Entero-Hepatic Circulation of 7α-^{3}H-DHEA-^{35}S-Sulfate, 7α-^{3}H-DHEA-^{14}C-Glucuronoside and Free 7α-^{3}H-DHEA in the Human

In recent experiments[1,2] the entero hepatic circulation of C_{19}-steroids has been demonstrated in the gunea-pig. Similar investigations in the human seemed of particular interest, since various C_{19}-steroid sulphates or glucuronosides could be isolated from human bile[3,4].

In order to follow the different metabolic processes, which eventually include a reconjugation of steroids, double labelled dehydroepiandrosterone-(DHEA)-sulphate or glucuronoside[3,5] were employed. In patients with a choledochus drain (Table I) the chromatographically pure compounds were infused by intraduodenal intubation or administered directly during operation into the lumen of different intestinal segments or into the portal vein. Then, plasma of the appropriate intestinal vein, peripheral venous plasma, bile and urine were assayed for free and conjugated single or double labelled steroids by established methods[1,2], thus securing an adequate isolation and identification of free metabolites as well as the corresponding sulphatides and glucuronosides. The quantitative analysis of ^{3}H, ^{35}S and ^{14}C was performed in a Packard 'Tricarb' Spectrometer 314-EX.

Following the injection of 7α-^{3}H-DHEA-^{35}S-sulphate (^{3}H/^{35}S = 1.93) into the duodenum (experiment 1), the 24-h urine contained 26.5%, the 24-h bile 0.5% of administered ^{3}H-activity. Such an excretion approximated to that found after infusion of the same substrate into the portal vein (experiment 2), indicating a complete reabsorption of the conjugate from the intestines. In both experiments the urinary steroid sulphates exhibited a ^{3}H/^{35}S ratio similar to that of the substrate (2.48 and 2.31 respectively Table II). Hence, the passage of steroid sulphate through the intestinal wall, its transport from the portal vein to liver, peripheral circulation, kidney and finally into urine must have occurred without substantial hydrolysis and reconjugation. However, any sulphoconjugated steroid reaching the bile underwent extensive hydrolysis and reconjugation, as demonstrated by a remarkable loss of ^{35}S in the fractions of tritiated steroid conjugates in bile. The analysis of steroid conjugates in intestinal vein blood after injection of 7α-^{3}H-DHEA-sulphate into the lumen of the ileum (experiment 3) revealed a continuous but slow reabsorption of the conjugate without apparent metabolism of the steroid moiety.

In contrast to these findings, the infusion of 7α-^{3}H-DHEA-^{14}C-glucuronoside (^{3}H/^{14}C = 2.02) into the duodenum (experiment 4) resulted in the isolation of 1.3% and 0.7% of administered ^{3}H-activity from the 24-h urine or 24-h bile respectively (Table II). Most radioactivity was contained in the fraction of single labelled steroid sulphates, while in the minute fraction of labelled steroid glucuronosides, too, no ^{14}C-activity could be detected. After injection of 7α-^{3}H-DHEA-^{14}C-glucuronoside into the portal vein (experiment 5), 25.4% and 6.7% of applied ^{3}H-activity were present in the 24-h urine and 24-h bile respectively, 95% of the ^{3}H-labelled urinary metabolites being excreted in form of double labelled glucuronosides with the original ^{3}H/^{14}C ratio (Table II). Of the ^{3}H-activity in bile only 25% were isolated as single labelled steroid glucuronoside, the rest as single labelled steroid sulphates. Therefore it can be assumed that merely 15–20% of the steroid glucuronoside were reabsorbed from the intestinal tract, and this only after complete hydrolysis. From the portal vein any glucuronoside present passed through liver, peripheral circulation and kidney into urine without any

1 P. KNAPSTEIN and G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *346*, 181 (1966).
2 P. KNAPSTEIN and G. W. OERTEL, Experientia *22*, 577 (1966).
3 G. W. OERTEL, P. KNAPSTEIN and L. TREIBER, Hoppe-Seyler's Z. physiol. Chem. *345*, 221 (1966).
4 M. KOHLE, P. KNAPSTEIN and G. W. OERTEL, in preparation (1967).
5 P. KNAPSTEIN, F. WENDLBERGER and G. W. OERTEL, Hoppe-Seyler's Z. physiol. Chem. *348*, 1 (1967).

Table I. Experimental conditions

Experiment No.	Patient (age; sex; diagnosis)	Injected substance (μg; Ipm ^{3}H/^{35}S or ^{14}C)	Injected into
(1)	55 years; female; 4th day after cholecystectomy (cholelithiasis), liver-tests in serum normal	0.192 μg 7α-^{3}H-DHEA-^{35}S-sulphate (6,710,000 Ipm ^{3}H/3,480,000 Ipm ^{35}S)	Duodenum by duodenal tube
(2)	62 years; female; cholecystectomy (cholecystitis), in serum bilirubin 4.9 mg%, alc. phosphatase 5.8 IU.	0.072 μg 7α-^{3}H-DHEA-^{35}S-sulphate (2,501,000 Ipm ^{3}H/1,296,000 Ipm ^{35}S)	Portal vein, directly
(3)	63 years; male; stomach resection (ulcus ventriculi) liver tests in serum normal	2.009 μg 7α-^{3}H-DHEA-sulphate (70,000,000 Ipm)	Lumen of the upper ileum through the intestinal wall
(4)	68 years; female; 8th day after cholecystectomy (cholelithiasis), liver-tests in serum normal	0.189 μg 7α-^{3}H-DHEA-^{14}C-glucuronoside (5,368,000 Ipm ^{3}H/2,660,000 Ipm ^{14}C)	Duodenum by duodenal tube
(5)	26 years; male; stomach resection (ulcus duodeni); in serum alc. phosphatase 18.5 IU, bilirubin normal	0.029 μg 7α-^{3}H-DHEA-^{14}C-glucuronoside (835,000 Ipm ^{3}H/413,000 Ipm ^{14}C)	Portal vein, directly
(6)	35 years; male; stomach resection (ulcus ventriculi), liver-tests in serum normal	0.288 μg 7α-^{3}H-DHEA (13,500,000,Ipm)	Lumen of the duodenum through the intestinal wall

Table II. Free and conjugated steroids in experiments Nos. 1–6

Experiment No.	Material (time after injection, volume)	Free steroids (Ipm ^3H)	Sulphoconjugated steroids Ipm ^3H (^{35}S)	Steroid-glucuronoside Ipm ^3H (^{14}C)	Total % of injected ^3H-activity
(1)	Bile (1–24 h, 563 ml)	–	28,710 = 5.3 (5,269)	5,510	0.506
	Urine (1–24 h, 1,120 ml)	–	1,779,900 = 2.4 (719,000)	5,300	26.499
(2)	Bile (1–24 h 483 ml)	–	5,084 = 8.4 (603)	1,225	0.262
	Urine (1–24 h, 1,420 ml)	–	727,200 = 2.3 (314,300)	42,150	30.621
(3)	Plasma of ileacal vein (1–120 min, 40 ml)	6,605	127,250	504	0.189
(4)	Bile (1–24 h, 566 ml)	–	24,430	15,110 (0)	0.740
	Urine (1–24 h, 475 ml)	–	56,475	15,070 (0)	1.341
(5)	Bile (1–24 h, 598 ml)	–	42,310	13,990 (0)	6.750
	Urine (1–24 h, 590 ml)	–	12,500	200,100 = 2.07 (97,080)	25.450
(6)	Plasma of duodenal vein (1–30 min, 40 ml)	177,840	577,300	106,390	4.190

noticeable hydrolysis. On the other hand, extensive hydrolysis and reconjugation preceeds the hepatic excretion of steroid glucuronoside, as evidenced by total loss of ^{14}C-activity of the steroid conjugates in bile.

When free 7α-^3H-DHEA was infused into the duodenum (experiment 6), a rapid and complete reabsorption of the steroid could be demonstrated by assay of intestinal venous plasma, 68.2% being conjugated with sulphuric acid and 12.4% with glucuronic acid (Table II). In the remaining fraction of free steroids only the unchanged substrate could be detected. 14% of the ^3H-activity of the steroid conjugates were DHEA-metabolites. While 3β-hydroxy-steroids were exclusively conjugated with sulphuric acid, 3α-hydroxy-steroids were predominantly coupled with glucuronic acid. Since, according to earlier experiments, no qualitative differences were observed in the metabolism of DHEA glucuronoside and DHEA sulphoconjugate (sulphate and sulphatide respectively)[3,5], the injected substrate must have undergone its metabolic changes in the steroid moiety prior to conjugation of the resulting metabolites. The latter processes appear to involve rather specific sulphokinases and glucuronyltransferases, as already demonstrated by Dahm and Breuer[6]. The isolation of small but significant amounts of estrogens as well as of androstendione from the fractions of steroid sulphoconjugates and glucuronosides may also be of interest[7].

Zusammenfassung. Während DHEA-sulfat, welches man bei Versuchspersonen in das Duodenum infundiert hatte, die Darmwand fast ohne Hydrolyse und Metabolismus passierte, erfuhr DHEA-glucuronid eine weitgehende Hydrolyse und Rekonjugation. Freies DHEA wurde hier zu 14% metabolisiert, wobei 3β-Hydroxy-Steroide mit Schwefelsäure, 3α-Hydroxy-Steroide mit Glucuronsäure konjugiert wurden. In der Leber unterliegen DHEA-sulfat und -glucuronid einem direkten Metabolismus.

P. Knapstein, F. Wendlberger and G. W. Oertel

Universitäts-Frauenklinik Mainz and Chirurgische Universitätsklinik Homburg/Saar (Germany), 5th December 1966.

[6] K. Dahm and H. Breuer, Hoppe-Seyler's Z. physiol. Chem. *345*, 139 (1966).

[7] These investigations were supported by the Deutsche Forschungsgemeinschaft, Bad Godesberg (Germany).

Failure of L-Phenylalanine to Prevent Benzene-Induced Pancytopenia

We have previously reported on a group of 60 rabbits that were intoxicated by means of s c. benzene injections[1]. With a dose of 300 mg/kg/day of pure benzene, peripheral pancytopenia could be induced in all the animals within 1–9 weeks. In the above mentioned study it was demonstrated for the first time radioautographically with ^3H-thymidine that the pancytopenia of benzene intoxication is due to a severe inhibition of the DNA-synthesis in the bone marrow cells.

Because of the fact that the chloramphenicol molecule contains a nitrobenzene-ring, one might reasonably as-