Figure 2 shows the activity of enzyme PNMT in the adrenals of foetal and new-born rabbits. The rate of methylation of noradrenaline to adrenaline was very high in the foetal adrenals of 25 days. This value declined by 37% in foetuses of 28 days ($P < 0.001$). At the age of 30 days, the foetal adrenals had the same level of PNMT activity as at 28 days. At 31 days an increase of 64% from that of 30 days occurred ($P < 0.001$). At the time of parturition, the methylation of noradrenaline to adrenaline was at the lowest level compared to all the values throughout the determination period ($P < 0.001$). At few hours post parturition, the enzyme activity showed nearly 3-fold increase from that at parturition.

Discussion. The active synthesis of adrenaline by foetal adrenals and paraganglia (extra adrenal chromaffin tissue) during last term of pregnancy in rabbits was shown by BRUNDIN et al.[27,28]. The results of the present investigation indicate marked variations in the release of adrenaline and noradrenaline in plasma of foetal and new-born rabbits. The determination of plasma noradrenaline and activity of enzyme PNMT in the adrenals shows that the release of both adrenaline and noradrenaline in the blood was directly affected by changes in noradrenaline methylation to adrenaline. Previous study has confirmed that most of the adrenaline in circulation is the product of adrenomedullary secretion[29]. The modifications in catecholamine release in blood and altered pattern of PNMT activity could be attributed to changes in concentration of corticosteroids[30–32] which are essential for induction of enzyme PNMT[33]. It was reported that hypophysectomy in animals caused a reduction in adrenal content of adrenaline and PNMT activity[34]. Similar effects were also confirmed in the foetus[14,15]. The reduction in both PNMT activity and adrenaline content of adrenals of hypophysectomized animals could be prevented after pretreatment with corticosteroids[34,35]. It was also shown that release of catecholamines in the circulation could also be greatly affected by changing the concentration of corticosteroids[36,37]. In the adrenal medulla, high concentrations of corticosteroid hormones are supplied via the direct vascular connections which have been found between the cortex and medulla[38]. Therefore any change in corticosteroid level will directly influence catecholamine biosynthesis and release. The detailed study about factors affecting release of catecholamines from adrenal medulla under different physiological conditions is extensively studied in the foetus by COMLINE et al.[10,12].

It is concluded that the changes in adrenaline and noradrenaline in plasma observed in foetus and new-born rabbits are induced directly by rate of adrenaline methylation from noradrenaline.

Zusammenfassung. Die Plasmaspiegel von Adrenalin und Noradrenalin bei Kaninchen-Foeten und neugeborenen Kaninchen wurde einige Tage vor Beendigung der Schwangerschaft sowie unmittelbar postpartal bestimmt. Die Resultate zeigten, dass die verschiedenen Plasma-Catecholaminwerte auf den Metabolismus der Catecholamin-Synthese zurückzuführen sind.

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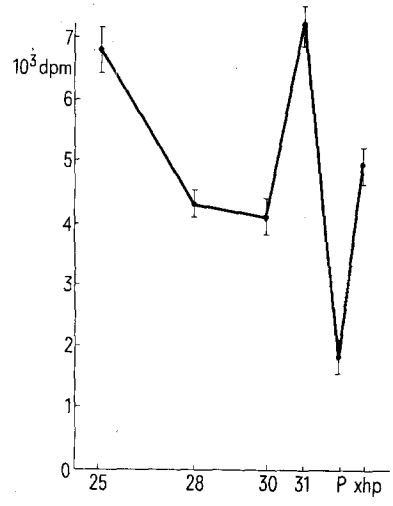

Fig. 2. Activity of enzyme phenylethanolamine-N-methyl transferase (PNMT) in adrenals of new-born and foetal rabbits. 25, 28, 30, 31 (days of pregnancy). P (parturition), XhP (4–8 h post parturition). All the groups contained at least 20 foetuses or new-born animals.

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Creatinine: A Precursor of Methylguanidine

We have previously demonstrated[1] that serum and urinary levels of methylguanidine (MG) are increased in uremia. Chronic administration of this substance produces an uremic-like syndrome in dogs[2] raising the possibility that MG is responsible for many of the manifestations of chronic renal insufficiency. The present investigation was designed to study the metabolic pathway for the synthesis of MG in the rat. In the present communication we have found that creatinine, for a long time believed to be metabolically inert[3], is a precursor of MG.

MG was determined by ion exchange column chromatography[1] and a modification of the VOGES-PROSKAUER reaction[4]. The biogenesis of MG was first studied by determining its urinary excretion in animals given unlabelled potential precursors. Sprague-Dawley rats, weighing between 150 and 300 g, were injected i.p. with

5 ml of 0.5 *M* arginine, urea and creatinine. The animals were housed in metabolic cages and a 24-h specimen of urine was collected. Next, these animals received 15 μCi of labelled potential precursors of MG i.p. Urea ^{14}C, S.A. 30–45 mCi/m*M*, arginine (guanido ^{14}C) S.A. 30–45 mCi/m*M*, guanidine ^{14}C S.A. 35–45 mCi/m*M*, creatinine ^{14}C (carbonyl) S.A. 5–15 mCi/m*M* and creatine ^{14}C, S.A. 5–15 mCi/m*M* were obtained from Schwarz/Mann, Orangeburg, New York. Creatinine $^{14}CH_3$ was purchased from Mallinckrodt Nuclear, St. Louis, Mo. After chromatography of the urine the radioactivity of the different fractions was determined by dissolving 1 ml of the effluent in 10 ml of Bray's solution and counting for 10 min in a liquid scintillation spectrometer.

The role of gastrointestinal bacteria in the formation of MG was evaluated by measuring 24-h urinary excretion of MG in animals treated with antibiotics for 1 week. 6 animals were given 60 mg of neomycin daily by nasogastric tube while another group of 6 animals was fed a mixture fo 150 mg of tetracycline, 10 mg of nystatin and 400 mg of sulfadiazine.

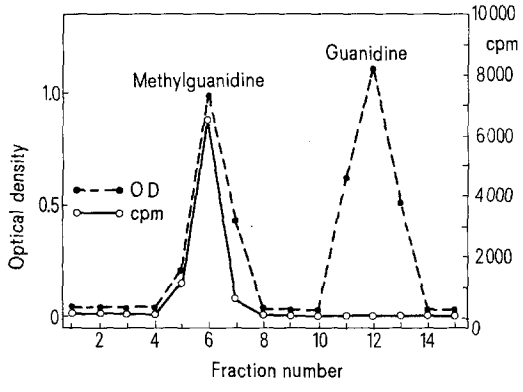
Creatinine administration orally or i.p. (Table I) resulted in a marked increase in the 24-h excretion of MG ($P < 0.02$, Student's *t*-test). The other compounds injected did not significantly change baseline MG excretion. Oral antibiotics failed to produce a significant change in urinary MG excretion (Table II). MG was present in normal amounts (86, 95, 106 μg/24-h) in 3 germ free rats obtained from Charles River Laboratories, Wilmington, Mass.

Table I. 24 h urinary excretion of methylguanidine (MG) following i.p. injection of 5 ml of 0.5 *M* solution of arginine, urea and creatinine values are given as mean ± SD

	N	MG (μg/24 h)
Control	(6)	78.3 ± 14.1
Arginine	(6)	79.8 ± 18.3
Control	(6)	84.8 ± 11.3
Urea	(6)	60.6 ± 28.1
Control	(6)	98.0 ± 10.2
Creatinine	(6)	246 ± 48.6

Table II. 24 h urinary methylguanidine (MG) excretion during control period and after 1 week of antibiotics given by nasogastric tube values are given as mean ± SD

	N	MG (μg/24 h)
Control	(6)	106 ± 16.4
Neomycin	(6)	109 ± 15.2
Control	(6)	108 ± 17.9
Tetracycline-Nystatin-Sulfadiazine	(6)	94.8 ± 19.5



Urinary radiochromatogram after i.p. administration of 15 μCi of $^{14}CH_3$-creatinine.

The other labelled urea cycle metabolites were given to these animals.

A typical urinary radiochromatogram after i.p. administration of 15 μCi of $^{14}CH_3$-creatinine is illustrated in the Figure. Total cpm in 6 24-h specimens ranged from 32,860 to 41,950. There was no recovery of labelled MG when creatinine ^{14}C (carbonyl) and the other labelled urea

The conversion of creatinine to MG in vivo was first postulated by PFIFFNER and MYERS[5]. The mechanism responsible for this conversion is not well understood. It is possible that MG arises from the degradation of creatinine by gastrointestinal bacteria. MILLER and DUBOS[6] first described a bacterial enzyme capable of degrading creatinine. VAN EYK et al.[7] identified MG and acetic acid when a strain of *Pseudomonas* was incubated with creatinine. More recently, JONES et al.[8] induced 'creatininase' activity in the gastrointestinal bacteria of rats fed creatinine. Although preliminary experiments in our laboratory suggest that rat colonic bacteria can synthesize MG from several precursors their contribution to overall MG production in animals on a standard diet is probably insignificant since this compound was present in normal amounts in the urine of antibiotic treated and germ free rats.

Our finding would explain the fate of creatinine previously unaccounted for in the anuric individual[9] and the observations of GIOVANETTI et al.[10], who recently confirmed the increased metabolic production of MG in uremia and demonstrated that there is a good correlation between serum creatinine and MG in patients with advanced renal insufficiency.

Zusammenfassung. Intraperitoneale Zufuhr von $^{14}CH_3$-Kreatinin in Ratten erhöht deren 24-h-Ausscheidung von radiomarkiertem Methyl-Guanidin im Urin, während Tiere, die andere radiomarkierte potentielle Vorläufer erhalten hatten, keine erhöhte Ausscheidung zeigten. Diese in-vivo-Konversion von Kreatinin zu Methyl-Guanidin konnte nicht der Aktion gastrointestinaler Bakterien zugeschrieben werden, da die Ausscheidung von Methyl-Guanidin sowohl in antibiotikabehandelten als auch in sterilen Ratten übereinstimmte.

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