high as 10^8 years is also consistent with the range of acceptable D values. Because our data do not preclude $D = 0$ for the Australian response, we cannot produce a lower bound on the time constant.

To sharpen the estimate of τ, it is necessary to obtain more accurate response functions and to calculate them for rugged regions more homogeneous in geological age; this last requirement runs counter to the one that a relatively large area be used to gain reliable estimates at the long wavelengths. However, we plan to do this for the North American data set, separating off the Appalachian Mountains and including the Canadian Rocky Mountains. Subdivision of the Australia data set seems less promising, owing to the relatively poor signal-to-noise ratio. It is unlikely that very precise estimates of τ will be possible with this approach because the effects of orogenic history and subsequent erosion make the behavior of the topographic load a very complex function of time and space.

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L-Dopa Methyl Ester: Prolongation of Survival of Neuroblastoma-Bearing Mice After Treatment

Abstract. *L-Dopa has been shown to demonstrate enhanced toxicity toward melanoma cells in vitro. Since melanocytes arise from the neural crest embryologically, the effect of L-dopa methyl ester, a soluble analog, on the murine C1300 neuroblastoma was studied. There was significant antitumor activity against the neuroblastoma, which was enhanced by combination with a dopa decarboxylase inhibitor, Ro4-4602. In vitro studies suggested inhibition of DNA synthesis as the principal site of action. A mechanism involving sulfhydryl compound scavenging is postulated.*

L-Dopa has been shown to possess enhanced toxicity toward melanoma cells both in vitro and in vivo (*1*). Since melanocytes arise embryologically from the neural crest (*2*) and share many attributes with neural tissue, it was of interest to examine the antitumor activity against the mouse C1300 neuroblastoma model. Finkelstein *et al.* (*3*) proposed the use of this system for the evaluation of potential chemotherapeutic agents for human disease. Since the cytotoxic concentration determined from the previous study (*1*) was approximately 6.0 mM, we elected to study the far more soluble derivative, L-dopa methyl ester.

Table 1 shows the results of treatment of tumor-bearing mice with L-dopa methyl ester. At 600 mg/kg, the maximum tolerated dose, there was a significant therapeutic effect ($P < .01$) with a 30 percent increase in median survival. Pretreatment with the dopa decarboxylase inhibitor Ro4-4602 [N^1-(DL-seryl)-N^2-(2,3,4-trihydroxybenzylhydrazine) (*4*, *5*) resulted in an increase in the maximum tolerated dose to 1000 mg/kg and a concomitant enhancement of the therapeutic response. L-Dopa itself did not demonstrate antitumor activity, although because of its insolubility equivalent doses could not be administered to mice.

Table 2 describes the effects of L-dopa and L-dopa methyl ester on macromolecule biosynthesis in the N2A clone of neuroblastoma in vitro. A marked inhibition of thymidine incorporation was observed, with somewhat lesser effects on

Table 1. Effect of L-dopa methyl ester on survival of C1300 neuroblastoma–bearing mice. L-Dopa and Ro4-4602 were gifts from Hoffmann–LaRoche & Co., Nutley, New Jersey; L-Dopa methyl ester was obtained from Sigma Chemical Co., St. Louis, Missouri; C1300 neuroblastoma was obtained from the Jackson Laboratory, Bar Harbor, Maine. Single cell suspensions of tumor cells were prepared in saline by repeated mincing, and 1×10^6 trypan blue–excluding cells were injected intraperitoneally into male A/J mice 6 to 8 weeks of age (ten animals per group). The assay procedures are identical to standard National Cancer Institute protocols (*11*), with treatment beginning on the first day after tumor inoculation and continuing daily for 12 days. Agents were given intraperitoneally in saline. Ro4-4602 was given 1 hour before treatment.

Treatment	Dose (mg/kg)	Survival time (days)*		ILS† (%)
		Range	Median	
Control		15–18	16.5	
L-Dopa	500	4–20	19.0	15
methyl	600	4–29	21.5	30‡
ester				
Ro4-4602	200	14–19	15.5	−6
L-Dopa	800+	9–36	24.0	45§
methyl	200			
ester +	1000+	12–36	33.0	100§
Ro4-4602	200			

*Measured from the day after tumor implantation. †Increase in life-span (ILS) was calculated as $100(t/c - 1)$, where t is the median survival time of the treatment group and c is the median survival time of the control group. ‡Significant at $P < .01$. §Significant at $P < .001$.

Table 2. Effect of L-dopa methyl ester on macromolecule biosynthesis in C1300 neuroblastoma in vitro. The C1300 neuroblastoma cells were a gift from L. Green, Harvard Medical School, and are designated clone N2A. The cells were grown in McCoy's 5A medium supplemented with 15 percent fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were plated in Linbro multiwell tissue culture trays. Exponentially growing cultures were aspirated and washed, and 1 ml of serum-free medium containing 2 μCi/ml of either [^{3}H]thymidine (specific activity, 2 Ci/mmole), [5-^{3}H]uridine (specific activity, 25 Ci/mmole), or [^{3}H]leucine (specific activity, 41 Ci/mmole) (New England Nuclear Co.) and drugs was added. After 60 minutes at 37°C the medium was removed, cells were washed once with saline, and 1 ml of 10 percent trichloroacetic acid was added. The precipitate was washed three times with saline and 0.5 ml of 1N KOH was added. After digestion at 37°C for 4 hours, a portion was added to scintillation fluid and counted. Values are expressed as percentage inhibition compared to controls and represent means ± standard errors of the means for triplicate samples.

Concentration (mM)	Inhibition (%)		
	Thymidine	Uridine	Leucine
	L-Dopa		
0.5	17 ± 3	11 ± 3	9 ± 5
1.5	51 ± 5	26 ± 5	26 ± 4
3.0	79 ± 5	36 ± 7	39 ± 3
	L-Dopa methyl ester		
0.5	9 ± 5	9 ± 5	5 ± 3
1.5	88 ± 4	36 ± 6	5 ± 1
3.0	98 ± 3	37 ± 8	4 ± 1

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uridine and leucine incorporation. L-Dopa, although displaying a similar pattern, was significantly less effective than the ester.

Enhancement of antitumor activity by combination with the dopa decarboxylase inhibitor Ro4-4602 was most likely due to a decrease in the formation of dopamine and a corresponding increase in the plasma concentration of the amino acid (*6*). Animals that did not receive pretreatment were tremulous and hyperactive and generally died within 1 to 2 hours of administration of the ester. Pretreatment with Ro4-4602 completely prevented the development of these signs and permitted delivery of much higher doses.

The mechanism of action of L-dopa methyl ester is unknown. Experimental and human tumors have a known requirement for sulfhydryl supplementation (*7*). The capacity of L-dopa to form stable adducts with sulfhydryl-containing residues such as cysteine (leading to production of 5-cysteinyl dopa) is well known (*8*). We therefore propose a mechanism involving sulfhydryl compound scavenging, by which enzymes central to DNA synthesis may be inactivated (*9*). Since L-dopa methyl ester is an *o*-quinol, it must first be oxidized to the quinone form before it can react with sulfhydryl residues. Melanoma cells possess the enzyme tyrosinase, which is capable of catalyzing this reaction, while peroxidase activity has been demonstrated in mast cells, eosinophils, and neurons (*10*). Enzymes in the catecholamine pathway might also facilitate this conversion. Alternatively, L-dopa methyl ester, a catechol, may autoxidize, generating free radicals (*1*) that result in disruption of cellular metabolism preferentially within tumor cells.

L-Dopa methyl ester has consistently demonstrated a greater ability to inhibit thymidine incorporation than L-dopa itself. This effect is most likely the result of its greater solubility, which may permit more facile intracellular entry.

Finally, there is the intriguing possibility that the phenomenon described here may also be involved in other biological effects of L-dopa, as in the therapy of Parkinson's disease. Extensive experience with L-dopa as a therapeutic agent in man should permit an early evaluation of the potential therapeutic implications of these results.

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Rearing Regimen Producing Piglet Diarrhea (Rotavirus) and Its Relevance to Acute Infantile Diarrhea

Abstract. *Piglets were weaned when 1 day old and thus were denied further access to the antibodies supplied by their dam's milk. They were placed in a nursery in which contamination by the ubiquitous rotavirus steadily increased with continuous use causing a progressive increase in the incidence of vomiting, diarrhea, and death among the piglets. A similar syndrome involving an antigenically related rotavirus and analogous management practices occurs in human infants.*

Acute infantile gastroenteritis is prevalent and costly. In India, 1.4 million infants die annually from noncholera diarrhea, and such disease accounts for a substantial amount of the morbidity among infants in the industrial West (*1*, *2*). Enterobacteria, particularly enterotoxigenic *Escherichia coli*, have been etiological suspects in this morbidity (*3*).

Recently, a reovirus-like agent (rotavirus) has been associated with most of the cases of infantile gastroenteritis [for a review see (*1*)]. Morphologically similar rotaviruses have been implicated as the cause of diarrhea in piglets, calves, mice, foals, and lambs (*1*, *4*, *5*).

In spite of the widespread distribution of rotavirus, little is known of the factors that exacerbate the disease. In this report we examine the practices that inevitably lead to vomiting and diarrhea in piglets, and discuss the relevance of these practices in the care of infants.

Piglets are born agammaglobulinemic (*6*), and acquire passive immunity from their dam's colostrum. Colostrum-deprived piglets, or piglets weaned precociously (at less than 2 weeks of age), are difficult to rear unless they are farrowed in a sanitary (*7*), isolated delivery room and reared in an isolated nursery. By using an isolated nursery equipped with an automatic feeding device (Autosow), we reared piglets farrowed under sanitary conditions and found that they increased their weight by about 3.5 times by 2 weeks of age and developed no symptoms of diarrhea (*8*).

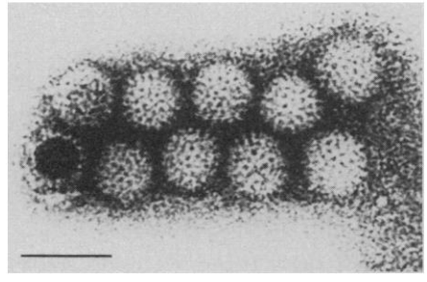

Fig. 1. Electronmicrograph of rotavirus in gut fluid from piglets with diarrhea. See (*6*) for details of preparation (scale bar, 100 nm).

Our interest in neonatal gastroenteritis resulted from a desire to develop a successful weaning regimen that could be adapted to existing farm conditions. The goal was to design a husbandry system that would decrease the high death rate of piglets reared on farms. About 20 percent of the piglets reared on farms die in the first week of life. Most of such deaths are due to the sow's inability to nurse adequately all the pigs in large litters.

Our plan was to wean the "extra" pigs in the large litters after they had nursed for approximately 1 day and had obtained passive immunity from their sow's colostrum (*6*). Day-old extra piglets would be taken from a well-managed, large, commercial farm to the isolated nursery containing the Autosow. According to this procedure, parturition would occur amid a farm environment surfeit with stale air and accumulated feces.

Over a period of 1½ months we reared a total of 93 piglets to 2 weeks of age in this manner. Approximately 70 percent of these piglets experienced vomiting

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