Table I. Effects of prostaglandins (PG) E_1, E_2 and $F_{2\alpha}$ on secretion of ^{3}H-labelled protein from rat exocrine pancreas

Additions	Release (%)			
	Final concentrations (M)			
	10^{-8}	10^{-7}	10^{-6}	10^{-5}
None 6.80				
PGE_1	7.28	6.70	6.68	8.10
PGE_2	5.73	6.13	6.20	6.15
$PGF_{2\alpha}$	5.77	7.48	6.48	7.25

is expressed as the percent total (medium + tissue) trichloracetic acid-insoluble radioactivity found in the medium. Experiments were performed at least 3 times.

Results and discussion. The effects of varying doses (10^{-8}–10^{-5} M) of PGE_1, E_2 and $F_{2\alpha}$ on secretion of ^{3}H-labelled protein from rat exocrine pancreas are described in Table I. None of the PG's altered the rate of basal secretion. Similar findings were observed in isolated, perfused cat pancreas infused with PGE_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in the presence of secretin[8]. Furthermore, the various PG's (10^{-5} M) did not affect the secretory response to either carbachol or dibutyryl cyclic AMP (Table II). Serotonin (10^{-7}–10^{4} M) also did not alter basal secretion (Table III)

Table II. Effects of prostaglandins (PG) E_1, E_2 and $F_{2\alpha}$ on carbachol and dibutyryl cyclic AMP stimulated secretion of ^{3}H-labelled protein from rat exocrine pancreas

Aditions	Release (%)			
	None	PGE_1 (10^{-5})	PGE_2 (10^{-5})	$PGF_{2\alpha}$ (10^{-5})
None	7.40	8.25	8.30	6.20
Carbachol (10^{-5})	22.88	23.70	25.70	26.97
Dibutyryl cyclic AMP (10^{-3})	13.55	15.63	14.33	13.85

Final concentrations (M) in parentheses.

Table III. Effect of serotonin (5-HT) on secretion of ^{3}H-labelled protein from rat exocrine pancreas

Additions	Release (%)			
	Final concentrations (M)			
	10^{-7}	10^{-6}	10^{-5}	10^{-4}
None 5.23				
5HT	5.48	6.10	5.30	5.60

and in a dose of 10^{-5} M was without effect on the relesae of protein stimulated by carbachol and dibutyryl cyclic AMP (Table IV).

It is concluded from these studies that though the vasoactive substances tested have a marked stimulatory effect on fluid and electrolyte secretion from exocrine pancreas[8] or salivary gland[9–10], they are without direct effect on the basal or stimulated release of protein from the pancreas.

Table IV. Effect of serotonin (5-HT) on carbachol and dibutyryl cyclic AMP stimulated secretion of ^{3}H-labelled protein from rat exocrine pancreas

Additions	Release (%)	
	None	5-HT (10^{-5})
None	6.10	5.91
Carbachol (10^{-5})	23.38	23.97
Dibutyryl cyclic AMP (10^{-3})	10.68	9.36

Final concentrations (M) in parentheses.

Résumé. Les effects de plusieurs prostaglandines et de la sérotonine sur la sécrétion des protéines du pancréas du rat ont été étudiés. Ces substances vasoactives n'agissent pas directement sur le pancréas pour provoquer un effet sécrétoire et n'affectent pas la sécrétion stimulée par carbachol ou l'analogue dibutyryl de l'AMP cyclique.

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Kinetics and Subcellular Distribution of S^{35}-Taurine Uptake in Rat Cerebral Cortex Slices

Taurine has been proposed as a possible neurotransmitter in rat cerebral cortex[1,2]. One of the criteria for such a function is a specific high affinity saturable transport mechanism at the cellular membrane, following Michaelis-Menten kinetics with K_m-values of the order of 10^{-5} M, as has been found, for example, for glycine, glutamate and γ-aminobutyrate (Gaba)[3]. K_m-values above 10^{-4} M do not seem to be involved in specific

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uptake[3]. Taurine appears to be transported in rat cerebral cortex by several different saturable and non-saturable mechanisms, for which K_m-values ranging from 5×10^{-5} to 1×10^{-2} M have variously been given[2,4,5]. In order to provide more evidence concerning the possible transmitter function of taurine, we have studied the uptake of S^{35}-taurine into rat cerebral cortex slices, determining the transport kinetics, as well as the initial uptake rate and the subcellular distribution compared to H^3-glycine and H^3-Gaba. A preliminary report has appeared in this journal[6].

Material and Methods. (a) *Uptake kinetics:* 50 mg cerebral cortex slices from male adult SIV Ivanovas rats were pre-incubated 5 min at 37°C in Krebs-Henseleit medium. After addition of S^{35}- (and cold) taurine to final concentrations between 9×10^{-8} M and 5×10^{-3} M in 2.0 ml incubation volume, the slices were incubated for 10 min at 37°C. The reaction was terminated in an icebath, the sedimented slices washed with 4 ml icecold medium and dissolved in 0.5 ml hyamine for liquid scintillation counting. Total uptake (v_{tot}) was calculated in nmoles taurine per min per g fresh weight. For each of 22 taurine concentrations, 5 separate determinations of v_{tot} on individual brains were made. The uptake kinetics were analysed by double reciprocal plotting, with linear regression lines fitted by the least squares method. The saturable transport component (v_{sat}) was determined by deduction of non-saturable (diffusion) transport at each substrate concentration: $v_{sat} = v_{tot} - K_D \times S$.

(b) *Subcellular distribution:* 120 mg cortex slices were incubated for 10 min at 25°C with 1×10^{-5} M S^{35}-taurine, (H^3-glycine and S^{35}-taurine: Radiochemical Centre, Amersham. H^3-Gaba: New England Nuclear), 4.5×10^{-7} M H^3-glycine and 4.5×10^{-7} M H^3-Gaba in 2.0 ml Krebs-Henseleit medium. After homogenization of the washed slices in 1.2 ml 0.32 M sucrose and sedimentation of the nuclear fraction at 184 g/10 min, 0.5 ml of the supernatant were layered on a 12.7 ml 10-step discontinuous sucrose density gradient designed for optimal separation of synaptosomes[7], and centrifuged for 90 min at 60,000 g; 30 fractions of 0.42 ml were collected for liquid scintillation counting. Fractions 1–7 contained mostly the mitochondria, Fractions 8–23 mainly the synaptosomes, and Fractions 28–30 the material not entering the gradient.

Results and discussion. In Table I uptake (v_{tot}) at 22 taurine concentrations is given. From these values, the unsaturable transport component was deducted, using a determined value of $K_D = 0.094$ min^{-1}. The Figure shows the double reciprocal plot for the saturable component (v_{sat}) in the range of 9×10^{-6} M to 9×10^{-4} M taurine. A single transport mechanism with a $K_m = 1.73 \times 10^{-4}$ M and a $V_{max} = 33.2$ nmoles/min per g can be calculated from these data, giving an excellent fit to the experimental points. This K_m corresponds to the low-affinity unspecific uptake mechanism found for glycine, glutamate, and Gaba[3]. In particular, glycine, which does not have a transmitter function in cortex, has a $K_m = 2.6 \times 10^{-4}$ M in cerebral cortex slices[8], similar to our value for taurine. Our kinetic data thus do not support a specific high-affinity uptake mechanism, such as has been described by KACZMAREK and DAVISON[2] and LÄHDESMÄKI and OJA[5]. This conclusion is based on the determination of the relative initial uptake rates, calculated as the distribution coefficient between the slices and the medium after 10 min incubation (Table II). Taurine is taken up much more slowly than glycine and Gaba. In particular, Gaba, an inhibitory neurotransmitter with a $K_m = 2 \times 10^{-5}$ M in cortex slices[3], has a 15-fold higher initial uptake rate than taurine. This too argues against a trans-

Table I. Total uptake rates of S^{35}-taurine into rat cortex slices at 37°C

S Taurine concentration (M)	V_{tot} Uptake rate (nmoles/min per g fresh wt.)
9.25×10^{-8}	0.0263 ± 0.0044
1.85×10^{-7}	0.0523 ± 0.0088
3.70×10^{-7}	0.104 ± 0.019
5.55×10^{-7}	0.152 ± 0.015
7.40×10^{-7}	0.206 ± 0.042
9.25×10^{-7}	0.269 ± 0.023
1.85×10^{-6}	0.549 ± 0.063
3.70×10^{-6}	1.082 ± 0.118
5.55×10^{-6}	1.34 ± 0.19
7.40×10^{-6}	1.96 ± 0.19
9.25×10^{-6}	2.54 ± 0.16
1.72×10^{-5}	4.25 ± 0.67
4.07×10^{-5}	10.1 ± 0.7
7.77×10^{-5}	17.7 ± 1.1
1.15×10^{-4}	23.3 ± 2.6
2.80×10^{-4}	45.3 ± 3.9
5.59×10^{-4}	77.2 ± 2.3
7.44×10^{-4}	98.2 ± 10.6
9.29×10^{-4}	117.0 ± 9.7
1.11×10^{-3}	120.0 ± 12.4
2.22×10^{-3}	210.9 ± 10.8
4.62×10^{-3}	429.1 ± 50.5

Each value is the mean of 5 determinations on individual animals

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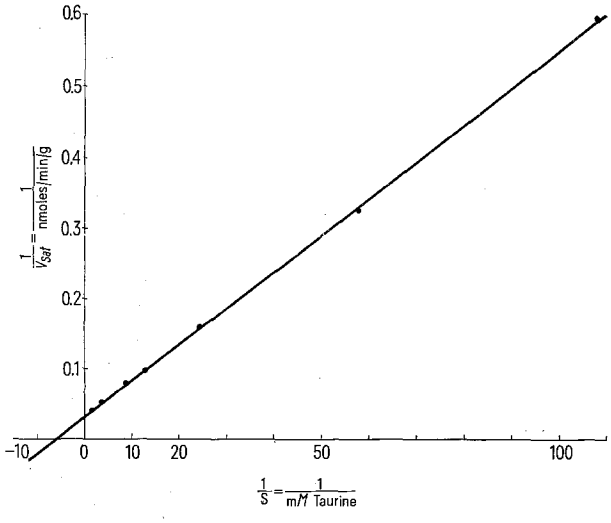

Double reciprocal plot of the saturable transport component (V_{sat}) of taurine uptake into rat cerebral cortex slices. The regression line fitted to the experimental points gives $K_m = 1.73 \times 10^{-4}$ M and $V_{max} = 33.2$ nmoles/min per g.

Table II. Tissue/medium ratio (relative initial uptake from the medium after 10 min incubation at 25 °C) for taurine, glycine and Gaba in rat cortex slices (calculated as cpm per 100 mg slices/cpm per 100 µl initial incubation medium)

		N	Tissue/medium ratio
^{35}S-taurine	1.0×10^{-5} M	7	0.73 ± 0.14
^{3}H-glycine	4.5×10^{-7} M	5	3.38 ± 0.36
^{3}H-Gaba	4.5×10^{-7} M	5	11.59 ± 0.75

Table III. Subcellular distribution of taurine, glycine and Gaba in rat cerebral cortex slices after 10 min uptake at 25 °C

		N	Fraction 1–7	Fraction 8–23	Fraction 24–27	Fraction 28–30
^{35}S-taurine	1.0×10^{-5} M	7	2.1 ± 0.5	27.4 ± 0.5	13.1 ± 1.3	57.3 ± 2.4
^{3}H-glycine	4.5×10^{-7} M	5	1.6 ± 0.3	40.6 ± 1.3	19.7 ± 1.1	38.0 ± 1.9
^{3}H-Gaba	4.5×10^{-7} M	5	1.4 ± 0.1	39.8 ± 1.2	19.1 ± 0.9	38.7 ± 1.0

Fractionation of homogenized slices (after sedimentation of cell nuclei at 184 g/10 min) on a 10-step discontinuous sucrose density gradient[7]. Figures give the precent of total cpm's applied to the gradient found in the 4 regions containing different subcellular structures.

Zusammenfassung. Die Kinetik der Aufnahme von S^{35}-Taurin in Rattencortex-Schnittchen wird im Konzentrationsbereich von 9×10^{-8} M bis 5×10^{-3} M untersucht. Nach Abzug des Transportes durch Diffusion ($K_D \times S$) findet man einen Mechanismus, der Michaelis-Menten Kinetik folgt (v_{sat}), mit $K_m = 1{,}73 \times 10^{-4}$ M. Ein solcher Transport liegt nicht im Bereich des spezifischen «uptake» der Neurotransmitter. Auch die sehr niedrige Aufnahme-Rate und die subzelluläre Verteilung nach «uptake» sprechen gegen eine Neurotransmitter-Funktion von Taurin.

mitter role for taurine. Furthermore, the subcellular distribution patterns of the 3 amino acids (Table III) shows that, while about 40% of glycine and Gaba are found in fractions 8–23 (synaptosomal), only 27% of the taurine label appears there. Most of the taurine remains in the supernatant (57%) compared with only 38% for glycine and Gaba.

Taurine is present in unusually high concentrations in mammalian brain[9] and retina[10]. In chicken retina, uptake kinetics have an apparent $K_m = 1.53 \times 10^{-3}$ M, falling into the low-specificity range[11], as we have found for rat cortex. Our preliminary experiments on rat retina and spinal cord slices (unpubl. observations) have shown even lower uptake rates than those in cortex. There are 3 lines of physiological evidence pointing to a transmitter function of taurine: (a) it inhibits neurones when applied to brainstem and spinal cord[12]; (b) it is released from chicken retina in response to light stimulation[13]; (c) it is released from cortex slices upon electrical stimulation[2]. In spite of this evidence, biochemical studies on a neurotransmitter role of taurine have so far had conflicting results[2, 4, 5, 14]. Our analysis of the kinetics and subcellular distribution of taurine uptake speaks against a neurotransmitter role for this amino acid.

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The Mechanics of Breathing in Respiratory Acidosis

The effect of respiratory acidosis on the airway diameter during bronchial contraction has not yet been sufficiently clarified. For this reason investigations on the influence of CO_2 and H^+ion concentration in the blood on the mechanical properties of the respiratory system were undertaken. Since it cannot be assumed, a priori, that the action of these two substances on the lungs and bronchial tree is the same, the influence of CO_2 and H^+ions was tested separately.

Material and methods. 22 rabbits weighing 2.60–3.25 kg were used. After immobilization, the animals were placed under light ether anesthesia and the trachea cut and connected to a Fleisch pneumotachometer. The jugular vein and carotid artery were cannulated with heparinized polyethylene catheters. A balloon was inserted into the oesophagus to record intraoesophageal pressure. 30 min after cessation of the anesthesia, the animal was placed in a body plethysmograph and gallamine triethiodide (Flaxedil) was injected into the jugular vein (4 mg/kg body wt.) to paralyze the respiratory muscles. As soon as respiratory movements ceased, controlled ventilation was started with a bellows connected to the plethysmograph. The velocity of respiratory air flow and changes of oesophageal pressure were measured for calculation of lung compliance (C_L) and total lung resistance (R). At the same time, a sample of arterial blood was taken for measurement of acid-base balance parameters. Then the animals were given an inspired gas mixture containing 10% CO_2 and 21% O_2, and after 10 min, the same measurements as those before inhalation were taken again. In 11