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IMMUNOSUPPRESSION BY PYRIMIDINE NUCLEOSIDE ANALOGS*

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Abstract—Pyrimidine nucleoside analogs have been examined in mice for their ability to suppress the primary immune response to the intraperitoneal injection of sheep erythrocytes. 5-Fluorodeoxyuridine was more effective than the corresponding 5-bromo-compound, while 5-iododeoxyuridine was essentially inactive in practical dosage levels. In all cases, the drugs were more effective when given intraperitoneally than subcutaneously, and this observation applied also to 6-mercaptopurine and to azathioprine. Cytosine arabinoside was the most potent agent tested, and its immunosuppressive effects could be inhibited by the concurrent administration of deoxycytidine. The combination of chloramphenicol with 6-azauridine exhibited potentiation of the weak immunosuppressive activity exerted by 6-azauridine, while no such activity was demonstrated by chloramphenicol under the conditions of the experiments.

DRUGS that suppress the immune response are currently being used to treat several "autoimmune" diseases and to modify the homograft reaction in tissue transplantation. Thus far, the most successful agents have been purine analogs, such as 6-mercaptopurine, 6-thioguanine, and azathioprine, which interfere with the metabolism of nucleotides and other precursors of nucleic acids[1, 2] and which were originally developed as antineoplastic agents. Analogs of pyrimidine derivatives have been less thoroughly studied with respect to the inhibition of antibody formation, although some are capable of disturbing normal nucleic acid synthesis,[3–5] and some are useful antineoplastic and antiviral agents. The few reports of experiments *in vivo*, in which such compounds were used as immunosuppressive agents, are not in complete agreement, probably because variable test systems have been employed. The results with *in vitro* systems are less divergent, but these are not necessarily in agreement with studies *in vivo*.[6]

The present studies were designed to evaluate the relative potency of pyrimidine nucleoside analogs in the intact mouse, under conditions in which relatively large numbers of animals would be used. In this paper are reported the results obtained in a standardized system with compounds in which (a) a halogen atom replaced a hydrogen in the 5-position of 2′-deoxyuridine, (b) a compound with a ring carbon (position 6) replaced by nitrogen (6-azauridine), and (c) a compound containing a normal pyrimidine base linked to arabinose (1-β-D-arabinofuranosylcytosine hydrochloride; cytosine arabinoside); these compounds have been compared to 6-mercaptopurine, azathioprine, and methotrexate.

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MATERIALS AND METHODS

Mice. Random-bred specific pathogen-free white Swiss CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Mass.), 7–9 weeks of age, and with an average weight of 30 g, were used. Approximately 100 animals of the same sex, age, and weight were employed in each experiment, in which a minimum of eight mice served as controls; each drug-test group contained from eight to ten animals.

Antigen. Sterile sheep blood in Alsever's solution was purchased (Probio, Inc., Nyack, N.Y.); usually this was used within 7 days of collection. Plasma was separated by centrifugation, and the cells were then washed three times with isotonic phosphate. buffered saline (pH 7·2), and resuspended in the same medium.

Procedure. Antigen was introduced in ways similar to those used by Nathan *et al.*,[7] except that the intravenous route was not used. Each mouse was injected by the intraperitoneal (i.p.) route in the morning of day 1 with 0·25 ml of a 30% suspension of sheep erythrocytes. Drug therapy was begun in the afternoon of the same day, using either the i.p. or the subcutaneous (s.c.) route, as indicated in the tables. Subsequent injections were given either once daily in the morning for 4 additional days, or, where indicated, twice daily (9.00 A.M. and 4.00 P.M.) for 4 additional days. On day 12, all animals were sacrificed by cervical dislocation, and blood was collected from each mouse separately by cutting the great vessels just above the heart. After clotting, the individual sera were used for determination of hemagglutinins.

Hemagglutination. The hemagglutinin titer was determined by serial twofold dilutions in 0·1% gelatin in phosphate-buffered saline beginning with a 1:32 (2^5) dilution and extending out to a 1:16,384 (2^{14}) dilution in covered Perspex trays. To each 0·5 ml of serum dilution was added 0·1 ml of a 1% suspension of thrice-washed sheep erythrocytes. After preparation, the trays were shaken on a Vortex mixer, allowed to stand for 3 hr at room temperature, and agglutination was scored in the lower dilutions as it became apparent. The plates were then left at 5° for 18 hr and rescored (on a 0 to 4+ basis, as described by Stavitsky[8]). The early scoring was necessary because the 1:32, 1:64, and 1:128 dilutions were often hemolyzed if not read early; higher dilutions were rarely if ever hemolyzed. The score changed but little between 3 and 18 hr, and the later score was always used unless hemolysis had occurred. Reduction by mercaptoethanol (ME) was preformed by incubating at 37° for 1 hr a 1:32 dilution of serum made 0·1 M with ME prior to carrying out serial two-fold dilutions.

Index. This was calculated exactly as described by Nathan *et al.*:[7] "The score for each tube was multiplied by the appropriate exponent of the 2-fold dilution series, i.e., a tube showing a 4+ agglutination at a dilution of 2^6 would have a value of 24. These values were then summed for each series. The index of drug effect was obtained as the ratio of these sums for the treated to the untreated controls:

$$\text{Antibody index} = \frac{\Sigma(S_1 + 2S_2 + 3S_3 + \ldots nS_n)T}{\Sigma(S_1 + 2S_2 + 3S_3 + \ldots nS_n)C}$$

where n refers to the exponent of the dilution, S is the agglutination score for the tube and T and C refer to treated and control series, respectively." Controls injected with saline or 1% methylcellulose in saline (1%MC) gave the same result and were grouped together. The Σ_C for 120 mice was 221 ± 40. An index value of 0·60 or lower was

chosen arbitrarily as a limiting value for activity. This is the same index value as that used by Bieber *et al.*,[9] and differs from the control average by greater than two standard deviations.

Drugs and abbreviations. The drugs were given i.p. or s.c. by the schedule indicated in the respective tables. A control without antigen and a control with saline as therapy were included in each experiment. The 5- iodo-2′-deoxyuridine (IUdR), lot 5272, was purchased from Nutritional Biochemicals Corp., the 5-iodo-2′-deoxycytidine (ICdR) was prepared by P. K. Chang of the Department of Pharmacology, Yale University School of Medicine;[10] the 5-bromo-2′-deoxyuridine (BUdR) was obtained from Schwarz BioResearch, Inc., lot DBU6002, through the Cancer Chemotherapy National Service Center; the 5-fluoro-2′-deoxyuridine (FUdR) was kindly supplied (RO 5-0360, lot 5411C) by Hoffmann-LaRoche, Inc.; the 6-mercaptopurine (6-MP, Purinethol), lot 45220, and the azathioprine (Imuran, BW 57–322), lot 45374, were obtained through the kindness of G. B. Elion of Burroughs Wellcome and Co., Inc., the methotrexate (MTX), lot J-0906, was obtained from the Lederle Laboratories Division of American Cyanamid Co.; cytosine arabinoside (CA) was obtained as U-19920 (Cytarabine), lot 14,866-2MZ, through the kindness of Dr. C. G. Smith of the Upjohn Co.; deoxycytidine (CdR) was purchased from Nutritional Biochemicals Corp.; 6-azauridine (6AzUR) was obtained from the Cancer Chemotherapy National Service Center as NSC 32074, lot 12-34-004; the chloramphenicol (CAP) was obtained through the kindness of Dr. A. C. Bratton, Jr., of Parke Davis and Co. and was used as the succinate sodium ester (CAP suc.). Whenever two drugs were used concurrently, they were administered by separate injections.

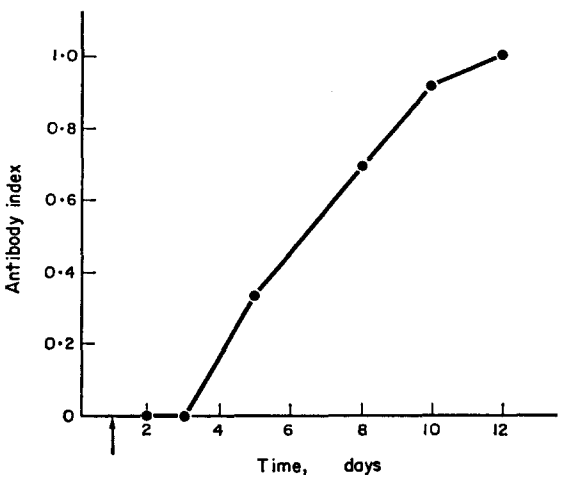

FIG. 1. Appearance of circulating antibody after administration of antigen on day 1.

RESULTS

The results of hemagglutinin titers determined on groups of 5 mice injected i.p. with antigen on day 1, and then i.p. with 0·2 ml of saline daily for 5 days or until sacrifice, are plotted in Fig. 1.

A similar study of groups of three mice in which the saline injections were omitted gave an essentially similar plot with plateauing after day 12 and extending to day 18.

These results are in agreement with those of Makinodan *et al.*[11] and Adler[12] and reflect the slower rise of antibody titers after i.p. stimulation, as compared to the more rapid rise observed by Nathan *et al.*[7] after the intravenous administration of antigen.

Of the halogenated pyrimidine nucleoside analogs tested, under the conditions employed, the immunosuppressive activity of FUdR was the most marked. As noted in Table 1, FUdR was highly effective at a level of 15 mg/kg given only once daily for

TABLE 1. EFFECT OF HALOGENATED PYRIMIDINE DEOXYRIBONUCLEOSIDES ON HEMAGGLUTINATION RESPONSE

Drug	Total daily dose (mg/kg)*	Route	No. of mice	Index
FUdR	10	i.p.	10	0·59
	15	i.p.	10	0·48
BUdR	33	i.p.	10	0·59
	33	s.c.	10	0·77
	66	i.p.	10	0·58
	66	s.c.	10	0·68
	75‡	s.c.	10	0·70
	100	i.p.	10	0·58
	100	s.c.	20	0·68
	100†	i.p.	18	0·52
	100†	s.c.	8	0·77
	120	s.c.	10	1·0
IUdR	100†	i.p.	17	0·66
	100†	s.c.	8	0·94
	120‡	s.c.	10	0·69
	300‡	s.c.	10	0·52
ICdR	300‡	s.c.	10	0·61

* Each drug was injected once a day for 5 consecutive days in saline solution with the following exceptions.
† Injected twice a day.
‡ Injected in 1% methylcellulose suspension.

5 days, although a dose of 10 mg/kg was just barely within the range considered effective. By contrast, BUdR was only slightly effective when given daily in doses as high as 100 mg/kg daily for 5 days in saline solution by the i.p. route. When given s.c. in saline or in 1% methylcellulose to decrease the rate of absorption, BUdR did not suppress the antibody response. When the compound was given in a total dose of 100 mg/kg in two divided doses daily, there was increased immunosuppression only if the injections were i.p., while no effect occurred if they were given s.c. Regardless of either the route or vehicle, IUdR was ineffective as an immunosuppressive agent in doses comparable to those of BUdR that were effective. When given s.c. at the maximal tolerated dose, 300 mg/kg daily, and suspended in 1% methylcellulose, IUdR caused immunosuppression analogous to that seen with two injections daily of BUdR in one third the total daily dose; at 400 mg/kg daily of IUdR, there was a 100% mortality rate. ICdR was ineffective in daily doses of 300 mg/kg.

To check the sensitivity of the method and to verify the discrepant results obtained with i.p. injections, in contrast to those given s.c., standard immunosuppressive agents were tested (see Table 2). As expected, 6-MP and azathioprine (purine analogs) were effective when given in adequate dosage, but only if the i.p. route, rather than the s.c.

one, was used. This dissociation of efficacy based on route of administration has been noted previously,[13] and 6-MP given by the s.c. route has been reported to be inactive.[4] Accordingly, the suggested route of administration in animal experiments has been intramuscular,[15] although excellent results also have been obtained by the i.p. route.[7] Methotrexate, a folic acid antagonist, was effective in daily doses of 2 mg/kg.

TABLE 2. EFFECT OF PURINE ANALOGS AND A FOLIC ACID ANALOG ON THE HEMAGGLUTINATION RESPONSE

Drug	Total daily dose (mg/kg)*	Route	Index
6-MP	50	s.c.	0·88
	75	s.c.	0·60
	75	i.p.	0·41
Azathioprine	33†	s.c.	0·77
	66	s.c.	0·90
	66	i.p.	0·56
	75‡	s.c.	0·69
	100	s.c.	1·0
	100	i.p.	0·56
MTX	2‡	s.c.	0·47

* Each drug was injected once a day for 5 consecutive days in saline solution into 10 mice with the following exceptions: † injected 9 mice; ‡ injected in 1% methylcellulose.

Cytosine arabinoside in a daily dose of 40 mg/kg for 5 days was the most potent drug tested in our studies (Table 3). It was approximately as active as an immunosuppressive agent when given by the i.p. as by the s.c. route, in confirmation of its antineoplastic activity, which was identical by the two routes.[16] Toxicity was minimal at this dose, with no deaths and no weight loss. A subacute toxicity study in groups of six to eight mice with five single daily i.p. injections was evaluated at 12 days. At doses of 225 mg/kg and less, all animals survived. The calculated LD_{50} was 340 mg/kg.

When CA was administered for only 3 days, its activity was less than that of a 5 day course, but the more important days appeared to be the 3rd and 4th after antigenic stimulation. The first day was less important, as reported by Frisch *et al.* with thioguanine.[17]

The immunosuppressive activity of CA could be inhibited by the simultaneous administration of twice the dose of CdR for a full 5 day period, but not for a 3 day period at this dose. If CdR was given simultaneously in four times the amount of CA, it was effective as an inhibitor of the activity of CA after a 3 day course, and even more inhibitory after a 5-day course; CdR by itself had no demonstrable effect on the titers of antibody formed.

The mercaptoethanol treated sera showed a reduction in titer during the early days after antigenic stimulation, but by day 12 there was no reduction. In contrast, the mice treated with CA, 40 mg/kg daily for 5 days, showed an average of a two tube reduction in titer, which suggests that 75% of the antibody was of the polymeric (primarily but not exclusively 19S) type (Table 4).

Although 6-AzUR exhibited immunosuppressive activity in mice when administered for 5 days in 1% methylcellulose in saline either once or twice daily, the magnitude of the effect was not great (Table 5). CAP suc. had no immunosuppressive activity when given in a total dose of either 500 or 1000 mg/kg in two divided doses daily. At the lower CAP suc. dose, there were two deaths among the twenty-five mice in the treatment group and, at the higher dose, six deaths among the eighteen mice injected.

TABLE 3. EFFECT OF CYTOSINE ARABINOSIDE AND DEOXYCYTIDINE, ALONE AND IN COMBINATION, ON THE HEMAGGLUTINATION RESPONSE

Drug	Total single daily dose (mg/kg)*	No. of days injected	Route	No. of mice	Index
CA	20	5	i.p.	16	0·56
	20	5	s.c.	10	0·41
	40	5	i.p.	43	0·19
	40	7	s.c.	10	0·18
	40†	5	s.c.	19	0·39
	40	3 (1–3)	i.p.	19	0·55
	40	3 (2–4)	i.p.	19	0·42
	40	3 (3–5)	i.p.	19	0·41
CA + CdR	20+40	5	i.p.	8	0·72
	20+80	5	i.p.	8	1·0
	40+80	5	i.p.	8	0·88
	40+80	7	s.c.	10	0·69
	40+80†	5	s.c.	19	0·68
	40† + 80	3 (1–3) + 5 (1–5)	i.p.	9	0·73
	40† + 80	3 (1–3) + 4 (2–5)	i.p.	9	0·53
	40† + 80	3 (1–3) + 3 (1–3)	i.p.	9	0·55
	40+160	5	i.p.	7	1·09
	40+160	3 (1–3)	i.p.	18	0·62
	40+160	3 (2–4)	i.p.	10	0·71
	40+160	3 (3–5)	i.p.	10	0·62
CdR	80	5	i.p.	8	1·05
	160	5	i.p.	10	0·91

* Each drug was injected once a day in saline solution with the following exceptions.
† Injected in 1% methylcellulose suspension.

TABLE 4. MERCAPTOETHANOL SENSITIVITY OF MOUSE SERA

Serum	No. of mice	Average titer without ME	Average titer with ME	Percent of polymeric antibody
Control day 2	5	<1:32	<1:32	
Control day 3	5	<1:32	<1:32	
Control day 5	5	1:4,096	1:128	97
Control day 8	5	1:16,384	1:4,096	75
Control day 10	5	1:16,384	1:8,192	50
Control day 12	10	1:16,384	1:16,384	0
CA 40 mg/kg × 5 (day 12)	10	1:1,024	1:256	75

There were no deaths in the 6-AzUR group given 180 mg/kg and only two deaths among forty-six mice given 270 mg/kg.

Combination therapy using both 6-AzUR and CAP suc. at their lower dosages did not result in potentiation; however, the combination of either drug in the lower dosage with the other in the higher dosage, led to a significant potentiation, more

TABLE 5. EFFECT OF 6-AZAURIDINE AND CHLORAMPHENICOL SUCCINATE SODIUM, ALONE AND IN COMBINATION, ON THE HEMAGGLUTINATION RESPONSE

Drug	Total daily dose (mg/kg)*	Route	No. of mice	Index
6-AzUR	180†	i.p.	18	0·74
	180	s.c.	20	0·60
	180	i.p.	26	0·58
	270†	i.p.	10	0·63
	270†	s.c.	9	0·77
	270†‡	s.c.	9	0·52
	270	i.p.	18	0·56
CAP suc.	500	i.p.	23	0·81
	1000	i.p.	12	0·79
CAP suc. + 6-AzUR	500† + 180	i.p. + i.p.	23	0·56
	500† + 270	i.p. + i.p.	17	0·36
	1000† + 180	i.p. + i.p.	13	0·50

* Each drug was injected twice a day for 5 consecutive days in saline solution or supension with the following exceptions:

† injected once a day;

‡ injected in 1% methylcellulose suspension.

marked when 6-AzUR was in the higher dosage. With combination therapy, there were three deaths among twenty-six mice given 6-AzUR, 180 mg/kg, with CAP suc., 500 mg; one death among eighteen mice given 6-AzUR, 270 mg/kg, with CAP suc., 500 mg/kg; and five deaths among eighteen mice given 6-AzUR, 180 mg/kg, with CAP suc., 1000 mg/kg.

The earlier antibody response to such a foreign antigen as sheep erythrocytes is characteristically expressed by the appearance of a 19S (γM) immunoglobulin. The level of γM begins to decrease after a few days as the level of γG (7S) antibody rises, and in the mouse the ratio of γM:γG may change from 10:1 to 1:35 with time.[12] The early macroglobulin antibodies are inactivated by treatment with mercaptoethanol which does not affect the late γG antibody. Accordingly, this technique has been used extensively to estimate the distribution of these two classes of immunoglobulins in sera. The sera in our early bleedings fell into the expected pattern. The control sera at 12 days were not mercaptoethanol sensitive, but the serum of CA-treated mice had its titer reduced by two serial dilutions, a finding which indicated that 75 per cent of the antibody is of the polymeric variety. Some early 7S antibody may be sensitive to mercaptoethanol.[18] The immunosuppression by CA during the period in which 19S antibody would ordinarily be formed has apparently led to a persistence of the 19S response and a suppression of the formation of 7S antibody. A similar pattern has

been demonstrated with 6-mercaptopurine[19] and would be expected with other potent immunosuppressive agents, although the mechanism of the effect has not yet been elucidated completely.

DISCUSSION

The formation of antibody in amounts measurable by current quantitative techniques, requires the multiplication of cells[20] and, therefore, nucleic acid synthesis. Agents that interfere with nucleic acid synthesis should inhibit antibody formation, and this has been well demonstrated, both *in vitro* and *in vivo*, by many investigators. Dutton *et al.*, however, have suggested that some antagonists like 6-MP, 8-azaguanine, 6-thioguanosine, and X-irradiation inhibit general cell metabolism, in contrast to thymidine analogs, which are specific inhibitors of deoxyribonucleic acid (DNA) synthesis, but otherwise are relatively nontoxic.[21] In early studies, BUdR proved to be an effective inhibitor of antibody formation *in vitro*[22] and later FUdR and IUdR were found to be similarly effective.[23] If these findings could be confirmed by observations *in vivo* these agents might be superior for clinical use to the general metabolic inhibitors now in use. The report of Bieber *et al.*[9] suggested that, in a mouse system with intravenous administration of sheep erythrocytes, BUdR in daily doses of 30 mg/kg for 4 days would significantly inhibit the immune response. Paradoxically, the same group found that IUdR was unable to inhibit the immune response in the same mouse test system.[24] Similar observations were made in rabbits with vaccinia as antigen.[25]

The effect of FUdR on antibody formation has been somewhat puzzling. Merritt and Johnson[26] found that in mice i.p. doses of 1 mg given daily for 7–12 days would delay and depress antibody formation, but 5 days of therapy would not. A single i.p. dose of 8 mg FUdR immediately before or after the antigen led to enhancement, but the same dose given 48 hr after antigen caused inhibition of antibody formation. Our study indicates that FUdR is the best immunosuppressive of the halogenated pyrimidines tested and deserves further study.

Of compounds so far studied, CA must be regarded as one of the most potent inhibitors of the immune response. As such it may provide another therapeutic approach to the treatment of "autoimmune" diseases and to the maintenance of homografts, if its toxicity is not permitted to become limiting. Since CdR does not readily reverse the immunosuppressive effects of CA if given later (Table 3), it may be possible to use this fact in the titration of the dosages and in timing, in order to reduce toxicity without marked impairment of immunosuppression. At present, all available evidence indicates that CA inhibits the formation of DNA, in part by inhibiting the conversion of cytidine to CdR[27] at the level of cytidine 5′-diphosphate[28]; also, however, it is incorporated into both RNA and DNA.[29] There is no evidence that RNA synthesis is impaired.[30]

In addition to inhibition of the hemagglutinin reaction to sheep erythrocytes, CA inhibits the hemolysis reaction, when given in a dose of 50 mg/kg daily for 7 days and tested on day 8.[31] The primary response of BALB mice to bovine γ-globulin (BGG) was inhibited similarly after doses of CA of 25 and 50 mg/kg daily for 12 days. When CA was injected at 8 mg per mouse (approximately 400 mg/kg), however, there was no suppression of antibody formation, and when the drug was administered 1 hr before BGG, a definite enhancement was seen at this dose.[32]

Chloramphenicol alone is known to inhibit the secondary immune response *in vitro*[33, 34] and the primary response *in vivo*[35, 36] Weisberger *et al.*[35] used 500 to 600 mg CAP/kg daily in suspension, injected in divided doses every 8 hr for 12 days, to achieve antibody suppression in rabbits. Butler and Coons had to use 1500 mg or more CAP suc./kg, given in divided doses every 6 hr for 10 to 12 days, to achieve a similar effect in mice.[36] The necessity for a higher dose in mice probably reflects, on the one hand, the more rapid uptake and excretion of the CAP suc. solution, as compared to the CAP suspension, and, on the other hand, the fact that the formation of the glucuronide of CAP, with resultant inactivation, occurs more rapidly in mice than in rabbits.[37] It is likely that in the various mammalian species, similar blood levels of CAP for comparable durations would produce approximately the same inhibition of antibody, but this has not yet been put to experimental test. In any case, it is clear that under the conditions of the experiment, with only two injections of relatively low doses of CAP suc., there was no immunosuppressive activity by the antibiotic agent alone.

The primary action of 6-AzUR is the reduction of RNA synthesis by inhibition of the *de novo*-pathway of pyrimidine biosynthesis. Refractoriness to 6-AzUR, when it develops, may be attributable to the induction of enzyme activities in the *de novo*-pathway of biosynthesis of pyrimidine-containing compounds.[38] Accordingly, it is conceivable that CAP, as an inhibitor of protein synthesis,[39, 40] may block the formation of induced enzymes and prevent refractoriness to 6-AzUR, as suggested by Cardoso and Jaffe,[41] both in neoplastic cells and in antibody-forming cells.

This report tends to confirm many of the earlier studies and to resolve some of the apparently conflicting observations. The cells that make antibody are in a process of rapid multiplication during the second, third, fourth, and fifth days after antigen injection and are most sensitive to inhibitors of the synthesis of DNA (or to the incorporation of such agents into DNA) in the period just preceding mitosis;[42] hence, high concentrations of drug must be present in the areas of cell activity during this critical period. When the drugs are given s.c., some local degradation occurs, and relatively little reaches the immunologically competent cells. After i.p. injection, however, uptake is more rapid and the drug goes in greater concentration to the lymph nodes involved. In addition, it is known that IUdR is metabolized very rapidly,[43, 44] so that in normal mice, three fourths of an administered dose is recovered in the urine within 4 hr.[45] FUdR is degraded less rapidly,[46] while BUdR is probably in an intermediate position, but appropriate comparative studies of the halogenated pyrimidine nucleoside analogs in the same species under strictly comparable conditions do not appear to have been performed. Maintenance of a higher dose level for a longer duration, by either repository injections or more frequent injections, almost certainly would increase the effectiveness of such drugs as immunosuppressive agents. Thus, in tissue culture,[23] in which the compounds were present for a long time and without enzymatic destruction, these agents were more nearly comparable in biological activity. In the intact animal, the differences in immunosuppression are marked and seem to be related largely to the differing rates of inactivation and elimination. Further studies of these several factors are strongly indicated.

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REFERENCES

1. G. H. HITCHINGS and G. B. ELION, *Pharmac. Rev.* **15**, 365 (1963).
2. M. C. BERENBAUM, *Br. med. Bull.* **21**, 450 (1965).
3. R. E. HANDSCHUMACHER and A. D. WELCH, *The Nucleic Acids*, Vol. 3, p. 453. Academic Press, New York (1960).
4. A. D. WELCH, *Cancer Res.* **21**, 1475 (1961).
5. A. D. WELCH, *Ann. N.Y. Acad. Sci.* **123**, 19 (1965).
6. R. S. SCHWARTZ, *Progr. Allergy* **9**, 246 (1965).
7. H. C. NATHAN, S. BIEBER, G. B. ELION and G. H. HITCHINGS, *Proc. Soc. exp. Biol. Med.* **107**, 796, (1961).
8. A. B. STAVITSKY, *J. Immunol.* **72**, 360 (1954).
9. S. BIEBER, G. B. ELION, G. B. HITCHINGS, D. C. HOOPER and H. C. NATHAN, *Proc. Soc. exp. Biol. Med.* **111**, 334 (1962).
10. P. K. CHANG and A. D. WELCH, *J. med. Chem.* **7**, 428 (1963).
11. T. MAKINODAN, N. GENGOZIAN and C. C. CONGDON, *J. Immunol.* **77**, 250 (1956).
12. F. L. ADLER, *J. Immunol.* **95**, 26 (1965).
13. R. SCHWARTZ and J. ANDRE, *Second International Symposium on Immunopathology*, p. 385. Benno Schwabe, Basel (1962).
14. J. H. HUMPHREY and J. L. TURK, *Immunology* **4**, 301 (1961).
15. H. SPIEGELBERG and P. MIESCHER, *Fedn. Proc.* **2**, 501 (1963).
16. J. M. VENDITOO, I. KLINE and A. GOLDIN, *Cancer Res.* (Suppl.) **24**, 830 (1964).
17. A. W. FRISCH, G. H. DAVIES and V. MILSTEIN, *J. Immunol.* **89**, 300 (1962).
18. F. L. ADLER, *J. Immunol.* **95**, 39 (1965).
19. Y. BOREL, M. FAUCONNET and P. A. MIESCHER, *J. exp. Med.* **122**, 263 (1965).
20. R. W. DUTTON, *Nature, Lond.* **192**, 462 (1961).
21. R. W. DUTTON, J. D. EADY and H. N. BULMAN, *Conceptual Advances in Immunology and Oncology*, p. 98. Hoeber Medical Div, Harper and Row, New York (1963).
22. R. W. DUTTON, A. H. DUTTON and J. H. VAUGHAN, *Biochem. J.* **75**, 320 (1960).
23. R. W. DUTTON and J. D. PEARCH, *Immunology* **5**, 414 (1962).
24. G. B. ELION, quoted, *Cancer Res.* **23**, 1270 (1963).
25. P. CALABRESI and R. W. MCCOLLUM. Unpublished observation (1964).
26. K. MERRITT and A. G. JOHNSON, *J. Immunol.* **91**, 266 (1963).
27. M. Y. CHU and G. A. FISCHER, *Biochem. Pharmac.* **11**, 423 (1962).
28. P. REICHARD, Z. N. CANELLAKIS and E. S. CANELLAKIS, *J. biol. Chem.* **236**, 2514 (1961).
29. M. Y. CHU and G. A. FISCHER, *Biochem. Pharmac.* **14**, 333 (1965).
30. J. H. KIM and M. L. EIDINOFF, *Fedn. Proc.* **24**, 331 (1965).
31. J. S. EVANS, E. A. MUSSER, L. BOSTWICK and G. D. MENGEL, *Cancer Res.* **24**, 1285 (1964).
32. H. H. BUSKIRK, J. A. CRIM, H. G. PETERING, K. MERRITT and A. G. JOHNSON, *J. nat. Cancer Inst.* **34**, 757 (1965).
33. C. T. AMBROSE and A. H. COONS, *J. exp. Med.* **117**, 1035 (1963).
34. S. E. SVEHAG, *Science.* **146**, 659 (1964).
35. A. S. WEISBERGER, T. M. DANIEL and A. HOFFMAN, *J. exp. Med.* **120**, 183 (1964).
36. W. T. BUTLER and A. H. COONS, *J. exp. Med.* **120**, 1051 (1964).
37. M. C. DUNN and P. E. THOMPSON, *J. infect. Dis.* **92**, 33 (1953).
38. H. L. ENNIS and M. LUBIN, *Biochim. biophys. Acta* **68**, 78 (1963).
39. A. S. WEISBERGER, S. WOLFE and S. ARMENTROUT, *J. exp. Med.* **120**, 161 (1964).
40. D. S. FISCHER, *Biochem. Pharmac.* **14**, 971 (1965).
41. S. S. CARDOSO and J. JAFFE, *Biochem. Pharmac.* **8**, 252 (1961).
42. T. F. O'BRIEN and A. H. COONS, *J. exp. Med.* **117**, 1063 (1960).
43. A. D. WELCH and W. H. PRUSOFF, *Cancer Chemother. Rep.* **6**, 29 (1960).
44. W. H. PRUSOFF, *Cancer Res.* **23**, 1246 (1963).
45. W. H. PRUSOFF, J. J. JAFFE and H. GUNTHER, *Biochem. Pharmac.* **3**, 110 (1960).
46. C. HEIDELBERGER and F. J. ANSFIELD, *Cancer Res.* **23**, 1226 (1963).