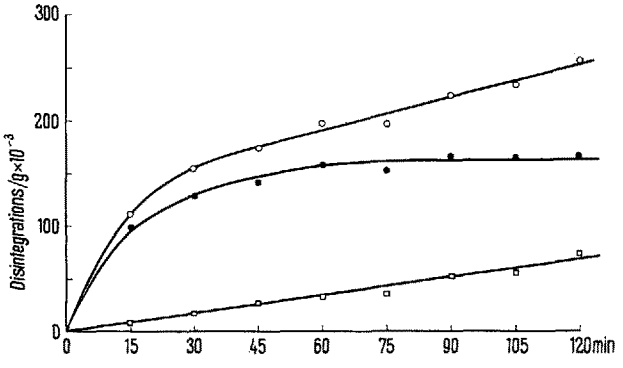
65°C. The clear and only slightly coloured solutions were diluted with dioxane, and aliquots of the resulting solutions added to counting vials containing a scintillation mixture of PPO and POPOP in toluene[5]. The samples were then refrigerated before counting in a Panax liquid scintillation equipment.

Results. The results of the experiments can be summarized as follows (Figure). Upon the administration of a gas mixture with a constant concentration of $^{14}CO_2$, the total tissue concentration of radioactive CO_2 increased roughly exponentially during the first 60 min, after that time the increase was roughly linear. The main part of the $^{14}CO_2$ was recovered in the acid-labile CO_2 fraction, and thus represented HCO_3^- and CO_2. The increase of radioactivity in this fraction was roughly exponential[4], a steady state being reached after about 60 min. The organic fractions took up $^{14}CO_2$ at a rate which was linear with time, the $^{14}CO_2$ fixed in organic compounds after 15, 30, 60 and 120 min amounted to 9, 17, 21 and 35%, respectively, of the total radioactivity of the tissue. Of these organic fractions, the main part of the radioactivity was recovered in the acid-soluble fraction, while of the remainder only the proteins were labelled to a significant degree. Thus, the labelling of the protein fraction at all times amounted to about 10% of the radioactivity which was fixed in organic compounds. During the time periods studied, labelling of the lipids and the nucleic acids was hardly significant.



The rate of incorporation of inspired $^{14}CO_2$ into the brain tissue of rats (unfilled circles) as well as into the acid-labile (filled circles) and the acid-soluble (squares) fractions of the tissue. The difference between the curve depicting the 'total' incorporation (unfilled circles) and the curve representing incorporation into the acid-labile compounds (HCO_3^- and CO_2, filled circles) is the total incorporation into organic tissue constituents.

Discussion. The results show that there is a rapid exchange between 'inorganic' C (CO_2 and HCO_3^-) and organic C in the acid-soluble compounds of the tissue. It is striking that within 15 min of exposure, about 10% of the total $^{14}CO_2$ content in the tissue is organically fixed. The findings confirm and extend the recent observations of a rapid labelling of dicarboxylic acids from infused $NaH^{14}CO_3$[3]. It is tempting to assume that the major part of the CO_2 fixed in our experiments has been incorporated into the dicarboxylic acids, and that the incorporation of these acids into proteins is responsible for the labelling of the protein fraction. It is proposed that the observations should be extended to a study of the incorporation of $^{14}CO_2$ into different acid-soluble tissue compounds, since the present technique of administering the $^{14}CO_2$ is more physiological and permits better quantitation than previous methods of administering radioactive bicarbonate by intraperitoneal, intravenous or intraarterial injections.

Zusammenfassung. Die $^{14}CO_2$-Fixation verschiedener Fraktionen des Rattengehirnes wurde untersucht. Die Ratten wurden 15–120 min in gasförmigem $^{14}CO_2$ exponiert und in flüssigem N_2 gefroren. Nach 15, 30, 60 und 120 min waren 9, 17, 21 und 35% vom Gesamt-$^{14}CO_2$ in der Gehirnsubstanz organisch gebunden. Der grösste Teil des organischen $^{14}CO_2$ fand sich in säurelöslicher Fraktion, während ein kleinerer Teil (etwa 10%) aus der Proteinfraktion isoliert werden konnte.

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Renal Excretion of Calcium-Disodium-Ethylenediaminetetraacetic Acid – A New Tubular Secretory Mechanism?

The rapid turnover of ethylenediaminetetraacetic acid (EDTA) in the organism, which, together with its metabolic inertia, is the cause of its very low toxicity, can be attributed chiefly to very rapid renal excretion. The first evidence that this substance is excreted by active secretion of the renal tubules, as well as by glomerular filtration, was the finding that its plasmatic clearance was the same as the clearance of diodrast[1]. We therefore undertook a detailed analysis of the mechanisms which participate in its excretion from the organism. Rats with a chronic urinary bladder fistula were anaesthetized and hydrated with 12% ethanol solution, the calcium-disodium salt of EDTA (50 mg/kg B.W. and inulin 25 mg/kg) was injected and the cumulative excretion of both substances in the urine was studied for $4^1/_2$ h and compared with the rate of excretion of sodium *p*-aminohippurate (PAH – a substance known to be excreted mainly by tubular secretion), administered in the same manner. Inulin was determined by means of *β*-indolacetic acid in a strongly acid medium[2], EDTA by titration with Bi^{+++} in 0.01 *N* HCl using xylenol orange as indicator[3], PAH by diazoting and coupling with N-1-naphthylethylendiamine[4].

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The results showed that the rate of excretion of the EDTA anion was about four times higher than the rate of simultaneous inulin excretion. After $4\,^1/_2$ h, an average of over 92% of the dose of EDTA had been excreted, while inulin excretion was only about 20%. The fact that after the same time interval a total amount of about 90% of the given dose of PAH was excreted, strongly supports the idea that tubular secretion also participates in the renal excretion of EDTA in rats.

Non-ionic pH-dependent diffusion does not play a significant part in the mechanism of EDTA excretion, since in similar experiments the rate of excretion of EDTA in the urine in a state of metabolic alkalosis (average pH of the urine 7.9) or acidosis (average pH of the urine 6.2) was practically the same.

At present there are two known basic mechanisms of tubular secretion in which any of the organic substances excreted can be included. These are the hippurate system, which chiefly includes organic acids, and the other system covering organic bases. DESPOPOULOS[5] concluded that substances containing a non-saturated double oxygen bond (C=O or S=O) in addition to a carboxyl and sulphone group belonged to the first system, while the mechanism of tubular secretion of organic bases chiefly included quaternary nitrogen and onium compounds. The structure of the EDTA anion does not correspond to the structural requirements of either of the above mechanisms. Mutual competitive inhibition exists between members of the individual transport system, however. In further experiments we therefore studied the possibilities of influencing EDTA excretion by means of known competitive inhibitors of the two transport systems. In experiments carried out on the same lines, however, it was reliably demonstrated that even the preadministration of large doses of *p*-aminohippurate, diodrast or probenecid (600 mg/kg, 350 mg I/kg, 100 mg/kg B.W. respectively) did not affect the rate of EDTA excretion in rats. Similarly, repeated large doses of EDTA (230 mg total dose/kg) did not influence the excretion of *p*-aminohippurate. These experiments indicate that the EDTA anion is not transported by the hippurate system. The rate of EDTA secretion did not even alter in animals to which quinine was administered beforehand as a powerful competitive inhibitor of transport of organic bases[6]; EDTA is thus not transported by the organic base system.

Avian kidneys receive their blood supply not only from the normal renal artery, but also from the renal portal vein, which collects blood from the ipsilateral lower limb. The blood from this portal system by-passes the glomerules and flows only through the peritubular capillary network. Tubular secretory mechanisms can therefore significantly increase the rate of excretion of a substance in the kidney on the side on which it is injected as compared with the control side, as was shown by SPERBER in 1948[7].

Further experiments were therefore carried out on chickens. The chickens (White Leghorn) were anaesthetized with pentobarbital sodium (30 mg/kg B.W.), the ureters were catheterized from the cloaca, $CaNa_2EDTA$ (500 mg/kg) and inulin (100 mg/kg) were injected unilaterally into the muscles of the area of the ipsilateral portal circulation and the rate of excretion of the EDTA anion and inulin were compared separately on each side. Adequate urine flow was induced by an intravenous infusion of hypertonic (5%) sodium chloride solution. In contrast to the results obtained in similar experiments with the intramuscular injection of PAH, in which the amount excreted by the ipsilateral kidney was significantly higher than on the control side, the amount excreted after administering $CaNa_2EDTA$ by the same technique was the same on both sides (Table).

A similar difference, as compared with PAH excretion, was also obtained in a study of transtubular transport in birds[8]. After the sudden, almost instantaneous intravenous administration into the renal portal system of the avian kidney of a substance crossing the tubular wall from blood into the tubular urine (e.g. *p*-aminohippurate), it appears in individual drops of urine collected from the ipsilateral ureter far sooner than simultaneously injected inulin, which has to pass *via* the systemic circulation into the renal artery. In complete agreement with the findings obtained on using SPERBER's technique, the first detectable traces of the EDTA anion in these experiments were demonstrated only in urine drops containing simultaneously administered inulin (Figure).

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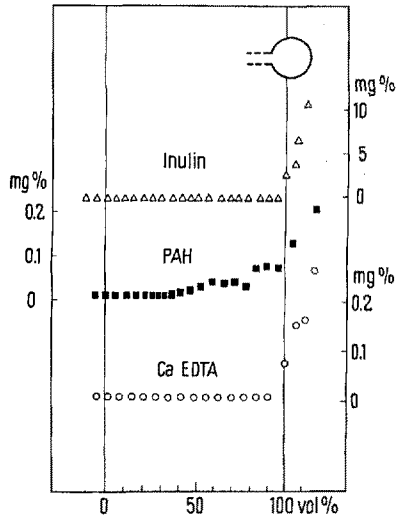
Rate of elimination of $CaNa_2EDTA$ and inulin by the right and left kidney in the chicken in comparison with the rate of elimination of sodium *p*-aminohippurate after intramuscular injection into the right leg

Time after injection	Right kidney (experimental) mg EDTA	mg PAH	mg insulin	Left kidney (control) mg EDTA	mg PAH	mg inulin
–	500 mg of $CaNa_2$ EDTA and 100 mg inulin i.m.					
10	0.417		0.85	0.53		0.93
20	1.09		0.92	1.97		1.23
30	3.60		0.85	3.90		0.91
40	3.90		0.87	2.04		0.52
Total	9.00		3.49	8.44		3.59
	Right/left ratio 1.067 for $CaNa_2EDTA$ and 0.972 for inulin resp.					
–	200 mg of sodium *p*-aminohippurate i.m.					
5		12.7			7.2	
10		27.7			11.1	
Total		40.4			18.3	
	Right/left ratio 2.21 for sodium *p*-aminohippurate					

The identical results of these two series of experiments which are, surprisingly, indicative of non-participation of tubular mechanisms in the excretion of EDTA by the avian kidney, were confirmed in further experiments comparing the rate of cumulative EDTA excretion with the rate of inulin excretion in the chicken. In experiments in which urine was collected from both kidneys together, however, and in which $CaNa_2EDTA$ and inulin (150 mg/kg and 25 mg/kg respectively) were injected simultaneously and intravenously, no difference was found in the rate of excretion of these two substances, thus indicating that they were excreted by the same renal mechanism, i.e. by simple glomerular filtration.



Excretion patterns of inulin, sodium *p*-aminohippurate and $CaNa_2$-EDTA in individual drops of urine collected from the ipsilateral ureter after instantaneous injection in the renal portal system in the chicken. Different time intervals of individual drops are substituted in the Figure by volume % of the urine volume contained in the kidney at the moment of injection.

The following conclusions may be drawn from these experiments: (1) in rats, the EDTA anion is excreted by tubular secretion as well as by glomerular filtration; (2) this tubular secretion is not dependent on the pH value of the urine; (3) it is not inhibited by the administration of sodium *p*-aminohippurate, diodrast, probenecid or quinine; (4) in the chicken EDTA anion is excreted by glomerular filtration only.

The only explanation of these results appears to be that EDTA is not excreted by either of the known tubular secretory mechanisms (i.e. neither by the hippurate system nor the organic base system), but by some other mechanism specific for mammals and not existing in birds.

Zusammenfassung. Die Ausscheidungsgeschwindigkeit nach intravenöser Injektion von $CaNa_2$ÄDTA bei Ratten war höher als die des Inulins und änderte sich weder mit dem pH-Wert des Harnes noch mit der Belastung durch hohe Dosen von PAH, Diodrast, Probenecid oder Chinin. Bei den Hühnern hingegen wurde kein wesentlicher Unterschied zwischen der Ausscheidungsgeschwindigkeit von $CaNa_2$ÄDTA und Inulin gefunden und eine tubuläre Sekretion der ÄDTA konnte sogar bei der Anwendung der Methode von SPERBER nicht bestätigt werden.

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Inhibition of Dog Fibrinolytic System in Experimental Tubular Necrosis of Kidney

Urokinase, a plasminogen activator excreted in urine, may be a product of kidney, as already suggested by some authors[1,2]. PAINTER and CHARLES[3] demonstrated an accumulation of soluble plasminogen activator during the growth of cultures of monkey and dog kidney cells in serum free media. A great fibrinolytic activity in venous renal blood has been found by BULUK et al.[4–6]. According to these authors, about 94% of urokinase is secreted by the kidney into the general circulation, and 6% only into urine.

It is well known that mercury chloride produces necrosis of kidney tubular cells, particularly of those in the Henle loops[7].

The purpose of this paper is to investigate the influence of mercury chloride intoxication upon the fibrinolytic system in dog.

Experiments were performed on 23 mongrel dogs. 14 dogs were injected with mercury chloride, subcutaneously, in a daily dose of 3 mg per 1 kg of weight during 5 days. Then blood was drawn from tibial and renal veins of those dogs under a general anaesthesia. Control dogs were treated in a similar way.

The following determinations were performed on dog plasma: prothrombin time[8], fibrinogen level[8], Factor V[9], Factor VII[10], Factor VIII[11], plasminogen and plasminogen proactivator[12], and antiplasmin[13]. Euglobulin fibrinolysis was measured using both test tube[14] and fibrin

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