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S-ADENOSYLHOMOCYSTEINE HYDROLASE ACTIVITY, DEOXYADENOSINE TRIPHOSPHATE ACCUMULATION, AND COMPETENCE OF THYMOCYTE AND SPLEEN LEUCOCYTE RESPONSE TO MITOGENS IN COFORMYCIN-TREATED MICE*

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Abstract—The inhibition of *S*-adenosylhomocysteine hydrolase and accumulation of dATP in thymus, spleen and other tissues of mice treated with the adenosine deaminase inhibitor coformycin were studied in parallel with the competence of thymocytes and spleen leucocytes to undergo mitogen-induced transformation. Newborn mice were lethally sensitive to daily injections of coformycin, 0.2 mg/kg, whereas adult mice were not. Developmental profiles of enzymes of nucleoside metabolism showed adenosine deaminase and purine nucleoside phosphorylase to be greatest in thymus around day 20 and to decrease for animals older than 60 days. The most notable change was a 3-fold increase in spleen leucocyte adenosine deaminase activity between days 10 and 30. Adenosine deaminase activity was reduced to less than 10% of normal in tissues of newborns treated with coformycin for 12–14 days. *S*-Adenosylhomocysteine hydrolase was also reduced to 5–40% of normal with no evidence of tissue specificity. Both thymocytes and erythrocytes of coformycin-treated mice accumulated dATP whereas spleen leucocytes did not. For coformycin-treated mice, spleen leucocyte and thymocyte response to concanavalin A (Con A) was reduced to 20 and 60% of controls respectively. Coformycin, 3.6 μM, also potentiated the *in vitro* toxicity of adenosine and deoxyadenosine toward thymocytes or spleen leucocytes by approximately an order of magnitude. Our observations are consistent with dATP being involved in impairment of thymocyte responsiveness; however, it appears unlikely that either dATP elevation or *S*-adenosylhomocysteine hydrolase inhibition is involved in the mechanism of impairment of spleen leucocyte response by coformycin.

The inherited human deficiency of adenosine deaminase (ADA‡, adenosine aminohydrolase, EC 3.5.4.4) has been found to be associated with severe combined immunodeficiency disease [1, 2]. This association has prompted investigation of the cytotoxic and immunosuppressive potential of the natural substrates and their analogs and of inhibitors of adenosine deaminase. These studies have been facilitated by the availability of potent and specific inhibitors of adenosine deaminase, namely coformycin [3, 4], deoxycoformycin [5], and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) [6]. The *in vivo* model of adenosine deaminase inhibition most well characterized is one involving the constant infusion of deoxycoformycin in mice [7]. Deoxycoformycin administration is highly lymphotoxic [8–11], produces an impairment of both B and T cell function [10–12], and yet does not eliminate the stem cell capacity of bone marrow [13].

Our objective has been to evaluate some of the biochemical events associated with immunosuppression which may be secondary to inhibition of adenosine deaminase activity. The accumulation of dATP and the inhibition of *S*-adenosylhomocysteine hydrolase (EC 3.3.1.1) were examined in coformycin-treated mice and correlated with the competence of spleen leucocytes and thymocytes to undergo mitogen-induced transformation. Among the mechanisms which may be involved in lymphotoxicity associated with a deficiency of adenosine deaminase is a disruption of DNA synthesis caused by an imbalance in deoxyribonucleotide concentrations. An accumulation of dATP has been observed in erythrocytes of adenosine deaminase deficient patients [14, 15], accompanied by the excretion of deoxyadenosine [16]. We have, therefore, examined dATP levels in coformycin-treated mice. In addition, *S*-adenosylhomocysteine hydrolase, which is inactivated by deoxyadenosine [17], has been found to be reduced in erythrocytes of adenosine deaminase deficient patients [18]. Inhibition of *S*-adenosylhomocysteine hydrolase may cause an accumulation of *S*-adenosylhomocysteine, thereby inhibiting methylation reactions [19]. No prior studies have examined the relationship between adenosine deaminase and *S*-adenosylhomocysteine hydrolase

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‡ Abbreviations: ADA, adenosine deaminase; EHNA, erythro-9-(2-hydroxy-3-nonyl) adenine; Con A, concanavalin A; RPMI 1640, Roswell Park Memorial Institute Medium 1640; PNP, purine nucleoside phosphorylase; and HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

activities both during and after administration of an adenosine deaminase inhibitor. In addition, the apparent sensitivity of newborn mice to coformycin administration caused us to examine the ontogeny of the activities of purine nucleoside metabolism in spleen leucocytes and thymocytes.

MATERIALS AND METHODS

The following radiochemicals were obtained from the Amersham-Searle Corp. (Oakville, Ontario, Canada): [8-^{14}C]adenosine, 59 Ci/mole; [G-^{3}H]deoxyadenosine, 21 Ci/mmole; [8-^{3}H]deoxyguanosine, 2.3 Ci/mmole; [Me-^{3}H]thymidine, 2.0 Ci/mmole; and [8-^{14}C]inosine, 52.5 Ci/mole. Coformycin was a gift from Dr. H. Umezama, Institute of Microbial Chemistry, Japan. Con A was obtained from Calbiochem (San Diego, CA, U.S.A.) and pokeweed mitogen was obtained from GIBCO (Montreal, Canada).

Assays. Adenosine deaminase and purine nucleoside phosphorylase (PNP) were assayed as previously described with radiolabeled substrates, adenosine (500 μM) and inosine (800 μM) [20]. Adenosine, deoxyadenosine and deoxyguanosine kinase were assayed under conditions previously established [21]. *S*-Adenosylhomocysteine hydrolase was assayed as described by Hershfield [17].

Coformycin administration and tissue preparation. Swiss–Webster mice (University of Calgary colony) were maintained on Purina rat chow in cages containing autoclaved bedding and filter caps (Lab Products Inc., Rochelle Park, NJ, U.S.A.). Coformycin (0.2 mg/kg) or 0.9% sodium chloride, was injected daily intraperitoneally. Heart, liver, brain and kidney homogenates were prepared from decapitated mice. Tissues were homogenized in 2 vol. sodium HEPES, 25 mM, pH 7.2, at 0° by four 10-sec pulses with a Polytron. Homogenates were centrifuged at 7200 *g* for 30 min, and supernatant fractions were stored at −70° for analysis. Erythrocytes were prepared from heparanized blood by washing three times in sodium HEPES, 25 mM, pH 7.2, containing NaCl, 154 mM, and resuspension in sodium HEPES, 25 mM, pH 7.2, for lysis. Thymus and spleen were removed aseptically, minced and allowed to settle, suspended, in minimum essential Eagle's medium containing HEPES, 25 mM, 10% fetal calf serum, and antibiotics. Cells were collected and washed once in Tris-buffered ammonium chloride, pH 7.2. Erythrocytes, thymocytes and spleen leucocytes were lysed by three freeze–thaw cycles in liquid nitrogen, followed by centrifugation for 30 min at 7200 *g* and recovery of supernatant fractions for assay or storage at −70°.

Mitogen response. Thymocytes or spleen leucocytes were suspended in Roswell Park Meorial Institute Medium 1640 (RPMI 1640) containing 10% fetal calf serum, 1% sodium pyruvate, antibiotics and mercaptoethanol (10 μM). Cells were cultured in 10% CO_2 in round bottom microtitre plates at 2×10^6 cells/ml, 0.250 ml/well, with and without mitogens for 24, 48 and 72 hr. Optimal mitogen concentrations were: Con A, 2.5 μg/ml for thymocytes or 1 μg/ml for spleen leucocytes; pokeweed mitogen, 10 μl/ml for spleen leucocytes. Cells were incubated for 3 hr with [^{3}H]thymidine, 2 μCi/ml, after which they were transferred to glass filters, successively washed with 0.85% saline, 5% trichloroacetic acid, and ethanol prior to liquid scintillation counting.

Nucleotide analyses. Cellular nucleotide concentrations were measured on a Spectra Physics 8000B equipped with a Whatman Partisil 1025 SAX column (Nutley, NJ). Separation was achieved using a two-component gradient: buffer A, 5 mM KH_2PO_4, pH 4.0; and buffer B, 1 M KH_2PO_4, pH 4.0. The gradient was 100 to 90% A from 0 to 12 min, 90 to 50% A from 12 to 47 min, constant at 50% A from 47 to 70 min, and 50% B until 70 min with a flow rate of 1.5 ml/min at 30°.

RESULTS AND DISCUSSION

As the immune dysfunction associated with human adenosine deaminase deficiency appears to increase in severity with age, we examined long-term coformycin administration in newborn mice. Daily administration of the tight binding adenosine deaminase inhibitor coformycin [22] could be tolerated by adult mice for at least 30 days at a dose of 0.2 mg/per kg/per day. The corresponding treatment of newborns caused many animals to die between days 7 and 10, though their weight gain was usually normal up to 1 day before death. Although the cause of death was not ascertained, the use of autoclaved bedding and filter caps on cages increased the survival of coformycin-treated newborns to essentially that of untreated controls.

In view of the sensitivity of newborn mice to daily injections of coformycin, we examined the developmental patterns of the principal activities of purine nucleoside metabolism. For thymocytes there were transient increases in adenosine deaminase, purine nucleoside phosphorylase, deoxyadenosine kinase and deoxyguanosine kinase at days 20–25 (Fig. 1).

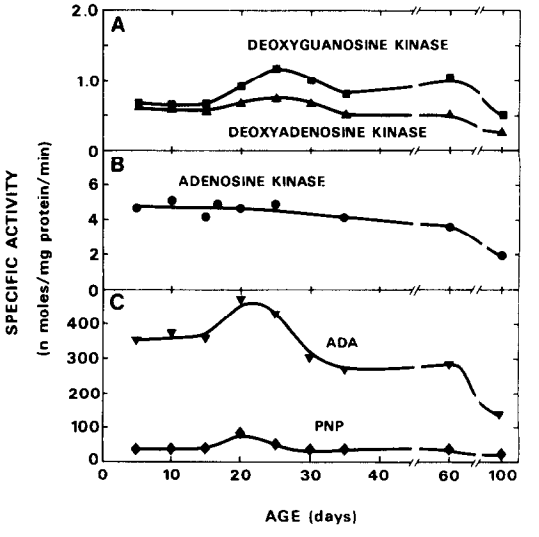

Fig. 1. Developmental changes in activities of purine nucleoside metabolism in mouse thymocytes. Each point represents the average of duplicate determinations for at least two animals.

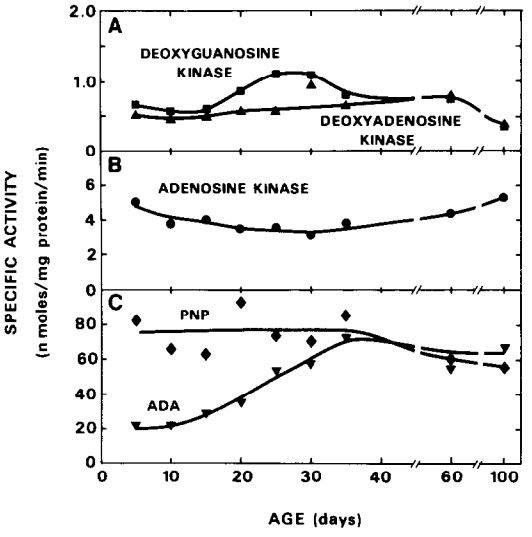

Fig. 2. Developmental changes in activities of purine nucleoside metabolism in spleen leucocytes. Each point represents the average of duplicate determinations for at least two animals.

Adenosine kinase showed little variation, but all thymus activities declined for animals older than 60 days which may reflect the general atrophy this organ undergoes. The cell mediated lympholysis response of thymocytes also begins to decline after 45 days [23]. The pattern of activities for spleen leucocytes is shown in Fig. 2. A transient increase in deoxyguanosine kinase and possibly deoxyadenosine kinase was observed between days 20 and 30, similar to the findings for thymocytes. Although weaning occurs around day 20, the increases in stomach and intestinal adenosine deaminase which also occur at this time in the mouse appear to be inherent rather than the result of dietary change [24]. The most remarkable change was a 3-fold increase in the specific activity of adenosine deaminase between days 10 and 35, whereas purine nucleoside phosphorylase and adenosine kinase remained relatively constant. Of corresponding temporal interest are observations indicating that the mixed leucocyte response of spleen cells is greater for mice of 28 days or older than for younger mice [23].

The inhibition by coformycin administration of adenosine deaminase activity for various tissues is given in Table 1. Treatment included daily injections of pregnant dams throughout gestation, 20 days, followed by cessation or continuation of treatment of the newborn for up to 21 days. Adenosine deaminase activity in treated mice was less than 10% of controls at 12–14 days continual treatment. Following 20–21 days sustained injection of coformycin, there was some evidence of adenosine deaminase becoming refractory to coformycin treatment (Table 1). The enzyme might become less sensitive to coformycin inhibition or protected by increased concentrations of adenosine and deoxyadenosine. Studies of 2-deoxycoformycin infusion suggest that such residual adenosine deaminase may be less sensitive to inhibition than the activity in previously untreated animals [7, 11]. There was no apparent difference between 20 and 21-day-old mice treated both prenatally and postnatally as compared to those treated postnatally. Cessation of coformycin treatment at birth resulted in partial recovery of adenosine deaminase activity by days 12–14, and several tissues including spleen leucocytes and thymocytes had greater than control activity at days 20–21. Perhaps related to these observations are findings that

Table 1. Adenosine deaminase activity in coformycin-treated mice*

Tissue	Controls [nmoles/(min·mg protein)]	Coformycin regimen (0.2 mg·kg^{-1}·day^{-1}) Prenatal and postnatal	Postnatal only (% of non-treated)	Prenatal only
(A) 12, 13, 14 days				
Brain	7.84 ± 0.22	8 ± 4 (3)	<2 (2)	25 ± 20 (3)
Heart	3.3 ± 0.3	24 ± 21 (4)	<2 (2)	60 ± 57 (3)
Kidney	16.6 ± 5.3	11 ± 6 (4)	4 (2)	67 ± 56 (3)
Liver	10.4 ± 7.1	13 ± 6 (4)	3 (2)	35 ± 33 (3)
Erythrocyte	1.93 ± 0.20	10 ± 5 (3)	<2 (2)	65 ± 51 (3)
Spleen leucocyte	18 ± 10	17 ± 6 (2)	ND†	100 ± 36 (2)
Thymocyte	192 ± 52	24 ± 1 (2)	9 (2)	85 ± 2 (2)
(B) 20, 21 days				
Brain	7.82 ± 0.30	9	7 ± 3 (2)	36 ± 2 (2)
Heart	4.3 ± 2.3	16 ± 3 (3)	16 ± 5 (4)	133 ± 28 (2)
Kidney	25 ± 12	10 ± 8 (3)	13 ± 5 (4)	ND
Liver	15 ± 10	16 ± 3 (3)	17 ± 4 (4)	116 ± 33 (2)
Erythrocyte	2.02 ± 0.19	10 ± 1 (3)	17 ± 6 (3)	74 ± 4 (2)
Spleen leucocyte	22.8 ± 5.2	30 ± 20 (3)	32 ± 5 (4)	164 ± 3 (2)
Thymocyte	249 ± 66	32 ± 20 (3)	35 ± 16 (4)	170 ± 31 (2)

* Results are expressed as specific activities for non-treated mice and as percentages of non-treated for coformycin-injected mice. Prenatal treatment involved injection of pregnant dams throughout gestation after timed matings. Animals were injected daily with a single dose of coformycin between 1:00 p.m. and 3:00 p.m. and were killed between 9:00 a.m. and 10:00 a.m. on the days indicated. Values are expressed as means ± S.D. (N).

† Not determined.

Table 2. *S*-Adenosylhomocysteine hydrolase activity in coformycin-treated mice*

Tissue	Controls [nmoles/(min · mg protein)]	Coformycin regimen (0.2 mg · kg^{-1} · day^{-1}) Prenatal and postnatal	Postnatal only (% of non-treated)	Prenatal only
(A) 12, 13, 14 days				
Brain	2.2 ± 1.2	21 ± 15 (3)	7 ± 2 (2)	13 ± 9 (3)
Heart	1.33 ± 0.17	40 ± 30 (4)	11	22 ± 12 (3)
Kidney	5.3 ± 13	52 ± 28 (4)	34 ± 2 (2)	39 ± 25 (3)
Liver	21 ± 10	37 ± 19 (4)	ND†	46
Erythrocyte	0.56 ± 0.30	10 ± 12 (3)	3 ± 1 (2)	38 ± 19 (3)
Spleen leucocyte	1.78 ± 0.52	32 ± 2 (2)	17 ± 3 (2)	45 ± 2 (2)
Thymocyte	3.14 ± 0.53	87 ± 20 (2)	26 ± 2 (2)	28 ± 5 (2)
(B) 20, 21 days				
Brain	1.54 ± 0.03	70	76 ± 13 (2)	93 ± 1 (2)
Heart	3.2 ± 1.9	40 ± 19 (3)	39 ± 20 (4)	29 ± 4 (2)
Kidney	8.0 ± 0.5	93 ± 8 (3)	98 ± 12 (3)	119 ± 2 (2)
Liver	66 ± 12	67 ± 6 (3)	98 ± 19 (3)	96 ± 33 (2)
Erythrocyte	0.69 ± 0.28	47 ± 24 (3)	59 ± 44 (3)	59 ± 14 (2)
Spleen leucocyte	4.91 ± 0.27	36 ± 42 (3)	47 ± 21 (4)	115 ± 4 (2)
Thymocyte	3.96 ± 0.61	93 ± 12 (3)	107 ± 11 (4)	90 ± 5 (2)

* Results are expressed as specific activities for non-treated mice and as percentages of non-treated for coformycin-injected mice. Coformycin treatment and sacrifice schedule were as described in the legend to Table 1. Values are expressed as means ± S.D. (N).

† Not determined.

infusion of EHNA at low doses promotes an increase of adenosine deaminase activity in lung, stomach and liver [7]. The kinetics of recovery of adenosine deaminase activity following cessation of coformycin treatment (Table 1) are similar to deoxycoformycin treatment [25] for blood, but differ for liver and spleen, perhaps reflecting the younger age of animals used in the present study. Brain appeared to be the slowest tissue to recover, having only 35% of control activity at 20–21 days.

There is evidence for the deficiency of adenosine deaminase causing a secondary inhibition of *S*-adenosylhomocysteine hydrolase [18], apparently mediated by suicide inactivation of the hydrolase by deoxyadenosine [17]. *S*-Adenosylhomocysteine hydrolase activity was also reduced in tissues of coformycin-treated mice (Table 2). Erythrocytes of treated mice had approximately 10% of control activity at 12–14 days. There appears to be a general correlation between the degree of inhibition of adenosine deaminase and the reduction in *S*-adenosylhomocysteine hydrolase activity. The ratio of the relative activities of adenosine deaminase to *S*-adenosylhomocysteine hydrolase was 0.34 ± 0.23 for all tissues in treated animals and 1.9 ± 1.1 after cessation of treatment (from Tables 1 and 2); these ratios are significantly different ($P < 0.01$). Thus, in actively treated animals the reduction in adenosine deaminase activity was greater than that of *S*-adenosylhomocysteine hydrolase. In mice no longer treated which had been born to dams treated with coformycin throughout gestation, adenosine deaminase activity was greater than *S*-adenosylhomocysteine hydrolase at the times examined, indicating a difference in the rates of recovery of these activities. There was no apparent advantage in treating

Table 3. Nucleotide concentration in coformycin-treated mice*

Coformycin (mg · kg^{-1} · day^{-1})	ATP	dATP (pmoles/10^6 cells)	GTP
Thymocyte			
0	85.9 ± 4.5 (6)	<0.5 (5)	13.3 ± 4.5 (5)
0.2	85.2 ± 5.1 (6)	2.7 ± 4.1 (5)	11.4 ± 1.7 (6)
Spleen leucocyte			
0	86.7 ± 6.9 (5)	<0.5 (5)	13.2 ± 6.9 (5)
0.2	85.6 ± 6.9 (5)	<0.5 (5)	12.5 ± 5.6 (5)
Erythrocyte			
0	93.2 ± 2.9 (5)	<0.5 (5)	5.9 ± 1.8 (5)
0.2	64 ± 11 (5)	18.0 ± 16 (6)	14 ± 6 (5)

* Coformycin treatment and sacrifice schedule were as described in the legend to Table 1. Results are the means ± 1 S.D. of the number of mice shown in brackets killed at 12–21 days of age. The ATP:ADP:AMP ratio was typically 10:1:<0.5 for thymocyte and spleen leucocyte extracts and 16:1:<0.5 for erythrocyte extracts.

dams throughout pregnancy as a means of lowering either adenosine deaminase of *S*-adenosylhomocysteine hydrolase in the newborn. We also found no evidence of the secondary inhibition of *S*-adenosylhomocysteine hydrolase being tissue specific which may argue against a specific impairment of the lymphoid system. The recovery of *S*-adenosylhomocysteine hydrolase activity, however, was less than that for adenosine deaminase, and it may be that under conditions of more complete inhibition of adenosine deaminase there may be some tissue specific effect on *S*-adenosylhomocysteine hydrolase activity and methylation reactions.

The presence of dATP in erythrocytes of adenosine deaminase deficient patients [14, 15] and the potential role of a dATP imbalance causing lymphotoxicity caused us to examine the nucleotide profile of coformycin-treated mice. Erythrocytes from coformycin-treated mice were found to have an elevated dATP pool accompanied by a one-third reduction in ATP and a 2-fold increase in GTP (Table 3). Deoxycoformycin treatment, either alone or in combination with deoxyadenosine, has been reported to produce hemolysis in mice also accompanied by an increase in erythrocyte dATP and a decrease in ATP [26, 27]. Thymocytes showed evidence of dATP accumulation in coformycin-treated mice without changes in ATP or GTP pools. Spleen leucocytes did not show any evidence of altered nucleotide levels with coformycin treatment. Small changes in the dATP pool may not have been observed by the liquid chromatographic analysis, which has a limit of detectability of approximately 0.5 pmole/10^6 cells.

The nucleotide findings are similar to those of a

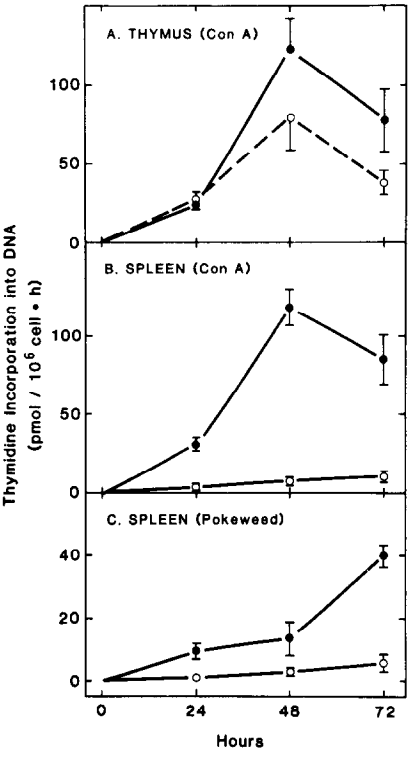

Fig. 3. Effect of coformycin treatment on mitogen responsiveness. The response of 12-day-old control (●) and coformycin-treated (0.2 mg/per kg/per day) (○) mice was examined for: (A) thymocytes with Con A; (B) spleen leucocytes with Con A; and (C) spleen leucocytes with pokeweed mitogen. Results are the means ± S.D. for three animals in each group.

Table 4. [^{3}H]Thymidine incorporation into DNA as a measure of thymocyte and spleen leucocyte response to mitogens for coformycin-treated mice*

Cell (mitogen)	Coformycin regimen (mg·kg^{-1}·day^{-1})	pmoles/(10^6 cells·hr)	% of control
(A) 12–15 days			
Thymocyte	0	92 ± 46 (6)	
(Con A)	0.2	54 ± 29† (8)	62 ± 33 (8)
Spleen leucocyte	0	95 ± 27 (7)	
(Con A)	0.2	15 ± 21‡ (8)	19 ± 28 (8)
Spleen leucocyte	0	41 ± 5 (5)	
(pokeweed)	0.2	19 ± 26† (8)	27 ± 30 (8)
(B) 20–22 days			
Thymocyte	0	95 ± 47 (5)	
(Con A)	0.2	86 ± 95 (6)	55 ± 56 (6)
Spleen leucocyte	0	114 ± 68 (5)	
(Con A)	0.2	47 ± 48† (5)	37 ± 36 (5)
Spleen leucocyte	0	62 ± 43 (5)	
(pokeweed)	0.2	39 ± 43 (6)	47 ± 29 (6)

* Cells were cultured in the absence and presence of mitogen as described in Materials and Methods and pulsed with [^{3}H]thymidine. Results are the averages ± 1 S.D. having subtracted the background of cultures in the absence of mitogen. Mitogen response for coformycin-treated mice was also expressed as a percentage of controls conducted on the same day.

† Significantly different from respective control ($P < 0.1$).

‡ Significantly different from respective control ($P < 0.01$).

previous study in which deoxycoformycin administration produced an increase in blood and thymus dATP, but none in spleen, liver, intestinal mucosa, bone marrow and skin [28]. Thymocytes and spleen leucocytes had comparable levels of deoxyadenosine kinase (Figs. 1 and 2), indicating a capacity to phosphorylate deoxyadenosine, but only thymocytes accumulated dATP in coformycin-treated animals (Table 3). The potential of a cell to accumulate dATP when adenosine deaminase is blocked may also be dependent on the relative activities of deoxyadenylate kinase and 5′-nucleotidase. The human T cell lines which have low 5′-nucleotidase activity have a greater capacity to synthesize dATP from deoxyadenosine than B cell lines which have higher 5′-nucleotidase activity [29, 30]. Few studies have addressed the question of the source of deoxyadenosine used for dATP formation *in vivo*; however, there is now indirect evidence for normoblast nuclei produced during erythropoiesis being a major source of deoxyadenosine [31].

In parallel with observations of enzyme activities and dATP levels, we also wished to assess the competence of thymocytes and spleen leucocytes to respond to mitogens for coformycin-treated mice. The response of spleen leucocytes to Con A was depressed significantly at both 12–15 and 20–22 days for mice treated from birth with coformycin (Table 4). A significant reduction in thymocyte response to Con A and spleen leucocytes to pokeweed mitogen was also observed at days 12–15. This difference may reflect the greater degree of inhibition of adenosine deaminase activity for the younger mice (Table 1). The time profile of mitogen response for treated and non-treated mice at 12 days is present in Fig. 3. The depression in splenocyte response to Con A is evident throughout the 72-hr course and for pokeweed mitogen at 72 hr. Consistent with the data of Table 4 is the partial depression of the thymocyte response to Con A for coformycin-treated mice. Although the total peripheral white cell count was essentially unchanged by coformycin treatment, the differential count revealed a reduction in lymphocytes from 65.2 ± 3.3 to $45.6 \pm 6.0\%$ in coformycin-treated mice at days 13–14. Neither spleen leucocytes nor thymocytes were grossly deficient in *S*-adenosylhomocysteine hydrolase activity in our studies (Table 2). These observations suggest that the depression in thymocyte responsiveness may be related to the elevation of dATP, whereas the basis for the depression in spleen leucocyte responsiveness is not yet evident.

The *in vitro* sensitivity of the thymocyte and spleen leucocyte response to adenosine and deoxyadenosine in the absence and presence of coformycin was examined. Thymocytes were approximately two orders of magnitude more sensitive to adenosine and deoxyadenosine in the presence of coformycin such that 10 μM nucleoside severely impaired mitogen responsiveness (Fig. 4). Spleen leucocyte sensitivity to the nucleosides was also attenuated by coformycin, and 10 μM adenosine effectively blocked the response

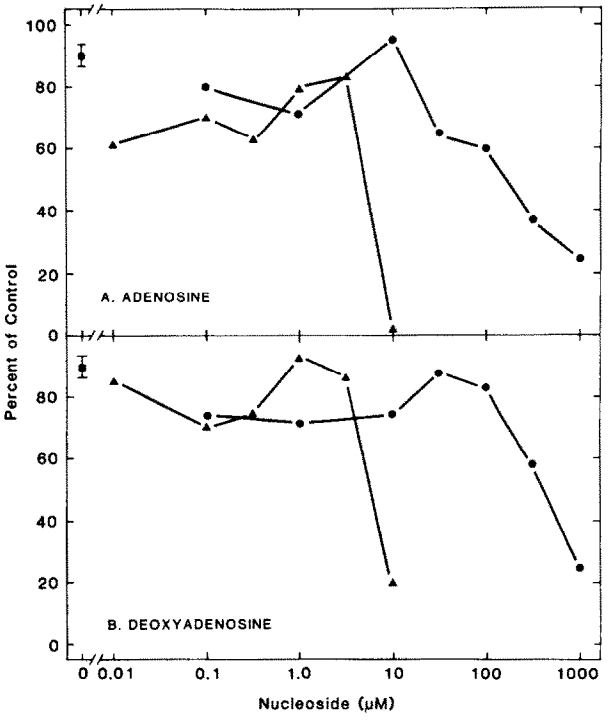

Fig. 4. *In vitro* effect of adenosine, deoxyadenosine and coformycin on thymocyte response to Con A. Thymocytes were cultured in the presence of Con A and pulsed with [^{3}H]thymidine at 48 hr as described in Materials and Methods. Incorporation into acid-insoluble material for control cultures was 95 pmoles per 10^6 cells per hr. Cells were cultured in the absence (●) or presence (▲) of coformycin (3.6 μM) or with coformycin alone (■).

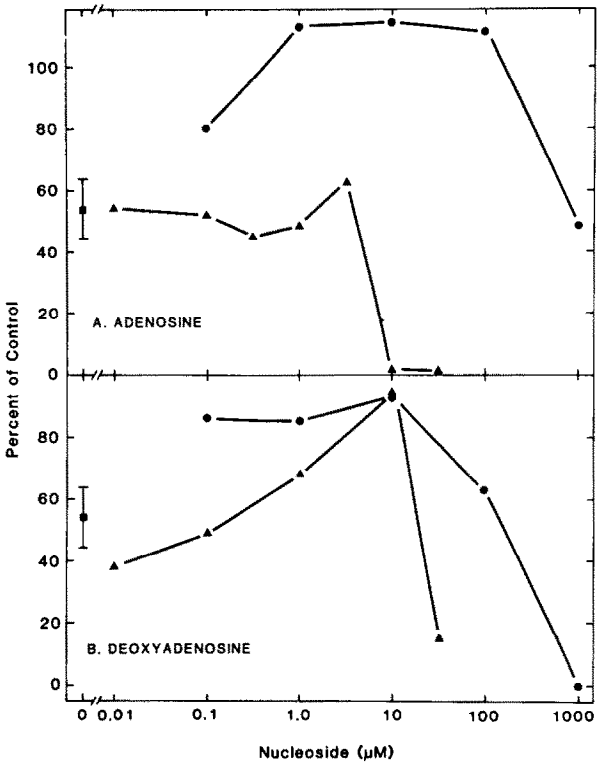

Fig. 5. *In vitro* effect of adenosine, deoxyadenosine and coformycin on spleen leucocyte response to Con A. Spleen leucocytes were cultured in the presence of Con A and pulsed with [^{3}H]thymidine at 48 hr as described in Materials and Methods. Incorporation into acid-insoluble material for control cultures was 115 pmoles per 10^6 cells per hr. Cells were cultured in the absence (●) or presence (▲) of coformycin (3.6 μM) or with coformycin alone (■).

whereas 10 μM deoxyadenosine had little effect (Fig. 5). Coformycin alone (3.6 μM) markedly reduced the mitogen response of spleen leucocytes to 55% of control but had little effect on thymocyte response (95% of control). A human lymphocyte precursor population has also been shown to be sensitive to a comparable concentration of coformycin [32]. Thus in the present study of coformycin administration, impairment of mitogen responsiveness appears to be correlated with the degree of completeness of adenosine deaminase inhibition and the *in vitro* sensitivity of the tissue to coformycin.

In summary, daily coformycin administration markedly reduced adenosine deaminase and to a lesser extent *S*-adenosylhomocysteine hydrolase activity in the newborn mouse. Coformycin administration was found to produce an accumulation of dATP in thymocytes and erythrocytes but not in spleen leucocytes. Spleen leucocyte response to mitogens was more severely impaired than that of thymocytes, though adenosine deaminase was reduced to the same extent in both cell populations. Our findings are consistent with accumulated levels of dATP being responsible for coformycin impairment of the thymocyte response. Although the most severe depression of mitogen responsiveness was found in spleen leucocytes of coformycin-treated mice, we obtained no evidence for dATP accumulation in these cells. Spleen leucocytes were found to be sensitive to coformycin alone *in vitro*, and the mechanism of spleen leucocyte sensitivity toward coformycin requires further investigation. Other effects of adenosine deaminase inhibitors on cellular metabolism include inhibition of adenylate deaminase [33–36] and adenosine kinase [37, 38]; however the *in vivo* significance of these effects has not yet been evaluated. Further *in vivo* studies with adenosine deaminase inhibitors may characterize the biochemical relationship between adenosine deaminase deficiency and immunodeficiency.

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