cyclic AMP independent mechanism to stimulate glycogen breakdown. Since different analogs can cause glycogenolysis and not stimulate DNA synthesis and *vice versa*, it is clear that there is no correlation between these two biochemical events.

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The effect of heating rat liver cytosol on oestrogen-induced tryptophan oxygenase activity

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Recently it has been shown that thermal activation of rat liver cytosol in the presence of tryptophan as stabilizer results in a considerable increase in the total enzyme units of tryptophan oxygenase [1]. During our studies on the effect of treatment with steroid hormones upon rat liver tryptophan oxygenase we initially compared the activities of non-activated and thermally activated tryptophan oxygenase. Besides large differences in the absolute values for the activities of the enzyme in the activated and non-activated state, we observed considerable differences in holoenzyme activation after induction of apoenzyme synthesis by oestrogens dependent on whether the liver cytosol had been heated or not (Table 1).

Female or male Wistar rats, weighing 180–200 g, were injected subcutaneously with 0·1 ml of an oestradiol benzoate solution in arachis oil (for doses see Table 1) for 15 days. The last injection was given one hour before decapitation. The livers were removed and homogenized in three volumes of 0·14 M potassium chloride containing 0·01 M L-tryptophan. Preparation of the cytosol and thermal activation (heating of the cytosol at 55° for 5 min) were carried out according to Schutz and Feigelson [1]. Estimation of tryptophan oxygenase activity was based on the combined rates of formylkynurenine plus kynurenine formation [1], which were measured by recording optical densities at 321 and 360 nm with a Zeiss PM QII spectrophotometer at 37°. The experimental design (complete randomization of rats and treatments) met the requirements for a statistical analysis of results by means of analysis of variance.

Treatment of female rats with oestradiol benzoate caused a highly significant increase in tryptophan oxygenase activity when the heated cytosol was used as the enzyme source,

Table 1. Activities of non-activated and thermally activated rat hepatic tryptophan oxygenase after treatment with oestradiol benzoate for 15 days

Treatment dose (μg/kg)	Tryptophan oxygenase activity (μmoles (formylkynurenine + kynurenine)/gm liver/hr ± SEM) Female rats		Male rats	
	Activated	Non-activated	Activated	Non-activated
Placebo (8)*	8·4 ± 0·6	2·6 ± 0·1	7·3 ± 0·3	2·1 ± 0·1
O.B.† (8) 50	13·5 ± 0·4‖	2·7 ± 0·1	11·9 ± 0·5‖	2·9 ± 0·2‡
O.B. (8) 100	13·3 ± 0·5‖	2·8 ± 0·3	12·7 ± 0·3‖	3·2 ± 0·2§

* Number of rats for each experiment.
† Oestradiol benzoate.
Statistical significance as compared to placebo values calculated by means of analysis of variance: ‡ $P < 0.05$, § $P < 0.01$, ‖ $P < 0.001$.

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

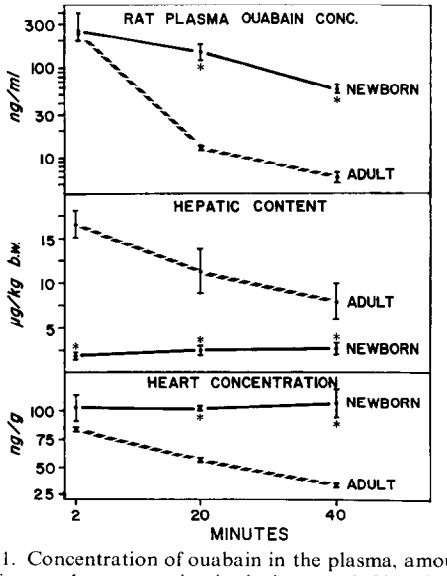

Fig. 1. Concentration of ouabain in the plasma, amount in the liver, and concentration in the heart at 2, 20 and 40 min after the i.v. administration of ouabain (0·08 mg/kg) to 7- and 39-day-old rats. Each value represents the mean ± S.E. of four rats. Asterisk indicates that the values are significantly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

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