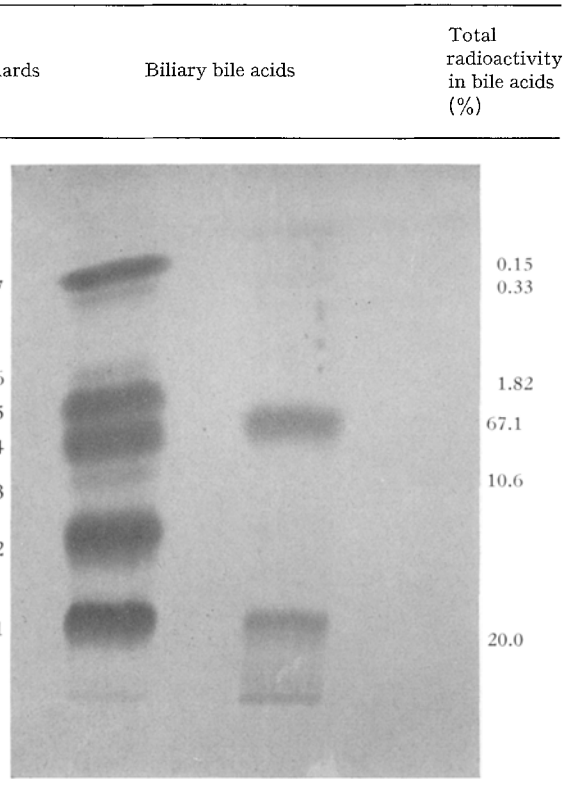

Distribution of radioactivity in biliary bile acids after injecting cholesterol-4-^{14}C (radioactivity expressed as percent of the total radioactivity in bile acids). Identification of bile acids: 1, cholic; 2, hyodeoxycholic; 3, ursodeoxycholic; 4, chenodeoxycholic; 5, deoxycholic; 6, 3β, 12α-dihydroxy cholanoic; and 7, lithocholic. Adsorbent = silica gel G. Solvent system, isoctane:isopropyl ether:acetic acid, 50:40:4 (v/v/v). The plates were stained with phosphomolybdic spray in methanol.

and crystallized to a constant specific activity with authentic cholestanol (Table). The contention that biliary cholestanol is of endogenous origin is supported by the observation that in vitro fecal cultures of the pigeon could not convert cholesterol-4-^{14}C into cholestanol[9].

The acidic fraction was subjected to thin-layer chromatography and the radioactivity present in various bile acid fractions was assessed (Figure). About 67.1% of the total radioactivity was present in the area corresponding to the authentic chenodeoxycholic acid. About 20% of the label was present in the cholic acid area. The rest of it was distributed between another dihydroxy bile acid (10.6%) and unidentified monohydroxy acids (2.3%). The formation of cholic and chenodeoxycholic acids has been demonstrated in many animal species[7]. However, it has not been shown in the pigeon to date. The identity of the label in chenodeoxy and cholic acids was confirmed by thin-layer chromatography of 2 different solvent systems[8] and crystallized to a constant specific activity. The identity of the other dihydroxy and monohydroxy bile acids could not be confirmed in this study.

Zusammenfassung. Es gelang, in Tauben die Umwandlung von injiziertem Cholesterol-C^{14} in Gallen-Cholestanol nachzuweisen, wobei Chenodeoxycholinsäure als Hauptmetabolit des Cholesterols-C^{14} in der Galle ermittelt wurde.

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Perinatal Changes of the Activity of Acyl-CoA: Monoglyceride Acyltransferase in the Rat Intestinal Mucosa

Dietary triglycerides are hydrolyzed in the lumen of the gastrointestinal tract into diglyceride, monoglyceride, free fatty acids and free glycerol[1]. In the small intestinal mucosa re-esterification of the absorbed lipolytic products occurs by two pathways, initiated either from α-glycerophosphate or monoglycerides. The monoglyceride pathway has been found to be more important in mammals, including man[2]. In this pathway acyl-CoA: monoglyceride acyltransferase catalyzes transacylation of activated fatty acids with absorbed monoglyceride forming diglycerides. It was found recently that a high fat diet evoked an increase[3] of this enzyme activity. The suckling period is naturally associated with a high fat intake, e.g., suckling rats' fat intake is 2–3 times higher than later in life[4]. In contrast, activity of pancreatic small intestinal lipase is approximately 10 times lower during the suckling period than in adult rats[5,6]. Thus, the question arose about the extent of the esterification processes in the small intestinal mucosa of suckling rats as compared to adult rats.

Methods. Studies were performed on rats of Charles River strain fed Purina Chow diet. Fetal rats (1 day before delivery) were obtained by caesarian section. 3 month-old males were used as adults. Microsomes from the mucosa (in fetuses and 3-day-old rats from the whole intestinal wall) from the entire jejunum and entire ileum were prepared from nonfasting rats according to Rodgers et al.[7]; materials from 8–9 fetal and suckling rats were always pooled and treated as 1 sample, adult rats were used individually. Activity of the acyl-CoA:monoglyceride acyltransferase was determined according to Rodgers[8],

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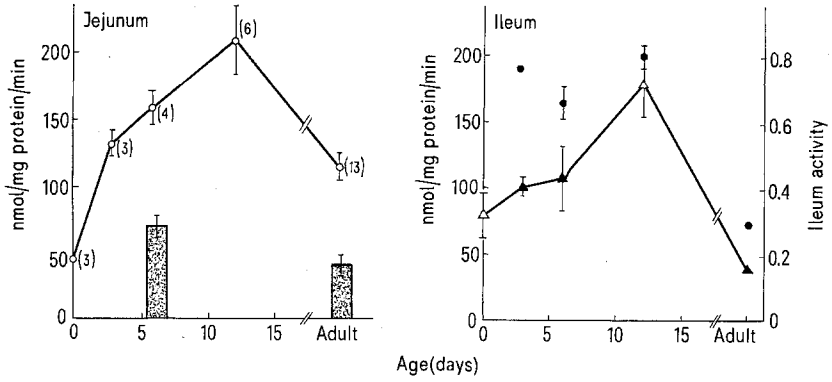

Acyl-CoA: monoglyceride acyltransferase. Circles denote activity in microsomes, columns in total homogenate of the jejunum. Short vertical lines denote 2 S.E.M. Not given if smaller than symbol used. Figures in parentheses indicate number of samples. Triangles denote activity in ileal microsomes. Full triangles indicate that values significantly differ from corresponding values in the jejunum. Large, full circles denote ileal activity expressed as percentages of jejunal activity.

protein according to LOWRY et al.[9] For statistical evaluation of differences *t*-test was used; *p* values 0.02 and lower were judged as significant.

According to determination of various markers for other cell fractions, the microsomal preparations were fairly standard. The protein yield (7–8% of the total homogenate protein) was similar to that described in adult rats[3,10]. Contaminations by microvilli indicated by the neutral β-galactosidase determined according to[11] was similar (around 10%) as has been observed by others[10,12]. Small amounts of acid β-galactosidase (according to[11]) were found also regularly in this fraction (4–7%), originating either from lysosomes or as an integral part of the microsomal fraction in the intestinal mucosa, as has been shown in the liver and kidney for another lysosomal enzyme, β-glucuronidase[13]. Succinodehydrogenase activity (determined according to[14]) and amounts of DNA (determined according to [15]) were always below 0.5% of the amount found in the total homogenate.

Results. Before the developmental changes of acyl-CoA: monoglyceride acyltransferase activity were studied, it was confirmed that the enzymatic reaction in the microsomal preparations from the jejunum and ileum of adult and 10-day-old rats proceeded linearly during the incubation period and was proportional to the amount of enzyme protein used. Furthermore, it was established that mutual mixing of preparations of the jejunal and ileal microsomes from 12-day-old and adult rats gave values corresponding to theoretical values. Therefore, the observed differences are due to the changes in activity of the enzyme studied and not due to changes in the amount of inhibiting or activating substances.

The Figure summarizes then the perinatal changes. Activity in the jejunum is low in fetuses and increases after birth, reaching highest values in 12-day-old animals. Values of adult rats were significantly lower than those found in 6- or 12-day-old rats. For comparative purposes activity was also determined in total jejunal homogenates of 6-day-old and adult rats. The specific activity was lower than of corresponding microsomal fractions, but again the activity found in suckling rats was significantly higher than found in preparations from adult rats. Perinatal changes of activity in microsomes prepared from ileum showed a fairly similar pattern as described in the jejunum. Values for jejunum and ileum of adult rats are in a very good agreement with data published by SINGH et al.[3]; activity in the ileum of our adult represents 30% of the activity in the jejunum. In suckling rats the relative activity of the ileum was significantly higher (between 66–80% of the jejunum), suggesting that this portion of the small intestine is relatively more involved in handling fat in the suckling period than in adult rats.

Discussion. Due to the low activity of pancreatic and small intestinal lipase[5,6], low hydrolysis of fat and therefore a low esterification process was assumed to be present in the small intestine of suckling rats. However, the presented suggest that esterification processes in suckling rat in the small intestine are even higher than in adult rats. In connection with this, the presence of lipolysis of fat in the stomach of suckling rats reported recently from various laboratories[16–18] must be taken in consideration. According to HELANDER and OLIVECRONA[17] lipolysis in the stomach preferentially liberates medium chain fatty acids and long chain diglycerides. Further experiments are thus needed to characterize the processes of handling fat, i.e., lipolysis and esterification in the gastrointestinal tract of suckling mammals[19].

Zusammenfassung. Die Aktivität der Acyl-CoA: Monoglycerid Acyltransferase, eines Enzymes, das in der Esterifizierung der freien Fettsäuren eine wichtige Rolle spielt, wurde während der perinatalen Entwicklungsperiode der Ratte in der Dünndarmschleimhaut bestimmt. Im Foetus ist die Aktivität niedrig, steigt nach der Geburt und zeigt in der Mitte der Säuglingsperiode die höchsten

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Werte, die 2–3mal höher sind, als bei Erwachsenen. Die Befunde werden im Bezug auf die niedrige Lipaseaktivität des gastrointestinalen Traktes sowie auf die hohe Fettaufnahme während der Säuglingsperiode diskutiert.

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Distribution of a Renin-Like Enzyme in the Bovine Adrenal Gland

The adrenal gland of the rabbit contains an enzyme that reacts in vitro with native renin substrate to form a vasopressor polypeptide, probably angiotensin I[1]. Recently, Hayduck, et al.[2], found a similar enzyme in the adrenal gland of the dog. Whether the renin-like enzyme reacts with renin substrate in vivo is not known. However, in view of the effects of angiotensin on the secretion of aldosterone and medullary catecholamines[3,4], the possibility exists that the enzyme influences specific adrenal gland functions. In the present investigation, a renin-like enzyme was found in bovine adrenal gland, and a study was made of its distribution.

Adrenal glands were dissected (at 4°C) as soon as possible (1–2 h) after slaughter. The glands were cut with a razor blade into cross-sections (2–3 mm thick). Each cross-section was cut to yield the following pieces: 1. cortex, 2. corticomedullary junction, 3. medulla and 4. central vein. Adherent capsular tissue was not removed from the cortical pieces. Similarly, it was not possible to separate the central vein from a small amount of medullary tissue.

The tissue pieces were weighed, homogenized, and then distilled water was added, 2 ml/g wet weight of tissue. The homogenates were frozen and thawed twice using a solid CO_2-ethanol slurry. The homogenates were centrifuged at $1{,}690 \times g$, at 4°C, for 30 min to remove tissue debris. The supernatants were dialyzed against Na_2HPO_4-citric acid buffer, pH 2.6, at 4°C, for 24 h, then against water for 6 h, and finally against 3 m*M* Na_2 · EDTA for 24 h. If, at the end of this time, the extracts were not free of catecholamines (estimated in terms of effects on mean arterial blood pressure[5]), dialysis was continued using 1 m*M* sodium phosphate buffer, pH 7.0, for another 24 h. Precipitates formed during dialysis were removed by centrifugation at $1{,}690 \times g$, at 4°C, for 30 min. Protein concentrations were measured by the method of Warburg and Christian[6].

One ml of dialyzed extract was added to 2 ml of rabbit renin substrate (2,000 ng of angiotensin content/ml) buffered at pH 6.0, the pH optimum of the reaction[1,7,8]. Soybean trypsin inhibitor, Na_2 · EDTA and dimercaprol were added to inhibit kallikrein and angiotensinase enzymes[7–9]. The reaction mixtures were free of angiotensinase activity. The incubation was carried out at 37°C. Samples were taken for assay after 1, 2 and 3 h of incubation. Renin-like activity was measured in terms of ng of angiotensin formed/ml/h. Angiotensin was assayed using the mean arterial blood pressure response of the pentolinium-treated, anesthetized rat[5]. Asn[1], Val[5]-angiotensin II was used as the standard reference compound.

Results are shown in the Table. Per g wet weight, the medulla contained 11-times more renin-like activity than did the cortex. Possibly because of the higher lipid content of cortex, the enzyme activities of cortex and medulla per mg of protein were much closer. The cortical pieces of tissue were not freed of adherent capsule, raising the possibility that the cortex itself has little or no renin-like activity. On this point, it may be relevant that Goormaghtigh and Handovsky[10] have described modified smooth muscle cells, similar to the juxtaglomerular cells of the renal afferent arterioles, in the capsule of the adrenal gland. Further support for the possibility that cortex contains little or no renin-like activity arises from the observation that the enzymic activity of the corticomedullary junction, per g wet weight, is not the mean of the cortical and medullary activities. In fact, the specific activity (activity/mg of protein) of the renin-like enzyme of the corticomedullary junction is less than that of either cortex plus capsule or medulla.

The central vein was of interest because of its large apical crescent of smooth muscle, which is penetrated by a variety of small veins draining the cortex and medulla. It was thought that a large concentration of renin-like enzyme in or around the apical crescent could, by releasing angiotensin, affect venous drainage and thereby either reduce flow or affect a redistribution of flow within the gland.

As shown in the Table, the renin-like activity of the central vein is about 65% that of medulla and thus is in great excess of that accounted for by trapped blood (average renin activity of 1 ng/ml/h). However, it was not possible to obtain central vein tissue without some

Distribution of the renin-like enzyme in bovine adrenal gland

Tissue	ng of angiotensin released per h	
	per g wet weight	per mg of protein
Cortex	18	15
Corticomedullary junction	37	10.5
Medulla	200	27
Central vein	128	14.5

Tissue segments were separated, extracted and assayed as described in the text. These values are the means of 4 experiments. The variation among experiments was less than 10%.

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