Gall stone disease without gall stones – bile acid and bile lipid metabolism after complete gall stone dissolution

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SUMMARY Although bile acid and bile lipid metabolism have been studied in established cholelithiasis, little is known about them in patients destined to develop gall stones, but in whom the stones have not yet appeared (prestone gall stone disease). After confirmed complete gall stone dissolution and withdrawal of treatment, gall stones recur frequently. Before the stones reappear, these patients have 'poststone gall stone disease'. In 13 such patients we confirmed complete gall stone dissolution with two normal cholecystograms and in 11 of the 13 by normal ultrasonography, measured bile acid and bile lipid composition in fasting duodenal bile, bile acid synthesis from marker corrected three day faecal bile acid excretion, bile acid pool size using an abbreviated isotope dilution technique, 'steady-state' bile lipid secretion using a duodenal amino acid perfusion system and then calculated the enterohepatic cycling frequency of the bile acid pool and the relationship between pool size and body weight. The results confirm that after withdrawal of treatment the biliary cholesterol saturation index reverts to levels ($1.6 \pm \text{SEM } 0.4$) comparable with those before dissolution therapy first began (1.6 ± 0.2 ; NS). The mean bile acid pool size in the 13 patients of 4.4 ± 0.5 mmol was comparable with that in untreated gall stone patients. Pool size was significantly smaller in the nine non-obese patients (3.5 ± 0.3), than in the four obese (6.0 ± 0.8 ; $p < 0.05$). It also correlated significantly with body weight ($r = 0.72$) and with %IBW ($r = 0.79$). The coefficients of variation for biliary bile acid, phospholipid and cholesterol secretion were high, but the mean hourly secretion rates were of the same order as those seen in untreated gall stone patients studied with the amino acid duodenal perfusion stimulus. These results provide a baseline for assessing the response to postdissolution treatment and may indicate metabolic events leading to gall stone formation.

Patients with cholesterol gall stones have abnormal bile acid and bile lipid metabolism. Their fasting gall bladder bile¹ and bile rich duodenal fluid² are supersaturated with cholesterol, their mean bile acid pool size is reduced³ and their biliary cholesterol secretion rate increased, either in absolute terms⁴ or relative⁵ to bile acid secretion.

Treatment with chenodeoxycholic acid (CDCA) or ursodeoxycholic acid (UDCA) enriches bile with

the conjugates of the prescribed bile acid,^{6–10} lowers biliary cholesterol secretion,^{11 12} reduces the cholesterol saturation of fasting duodenal bile^{6 7} and, in selected patients, dissolves cholesterol rich gall stones.^{9 13–15}

When gall stones have been dissolved completely and bile acid treatment is stopped, biliary bile acid composition returns to its pretreatment state⁷ and bile resaturates with cholesterol.^{7 16} Without further treatment, gall stones recur in up to 50% of patients by five years.^{17–19}

The British Gallstone Study Group's postdissolution trial was designed to test the efficacy of three different treatments in preventing gall stone recurrence after confirmed complete gall stone dissolution – (i) a diet high in fibre and low in refined

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carbohydrate (ii) low-dose, continuous bile acid treatment (3 mg UDCA kg⁻¹ day⁻¹) and (iii) placebo – the second and third of these treatments being given double blind.

In addition to the clinical study of gall stone recurrence, a subset of patients underwent more detailed studies of bile acid and bile lipid metabolism. These investigations were carried out after complete gall stone dissolution had been confirmed, when treatment had been stopped and before the postdissolution treatment had been started. The aim of these studies, therefore, was to determine the mechanism for the increase in biliary cholesterol saturation seen after withdrawing dissolution therapy by measuring biliary lipid secretion, bile acid composition, bile acid pool size and synthesis and the enterohepatic cycling frequency of the bile acid pool.

Methods

PATIENTS

Of the 58 patients so far admitted to the post-dissolution trial,* 13 (11 women and two men) agreed to the detailed bile acid and bile lipid studies described below. The studies were approved by the Ethical Committees of Guy's Hospital, London, the Ninewells Hospital, Dundee, and the Bristol Royal Infirmary. All patients gave their informed consent.

Clinical details of these 13 patients are outlined in Table 1. Nine were non-obese (arbitrarily defined as <130% ideal body weight – IBW)²⁰ and four were obese (>130% IBW). In all 13 patients, body weight had remained stable (<10% variation) over

*In June 1983; by March 1986 the number was 103.

the preceding six months. No patient was taking drugs known to influence biliary cholesterol saturation²¹ and none was hyperlipoproteinaemic.²² One had non-insulin dependent diabetes mellitus.²³

All patients had had radiolucent, presumed cholesterol rich gall stones. They had been treated with either CDCA (n=10) or UDCA (n=3) for periods ranging from nine to 39 months. Complete gall stone dissolution was confirmed by two consecutive normal oral cholecystograms, not less than three months apart during continued bile acid treatment, supplemented, in 11 of the 13 patients, by normal real time ultrasonography. Bile acid treatment was then withdrawn. The patients were investigated not less than six weeks⁷ after stopping dissolution therapy.

STUDY DESIGN

All patients completed the full set of studies outlined below, with the exception of two patients who did not provide stool samples. The lipid composition of fasting duodenal bile – that is, gall bladder bile rich duodenal contents, was also measured before and during the initial gall stone dissolving treatment in seven of the 13 patients. The pre- and on-treatment bile lipid results in these seven patients were related to the post-dissolution findings in the overall group of 13 patients.

BILE LIPID AND BILE ACID COMPOSITION

The lipid composition of fasting duodenal bile was measured and the saturation indices (SI's) calculated, as previously described.^{9 24-26} Bile acid composition was measured by gas liquid chromatography of the trifluoroacetate derivatives.²⁷

Table 1 Clinical details of the 13 patients studied. Mean results±SEM's are given for all patients (bottom line) and for the nine non-obese and four obese individuals.

	Patient	Sex	Age (yrs)	Body weight (kg)	% IBW	Body mass index (kg m ⁻²)
Non-obese patients	1	F	63	64	112	24.4
	2	F	32	65	120	26.7
	3	F	59	65	118	26.4
	4	F	72	56	104	23.0
	5	F	37	46	88	19.4
	6	F	44	75	115	25.0
	7	F	54	56	103	23.3
	8	F	55	69	119	26.0
	9	M	52	71	100	23.2
	Mean±SEM		52±4	63±3	109±4	24.2±0.8
Obese patients	10	F	65	76	133	32.9
	11	F	36	101	160	37.1
	12	F	34	93	158	37.3
	13	M	63	84	133	30.9
	Mean±SEM		50±8	89±5	146±8	34.6±1.5
All patients	Mean±SEM		51±4	71±4	120±6	27.4±1.5

BILE ACID POOL SIZE

This was measured during the intestinal perfusion studies (see below) using the abbreviated isotope dilution technique.²⁸ Five microcuries ¹⁴C-cholic acid were given intravenously immediately before the intestinal intubation, four to five hours allowed for mixing and then the pool size derived from the samples obtained between hour four and hour five of the perfusion study.

BILE LIPID SECRETION

The 'steady state' bile lipid secretion was determined using a modified* Shaffer and Small amino acid perfusion technique.⁵ After a three to four hour equilibration period, duodenal fluid was aspirated hourly over the next four to five hours and 5 ml aliquots retained and stored for bile lipid analysis⁹ and measurement of BSP content.²⁹

BILE ACID SYNTHESIS

It was assumed the bile acid synthesis would equal faecal bile acid loss and for these studies, the patients took 15 inert radioopaque plastic recovery markers/day for seven days before and during the three days of the stool collection. The collections were then homogenised, the bile acid's extracted,³⁰ solubilised and their concentrations measured enzymatically.

Previous studies have suggested that there are significant relationships between the degree of obesity, bile acid pool size and the cholesterol content of bile.^{3 31-33} As a subsidiary aspect of this study, therefore, we looked, by linear regression analyses, at the possible relationships between: - (i) body weight and moles % cholesterol in bile (ii) body weight and biliary cholesterol SI's (the moles % cholesterol and the SI's being based on the fasting duodenal bile samples), (iii) bile acid pool size and body weight (iv) bile acid pool size and % IBW, and (v) bile acid pool size and SI's.

EXPRESSION OF RESULTS AND STATISTICAL METHODS

Results (means±SEM's with ranges) are given for all 13 patients together, and separately for the two subgroups of patients - the nine non-obese and the four obese.

The significance of differences between the groups was tested by the Mann-Whitney U-test. Linear regression analysis was by the method of least squares.

*The modification involved the use of a different commercial amino acid solution ('Vamin-N', Kabi Vitrum, Stockholm, Sweden) diluted 1:3 with water and supplemented with methionine 21.95 mmol/l, phenylalanine 22.45 mmol/l, and valine 51.83 mmol/l.

Results

BILE LIPID COMPOSITION

The results of biliary cholesterol SI's before the initial dissolution therapy began, after not less than six weeks of treatment and again after discontinuing the bile acid therapy for not less than six weeks, are shown in Figure 1.

In six of the seven patients in whom SI's had been measured before the initial gall stone dissolving treatment began, the bile was supersaturated with cholesterol (mean SI $1.6 \pm \text{SEM } 0.2$; range 0.9 to 2.1). During gall stone dissolution treatment, all but one of the seven patients developed unsaturated bile with a mean SI of 0.8 ± 0.1 (range 0.6-1.3). After stopping the bile acid therapy, the mean biliary cholesterol SI's in the 13 patients reached a level similar to that seen before treatment began (1.6 ± 0.4 ; range 0.9-2.2) with comparable results in the four obese (1.4 ± 0.1) and the nine non-obese (1.6 ± 0.1) patients.

There was no significant relationship between body weight and either saturation index ($r=0.26$; $p>0.10$) or moles % cholesterol in bile ($r=0.30$; $p>0.10$).

BILIARY BILE ACID COMPOSITION

The bile acid composition results are given in Table 2. In keeping with the findings in previous studies, cholic, chenodeoxycholic and deoxycholic acids were the major bile acids found in bile, together with small amounts of lithocholic and ursodeoxycholic acids. Unusual bile acids accounted for less

Fig. 1 Biliary cholesterol saturation indices (SI's) measured in fasting duodenal bile, before, during and not less than six weeks after stopping gall stone dissolution therapy. The broken horizontal line indicates a saturated solution according to the cholesterol solubility limits defined by Hegardt and Dam. The E shaped symbols indicate mean values±SEM's.

Table 2 Bile acid composition in fasting bile rich duodenal fluid: results are mean percentage values $\pm$ SEM's with ranges. CA=cholic acid; CDCA=chenodeoxycholic acid; DCA=deoxycholic acid; LCA=lithocholic acid; UDCA=ursodeoxycholic acid conjugates.

CA	CDCA	DCA	LCA	UDCA	Others*
38.0 $\pm$ 2.4 (29.0–56.0)	25.0 $\pm$ 3.0 (13.8–50.4)	27.0 $\pm$ 3.6 (2.0–47.7)	4.0 $\pm$ 0.5 (0.2–5.0)	2.1 $\pm$ 0.7 (0–4.4)	<5.0 (2.1–10.0)

* 3 α 7 keto-, 3 β -, 3 β 12 α -, 3 β 12 keto-, 3 α 7 α 12 keto-, 5-cholanic acids

than 5% of the total – with the exception of one patient who, as judged by the relative retention times on the GLC tracing, had 10.5% isodeoxycholic acid (3 β , 12 α di-hydroxy 5- β cholan-24-oic-acid). This unusual finding requires confirmation by mass-spectral analysis (GC-MS).

BILE ACID POOL SIZE

The total bile acid pool sizes for all 13 patients, and for the four obese and nine non-obese individuals, are shown in Table 3. For the group as a whole, the mean bile acid pool size was 4.4 $\pm$ 0.5 mmol (range 2.6–7.4). The obese patients had significantly larger pools (6.0 $\pm$ 0.8) than the non-obese (3.5 $\pm$ 0.3; p <0.05). As expected, however, when pool size was expressed/kg BW, this difference was no longer significant. Indeed, there were significant correlations (Fig. 2) between pool size and body weight (r =0.72; p <0.05) and between pool size and % IBW (r =0.79; p <0.01). In these 13 patients, however, there was no significant relationship between the bile acid pool size and the saturation index of fasting duodenal bile.

BILE LIPID SECRETION

The steady-state secretion rates of cholesterol, phospholipids and bile acids, expressed both in absolute terms and as a function of body weight, are shown in Table 4.

Fig. 2 Relationship between total bile acid pool sizes, measured by an abbreviated isotope dilution technique, and body weight expressed in kg (solid circles) as the percentage of ideal body weight (IBW; open circles).

In all 13 patients, the mean secretion rates (mmol/h) for bile acids, (1.1 $\pm$ 0.19), phospholipids (0.5 $\pm$ 0.08) and cholesterol (0.2 $\pm$ 0.03) differed somewhat from, but were, nonetheless, of the same order as, those found previously in untreated gall stone patients^{4,5} in whom bile secretion had been stimulated with amino acid perfusates. Furthermore, in keeping with some previous reports^{5,34} there were significant linear relationships between the secretion rates of cholesterol and bile acids

Table 3 Bile acid (BA) pool size, enterohepatic cycling frequency and synthesis rates (based on marker-corrected, three day faecal BA excretion): mean values $\pm$ SEM's for all 13 patients and for the obese and non-obese sub groups.

	BA pool size		Cycling frequency of BA pool		BA synthesis rate	
	mmol	μ mol/kg	Fraction of pool cycling/h	Cycles/d	mmol/d	μ mol/kg/d
All (n=13)	4.4 $\pm$ 0.5 (2.6–7.4)	60.1 $\pm$ 4.3 (35.0–83.0)	0.25 $\pm$ 0.04 (0.06–0.49)	5.9 $\pm$ 0.85 (1.5–11.7)	0.66 $\pm$ 0.14 (0.14–1.60)	9.6 $\pm$ 2.20 (1.7–28.0)
Obese (n=4)	6.0 $\pm$ 0.8 (3.7–7.4)	66.3 $\pm$ 7.3 (49.0–83.0)	0.22 $\pm$ 0.04 (0.12–0.30)	5.3 $\pm$ 0.90 (2.9–7.1)	0.45 $\pm$ 0.18 (0.14–0.90)	4.8 $\pm$ 1.7 (1.7–9.0)
Non-obese (n=9)	3.5 $\pm$ 0.3 (2.6–5.0)	55.4 $\pm$ 4.6 (35.0–77.0)	0.26 $\pm$ 0.05 (0.06–0.49)	6.2 $\pm$ 1.2 (1.5–11.7)	0.77 $\pm$ 0.18 (0.18–1.60)	11.9 $\pm$ 2.9 (2.4–28.0)

BA=bile acid.

Table 4 Mean biliary lipid secretion rates (obtained during the secretion/perfusion studies) $\pm$ SEM's and ranges.

	Cholesterol		Phospholipids		Bile acids	
	mmol/h	μ mol/kg/h	mmol/h	μ mol/kg/h	mmol/h	μ mol/kg/h
All (n=13)	0.2 $\pm$ 0.03 (0.1–0.4)	2.7 $\pm$ 0.40 (1.1–5.6)	0.5 $\pm$ 0.08 (0.1–1.0)	5.0 $\pm$ 1.82 (1.6–11.9)	1.1 $\pm$ 0.19 (0.2–2.2)	15.2 $\pm$ 2.46 (3.6–32.3)
Obese (n=4)	0.2 $\pm$ 0.04 (0.1–0.3)	2.6 $\pm$ 0.50 (1.1–3.6)	0.5 $\pm$ 0.17 (0.2–1.0)	5.5 $\pm$ 1.68 (2.2–10.0)	1.4 $\pm$ 0.37 (0.7–2.2)	15.4 $\pm$ 3.77 (7.5–22.0)
Non-obese (n=9)	0.2 $\pm$ 0.03 (0.1–0.4)	2.8 $\pm$ 0.49 (1.3–5.6)	0.5 $\pm$ 0.10 (0.1–1.0)	4.8 $\pm$ 1.00 (1.6–11.9)	0.9 $\pm$ 0.21 (0.2–2.1)	15.1 $\pm$ 3.28 (3.6–32.3)

($y=0.11x+0.06$; $r=0.66$; $p<0.01$), and, to a lesser extent, between cholesterol and phospholipids ($y=0.21x+0.09$; $r=0.49$; $p<0.01$). Again as noted by others,^{5 34 35} there was also a linear relationship between phospholipids and bile acids ($y=0.36x+0.08$; $r=0.90$; $p<0.01$). The mean bile acid:cholesterol molar ratio in the secreted bile was 5.8:1, which is somewhat less than that noted in previous studies.^{4 5}

Unlike our previous results,⁴ in the present study we found no significant differences in cholesterol and phospholipid secretion rates between the obese and non-obese subgroups. The number of patients in the obese group was small, however, which limits the statistical power of any comparison between the obese and non-obese patients. Moreover, although the mean bile acid secretion rate in the obese was 60% greater than that in the non-obese, because of a considerable scatter in the results, this difference was not statistically significant. In fact, as shown by the size of the SEM's and ranges, not only were there obvious differences in the secretion rates for all three lipids from patient to patient, but the within patient, hour-to-hour, mean coefficients of variation were high – 48% for cholesterol, 52% for phospholipids, 59% for bile acids.

ENTEROHEPATIC CYCLING FREQUENCY OF THE BILE ACID POOL

Despite the non-physiological conditions of the perfusion study, the extrapolated mean 24 hour enterohepatic cycling frequency (Table 3) of approximately 6 cycles/day was comparable with that described in previous studies.³⁴ The large bile acid pool in the four obese patients cycled somewhat more slowly (5.3 ± 0.9) than that in the nine non-obese (6.2 ± 1.2), but this difference was not statistically significant.

The fraction of the bile acid pool cycling/hour was 0.25 ± 0.04 and although the obese patients had larger pools, the fraction of the pool cycling/hour was comparable in the obese and in the non-obese subgroups.

BILE ACID SYNTHESIS RATE

The faecal bile acid excretion results, again expressed both in absolute terms and as a function of body weight, are also shown in Table 3. The mean value for faecal bile acid output of 0.66 ± 0.14 mmol/day was similar to that found previously both in control subjects³⁰ and in untreated gall stone patients.³³ Once again, however, there were large differences between individual patients in their faecal bile acid outputs.

Although the obese patients had a significantly larger bile acid pool than the non-obese, this pool cycled more slowly which may explain, in part, why they had a 42% smaller mean faecal bile acid output.

Discussion

The present results confirm that after complete gall stone dissolution and withdrawal of treatment, the bile acid and bile lipid composition of fasting duodenal bile revert to levels comparable with those seen before treatment began.^{7 16} We have extended these observations by providing, for the first time in postdissolution patients, data on bile lipid secretion and on bile acid pool size, EH cycling frequency and synthesis rate. Ideally, all these variables should have been measured sequentially in the same individuals studied before, during and after treatment but for practical and ethical reasons, this was not possible. For the same reasons, these detailed studies were not carried out in normal controls or in untreated gall stone patients. Nonetheless, it seems reasonable to compare the results obtained in the present investigation, with our own previous data⁴ and those reported by others who have studied control subjects^{5 36} and gall stone patients,⁵ using similar techniques. Ideally, the results in non-obese, postdissolution subjects should only be compared with those in non-obese subjects who either have an intact gall bladder and no gall stones^{4 5 36} or an intact gall bladder with stones.^{4 5}

BILE ACID POOL SIZE

There have been no previous measurements of bile acid pool size in patients whose stones have been dissolved, but the present findings are in close agreement with the results of many previous reports which show that the pool size is small in untreated gall stone patients of normal weight^{3, 37} although it may be normal or even greater than normal in very obese patients.⁵ In theory, abbreviated isotope dilution techniques, such as that used here, may underestimate bile acid pool size either because of incomplete mixing of the label within the endogenous bile acids due to incomplete gall bladder emptying^{28, 38} or because the individual bile acids within the pool cycle at different rates.³⁹

In agreement with previous studies in normal subjects⁴⁰ and in patients with gall stones,⁴¹ we found no relationship between bile acid pool size and biliary cholesterol saturation index.

BILE LIPID SECRETION

For the studies of bile lipid secretion, we used Shaffer and Small's continuous amino acid perfusion technique.⁵ This method yields different absolute secretion rates from those obtained using liquid formula perfusions or intermittent liquid meals. It seems likely, however, that the different patterns of bile lipid secretion seen in various patient groups will remain the same, despite different secretory stimuli. Indeed, when Everson and colleagues³⁶ measured bile lipid secretion in the same individuals on two separate occasions using first the amino acid perfusion technique and then the liquid formula, they found a broadly comparable pattern of results – even if the absolute secretion rates obtained were different with the two stimuli. In fact, they found that the liquid formula, 40% fat infusion was associated with (i) more complete, tonic gall bladder emptying; (ii) shorter small intestinal transit time; (iii) greater hormonal stimulation; (iv) higher biliary lipid secretion rates (bile acids > cholesterol) and (v) a lower molar percentage cholesterol in the secreted bile.

Despite this, it seems likely that the ideal of steady state conditions is never achieved during such perfusion studies – whatever the secretory stimulus. Indeed, the coefficients of variation noted here were not appreciably different from those reported by others who used liquid formula perfusates. Mabee *et al.*,³² for example, found coefficients of variation for cholesterol secretion ranging from 19.2 to 58.3%, for phospholipids from 22.6 to 58.3% and for bile acids from 20.2 to 70.3%. Similarly, Grundy and Metzger found coefficients of variation for cholesterol secretion of up to 30% and for bile acid secretion of up to 60%.³⁵

As stated above, although the absolute secretion rates for the three major lipids were of the same order as those reported previously by others who used perfusion stimuli, the outputs of bile acids, phospholipids and particularly of cholesterol were higher in the 13 patients studied here, than in both obese⁵ and non-obese⁵ gall stone patients. After cholecystectomy, however, Shaffer and Small⁵ found that the bile acid secretion rate increased to levels comparable with those stated here. Despite this, there are other differences in the pattern of bile lipid secretion between cholecystectomised and postdissolution patients: in particular, for every mole of bile acid secreted, biliary cholesterol output was much higher in the postdissolution patients than after cholecystectomy. There are no comparable results for bile lipid secretion in post-dissolution gall stone patients studied with amino acid perfusion techniques. The molar ratio of bile acids:phospholipids:cholesterol in the secreted bile was, however, roughly similar to that described by others^{5, 36} in untreated gall stone patients – even if the molar ratio of bile acids:cholesterol in the present study was less than that found by others,^{5, 34} possibly because of the relatively high biliary cholesterol secretion rate. Furthermore, the linear relationship between the secretion rates of bile acids and cholesterol noted in the present study, held true over a 20-fold range in bile acid output. This indirect evidence suggests that in poststone gall stone disease, not only is there an absolute or relative hypersecretion of biliary cholesterol (a fact well established in untreated gall stone patients)^{2, 42} but also that there may have been an 'overshoot' phenomenon in biliary cholesterol output.

Bile acid secretion rate is the product of pool size times EH cycling frequency. Because the mean bile acid pool size in the nine non-obese patients was significantly smaller than that seen in the four obese while their EH cycling frequencies were similar, it follows that the mean bile acid secretion was 36% lower in the non-obese, than in the obese, patients. This observation confirms the results of previous studies from our own group⁴ and elsewhere⁵ showing that non-obese gall stone patients hyposecrete bile acids, but they do not support the controversial claim that small bile acid pools cycle more frequently than normal.⁴³

We,⁴ and others,⁴² have previously shown that obese gall stone patients secrete more biliary cholesterol than the non-obese but this difference was not seen in the present study – mainly because the mean biliary cholesterol output in our non-obese patients was high, perhaps as a result of the 'overshoot phenomenon' discussed above.

BILE ACID SYNTHESIS

The observation that the mean bile acid synthesis rate (faecal bile acid excretion) was 42% lower in the obese than in the non-obese patients, is surprising and unexplained. Given the differences in mean bile acid pool sizes between the two groups, if the number of EH cycles per day really is comparable, this implies that the fraction of the small pool reabsorbed is less in the non-obese than in the obese.

SIGNIFICANCE OF FINDINGS IN RELATION TO GALL STONE FORMATION

There is now good evidence that when treatment is stopped, gall stones will ultimately recur in the majority of patients whose stones dissolved.¹⁷⁻¹⁹ Our results suggest that the pathophysiology of 'post-stone gall stone disease' is similar to that of prestone gall stone disease.⁴⁴ The relative importance of the different factors involved – including those not assessed in the present study such as diurnal changes in biliary cholesterol saturation,⁴⁵ changes in gall bladder mucosal⁴⁶ and motor⁴⁷ function, nucleating agents⁴⁸ and crystallisation inhibitors^{49 50} – is not known.

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