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*).

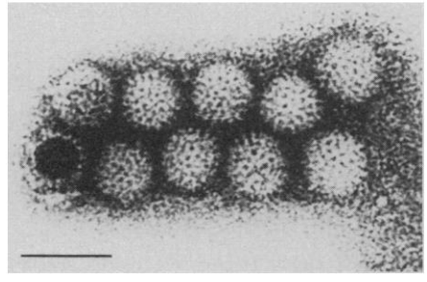

Fig. 1. Electronmicrograph of rotavirus in gut fluid from piglets with diarrhea. See (*6*) for details of preparation (scale bar, 100 nm).

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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and diarrhea after being in the nursery about 1 week. Forty-three piglets were treated orally with 25 ml of cow's colostrum three times a day for five successive days (*5*, *8*), and 7 percent of these died. Fifty of the pigs were untreated; 29 percent of these died. Weight gains were poor in that the surviving 2-week-old piglets were 2.5 times their birth weights instead of the expected three to four times.

We conducted the following experiment to determine whether the nursery could be used continuously if piglets were farrowed in a sanitary environment. Two sows were farrowed in the isolated delivery room about once every 10 days. Their progeny, after 1 day's nursing, were placed in the nursery for 2 weeks. In this way, there was an overlap of younger and older piglets in the nursery, with no opportunity to clean completely and fumigate the nursery between groups.

This procedure led to a repeatable pattern of disease development. To wit, the first groups of pigs were reared without incident and they gained about three to four times their birth weight by 2 weeks of age. However, at about the time the eighth litter of pigs was taken to the nursery (approximately 70 pigs and after 5 weeks of continuous operation), a mild, transient diarrhea usually lasting less than 1 day was noticed in the older pigs. As additional litters of pigs were moved in (after 5 to 7 weeks of continuous operation), the diarrhea became more pronounced, of longer duration, and affected more pigs. Often it was accompanied initially by some vomiting, but the piglets did not become dehydrated, and there were no deaths.

Between 7 and 9 weeks of continuous operation of the nursery, all the entering piglets started vomiting at 3 to 5 days of age. Then they underwent dehydration and at least 50 percent of these piglets died. Surviving piglets gained about 2.5 times their birth weight by 2 weeks. The problem was now severe.

After 9 weeks of continuous operation of the nursery, the piglets started vomiting 2 to 4 days after entry and were dead by 10 days of age. Bacteria-free infectious material (*5*) containing large numbers of rotavirus ($\sim 10^9$/ml), was obtained from diarrheal fluid (Fig. 1). At this time, all pigs were removed from the nursery and the area was thoroughly cleaned, fumigated, and left unoccupied for about 1 week. The next group of pigs that went into the system did exceptionally well. This continued until about the eighth litter, at which time the cycle of increasingly severe diarrhea repeated itself. Over a period of 2 years this cycle was observed six times. Sometimes the cycle was shorter, but it was always one of increasing severity. From time to time, litters were divided and half of the piglets were placed in a recently fumigated isolation room. These pigs developed no diarrhea, whereas their littermates in the nursery expressed disease to a degree that corresponded to the length of continuous use of the nursery.

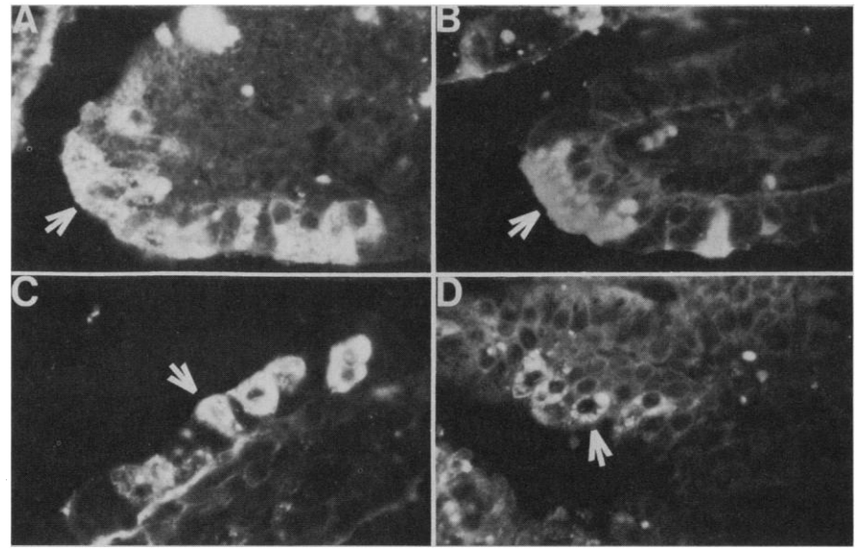

Fig. 2. Jejunum from colostrum-deprived piglet experimentally infected with rotavirus (*6*). (A) Jejunum reacted with serum from a 1-day-old nursing pig, then with fluorescent antibody to porcine gamma globulin. (B) Jejunum reacted with cows' colostral whey diluted 1 : 32, then with fluorescent antibody to bovine gamma globulin. (C) Jejunum reacted with antiserum to human rotavirus (obtained from a convalesced experimentally infected gnotobiotic calf), then reacted with fluorescent antibody to bovine gamma globulin. (D) Jejunum reacted with antiserum to bovine rotavirus (rabbit), then reacted with fluorescent antibody to rabbit gamma globulin. Arrows point to fluorescing enterocytes (× 135).

This pattern of increasing severity suggests that initially there is a postpartum transmission from dam to young of a small amount of virus or of a virus with low infectivity. This mild assault is contained by the colostral antibody still in the piglet's gut and causes an asymptomatic infection of little consequence unless the nursery contains susceptible piglets capable of amplifying the infection. Such would be the case if piglets were in residence that had been weaned for some days. These piglets would be free of the protective antibody that bathes enterocytes, and would, therefore, be capable of amplifying the infection. Thus, as piglets are continually added to the nursery, an infection of increasing severity passes from young to young.

Bacterial numbers in the air in the nursery were monitored to determine whether they would serve as indicators of increasing fecal contamination of the environment and of impending diarrhea (*9*). There was no correlation between the number of airborne bacteria, length of continuous use of the nursery, and the initiation of diarrhea. The total count fluctuated between 500 to 1000 bacteria per cubic meter of air. Most of the bacteria were enterostreptococci and staphylococci, followed by about three coliforms per cubic meter of air.

The prevalence of rotavirus in the North Carolina swine community was established by surveying the sow population from 1968 to 1977 for the presence of antibodies to porcine rotavirus. The antibodies were identified by an indirect immunofluorescence technique. We used serums from 1-day-old nursing piglets (Fig. 2A), assuming that the antibodies in the sow would be concentrated in the colostrum which, in turn, would become concentrated in the serums of the piglets (passively acquired immunity). Eighty-eight percent of 150 sows in 14 herds had antibodies to pig rotavirus; no herds were negative. The number of positive sows in a herd ranged from 72 to 100 percent.

Because cows' colostrum protects piglets (*5*, *8*) from rotaviral diarrhea, we examined ten samples of pooled cows' colostrum (collected over a 10-year period) for the presence of antibodies to this virus. Antibodies to pig rotavirus were found in all samples (Fig. 2B). Antibodies to calf and infant rotavirus also cross-reacted with pig rotavirus (Fig. 2,

C and D). Others have observed antigenic similarities between human, calf, and pig rotavirus (*1*).

We conclude from these and other (*1*, *4*, *5*) data that rotavirus occurs in most mammals, but it is generally benign. Our results suggest that rotavirus owes its benignity to its high prevalence which inevitably leads to the neonate encountering the virus while being passively protected (*5*, *8*) by local antibody in the gut provided by the mother's milk. Active immunity, without the burden of overt disease, probably issues from this constant encounter. This is analogous to the sequence of subclinical infection and immunity seen with infantile paralysis (poliomyelitis) in primitive societies (*10*).

Problems arise when the dose of the virus exceeds the capacity of the immune system to contain viral growth. According to our results, this occurs when the highly susceptible piglet (less than 1 week of age) is exposed to large doses of rotavirus while being reared artificially. At this time, the piglet is weaned and no longer protected by antibodies in the milk, and the virus is free to multiply. The disease can be prevented by cleaning the delivery room (thus lowering the dose) and rearing the piglets in a nursery that can be entirely cleaned between groups (thus preventing the buildup of the infecting dose). Symptoms can be ameliorated by promptly feeding antibodies, for example, cows' colostrum.

Most children by 6 years of age have antibody to rotavirus. Rotaviral diarrhea peaks in the winter months in children ½ to 1½ years of age, and is often acquired in infant wards. Nursing infants, in comparison to infants fed artificially, have a much lower incidence of the disease in hospitals (*1*, *2*, *11*). In view of our findings, these data on human infants suggest (i) that the virus is prevalent in the community and the disease exacerbates when dose is increased (because of crowding, decreased ventilation in the winter, continuous use of nurseries), and (ii) that the weaned infant is removed from protective antibody in milk. These circumstances, when coupled with lower levels of sanitation and nutrition, could account for the high number of infant deaths in the Third World. Thus the management practices that precipitate rotaviral diarrhea in piglets may be similar to those used for some infants.

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Binding of Benzo[*a*]pyrene 7,8-Diol-9,10-Epoxides to DNA, RNA, and Protein of Mouse Skin Occurs with High Stereoselectivity

Abstract. *The formation, stereostructure, and cellular reactions of the 7,8-diol-9,10-epoxide metabolites of the carcinogen benzo[a]pyrene have been examined after topical application of benzo[a]pyrene to the skin of mice. In this known target tissue, polymer adducts from diastereomeric diol epoxides, (+)-(7S, 8R, 9R, 10R) and (+)-(7R, 8S, 9R, 10R), were formed stereospecifically from their corresponding 7,8-dihydrodiols. Both diol epoxides bind with proteins, RNA, and DNA in vivo. For the nucleic acids, binding occurs preferentially at the 2-amino group of guanine in cellular RNA and DNA in vivo. Methods for establishing the structure of the cellular adducts as well as the possible biological implications of their formation are discussed.*

Since the initial studies indicating that metabolites of benzo[*a*]pyrene (BP) 7,8-dihydrodiol are bound to DNA to a much greater extent than any other metabolites of BP (*1*), substantial evidence has accumulated suggesting that the diastereomeric BP 7,8-diol-9,10-epoxides (diol epoxides **1** and **2**) are ultimate carcinogenic metabolites of BP. For example, BP 7,8-oxide is a potent skin carcinogen although weaker than BP, BP 7,8-dihydrodiol is slightly more carcinogenic than BP on mouse skin, and BP 7,8-oxide and BP 7,8-dihydrodiol are noncarcinogenic when the 9,10 double bond of the molecule is hydrogenated (*2*). In addition, both BP 7,8-dihydrodiol and diol epoxide **2** are much more potent car-

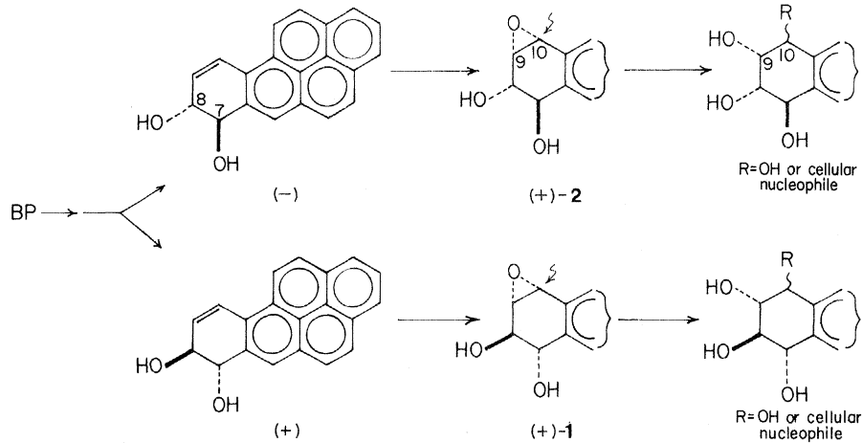

Fig. 1. Metabolic activation of BP in mouse skin. The absolute stereochemistry shown for the BP 7,8-dihydrodiols has been established by exciton chirality studies (*15*, *16*).

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