Observations on the Smooth Muscle and Contractile Activity of the Common Bile Duct

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THE EARLY American and European literature containing descriptions of the smooth muscle in the extrahepatic biliary system was summarized in 1898 by Hendrickson.[19] Controversy was evident, with some investigators even questioning the presence of a muscular tunic in the gallbladder. Hendrickson demonstrated longitudinal and transverse fibers of smooth muscle in varying arrangements throughout the entire biliary tree of the dog, rabbit and man. Burden,[6] in 1925 noted both longitudinal and circular smooth muscle bundles in the human common bile duct. From an anatomic standpoint, he felt that peristalsis in the human bile duct was possible.

More recently, Myers *et al.*[29] did not see any smooth muscle in microscopic sections of the human common bile duct. Coupling this with cinefluorographic observations, they concluded that the common bile duct of man was a passive, connective tissue conduit. On the other hand, Burnett and Shields [8] described longitudinal smooth muscle in the human bile duct, and with the aid of the image intensifier observed peristaltic waves progressing toward the duodenum at the rate of 3 to 5 per minute. In view of these diverging opinions the present study was undertaken in man, monkey and dog.

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Methods

Histology. Specimens of the extrahepatic biliary tree were obtained at necropsy from 20 patients without hepato-biliary disease. Similar specimens from 13 dogs of mixed breed and 6 Macaca mullata monkeys were used. All tissues were fixed in 10% formalin and serial sections obtained in both the circular and longitudinal axes. The common bile ducts were stained with one or more of the following stains: hematoxylin, eosin and saffron; Masson's trichrome; hematoxylin phloxine and saffron: Heidenhain's aniline blue; Verhoeff's elastic stain.

In Vitro. Thirty-two Macaca mullata monkeys and 6 dogs of mixed breed were anesthetized with sodium pentobarbital, 30 mg./Kg. intravenously. Some of the monkeys had been previously utilized in pharmacologic experiments, but were considered essentially normal as they had stopped receiving drugs several months prior to this study. The supraduodenal portion of the common bile duct from immediately distal to the cystic duct to just proximal to the sphincter was carefully excised. The entire specimen was suspended in the longitudinal axis in a 100-ml. isolated organ bath at 37° centigrade. The bath contained a balanced salt solution ** which was supplied with 95% O_2–5% CO_2 mixture. The re-

** Electrolyte composition of one liter of salt solution: NaCl—7 Gm., KCl—0.354 Gm., $CaCl_2 \cdot 2H_2O$—0.350 Gm., $MgSO_4 \cdot 7H_2O$—0.147 Gm., $NaHCO_3$—2.1 Gm., K_2HPO_4—0.081 Gm., glucose—0.9 Gm.

cording lever consisted of two small foil strain gages *** bonded to a previously tempered 0.008-inch-thick 2% beryllium-copper clip. This unit was firmly supported at one end, while the opposite end contained the suture suspending the tissue in the bath, allowing essentially isometric contractions to be recorded. Lead wires from the strain gages were connected to an oscillograph † for recording contractions.

Results

Man. Well defined layers of smooth muscle as seen in the wall of the intestinal tract do not exist in the common bile duct. In the upper one-third of the duct, there are only sparse longitudinal muscle fibers. In the mid-portion of the duct the fibers become more prominent, and as one progresses toward the Sphincter of Oddi the muscle becomes organized into a longitudinal layer (Fig. 1). Very little, if any circular smooth muscle is present. The longitudinal muscle fibers are surrounded by abundant collagenous connective tissue. Fine fibers of elastic tissue are diffusely distributed through the wall. In summary, the common bile duct of man is composed of an inner epithelium, a subepithelial layer of scattered longitudinal smooth muscle, elastic and collagenous connective tissue and an outer loose connective tissue layer.

Monkey. Smooth muscle is present throughout the length of the common bile duct. It is in the subepithelial layer and is more abundant than that which occurs in man. The muscle is primarily longitudinal in orientation (Fig. 2), although, as one approaches the sphincter circular fibers can be found. A similar amount and diffuse distribution of elastic tissue as seen in man is present in the monkey's common bile duct. The remainder of the subepithelial layer consists of collagenous connective tissue. Although no attempt was made to delineate the pattern of nervous tissue in the duct, cells of autonomic ganglia could readily be seen with the stains used.

Dog. The majority of the canine common bile ducts have a subepithelial layer devoid of significant smooth muscle (Fig. 3). Only rarely can a few fine fibers of smooth muscle be seen. The wall is primarily collangenous connective tissue with somewhat more elastic tissue than demonstrable in the other species.

In Vitro. None of the common bile ducts obtained from the dog exhibited spontaneous contractions. Repeated washings and the utilization of drugs failed to induce contractile activity. Most significant, was the absence of contraction to a strong solution of potassium chloride. It must be concluded from this evidence and the microanatomical appearance that the dog's bile duct does not contain a significant amount of smooth muscle.

In contrast to this, the monkey's common bile duct exhibits an excellent and characteristic contractile pattern (Fig. 4). Once placed in the isolated organ bath with the aforementioned salt solution, the bile duct requires a mean of 18.2 ± 3.9 minutes before it begins spontaneous contraction. The mean duration of spontaneous activity once initiated is 168.7 ± 20 minutes. The frequency of contraction is slow, having a mean value of 1.8 ± 0.1 per minute. The mean maximum amplitude developed during spontaneous activity was 39.7 ± 4.2 mm. At the termination of each experiment the muscle cells of the bile ducts were depolarized with potassium chloride producing a mean maximum amplitude of 42.4 ± 4.6 mm. In comparing this value with the mean maximum amplitude achieved during spontaneous activity we see that the ducts were capable of producing a near-maximal response during the time of spontaneous contraction.

*** MD-DY-031-DE-350 Micro-Measurements, Inc., Romulus, Michigan.

† Offner type R Dynograph, Spinco Division, Beckman Instruments, Lincolnwood, Illinois.

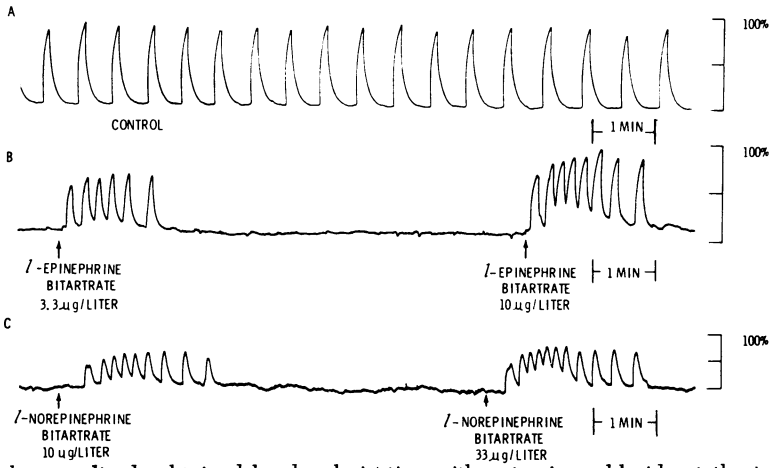

FIG. 4. Control and catecholamine induced responses of isolated monkey common bile duct. Control record (A) is typical of spontaneous contractions at a frequency of 1.8 per min. Responses to *l*-epinephrine (B) and *l*-norepinephrine (C) were obtained following subsidence of the spontaneous activity. In this and subsequent figures; A, B, and C refer to records from separate preparations; drug concentrations are expressed in terms of the salt; % amplitude is related to 100% being that amplitude obtained by depolarization with potassium chloride at the termination of each experiment.

The use of strain gages in the recording lever allows a reasonable estimation of the force developed by a contraction. Calibration of the strain gage is obtained by suspending several known weights and calculating the grams necessary for one millimeter of pen deflection. With this technic a mean value of 14.3 ± 1.5 grams is the total force developed by the monkey common bile duct during a near-maximum contraction.

Utilizing the interval of time (peak time) from the onset of a contraction to the point of maximum amplitude, an expression of contractile velocity is achieved. Although the data is insufficient for a detailed analysis, in general contractions of large amplitude occurred with a fast frequency and a fast velocity of contraction. As an example, a 60-mm. contraction with a frequency of 2.5 per minute had a peak time of 4.8 seconds while a 30-mm. contraction at 1.2 per minute had a peak time of 8.4 seconds. During the course of spontaneous activity this smooth muscle does vary somewhat in frequency and the force of contraction. Having characterized several parameters of the monkey's spontaneous common bile duct contractions, a limited pharmacologic evaluation was performed on these preparations.

This smooth muscle is stimulated by the catecholamines. Figure 4 shows the response to *l*-epinephrine bitartrate and *l*-norepinephrine bitrate * in common bile ducts whose spontaneous contractions have ceased. These drugs consistently produce a burst of contractions superimposed on an increase in tone. The threshold concentration of *l*-norepinephrine bitartrate is in the range of 10 to 33 µg./liter. Both duration and amplitude of the response are dose dependent. Tachyphylaxis develops quickly upon repeated administration of the same concentration of the drug, however, increasing the concentration restores the response. No significant difference between *l*-epinephrine and *l*-norepinephrine was evident, however, *l*-isoproterenol in levels up to 500 µg./liter was given to several preparations with no evidence of a response.

Three monkeys received reserpine acetate 1 mg./Kg. I.M. 24 hours prior to use. Two other monkeys received guanethidine 10 mg./Kg. I.M. for two days. One of the animals who was pretreated with guanethidine exhibited spontaneous bile duct contractions for only 28 minutes. All of the

* All drug concentrations used in this study are expressed in terms of the salt.

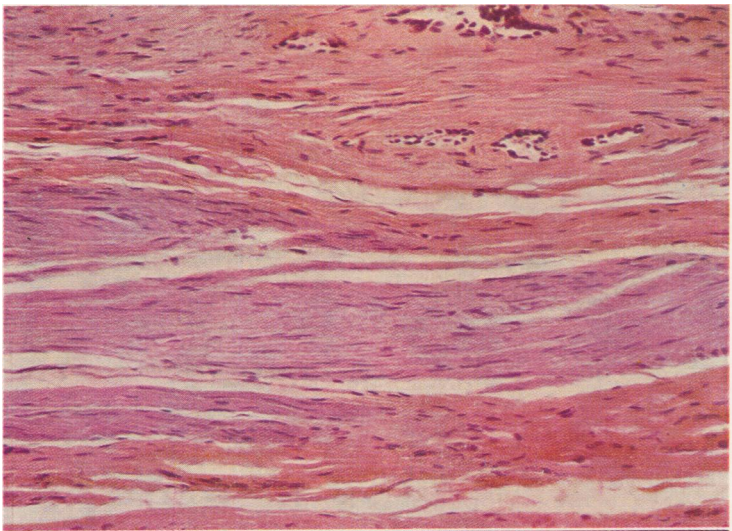

FIG. 1. (Top) Human common bile duct. Longitudinal section; hematoxylin, eosin, and saffron. Note the subepithelial position of the longitudinal smooth muscle fibers. Significant circular smooth muscle is not found in this structure. (Majority of epithelium absent due to postmortem change.)

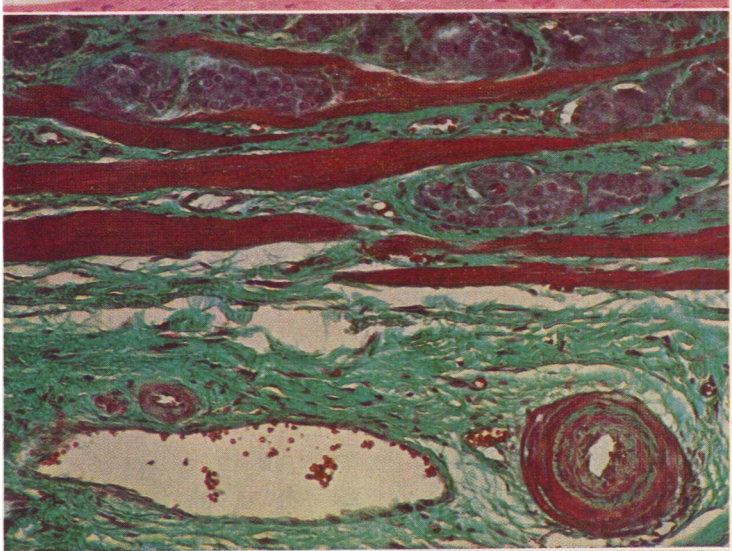

FIG. 2. (Center) Macaca mullata common bile duct. Longitudinal section; Masson's trichrome. The longitudinal smooth muscle bundles are located subepithelially as in man. The amount of smooth muscle is somewhat more than seen in the human bile duct.

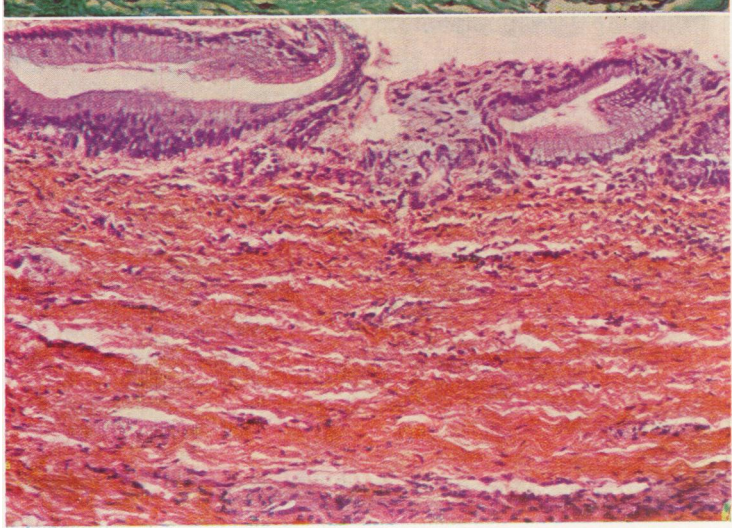

FIG. 3. (Bottom) Canine common bile duct. Longitudinal section; hematoxylin, eosin, and saffron. This is the usual collagenous architecture of the bile duct wall. There are no circular smooth muscle fibers and only rarely can small longitudinal muscle fibers be seen.

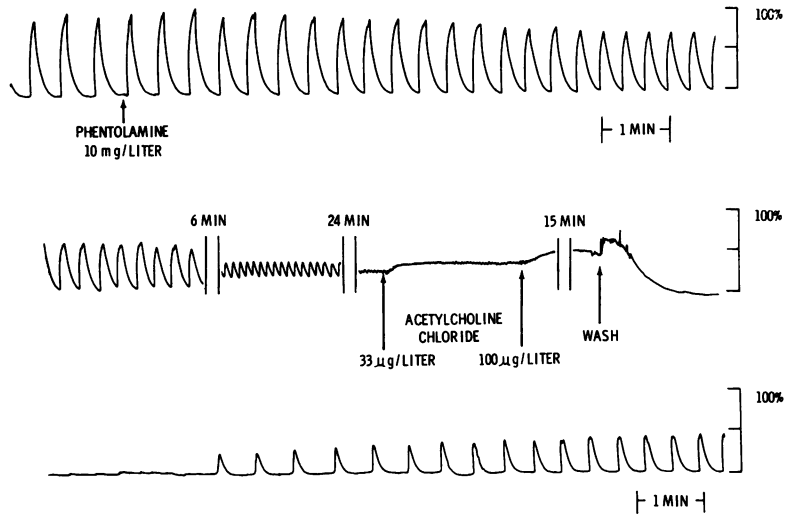

FIG. 5. The effect of phentolamine on the spontaneous contractions of the monkey common bile duct. Note that with phentolamine blockade in force, acetylcholine is unable to induce contractions. Phentolamine is readily washed from the bath with return of spontaneous contractions within 10 minutes. (Calibration as in Figure 4.)

other bile ducts failed to display the usual spontaneous contractile activity, indicating that this contractile system is very sensitive either to catecholamine depletion or adrenergic anti-release treatments alone.

With the indication that the duct was principally innervated by an adrenergic system, the response to phentolamine methane-sulfonate was tried. Upon addition of 10 mg./liter of this drug to the bath, spontaneous contractions of various preparations gradually diminish over a period of 10 to 78 minutes until 2 per cent or less of the activity remains (Fig. 5). When phentolamine is washed from the bath, spontaneous contractions readily return in a period of 5 to 10 minutes.

Acetylcholine chloride also causes stimulation of the bile duct muscle (Fig. 6). The amplitude and duration of response are dose dependent, with a threshold level between 1 and 3.3. µg./liter. If a spontaneously contracting duct is allowed to stop, and is then stimulated with acetylcholine, this induced contractile activity is blocked by phentolamine (Fig. 6). Also, when spontaneous contractions have been stopped with phentolamine, acetylcholine is unable to induce contractions while the adrenergic receptor blockade is in effect (Fig. 5). Of further interest is the fact that one reserpinized preparation did not respond to acetylcholine, and another required 33 µg./liter for a poor low amplitude, 60 second response.

Hexamethonium chloride in a concentration of 10 mg./liter was given to three common bile ducts shortly after the onset of spontaneous contractions (Fig. 7). There was no effect and these preparations continued to contract spontaneously for a mean of 164.6 ± 4 minutes after administration of the drug. This mean duration of spontaneous contraction is not significantly different from that obtained in the control ducts (168.7 ± 20 minutes).

Atropine sulfate at a level of 10 µg./liter abolishes the spontaneous contractions of the bile duct (Fig. 7). When this drug is washed from the bath, administration of the same concentration again blocks the spontaneuos activity indicating that tachyphylaxis does not develop in this situation. In several experiments, atropine sulfate 10 µg./liter was placed in the bath prior to the use of acetylcholine or *l*-norepinephrine. Atropine completely blocked the acetylcholine-induced response, whereas it

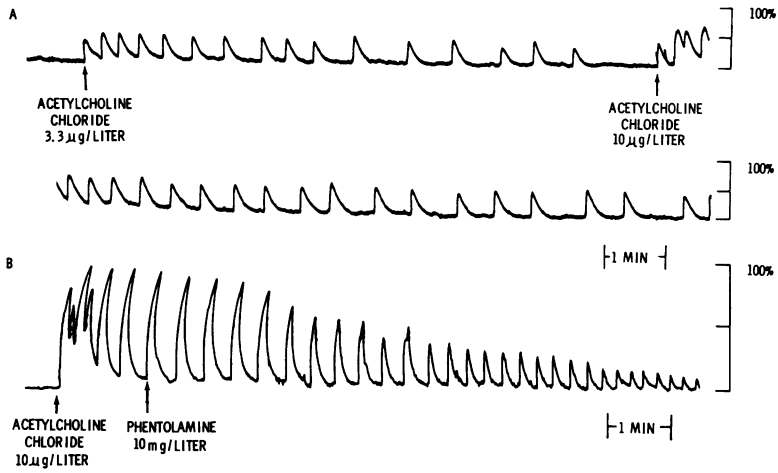

FIG. 6. Acetylcholine induced contractions of the monkey common bile duct. Compare the typical contractile response to acetylcholine (A), with the blockade of this expected long stimulation by phentolamine (B). (Calibration as in Figure 4.)

failed to block the contractile activity induced by *l*-norepinephrine (Fig. 8).

To further explore this contractile system, the unusual sympathetic ganglionic stimulant, McNeil-A-343,* was utilized. As Roszkowski [34] has shown, the effects of the drug are at the ganglionic level and are blocked by atropine but not by hexamethonium. McNeil-A-343 in a concentration of 10 to 33 µg./liter given to a spontaneously contracting common bile duct results in a distinct increase in contractile frequency (Fig. 7). When the common bile duct is allowed to contract spontaneously until the preparation becomes inactive, a larger dose (0.5 mg./liter) of McNeil-A-343 is now required to produce a contractile response. The McNeil-A-343 induced contractile response is blocked either by phentolamine

* McNeil-A-343, 4(*m*-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride, McNeil Laboratories, Inc., Philadelphia, Pennsylvania.

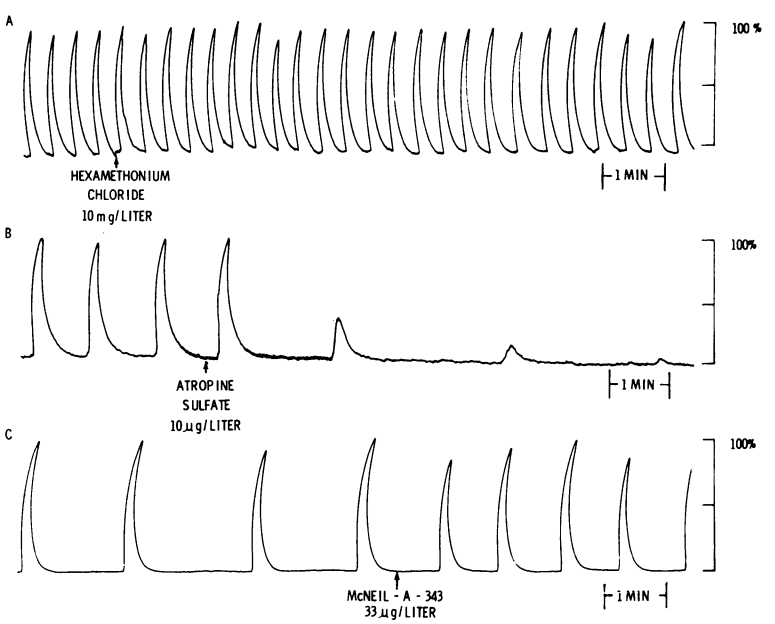

FIG. 7. Hexamethonium chloride in a concentration of 10 mg./liter has no effect on the spontaneous bile duct contractions (A), while atropine sulfate at a level of 10 µg./liter consistently abolishes the spontaneous activity (B). McNeil-A-343 given to a spontaneously contracting duct causes a significant increase in the frequency of contraction (C). (Calibration as in Figure 4.)

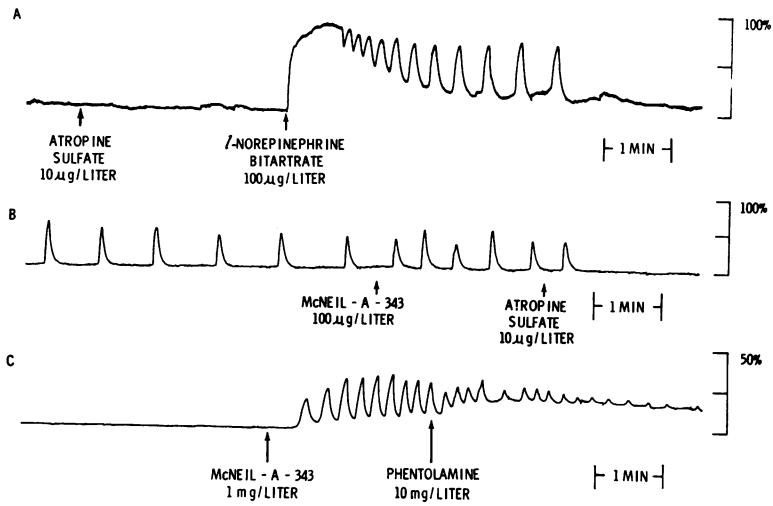

FIG. 8. *l*-Norepinephrine is capable of inducing a typical contractile burst in the presence of atropine sulfate blockade of spontaneous contractions. The contractile response induced by McNeil-A-343 is blocked by atropine (B) or phentolamine (C). (Calibration as in Figure 4.)

or atropine (Fig. 8). The common bile duct of a reserpinized monkey, as previously noted, exhibits no spontaneous contractions. In addition, McNeil-A-343 in a dose as high as 1 mg./liter has no effect on the common bile duct of a reserpine pretreated animal.

Several additional compounds were tested for activity on the common bile duct. Cholecystokinin * in levels up to 30 units/100 ml. produced no response. Histamine diphosphate in a concentration of 100 μg./liter results in a contractile stimulus. A burst of contractions superimposed upon a tone change is consistently seen with angiotensin at a level of 50 μg./liter. Vasopressin (Pitressin) does not always stimulate the duct to contract even when an excessive concentration of 6 units/100 ml. is attained.

Discussion

The microscopic anatomy of the common bile duct varies with the species being studied. Despite the fact that several investigators [3, 19] have described smooth muscle in the dog's common bile duct, the present study does not support this contention from an anatomical viewpoint. Further, the absence of significant smooth muscle in the dog's common bile duct is substantiated in this study from a physiological viewpoint, i.e., by the consistent inability to cause even the slightest contraction by depolarization with a saturated solution of potassium chloride in the isolated organ bath. On the other hand, definite longitudinal smooth muscle fibers are demonstrable in the common bile ducts of man and the Rhesus monkey. The amount of smooth muscle present in the human common bile duct is relatively small. In contrast to the relative sparseness of smooth muscle found in the human bile duct in this study; Burden [6] felt from an anatomic standpoint that the common duct was capable of peristalsis. Burden's opinion is open to question for the following reasons. In his description, muscle bundles were located in the loose connective tissue or outer advential layer. From this location and the photomicrograph in his paper, the author concludes that he was in fact confusing large autonomic nerve fibers for smooth muscle. The longitudinal smooth muscle fibers in both man and monkey, as observed in this investigation, are part of the

* Cholecystokinin-Cecekin-Vitrum Co., Stockholm, Sweden.

middle or subepithelial coat of the common bile duct.

Because of the relative lack of smooth muscle in the canine common bile duct and the limited availability of fresh human tissue, the Rhesus monkey was used in this study. It is well known that the external muscle layers of the intestinal tract relax when epinephrine, norepinephrine or isoproterenol are administered. To the contrary, the smooth muscle of the common bile duct uniformly responds with a burst of contractions to epinephrine or norepinephrine and does not respond to isoproterenol. In a classic study, King and Robinson [22] described the pharmacology of the intestinal muscularis mucosae. They found that this type of smooth muscle contracted to both acetylcholine and epinephrine. These responses are similar to those found in the present investigation, and lead to the conclusion that the smooth muscle of the common bile duct is pharmacologically of the muscularis mucosae type of gastro-intestinal smooth muscle. The muscularis mucosae of the intestine has been described as contracting *in vivo* at a frequency of 3 to 4 per minute.[15] The *in vitro* common bile duct contractile frequency of 1.8 ± 0.1 per minute further supports the view that the smooth muscle of the bile duct is physiologically of the muscularis mucosae type.

Crema, Benzi, and Berte [2, 12, 13] while studying the isolated Sphincter of Oddi have noted that contraction is produced both by acetylcholine and epinephrine. Although they did not mention the possibility of this smooth muscle being physiologically and pharmacologically of the muscularis mucosae type, their evidence would suggest that this is the case. Concerning the embryologic origin of the sphincter musculature, Boyden [35] has described this as arising *de novo* from surrounding mesenchyme four weeks after the external intestinal muscle layers have formed. It seems reasonable to the author that the muscle of the sphincter and the bile duct have a common histogenesis and represent a muscularis mucosae type of smooth muscle.

Further evidence supporting the concept that the bile duct smooth muscle is not the same as the external muscle layers of the intestine is found in the responses of this muscle to atropine and hexamethonium. Tachyphylaxis to atropine develops in the isolated and intact intestine.[1, 18] This does not occur in the isolated common bile duct. Further, hexamethonium in a concentration up to 10 mg./liter has no effect on isolated bile duct smooth muscle, while much lower doses profoundly affect the isolated intestine of many species.[30]

In this study it has been shown that spontaneous contractile activity of the monkey's common bile is abolished following pretreatment with reserpine. Similarly, pretreatment of the animal with guanethidine markedly suppresses spontaneous contractile activity. Phenotolamine abolishes the spontaneous contractions of the duct. Isoproterenol, however, in concentrations up to 500 μg./liter had no effect on the isolated common bile duct. It appears that adrenergic *alpha* but not *beta* receptors are responsible for contractile stimulant activity in this system.

Acetylcholine, like epinephrine or norepinephrine is capable of stimulating contractile activity in the isolated bile duct. Atropine blocks the contraction induced by acetylcholine but not that of norepinephrine. The spontaneous bile duct contractions are blocked by either atropine or phentolamine. McNeil-A-343 causes an increased contractile frequency of common bile duct contractions by ganglionic stimulation.[34] The effects of McNeil-A-343 and of acetylcholine are blocked by either atropine or phentolamine. This indicates that both acetylcholine and atropine exert their effects at the ganglionic level in this system. Hexamethonium has no effect on the

spontaneous bile duct contractions. This evidence suggests that the bile duct smooth muscle contains ganglia which appear to consist primarily of cells or receptors stimulated by acetylcholine or McNeil-A-343, blocked by atropine, but unaffected by hexamethonium. Norepinephrine appears to be the predominant, if not the only, peripheral autonomic neurotransmitter in this system (Fig. 9).

The investigations of Dunphy and Stephens [16] with prosthetic segments of artery, vein and Teflon in the common bile duct indicated that physiologic rather than anatomic factors could be responsible for the failure to replace a portion of this organ. The presence or absence of "peristalsis" in the common bile duct has been considered by several investigators.[8, 16, 28, 29] From a microscopic and physiologic standpoint, it does not appear correct to apply this term to the bile duct in the same manner that it is used in describing intestinal motility. Having predominantly one direction of the fibers, the musculature of the bile duct is undoubtedly more involved with maintainence of tone in the wall, rather than propulsion of intraluminal contents. The intrinsic autonomic nervous system of the biliary tree probably closely controls the activity of the smooth muscle through its ganglia which receive continuous information from multiple pressure receptors. Admittedly, the physiological and pharmacological evaluations in the present study have not been correlated with *in vivo* function, but it is conceivable to the author that our inability to replace a segment of the common bile duct may be related to more complex physiology than previously appreciated. *In vivo* investigations are currently in progress in this laboratory,[27] and it is hoped that several of these aspects will be directly applicable to further understanding of the human biliary tree and especially to the problem of reconstructing the common bile duct.

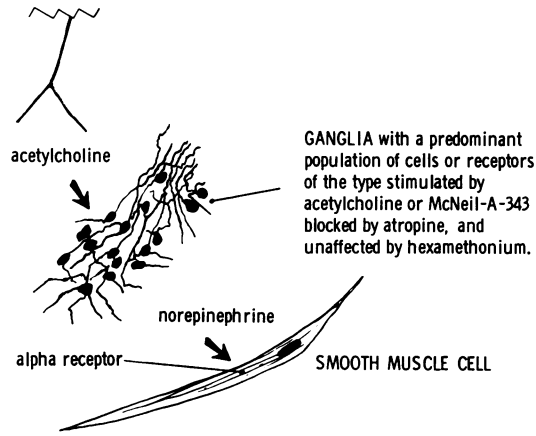

FIG. 9. Proposed autonomic innervation of the isolated monkey common bile duct.

Summary

1. Anatomic and physiologic evidence is presented to show longitudinal smooth muscle fibers are present in the common bile duct of the Rhesus monkey, but not to as significant a degree as in that of the dog. Anatomic evidence of longitudinal smooth muscle in the human bile duct is also presented.

2. Physiologic and pharmacologic evidence is presented which proves that the smooth muscle of the monkey's common bile duct is of the muscularis mucosae type.

3. The autonomic innervation of the bile duct smooth muscle appears to contain ganglia which are stimulated by acetylcholine or McNeil-A-343, blocked by atropine, and unaffected by hexamethonium. The predominant peripheral neuro-transmitter appears to be norepinephrine acting on an *alpha* receptor to produce contraction.

Acknowledgment

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