

## The muscle content and contractile capability of the common bile duct

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### Summary

*The supraduodenal portion of the human and canine common bile duct has been shown by indirect immunofluorescent staining with anti-actin sera to contain scattered bundles of smooth muscle arranged predominantly in a longitudinal direction with respect to the axis of the bile duct. Isolated strips of canine common bile duct in vitro can contract in response to stimulation with cholecystokinin. The force of contraction in a longitudinal direction is about three times that in a transverse direction but only 3% of that produced by isolated gallbladder strips. There appears to be a roughly proportional relationship between the amount of muscle demonstrated and the force of contraction in response to cholecystokinin.*

### Introduction

Although some workers have concluded that the supraduodenal portion of the *human* bile duct is practically devoid of smooth muscle<sup>1-5</sup>, others have found scattered bundles of muscle present in small but significant amounts<sup>6-9</sup>. There has also been disagreement about the orientation of the muscle fibres. Hendrickson<sup>6</sup> and Burden<sup>7</sup> describe both circular and longitudinal fibres in fairly similar amounts, while Ludwick<sup>9</sup> found all the smooth muscle to be arranged longitudinally with respect to the bile duct and this view is shared by Hand<sup>10</sup>.

According to Hendrickson<sup>6</sup> the *dog* common bile duct also contains isolated smooth muscle fibres in both circular and longitudinal orientation, but in this animal Ludwick<sup>9</sup> was unable to see any significant amount of muscle.

Even if there was agreement on the histology this would not, however, answer the

fundamental question how much contractile force this muscle, if present, can produce. Can it generate a peristaltic wave? Could spasm produce biliary colic? Burnett and Shields<sup>8</sup> studied movements in the *human* common bile duct using T-tube cholangiography with an image intensifier and claim to have seen definite peristaltic waves travelling down the duct. Myers *et al.*<sup>4</sup> were unable to see any such peristaltic waves and this view is shared by many others<sup>11-15</sup>. Under in-vitro conditions, longitudinal strips of human common bile duct have been shown to contract<sup>16</sup>.

In a study of *canine* common bile duct dynamics Watts and Dunphy<sup>17</sup> were able to elicit increased pressure changes from a cannula inside a closed segment of common bile duct in response to several agents, including cholecystokinin. In vitro they observed contraction of isolated canine common bile duct strips cut in longitudinal but not transverse orientation. As an opposing view, however, Ludwick<sup>9</sup> was unable to demonstrate any contraction of the isolated canine common bile duct in vitro.

The present study was undertaken to reassess the existence of smooth muscle in the common bile duct of man and dog using a newer staining technique. An attempt has also been made to correlate the amount of muscle seen with the contractile force which can be produced in vitro.

### Methods

#### HUMAN MATERIAL

Five bile ducts were obtained at postmortem from subjects free from any apparent biliary tract disease. Histological sections were cut from the supraduodenal portion of each in both a longitudinal and transverse direction with respect to the line of the bile duct.

Each section was stained for muscle fibres by an indirect immunofluorescent method using specific

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anti-actin sera<sup>18</sup>. The latter were obtained by immunising rabbits with purified actin prepared from rabbit or pig skeletal muscle and emulsified in Freund's complete adjuvant. The specificity of these antisera for actin was tested by indirect immunofluorescence and immunoperoxidase staining of the smooth muscle in rat stomach and the glomeruli in rat kidney. They also gave I-band staining of glycerinated stretched skeletal muscle fibres. Several sections were stained with haematoxylin and eosin and also by the van Gieson method for comparison with anti-actin staining.

#### CANINE MATERIAL

Eight bile ducts were removed from anaesthetised healthy greyhounds and 2 strips were cut from each, one longitudinally and one transversely with respect to the bile duct. Each strip measured approximately  $10 \times 2.5$  mm and was mounted in an isolated organ chamber of the type described by Bennett<sup>19</sup>, bathed in a modified Krebs solution, bubbled with 95% O<sub>2</sub> and 5% CO<sub>2</sub>, and maintained at 37°C. One end of each strip was fixed and the other connected via silk thread to a strain gauge. The recording system was near-isometric and was set to an initial resting tension of  $5 \times 10^{-3}$  N. Recording was made of the force of contraction in response to a single dose of 3.6 U/ml cholecystokinin (CCK) (Boots) injected into the bath-

ing fluid. This dose had previously been shown to produce the maximal response for CCK.

The length and weight of each strip was measured and the cross-sectional area thus calculated. The force of contraction of each strip was expressed as newtons/square metre cross-sectional area (N.m<sup>-2</sup>).

Each strip of canine bile duct was then subjected to histological section and actin staining as for the human material.

## Results

#### HISTOLOGY

Four out of 5 human and 6 out of 8 canine common bile ducts were demonstrated by actin staining to contain some muscle. Overall, there was slightly more muscle in the human than the dog. Virtually all the smooth muscle in both human and dog appeared orientated longitudinally along the bile duct (Fig. 1). The orientation of muscle fibres seen in Figure 1 is representative of all sections.

#### FORCE OF CONTRACTION

The force of contraction of the strips of dog common bile duct can be seen in summary in

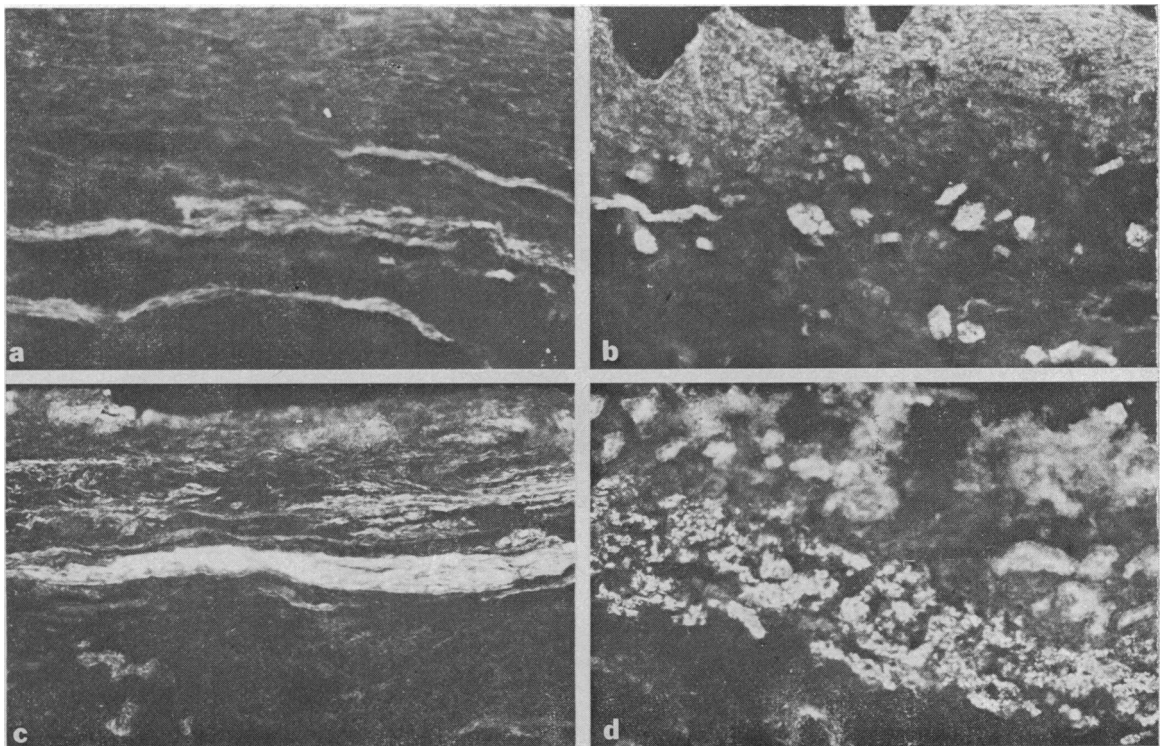


FIG. 1 Common bile duct sections stained with antiserum for actin by an indirect immunofluorescent method. Mucosa, largely autolysed, is uppermost in each. Muscle stains as bright areas of fluorescence. (Magnification  $\times 130$ ). (a) Human (longitudinal section). (b) Human (transverse section). (c) Dog (longitudinal section). (d) Dog (transverse section).

*Force of contraction (N.m<sup>-2</sup>) in response to CCK (3.6 U/ml)*

	<i>Mean response</i>	<i>Range</i>	<i>SEM</i>	<i>No of observations</i>
Canine common bile duct—longitudinal	240	0–690	100	8
—transverse	85	0–280	41	8
Canine gallbladder	7700	3500–12 300	1000	7

the table. This table also includes the force of contraction of isolated, obliquely cut strips of greyhound gallbladder recorded under the same conditions. The gallbladder results form part of a separate study (to be published) but are included here for comparison.

#### CORRELATION BETWEEN ACTIN STAINING AND FORCE OF CONTRACTION

Because of technical difficulties and variation in the distribution of muscle bundles in any one strip it proved impossible precisely to quantitate the amount of muscle seen histologically. However, when the longitudinal strips of canine bile duct were considered an approximate correlation between muscle content and force of contraction was seen. Of these 8 strips, 2 contained no visible muscle and produced no tension in response to CCK, 3 contained occasional scattered muscle fibres and produced tensions of 0, 50, and 84 N.m<sup>-2</sup> respectively, while in 3 strips discrete muscle bundles were seen in at least 10% of high-power fields and these produced tensions of 500, 610, and 690 N.m<sup>-2</sup> respectively.

#### COMPARISON WITH OTHER STAINING METHODS

Figure 2 shows a longitudinal section of canine common bile duct stained with (a) anti-actin sera, and (b) haematoxylin and eosin. We found that muscle bundles were better seen when stained by anti-actin sera than by either haematoxylin and eosin or the van Gieson method, and immunofluorescent methods are particularly suitable for reproduction in black and white.

### Discussion

It would appear that in both man and dog the supraduodenal portion of the common bile duct usually contains a small but variable amount of muscle. The amount of muscle roughly correlated with the contractile force observed in response to CCK, suggesting that the observed contraction was produced from this source and not, for example, from arteriolar muscle or myoepithelial cells.

Although from the histological point of view practically all the muscle was orientated longitudinally, there was still a measurable contractile effect in the transverse or circular muscle. Possibly there was some degree of obliquity of the fibres and occasionally oblique fibres were seen in the sections.

The significance of the presence of any smooth muscle in the common bile duct has long been the subject of dispute. We have shown that longitudinal strips of canine common bile duct produce a mean force of contraction of 240 N.m<sup>-2</sup>. If we assume that the human bile duct can produce a similar order of contractile force, then a common bile duct of 5 mm diameter and 0.5 mm wall thickness would be able to contract against a weight of up to 200 mg applied in its longitudinal axis.

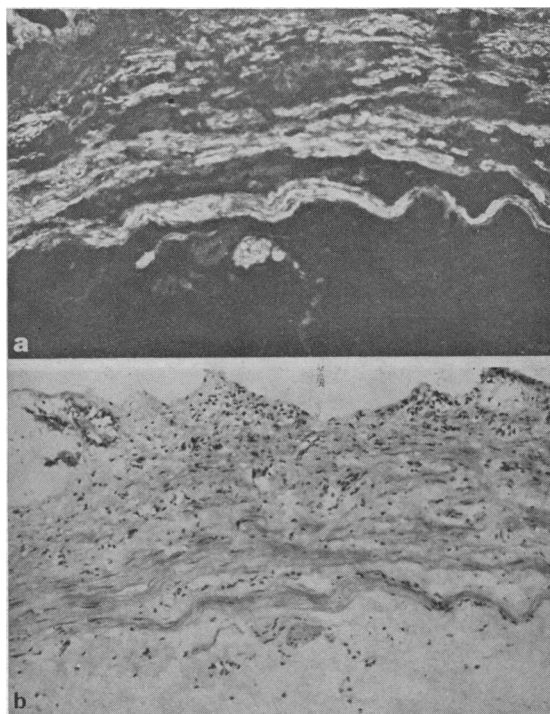


FIG. 2 *Longitudinal section of canine common bile duct stained with (a) anti-actin sera, (b) haematoxylin and eosin.*

It would seem unlikely that spasm of this very weak muscular apparatus could lead to the symptom of colic in a way that may happen in other hollow viscera, although this cannot be totally excluded without more knowledge about sensory perception from the bile duct. Nevertheless, it seems likely from both our histological and in-vitro physiological evidence that muscular contraction of the bile duct occurs at least in the dog.

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