as described in previous papers[4,5]. Kö 592, LB 46 and ICI 50 172, which did not have any influence on the active transport of calcium into mitochondria, were less effective here, too. Only very high concentrations of these drugs ($\leqq 1.5 \times 10^{-3} M$) produced an inhibition.

In order to test the affinity of these drugs to the phospholipids of the mitochondrial membrane, we investigated whether the delay of calcium uptake in isolated mitochondria, which is induced by the drugs, is reactivated by adding special phospholipids. Figure 2 shows our results. The calcium transport of rat liver mitochondria was inhibited by $10^{-3} M$ propranolol. On adding rising amounts of phosphatidylserine or lecithin, there was a reactivation of the uptake velocity, phosphatidylserine being much more effective than lecithin. While phosphatidylserine slightly increased the rate of uptake also in the controls, lecithin had no influence. Phosphatidylaethanolamine or phospholipid fragments, such as serinphosphate or serine, had no reactivating capacity in a final concentration from $0.5–20 \times 10^{-4}$ g/ml at all. In addition to these findings in isolated mitochondria, the slowing of the velocity of ADP uptake induced by $10^{-3} M$ propranolol could be restituted by adding phosphatidylserine and lecithin, too. These results indicate that the drugs tested may react in a similar way with phospholipids of the mitochondrial membrane in vivo.

β-receptor blocking agents and some related cardioactive compounds (quinidine, tetracain) have a pronounced negative inotropic effect on the myocardium. This effect might be brought about by the reaction of the drug in a cationic form with anionic binding sites of specific phospholipids in competition with cations occupying the carriers for translocating calcium, potassium or hydrogen ions. On the basis of this kinetic pattern, an interaction between the phospholipids of the mitochondrial membrane and the effective drug might inhibit or impede the intracellular transport of calcium and adeninenucleotides. With respect to β-receptor blocking agents, this would result in a depression of the cardiac activity and metabolism which, in accordance with FLECKENSTEIN et al.[11], is not correlated to the specific β-blocking activity.

50% inhibition of lipid-facilitated calcium transport

Drug	$\times 10^{-4} M$
D-alprenolol	0.69
L-alprenolol	0.70
DL-propranolol	2.55
Quinidine	3.45
DL-oxyfedrin	2.60
L-oxyfedrin	3.10
Kö 592	15.0
LB 46	30.0
ICI 50 172	> 50.0

Zusammenfassung. β-Rezeptorenblocker hemmen den aktiven Kalziumtransport an isolierten Herzmitochondrien und den phospholipidvermittelten Kalziumtransport in Ausschüttelungsversuchen. Die Hemmung des mitochondrialen Kalziumtransportes lässt sich durch Phosphatidylserin und weniger stark durch Lecithin ($\geqq 10^{-4}$ g/ml) aufheben, während andere Phospholipide und deren Bausteine wirkungslos sind.

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[12] Parts of this study were presented at the Symposium on Calcium and the Heart of the International Study Group for Research in Cardiac Metabolism held at London on the 6th September 1970.

Chronic Urea Intoxication in Dogs

Chronic toxicity of urea has as yet still to be properly defined and consequently its role in causing uremic symptoms is not clear. Many experiments aimed at shedding light on this problem are based on the acute administration of urea to animals (JAVID et al.[1], STEVENSON et al.[2]) or to volunteers (PERKOFF et al.[3], EKNOYAN et al.[4]), while others have been based on the study of uremic animals (GROLLMANN et al.[5]) and of patients with renal failure (MERRILL et al.[6], HUTCHINGS et al.[7]) dialyzed with urea in the dialyzate.

Neither of these experimental schedules allow clear-cut conclusions on chronic toxicity of urea. This may be assessed only by studying animals in which the only blood chemical abnormality is a high blood urea level maintained for long periods of time. The study of dogs in this condition is the subject of the present paper.

Materials and methods. After a control period of 10–15 days, 12 mongrel dogs, in which 1 kidney had been previously removed, were injected s.c. every 8 h with a 10% urea solution in each single dose of 30–40 ml/kg body wt. The fluid injected was rapidly absorbed, did not cause local side effects and induced plasma urea levels ranging from 600–700 mg/100 ml (20–30 min after the injection) to 200–350 mg/100 ml immediately before the subsequent administration).

In 4 of the dogs studied the spontaneous movements were continuously recorded for 3 days during the control period, and for 3 days during intoxication. This was done by connecting the cages containing each of the 4 animals with an electrokimograph. In 2 other animals permanent electrodes were implanted for registration of the EEG.

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Haematocrit and platelet counts were performed in 5 animals, during the control period and during intoxication at intervals of 5 days. In 3 animals the bleeding time was controlled by inducing a superficial wound in an ear. At the end of intoxication, which was prolonged for 30–45 days, all the animals were killed with an i.v. injection of pentobarbital and submitted to necroscopy.

Results. The only symptom which appeared following the administration of urea was a mild drowziness with reduction in the spontaneous movements. The EEG recorded during intoxication revealed minimal changes similar to those induced by tranquillizing drugs.

No other abnormalities were observed. Dogs did not suffer from g.-i. disturbances but continued feeding regularly and, at the end of intoxication, their weight had not changed. Diuresis was, of course, increased due to the diuretic action of the urea, and consequently the ingestion of water also increased. Haematocrit, platelet counts and bleeding time did not change.

Necroscopy was completely negative, and histological examination did not reveal any significant abnormality in the liver, heart, kidney, stomach and duodenum.

Conclusions. The conclusion to be drawn from these results is that urea does not induce any severe toxic symptom in dogs, not even when its plasma concentrations were higher than those found in severe chronic uremics. Of all the typical symptoms of human uremia, only drowziness occurred, and even this disturbance disappeared as soon as administration of urea was interrupted. It seems safe to conclude that urea cannot be considered an important uremic toxin[8].

Riassunto. Le concentrazioni plasmatiche dell'urea di 12 cani normali venivano mantenute fra 250 e 650 mg/100 ml per 25–30 giorni mediante iniezioni ravvicinate di una soluzione al 10%. Nessun sintomo veniva obbiettivato ad eccezione di un lieve torpore e l'esame necroscopico era del tutto negativo.

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[8] This investigation was supported by Public Health Service research contract No. PH-43-681466.

Hemmung der Kininbildung im Blut durch *p*-Amidinophenylbrenztraubensäure

In den vorangegangenen Untersuchungen über die Hemmung der kininbildenden Fermente (Kallikreine) durch Benzamidinderivate konnte gezeigt werden, dass die als kompetitive Hemmstoffe des Trypsins bekannten synthetischen Inhibitoren eine starke Hemmung des Serumkallikreins bewirken, während das Pankreaskallikrein bei gleicher Inhibitordosis nicht gehemmt wurde[1,2]. Als stärkster Hemmstoff des Serumkallikreins hatte sich die von GERATZ[3] als Trypsinhemmstoff beschriebene *p*-Amidinophenylbrenztraubensäure (APPA) erwiesen. Diese in vitro erhobenen Befunde sind von besonderem Interesse, da die pharmakologische Beeinflussung des Kallikrein-Kinin-Systems an Bedeutung gewinnt und zur Klärung der physiologischen und pathologischen Rolle dieser proteolytischen Reaktionskette beitragen kann. Wir haben daher geprüft, ob sich mit Hilfe von APPA auch die Kininbildung im Blut beeinflussen lässt.

Es wurde zunächst der Einfluss von APPA auf die durch Pankreaskallikrein (Präparat aus Schweinepankreas mit einer Aktivität von 525 KE/mg) und Serumkallikrein (nach dem Verfahren von HABERMANN[4] aus Schweineserum isoliertes Präparat; Aktivität entspricht 1 KE/mg) bewirkte Kininbildung im Serum untersucht. Die Bestimmung der gebildeten Kininmenge erfolgte durch Messung ihrer kontraktionsauslösenden Wirkung am isolierten Meerschweinchenileum (Versuchsbeispiel Figur 1). Demnach wird die Kininbildung durch Serumkallikrein in Abhängigkeit von der APPA-Konzentration gehemmt ($I_{50} = 6 \times 10^{-6} M$). Die Kininfreisetzung durch Pankreaskallikrein wird dagegen in Übereinstimmung mit Untersuchungen anhand der Hydrolyse von synthetischen Substraten erst bei sehr viel höheren APPA-Konzentrationen beeinflusst ($I_{50} > 10^{-3} M$).

Zum Nachweis der Antikallikrein-Wirkung in vivo wurde versucht, die durch Injektion von Serum- oder

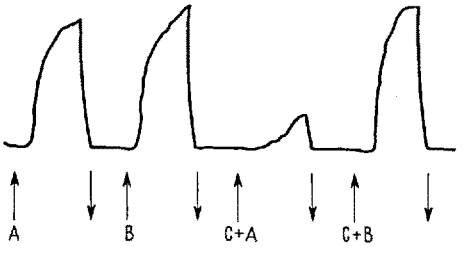

Fig. 1. Einfluss von 4-Amidinophenylbrenztraubensäure (APPA) auf die Kininbildung im Serum durch Serumkallikrein (SK) und Pankreaskallikrein (PK). Messung der Kininwirkung am isolierten Meerschweinchenileum in 10 ml Tyrodelösung mit 0,5 ml Humanserum. A) Zugabe von 0,5 Einheiten SK. B) Zugabe von 0,5 Einheiten PK. C) Zugabe von 0,1 µMol APPA.

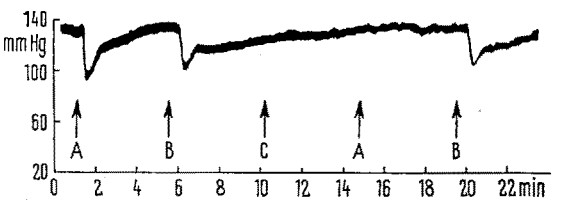

Fig. 2. Wirkung von APPA auf den blutdrucksenkenden Effekt von Serumkallikrein (SK) und Pankreaskallikrein (PK) beim Kaninchen (Blutdruckmessung in der A. carotis communis). A) i.v. Injektion von SK 3 KE/kg. B) i.v. Injektion von PK 2 KE/kg. C) i.v. Injektion von APPA 1,0 mg/kg.

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