androsterone) and androst-5-en-3β,16α,17β-triol (androstentriol) were separated from the polar glucuronoside fraction. For further characterization of the individual compounds, all steroids were diluted with the appropriate non-labelled standard and purified to constant specific activity, the steroid concentration being quantitated by means of the 2,4-dinitrophenylhydrazine reaction[9] (ketonic steroids) or the OERTEL–EIK-NES reaction[10] (androstendiol and androstentriol) (Table II). The former procedure not only allowed a repeated chromatography and ensuing quantitation of the coloured derivative but provided the separation of traces of 5α-androstan-3,17-dione and 5β-androstan-3,17-dione (androstandione and etiocholandione) from the androstendione fraction.

Table I. C_{19}-steroid glucuronosides, isolated from 24 h urine

3-glucuronoside of	dpm ^3H	dpm ^{14}C	$^3H/^{14}C$
Dehydropeiandrosterone	7,850	3,760	2.09
Testosterone	27,100	13,400	2.02
Androsterone	7,700	3,670	2.10
Etiocholanolone Epiandrosterone	58,180	27,800	2.10
Androstendione Androstandione Etiocholandione	6,100	3,020	2.02
Polar steroids	124,100	58,100	2.14

Table II. Specific activity of free steroids after enzymic hydrolysis of 3-glucuronosides and reverse isotope dilution

Steroid	Specific activity (dpm/μg) after chromatography			
	Free		2,4-DNPH-derivative	
System	A	B	C	D
Dehydroepiandrosterone		394	380	383
Androstendione		35.8	33.2	30.4
Testosterone		119	98.6	98.2
Androsterone		156	145	142
Etiocholanolone		36.6	34.5	35.0
Epiandrosterone		429	415	420
Androstendiol	178		175[a]	
16α-Hydroxy-dehydroepiandrosterone	156	150	144	148
Androstentriol	20.0		18.5[a]	

A = paper chromatography in propylene glycol/toluene; B = paper chromatography in propylene glycol/methylcyclohexane; C = thin layer chromatography on silica gel G in chloroform-dioxane (94:6 v/v); D = thin layer chromatography on silica Gel in chloroforme.
[a] As free compound.

From these results, it becomes evident that the direct metabolism of C_{19}-steroid glucuronosides, such as reduction in ring A, is not limited to 17β-glucuronosides but may occur with 3β-glucuronoside also. Hence, the conversion of dehydroepiandrosterone 3β-glucuronoside to the glucuronosides of androsterone or etiocholanolone necessarily involves the intermediate formation of 3,5-dienol glucuronoside(3), as reflected by the isolation of androstendione and testosterone and demonstrated by other authors[11]. Comparing the direct metabolism of dehydroepiandrosterone glucuronoside with that of dehydroepiandrosterone sulphate or sulphatide respectively[12], it seems noteworthy that the mode of conjugation does not seriously affect the metabolic changes in the steroid moiety, at least with regard to metabolites formed[13].

Zusammenfassung. Es wird gezeigt, dass beim Menschen Steroide in Form ihrer 3-Glucuronoside metabolisiert werden können, und zwar in ähnlicher Weise wie dies schon für entsprechende 17-Glycoside nachgewiesen worden ist.

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Role of Bacterial Endotoxins in Intestinal Ischemic (SMA) Shock

Recently, a hypothesis has been advanced that impairment of reticulo-endothelial system (RES) function with overwhelming endotoxemia is the cause of experimental irreversible (refractory) shock[1]. Evidence in support of this thesis is that RES blockade by different means has been demonstrated to induce a loss of endotoxin tolerance[2] and to render animals more susceptible to bacterial endotoxins[3]. In addition, injection of various types of gram-negative bacterial cultures caused an increased incidence of mortality in hemorrhagic and tourniquet shock[4]. These latter investigators also demonstrated that rabbits recovering from reversible hemorrhagic shock

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were extremely sensitive to bacterial endotoxin; the lethal dose being 1/100,000 of that used in control animals[5].

However, other investigators have questioned the role of bacterial endotoxin in irreversible experimental shock for several reasons: (1) the failure to demonstrate a difference in the course or ultimate outcome of animals pretreated with non-absorbable antibiotics for several days prior to hemorrhagic shock[6], shock due to ligation of the superior mesenteric artery (SMA)[6,7] or in endotoxic shock[8]; (2) the failure to detect any difference in germ-free animals versus normal controls in response to bleeding, duration of hypotension, pathology and ultimate outcome[9,10]; and (3) the failure to detect bacterial endotoxin in the blood of rabbits subjected to SMA shock[11].

More recently other work has demonstrated: (1) a 'RES depressing substance' in the ischemic intestine of rats which is not bacterial endotoxin[12]; (2) a marked and progressive functional depression of the RES of rats subjected to *mild* intestinal ischemic (SMA) shock[13,14]; (3) increased survival rates in SMA shocked animals being correlated to a hyperfunctional RES[13,14]; and (4) an increased susceptibility of rats exhibiting prior 'RES blockade' to SMA shock[15]. On the basis of these observations it was suggested that the RES does, indeed, play an important role in the progression of the experimental shock syndrome[15]. However, such studies may or may not implicate bacterial endotoxemia as the cause of the ultimate demise of an animal subjected to experimental shock.

Since the RES is known to be depressed in animals subjected to *mild* SMA shock[13,14] then an LD_{50} injection of bacterial endotoxin in such *mildly* shocked animals should exacerbate the SMA shock picture and lead to a greater incidence of shock mortality, similar to that previously seen in hemorrhagic and traumatic shock[4], if gram-negative bacteria are, indeed, elaborated by the ischemic intestine[16] and are responsible for irreversibility in all types of experimental shock[1].

1 group of Wistar strain female rats (average weight 150 g) was anesthetized with pentobarbital (30 mg/kg) and received an i.v. LD_{50} dose of *S. enteritidis* lipopolysaccharide endotoxin (Difco). A second group was similarly anesthetized and received a *sham* SMA operation (laparotomy plus *loose*, unoccluded, ligature around the SMA for a period of 20 min). A third anesthetized group of animals was subjected to a temporary ligation of the superior mesenteric artery for a period of 20 min using a previously described technic[17]. Both of the latter groups of animals received an i.v. LD_{50} dose of *S. enteritidis* endotoxin 3 h after release of the *sham* ligatures and ligated SMAs. This time interval was selected as other studies in 20 min SMA animals showed *phagocytic indices* which were 33% *depressed* over sham-operated or normal rats[13,14]. All 3 groups of rats were then observed for 48 h for survival. The results of these experiments are shown in the Table. It is quite evident from these data that endotoxemia does not exacerbate the incidence of mortality in the intestinal ischemic shocked animals or vice versa.

These results when taken together with: (1) the previously cited evidence[6-11]; (2) direct in vivo microscopic observations of mesenteries of rats subjected to SMA shock[17], showing an entirely different pattern of vascular behavior from mesenteries of rats subjected to lethal doses of endotoxin[18,19]; and (3) the inability of endotoxin tolerant rats to tolerate SMA shock[7] (a circumstance in itself arguing against endotoxemia as being the primary factor in cardiovascular collapse following SMA occlusion), although not questioning the role of gram-negative bacteria in irreversible hemorrhagic or traumatic shock do strongly suggest the need for a re-evaluation of the role of these bacteria in intestinal ischemic shock[20].

Influence of bacterial endotoxemia on survival after *mild* intestinal (SMA) ischemic shock

Group	Survivors/total rats	Survival (%)[a]
Endotoxin alone[b]	11/24	46
Sham SMA + endotoxin[c]	21/37	57
SMA + endotoxin[c]	18/35	51

[a] Survival determined at 48 h in all cases. [b] *S. enteritidis* administered i.v. in a dose of 2.0 mg/ 100 g body weight. [c] *S. enteritidis* administered i.v. in a dose of 2.0 mg/100 g body weight 3 h after sham or SMA procedure.

Zusammenfassung. Es werden Versuche zur Aufklärung der Rolle der gram-negativen Bakterien im irreversiblen ischämischen Darmschock durchgeführt: Ratten erweisen sich unter dem Einfluss einer milden Form von ischämischem Darmschock keinesfalls für Endotoxemia empfindlicher als falsch operierte oder normale Tiere, die dieselben Dosen von bakteriellem Endotoxin erhalten haben. Dadurch wird die Rolle der Endotoxemia in der Pathogenese des irreversiblen Darmschocks fragwürdig.

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