A series of test solutions with ascending concentration were dropped on the cuticular surface where a number of setae stood out. An example of the response is illustrated in Figure A. Two distinct responses were evoked at concentrations below and above $^1/_2M$ NaCl respectively. Responses to varying concentrations of NaCl and sucrose are shown in the graphs of Figure B. While response magnitude shown as impulse frequency decreased with increasing concentration of hypotonic solutions, it increased with increasing concentration of hypertonic solutions. These responses disappeared in $^1/_2M$ NaCl or $1M$ sucrose which was the isotonic concentration for marine Decapods. Similar response characteristics were observed with other solutes, choline-Cl and glycerol. Since the solutes tested were electrolyte and non-electrolyte, it is unreasonable to think that a specific substance elicited the effect on the receptor. The water sensitive setae did not respond to tactile and chemical stimulations. Although mechanoreceptor impulses also appeared for a brief period just when test solutions were applied, the discrimination between the two responses was made by differences in their time course and impulse amplitude. Mechanical stimulation given to individual hairs revealed that mechanoreceptor responses came from other hair sensilla and adapted rapidly. These sensilla were not sensitive to water. It is unlikely, therefore, that mechanosensory hairs respond to osmotic concentration changes.

The result suggests that the setal organ on the antenna is an osmoreceptor because a parameter of the test solutions correlated to the response is osmotic pressure.

The water receptors in the cat[5] and frog[6] are not associated with osmotic sensitivity, and the water sensitive receptor in the blowfly[7] does not behave as an osmoreceptor to salts or non-electrolytes other than sucrose. The responses described in the present study are different in nature from the water responses in the organs mentioned above. It is considered that the two receptor cells sensitive to changes in osmotic pressure may occur in a single seta because two types of response took place at hypotonic and hypertonic concentrations respectively. The activity of individual setae should be further studied for clearing this point.

Zusammenfassung. Es wurden an den grossen Antennen des marinen Hummers, *Panulirus japonicus*, die afferenten Antworten auf Änderungen in der osmotischen Konzentration von verschiedenen elektrolytischen und nichtelektrolytischen Lösungen untersucht. Die Borstenorgane der Antennen scheinen Änderungen des osmotischen Druckes der Badeflüssigkeit wahrzunehmen.

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Determination of the Biliary Dead Space Using ^{14}C-Taurocholate as a Marker

Whereas organ structure and its relation to function has been extensively explored for the kidney[1], relatively little is known about such relationships in the liver. Clearance measurements with easily diffusible solutes, like erythritol, have allowed to differentiate between canalicular and ductular bile formation. By contrast, knowledge concerning the dimensions and the functional importance of the biliary spaces is scarce. About 10 years ago Barber-Riley[2] introduced a method to measure the volume of the biliary tree by determining the washout volume of bile after rapid i.v. injection of BSP. However, as demonstrated by Sicot et al.[3], this method permits at best rough approximations, and probably overestimates the true value. The limitations of such measurements arise from the unknown transit time of the marker between its injection and appearance in bile. These transit times have been neglected in previous studies. The use of a marker substance which signals its appearance in bile by producing an increase of bile flow should permit us to eliminate these variables. An approach using ^{14}C-taurocholate as a marker substance was therefore employed to determine the biliary dead space.

Materials and methods. Male Sprague-Dawley rats weighing 280 to 370 g, maintained under standard laboratory conditions, were used. The common bile duct was cannulated under pentobarbital (Nembutal®) anesthesia (5 mg/100 g body wt. i.p.) with PE 10 polyethylene tubing just below the bifurcation. The body temperature was maintained between 36.5 and 37.5 °C by using a warming lamp and a heated operating table. After a control period of 15 min, sodium-24-^{14}C-taurocholate[4] (8 µmoles/100 g body wt., 37.5 µCi/mmole) dissolved in a volume of 0.3 ml of saline was rapidly injected in 15 rats via a jugular catheter and flushed with 0.3 ml of isotonic saline. Bile was collected in 20 sec periods up to 300 sec and in 1 min periods up to 480 sec and weighed. Thereafter 5 ml of Instagel® were added and radioactivity was measured in a Packard Tricarb liquid scintillation counter. The counting efficiency was determined by the channel ratio method employing an external standard.

The volume of the biliary dead space was calculated in 2 ways: *Method I: Calculation according to* Barber-Riley *as modified by* Sicot[3]. By this approach the biliary dead space is calculated as the volume of bile collected between i.v. injection and appearance of a marker in the collected bile. Since the marker does not appear in bile as a flat concentration front, the volume of the biliary dead space (BDS) is calculated in the following way:

$$\mathrm{BDS} = \mathrm{Vol}_{max} - \sum_{i=1}^{i\mathrm{C}_{max}} \frac{\mathrm{C}_i}{\mathrm{C}_{max}} \cdot \mathrm{Vol}_i - \mathrm{Vol}_{catheter}$$

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 $i\mathrm{C}_{max}$ 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 II: Modified approach for calculation of biliary dead space using a choleretic marker substance. This approach is based on the assumption that the increase of bile flow, which follows the administration of tauro-

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cholate indicates the time of appearance of injected bile salts in the canalicular lumen. The same formula as in method I was used with the only difference that Vol_{max} represented the cumulative volume of bile collected from the first increase of bile flow until maximal concentration of the marker in bile was reached. This should allow one to eliminate errors in estimation of the biliary dead space due to circulation delay and due to the transit time of the marker through the hepatocyte.

Results and discussion. Bile flow and bile radioactivity following the injection of ^{14}C-taurocholate in a representative experiment are depicted in Figure 1. Whereas bile flow increased rapidly in the third 20-sec period and reached its maximum 150 sec after the injection, radioactivity was measurable in the fourth collection period and reached its peak after 210 sec. In Figure 2 the radioactivity expressed as fraction of the maximal radioactivity reached in bile is plotted against cumulative bile volume. The shaded area represents the biliary dead space as calculated by method I. The biliary dead space volume calculated by method II corresponds to the part of the shaded area beginning at the moment when bile flow started to increase (arrow).

The mean volumes of the biliary dead space obtained with either method are given in the Table. A remarkable discrepancy may be noted between the values reported by BARBER-RILEY[2] (5.1 µl/g liver) and our findings. Even using the same method of calculation (method I) our values obtained with ^{14}C-taurocholate as test substance are only 63% of those obtained with BSP by BARBER-RILEY. This difference in the biliary dead space, depending on the type of marker substance used, could be substantiated in the present study by sequential injections of BSP and ^{14}C-taurocholate. Whereas the dead space was comparable to the value reported by BARBER-RILEY

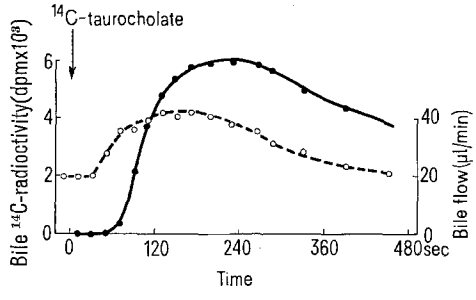

Fig. 1. Bile ^{14}C-radioactivity (●—●) and bile flow (○—○) after rapid i.v. injection of 8 µmoles 24-^{14}C-taurocholate (37.5 µCi/mmole) in a representative experiment (310 g rat).

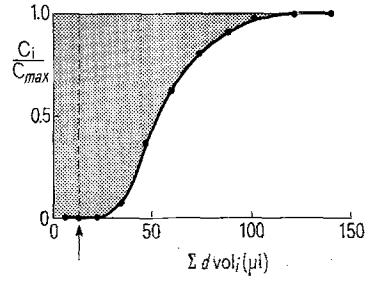

Fig. 2. Graphic illustration of the calculation of biliary dead space. The radioactivity of 24-^{14}C-taurocholate (C_i) expressed as fraction of the maximal radioactivity reached in bile (C_{max}) is plotted against cumulative bile volume. The arrow indicates the volume of bile secreted before the bile flow started to increase.

	Biliary dead space	
	µl/100 g body weight	µl/g liver weight
Method I (n = 15)	11.6[a] ± 0.7	3.2 ± 0.14
Method II (n = 15)	8.3 ± 0.5	2.3 ± 0.11

[a] $\bar{X}$ ± SEM.

when BSP was used, it was significantly smaller when ^{14}C-taurocholate was employed as marker substance. Comparing the results obtained by rose bengal, BSP and ICG in the rabbit, SICOT et al.[3] have previously shown that the calculated dead space depends on the type of marker used. Since all these markers are believed to be secreted by the hepatocyte, this difference may be attributed to different transit times of the marker through the hepatocyte. Our finding of a smaller biliary dead space when taurocholate is employed as test-substance may be interpreted by a shorter hepatocytic transit time of this marker.

Since no correction for the transit time between the site of injection and the hepatocyte is made, method I presumably leads to an overestimation of the real value, even if an ideal marker with negligible transit time through the hepatocyte is used. This difficulty should be overcome by method II.

It is assumed that the secretion of taurocholate into the bile canaliculus is accompanied by osmotically obligated water, which leads to an immediate increase of bile flow. The increase of bile flow following taurocholate injection may therefore be regarded as signal for the first appearance of the injected bile salt in the bile canaliculi. For the reasons discussed, the prebiliary transit time of the marker is not included in the calculation of the dead space when method II is used. This time corresponds to the time which elapses between the injection of taurocholate and the first increase of bile flow. It was about 30 sec in the present experiments. Thus, an important factor leading to overestimation of the biliary dead space may be excluded. As expected, the biliary dead space obtained using this method (2.3 µl/g liver) was significantly smaller than that determined by method I (Table). It was smaller than the 'upper limit' estimated by FORKER[5] for the biliary dead space (3.2 µl/g liver).

In the present studies, the volume of bile was measured which was contained within the biliary tree before secretion of the marker. A distention of the biliary tree as a result of the choleresis induced by the marker should therefore have no effect on the calculation of the biliary dead space. It is assumed in these calculations that no net changes of bile flow occur within the bile ductules. Although it has been shown in the dog, that both secretion and absorption of bile water occur within the biliary tree[6], in the rat, no evidence for such ductular mechanisms has so far been obtained under conditions similar to those of our study. Thus, erythritol clearance practically equals total bile flow, and the erythritol bile to plasma ratio in the rat was close to one under different rates of bile flow[7,8].

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Although it appears reasonable to assume that the measured volume corresponds to biliary dead space, it is not possible to define this space in strict anatomical terms. Presumably, the measured volume represents all or a large part of the bile duct system. Further investigations combined with stereological studies of the structures involved will be needed for a better definition of this space. In the absence of precise methods to determine prebiliary delay, ^{14}C-taurocholate may represent a relatively ideal marker substance for measurement of the biliary dead space; firstly it exhibits a much shorter prebiliary transit time than BSP and secondly it signals its appearance in the bile canaliculi by its potent choleretic effect.

Zusammenfassung. Mit der von BARBER-RILEY angegebenen Methode wird das Gallengangsvolumen auf Grund der unbekannten Transitzeit der verwendeten Testsubstanz vom Ort der Injektion bis zum Gallencanaliculus (präbiliäre Transitzeit) überschätzt. Es wird eine Methode angegeben, bei der ^{14}C-Taurocholat als Testsubstanz benützt wird. Auf Grund der choleretischen Wirkung dieses Gallensalzes ist es möglich, den Zeitpunkt des ersten Erscheinens von ^{14}C-Taurocholat in den Gallencanaliculi zu ermitteln und damit den bisher durch die unbekannte präbiliäre Transitzeit gegebenen Fehler zu eliminieren. Der mit dieser Methode bestimmte Gallengangtotraum der Ratte betrug 2.3 ± SEM 0.11 µl/g Leber.

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30 April 1973.

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Transmembrane Potentials of the Ductus Arteriosus

Using intracellular microelectrodes in taenia coli, BÜLBRING[1] recorded reductions in membrane potentials as well as action potentials in the presence of either stretch or acetylcholine-induced contraction. In addition, electrical recordings[2-4] have provided evidence of attenuation of membrane potentials associated with contraction of vascular smooth muscle. Since reduction in transmembrane potential has been shown to accompany contraction in vascular smooth muscle, it was of interest to determine if the ductus arteriosus fit the pattern, of electrical and mechanical response to the same stimulus, seen in other vascular smooth muscle. Physiological closure of the ductus arteriosus is initiated by an increase in blood oxygen tension which occurs at birth[5-8]. Events occurring at the cell membrane of smooth muscle cells of the ductus arteriosus which lead to its closure remain unknown. The purpose of this study is to describe the state of the transmembrane potential during exposure to a level of oxygen which stimulates contraction in a normal ductus.

Materials and methods. Ducti were obtained from goat foetuses used in another laboratory involved in fetal pulmonary research. The ducti were surgically removed by cutting the ductal insertions into the pulmonary artery and aorta. The ducti were then cut into longitudinal strips and placed in Krebs-Henseleit buffer bubbled with 95% N_2 and 5% CO_2, at 37 °C, for at least 1 h. After this period of equilibration, a strip of ductus was anchored in the recording chamber of the bath with stainless steel pins. To record transmembrane potential changes in response to oxygen, the perfusing gas was switched from 95% N_2, 5% CO_2 to 95% air, 5% CO_2.

Capillary microelectrodes similar to those described by LING and GERARD[9] were used to record transmembrane potentials. Only electrodes with a tip diameter too small to be resolved by a dissecting microscope were used (average tip diameter = 0.9 µm). A Grass P-18 D.C. amplifier was used as a cathode-follower stage to a Tektronix 564 B storage oscilloscope. Records were photographed with a PC-2 A Nihon Kohden continuous recording camera. Criteria for evaluating an impalement were: 1. clean, rapid shift of the potential; 2. stability at the new level, and 3. clean, rapid return to baseline on withdrawal. Transmembrane potentials were measured in tissue superfused by oxygenated Krebs-Henseleit buffer (95% air, 5% CO_2) or deoxygenated Krebs-Henseleit buffer (95% N_2, 5% CO_2).

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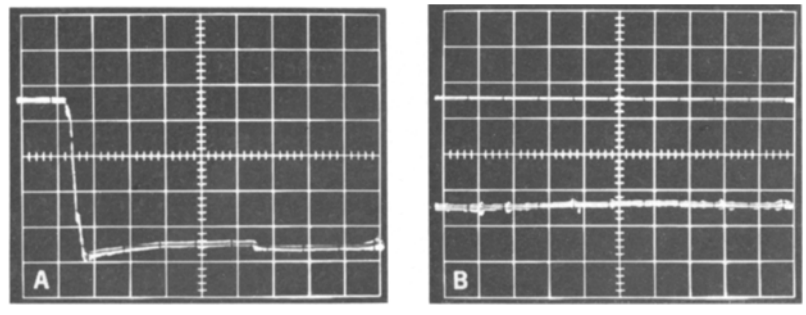

Fig. 1. Potentials obtained on a typical impalement of a cell in a fetal goat ductus arteriosus. In 1A the preparation is super-perfused with 95% nitrogen, 5% CO_2 and buffer; resting potential is −90 mV. In 1B, the buffer was gassed with 95% air, 5% CO_2; new resting potential is −60 mV. Calibration is 20 mV per horizontal division and 100 msec per vertical vision.