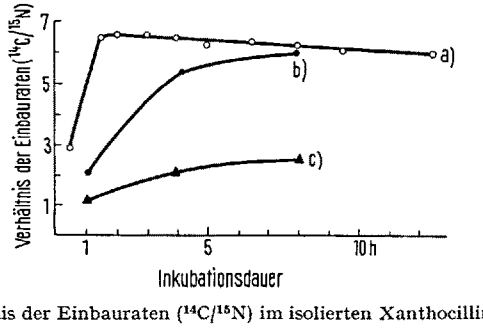
cursor übertragen werden, oder die Transaminierung führt lediglich zu einer Isotopenverdünnung im Aminstickstoff des Tyrosins, das dann als intakte C_6-C_2-N-Einheit in I eingebaut wird.

Um eine Entscheidung zwischen den eingangs aufgezeigten Alternativen zu ermöglichen, wurde der Einbau von Tyrosin-2-^{14}C-^{15}N in I genauer untersucht: a) in Abhängigkeit von der Inkubationsdauer; b) in Gegenwart von L-Alanin; c) in Gegenwart von 4-Hydroxyphenylbrenztraubensäure. Die Zusätze in den Versuchen b) und c) sollten durch Produkthemmung die Transaminierung unterdrücken.

Material und Methoden. Die Fermentation von *Penicillium notatum* Westling wie auch die Isolierung des Xanthocillins erfolgte in der früher angegebenen Weise[1]. DL-Tyrosin-2-^{14}C-^{15}N erhielten wir durch Umkristallisieren einer Mischung von DL-Tyrosin-2-^{14}C (The Radiochemical Centre, Amersham) mit DL-Tyrosin-^{15}N[5].

Die Fermentationsansätze (300 ml) wurden am 5. Tage mit jeweils 45 mg DL-Tyrosin-2-^{14}C-^{15}N inkubiert. Aufarbeitung erfolgte nach verschiedener Inkubationsdauer. Bei den Hemmversuchen wurden ausserdem zugesetzt: 225 mg L-Alanin (Versuch b)) bzw. 135 mg 4-Hydroxyphenyl-brenztraubensäure und 27 mg Phenyl-brenztraubensäure-Na (Versuch c)).

Zur Bestimmung der Einbauraten wurde isoliertes Xanthocillin X zum Dimethyläther umgesetzt. Die Radioaktivitätsmessungen führten wir mit einem Scintillationsspektrophotometer (Typ Tricarb) durch. Der ^{15}N-Gehalt wurde massenspektrometrisch durch Peakhöhenmessung am Molekülion bei 17 eV ermittelt (Varian-MAT SM1B).



Verhältnis der Einbauraten (^{14}C/^{15}N) im isolierten Xanthocillin nach verschiedener Inkubationsdauer. a) —○—○—○—, ohne Hemmer; b) —●—●—●—, mit Zusatz von L-Alanin; c) —▲—▲—▲—, mit Zusatz von 4-Hydroxyphenyl-brenztraubensäure.

Resultate und Diskussion. Für isoliertes Xanthocillin wurde das Verhältnis der Einbauraten (^{14}C/^{15}N) bestimmt und in Abhängigkeit von der Inkubationsdauer dargestellt (Figur).

Wie die Werte zeigen, isoliert man ohne Zusatz von Hemmern (Versuch a)) 30 Minuten nach der Inkubation ein Xanthocillin, bei dem der α-Kohlenstoff aus Tyrosin 3 mal besser eingebaut wird als der Stickstoff. Bereits eine Stunde später ist das Verhältnis ^{14}C/^{15}N in I auf 6,5 gestiegen und ändert sich dann innerhalb der Messzeit (bis zu 13 h) nicht mehr.

Diese Ergebnisse lassen sich nur plausibel erklären, wenn Stickstoff aus Tyrosin als intakte C_6-C_2-N-Einheit in I eingebaut wird. Wir stellen uns vor, dass nach Inkubation mit Tyrosin-2-^{14}C-^{15}N im Fermentationsansatz sehr schnell ein Stickstoffaustausch einsetzt, der innerhalb von 1,5 h zu einer Gleichverteilung der markierten Atome führt über alle Pools, die mit Vorstufen von I in Verbindung stehen. Dabei wird der Stickstoff aus Tyrosin stärker verdünnt als der α-Kohlenstoff.

Dass dieser Stickstoffaustausch über Transaminierungs-Mechanismen verläuft, legen die Ergebnisse der Einbauversuche in Gegenwart von Hemmern nahe: Bei kurzer Inkubationszeit unter Zusatz von inaktivem L-Alanin (Versuch b)) – als NH_2-Donator zur Unterdrückung der Desaminierung des eingesetzten Tyrosins – sinkt das ^{14}C/^{15}N-Verhältnis auf 2 ab.

Dieses Verhältnis nähert sich mit 1,2 dem theoretischen Wert 1, wenn durch Zusatz von *p*-Hydroxyphenylbrenztraubensäure die intermediäre Bildung von einfachmarkiertem Tyrosin-2-^{14}C gehindert wird[6].

Summary. The incorporation of tyrosine-2-^{14}C-^{15}N into xanthocillin X (I) by *Penicillium notatum* Westling has been investigated showing that nitrogen of the isocyano groups originates from tyrosine, which is incorporated as C_6-C_2-N-unit.

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Carotid Occlusion and Renal Vein Free Fatty Acids

Sympathoadrenomedullary activity during emotional stress contributes to an increased mobilization of free fatty acids (FFA) from adipose tissue[1]. The relationship between hypertension, emotional upset and various anxiety factors to plasma FFA levels has been explored by other investigators[2–4] and FFA levels have been shown to increase significantly after most stressful situations. The peak increase in FFA levels has been demonstrated to occur 15–20 min[3] after the application of the stress factor. This increase in plasma FFA levels may be attributed to increased catecholamine production, an elevated hormonal activity or perhaps increased renin release.

Bunag et al.[5] and Hodge et al.[6] have shown that renin release follows carotid occlusion. Brubacher and Vander[7] have demonstrated increased renin levels in sodium depleted dogs in the resting state. Nash[8] has hypothesized a mechanism whereby renin release is dissociated from renal hemodynamics but not from the action of sodium. This suggested that the filtered load and actual so-

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dium excretion were more important parameters to be considered than plasma circulating sodium. Carotid occlusion, as well, has been shown to increase primarily norepinephrine excretion, which would in turn enhance the mobilization of FFA[9]. The purpose of the present study was to investigate the effects of carotid occlusion and sodium depletion upon the levels of free fatty acids in the renal artery.

Methods and procedures. Fifteen mongrel dogs, weighing 15.4–26.6 kg were divided into 3 groups: group A, controls; group B, sodium depleted controls; group C, sodium depleted with carotid occlusion. The control animals were fed a standard kennel chow containing 0.5–1.5% NaCl preoperatively with water being available at all times. The other 2 groups were given 50 mg of hydrochlorothiazide 7 days prior to the day of operation and then fed a low sodium diet (prescription diet®, Hill Packing Co., Topeka, Kansas) containing less than 1 mEq per 100 g of chow. A 24 h urine collection was obtained immediately prior to surgery by confining the dogs to metabolic cages overnight. Any urine remaining in the bladder was aspirated at the time of surgery. Animals with 24 h sodium excretions of less than 7.0 mEq were considered to be experimentally sodium depleted. The experimental procedure is outlined in the Figure. The methodology and surgical techniques were standard for positive pressure respiration (air), renal catheterization, carotid occlusion, renal flow measurement and blood sampling. During surgery dogs from groups A and B were given 400–1500 cm³ of 5% dextose in H_2O and pentobarbital i.v. as needed. The experimental animals (Group C) received 900–1400 cm³ of dextose i.v. in an attempt to hydrate them without sodium replacement.

Following a 1 h stabilization period the carotid arteries of the experimental animals were occluded during the 0–30 min interval. After a further stabilization time of 1 h the carotids were again occluded during the 90–120 min interval with the perfusion pressure being rapidly con-

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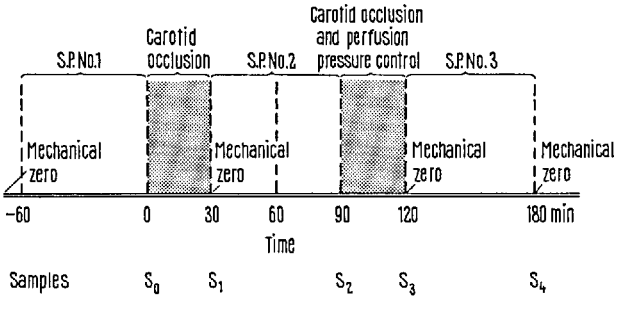
Table I. Descriptive data for the control and experimental dogs

Group	*n*	Age (years)	Weight (kg)	Sex	Weigth Loss during Na + Depletion (kg)
A	3	2.0 ± 1.0	18.78 ± 0.6	2♀ 1♂	—
B	3	7.1 ± 0.3	16.36 ± 1.4	3♂	1.55 ± 0.25
C	9	4.2 ± 1.1	22.00 ± 2.1	2♀ 7♂	1.50 ± 0.63



Experimental timetable.

Table II. Mean hemoglobin, hematocrit, plasma sodium and 24 h urine sodium values for control and experimental animals

Group	*n*	Hemoglobin (g/100 ml)	Hematocrit (%)	Plasma sodium (mEq/wt./l)	24 h urine sodium (mEq/l)
A	3	16.6 ± 1.0[a,b]	51 ± 4.0	143 ± 8.0	34.33 ± 4.12
B	3	17.0 ± 2.0	51 ± 4.0	143 ± 8.0	1.91 ± 1.22[c]
C	9	16.5 ± 1.5	51 ± 4.0	139 ± 7.0	1.74 ± 0.61[c]
Range		11–18	38–53	135–160	—
$\bar{x}$		16.7	51	142	—

[a] Values are means ± S.E.M. [b] All values represent the mean of 4 determinations on each dog. [c] Sodium depleted vs. control animals ($P < 0.01$).

Table III. Free fatty acid mobilization under experimental conditions for control and sodium depleted dogs

Group	*n*	1st Stabilization period	Carotid occlusion +30 min	2nd Stabilization period	Carotid occlusion and perfusion pressure control +30 min	3rd Stabilization period
A	3	0.61 ± 0.06	0.63 ± 0.11	0.73 ± 0.05	0.62 ± 0.12	0.63 ± 0.04
B	3	0.72 ± 0.04	0.69 ± 0.04	0.78 ± 0.02	0.65 ± 0.09	0.69 ± 0.04
C	0	0.60 ± 0.06	0.85 ± 0.02[a]	1.00 ± 0.06[a]	0.49 ± 0.04	0.62 ± 0.05

Values are means ± S.E.M. μg/ml. [a] Experimental vs. control dogs ($P < 0.01$).

trolled. A 3rd stabilization period of 1 h followed. 5 ml samples of blood were withdrawn from the left renal vein at the noted times (Figure) and measurements of plasma FFA was analyzed by the method of DOLE and MEINERTZ[10]. Sodium, hemoglobin and hematocrit estimations were determined from 50 ml blood samples taken from the left renal vein. The data were treated with the analysis of variance technique with the significance of the difference between individual means being determined by means of the Tukey's 'w' test[11].

Results and discussion. Table I contains the descriptive data for the experimental and control dogs. Most of the dogs in this study were somewhat dehydrated clinically although the dogs designated sodium depleted had lower plasma sodium levels. In spite of this observation it was difficult to demonstrate hemoglobin values outside of that range of values considered normal (Table II). The 12 animals which were subjected to the low sodium diet had 24 h urine sodium excretions of less than 7 mEq whereas the normal values are considered to be 20–50 mEq[7,8,12]. Urine collections were made during the procedure to ensure that urine formation was occurring, that renal function was not impaired, and that sodium depletion was evident.

Table III contains experimental renal vein plasma FFA level data. No differences were found between groups after surgical stabilization. Occlusion of the carotid artery caused increases in plasma FFA levels in the renal vein of the experimental animals. This increase persisted throughout the second stabilization period. Carotid occlusion and controlled perfusion pressure resulted in a drastic drop in FFA levels which were gradually returned to resting levels after the 3rd stabilization period. Limited variation was noted throughout the experimental cycle for the control animals. The hemodynamic changes which occurred secondary to carotid occlusion were predictable. During the phase of sympathetic response with control of the renal perfusion pressure, most dogs demonstrated decreases in renal blood flows. There is some experimental evidence[13–15] that intrarenal redistribution of blood flow can favour an increase in renal medullary flow and that changes in renal blood may not necessarily reflect a change in glomerular filtration rate. CASTENFORS[15] recently found that subjects under the stress of exercise decreased renal blood flow (RBF) more than glomerular filtration rate (GFR). It would seem that changes in RBF do not necessarily reflect changes in GFR. SAKAI et al.[16] demonstrated that noradrenaline infusion induced an immediate rise in the perfusion pressure which was sustained until the infusion was withdrawn whereby the resting level was again approached. No direct evidence was available to indicate catecholamine release as the renal response to ischemia.

The renal vein was selected as the site for the collection of blood to measure plasma FFA levels as the relationship between elevated renin levels after exercise[17] and catecholamine infusion[18] have been previously investigated under similar surgical procedures[19]. Angiotension stimulates the release of catecholamines from the adrenal medulla[20] which in turn affects the mobilization of FFA and renin release[21]. The relationship between the release of renin and FFA distribution to the kidneys is an interesting feedback reaction, however, the physiological reasons remain unanswered at this time.

The results indicate that the sympathetic nervous system (SNS) can control the release of FFA to the kidneys under the conditions of this study. The response to the SNS stimuli requires more than 10 min to increase renal vein plasma FFA levels. Such a lag phase would suggest that the stimulus is weak or that FFA are not a prime source of energy for the kidney under surgical trauma. It has been suggested[4] that a lipase, activated by circulating catecholamines, may continue to catalyze FFA mobilization after the application of the stressor with no quantitative relationship to the plasma norepinephrine level or the magnitude of the original stressor.

It may be concluded that the sympathetic response following carotid occlusion in the mongrel dog stimulates the release of FFA. Sodium depletion apparently does not affect the resting FFA levels in the renal vein[22].

Résumé. L'occlusion de la carotide, l'épuisement du sodium du plasma et l'effet de la pression de perfusion rénale ont été utilisés pour étudier les niveaux d'acide gras non-estérifié dans la veine rénale. Dans la présente recherche, le système nerveux sympathique controlait la libération vers les reins de l'acide gras non-estérifié. Apparemment, l'épuisement du sodium n'affecte pas les niveaux normaux de repos de cet acide dans la veine rénale.

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The Nucleoside-Copper (II) Interaction and the Tautomeric Forms of the Nucleosides[1]

Copper(II) ions are known to interact with some nucleosides, nucleotides, polynucleotides and the corresponding derivatives of the 2'-deoxyribose[2–4]. In some papers an enolic tautomer of guanosine is postulated for the copper (II) complex, both in the solid state and in aqueous solution[5,6]. The knowledge of the tautomeric forms of the nucleosides in their copper(II) complexes is necessary for proposing a correct model of the DNA-copper(II) inter-