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EXPERIMENTAL STUDIES OF GASTROINTESTINAL CONJUGATION FUNCTIONS

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Abstract—Experimental (duodenal) ulcer can be caused by feeding excessive amounts of cinchophen to dogs. Studies on the mechanism of ulcer formation revealed the importance of conjugation systems since it was possible that cinchophen depleted the liver of glucuronic acid. The ability of liver slices taken from cinchophen intoxicated animals to carry out glucuronide synthesis necessitated a search for other organs capable of carrying out glucuronide conjugation reactions. Negative results were obtained with a wide number of tissues and apart from liver and kidney only slices taken from the mucous membrane of stomach, duodenum, ileum and colon gave high values for glucuronides synthesis. Other results showed that a number of substances were conjugated in the gastrointestinal tract; the significance of these results in relation to ulcer formation is discussed.

THE writer became interested in the detoxication mechanisms and specifically on the glucuronide formation in connexion with our previous studies of the mechanism of the experimental cinchophen (atophan) ulcer. It has been shown by van Wagoner and Churchill[1] that excessive feeding of cinchophen to dogs results in ulcer formation in the pyloric and duodenal regions. This method is one of the most reliable ways of producing an experimental ulcer.

In our own studies we were able to show that cinchophen causes a rather specific reduction and even cessation in the mucus secretion by the pyloric and duodenal (Brunners) glands[2, 3] in dog. The same was shown to be true also for the secretion by the pyloric glands.[4] Mucoproteins, which are secreted in the mucus, contain glucuronic acid and our interest was aroused by the fact that cinchophen is excreted and detoxicated as a glucuronide conjugate. According to the ideas of most authorities at that time—I would like to refer to the monograph of Williams[5] glucuronide conjugation is carried out by the liver and to a lesser extent by the kidney. The possible depletion resources of glucuronic acid of the liver by cinchophen feeding was the first working hypothesis. It was, however, found, that the whole organism[6] as well as liver slices taken from cinchophen toxicated animals[7, 8] were capable of carrying out glucuronide synthesis even after ulcers had developed in the same animal.

The next step in our work[9, 10] was to find out whether the liver and kidney really were the only important sites for the glucuronide conjugation reactions, or was it possible that other organs could also participate in this activity.

The studies were conducted in *in vitro* conditions. Tissue slices were incubated in the Warburg apparatus in the presence of *o*-aminophenol. The glucuronide conjugate of

this compound is easy to detect by colour reactions and can be quantitatively assessed according to the method described by Levvy and Storey.

Table 1 gives the results produced by liver and kidney slices taken from various animal species.

TABLE 1. GLUCURONIDE SYNTHESIS OF THE LIVER AND KIDNEY IN VARIOUS ANIMAL SPECIES. γ *o*-AMINOPHENOL CONJUGATED (100 MG DRY WEIGHT OF TISSUE) 90 MIN

Organ	Animal	Range	Mean
Liver	Rabbit	38–236	123
	Rat	24–195	65
	Guinea pig	69–269	124
	Dog	40–110	66
	Cat	trace?	—
Kidney	Rabbit	38–138	88
	Rat	24–156	79
	Guinea pig	43–96	80
	Dog		
	Cat	trace?	—

TABLE 2. LIST OF ORGANS TESTED FOR GLUCURONIDE SYNTHESIS

Organ	Animal	Results
Pancreas	Rabbit, rat, cat, guinea pig	negative
Spleen	Rabbit, rat, dog, guinea pig	negative
Adrenals	Rabbit, rat, dog, guinea pig	negative
Ovary	Rabbit, rat, dog, guinea pig	negative
Testicle	Rabbit, rat	negative
Placenta	Rabbit, rat, human	negative
Peritoneum	Rabbit, rat	negative
Muscle		
diaphragm	Rabbit, rat	negative
skeletal	Rabbit, rat	negative

TABLE 3. GLUCURONIDE SYNTHESIS. LIST OF ORGANS KNOWN TO CONTAIN OR PRODUCE GLUCURONIC ACID AND TESTED FOR THEIR ABILITY TO CONJUGATE *o*-AMINOPHENOLGLUCURONIDE *in vitro*

Organ or tissue	Animal	Result
Synovial membrane	Rabbit, rat	negative
Umbilical cord	Rabbit	negative
Excised eye, various parts	Rabbit, rat	negative
Trachea, mucous membrane	Rabbit, rat	negative
Ureter, mucous membrane	Rabbit, rat, guinea pig	negative
Urethra, mucous membrane	Rabbit	negative
Urinary bladder, mucous membr.	Rabbit, rat, dog, cat	trace?

Table 2 shows the negative results obtained with a variety of other organs. In the next series (Table 3) those organs were tested which either produce glucuronic acid containing compounds (e.g. hyaluronic acid) or which otherwise contain it. As can be seen, the results were negative.

The studies were then continued by taking slices from various parts of the alimentary canal. The results are listed in Table 4. It can be seen that slices taken from the mucous membrane of the stomach, duodenum, ileum and colon all gave markedly high values for glucuronide synthesis. These findings are thus in agreement with some earlier work which indicate that the sulphate conjugation takes place also in the gastrointestinal tract.[11, 12]

TABLE 4. GLUCURONIDE SYNTHESIS BY THE MUCOUS MEMBRANE OF THE GASTROINTESTINAL TRACT. *o*-AMINOPHENOL CONJUGATED (100 MG DRY WEIGHT TISSUE) 90 MIN

Organ	Cat	Dog	Rabbit	Rat
Stomach				
greater curvature	—		10–100	62– 71
lesser curvature	—		25–217	–150
pyloric	—	32–145	69–240	30–511
Duodenum	—	55–160	28–568	48–567
Ileum	—	–175	82–328	40–208
Colon	—		29–300	27–657

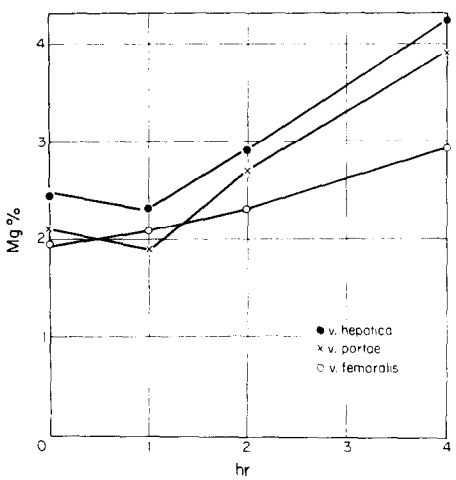

FIG. 1. Serum glucuronides after intragastric feeding of 500 mg cinchophen in dog. D. choledochus was ligated.[17]

Independently of these studies, and at the same time, two other groups had arrived at the same conclusion. Zini[13] and Shiray and Ohkubo[14] reported that significant glucuronide synthesis occurs in adult stomach and intestine. All these studies were later confirmed by Dutton[15, 16] who was also able to demonstrate the presence of the same enzyme machinery in the mucosal elements.

All these studies have been performed with isolated tissue slice techniques, We have also performed studies in whole animals. The portal blood, venous hepatic blood and systematic blood were analysed after feeding of a glucurogenic substance, phenolphtalein or cinchophen, to dogs. It was found (Fig. 1) that the glucuronide content in

the portal blood rose by approximately 100 per cent, even when the escape of bile into the intestine was prevented by ligation of the common bile duct.[17]

Similar studies have been performed for foetuses.[18] Intragastric feeding of phenolphtalein phosphate to nearly full term guinea pig foetus *in utero* resulted in the appearance of easily detectable amounts of phenolphtalein glucuronide both in the foetal and maternal blood (Fig. 2).

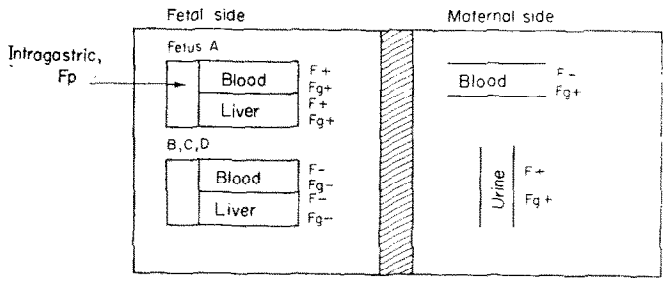

FIG. 2. Studies of the intrauterine gastric feeding of 250 mg of phenolphtalein phosphate (Fp) in the guinea pig. F, free phenolphtalein, Fg, phenolphtalein glucuronide.[18]

We have started a series of experiments in order to find out which kind of substances undergo glucuronide conjugation in the gastrointestinal tract. Some of the results are listed in Table 5.[19–21] In these studies the substances were incubated with the tissue slices, after which the substrate and metabolites were extracted with specific solvents. The final identification was then performed by chromatographic analyses.

TABLE 5. DUODENAL GLUCURONIDE SYNTHESIS

Compound	Glucuronide formation
Estrone	+
Estriol	+
Estradiol	+++
Equilin	++
Stilboestrol	++
Progesterone	–
Pregnandiol	–
Androsterone	–
Testosterone	–

As can be seen (Table 5) only those steroids which possess oestrogenic activity are conjugated, whereas the androgens are not. All the steroid hormones tested so far and which have been found to be conjugated to glucuronides contain a three carbon phenol hydroxyl in their molecule. None of the unconjugated steroids have this characteristic. This appears to hold for the other steroids tested so far. Thus neither cortisone or hydrocortisone are conjugated by the mucous membrane. Our present work is also partly concentrated on the probable molecular specifity of the glucuronide conjugations.

Since it has been well established that the glucuronide conjugation is not solely confined to the liver and kidney but that it is also carried out by the gastrointestinal

tract, we are left to discover the relationship between the hepatic and intestinal synthesis, and the physiological meaning of these processes in the gastrointestinal tract.

As to the behaviour of the hepatic and intestinal processes under varying experimental conditions Table 6 illustrates few of the results obtained so far.[22–24] It can be seen that the hepatic and duodenal conjugation are not affected in the same way by the same factors. This can also be seen in Figs. 3 and 4. In these studies a 400 r or 1200 r

TABLE 6. EFFECT OF VARIOUS AGENTS ON GLUCURONIDE SYNTHESIS

Agent added *in vivo*	Intestinal synthesis	Hepatic synthesis
Thyroxin	*	+
I^{131}	*	—
Cortisone	—	*
Cinchophen	*	*
Carbontetrachl added *in vivo*	*	—
Heparin	*	*
Histamin	*	*
Serotonin	*	*
"Intrinsic factor"	*	*
B_{12}	*	*

* = no effect.
\+ = stimulation.
— = depression.

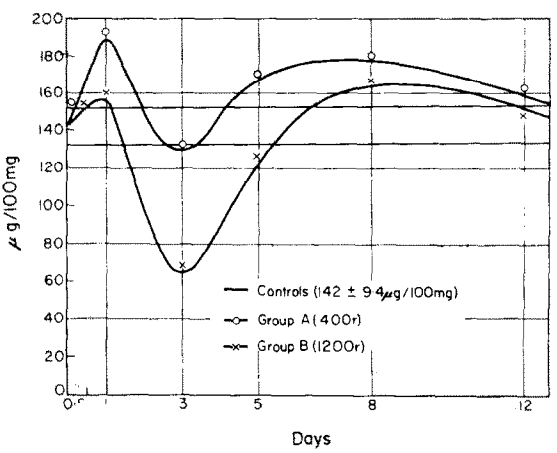

FIG. 3. Effect of x-ray irradiation on the *o*-aminophenolglucuronide synthesis by the liver parenchyma. Results expressed as ug *o*-aminophenolglucuronide per 100 mg dry weight liver tissue.[26].

x-ray dose has been applied locally to the exposed liver or stomach, after which the synthetic capacity was measured at certain intervals.[25, 26] It appears that the liver conjugation mechanism is rather resistant towards irradiation compared with the gastric mucous membrane. In both cases the effect is, however, reversible.

What is the meaning of the gastrointestinal conjugations? The answer cannot be given yet, though several possibilities are at hand. First, that it is a true detoxication

reaction by which organic compounds are made less toxic or active. Another alternative is that these reactions serve the purpose of active transport across cellular membrane. It is known that for many substances the conjugation leads to a better solubility in water and biologic fluids. If so, then these reactions would actively help the selective adsorption from the gastrointestinal tract. For many of these substances diffusion in the free state, rather than selective absorption in the conjugated form, seems to be

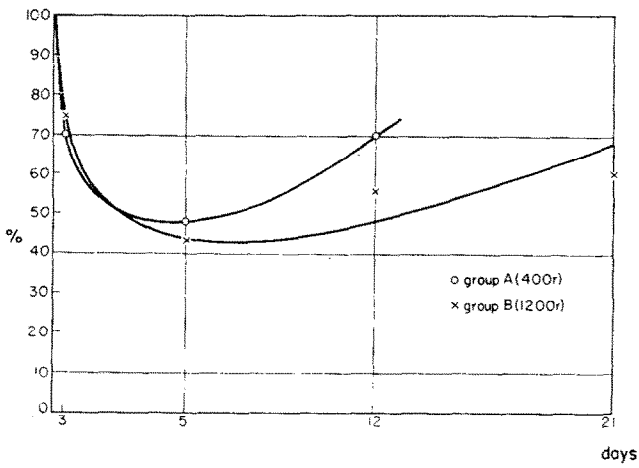

FIG. 4. Effect of x-ray irradiation on the *o*-aminophenolglucuronide synthesis by the gastric mucous membrane. Results expressed as percentage changes from the simultaneous control values.[25]

more likely. I would like to refer to the work of Schachter who has found that after feeding of salicylates the resulting concentration of unconjugated salicylate in the plasma was 100–200 times the concentrations of the salicyl glucuronides. More experimental work is needed to answer these questions.

Even so, there are some aspects which already might be of interest for us.

When performing synthetic reactions in general one part of the molecule synthetized is provided by the organism—in this case glucuronic acid. When the dose of the foreign compound is not excessive the conjugating agent may be provided from waste material or from the tissues, without strain upon the resources of the animal. With excessive doses, however, the conjugating agent may be utilized for detoxication at the expense of material required for the well-being of the organism. This has been illustrated by the case of bromobenzene,[5] which when fed to growing animals in excess of a certain dose, causes cessation of growth, apparently because the animal is unable to provide sufficient cysteine for both growth and the detoxication of the excessive amounts of bromobenzene. A similar condition seems to exist during glucuronide conjugation. It was actually the side-effect, i.e. the depletion of the mucoprotein production by the mucous membrane, which led us to the discovery of the local gastrointestinal glucuronide synthesis. Two competitive functions, both carried by the cellular components of the mucous membrane, may be taking place simultaneously. With excessive doses of cinchophen, for example, mucous secretion is inhibited. One function of the mucus is to protect the epithelial surface against chemical and mechanical

injuries. When this protection is lacking other consequences may follow. In the case of excessive doses of cinchophen the result may be erosions or even actual ulcer formation. If this hypothetical line of events actually takes place, we would then be able to explain and understand some of the frequent gastrointestinal manifestations provoked by many of our drugs. I would particularly like to draw attention to the gastrointestinal side-effects, including ulcers, caused by the widely used salicylates. It has been claimed[27] that the carboxyl and phenol groups of salicylate are conjugated with glucuronic acid by intestine slices.

As to the probable competition with some other functions in the gastrointestinal tract, it appears that simultaneous administration of a glucurogenic substance, e.g. cinchophen, does not interfere with the absorption rate of glucose. On the other hand glucose seems to enhance the absorption of a glucurogenic substance.

We have also been quite concerned about the role of β-glucuronidase in the conjugation processes. An experimental condition was sought which would allow parallel analyses of the glucuronide conjugation and β-glucuronidase activity of the same

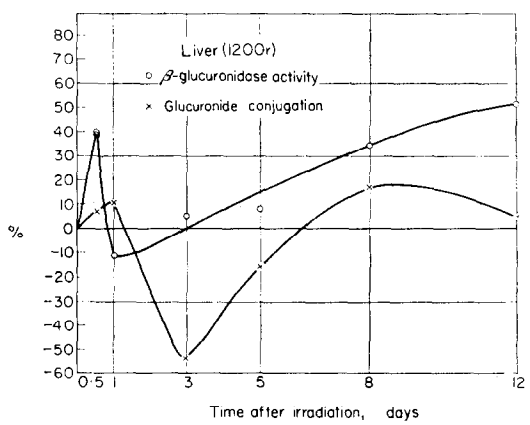

FIG. 5. Comparison of the hepatic β-glucuronidase activity (○–○) and the glucuronide conjugation capacity (×–×) after a local 1·200 r x-ray dose.[28]

tissue when changes are caused in the former. In our previous x-ray irradiation studies we had an opportunity of causing constant changes in the synthetic activity. These experiments were continued, and from the results (Figs. 5 and 6) it is evident that the β-glucuronidase activity curves are different in the two organs after the irradiation. In the stomach the activity parallels the simultaneous glucuronide synthetic capacity, whereas in the liver, there is a rise and depression of the enzyme activity similar to the glucuronide conjugation capacity. Nevertheless there is no direct relationship between these two functions. There is a certain time lag in the β-glucuronidase curve that the maximum and minimum points of the two curves do not coincide with each other.

As to the question of the possible role of β-glucuronidase in the synthetic reactions, these results may be taken as an indication there is no such relationship.

We are still not satisfied with our present knowledge of the physiological role that β-glucuronidase plays in the conjugation processes. We have tried to attack this

problem from another angle. We turn back to our original gastric lesion problems. Fig. 7 shows the preliminary results which have been obtained after attempts to inhibit the cinchophen effect in animal.[29] Chicks have been chosen for trials first because cinchophen also causes easily detectable gastric ulcers in this species, and secondly because it is a cheap animal which permits the use of large-groups.

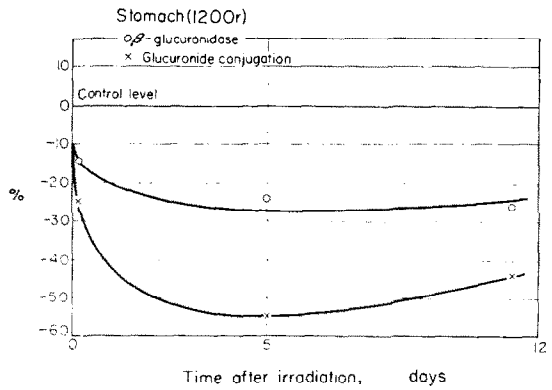

FIG. 6. Comparison of the β-glucuronidase activity (○–○) and the glucuronide conjugation capacity (×–×) of the gastric mucosa after a local 1·200 r x-ray dose.[28]

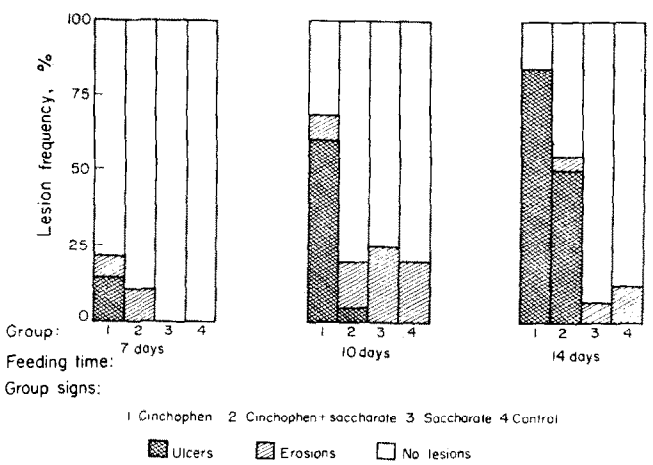

FIG. 7. Effect of saccharate on the frequency of the cinchophen provoked gastric lesions in chicks.[28]

We have administered a β-glucuronidase inhibitor, saccharic acid lactone in connexion of the cinchophen feeding. It can be seen that simultaneous administration of this inhibitor with the glucurogenic substance, cinchophen is able markedly to reduce the ulcer frequency. At the 10 day point there are only 8 per cent with ulcers in this group as compared to 65 per cent in the group without the inhibitor. On the fourteenth day the same figures are 50 per cent against 85 per cent. I want to stress that these are only preliminary studies. More work is needed for control and understanding of the immediate complex mechanism underlying these effects. We know that the stomach of the chick is able to synthetize *o*-aminophenol and there is also large

amounts of β-glucuronidase in it. These experiments may be taken as a further indication that the cinchophen ulcer might be related to the glucuronide formation machinery and that the competitive functions between the glucuronide synthesis and the mucopolysaccharide involving protective mucous production could be involved in these processes.

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