does not abolish the natriuresis induced by saline loading. Sodium excretion continued above control levels despite a local increase in protein concentration and a decrease in the filtered load of sodium. The decreases in filtered sodium which occurred during the protein infusion exceeded the associated decreases in excreted sodium, so that *net tubular reabsorption* was even further reduced during the protein infusion. Thus, it appears unlikely that the apparent decrease in tubular reabsorption of sodium observed in these studies resulted from the dilution of plasma protein associated with the saline infusion. This conclusion is in agreement with that of Levinsky and Lalone, who found that the natriuresis resulting from infusions of "plasma" continued in the presence of a hemodynamically induced decrease in filtration rate (14). The present studies do not exclude the possibility that once induced, the natriuresis of extracellular volume expansion may continue despite normal plasma oncotic pressure and a decreased filtered load of sodium, even though these factors may be of importance in initiating the natriuresis.

The observation that renal arterial infusions of hyperoncotic albumin uniformly reduced glomerular filtration rate is consistent with the premise that the oncotic pressure of glomerular capillary blood may be a significant factor determining rate of filtration. Increasing the concentration of impermeant solute would increase resistance to filtration, and if other factors (hydrostatic pressure, permeability) were unchanged, filtration rate should decrease. However, the possibility cannot be excluded that the presence of alien protein or unrecognized constituents of the albumin solution produced a fall in glomerular filtration rate by some non-specific mechanism.

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Slow Elimination of Urethan in Relation to its High Carcinogenicity in Newborn Mice.* (29220)

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It now seems well established that newborn mice are more sensitive to urethan carcinogenesis than adults. This has been most strikingly demonstrated for leukemogenesis, which is very pronounced when urethan is given soon after birth(1,2,3), but weak or absent when adults are treated, unless very

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high doses are used(4,5), or unless associated with X-irradiation(6,7). Newborn mice have also recently been reported to be more sensitive than adults to the action of urethan as carcinogen to the lung(8) and liver(9), and as initiator of skin tumors, with croton oil as promoter(9). However, other workers earlier reported that newborn mice were *less* sensitive to lung carcinogenesis by urethan than one-week-old mice(10).

The age difference in response could be due to an increased sensitivity of the immature organs(11). Kaye(12) proposed an alternative explanation, that urethan is less rapidly eliminated from very young mice, and therefore has a longer time to act, to explain a correlation between a slower elimination of urethan and a somewhat greater carcinogenicity in 2-week-old Swiss mice as compared with adults. In support of this view, more striking differences are now reported between rates of urethan elimination in newborn and adult mice of the closely related SWR strain. A similar correlation was recently reported by Domsky *et al*(13) for the action of 9,10-dimethyl-1,2-benzanthracene in newborn Swiss mice.

Material and methods. Male and female SWR mice less than 24 hours old served as "newborn," and those 9-10 weeks old (predominantly female) as "adults." The animals were kept at 22-25°, and fed Purina Laboratory Chow and tap water *ad libitum*. Radioactive urethan (carboxyl-labeled ethyl carbamate-^{14}C), obtained from the New England Nuclear Corp., Boston, was injected intraperitoneally at a dose level of 0.5 mg urethan per g body weight, using 1.25% urethan in 1% saline containing 0.5 μc/ml of ^{14}C-urethan. In the newborn, the needle was passed through the gluteal muscles to prevent leakage, the exact volume injected was confirmed by weighing the animals before and after injection, and the mice were then returned to their mothers.

After varying intervals (1-72 hours), the mice were killed and the livers analyzed for residual ^{14}C-urethan by the "alkaline-labile" method of Berenblum *et al*(14). Weighed whole livers (newborn) or 40-60 mg portions thereof (adult) were treated with 1 ml 5 N NaOH at 100°C for 15 minutes. The resulting $^{14}CO_2$ was collected using diffusion for 48 hours instead of distillation, counted as $Ba^{14}CO_3$ in a "Tracerlab" automatic windowless gas-flow counter, and corrected for self-absorption. The urethan present in 50 mg liver one hour after injection gave about 450 counts/min. In some instances, estimations were also carried out on 0.025 ml blood, collected from the neck after decapitation (newborn) or the supra-ocular sinus (adult), and on aliquots of the remaining carcass (newborn only), the latter after 30 minutes boiling in 5 N NaOH and filtration.

Results. The livers, rather than blood, were mainly used for analysis because of the greater difficulty in obtaining blood from newborn mice. The liver:blood ratios for ^{14}C-urethan concentration, using groups of 4-6 mice, were 0.96 $\pm$ 0.09 (mean $\pm$ S.D.) and 0.94 $\pm$ 0.08 for newborn mice, measured 1 and 24 hours after injecting urethan; and 0.83 $\pm$ 0.04, 0.84 $\pm$ 0.08 and 0.71 $\pm$ 0.09 for adult mice, measured after 1, 4 and 6 hours (ratios were calculated separately for each mouse). Liver values thus paralleled blood values, but were consistently a little lower, especially in adults (suggesting exclusion of urethan from the more fibrous components in the adult organ). This is in conformity with earlier results(14,15) pointing to a fairly uniform distribution of urethan in the animal body, and shows that liver analyses may be used as a basis for comparing newborn and adult mice. The corresponding liver:carcass ratio was 0.99 $\pm$ 0.03 for 4 newborn mice analyzed 24 hours after injection.

Rates of elimination of ^{14}C-urethan from the livers of newborn and adult SWR mice are compared in Fig. 1. While in the newborn mice only 20% of the dose of 0.5 mg urethan per g disappeared after 24 hours, in the adults 75% disappeared after 6 hours. In the newborn, 62 $\pm$ 18% of the urethan disappeared after 48 hours (in 7 mice), and virtually all (98 $\pm$ 1% for 7 mice) after 72 hours. In contrast, virtually complete elimination in the adults required only 8 hours. Earlier reports on adult mice give similar periods for complete elimination of urethan

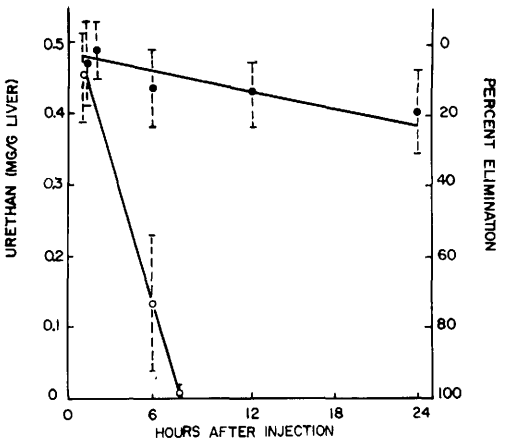

FIG. 1. Urethan content of liver and percent elimination of urethan at varying intervals after injection to SWR mice. Means ± S.D. are shown for groups of 6-9 mice. ●——● = newborn mice; ○——○ = adults.

to those found here, *e.g.* 10 hours for a dose of 0.75 mg/g given to adult Swiss mice(12). The approximately 10-fold difference in rates between newborn and adult mice may be compared with the 1.5-fold difference observed between 2-week-old and adult Swiss mice(12).

It has previously been checked that the "alkaline labile" $^{14}CO_2$ represents urethan remaining in the body, using paper chromatography with aqueous butanol of plasma and liver homogenates, obtained after injection of adult mice with ^{14}C-urethan; only a single peak of radioactivity was demonstrated, corresponding to urethan itself(16). A similar check on blood withdrawn 24 hours after injecting ^{14}C-urethan to newborn mice, gave the same result.

Discussion. The present results could well explain the reported greater sensitivity of newborn mice to the various forms of urethan carcinogenesis, on the basis that the carcinogen has a longer time to act, though this may not be the only factor involved.

The results appear also to explain the phenomenon that whereas lung tumors develop in fair numbers in the offspring of pregnant mice injected with urethan several days before delivery(17,18,19,20), the number of these tumors is far greater (up to 5-fold) when the injection is made less than 24 hours before delivery(17,18,19). In the latter case, the young were born, presumably, with unmetabolized urethan still present in the body, which they were unable to metabolize for several days; while in the former case, all the urethan would have been metabolized by the mother at the usual rate for adults, before giving birth. The exceptionally high incidence of lung tumors in offspring delivered by caesarean section 1-5 hours after injecting urethan to the mothers(18,19), is also in keeping with this explanation.

As urethan is eliminated from adult mice 90% by metabolism to CO_2(21), the slower elimination in the newborn mice is presumably due to a slower metabolism. Preliminary results indicate a lack in the newborn mouse of the esterase which metabolizes urethan to CO_2 and occurs in adult liver microsomes (*22*), in line with the general lack of liver microsomal enzymes in newborn rodents(23).

Summary. Urethan was found to be eliminated from newborn SWR mice at about one-tenth of the rate found in adults, when injected at a dose of 0.5 mg/g body weight. The longer time during which urethan remains in the body may explain the greater carcinogenic action in newborn mice as compared with adults.

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Fluorometric Microanalysis of Plasma Hippuric Acid and Comparison of Hippurate with P-Aminohippurate Clearances in Dogs.* (29221)

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The relationship of the clearance of hippuric acid to p-aminohippuric acid has never been determined(1), although there are numerous studies in the literature of the clearances of the congeners of hippuric acid. It has been assumed that the clearances of p-aminohippurate and the endogenous metabolite hippuric acid are identical. Limitations of the methodology for hippuric acid have prevented an accurate, direct comparison of the relationship between these two substances. This report is concerned with the development of a micromethod for analysis of plasma hippuric acid; and comparison of the clearances of hippuric with p-aminohippuric acid in the same dog under similar experimental conditions. Knoefel and Huang(2) have reported that hippuric acid is secreted by the renal tubules in the dog, and that it exhibits quantitative competition for transport with p-aminohippurate. Sodium hippurate has been shown to depress the phenol red/creatinine clearance ratio by Smith *et al*(3). Wu and Elliott(4) have shown that in man the excretion of hippuric acid following administration of small amounts of benzoate or hippurate can be described by first order kinetics; a relationship essential for clearance data according to Bray and White(5).

Methods. The methods used for analysis of creatinine, p-aminohippurate, inulin and urine hippuric acid are the same as those previously reported(4,6). The method for hippuric acid in plasma is based on the work of Ellman *et al*(7) who described the fluorescence of hippuric acid in 70% sulfuric acid.

Plasma hippuric acid was analyzed as follows: A protein-free filtrate of plasma is made by the slow, dropwise addition with vigorous stirring (Vortex, Scientific Industries) of 1.0 ml of 20% trichloroacetic acid to a dilution of 0.5 ml of plasma in 3.5 ml of distilled water contained in a 15 × 125 mm test tube. This vigorous treatment is necessary to release the protein bound hippurate. 60-80% of the hippurate in the plasma is protein bound at the concentrations indicated in Fig. 1. The percent binding was determined by ultrafiltration using a Filterfuge Tube† with a cellophane membrane at 2400 rpm and 5°C for 4 to 6 hours.

The mixture is centrifuged, and 0.1 ml of the filtrate is added to 3.0 ml of 70% sulfuric acid in a quartz cuvet.‡ A blank is prepared containing an equivalent amount of trichloroacetic acid in 70% sulfuric acid. The solutions are mixed slowly but thorough-

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† International Equipment Co., Needham Heights, Mass.

‡ Corning Vycor #7910, 13 x 75 mm.