Negative numbers signify that the onset and termination of the center spot precedes the annulus, whereas positive numbers indicate that the annulus precedes the spot in time; zero delay means that the spot and annulus were presented simultaneously for the full duration of the stimulus. The delay (in msec) between the spot and annulus which leads to the greatest amount of suppression of the on-discharge will, henceforth, be referred to as the *best delay* for the cell. The best delay for the sustained cell of Figure 1 is zero. It can also be seen from the figure that the amount of suppression diminished for delays that are either shorter (spot leading annulus) or longer (annulus leading spot) than the best delay. The mean best delay for 51 sustained cells was found to be 7.32 msec (S.D. = 3.8), with the annulus leading the spot.

The average response histograms of Figure 2 illustrate the effect of stimulus timing upon the responses of a typical transient cell. As was the case for sustained cells there is a clearly defined best delay and there is an increase in response strength of the on-discharge as delays become shorter or longer than the best delay. The best delay for the cell in Figure 2 is 40 msec, with the annulus leading the spot. The mean best delay for 46 transient cells was determined to be 38.4 (S.D. = 5.9).

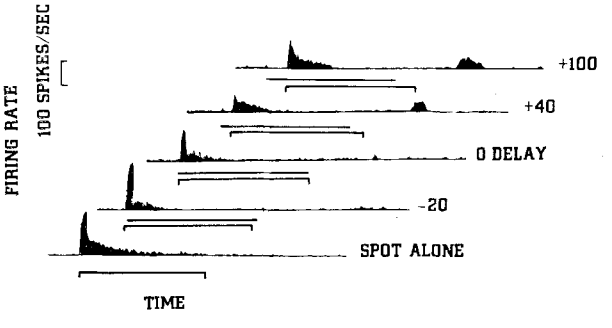

Fig. 2. Time series for typical transient cell. Refer to Figure 1 for details of stimulating procedure.

This mean is significantly ($p < 0.001$) larger than the mean best delay for the sustained cells. Although there is a clear difference between the best delay for suppression of the on-discharge, we were not able to find systematic differences between the off-discharges of these two cell types.

The results of several studies[3,6,11] suggest that the peripheral inhibition is stronger in sustained cells than in transient cells. In view of the findings of the present study, these earlier results may require reinterpretation. For example, FUKADA[3] reports that sustained cells give weaker responses to diffuse light than transient cells. The natural inference from this finding is that peripheral inhibition is weaker in transient cells. It must be pointed out, however, that the stimulus conditions of the FUKADA study favor peripheral inhibition in the sustained cell because the center and surround were stimulated simultaneously. If the periphery had been stimulated 40 msec earlier than the center, the transient cells would probably appear to have stronger surround inhibition. We find no evidence for differences in surround inhibition in transient and sustained cells when cells are compared at their best delays.

Zusammenfassung. Die Resultate deuten darauf hin, dass nicht, wie bisher vermutet wurde, die Stützzellen schwach auf Diffuslicht reagieren, sondern dass unter bestimmten Umständen die Inhibition stärker bzw. schwächer sein kann.

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Different Pathways for Hepatic Uptake of Taurocholate and Indocyanine Green

Transport across the sinusoidal membrane of the hepatocyte represents the first and least well known step in the biliary excretion of many organic anions. Studies on the hepatic handling of sulfobromophthalein in the dog[1] and of indocyanine gree in the rat[2,3] indicate that the hepatic uptake of anionic dyes is dependent on a saturable transport system obeying MICHAELIS-MENTEN kinetics. Although bile acids may be regarded as the most important organic anions which are excreted into the bile, the mechanisms governing their removal from the blood are insufficiently understood. Only recently it has been shown that the kinetics of hepatic bile acid uptake are also compatible with carrier-mediated transport[4-6]. It is not clear, however, whether anionic dyes and bile acids enter the hepatocyte via one or more pathways. Multiple mechanisms have been suggested for the biliary excretion of organic anions[7]. Thus, it appears possible that different pathways exist for the hepatic uptake of bile acids and anionic dyes. To test this hypothesis, the kinetic parameters of hepatic uptake of taurocholate and of indocyanine green were compared in the perfused rat liver, and it was investigated whether competitive inhibition phenomena are present when both anions are administered together.

Materials and methods. Male SPF-rats of the Sprague Dawley strain weighing 344 ± SD 37 g and maintained on a standard rat diet (Altromin 300 R) were used as liver donors. The liver weight averaged 3.4 ± SD 0.3% of the body weight.

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Liver perfusion studies were performed according to a method described previously[8]. The livers were perfused in situ with a perfusate consisting of Krebs-Ringer-bicarbonate buffer containing 2 g/100 ml of dried purified bovine albumin (Behringwerke AG, Marburg/Lahn, Germany) and 20% (v/v) bovine erythrocytes.

During each of 14 perfusion experiments, 3 to 6 doses of ^{14}C-labelled taurocholate[9] (75 to 25000 nmoles), indocyanine green (ICG)[10] (60 to 5000 nmoles), or mixtures of varying doses of taurocholate (75 to 25000 nmoles) with a constant dose of ICG (1000 nmoles), were rapidly injected into the portal vein. Prior to administration, each dose of ICG was mixed with ^{51}Cr-labelled erythrocytes (intravascular reference substance) and ^{99}Tc^{m}-albumin (extravascular reference substance). Taurocholate and mixtures of taurocholate and ICG were injected together with ^{99}Tc^{m}-albumin only, and the intra- and extravascular space of the liver was determined separately at the beginning and at the end of each perfusion experiment. Following the injection of the indicators, total hepatic venous outflow was collected in 2 sec intervals in tared tubes and weighed. ^{99}Tc^{m}-albumin and ^{14}C-taurocholate radioactivity were counted in the supernatant, the latter after the ^{99}Tc^{m}-radioactivity had decayed for at least 10 half-life-times. ^{51}Cr-radioactivity was assessed in hemolyzed blood after decay of the ^{99}Tc^{m}-radioactivity. ICG was measured in the supernatant by spectrophotometry at 800 nm.

The dilution curves were analyzed according to GORESKY[1,11] to obtain the initially available extravascular doses of taurocholate and ICG and their initial removal rates from the extravascular space.

The relationship between uptake rate and dose of extravascular available taurocholate or ICG was analyzed using the MICHAELIS-MENTEN equation. The maximal uptake rate (V_{max}) and the apparent half-saturation constant (Km) were calculated non-linearly in a weighted form according to WILKINSON[12]. For the statistical comparison of the transport parameters obtained in different sets of experiments, a linear transformation of the MICHAELIS-MENTEN equation[13] was used and tested with a modification of Student's t-test[14].

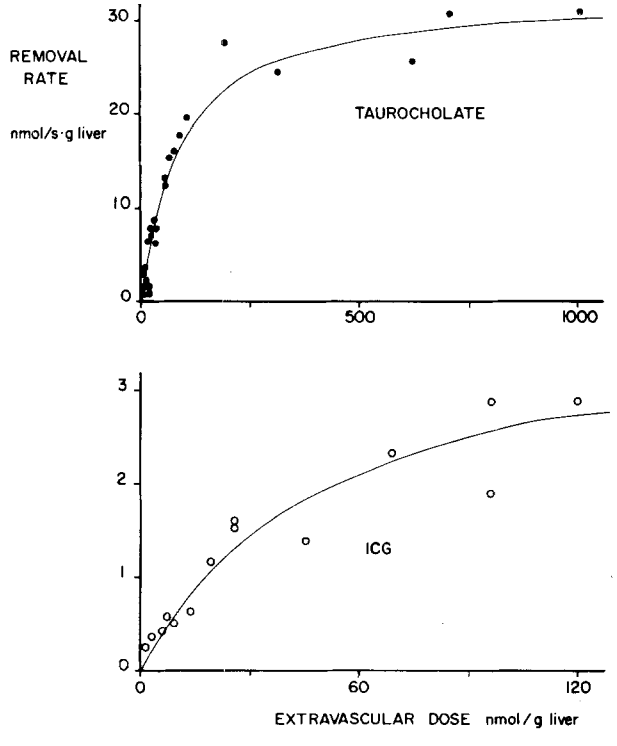
Results and discussion. The taurocholate dilution curves were always contained within the albumin curves but were reduced in magnitude in a dose-dependent fashion. This behaviour indicated that taurocholate and albumin were distributed in equal spaces and that, depending on the dose, a certain fraction of taurocholate was removed by the liver. The equality of the space of distribution of taurocholate and of albumin could be substantiated by the finding of a straight line when the logarithm of the ratio albumin to taurocholate outflow fraction was plotted against time.

Similar dilution curves were obtained with ICG. Also, for this organic anion, equality of its space of distribution with the albumin space was found. From the dilution curves, the extravascular available dose of taurocholate or ICG and the respective removal rates of these anions could be computed according to GORESKY[1,11].

The initial uptake rate of both taurocholate (Figure, top) and ICG (Figure, bottom) increased with the extravascular dose in a non-linear fashion exhibiting saturation kinetics. Analysis of these data revealed compatibility with MICHAELIS-MENTEN kinetics for both anions. These findings are in agreement with previous studies of ICG uptake in the rat[2,3] employing a different methodology, and with a recent report on the kinetics of taurocholate uptake in the dog[4]. It is noteworthy that the maximal uptake capacity (V_{max}) of taurocholate, the major bile salt in the rat, (32.5 $\pm$ SD 1.4 nmol/s·g liver) exceeded the V_{max} of the anionic dye ICG (3.8 $\pm$ SD 0.5 nmol/s·g liver) approximately by a factor of 9. This difference is much smaller than that between the steady state excretory transport maxima (Tm) of taurocholate (1310 nmol/min·100 g body weight)[15] and ICG (20 nmol/min·100 g body weight)[16] observed in the intact rat.

The apparent half-saturation constants (Km) for the hepatic uptake of taurocholate and ICG were 90.6 $\pm$ SD 10.6 and 50.0 $\pm$ SD 15.1 nmol/g liver, respectively.

To investigate whether taurocholate and ICG compete for a common receptor or carrier of the hepatocyte, taurocholate uptake was studied in the presence of ICG. No significant influence of ICG on taurocholate uptake could be detected when 1000 nmoles ICG (a dose being removed at a rate corresponding to $V_{max}/2$) were injected together with varying doses of taurocholate. No significant difference was found when the transport parameters V_{max} and Km obtained for taurocholate uptake in the presence and in the absence of ICG were compared, using the linear transformation of the MICHAELIS-



Relationship between extravascular dose and removal rate of taurocholate (top) and ICG (bottom) in the perfused rat liver.

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MENTEN equation according to WOOLF[13]. V_{max} was 31.5 ± SD 1.3 and 32.5 ± SD 1.4 nmol/s·g liver in the presence and in the absence of ICG, respectively. The corresponding *Km* values were 102.8 ± SD 14.4 and 90.6 ± SD 10.6 nmol/g liver. (Difference of slopes $t = 0.69184$; $0.5 > p > 0.4$; difference of regression lines $t = 1.75768$; $0.1 > p > 0.05$). Thus, no significant competition between taurocholate and ICG for their uptake by the hepatocyte could be demonstrated. It may, therefore, be assumed that different pathways exist for the transport of bile acids and anionic dyes from the blood into the hepatocyte. These findings substantiate previous results obtained with a different technique in the intact rat[2,3], and parallel the demonstration of multiplicity of hepatic excretory mechanisms for organic anions by ALPERT et al.[7].

Hepatic transport of organic anions from the blood into the bile may be regarded as serving mainly two purposes: the generation of bile flow, and the elimination of a variety of substances from the blood. It may offer a biological advantage that these two main functions are not dependent on the same transport system. Thus, under certain pathological conditions, the hepatic uptake of some organic anions could be disturbed while the transport of bile acids and, with it, bile flow are maintained[17].

Zusammenfassung. Mittels der Indikatorverdünnungsmethode nach GORESKY[1,11] konnte gezeigt werden, dass die hepatische Aufnahme von Taurocholat und Indocyaningrün (ICG) der MICHAELIS MENTEN Kinetik folgt. Dies ist mit der Annahme eines Carrier-Transportes vereinbar. Zwischen Taurocholat und ICG konnte keine Kompetition um die Aufnahme in die Leber nachgewiesen werden. Dies weist darauf hin, dass für den Transport von Gallensäuren und anionischen Farbstoffen vom Blut in den Hepatozyten verschiedene Transportwege existieren.

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Rhythmic Neuronal Activity in Tissue Culture[1]

Rhythmic variations in neuronal spike production have been obtained in explants taken from chick brains following a few hours of incubation[2]. A similar rhythmicity was described in fetal rodent cultures[3] and more recently from cerebellar explants by GÄHWILER[4] and CALVET[5]. During the course of investigating spreading depression in tissue culture[6], the observation of rhythmicity was expanded to several brain areas each having a different, yet characteristic, burst pattern and some having extraordinarily long periodicities measured in minutes[7].

Methods. The technique of culturing and recording is described fully elsewhere[6]. Explants from midbrain, colliculi and cerebellum were taken from newborn rats of more than 24 but less than 72 h of age. These were placed on a glass cover slip and held by a clot over a hole (averaging 200 μm in diameter) drilled through the cover slip. The cultures were maintained by the 'flying cover slip' method in roller tubes for at least 2 weeks by which time the tissue had proliferated and migrated into the hole in the side. The cover slip was placed on a chamber which permitted the addition of Gey's balanced salt solution above and below the tissue with contiguity of the fluid only at the hole. Salt-agar bridges carrying Ag·AgCl electrodes were immersed in the fluid above and below the tissue; thus, no perturbation of the tissue occurred from manipulation of the electrodes. The tissue was grown, so to speak, in the tip of the recording electrode. One electrode was at ground and the other recorded neuronal activity and direct current (dc) difference between upper and lower chambers. The dc signal was amplified and displayed on a penwriter. Neuronal activity was monitored by a digital frequency meter which emitted a pulse for each spike over 20 μV. The quantity of spikes per unit of time (generally 1.0 sec) was displayed on the penwriter. In addition the preparation was made to form one arm of an alternating current (ac) Wheatstone's bridge from which impedance imbalance was recorded and over which shocks to the preparation were delivered.

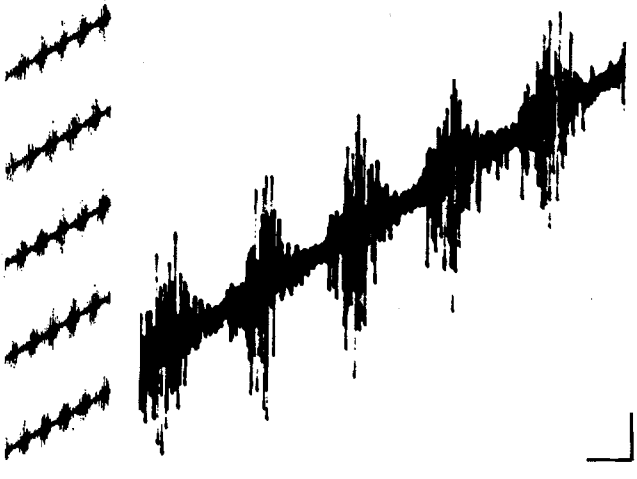

Fig. 1. Bursts of neuronal spikes photographed from the oscilloscope during the recording of neuronal activity in an explant of cerebellum cultured for 26 days. To the left is a print of a strip of film indicating the regularity of these bursts and to the right is an enlargement. Vertical mark indicates 20 μV and the horizontal 50 msec. Hole measured 190 μm in diameter.

Results and discussion. Using these techniques spontaneous rhythms were observed in explants from the several brain areas yielding distinctly different patterns of

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