release of ACTH in the morning and that ACTH stimulates the activity of adenyl cyclase to produce cyclic AMP which, in turn, stimulates corticosterone production. This is further supported by observations that the adenyl cyclase rhythm, like the corticosterone rhythm[1], has the properties of a circadian rhythm and, in addition, is eliminated by hypophysectomy[11].

The animals with hypothalamic lesions provide further information on the central pathways controlling the rhythms. Both the corticosterone (Figure 1) and adenyl cyclase (Figure 2) rhythms are abolished by bilateral transection of the MFB. In these operated animals the corticosterone levels are all below the normal levels but the differences between the two groups are only significant at the points around the peak in the normal curve. The MFB animals appear to show an attenuated curve but the difference between the low (19.00 h) and high (13.00 h) points only approaches statistical significance ($p < 0.10 > 0.05$; two-tailed t-test). The adenyl cyclase levels in the MFB animals show minor fluctuations in a range around the mid-point in the normal cycle but the curve is nearly flat. Thus, both rhythms are eliminated by section of the MFB; an effect identical to that of bilateral MFB lesions on pineal rhythms of serotonin and the melatonin-forming enzyme, hydroxyindole-*O*-methyltransferase (HIOMT)[12]. The alteration of the HIOMT rhythm can be attributed to section of a visual pathway, the inferior accessory optic tract, which runs among the fibers of the MFB in the rat[13], but this will not account for the effects of MFB section on the pineal serotonin or the adrenal rhythms, since elimination of visual input should not abolish these circadian rhythms but only cause them to become free-running[1,11]. Lesions transecting the medial forebrain bundle are known to produce substantial decreases in brain monamines[14] and should destroy, in particular, serotonin-containing axons arising in the brainstem raphe nuclei to traverse the medial forebrain bundle before innervating the suprachiasmatic nuclei[15]. It has been suggested recently that the circadian corticosteroid rhythm is mediated by serotonergic neural mechanisms[16]. If this is the case, and the serotonergic innervation to the suprachiasmatic area is essential, raphe lesions should be equivalent to MFB lesions in their effects on adrenal rhythms. The MFB lesion effect on the adrenal corticosterone rhythm is very similar to that of anterior deafferentiation of the medial hypothalamus[10], suggesting that both lesions might interrupt a critical pathway to the tuberal hypothalamus. Regardless of the mechanism of the MFB lesion effect, it is evident that this pathway participates in the neural regulation of circadian rhythms in the rat adrenal gland[17].

Zusammenfassung. Nachweis, dass die Nebennierenrinde von Ratten nicht nur eine rhythmische Tagesschwankung in ihrem Gehalt an Corticosteron, sondern auch eine Schwankung im Gehalt an Adenyl-Cyclase aufweist. Diese Rhythmen können durch eine stereotaktisch ausgeführte Trennung des medianen Vorderhirnbündels, welches zum Hypothalamus führt, aufgehoben werden.

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A Comparison of L-Asparaginase from *Erwinia aroideae* and from *Escherichia coli*: Biochemical and Biological Properties

In search of L-asparaginases from sources, other than *E. coli*, which were suitable for a large scale production WADE[1-3] and PETERSON[4,5] found several strains of the plant pathogens *Erwinia* producing L-asparaginase in high yield. Treatment of mice bearing 6 H3 HED tumors with L-asparaginase from *Erwinia* caused regression of the tumors. NORTH[6] described the crystallization of the enzyme and some physical data.

Since there is no immunological cross-reaction between L-asparaginases from *Erwinia* and from *E. coli* (Figure 1), their exchange during leukaemia therapy might be favorable when allergic reactions are to be expected.

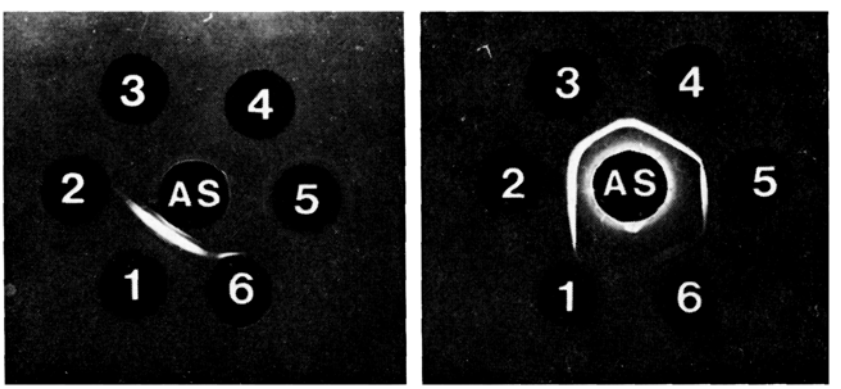

Fig. 1. Immunodiffusion according to Ouchterlony. Antisera from rabbits are applied to the center wells. The left exposure shows the precipitation with antiserum against L-asparaginase from *Erwinia aroideae*, the right exposure the precipitation with antiserum against L-asparaginase from *E. coli*. In both exposures sample 1 represents L-asparaginase from *E. aroideae*, sample 2–6 are EC2 L-asparaginases from various strains of *E. coli*. There is no immunological crossreaction between the 2 L-asparaginases.

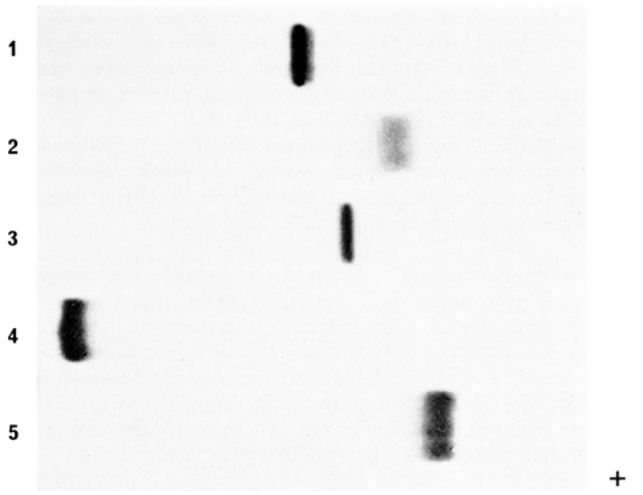

Fig. 2. PAA gel electrophoresis, pH 8.6, 10 V/cm, 3 h. 1. EC2 L-asparaginase A, *E. coli*, isoelectric point (IEP): pH 5.0. 2. EC2 acetyl L-asparaginase A, IEP: pH 4.5. 3. EC2 L-asparaginase B, IEP: pH 4.8. 4. L-asparaginase, *E. aroideae*, IEP: pH 8.2. 5. Acyl L-asparaginase, *E. aroideae*. The IEP is estimated to be in the vicinity of pH 4.5. Isoelectric focussing is not possible, since the substance decomposes below pH 5.

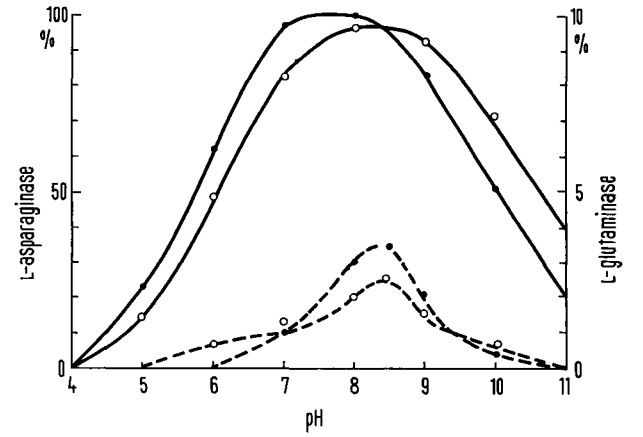

Fig. 3. L-asparaginase and L-glutaminase activities at various pH-values. ●—●, L-asparaginase from *E. aroideae*; ○—○, acyl L-asparaginase from *E. aroideae*. The dotted diagrams and the right ordinate illustrate the activities with L-glutaminase as the substrate.

Erwinia aroideae NRRL 138 was kindly supplied by Dr. PETERSON. The bacteria were grown in liquid media and harvested by centrifugation. The isolation of L-asparaginase is shown in Table I.

L-asparaginase from *Erwinia aroideae* is a basic protein with an isoelectric point of pH 8.2. However, by acylation of free amino groups, the isoelectric point shifts into the range of pH 4.5. The acylated enzyme retains 75% of the original activity (Figure 2).

Table I. Purification of L-asparaginase from *Erwinia aroideae*

Preparation		Specific activity (IU/mg protein)	Recovery (%)
1	Homogenized cells	1.1	(100)
2	Batchwise adsorption on cation exchanger, followed by elution	48	75
3	Concentration by ultrafiltration	142	53
4	Fractionated precipitation	264	43
5	Chromatography on cation exchanger	495	33
6	Gelfiltration	600	25

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Table II

	Specific activity IU/mg protein	N-terminal amino acid	S° 20, w SVEDBERG units[a]	Molecular[a] weight
L-asparaginase, *Erwinia aroideae*	550–600	L-Ala	7.0	128,000–142,000[6] 122,500 ± 4,500
Acyl-L-asparaginase, *Erwinia aroideae*	410–450	L-Ala, acylated	not estimated	not estimated
EC2 L-asparaginase A, *E. coli*	270–300	L-Leu plus 13 consecutive amino acids[11]	7.5	139,000[9] 255,000, 132,000[10] 240,000, 120,000[11] 140,000[12]
EC2 Acetyl-L-asparaginase A, *E. coli*	190–200	L-Leu, acetylated	7.5	140,000 ± 4,000
EC2 L-asparaginase B	270–300	L-Leu	7.5	240,000, 120,000[11]

[a] Our methods for the determination of molecular weight were equilibrium sedimentation[13] and gelfiltration[14], using a column of Sephadex® G 150, equilibrated with 0.1 *M* *Tris*/HCl, pH 8.0 plus 1 *M* NaCl. There was good accordance with both methods.

L-asparaginase from *Erwinia aroideae* contains no carbohydrate. The N-terminal amino acid is L-alanin, as determined by dansylation of the pure enzyme followed by hydrolysis and chromatography[7]. 3.3% glutaminase activity was found at pH 8.5 with the original enzyme, and 2.5% with the acylated enzyme. The dependence of the activity upon pH is shown in Figure 3. L-asparaginase from *Erwinia aroideae* loses its enzyme activity rather quickly in plain buffer, between pH 4 and pH 9, when exposed to temperatures above 30 °C; however at pH 10 it is rather stable under the same conditions (Figure 4). Data from L-asparaginases from *Erwinia aroideae* and from *E. coli* have been compiled for comparison in Table II.

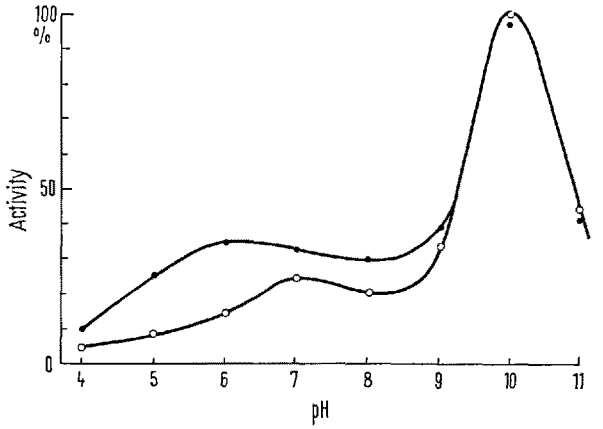

Fig. 4. Heat stability of L-asparaginase from *E. aroideae*. Residual activity was measured after exposure to 37 °C for 1 h at pH 4–11. ●—●, L-asparaginase; ○—○, acyl L-asparaginase.

The clinical application of L-asparaginase from *Erwinia* will show whether this enzyme is an agent as suitable as L-asparaginase from *Escherichia coli* for the treatment of certain types of leukaemia[15].

Zusammenfassung. L-Asparaginase wurde aus *Erwinia aroideae* gewonnen und durch Fraktionierung wie Fällungsschritte und Chromatographie um das 500fache angereichert. Das Enzym zeigt keine immunologische Kreuzreaktion mit L-Asparaginase aus *Escherichia coli*. Durch Acylierung lässt sich das basische Protein L-Asparaginase aus *E. aroideae* in ein saures Protein unter weitgehendem Erhalt der Enzymaktivität überführen.

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[15] Acknowledgments. We are grateful to Dr. T. KRANZ for running the ultracentrifugal analysis and to Dr. P. STEIN for the suggestion of the method for acylating L-asparaginase from *Erwinia aroideae*. The skilled technical assistance of M. SALLERMANN and K. WIEGAND is greatly appreciated.

Effect of Anesthetics on the Organic Acid Transport System of Renal Tubules

Although the effects of anesthesia on renal function are often measured by determining the rate at which compounds are secreted by the organic acid transport system of proximal convoluted tubules, data are not available on the effects of anesthetics on this transport system. Using the method of FORSTER and TAGGART[1] we evaluated in vitro the response of this system to clinically effective concentrations of several anesthetics.

Adult goldfish (*Carassius auratus*) were decapitated and both kidneys rapidly removed and placed in chilled fish Ringer's solution (NaCl 100.0, KCl 2.5, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.5, and $NaHCO_3$ 10.0 mM/l) where they were teased apart into individual tubules which were then placed in split Petri dishes containing $10^{-5}M$ chlorphenol red in 24 °C fish Ringer's. One side of the dish served for control studies (without anesthetic) while tubules from the same kipney were simultaneously exposed to anesthetic on the other side of the divider. Control observations confirmed that functional viability persisted for over 6 h. Nitrous oxide (80% in oxygen) was compared to nitrogen (80% in oxygen) while halothane (2.0%) and methoxyflurane (1.2%) in oxygen were compared to paired controls exposed to 100% oxygen. Thiopental studies were performed during exposure to room air. Because of the high pH of thiopental solutions, $0.01M$ secondary-tertiary sodium phosphate buffer, pH 7.4, was added to the fish Ringer's solution and comparisons were made between effects of buffer alone and buffer plus thiopental. Anesthetics were insufflated through the media for 1 h before the tubules were added. The time required for the first discernable appearance of dye intraluminally was determined visually using low power (×50–400) light microscopy, together with the time required before attainment of maximal concentration. Means and standard deviations were calculated for each series and its paired controls. The two-sample t-test was used to determine the statistical significance of the difference between two means. Significance was assumed if resulting p values were 0.05 or less.

As shown in Tables I and II halothane had no effect on the rate at which dye was transported by renal tubules. Methoxyflurane did not affect the time required for appearance of detectable amounts of intraluminal dye but did prolong the time for maximal concentrations to be achieved. Nitrous oxide increased the time to first appearance of dye but had no effect on the time required for peak dye concentrations to be reached. Both concentrations of thiopental significantly prolonged the time of first dye appearance, as well as the time of peak concentration.

The fact that nitrous oxide and methoxyflurane had different effects on the 2 measurements (time of dye appearance and time for attainment of peak concentration) suggests that different anesthetics may act in different ways. The fact that halothane had no effect implies that inhibition of this tubular transport system is not a characteristic of all anesthetics.

The concentrations of anesthetics used in the present study correspond to concentrations used in clinical

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