Degradation of Haemoglobin-^{14}C and Urinary Excretion of Bilirubin-^{14}C by the normothermic Perfused Isolated Dog Kidney

A few years ago[1,2], we observed that when the free plasma haemoglobin concentration was higher than 50 mg/100 ml, 82% of the experimental dogs showed a marked increase of the urinary bilirubin excretion. There was never a significant rise in the serum conjugated bilirubin levels. We believed that the increased urinary bilirubin excretion was an auxiliary mechanism, by which the organism was able to change the accumulated plasma haemoglobin in the kidney to bilirubin, which is immediately excreted in the urine. In these situations there was a significant difference between male dogs and bitches[3]. All male dogs had a marked increase of the urinary bilirubin excretion. In these conditions we studied the metabolism of bilirubin in isolated perfused dog kidney systems[4]. If the free plasma haemoglobin level was higher than 50 mg/100 ml during perfusion, all male and a few female kidneys formed significant amounts of bilirubin. The male dog kidneys excreted this conjugated bilirubin in significant amounts in the urine, the female kidneys did not do so.

Radioactive haemoglobin was prepared according to the method of CUSTER et al.[5] with both dog or duck whole blood and with glycine-^{14}C, and then purified by gel chromatography with sephadex G-50. We used perfusion technique of a kidney with homologous blood. At the beginning of the perfusion all plasma haptoglobin was bound with unlabeled dog haemoglobin till an haemoglobinuria resulted. $^1/_2$ h later, 500 mg of labeled haemoglobin was given. The initial free plasma haemoglobin

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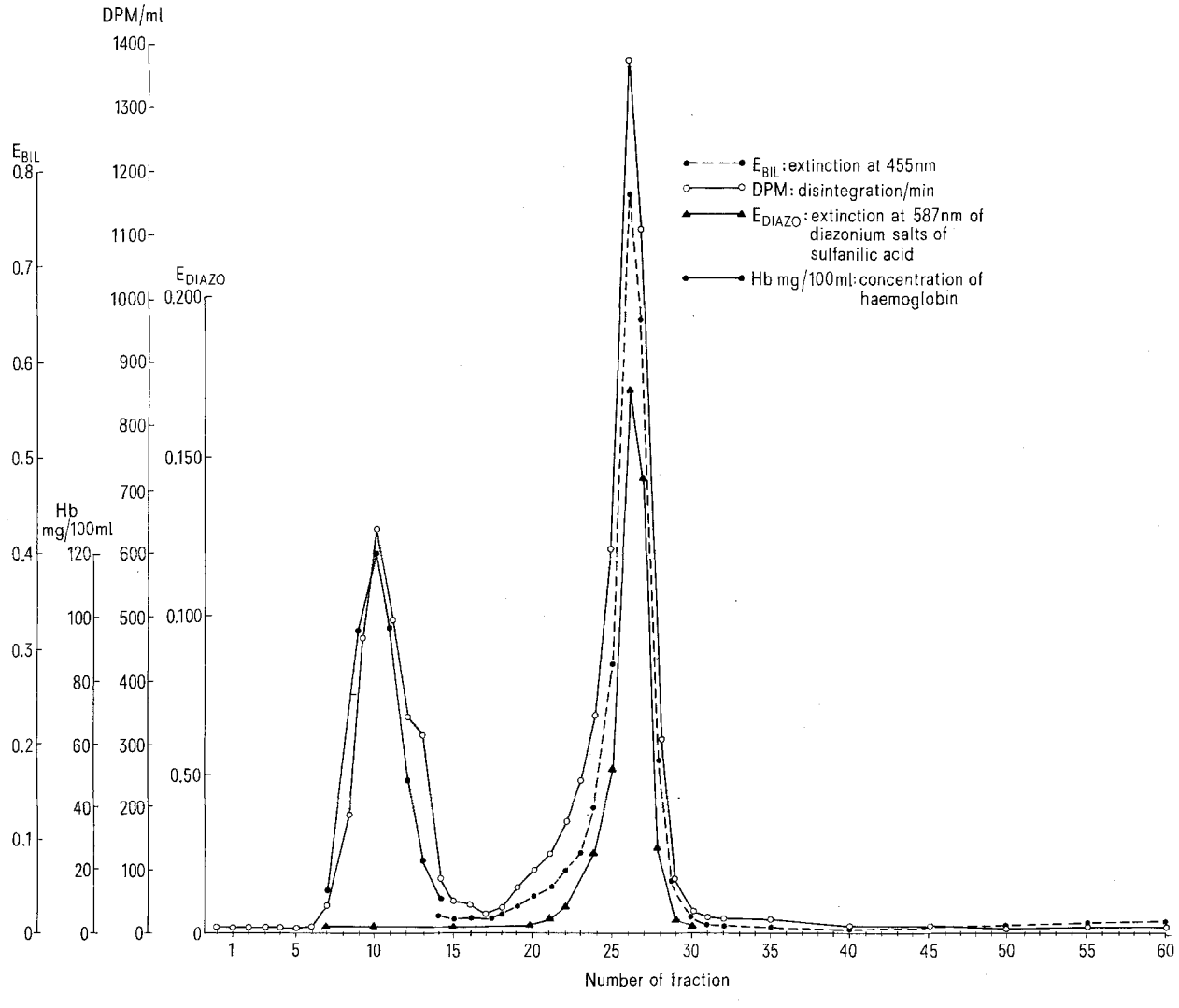

Gel chromatographic separation (Sephadex G 50) of haemoglobin and bilirubin in the urine of the 4th perfusion h of an isolated normothermic male dog kidney system after administration of 500 mg haemoglobin-2-^{14}C.

level was between 150 and 250 mg/100 ml. In 5 cases haemoglobin-2-^{14}C and in 3 cases haemoglobin-1-^{14}C was administered. The experimental conditions were the same as in the previous report[4]. Urine samples were collected at hourly intervals. All 8 experiments were done with male dog kidneys. In order to separate the labeled urinary haemoglobin and bilirubin, gel chromatography with sephadex G-50 was performed (according to VAN DER STOCK and DE SCHEPPER, in preparation), using caffeine sodium benzoate 0.1 *M* as the elution solvent. A typical result is given in the Figure. In each eluted fraction we measured the radioactivity (DPM/ml), the haemoglobin concentration, the extinction of bilirubin at 455 nm, the extinction at the 587 nm of the sulfanilic diazonium salts of bilirubin and the extinction at 530 nm of the extracted azopigments of ethylanthranylate with aethylpropylketone/butylacetate (17 : 3).

In the first radioactive fractions (No. 7 to 15), we found the radioactive haemoglobin in the void volume of the column. After this, a yellow product with an absorption maximum at 455 nm was eluted with a radioactive maximum at fraction number 26. In all fractions between fraction 24 and 28, there was a very good correlation between the course of the results of the total radioactivity, of the extinction at 455 nm and the extinction at 587 nm of the diazonium salts of sulfanilic acid. In another run of the same urine sample, we found at the same place a close correlation between the course of the results of the radioactivity, of the extinction at 530 nm, of the extracted azopigments of ethylanthranylate and of the radioactivity of the extracted azopigments of ethylanthranylate. In the 3 experiments with haemoglobin-1-^{14}C from which we know that the carboxyl carbon atom is not utilized for synthesis of the haeme group[5], the results were in complete agreement, except that there was no radioactivity at the extinction 455 nm fraction.

The absorption characteristics in the visible range, the positive significant correlation at the highest observations (fraction 24 to 28) after the administration of haemoglobin-2-^{14}C between the course of the results of the total radioactivity, of the extinction at 455 nm, of the extinction at 587 nm, of the diazonium salts of sulfanilic acid, of the extinction at 530 nm of the extracted azopigments of ethylanthranylate and of the radioactivity of the extracted diazonium salts of ethylanthranylate, as also the fact that the yellow eluate is not radioactive after administration of haemoglobin-1-^{14}C, indicates that the measured yellow substance was identical with bilirubin, from which we know that it is a radioactive decomposition product of haemoglobin-^{14}C[6]. So our results prove the formation of bilirubin from haemoglobin in the male dog kidney during haemoglobinuria.

Résumé. L'hémoglobine libre du sang, filtrée par les glomérules rénaux et partiellement absorbée par les tubules, est métabolisée par le tissu renal. A l'aide de l'hémoglobine marquée en ^{14}C, formée à partir de la glycine-2-^{14}C et de la glycine-1-^{14}C nos résultats prouvent que chez le chien mâle les reins métabolisent l'hémoglobine en bilirubine conjugée et excrètent cette dernière dans les urines.

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Transport synaptosomal du Tryptophane et de la Tyrosine cérébrale. Existence de systèmes de capture d'affinité différente

L'analyse des facteurs qui contrôlent les concentrations intraneuronales des précurseurs des monoamines représente un aspect fondamental de l'étude de la régulation de leur synthèse : c'est ainsi que des études récentes suggèrent l'importance des mécanismes de transport du tryptophane dans la régulation de la synthèse de la sérotonine (5HT)[1–6]. Ces phénomènes de capture ont déjà été étudiés sur des fractions purifiées de synaptosomes isolées à partir de cerveaux de rats[2,7]. Cependant les mécanismes de la régulation de ce transport sont encore mal connus. Ce travail consiste en une étude des variations des caractéristiques cinétiques du système de capture du tryptophane (Trp) et de la tyrosine (Tyr) par les synaptosomes consécutives aux changements de concentrations extra-cellulaires de ces amino-acides.

Matériel et méthode. Ces expériences sont réalisées sur des rats mâles de souche OFA pesant 200 à 250 g. Les animaux sont sacrifiés par décapitation et leur cerveau prélevé. Le mésencéphale est rapidement disséqué[8]. A partir de 5 à 10 de ces structures rassemblées, une fraction purifiée de synaptosomes est préparée par centrifugation sur gradient de densité de saccharose[2,9]. Après son isolement la fraction particulée contenant les synaptosomes est mise en suspension dans un milieu physiologique à pH 7,4 contenant: NaCl, 0,12 *M*; KCl, 2,4 m*M*; $CaCl_2$, $2H_2O$, 1,1 m*M*; $MgCl_2$, $6H_2O$, 0,83 m*M*; KH_2PO_4, 0,5 m*M*; Na_2SO_4, 0,51 m*M*; acide ascorbique, 0,56 m*M*; glucose, 5,35 m*M*, $NaHCO_3$, 0,028 m*M* pour 1 l d'eau bidistillée. Ce milieu est saturé d'un mélange d'O_2–CO_2 (96-4 V/V). 500 µl de cette suspension sont placés dans un bain-marie à 37 °C et préincubés à l'air durant 1 min. 500 µl du milieu à 37 °C et contenant du L-(méthylène ^{14}C) tryptophane (46 mCi/m*M*) ou de la L-tyrosine ^{14}C (10 mCi/m*M*) (The Radiochemical Centre, Amersham) sont ensuite ajoutés au milieu d'incubation. Si cela est nécessaire, la concentration initiale du Trp est ajustée à l'aide de Trp non radioactif (Calbiochem).

A la fin de la durée de l'incubation les synaptosomes sont séparés du milieu par filtration sur filtre millipore d'une porosité de 0,65 µm[2]. Les filtres sont ensuite lavés par 2 fois 2 ml de la solution physiologique à 2 °C. Leur radioactivité est alors déterminée par scintillation liquide[2]. Une analyse chromatographique réalisée à partir des

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