to prove (*16*), experimental ethylene production in *Gloeocapsa* is doubly verified. This supportive evidence is offered by (i) the failure of samples to overcome nitrate repression within 18 hours since bacterial recovery from combined nitrogen repression should have been effected earlier (*17*); (ii) no acetylene reduction in dark bottles; and (iii) the 8-hour treatment in darkness that inhibited ethylene production in samples incubated in light for at least 60 minutes. Although this last-mentioned lack of response probably would not have been recorded in cultures unaccustomed to continuous lighting, it does indicate that acetylene reduction in the *Gloeocapsa* samples is truly light-dependent.

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Maturation of Renal Organic Acid Transport: Substrate Stimulation by Penicillin

Abstract. *Renal* p-*aminohippurate transport in rabbits increased rapidly from birth to 4 weeks of age and then declined to that observed in adults. Penicillin administration to pregnant does or newborn rabbits stimulated the developing transport system, but did not increase the peak observed at 4 weeks. Therefore the continued presence of substrate (penicillin) during development significantly enhances the rate of maturation.*

The kidneys of newborn rabbits are histologically and physiologically immature (*1*). This is exemplified by the presence of a nephrogenic zone in the outer cortex and the development of a brush border in the proximal convoluted tubules during the first 4 to 5 weeks of life (*1*). Physiologically, it has been demonstrated that the renal transport mechanisms for organic ions in rabbits and several other species are not fully developed at birth (*2–4*). However, few attempts have been made to follow the development of specific kidney functions during the neonatal period. In contrast, investigation of the development of glycolytic and drug-metabolizing enzymes in the liver has been extensive (*5*). For example, it has been shown that the activity of hepatic drug-metabolizing enzymes is low at birth, with adult activity being reached at various times in the neonatal period (*5*). The mechanisms responsible for the increase in enzyme activity after birth have not been elucidated, although Dawkins (*5*) has suggested that one possible factor is substrate-induced stimulation of the enzymes. In this regard, it has been shown that a wide variety of drugs stimulate hepatic drug-metabolizing enzymes by a mechanism involving substrate stimulation (*6*). By analogy, it should be possible to stimulate a specific function of the kidney by challenging it with a substrate during the period of development. Thus, the objectives of this investigation were twofold: (i) to quantitate the maturation of renal organic acid transport in the newborn rabbit, and (ii) to stimulate the maturation of transport by treating either the pregnant doe or the newborn with a suitable substrate.

The technique developed by Cross and Taggart (*7*) was used to study in vitro the ability of renal tubules to actively transport the organic acid *p*-aminohippurate. Thin slices of kidney cortex were incubated in oxygenated media containing buffered salts and *p*-aminohippurate. At the end of the incubation period (90 minutes), the *p*-aminohippurate content of the slices and of the media was analyzed, and the transport that occurred was reported as the ratio of concentration in the slice to that in the medium (S/M) (micromoles of *p*-aminohippurate per gram of tissue divided by micromoles of *p*-aminohippurate per milliliter of medium). An S/M ratio greater than unity is indicative of active transport (*7*).

The pattern of development of organic acid transport was followed in young rabbits (ranging in age from 1 day to 8 weeks) and in adults. The S/M ratio of *p*-aminohippurate increases slowly from 1 day of age to about 2 weeks, and from this point to about 4 weeks of age the ratio increases rapidly, as does body weight. However, body weight continues to increase beyond 4 weeks of age, while the S/M ratio begins to decline until it reaches adult value. Rennick *et al.* (*2*) observed a similar peak in *p*-aminohippurate transport in 4-week-old puppies. At the present time we have no explanation for the significant increase in the S/M ratio at 4 weeks of age. In this regard, New *et al.* (*4*) indicated that the difference in *p*-aminohippurate accumulation in slices from newborn and adult rabbits is not due to differences in tissue water content. A rapid increase in activity during enzymatic maturation with a subsequent decline to adult activity is not a unique observation. Zorzoli (*8*) reported that the activity of various glycolytic enzymes was greater in kidney cortical tissue obtained from mice at 2 to 4 weeks of age than it was from adults.

Since a major excretory pathway of penicillin is active tubular secretion (*9*), it was thought that the prolonged presence of this drug in the body con-

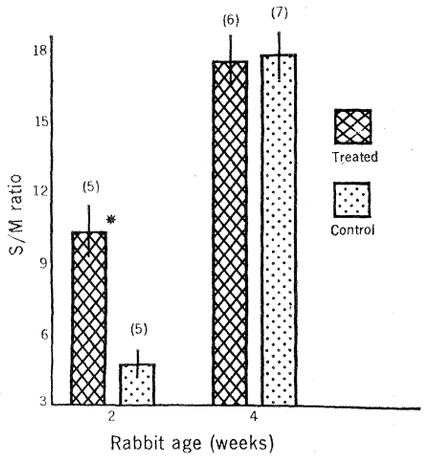

Fig. 1. The S/M ratios of *p*-aminohippurate in 2- and 4-week-old rabbits after subcutaneous injection of procaine penicillin G (30,000 I.U.) twice daily for 3 days. Rabbits were killed by a blow on the head, the kidneys removed, and thin cortical slices prepared freehand. Slices (about 100 mg) were added to beakers containing *p*-aminohippurate media and then incubated for 90 minutes at 25°C. The *p*-aminohippurate content of the slices and media was determined by the method of Smith *et al.* (*12*). Each bar represents the mean (± standard error) determined in five to seven rabbits. The asterisk indicates a significant difference from control ($P < .05$). The numbers in parentheses indicate the number of animals tested.

stantly challenging the secretory mechanism would stimulate the maturation of developing transport sites. Procaine penicillin G [60,000 international units (I.U.)] was administered subcutaneously twice daily for 3 days to 2- and 4-week-old rabbits. Control animals received saline, and all animals were killed 24 hours after the last injection. The *p*-aminohippurate transport was increased in the 2-week-old rabbits after penicillin administration, but not in the 4-week-old rabbits (Fig. 1). It appears that penicillin increases organic acid transport during the developmental period, but has no effect when transport development is complete. Although not shown, penicillin treatment increased kidney weight in 2-week-old, but not in 4-week-old rabbits.

The developing organism is particularly sensitive to drugs which readily cross the placenta (*10*). Significant blood concentrations of penicillin are maintained for long periods of time in the fetus when the drug is administered to the pregnant female (*11*). Pregnant rabbits were treated intramuscularly with procaine penicillin G (60,000 I.U.) daily during the last half of pregnancy (days 16 to 30) to determine if transport of *p*-aminohippurate in the offspring would be affected. The S/M ratios were measured in the offspring at ages ranging from 1 day to 4 weeks (Fig. 2). The results suggest that the presence of penicillin in the pregnant rabbit (and presumably in the fetus) stimulates maturation of tubular transport in the fetal and newborn rabbit. This stimulation of *p*-aminohippurate transport was present for about 2 weeks after birth. Apparently the rapid increase in the S/M ratio normally observed between 2 and 4 weeks of age exceeds any stimulation of *p*-aminohippurate secretion during this time caused by penicillin treatment before birth. The stimulatory effect of penicillin may be caused by enhanced development of existing tubular transport processes or the synthesis of new enzyme proteins responsible for organic acid transport. The data favor the former because treatment with penicillin either before or after birth did not enhance the maximum transport capacity seen at 4 weeks. However, it is also possible that penicillin may stimulate *p*-aminohippurate transport by nonspecifically or indirectly enhancing the maturation of the outer renal cortical cells. In this regard, Rennick *et al.* (*2*) have shown that maturation of the undeveloped outer cortex of piglet and puppy kidneys results in an increased capability of these cells to transport *p*-aminohippurate.

It should be pointed out that the S/M ratio actually represents the ability of the tissue to maintain a concentration gradient in a steady-state system. Therefore, not only is active transport of *p*-aminohippurate involved, but protein binding and diffusion are as well. However, inasmuch as the only parameter that would be expected to demonstrate a high degree of specificity is the active transport mechanism, these data strongly support the hypothesis that penicillin treatment stimulated the active transport of *p*-aminohippurate.

Enhancement of kidney function by substrate stimulation of organic acid transport may be of benefit to prematurely born animals in adapting to life after birth. Of more immediate benefit is the opportunity to use this information in the study of renal tubular transport. Selective stimulation of this system can be employed in the search for enzymes and proteins specifically associated with this function.

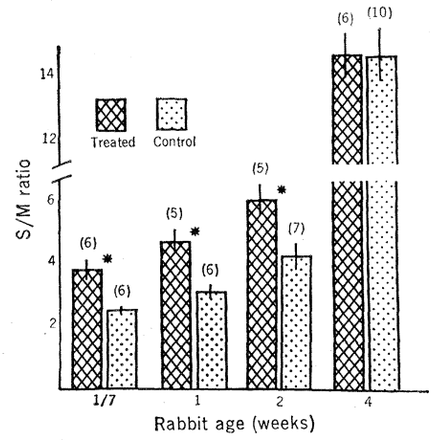

Fig. 2. The S/M ratios of *p*-aminohippurate in rabbit offspring after daily intramuscular injection of the pregnant doe during the last half of pregnancy with procaine penicillin G (60,000 I.U.). Newborn were studied at 1 day and 1, 2, and 4 weeks of age. Each bar represents the mean (± standard error) determined in five to ten rabbits. Asterisks indicate those values that are significantly different ($P < .05$) from their respective controls. The numbers in parentheses indicate the number of animals tested. The experimental procedure used is given in the legend for Fig. 1.

Last, in addition to its effectiveness in inhibiting bacterial growth, it appears that in mammals penicillin possesses specific trophic effects on kidney growth as well.

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