

Diurnal variations in the pH of pathological gallbladder bile

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SUMMARY The pH of gallbladder and common duct bile from patients undergoing surgery for cholecystectomy has been measured. The bile was collected and kept anaerobically at 37°C, and the pH measured at 37°C, generally within 30-60 minutes of the bile being taken from the patient. Only data from patients having functioning or poorly-functioning gallbladders were included in the calculations. The pH of common duct bile was always greater than that of the corresponding gallbladder bile, and the data available suggest that it does not vary diurnally. The mean pH of gallbladder bile from patients undergoing their operation at different times during the day shows a diurnal variation, the bile becoming more acidic with time. Furthermore, there is also a diurnal variation in the activity of the acidifying process. Data for the patients suggest this is retarded by sleep, inactivity, or lying down.

The pH of both pathological and normal human bile has been measured by several workers, but most of these investigations were carried out before 1932, and data given in biological handbooks are mainly taken from this period. Tables in Sobotka (1937) list most of these studies, and a typical set of results is 5.6 to 8.0 for the pH of gallbladder bile and 6.2 to 8.5 for the pH of liver bile. These values are quoted by Altman (1961) in a biological handbook. In a more recent study, Crawford and Brooke (1955) report a range of 6.5 to 9.0 for the pH of normal gallbladder bile, and 6.9 to 8.0 for that of abnormal gallbladder bile from patients with gallstones or with malignant deposits in the liver.

Later investigations were concerned with the effect of stratification in bile. During a study on gallbladder bile taken from cattle after slaughter, Campbell and Burton (1949) found that the hydrogen ion concentration showed layer formation. Tera (1960) carried out a detailed study of stratification in normal and pathological human gallbladder bile and found a variation in pH from the thinnest to the thickest layers, and also in corresponding layers from different gallbladders. The variations in pH resulting from stratification do not, however, account for all the extreme values reported by other workers.

In most of the studies on the pH of bile, essential experimental information necessary to assess the

accuracy and validity of the results is lacking. Occasionally, samples were stored in a refrigerator which could cause precipitation and other changes. As far as we have been able to ascertain, most measurements were not made on fresh bile at 37°C, and generally the bile was not kept anaerobically until the pH was determined. Furthermore, the factors which determine and affect the pH of bile have not been found apart from the stratification effect and the change in pH when carbon dioxide is lost. A new investigation into the pH of bile seemed long overdue, and we have therefore measured the pH of human bile under controlled conditions, but, unfortunately, only pathological bile was available for this study. Values so obtained are also being compared with other measurements made on the bile and with data for the patients, and in this paper we have investigated diurnal variations in the pH.

Methods

Bile was obtained from the gallbladder and common duct of patients undergoing operation for cholecystectomy at University College Hospital over a period of 18 months. Care was taken to avoid contamination of common duct bile by gallbladder bile, and each bile was collected anaerobically in a syringe by drawing in the sample, immediately expelling any air bubbles and sealing the syringe by bending the needle. The gallbladder was completely aspirated to eliminate stratification effects (Tera,

1960). For technical reasons, common duct bile was not always obtained. The syringes of bile were insulated to prevent cooling and almost immediately placed in a constant temperature box at 37°C where the pH was measured using a Radiometer pH meter and microelectrodes which require only 30 μ l of sample. The meter was standardised with a Radiometer buffer, pH = 7.38 \pm 0.02 at 38°C. Two measurements were made on each bile, and, in between, the glass electrode was flushed out with saline followed by water, and the standardisation of the meter was checked. In general, the pH was measured within 30-60 minutes of the bile being taken from the patient. White bile, green bile, and bile containing blood were excluded from this study.

The patients were assigned to one of four groups depending on whether their operation began at about 8.30, 10.30, 14.00, or 15.30. The first two groups had no food or liquid after 22.00 hours on the evening preceding surgery. The afternoon groups were allowed a piece of toast and cup of tea before 6.00 on the day of operation.

The gallbladder function was usually known and had been assessed by oral x-ray cholecystography.

Statistical significance was determined using nonparametric tests. The Mann-Whitney U-test was used for testing differences between independent samples, and the Wilcoxon test was applied to differences between related samples—that is, gallbladder and common duct bile from the same patient (Siegel, 1956).

Results

The pH of gallbladder bile from patients with functioning, poorly-functioning, and non-functioning gallbladders is plotted against hour of operation in the Figure. The estimated error in the pH measurements is \pm 0.05. The extreme values of pH observed—namely, 6.55, 6.65 and 7.85—are for bile from non-functioning gallbladders. In the following calculations, bile from functioning and poorly-functioning gallbladders has been grouped together, but that from non-functioning gallbladders has not been included, as it would not be expected to provide information on diurnal variations in pH. Table 1 gives the length of fast before operation and the mean pH of the bile for the four groups of patients with operations at 8.30, 10.30, 14.00, and 15.30. The mean pH at 8.30 was significantly higher than at 10.30, 14.00, and 15.30 ($P < 0.002$, < 0.01 , and < 0.002 respectively). The mean pH at 15.30 was significantly lower than at 10.30 and at 14.00 ($P < 0.05$ and < 0.04 respectively).

The pH values for corresponding common duct and gallbladder bile from patients with functioning

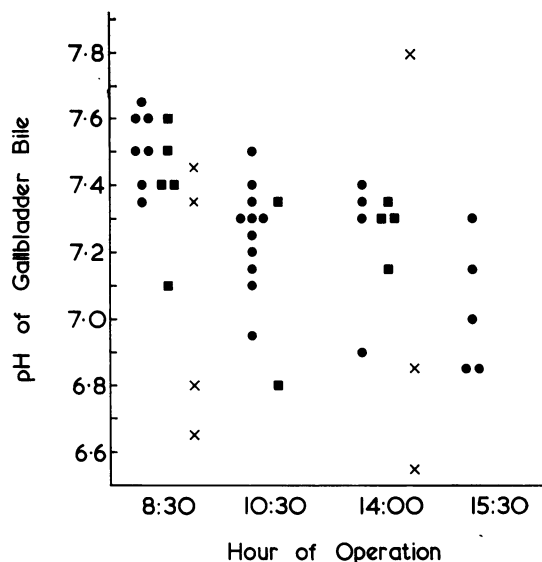


Figure 1 The pH of gallbladder bile plotted against the hour of operation for patients with functioning (●), poorly-functioning (■), or non-functioning (×) gallbladders.

and poorly-functioning gallbladders are listed in Table 2, but common duct samples were available in sufficient number for comparison purposes only from the morning operations. The mean values for common duct bile are 7.76 and 7.71 for patients undergoing surgery at 8.30 and 10.30 respectively, and there is no significant difference between these two averages. The range in values is greater, however, for patients in the former group. In both these groups of patients, the mean pH of common duct bile is significantly greater than the mean pH of the gallbladder bile ($P < 0.01$ and $P < 0.02$ for 8.30 and 10.30 operations respectively) and individual values for each patient show the same trend. Unfortunately, common duct bile was only obtained from two patients undergoing surgery in the afternoon and having either a functioning or a poorly-functioning gallbladder. These values of 7.70 and 7.80 are within the range of pH values for common duct bile from patients undergoing surgery in the morning.

The pH of common duct bile was measured for three patients with non-functioning gallbladders. The values are 7.60 (operation at 8.30), 7.60 (operation at 10.30), and 8.05 (operation at 14.00). The pH of common duct bile from one patient whose gallbladder function had not been determined is 7.50 (operation at 10.30).

Most of the patients underwent cholecystectomy

Table 1 Mean pH of gallbladder bile and duration of fast for four groups of patients with different times of operation

	Hour of operation			
	8.30	10.30	14.00	15.30
Number in group	12	13	8	5
Mean pH	7.47	7.23	7.26	7.03
Length of fast (h)	10½	12½	8	9½

Table 2 pH of gallbladder and common duct bile from patients classified according to their hour of operation

8.30 a.m. operation		10.30 a.m. operation	
Gallbladder bile (pH)	Common duct bile (pH)	Gallbladder bile (pH)	Common duct bile (pH)
7.65	7.90	7.50	7.70
7.60	7.85	7.35	7.65
7.60	7.75	7.30	7.75
7.50	7.80	7.30	7.80
7.50	7.65	7.15	7.55
7.40	7.50	6.95	7.75
7.40	7.65	6.80*	7.80
7.35	8.05		

*Poorly-functioning gallbladder; all other values are for patients with functioning gallbladders.

because of gallstones. The different groups contain roughly similar proportions of patients with stones composed of 100% cholesterol, cholesterol + calcium salts in any proportion, and 100% calcium salts (Sutor and Wooley, 1973) and so stone composition should not affect the results obtained. As yet, the pH of bile cannot be correlated with the stone composition of patients in the different operation groups because of insufficient data.

Discussion

The pH values for common duct bile obtained in the morning range from 7.50 to 8.05, but the mean values are the same for patients undergoing operation at 8.30 and 10.30. There are insufficient measurements at other times to determine whether there is a diurnal variation in the pH of common duct bile. In the case of every patient, the pH of common duct bile is higher than that of the corresponding gallbladder bile and mean values are significantly different. The pH of duct bile is therefore altered in the gallbladder and this could result from any concentrating action of the gallbladder on the bile salts and other acids, the transport or diffusion of certain ions like bicarbonate across the gallbladder wall, or the secretion or diffusion of hydrogen ions into the gallbladder.

Our results show a diurnal variation in the pH of gallbladder bile from the fasting patients comprising this study. Patients operated on at 8.30 and 10.30 have mean pH values of 7.47 and 7.23, respectively, which are significantly different. These patients fasted overnight, and the only difference in their behaviour during the fast was that those in the 10.30 group waited longer for surgery. Patients operated on at 14.00 and 15.30 have mean pH values of 7.26 and 7.03, respectively, which are also significantly different. These patients fasted during the day and the only difference in their behaviour was that those in the 15.30 group waited longer for surgery. It can therefore be concluded that bile left in a functioning gallbladder becomes more acidic with time.

The results also suggest a diurnal variation in the particular process responsible for the change in pH of gallbladder bile. Patients operated on in the afternoon fasted for several hours less than patients undergoing surgery in the morning, but bile for the two afternoon groups is significantly more acidic than that for the two morning groups except for patients operated on at 10.30 and 14.00, when the mean pH values are comparable. The morning and afternoon groups, however, behaved differently during their fast. The first probably slept or rested much of the time because the fast was overnight, whereas the second was more physically active during this period because the fast was during the day. The acidification of bile is, therefore, apparently retarded during sleep, inactivity, or lying down. Johnston *et al.* (1932) found a diurnal variation in the concentration action of normal gallbladders in dogs. Water was absorbed more slowly when the animals slept, and it was also concluded that inactivity and not feeding was responsible for the variation. From our data it is not possible to say which process varies diurnally because it is not known what determines the pH of gallbladder and common duct bile.

The patients with non-functioning gallbladders had common duct bile with pH values within the range of those for patients with functioning and poorly-functioning gallbladders. The pH of gallbladder bile varied considerably, did not follow any pattern, and presumably showed the very variable conditions that can exist in non-functioning gallbladders.

Unfortunately, our patients could not be compared with normal controls to discover if diurnal variations in pH are enhanced or diminished in gallstone disease.

Our pH values for bile from functioning and poorly-functioning gallbladders range from 6.80 to 7.65. In this and our continuing study of bile, there

is no evidence for the extreme values reported in other studies. This is also the case for common duct bile where our values range only from 7.50 to 8.05. The measurements were made on pathological bile under carefully controlled conditions, but Crawford and Brooke (1955) found that the ranges for normal and abnormal bile were similar. These data suggest that some values given in the biological handbooks should be viewed with caution.

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