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Received July 29th, 1963

PN 1296

Changes in the occurrence of different lipid classes during postnatal development of the rat

It has been pointed out previously (HAHN *et al.*[1], KOLDOVSKÝ *et al.*[2]) that milk is a high-fat diet. It appears that the suckling mammal is well adjusted to such a diet, since fat utilization is high (HAHN AND KOLDOVSKÝ[3]) and fat absorption and transport also differ from the same processes in adult animals (KOLDOVSKÝ *et al.*[4]). There are some indications that the lipid composition of some organs also changes during development (YARBRO AND ANDERSON[5], NOVÁK *et al.*[6]).

In the present work the lipid composition of the livers, lungs, brown interscapular fat and small intestine of rats aged 1 and 10 days postnatally and in adult animals was studied. Thin-layer chromatography on silica gel G (PEIFER[7]) was used. Infant rats were taken directly from the mother rat and adult animals were in the fed state.

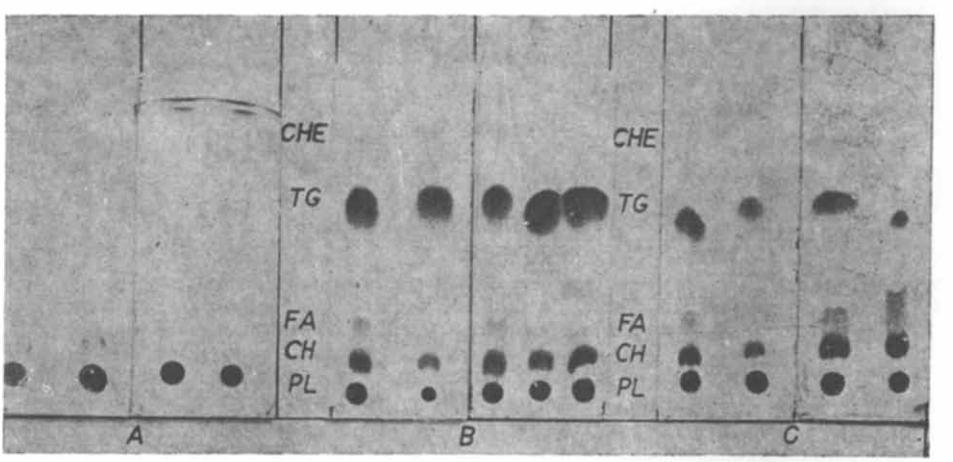

Fig. 1. Thin-layer chromatograms showing the distribution of lipids in the rat intestine. A, 1-day-old rats; B, 10-day-old rats; C, adult rats (200 g females). For 1-day-old rats 3–4 animals and for 10-day-old rats 2 animals were pooled for 1 determination. Abbreviations: PL, phospholipids; Ch, cholesterol; FA, free fatty acids; TG, triglyceride; ChE, cholesterol esters.

Immediately after sacrifice the tissues to be analysed were removed and homogenized with sea sand (*pro analysi*). For 500 mg tissue 25 ml ethanol–diethyl ether (3:1, v/v) were used. In the case of the small intestine the organ was rinsed with saline and the central part only (about one-third) was analysed. The ethanol–diethyl ether extract was filtered into ampoules weighed previously. It was evaporated to dryness under N_2 and the fat left was weighed. The lipids were then dissolved in chloroform–methanol (2:1, v/v) to give a final concentration of 1 mg lipids per 0.1 ml. To the thin-layer chromatogram 0.01 ml containing about 100 μg were applied. The chromatograms were developed in hexane–diethyl ether–ethyl acetate (40:10:1.5, v/v) for 5–7 min. They were detected by charring after having been sprayed with 25%

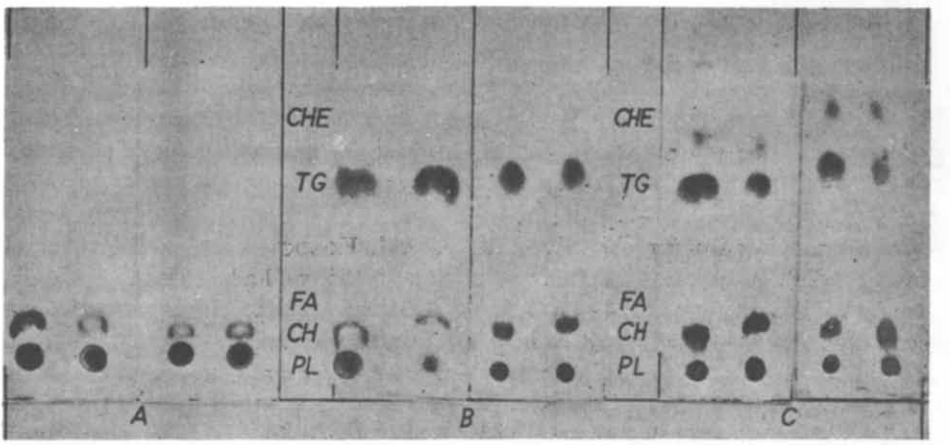

Fig. 2. Thin-layer chromatograms showing the distribution of lipids in the rat lung. A, 1-day-old rats; B, 10-day-old rats; C, adult rats (200 g females). For 1-day-old rats 3–4 animals and for 10-day-old rats 2 animals were pooled for 1 determination. Abbreviations: PL, phospholipids; Ch, cholesterol; FA, free fatty acids; TG, triglyceride; ChE, cholesterol esters.

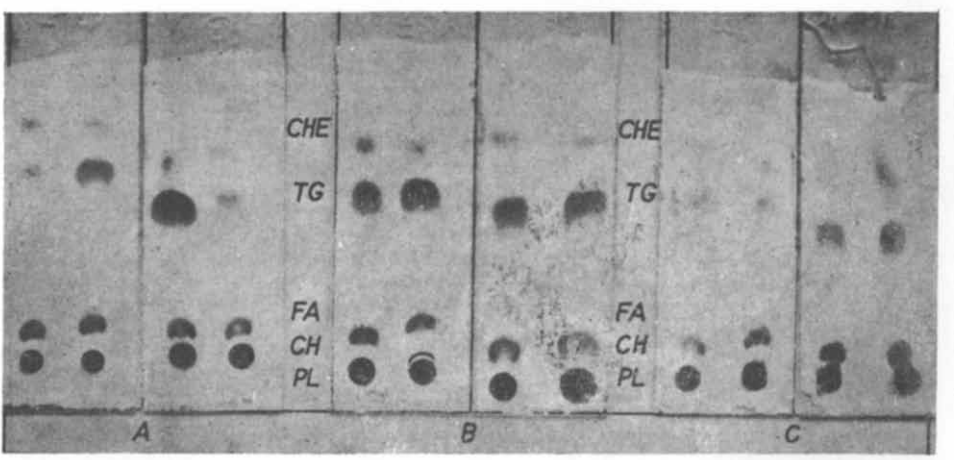

Fig. 3. Thin-layer chromatograms showing the distribution of lipids in the rat liver. A, 1-day-old rats; B, 10-day-old rats; C, adult rats (200 g females). For 1-day-old rats 3–4 animals and for 10-day-old rats 2 animals were pooled for 1 determination. Abbreviations: PL, phospholipids; Ch, cholesterol; FA, free fatty acids; TG, triglyceride; ChE, cholesterol esters.

perchloric acid in water and then heated so as to give adequate spots. The remainder of the lipids were separated into lipid classes and these were analysed for their fatty acids by gas–liquid chromatography and results of that part of this work will be published separately. Details of the method are given elsewhere (DOBIÁŠOVÁ[8]).

The figures illustrate the results. In the small intestine (Fig. 1) of 1-day-old rats phospholipids seem to be the only lipid class present. This is no longer the case on the 2nd day (not shown) when evidently fat from milk has changed the picture. On the 10th day the triglyceride content of the intestine is high, higher than in adult animals. Again this may be related to the high fat content of the milk and the lesser extent of hydrolysis of triglycerides in the intestinal tract (ROKOS *et al.*[9]).

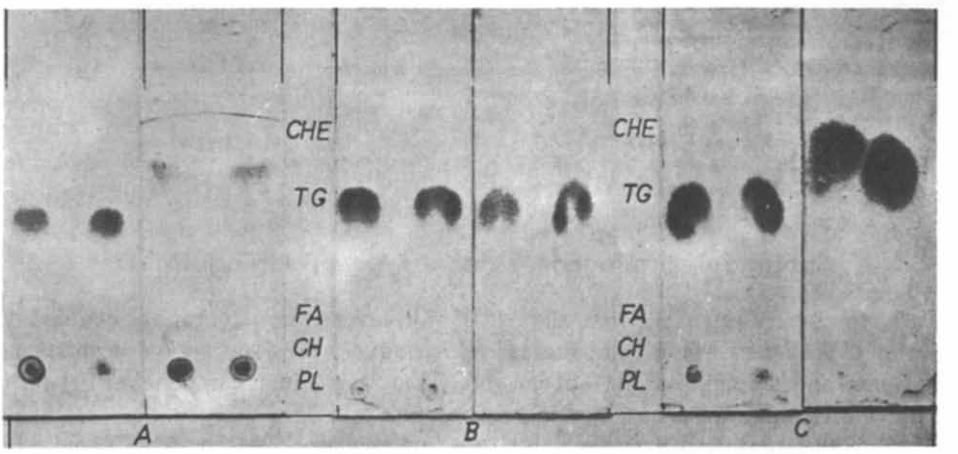

Fig. 4. Thin-layer chromatograms showing the distribution of lipids in the rat brown fat. A, 1-day-old rats; B, 10-day-old rats; C, adult rats (200 g females). For 1-day-old rats 3–4 animals and for 10-day-old rats 2 animals were pooled for 1 determination. Abbreviations: PL, phospholipids; Ch, cholesterol; FA, free fatty acids; TG, triglyceride; ChE, cholesterol esters.

In the lungs the picture (Fig. 2) is somewhat similar. On the 1st day only phospholipids and cholesterol are found, while later in life triglycerides make their appearance. Cholesterol esters in a larger amount are present in adult rats only. This is being further studied since previous work has shown that both histologically (VACEK *et al.*[10]) and chemically (NOVÁK *et al.*[6]) free fatty acids appear to play a special role in suckling rats. They are present in amounts which do not permit them to be evaluated by the present method.

In the liver (Fig. 3) no particular differences were found to occur with age and a definite answer will have to wait until more quantitative methods have been applied.

Finally, in the brown interscapular adipose tissue (Fig. 4) again phospholipids appear to predominate in 1-day-old rats while the triglyceride content is highest in adult animals. This is in agreement with the results of YARBRO AND ANDERSON[5].

It is attractive to speculate that the dietary fat has a profound effect on the early postnatal changes in lipid distribution and this hypothesis is being put to the test.

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Received May 31st, 1963

Biochim. Biophys. Acta, 70 (1963) 713–716

PN 1298

Sphingoplasmalogens. A new type of sphingolipids

We have already reported[1] that the use of thin-layer chromatography[2] enabled detection of some new minor components in the brain cerebroside fraction purified from phospho- and sulpholipids. It appears now, that the minor components belong to a previously unknown class of sphingolipids.

Our first tentative proposal[1] was that the compounds differ from usual cerebrosides only in the presence of a new fatty acid, bound to the amino group of sphingosine. In contradiction to this suggestion, it appeared that *N*-palmitoylsphingosine galactoside* had the same mobility by thin-layer chromatography (R_F 0.21) as the natural or synthetic cerasine and dihydrocerasine. Hence, R_F values (0.62 and 0.56) of the minor components considerably higher than that of cerasine (0.21) could be explained only by substitution of one of the free hydroxyl groups by a long alkyl residue. This view was supported by the R_F values of minor components close to those of mono-*O*-palmitoylcerebrosides, obtained by acetylating cerebrone and cerasine (2 moles of palmitoyl chloride, pyridine, for 2 h at room temperature). However, the natural minor components differed from the synthetic mono-*O*-palmitoylcerebrosides in that the ester carbonyl band (1627 cm^{-1}) of their infrared spectra was absent (Fig. 1). The other difference was the complete stability of the minor components towards alkaline hydrolysis (0.5 N NaOH, 40°, 3 h). Analogous treatment of mono-*O*-palmitoylcerebrosides resulted in their complete destruction to the corresponding cerebrosides. This suggested that the minor components are not cerebroside *O*-acyl derivatives.

We isolated minor components from fraction A (ref. 2) on a preparative scale using thin-layer chromatography with the solvent system chloroform–methanol (85:15, v/v) (column chromatography still gives poor resolution[1]). The zones were detected by spraying the dry chromatogram with water[4]. Some 10 mg of each minor

* The substance was obtained from psychosine by the method of WEISS AND RAISMAN[3].