interaction (−0.77), the energy of activation per methylene group in all cases (Table I) has been found to be much closer to that which corresponds to the adsorption of the methylene group to an air–water interface.

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Absorption of Chlormadinone Acetate and Norethindrone from In Situ Rat Gut

KATHLEEN S. PELZMANN

Abstract ☐ The GI absorption of chlormadinone acetate and norethindrone was studied in a rat in situ preparation. The data evaluated with respect to the absorption half-life in the ligated stomach and intestine suggest that chlormadinone acetate is absorbed slightly more rapidly than norethindrone and that both steroids are absorbed to a greater extent in the intestine. The effects of bile duct cannulation, ethanol, and exogenous bile salts were investigated. The presence of bile significantly altered the absorption and metabolism of chlormadinone acetate. Ethanol, which was used as the drug vehicle, did not improve or depress the absorption of either steroid. Tissue accumulation experiments indicated that the jejunum of the intestine and the pyloric and cardiac area of the stomach accumulate the steroids to a greater extent than other GI tissues. Both compounds exhibited biexponential absorption with significant membrane accumulation.

Keyphrases ☐ Chlormadinone acetate and norethindrone—absorption from in situ rat gut ☐ Norethindrone and chlormadinone acetate—absorption from in situ rat gut ☐ Absorption, GI—norethindrone and chlormadinone acetate, in situ rat gut

Although the oral administration of steroids is common today, relatively little is known about their GI absorption characteristics. Most published studies measure absorption indirectly by plasma or urine levels of a steroid and its metabolites. The present investigations were undertaken to study the GI absorption of two progestins, chlormadinone acetate and norethindrone, and to develop an animal model for these studies.

Some investigators have used the in vitro rat intestine preparation (1, 2) or the everted intestinal sac method (3, 4). The absorption rates measured in these experiments are not influenced by blood and blood pressures, lymph, nerves, and GI secretions. Levine et al. (5) reported the loss of structural integrity and cellular death occurring in the intestinal mucosa within 10–15 min. after the preparation of an everted intestine. Consequently, the only observation that can be made is the movement of drug across a semiviable membrane.

Searching for a less traumatic intestinal preparation, Schanker et al. (6) used in situ perfusion techniques consisting of a single pass or a recycling of the drug solution through the cannulated intestine. Doluisio et al. (7) described an in situ technique with duodenal and ileal ends of the intestine cannulated with L-shaped cannulas. Stopcocks and syringes were attached to both cannulas for instilling and sampling the drug solution. Although Doluisio et al. reported very reproducible results, this preparation was found cumbersome by the present author.

Only a few experiments involving steroid intestinal absorption in animals have been published. Symchowicz

et al. (8), using in-dwelling intestinal cannulas, studied the absorption of betamethasone in various sections of the dog gut. Meli *et al.* (9) studied the possible active transport of estrogens by the rat small intestine, and Collins and Forist (10) inferred the absorption properties of hydrocortisone in the dog from plasma levels. The most comprehensive study was reported by Schedl and Clifton (11) on the absorption of 22 androgens, estrogens, and corticosteroids. Their data were obtained with the rat gut intestinal perfusion technique; the percentage of absorbed steroid was independent of concentration over a wide range. It was concluded that steroids seem to be absorbed by passive diffusion.

In this paper, the absorption characteristics of chlormadinone acetate and norethindrone from the GI tract are presented in detail. The animal preparation described is a simple and an improved *in situ* method. Intestinal and gastric absorption rates can be described by sampling the lumen contents as well as the bile and plasma with a minimum of apparatus and trauma to the animal.

EXPERIMENTAL

Animal Preparation—Sprague-Dawley female rats, weighing 200-250 g., were fasted 16-18 hr. with water *ad libitum* prior to surgery. They were housed in false-bottom cages to prevent coprophagy. The animals were anesthetized with pentobarbital, 50 mg./kg. i.p., and the viscera were exposed by midline abdominal incision. To prevent enterohepatic circulation, the bile duct was cannulated with 11 cm. of polyethylene tubing[1]. The portal vein was cannulated to permit blood sampling (12), and the pylorus was closed with clamps or sutures. In some experiments, the jejuno-ileal junction was ligated. In experiments in which the jejunum was not restricted, 10 ml. of the drug solution was instilled into the duodenum with a 23-gauge needle and a disposable syringe at the rate of 5 ml./min. In experiments that were restricted to certain ligated sections, the amount of solution varied, depending on the volume required to fill, but not distend, the intestinal section, usually 3-5 ml. The gut was not rinsed previous to the instillation. To sample the intestinal contents, a 27-gauge needle was inserted through the intestinal wall along the ligated section, and at each time point a 0.1-ml. sample was withdrawn into a tuberculin syringe. Intralumenal bleeding was not a problem, because care was taken not to sample in the vascular lateral regions of the intestine. The bile samples, approximately 0.1 ml., were an accumulation of flow over 5-min. intervals. Blood samples of 0.3 ml. were taken from the portal vein at specific times, and 0.1 ml. of plasma was used for the drug assay.
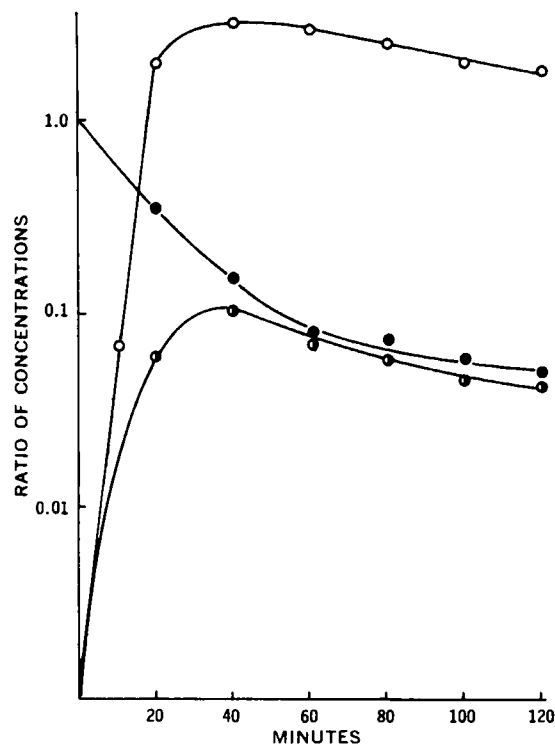
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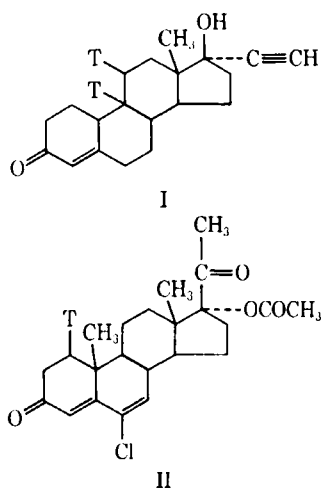
Figure 1—*Time course of norethindrone absorption in the intestine* (●) *and its appearance in the plasma* (◑) *and the bile* (○). *Ratio of concentrations is calculated in terms of* (*drug at* $t_n/0.1$ *ml. sample*)/ (*drug at* $t_0/0.1$ *ml. sample*). *Norethindrone is highly concentrated by the liver and, consequently, the rise above 1.0 is not unreasonable.*

For gastric absorption studies, the stomach was exposed. The muscular area was made ready for fistulation by purse string sutures in the least vascular pyloric area. An incision was made through the muscle, and a polyethylene cannula was inserted and secured by purse string sutures. The pylorus was clamped securely, the animal's head was raised slightly, and 2.5 ml. of the drug solution was instilled through the cannula into the stomach. Samples of 0.1 ml. were taken through the cannula at regular intervals. Throughout the experiments, the viscera were kept warm with a high-intensity lamp and moist with gauze and saline.

Drug Solutions—The compounds studied were the tritiated progestogens, norethindrone (I) and chlormadinone acetate (II). Tritiated steroid was coprecipitated with unlabeled steroid in cold acetone and water to make a specific activity of 4 mc./mmole. The dose per rat was 0.4 mg./kg., approximately 30 times the normal human dose. Because of the low aqueous solubility of these steroids, the 100-mcg. dose was dissolved in 1 ml. absolute ethanol. Just prior to the experiment, the alcohol solution was diluted with 9 ml. of Sørensen's buffer, pH 6.3, or 9 ml. of Clark and Lub's potassium chloride-hydrochloric acid buffer, pH 1.6, and made isotonic.

Water diffusion out of the intestine has a considerable effect after 45-60 min. Phenol red was used as a standard to correct for changes in steroid concentration resulting from water absorption (13).

The intestinal drug solution volume was 10 ml., although solutions of 8, 5, and 3 ml. were also used depending on the length of the intestinal section studied. The final ethanol concentration was 10%. In gastric studies, 2.5-ml. solutions of varying ethanol concentration were used. A double-tracer experiment was used to follow the absorption of ^{14}C-ethanol and ^{3}H-steroid simultaneously.

All samples of lumen contents, bile, and plasma were added to Bray's solution (14) and counted on a scintillation counter[2]. Several bile samples were pooled and extracted with ether. The drug remaining in the water phase was considered to be metabolite. No further determination of the metabolites was made. All data reported are total radioactivity. To account for quenching, a "spike" of ^{3}H-toluene was added to each vial and the vial was recounted. The

[1] Clay-Adams Intramedic PE 50 polyethylene tubing.

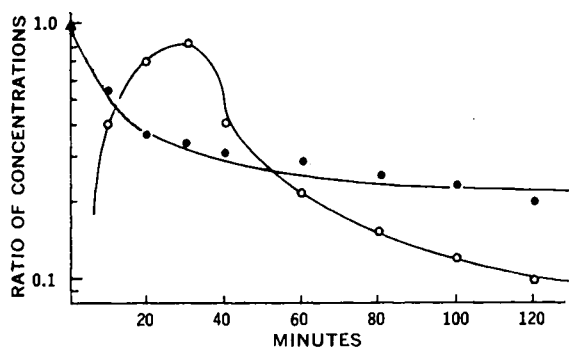[2] Packard Tri-Carb scintillation counter, model 3365.

Figure 2—*Time course of norethindrone in the stomach (●) and its appearance in the bile (O).*

disintegrations per minute and amount of drug recovered from each sample were calculated and used to determine the percent dose remaining in the gut or entering the bile.

Site of Accumulation—Experiments were done to determine if steroids are absorbed uniformly in all sections of the GI tract. Drug solutions (25 mcg./2.5 ml. or 100 mcg./5 ml.) were instilled into the stomach or the ligated duodenum, jejunum, or ileum. The solutions remained in the stomach 30 min. before the rat was sacrificed. The stomach was removed, washed, and divided into four parts: cardiac, pyloric, and two fundus portions. In the intestinal experiments, the animals were sacrificed 1 hr. after the drug solution was instilled into the ligated section. The intestine was excised, washed with saline, and cleaned of mesentery and fat. The intestinal sections were divided into 1-cm. pieces.

All tissue sections were dried overnight at 60° and combusted using a tissue oxidizer[3]. The samples were assayed for tritium by scintillation counting.

Bile Effect—The intestinal absorption of each steroid was studied with and without bile duct cannulation. To investigate the effects that bile might have in drug absorption, 1 ml. of the exogenous bile salt, sodium dehydrocholate (100 mM), was used to stimulate bile flow in uncannulated animals. The bile salt was administered by placing the solution in the exposed peritoneal cavity beneath the portal vein immediately prior to injecting the drug solution. Drug was then instilled into the duodenum; bile and lumen samples were taken as previously described.

To extend the bile study, dual bile duct cannulation experiments were devised. Two rats were prepared as described in the previous section and both bile ducts were cannulated. Rat 1 supplied fresh bile to Rat 2 by placing the first cannula into the duodenum of Rat 2. In the first experiment, drug solutions were instilled into the duodenum of Rat 2 with bile samples collected from Rat 2. In the second experiment, drug solutions were placed in the duodenum of Rat 1 with a few bile samples from Rat 1 and continuous bile sampling from Rat 2.

Binding Affinities of Steroids to Gastric Juices—Human gastric juice and gastric mucin[4] were used to determine the possible effect of steroid binding on absorption characteristics. Human gastric juice (pH 1.6) was obtained from hospital patients undergoing surgery for other than GI ailments. Simulated gastric fluid was made from 2.6 g./l. mucin, 2.0 g./l. NaCl, and 80 ml./l. concentrated HCl. The final pH was 1.3. By using a microequilibrium dialysis apparatus, 100 μl. of drug solution in Sørensen's buffer was placed in one well-half and 100 μl. of binding substance was placed in the other, separated by cellulose dialysis membrane. The drug solutions ranged from 10^{-10} to 10^{-6} M.

The dialysis apparatus was shaken for 24 hr. at 37°. Samples of 50 μl. were taken from each well-half with a syringe[5]. The samples were placed in Bray's solution for scintillation counting. The binding constants and bound-to-free ratios were determined by a computer program written from Scatchard's original binding calculations (15). Human serum albumin was used as a standard.

Partition Coefficient—The partition coefficients of ³H-chlormadinone acetate and ³H-norethindrone were determined in a
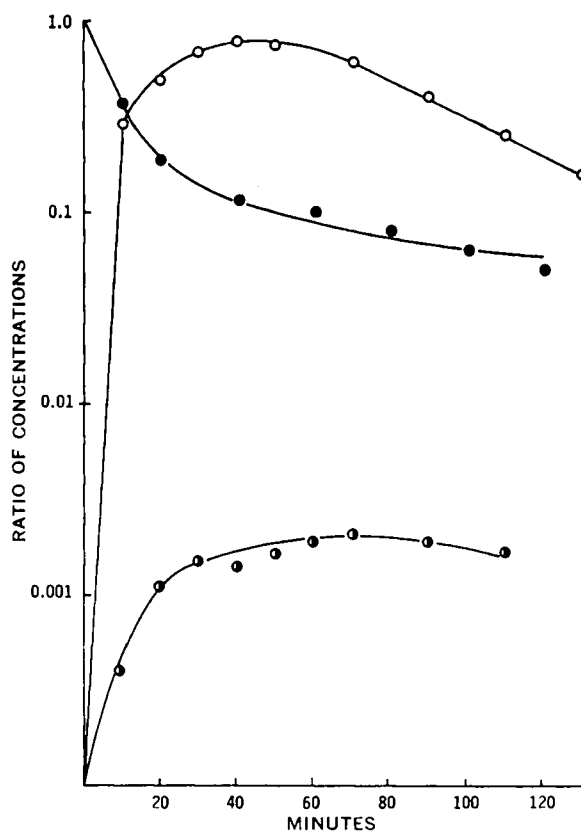

Figure 3—*Time course of chlormadinone acetate in the intestine (●) and its appearance in the plasma (◑) and the bile (O).*

two-phase system of 10% ethanol-Sørensen's buffer at pH 6.3 and isopropyl myristate. The phases were first equilibrated at 37° to ensure saturation of each phase with the other. The drug solution (0.1 μc. in 1 μl. ethanol) was added to the ethanol-buffer solution. The phases were shaken 5 min. and stored overnight at 37°. Duplicate 1-ml. samples were taken from each phase and assayed by scintillation counting. Four separate determinations were made.

RESULTS

In the present experiments, the absorption of two steroids from the stomach and small intestine of the rat was studied under physio-
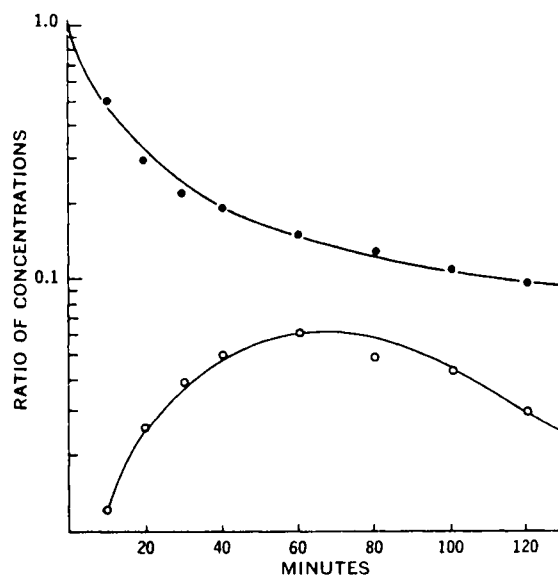

Figure 4—*Time course of chlormadinone acetate in the stomach (●) and its appearance in the bile (O).*

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[3] Packard Tri-Carb oxidizer.
[4] Nutritional Biochemical Corp., Cleveland, Ohio.
[5] Hamilton.

Table I—Intestinal and Gastric Absorption Data

Drug and Vehicle	Animal Number	$t_{1/2}L$, min.	$t_{1/2}B$, min.	Time to Apparent Second Phase, min.	Time to Bile Peak, min.	Plasma Maximums, mcg./ml. (min.)
Intestinal						
Norethindrone and	1	5	8	21	20	0.95 (20)
10% ethanol–buffer	2	6	8	24	15	1.1 (20)
	3	5	7	23	21	0.92 (15)
	4	8	8	24	20	0.98 (20)
Chlormadinone	2	4	—	10	—	0.31 (30)
acetate and	5	4	10	15	15	0.29 (30)
10% ethanol–buffer	6	3	12	10	20	0.25 (35)
	8	4	10	11	18	0.48 (33)
	10	3	11	13	18	0.20 (35)
	11	4	10	14	15	0.19 (35)
Gastric						
Norethindrone:						
10% ethanol–buffer	5	6	11	30	15	
	2	11	14	30	30	
	3	13	14	30	30	
5% ethanol–buffer	1	10	—	30	40	
	4	13	36	45	45	
0% ethanol–buffer	6	11	15	15	15	
Chlormadinone acetate:						
10% ethanol–buffer	1	16	—	45	—	
	2	7	na[a]	45	55	
	5	10	na[a]	45	30	
20% ethanol–buffer	6	6	na[a]	45	60	
30% ethanol–buffer	8	6	60	45	60	

[a] na = not attained.

logical conditions. The absorption curves showed that the absorption of these progestins was not described by simple first-order kinetics but was biexponential in all sections of the gut. This absorption was not only dependent on the concentration of drug in any one tissue compartment but was also regulated by the membrane structure, apical and basal; by the mesentery blood flow, pressure, and volume; and by tonicity of the instilled solution.

Chlormadinone acetate and norethindrone were readily absorbed from both the intestine and the stomach, as measured by the appearance in the plasma and bile and by the disappearance from lumen and gastric contents. Both steroids showed biexponential disappearance from the gut.

As shown in Fig. 1, when 0.1 mg. norethindrone in 10% ethanol–buffer solution was instilled into the intestinal lumen, the time to reach the apparent second phase of absorption was approximately 20–25 min. The maximum concentration of drug in the bile was also found within this time range. The time required for the original drug concentration to decrease to half in the lumen $(t_{1/2}L)$ was less than 10 min.; 8–10 min. was required for one-half the original drug concentration to appear in the bile $(t_{1/2}B)$. These data were corroborated by experiments conducted in another laboratory using the Doluisio technique[6]. The $t_{1/2}L$ was 10 min. in those intestinal absorption experiments.

In the stomach, the absorption of norethindrone was not as rapid or as complete as from the intestine (Fig. 2); $t_{1/2}L$ ranged from 10 to 13 min., and $t_{1/2}B$ was about 15 min. The time to reach the apparent second phase of lumenal absorption was 20–30 min., and the peak drug concentration in the bile appeared at 30–40 min.

Chlormadinone acetate was absorbed slightly more rapidly than norethindrone (Figs. 3 and 4). In the intestine, the $t_{1/2}L$ was less than 5 min. and the time to reach the apparent second phase was 10–15 min. The bile concentration peaked at 15–20 min., with one-half the original drug concentration appearing in the bile from 9 to 12 min. Chlormadinone acetate absorption from the stomach was considerably less than from the intestine. The $t_{1/2}L$ ranged from 6 to 16 min. The drug concentration in the bile reached a maximum value at 50–60 min., but the biliary concentration did not reach one-half the original lumen concentration. This low response of the liver to the

clearance of chlormadinone acetate from the blood is quite remarkable when compared to the rapid $t_{1/2}B$ reported for norethindrone. Table I is a summary of the absorption data.

From the lumenal and plasma data, rate constants were determined. The feathering method was used to determine the rate constants from graphical estimations as shown in Fig. 5.

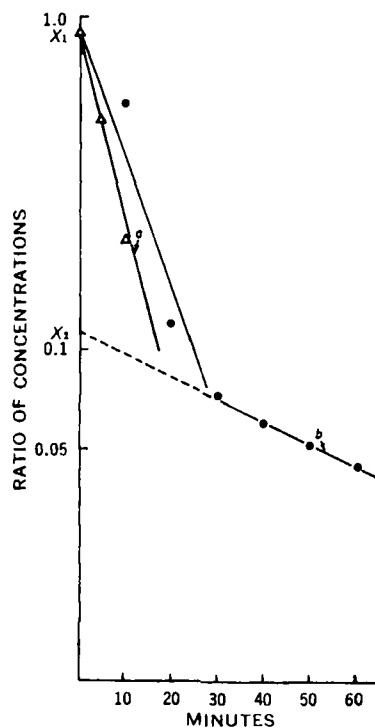There is apparently no unique site of absorption for these steroids

Figure 5—Feathering procedure. The slope of line a is determined by extending the second phase b and subtracting second phase values from the first phase values.

Table II—Site of Accumulation

Section	Disintegrations per Minute Ratio[a]
Intestinal	
Duodenum	4-9
Jejunum	2-3
Ileum	1
Gastric	
Cardiac	538-563
Pyloric	473-491
Fundus	1

[a] The ratios include values for ³H-norethindrone and ³H-chlorma-dinone acetate obtained from eight rats. Both steroids behave similarly with respect to the location of accumulation.

in the stomach or in the intestine, but there is a possibility that the stomach is more selective. Compared to the fundus sections, the pyloric and cardiac regions of the stomach exhibited considerable affinity to norethindrone and chlormadinone acetate. The large amount of drug absorbed by the pyloric region is easily understood because of the ample supply of blood and the binding properties of both drugs to the gastric fluids produced there. This does not explain the high accumulation observed in the less vascular area of the cardiac region. In the intestine, the duodenum showed greater affinity for the steroids than did the jejunum, and the ileum absorbed drugs to the least extent. Along with the tissue combustion data and the biexponential disappearance of the steroids from the lumen, these results led to the conclusion that accumulation or storage of the drugs in the intestinal or gastric membranes was occurring. This can account for the rapid distribution of drugs from the lumen solution into the membrane, with a slower appearance of the drug into the plasma (Table II).

The binding studies (Table III) were designed to show any correlation between absorption rates and affinities of steroids to gastric juice and gastric mucin. It is obvious from these data that chlormadinone acetate bound much more to the gastric mucin and natural gastric juice than did norethindrone. Binding of norethindrone appears to occur mainly with gastric mucin, whereas the binding of chlormadinone acetate is only partially accounted for by mucin. Chlormadinone acetate possibly binds to other components of
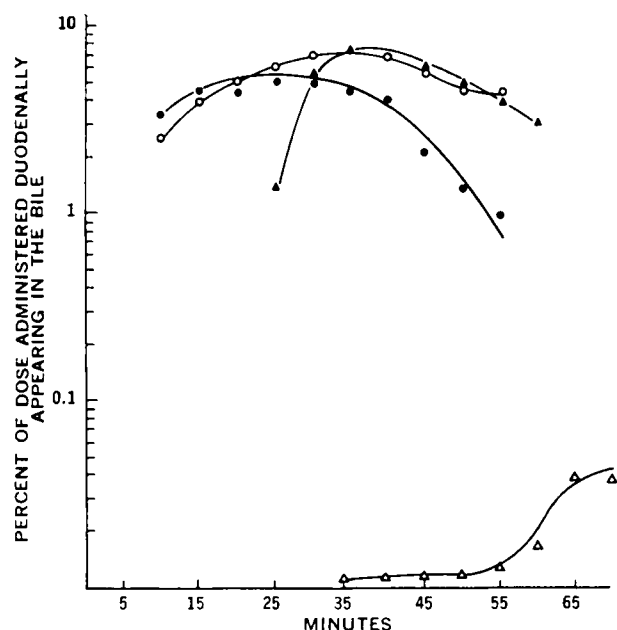
Table III—In Vitro Binding Properties of Chlormadinone Acetate and Norethindrone

Drug	Binder	Ratio Bound/Free	Percent Bound
Norethindrone	0.4% Human serum albumin	0.92	48.0
		1.09	52.2
		1.38	58.1
	Natural gastric juice	0.07	6.6
		0.10	9.2
		0.13	11.4
	Synthetic gastric juice	0.08	7.0
		0.04	3.8
		0.18	14.9
Chlormadinone acetate	0.4% Human serum albumin	1.19	54.3
		1.31	56.7
		1.43	58.8
	Natural gastric juice	0.68	40.6
		0.69	41.0
		0.83	45.6
		1.01	50.3
	Synthetic gastric juice	0.34	25.5
		0.41	28.9
		0.42	29.4

natural gastric juice such as chondroitin sulfate or other low molecular weight mucoproteins which serve as the "intrinsic factor."

Extending the in vitro comparison of chlormadinone acetate and norethindrone in GI absorption, the partition coefficients of the two steroids were found to be 65 for chlormadinone acetate and 48 for norethindrone. The partition coefficient data support results previously given in this paper which imply that chlormadinone acetate is absorbed to a greater extent and at a faster rate than norethindrone in gastric preparations. The in vivo absorption rates did not indicate such a large difference as might be expected by the differences in the partition coefficients and binding affinities. Consequently, the estimation of in vivo absorption rates from the partition coefficient and other in vitro methods should be made with caution.
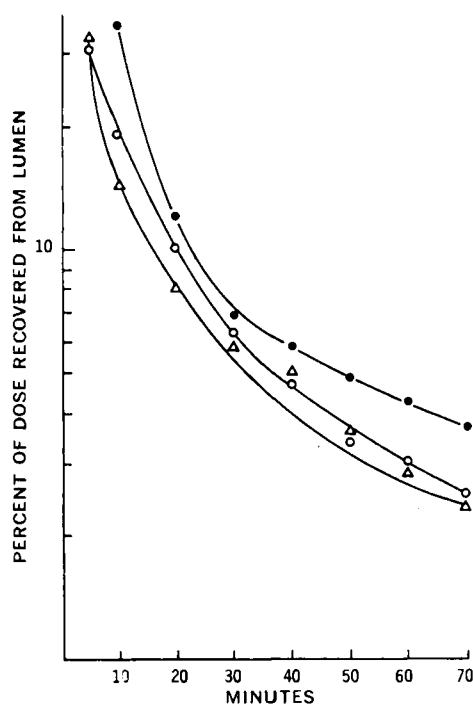

Figure 6—Metabolite appearance in the bile. No change was seen in metabolite output when norethindrone was instilled into the duodenum with and without bile (●, dual cannulation; ▲, bile cannulation). When chlormadinone acetate was instilled in the presence of bile, the drug was metabolized to a great extent (O, dual cannulation; △, bile cannulation).

Figure 7—Intestinal absorption of norethindrone under various conditions. Key: O, no bile cannulation or stimulation; ●, animals with bile duct cannulation; and △, animals with exogenous sodium dehydrocholate stimulation. The intestinal absorption is affected only slightly.

It is recognized that bile salts play an important role in the intestinal absorption of lipids and other substances and that they themselves are absorbed by active transport in the ileum. Because of the bile cannulation in the preparations, absorption was unaided by bile salts in most experiments. However, several experiments were devised to ascertain the effects of bile on chlormadinone acetate and norethindrone absorption. The bile appeared to have a significant effect on the absorption and metabolism of chlormadinone acetate, but it did not appear to influence norethindrone. As seen in Fig. 6, bile greatly enhanced the output of chlormadinone acetate or its metabolite from the liver. In cannulated animals, less than 1% radioactivity was recovered in the bile as compared to 40% recovered after the intestine received fresh bile from the donor rat. Rates of lumenal absorption were not altered significantly by the presence or absence of bile. Bile flow stimulation through the use of exogenous sodium dehydrocholate did have an effect on the lumenal absorption rates of chlormadinone acetate and norethindrone, but the enhanced absorption was not significant (Fig. 7).

These results are in contrast with data obtained previously (16) using sulfadiazine, which showed the exogenous stimulation greatly increased the bile acidity and consequently the solubility of sulfadiazine. This result possibly increased the absorption of the drug. Kakemi et al. (17) found from perfusion experiments that unionized and poorly absorbed drugs are hardly affected by bile salts. This is consistent with the findings on the two progestins. Kakemi et al. also suggested that any enhanced absorption of poorly absorbed drugs may be due to the bile salt action on the mucosal membrane and not to the direct drug–bile salt interaction.

Because the steroids were administered in an alcoholic solution, experiments were conducted to determine the effect of the vehicle. The percent of ethanol in the drug solutions was varied, and simultaneous ^{14}C-ethanol–^{3}H-drug solution absorption curves were plotted. In the gastric preparations, the variation of ethanol in the drug solutions did not have an effect on the drug absorption; the "solubilizing function" proposed by Tappeiner (18) in 1880 was ruled out. In some instances, however, there appeared to be a delay in the appearance of metabolites in the bile. Ethanol is known to cause irritation to organic tissue. In concentrations exceeding 15% in the stomach, the solution was so strong that marked congestion and ulceration often occurred. It is possible, therefore, that the delay in metabolite appearance was the result of ethanolic irritation of the liver and interaction with drug metabolism as well. In the few experiments done, no evidence was found to indicate any difference whatsoever in the intestinal absorption of chlormadinone acetate and norethindrone using ethanol concentrations of 0–15%. Magnussen (19) reported that ethanol aids gastric absorption of only a few drugs, these drugs being already well absorbed without ethanol.

The absorption of the drugs is not affected by the presence or absence of ethanol or bile. Chlormadinone acetate does show greater binding properties, with higher partition coefficient values and greater general intestinal absorption, than norethindrone. According to Schedl and Clifton (20), the absorption is predictable from the chemical structure: the less polar the steroid, the greater is the absorption rate. The wall of the intestinal wall is lipoidal and substances that are lipid soluble pass into the wall more readily than do some hydrophilic compounds. The structures of norethindrone and chlormadinone acetate are so similar that this comparison is difficult to make. However, chlormadinone acetate is slightly more hydrophobic, resulting in the small differences seen in the GI absorption of the two steroids.

The data from the intestinal absorption experiments show a biexponential profile which suggests that the drug is rapidly distributed between the gut lumen and the intestinal wall, followed by a slower release of the drug from the membranes into the blood. The initial absorption of the drugs into the intestinal membranes and the consequent distribution into the blood is not simple first-order kinetics. The absorption did appear to be via diffusion without an energy requirement or the mediation of an enzyme system.

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