The reaction mixture of 2 ml contained 20 μmoles of DL-isocitrate; 100 μmoles of phosphate buffer (pH 7.4); 4 μmoles of INT (2-p-iodophenyl-3-nitrophenyl tetrazolium chloride); 10 μmoles of $MgCl_2$; 0.2 μmoles of ADP and 0.2 μmoles of NAD^+. The reaction was initiated by the addition of 0.5 ml of supernatant. The control reaction mixture received 0.5 ml of sucrose in the place of supernatant solution. After an incubation of 30 min at 37 °C, the reaction was stopped by the addition of 5 ml of glacial acetic acid and the formazan formed due to reduction of the dye was extracted into 5 ml of toluene for overnight in cold, and colour was read in UV spectrophotometer (Hilger & Watts, England) at 495 nm using silica cuvette of 10 mm path length. Individual zero time controls were maintained for all the samples by the addition of glacial acetic acid to the reaction mixture prior to the addition of the enzyme and incubation. The enzyme activity was expressed as μmoles of formazan/mg protein/h. Protein levels were determined by the method of Lowry et al.[9]. The data was subjected to statistical processing according to standard procedures (Pillai and Sinha)[7].

Results and discussion. The activity levels of isocitrate dehydrogenase were found to range from 0.120 ± 0.014 to 0.158 ± 0.020 μmoles of formazan/mg protein/h in the pedipalpal muscle and 0.071 ± 0.013 to 0.111 ± 0.026 μmoles of formazan/mg protein/h in hepatopancreas (figure). In both the tissues, the maximal activity was recorded at 20.00 h and minimal activity was recorded at 08.00 h. In both tissues the difference between the maximal (20.00 h) and minimal (08.00 h) was significant ($p < 0.001$ for muscle; $p < 0.001$ for hepatopancreas). But the pattern of rise and fall in isocitrate dehydrogenase activity in between these times was different in the 2 tissues studied. In both the tissues, eventhough the peak enzyme activity is found at 20.00 h, the enzyme activity remains relatively high between 16.00 h and 04.00 h.

The higher isocitrate dehydrogenase activity in the muscle perhaps reflects increased channeling of the substrates to the citric acid cycle for energy requirements during muscle contraction. Hepatopancreas is known to be the main organ for storage of nutrients. Synthesis and break down of metabolites are related to the metabolic needs of the animal. The scorpion is a nocturnal animal and shows a significant increase in locomotor activity at night[1]. The metabolic rate is also high between 16.00 h and midnight (24.00 h) with a peak around 20.00 h[1]. The necessary energy is perhaps made available through increased metabolic degradation of glycogen, as evidenced by increased phosphorylase activity[4] and blood glucose[6]. The nocturnal peak of the isocitrate dehydrogenase activity at 20.00 h may signify increased channeling of substrates through the Krebs cycle to sustain the raised energy requirements due to the increased locomotor activity, involving higher rate of muscle contraction of the animal during night.

It is known in mammals that epinephrine induces greater production of cyclic-AMP in muscle, which leads to the acceleration of breakdown of glycogen in liver and muscle[11]. Circadian rhythm in the neurosecretions in the scorpion has also been reported[2]. It is probable that the active principle from scorpion neurosecretory system with epinephrine-like action is responsible for increased channeling of substrates to the Krebs cycle for mobilization of energy through the activation of isocitrate dehydrogenase.

Thus the peak activity of the isocitrate dehydrogenase activity at 20.00 h, coinciding with the nocturnal habit of the scorpion, appears to be significant in view of the raised energy requirements to sustain the nocturnal increase of locomotor activity.

9 O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
10 S. K. Pillai and H. C. Sinha, Statistical methods for biological Workers. Ram Prasad and Sons, Agra 1968.
11 Bela Issekutz, Jr, and M. Allen, Metabolism *21*, 48 (1972).

Measurement of the biliary tree volume in the rat under T_m-conditions

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Summary. Biliary tree capacities determined under T_m-conditions using ^{35}S-sulfobromophthalein sodium, ^{35}S-sulfobromophthalein glutathione, and ^{14}C-taurocholate sodium as markers exceeded markedly the biliary volumes reported until now. This may have been caused at least partly by a more homogenous distribution of the marker substance throughout the liver parenchyma in contrast to simple bolus injection studies.

About 15 years ago, Barber-Riley[1] estimated the capacity of the rat biliary as the volume of bile collected between the time of i.v. injection of sulfobromophtalein and the time at which the concentration of this dye in bile reached one-half maximal excretion rate. However, it now appears that Barber-Riley's method may give erroneous estimates of capacities in that a time lag occurs between the i.v. administration of the dye and its excretion into the bile. Häcki and Paumgartner[2] have largely overcome this transit time problem by using ^{14}C-taurocholate as a marker, and they reported nondistended biliary tree capacities of a rat liver weighing 10 g of about 23 μl. The retrograde push-back technique of Peterson et al.[3] also does not contain a transit time artifact and gives 37 μl as the estimate for biliary tree capacities in the distended state in contrast to 121 μl found by Barber-Riley[1]. Although different methods were used to determine the biliary tree in its nondistended or distended state, they are based on the same theoretical consideration that the dye injected is distributed or washed out homogenously throughout the biliary system, and thus may permit measurement of the biliary tree volume. This assumption has not been proved yet. An approach to determine the biliary tree capacity under T_m-conditions was introduced using ^{35}S-sulfobromomophthalein, ^{35}S-sulfobromophthalein glutathione, and ^{14}C-taurocholate as markers.

1 G. Barber-Riley, Am. J. Physiol. *205*, 1127 (1963).
2 W. Häcki and G. Paumgartner, Experientia *29*, 1091 (1973).
3 R. E. Peterson, J. R. Olson and J. M. Fujimoto, Toxic. appl. Pharmac. *36*, 353 (1976).

Methods and materials. Male Wistar rats weighing 350–400 g maintained under standard laboratory conditions were used. The common bile duct and jugular vein were cannulated under urethane anaesthesia (1.25 g/kg b. wt i.p.). The bile catheter with a dead space of 10 µl was positioned just below the bifurcation. After surgery a thermistor probe was inserted into the rectum and body temperature was maintained at 37.5–38 °C by a 'Temperaturregler', Sachs, W. Germany. Unlabelled sulfobromophthalein sodium and taurocholate sodium were infused at constant rates of 2.5 µmoles/min kg b.wt[4] and 1.6 µmoles/min 100 g b.wt[5] respectively until T_m-values were achieved. Then ^{35}S-sulfobromophthalein sodium (^{35}S-BSP)[6], ^{35}S-sulfobromophthalein glutathione (^{35}S-BSP-GSH), and 24-^{14}C-taurocholate sodium[6] (0.4 µmoles/100 g b.wt) dissolved in a volume of 300 µl of saline were rapidly injected i.v. in 8 rats and flushed with another 300 µl of isotonic saline. Bile was collected in 20 sec periods up to 300 sec and in 30 sec periods up to 600 sec and weighed. Thereafter dye concentration of each bile sample was calculated using a Packard Tricarb liquid scintillation counter (model AAA 544). The volume of the biliary capacity was calculated in 2 ways: Method 1: Calculation according to Barber-Riley[1] as modified by Sicot[7] and recently described by Häcki and Paumgartner[2]. Since the marker does not appear in bile as a flat concentration front, the volume of the biliary capacity is calculated in the following way:

$$BTC = Vol_{max} - \sum_{i=1}^{iC_{max}} \cdot \frac{C_i}{C_{max}} \cdot Vol_i - Vol_{catheter}$$

Vol_{max} stands for cumulative volume of bile collected from the time injection until maximal concentration (C_{max}) of the marker in bile is reached. i = 1 denotes the first sample after injection and iC_{max} the sample in which C_{max} is reached. C_i represents the concentration of the marker in the single sample i, and Vol_i the volume of sample i. Method 2: Modified approach for calculation of the biliary volume using a labelled marker. This determination is based on the assumption that measurable radioactivity probably indicates, with only very little delay, the time of appearance of the injected dye in the left and right hepatic duct. Thus the same formula as in method 1 was used with the only differences that (1) Vol_{max} represents the cumulative volume of bile collected from the first measurable radioactivity in bile until

4 W. Grote, A. Schmoldt and H. G. Dammann, Biochem. Pharmac. *24*, 1121 (1975).

5 G. Paumgartner, K. Sauter, H. P. Schwarz and R. Herz, in: The Liver. Quantitative Aspects of Structure and Function, Karger, Basel 1973.

6 Source of supply: Amersham-Buchler, Braunschweig, Federal Republic of Germany.

7 C. Sicot, A. Richard and J. P. Benhamou, Revue fr. Etud. clin. biol. *13*, 270 (1968).

Table 1

	n	t_o (sec)	t_{max} (sec)	C_{max} (cpm × 10^3)	Vol_{max} Method 1 (µl)	Method 2 (µl)
A	1	80	480	107	169	148
	2	60	360	137	178	146
	3	80	390	118	189	158
	4	60	360	139	131	112
	5	80	420	112	171	147
	6	60	360	135	176	149
	7	80	390	120	182	157
	8	60	420	140	137	111
	Median value	70	390	128	174	148
	Mean ± SD	70 ± 10.7	397.5 ± 41.7	126.0 ± 13.2	166.6 ± 21.1	141.0 ± 18.7
B	1	40	390	270	235	220
	2	40	280	297	151	138
	3	60	480	230	284	266
	4	40	400	277	160	147
	5	40	420	212	229	218
	6	40	390	270	165	153
	7	40	480	297	283	265
	8	60	280	230	157	145
	Median value	40	390	270	197	186
	Mean ± SD	45.0 ± 9.3	390.0 ± 76.9	260.4 ± 32.4	208.0 ± 56.8	194.0 ± 54.6
C	1	100	600	879	226	193
	2	60	600	1116	325	302
	3	80	570	1138	240	213
	4	80	780	681	379	344
	5	60	600	1189	309	277
	6	80	570	1138	240	213
	7	100	600	879	226	193
	8	80	780	681	379	344
	Median value	80	600	1009	275	245
	Mean ± SD	80.0 ± 15.1	637.5 ± 88.9	962.6 ± 210.1	290.5 ± 66.1	259.9 ± 65.0

Main data of each experiment using ^{35}S-sulfobromophthalein glutathione (A), ^{14}C-taurocholate sodium (B), and ^{35}S-sulfobromophthalein sodium (C) as test substances for the determination of the biliary tree capacity under T_m-conditions. t_o = time at which ^{35}S-radioactivity is first detected in the bile, t_{max} = time until maximal concentrations are reached, C_{max} = maximal concentration, Vol_{max} = cumulative volumes of bile collected according to method 1 and 2. Median values and means ± SD are indicated.

Table 2

		Biliary tree capacity µl/rat liver		µl/g liver wet wt	
A	Method 1 (n = 8)	69.7 ± 8.08	$p < 0.001$	4.47 ± 0.40	$p < 0.001$
	Method 2 (n = 8)	54.4 ± 5.25		3.52 ± 0.58	
B	Method 1 (n = 8)	73.2 ± 11.37	Not	4.60 ± 0.70	Not
	Method 2 (n = 8)	70.2 ± 11.78	significant	4.42 ± 0.75	significant
C	Method 1 (n = 8)	136.1 ± 16.82	$p < 0.001$	9.03 ± 0.57	$p < 0.001$
	Method 2 (n = 8)	115.1 ± 14.56		7.63 ± 0.52	

Mean nondistended capacities ± SD of the biliary tree under T_m-conditions using ^{35}S-sulfobromophthalein glutathione (A), ^{14}C-taurocholate sodium (B), and ^{35}S-sulfobromophthalein sodium (C) as test substances.

maximal concentration of the marker was reached and that (2) $Vol_{catheter}$ was not substracted since it was suggested that the bile cannula itself has no remarkable effect on the detection of the test substance in bile, except a time delay which is caused by the additional passage through the catheter.

Results and discussion. Bile flow and radioactivity following the injection of ^{35}S-BSP under T_m-conditions in a representative experiment are depicted in figure 1. Radioactivity was first measurable in the 4th 20 sec bile sample (mean ± SD: 80.0 ± 15.1 sec) and reached its peak after 720 sec (637.5 ± 88.9 sec). In the case of ^{35}S-BSP-GSH injected after T_m-values with sulfobromophthalein sodium had been achieved, radioactivity was also first detected in the 4th 20 sec period but reached its maximum after 390 sec (means ± SD: 70.0 ± 10.7 and 397.5 ± 41.7 sec respectively). In contrast to these experiments ^{14}C-taurocholate (figure 2) appeared under T_m-conditions already in the second 20 sec bile sample (mean ± SD: 45.0 ± 9.3), however the peak was also established after 390 sec (390.0 ± 76.9 sec). The mean volumes of the biliary capacity obtained with either experimental procedure and calculated according to both methods are summarized in table 2. A remarkable discrepancy may be noted between the values reported so far and our findings. Even using the same marker substance, our values are more than 44 and 92% larger than those published by Häcki and Paumgartner[2]. Interestingly the biliary capacities estimated with ^{35}S-BSP-GSH and ^{14}C-taurocholate according to method 2 differ not so extremely from those found by Barber-Riley[1] (+ 3 and 13% respectively). Using ^{35}S-BSP-GSH only BSP-T_m-conditions were established. Results might be different if it were possible to perform BSP-glutathione–T_m-values. When ^{35}S-BSP was used as marker substance and injected under T_m-conditions, the biliary capacities exceeded those of Barber-Riley[1] significantly and also those estimated with ^{35}S-BSP-GSH and ^{14}C-taurocholate in this study. It should be noted, however, that in this study a slight distention of the biliary tree as a result of the choleresis induced by T_m-conditions cannot be excluded. Furthermore, it remains an unresolved question by which mechanisms prolonged times, at which maximal concentrations were seen, are established. On the basis of kinetic considerations, one would assume that mean hepatocellular transit time (prebiliary transit time) of a marker substance is increased under T_m-conditions. As would be expected T_m-conditions, which indicate the functional maximum of the liver, do not prolong the appearance of the marker substances in the bile. However, both factors – distention of the biliary tree by osmotic choleresis and increased prebiliary transit time – may lead to an overestimation of the biliary tree capacity. On the other hand, Barber-Riley[1] tried to explain the sigmoid shape of the biliary excretion curve in the main with different lengths of the various bile channels. If the latter point were to play a major role in producing prolonged t_{max}-times, it might be supposed that possibly under T_m-conditions the very periphery of the biliary tree is involved in the excretion of the test substances, since the slope of the sigmoid curves (figures 1 and 2) is much slower in contrast to simple bolus injection studies. Thus, it may be hypothesized that the biliary excretion of a bolus injection involves only a part of the liver and the excretion curves may represent biliary capacities only partly. This hypothesis may be supported by the zonal relationship between the cells constituing the acini and their blood supply[8].

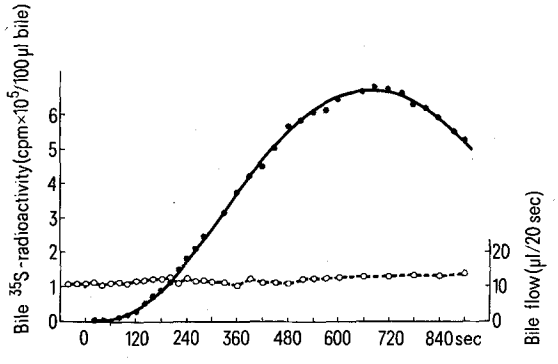

Fig. 1. Bile ^{35}S-radioactivity (●—●) and bile flow (○—○) after rapid i.v. injection of ^{35}S-sulfobromophthalein sodium (0.4 µmoles/100 g rat) under T_m-conditions in a representative experiment.

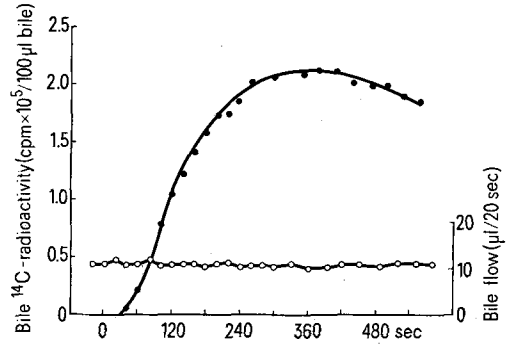

Fig. 2. Bile ^{14}C-radioactivity (●—●) and bile flow (○—○) after rapid i.v. injection of ^{14}C-taurocholate sodium (0.4 µmoles/100 g rat) under T_m-conditions in a representative experiment.

8 A. M. Rappaport, in: The Liver. Ed. Ch. Rouiller. Academic Press, New York and London 1963.