trimethylbenzoate esters, although quite insoluble, were essentially inactive.

Increasing substitution in the aromatic series resulted in a severe decrease in activity. For instance, the benzoate and 4-methoxybenzoate esters were quite active, the 2,6-dimethylbenzoate ester possessed fair activity, while the 2,4,6-trimethylbenzoate and 3,4,5-trimethoxybenzoate esters were much less active.

The situation was somewhat different with respect to the aliphatic acid esters in that increasing the number of carbon atoms resulted in an increase in activity. Thus, the stearate, palmitate and adamantoate esters were far more active than the octanoate and acetate esters. The only compounds tested which were totally inactive under every test condition were the sulfonate esters, i.e. neither displayed any immunosuppressive or antitumor activity, nor was any toxicity observed.

TABLE 1. COMPARISON OF SELECTED CYTARABINE DERIVATIVES IN THE TREATMENT OF L1210 LEUKEMIA

Dose (mg/kg/day)	Route	Schedule	% ILS (cures)*	
			Adamantoate	Palmitate
20	i.p.	Days 1–5	285 (1/8)	486 (3/8)
100	i.p.	Day 1 only	– (4/8)†	– (4/8)†
20	s.c.	Days 1–5	177 (0/8)	246 (0/8)
100	s.c.	Day 1 only	185 (1/8)	270 (0/8)
100	i.p.	Daily 1–5	Cytarabine	107 (0/8)

* Mice were inoculated intraperitoneally on day 0 with 10^6 L1210 cells per mouse. The per cent ILS (increase in life span) was calculated from median survival of treated and control groups; the latter was 6·5 days. Cures were defined as 45-day survivors.

† Per cent ILS could not be calculated in these groups because half the animals were cured.

Comparison of the antitumor activities of the adamantoate and palmitate esters

Table 1 shows that the palmitate ester possessed at least as much antitumor activity as the adamantoate, regardless of the route or number of injections employed. In general, the intraperitoneal route was more effective than the subcutaneous route. It is interesting to note that, regardless of the route, the therapeutic effect obtained with either compound was primarily dependent upon the total dose employed. For instance, considering the palmitate ester, there were similar numbers of cures when a total dose of 100 mg/kg was administered singly or as five daily injections (3/8 and 4/8 respectively). In other experiments not reported here, it was also found that suppression of the hemagglutinin response by the palmitate ester, as well as the other active esters (including the adamantoate[27]), was dependent upon the total dose rather than the dose schedule.

Immunosuppressive effects in hamsters

The immunosuppressive effects of injecting selected derivatives into hamsters 1 day after the injection of sheep erythrocytes are shown in Table 2. Hamsters were studied because, of all the species examined,[31] only hamsters possessed levels of liver deoxycytidine aminohydrolase activity comparable to that found in man and because this is most likely the same enzyme responsible for the deamination (and inactivation) of cytarabine in man and other species.[32,33] Surprisingly, however, the results in hamsters were essentially the same as those in mice, i.e. the most potent derivatives were the palmitate, adamantoate and benzoate esters. The latter two compounds were, however, quite toxic at the doses shown (200 mg/kg, intraperitoneally, represented an LD_{50} dose). Lowering the dose resulted in less toxicity, and immunosuppressive effects were still observed with the two compounds. The derivatives which displayed little or no activity in mice were also inactive in hamsters. These included the cyclohexanecarboxylate, the trimethylbenzoate and the two sulfonate esters.

As Table 3 shows, when the compounds were compared on a daily injection basis, the palmitate and adamantoate esters were still more immunosuppressive than cytarabine.

TABLE 2. EFFECT OF SINGLE INJECTIONS OF SELECTED CYTARABINE DERIVATIVES ON HAMSTER HEMAGGLUTININ FORMATION

Compound (200 mg/kg, i.p. day 1)	Hemagglutinin titer*
Control	7·8 ± 0·6
Trimethylbenozate	7·7 ± 1·4
4-Methylbenzenesulfonate	6·6 ± 1·1
Triisopropylbenzenesulfonate	8·5 ± 0·8
Cyclohexanecarboxylate	6·2 ± 1·0
Cytarabine	7·2 ± 1·0
Palmitate	1·4 ± 1·3
Benzoate	0†
Adamantoate	0†

* The titers represent the average of six hamsters ± 1 mean deviation. The titers were determined on sera from blood drawn on day 7.

† No hemagglutinin titers were detected in the survivors (2/5 survivors with the adamantoate, and 4/5 survivors with the benzoate).

Immunosuppressive effects in rat skin graft rejection

Figure 2 shows that the median survival time of transplanted skin grafts was prolonged to 35 days by the palmitate ester, to 25 days with the adamantoate ester, and to only 15 days with cytarabine, when the compounds were injected under identical conditions.

Antiviral effects in mice

Inspection of the data in Table 4 shows that the adamantoate, benzoate and palmitate derivatives were effective anti-herpes agents when administered intracranially

TABLE 3. EFFECT OF DAILY INJECTIONS OF SELECTED CYTARABINE DERIVATIVES ON HAMSTER HEMAGGLUTININ FORMATION

Compound	Dose (mg/kg, i.p., days 1–6)	Hemagglutinin titer*
Controls	—	5·7 ± 1·0
Cytarabine	10	4·7 ± 0·8
Palmitate	10	2·8 ± 2·2
Adamantoate	10	2·5 ± 0·9
Cytarabine	20	2·5 ± 1·2
Palmitate	20	1·2 ± 1·4
Adamantoate	20	1·6 ± 0·9

* The titers represent the average of six hamsters ± 1 mean deviation. The titers were determined on sera from blood drawn on day 7.

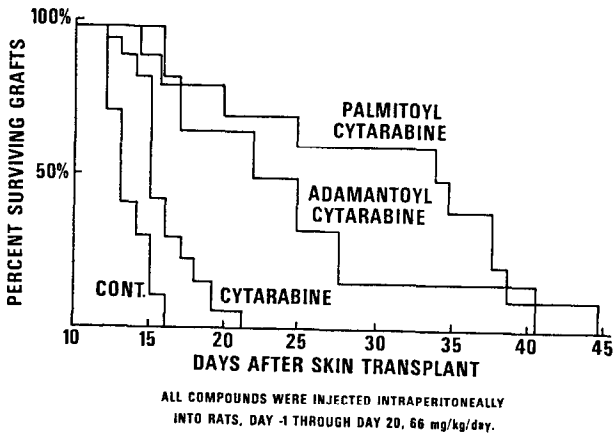

FIG. 2. Immunosuppressive effects of selected cytarabine derivatives on transplanted skin graft rejection in rats. The compounds were injected as shown above into groups of at least 16 Lewis rats receiving LBN rat skin grafts. Graft rejection was considered complete when the grafts became thickened and hard.

either 3 hr before or 3 hr after virus infection. In contrast, cytarabine was active only when injected after virus infection. In other experiments (data not presented here), no antiviral activity was achieved when the above derivatives or cytarabine was injected subcutaneously or intraperitoneally, using single or multiple injection schedules.

Inhibition of thymidine incorporation in PHA-stimulated human lymphocytes

The results in Table 5 show that, as in the case of the animal experiments, the adamantoate and palmitate derivatives demonstrated a long-lasting inhibitory effect when compared with cytarabine *in vitro*. Both compounds inhibited thymidine incorporation in PHA-stimulated lymphocytes at concentrations roughly ten times less

TABLE 4. EFFECTS OF CYTARABINE AND SELECTED CYTARABINE DERIVATIVES ON HERPES SIMPLEX VIRUS INFECTION IN MICE

Compound	Dose (mg/kg)	Treatment schedule* (hr)	% Survivors at 7 days
Cytarabine	50	−3	0
Cytarabine	50	+3	55
Palmitate	133	−3	47
Palmitate	133	+3	60
Adamantoate	66	−3	44
Adamantoate	66	+3	60
Benzoate	66	−3	58
Benzoate	66	+3	68
Vehicle		−3	0
Vehicle		+3	5

* The 100 LD_{50} dose of herpes simplex virus was administered intracranially at 0 hr into groups of 20 mice.

than that observed with cytarabine when the compounds were added at the beginning of the 64-hr incubation.

TABLE 5. INHIBITORY EFFECTS OF CYTARABINE AND SELECTED DERIVATIVES ON THYMIDINE INCORPORATION INTO PHA-STIMULATED HUMAN LYMPHOCYTES

Compound	ID_{50} Range, mμM (mμg/ml)*			
	Present during the entire 64-hr incubation		Present during the last 8 hr of incubation only	
Cytarabine	3200–8900	(900–2500)	10–18	(3–5)
Palmitate	390–770	(200–400)	39–77	(20–40)
Adamantoate	230–1100	(100–500)	23–45	(10–20)

* Inhibitory dose, 50% (ID_{50}), means the concentration at which thymidine incorporation was inhibited by 50% in PHA-stimulated human lymphocytes. The range above represents the values obtained with lymphocytes from four different individuals.

Indirectly supporting the idea that these derivatives represent a slow-release form of cytarabine was the finding that the derivatives were less active than cytarabine when the compounds were compared for their "acute" inhibitory effects, i.e. when they were added during the last 8 hr of incubation. In this situation, cytarabine was an effective inhibitor at a dose one-third to one-fourth that of the derivatives.

DISCUSSION

Although cytarabine has been reported to be a potent immunosuppressant and antitumor agent, the use of complex dose schedules was necessary to achieve maximum inhibitory effects.[5,6,15–17] Thus, a program to investigate various derivative forms of of cytarabine was undertaken, and many chemically interesting compounds were synthesized and described previously.[34,35] The first derivative which was clearly more potent than cytarabine (synthesized by P. F. Wiley of these laboratories) was the 5′-adamantoate. While this compound was of immediate interest because of its greater potency,[26–29] it posed several questions which led to the synthesis of additional derivatives. One question dealt with the possibility that the adamantane moiety, which has been exhaustively studied by Gerzon *et al.*,[36–38] possessed unique and novel pharmacologic properties. While the addition of the adamantane moiety did change some of the characteristics of cytarabine,[26–29] the current data show that a variety of other substitutions was also as effective, e.g. the palmitate, stereate, benzoate and 4-methoxybenzoate derivatives.

The possible reasons for the enhanced potency of the adamantoate derivative were stated previously.[27,29] Since direct biochemical evidence has now been obtained in support of these postulates,[39] the explanation for the potency of the newer derivatives is as follows. After injection of these insoluble derivatives, there is a finite time required for dispersion and solubilization. The freely circulating derivatives may pos-

sess a different tissue distribution because of their increased lipophilicity. More importantly, however, these derivatives should be more resistant to deamination which, in the case of the parent compound, leads to the formation of an inactive metabolite (*ara*-uridine). After enzymatic hydrolysis of the derivative to the corresponding acid and cytarabine, the latter is then free to exert its inhibitory activities. The net effect, therefore, of administration of these derivatives is the maintenance of relatively constant low levels of cytarabine for long periods of time (which was clearly the case with the adamantoate[39]).

What, then, would be the effect of maintaining low levels of cytarabine by injection of these sustained-release forms? One effect was obvious. These derivatives were capable of achieving certain desired biological effects, e.g. prolongation of transplanted skin graft survival in specific situations where the parent compound had only minimal effect. Similarly, in the herpes simplex-infected mice, cytarabine was only active when administered after virus infection, whereas the derivatives were active when administered either before or after infection. Thus, there was a practical advantage to the use of these derivatives.

This is not to say that cytarabine would not be capable of inducing the desired effect, but rather that the most efficacious injection procedure may not have been employed. For instance, approximately the same number of L1210 "cures" (45-day survivors) can be obtained with the derivatives and with cytarabine, but only if the latter is employed in complex injection schedules.[15] Moreover, it should be noted that in all the current experiments, the desired inhibitor effects were attained with derivatives which make available approximately 50 per cent less cytarabine than when cytarabine itself is employed.

The experiments *in vitro* with PHA-stimulated human lymphocytes demonstrate the importance of carefully describing the nature of the experiment in which the compounds were compared. Under one set of conditions, in which the compounds were present throughout the period of PHA stimulation, the derivatives were far more inhibitory than cytarabine. Conversely, during a short period of incubation, cytarabine was more inhibitory than the derivatives. Irrespective of any attempt to compare these compounds directly, however, these experiments did show that the adamantoate and palmitate esters were capable of providing a reservoir of active cytarabine in a system *in vitro* utilizing human cells.

The biochemical effect of maintaining low levels of cytarabine may be stated as follows. It has been shown that cytarabine must not only be phosphorylated intracellularly[40–46] to inhibit DNA synthesis,[47] but it must also be present in the extracellular environment at the time the cell is beginning the S-phase,[48–51] with maximum inhibitory efficacy in the latter half of the S-phase and beginning half of the G_2-phase.[52] Thus, inhibition of both antigen-stimulated lymphoid cells and actively dividing leukemia cells should require the prolonged maintenance of effective cytarabine levels in their environment because, in their natural state, they are asynchronously proliferating cell populations.

One criterion which is of paramount importance in determining whether the derivatives offer any advantage over the parent compound is that of therapeutic index. Unfortunately, this simple question is difficult to answer for a simple reason. Whereas the (active) derivatives possessed good activity in a variety of test systems, under the same conditions the parent compound possessed little or no activity. For instance,

daily cytarabine administration had only marginal activity in prolonging skin graft survival in rats (even at doses as high as 500 mg/kg/day) and, even at lethal doses in mice, no prolongation of skin graft survival has been observed in these laboratories.[26] Thus, it would be completely misleading to compare the lack of activity with cytarabine to good activity with cytarabine derivatives and try to determine the relative therapeutic index (which in this case, would be infinitely in favor of the derivatives). Another example which militates against a general statement concerning the relative therapeutic index was the comparison between cytarabine and its derivatives when single injections were employed. In this case, the parent compound was essentially devoid of all activity, whereas the (active) derivatives were very active under these conditions.

Similar problems emerged when the other parameter of the therapeutic index was examined, viz. toxicity. Cytarabine was essentially devoid of toxicity in rats and mice when one single injection was employed at doses of 1000 mg/kg. The active derivatives (e.g. the adamantoate, palmitate and benzoate esters), on the other hand, were toxic at single doses in mice ranging from 150 to 300 mg/kg. Similarly, the 5-day LD_{50} dose for cytarabine in mice was about 250 mg/kg/day, whereas the 5-day LD_{50} dose for the active derivatives was approximately 25–50 mg/kg/day. Thus, the derivatives are clearly more toxic than the parent compound, but to view these data separately from activity measurements would be completely erroneous.

Any speculation as to the potential clinical advantages of these derivatives as immunosuppressants, antiviral agents, or antitumor agents simply must await further study. Any of the parameters alluded to above may greatly influence activity in man. For instance, it may be that a particular derivative will not dissolve at a sufficient rate, or perhaps, the ester might be hydrolyzed too slowly to provide effective blood levels. Regardless of the final outcome, these results show that the activity of a potent immunosuppressant, antiviral agent, and antitumor agent can be profoundly influenced by simple chemical modifications.

REFERENCES

1. C. G. SMITH, *Proc. Third Int. Pharmac. Meeting* (Ed. A. D. WELCH), Vol. 5, p. 33. Pergamon Press, Oxford (1968).
2. H. H. BUSKIRK, J. A. CRIM, H. G. PETERING, K. MERRITT and A. G. JOHNSON, *J. natn. Cancer Inst.* **34,** 747 (1965).
3. E. M. GLENN, *Proc. Soc. exp. Biol. Med.* **129,** 127 (1968).
4. R. O. GORDON, M. E. WADE and M. S. MITCHELL, *J. Immun.* **103,** 233 (1969).
5. G. D. GRAY, J. A. CRIM and M. M. MICKELSON, *Transplantation* **6,** 818 (1968).
6. G. D. GRAY, M. M. MICKELSON and J. A. CRIM, *Transplantation* **6,** 805 (1968).
7. D. S. FISCHER, E. P. CASSIDY and A. D. WELCH, *Biochem. Pharmac.* **15,** 1013 (1966).
8. G. D. GRAY, R. J. PERPER, M. M. MICKELSON, J. A. CRIM and C. F. ZUKOSKI, *Transplantation* **7,** 183 (1969).
9. J. E. HARRIS and E. M. HERSH, *Cancer Res.* **28,** 2432 (1968).
10. M. S. MITCHELL, S. R. KAPLAN, and P. CALABRESI, *Cancer Res.* **29,** 876 (1969).
11. M. E. WADE, R. O. GORDON, and M. S. MITCHELL, *Am. J. Obstet. Gynec.* **106,** 286 (1970).
12. G. E. UNDERWOOD, *Proc. Soc. exp. Biol. Med.* **111,** 660 (1962).
13. H. N. PRINCE, E. GRUNBERG, M. BUCK and R. CLEELAND, *Proc. Soc. exp. Biol. Med.* **130,** 1080 (1969).
14. J. S. EVANS, E. A. MUSSER, L. BOSTWICK and G. D. MENGEL, *Cancer Res.* **24,** 1285 (1964).
15. H. E. SKIPPER, F. M. SCHABEL and W. S. WILCOX, *Cancer Chemother. Rep.* **51,** 125 (1967).
16. I. KLINE, J. M. VENDITTI, D. D. TYRER and A. GOLDIN, *Cancer Res.* **26,** 853 (1966).
17. I. KLINE, D. D. TYRER, M. GANG, J. M. VENDITTI and A. GOLDIN, *Cancer Chemother. Rep.* **52,** 399 (1968).

18. M. S. MITCHELL, M. E. WADE, R. C. DECONTI, J. R. BERTINO and P. CALABRESI, *Ann. intern. Med.* **70,** 535 (1969).
19. T. C. HALL, C. WILFERT, N. JAFFE, D. TRAGGIS, S. LUX, P. ROMPF and S. KATZ, *Trans. Ass. Am. Physns.* **82,** 201 (1969).
20. E. M. MCKELVEY and H. C. KWAAN, *Blood* **34,** 706 (1969).
21. B. E JUEL-JENSEN, *Br. med. J.* **2,** 154 (1970).
22. S. A. PLOTKIN and H. STETLER, *Antimicrob. Agents Chemother.* **372** (1970).
23. E. FREI III, J. N. BICHEUS, J. S. HEWLETT, M. LANE, W. V. LEARY and R. W. TALLEY, *Cancer Res.* **29,** 1325 (1969).
24. J. J. WANG, O. S. SELAWRY, T. J. VIETTI and G. P. BODET, Sr., *Cancer, N.Y.* **25,** 1 (1970).
25. P. J. BURKE, A. H. OWENS, JR., J. COLSKY, B. I. SHNIDER, J. H. EDMONSON, A. SCHILLING, H. S. BRODOUSKY, H. J. WALLACE, JR. and T. K. HALL, *Cancer Res.* **30,** 1512 (1970).
26. G. D. GRAY and M. M. MICKELSON, *Transplantation* **9,** 177 (1970).
27. G. D. GRAY, M. M. MICKELSON and J. A. CRIM, *Biochem. Pharmac.* **18,** 2163 (1969).
28. G. D. GRAY and M. M. MICKELSON, *Immunology* **19,** 417 (1970).
29. G. L. NEIL, P. F. WILEY, R. C. MANAK and T. E. MOXLEY, *Cancer Res.* **30,** 1047 (1970).
30. D. T. GISH, R. C. KELLY and W. J. WECHTER, *J. med. Chem.* in press.
31. ZÍCHA and L. BŮRIČ, *Science, N.Y.* **163,** 192 (1969).
32. G. W. CAMIENER and C. G. SMITH, *Biochem. Pharmac.* **14,** 1405 (1965).
33. W. A. CREASY, R. J. PAPAC, M. E. MARKIW, P. CALABRESI and A. D. WELCH, *Biochem. Pharmac.* **15,** 1417 (1966).
34. W. J. WECHTER, *J. med. Chem.* **10,** 762 (1967).
35. C. G. SMITH, H. H. BUSKIRK and W. J. LUMMIS, *J. med. Chem.* **10,** 774 (1967).
36. K. GERZON and D. KAU, *J. med. Chem.* **10,** 189 (1967).
37. K. GERZON, E. V. KRUMKALNS, R. L. BRINCLE, F. J. MARSHALL and M. A. ROOT, *J. med. Chem.* **6,** 760 (1963).
38. R. T. RAPALA, R. J. KRAAY and K. GERZON, *J. med. Chem.* **8,** 580 (1965).
39. G. L. NEIL, H. H. BUSKIRK, T. E. MOXLEY, R. C. MANAK, S. L. KUENTZEL and B. K. BHUYAN, *Biochem. Pharmac.* **20,** 3295 (1971).
40. J. P. DURHAM and D. H. IVES, *Molec. Pharmac.* **5,** 358 (1969).
41. D. KESSEL, *J. biol. Chem.* **243,** 4739 (1968).
42. D. KESSEL and S. B. SHURIN, *Biochim. biophys. Acta* **163,** 179 (1968).
43. D. KESSEL, T. C. HALL and I. WODINSKY, *Science, N.Y.* **156,** 1240 (1967).
44. D. KESSEL, T. C. HALL and D. ROSENTHAL, *Cancer Res.* **29,** 459 (1969).
45. A. W. SCHRECKER, *Cancer Res.* **30,** 632 (1970).
46. K. UCHIDA and W. KREIS, *Biochem. Pharmac.* **18,** 1115 (1969).
47. J. J. FURTH and S. S. COHEN, *Cancer Res.* **28,** 2061 (1968).
48. M. Y. CHU and G. A. FISCHER, *Biochem. Pharmac.* **11,** 423 (1962).
49. M. KARON and S. SHIRAKAWA, *Cancer Res.* **29,** 687 (1969).
50. L. LENAZ, S. S. STERNBERG and F. S. PHILLIPS, *Cancer Res.* **29,** 1790 (1969).
51. R. S. K. YOUNG and G. A. FISCHER, *Biochem. biophys. Res. Commun.* **32,** 23 (1968).
52. W. F. BENEDICT, N. HARRIS and M. KARON, *Cancer Res.* **30,** 2477 (1970).