while no effect was seen with the unheated cytosol. With the male rats a smaller increase in tryptophan oxygenase activity was observed with the unheated than with the heated cytosol. Although the effect of oestradiol benzoate on tryptophan oxygenase activity is well known [2], the use of unheated instead of heated cytosol could explain some reported discrepancies [3, 4] and difficulties [5] encountered in explaining the effects of oestrogens on tryptophan oxygenase.

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Hepatic uptake of cardiac glycosides in newborn rats, rabbits and dogs*

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It has been widely recognized for over a decade that the newborn cannot metabolize drugs as efficiently as the adult [1, 2], but relatively little is known about the hepatic excretory mechanism of the liver of the newborn. Ouabain is at least 40 times more toxic to newborns than to adult rats [3], is not metabolized prior to its excretion [4–6], is excreted from the body almost entirely via the bile [5], and thus is ideal for the study of the hepatic excretory mechanism in newborn rats.

In rats from 3 to 12 days of age, the toxicity of ouabain decreased gradually, but a rapid decrease was observed between 12 and 21 days of age. After 30 days of age, the toxicity of ouabain remained constant [7, 8]. Ouabain disappeared very slowly from the plasma of the 7-day-old, having a half-life of 30 min. The half-life in the adult is approximately 5 min. The longer half-life of ouabain in the newborn is due to the inability of its liver to remove ouabain from plasma. The concentration of ouabain in the liver of an adult is 50 times that of the plasma, whereas the liver of the newborn does not have any capacity for concentration. The ability of the liver to extract ouabain from the plasma and to concentrate it develops concurrently with the increase in LD_{50}. It appears that the immaturity of the liver to extract the ouabain from the plasma and to excrete it in bile results in a prolonged high plasma ouabain concentration which is associated with a higher toxicity [7, 8].

Since digoxin and digitoxin have also been shown to be more toxic in the newborn rat than in the adult [3], it was of interest to determine whether the ability of the liver of the newborn rat to concentrate these glycosides is also low. Newborn rabbits and dogs were also studied. Only digoxin was studied because in these species ouabain is excreted to a low extent [6] and digitoxin is extensively metabolized before excretion into the bile [9, 10].

Rats 7 and 39 days of age were used as representatives of newborn (13–15 g) and adult rats (130–190 g). It has previously been demonstrated that the livers of 7-day-old rats are immature in their ability to remove ouabain from plasma, while this ability in 39-day-old rats is fully developed. The rats were born in our laboratory and were the offspring of untreated Simonsen Sprague–Dawley rats. The mother and offspring were kept in "shoebox" cages for 1 month before removing the mother. The rats had access to food and water at all times.

New Zealand White rabbits (1·3–1·7 kg) and 6-day-old rabbits (100–160 g) were used as adult and newborn. The newborn rabbits were born in our laboratory and were the offspring of untreated New Zealand White rabbits. Adult mongrel dogs (12–15 kg) and newborn dogs (5 days old, 300–400 g) born in our animal facilities were used as well.

Randomly labeled tritiated ouabain, digoxin and digitoxin were obtained from New England Nuclear Corp. (Boston, Mass.) and mixed with their respective nonradioactive glycosides (obtained from Sigma Chemical Co., St. Louis, Mo.). Ouabain was dissolved in saline, digoxin in pyridine and diluted 40- to 100-fold with saline, and digitoxin in ethanol and diluted 100-fold with saline. All glycosides were administered at a dose of 0·08 mg/kg.

The glycosides were administered i.v. to rats (2 ml/kg) via the distal portion of the femoral vein, to dogs (0·5 ml/kg) via the cephalic vein of the foreleg or femoral vein of the hindleg, and to rabbits (2 ml/kg) via the marginal ear vein or

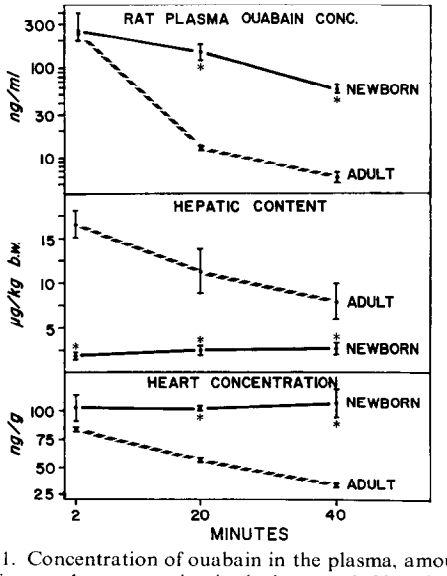

Fig. 1. Concentration of ouabain in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of ouabain (0·08 mg/kg) to 7- and 39-day-old rats. Each value represents the mean ± S.E. of four rats. Asterisk indicates that the values are significantly different ($P < 0·05$).

* This work was supported by U.S. Public Health Service Grant AM 14513.

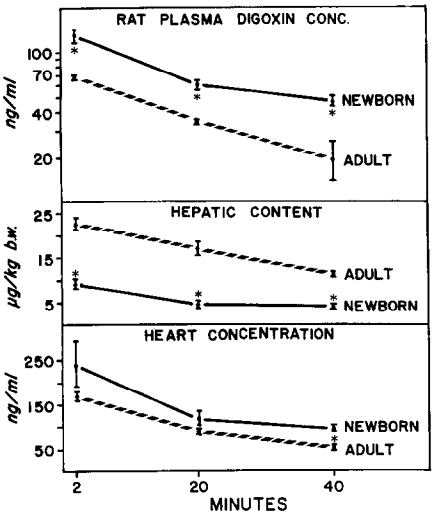

Fig. 2. Concentration of digoxin in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of digoxin (0·08 mg/kg) to 7- and 39-day-old rats. Each value represents the mean ± S.E. of four rats. Asterisk indicates that the values are significantly different ($P < 0.05$).

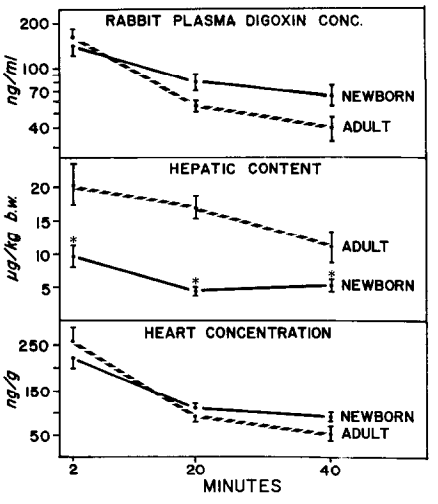

Fig. 4. Concentration of digoxin in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of digoxin (0·08 mg/kg) to newborn and adult rabbits. Each value represents the mean ± S.E. of five newborn or three adult rabbits. Asterisk indicates that the values are significantly different ($P < 0.05$).

femoral vein. At 2, 20 or 40 min after glycoside administration, the rats and rabbits were anesthetized with ether, and dogs with sodium pentobarbital (30 mg/kg). A blood sample was taken by cardiac puncture with a heparanized syringe, and the liver and heart were excised. Radioactivity was measured as previously described [7].

The data were compared by the Student's *t*-test [11]. $P < 0.05$ was considered significant.

The concentration of ouabain in plasma and heart and the amount in the liver were measured at 2, 20 and 40 min after the i.v. administration of ^{3}H-ouabain (0·08 mg/kg) to 7- or to 39-day-old rats (Fig. 1). The concentration in the plasma was similar in the two groups at the 2-min time interval, but ouabain disappeared from the plasma much more slowly in the newborn than in the adult rats. At the 20-min time interval, the concentration of ouabain in the plasma of the newborn was more than 10 times that in the adult.

The amount of ouabain in the liver of the newborn was always much lower than in the adult. At the later time intervals, the difference was not as great because there was little available ouabain remaining in the body to be concentrated in the liver. The lower amount in the liver was not due solely to the smaller liver of the newborn (newborn 2·9 per cent of body wt and adult 4·4) but was also due to a lower concentration of ouabain in the liver. An almost entire lack of ability of the newborn liver to concentrate ouabain was demonstrated.

The concentration of ouabain in the hearts of the two groups was not different at the 2-min interval, but the concentration in the heart did not decrease in the newborn during the 40-min time interval as it did in the adult, which resulted in a higher concentration in the heart of the newborn at the later time intervals.

The disposition of digoxin in newborn and adult rats is shown in Fig. 2. The concentration of digoxin in the plasma was higher in the newborn than in the adult at all times after i.v. administration (0·08 mg/kg). The amount of digoxin in the liver of the newborn was always lower than in the adult. The concentration of digoxin in the heart always tended to

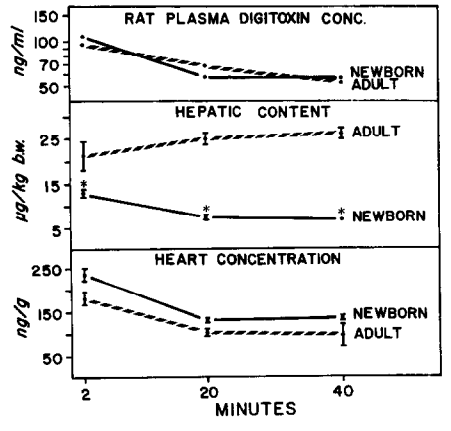

Fig. 3. Concentration of digitoxin in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of digitoxin (0·08 mg/kg) to 7- and 39-day-old rats. Each value represents the mean ± S.E. of four rats. Asterisk indicates that the values are significantly different ($P < 0.05$).

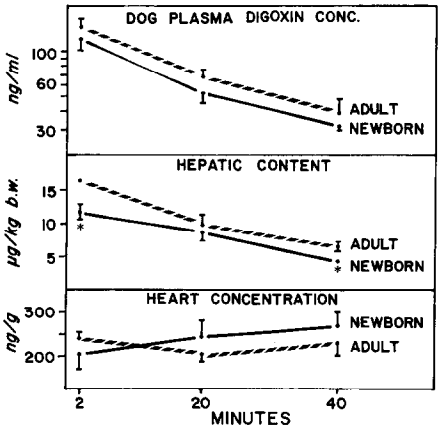

Fig. 5. Concentration of digoxin in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of digoxin (0·08 mg/kg) to newborn and adult dogs. Each value represents the mean ± S.E. of four to five newborn or three adult dogs. Asterisk indicates that the values are significantly different ($P < 0.05$).

be higher in the newborn than in the adult but was significantly so only at the last time interval.

Comparison of the disposition of digitoxin in newborn and adult rats is depicted in Fig. 3. Digitoxin disappeared at a similar slow rate from the plasma of both groups. However, the liver of the newborn contained a significantly lower amount of digitoxin than that of the adult at all time intervals. The lower amount of digitoxin in the liver of the newborn was due both to the smaller liver and to the lower concentration of glycoside in the liver. However, the liver of the newborn was able to concentrate the digoxin over that in plasma but not to the extent observed in the adult liver.

The disposition of digoxin in newborn and adult rabbits is shown in Fig. 4. The concentration of digoxin in the plasma or heart of the two groups was not significantly different. The amount of digoxin in the liver was lower in the newborn than the adult at all time intervals. This was mainly due to the difference in the ability of the livers of the two groups to concentrate digoxin.

Corresponding data obtained in the dog are shown in Fig. 5. Digoxin disappeared from the plasma at similar rates in the two groups. The amount of digoxin in the liver was significantly less in the newborn dogs at the 2- and 40-min time interval. Since the newborn dogs had relatively larger livers (4·1 per cent of body wt) than the adult dogs (3·3 per cent of body wt), the concentration difference was even greater. Both groups of dogs were able to concentrate digoxin in their livers but the adult could do this to a greater extent. No significant difference in the heart digoxin concentration was detected.

In conclusion, a decrease in the uptake of cardiac glycosides into the liver in newborn animals definitely is not limited to one cardiac glycoside (ouabain) or one species (rat). Although the largest difference was seen with ouabain in the newborn rat, differences existed with other glycosides and in other species as well. In newborn animals, the decreased rate of removal of cardiac glycosides probably results in a longer duration of their pharmacological action and may produce increased sensitivity to their toxic effects as well.

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Transcortin levels in the blood of arthritic rats

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Cortisol is extensively used in the treatment of rheumatoid arthritis and is also effective in controlling the polyarthritis in the adjuvant rat [1]. Most of the endogenous cortisol in circulating blood is protein-bound to transcortin and albumin [2]. The binding between albumin and cortisol is weak (association constant 10^3–$10^5 M^{-1}$) and non-specific [2]. In contrast, transcortin binds cortisol firmly (association constant 10^7–$10^8 M^{-1}$) and is relatively specific [2]. Traditionally transcortin bound cortisol was thought to be inactive, but recent evidence suggests that it may be active in some tissues [3].

The inflammatory response in both rheumatoid arthritis [4] and adjuvant arthritis [5] affects protein biosynthesis *in vivo*. In this communication we have examined the effect of inflammation on the levels of transcortin in the blood of adjuvant arthritic rats.

Experimental. Arthritis was induced in Wistar strain male and female rats (150–200 g) by a method previously described [6]. Human strains (C, DT and PN) of tubercle bacilli were kindly supplied by the Ministry of Agriculture Veterinary Laboratories, Weybridge, Surrey. The adjuvant was introduced into the left hind foot-pad. The intensity of the induced inflammation was measured in both hind feet by immersing them separately to the hair line in a mercury bath connected to a pressure transducer linked to a Devices recorder. The initial measurements of foot volume were taken prior to injection of the adjuvant and subsequent measurements prior to collection of blood samples on the appropriate day.

Blood was drawn from the aorta of anaethetised animals and the serum was dialysed for 24 hr against saline at 4° to remove free cortisol. Serum transcortin levels were determined by the method of Milgrom [7]. In this method, 0·5 ml serum was incubated at 37° for 15 min with a large excess of [^{3}H]cortisol (1·9 μCi, 2·3 μg) to displace any endogenous cortisol remaining in the binding sites. Two ml. of a dextran coated charcoal suspension (0·25 g charcoal and 0·025 g dextran in 100 ml Tris–HCl buffer, pH 7·4) was added and the mixture shaken vigorously at 4° at a constant speed. At various intervals portions were removed, and after centrifuging to remove the charcoal, 0·2 ml of the supernatant was added to 5 ml of Bray's reagent [8] and the radio-