



Fig. 1. Distribution of ^{90}Y between a solution of 0.1 M DBP in chloroform and 0.1 M HNO_3 as a function of the conc. of hexol in the chloroform phase. The curve is calculated with equations in the text assuming that the lowering of q is due to formation of a complex between DBP and hexol.

with 5 ml of 0.1 M DBP in alcohol-free chloroform. The amount of ^{90}Sr extracted is less than $10^{-2}\%$. This amount may be reduced by a factor of 10^4 by washing the organic layer with 5 ml of 0.1 M HNO_3 ; 1% of ^{90}Y is lost for each wash. The ^{90}Y is quantitatively back-extracted into 5 ml of 1 M HNO_3 after the addition of 1 ml of methyl isobutyl carbinol (hexol). The aqueous phase then contains about 0.0003 M DBP. The DBP conc. can, however, be lowered by a factor of 400 by washing the 1 M HNO_3 phase with 1 ml of hexol + 5 ml of chloroform. In this way the conc. of DBP can be reduced to a very low level and the final solution will only contain volatile substances (HNO_3 , water, chloroform and hexol) in addition to the ^{90}Y .

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1. Dyrssen, D. *Acta Chem. Scand.* **11** (1957) 1277.
2. Peppard, D. F., Mason, G. W. and Moline, S. W. *J. Inorg. & Nuclear Chem.* **5** (1957) 141.
3. Peppard, D. F. et al. *J. Inorg. & Nuclear Chem.* **4** (1957) 334; **7** (1958) 276.
4. Dyrssen, D. *Acta Chem. Scand.* **11** (1957) 1771.
5. Dyrssen, D. and Liem Djiet Hay. *Acta Chem. Scand.* **14** (1960). *In the press.*

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The Composition of Rat Milk Fat

AAGE JART, J. P. FUNCH and H. DAM

*Danish Fat Research Institute,
Copenhagen, Denmark*

Rats are extensively used in experiments on nutrition, e.g. for the study of the role of fats. It is therefore of interest to know the composition of the fat in the milk consumed by the suckling young rat.

Investigations on this problem have been carried out by Hallanger and Schultze¹, who found it possible to increase the content of polyenoic acids (mainly dienoic) by increasing the content of such fatty acids in the diet.

The aim of the present study was to determine not only the polyenoic fatty acids but also the chain length of the fatty acids in milk fat of rats receiving the stock diet commonly used in our laboratory.

To study this problem the fat fraction of rat milk was isolated and converted into methyl esters which were analyzed by means of gas-liquid chromatography². The column consisted of 0.9 m Celite 545 with silicone elastomer E 301. The carrier gas used was nitrogen at a rate of 1 l per hour, and the temperatures applied ranged from 160°C to 225°C. Assuming the areas lying under the recorded curves to be proportional to the molar percentages of the individual components, the following composition (Table 1) was found (in per cent w/w of the fatty acid mixture).

Table 1.

Acid	%
C ₇	<0.05
C ₈	2
C ₉	0.1
C ₁₀	6
C ₁₁	0.15
C ₁₂	4
C ₁₃	0.1
C ₁₄	6
C ₁₅	0.5
C ₁₆	22
C ₁₇	0.1
C ₁₈	59

It will be seen that the analyzed fat from rat milk contains about 1 % of fatty acids with an uneven number of carbon atoms in the chain, and that it is a characteristic feature that it contains appreciable quantities (about 0.5 %) of a fatty acid with a 15-carbon atom chain. In agreement with other investigations³, butyric acid and caproic acid were not found in the rat milk fat.

To determine the content of unsaturated fatty acids the fats isolated from another milk sample (with approximately the same distribution of the fatty acids as regards chain length) was examined by alkali isomerization analysis⁴. The following results were obtained (unsaturated linkages being assumed to occur chiefly in the C₁₈-fraction):

linoleic acid	11 %
linolenic acid	4 »
conjugated dienoic acids	0.9 »

It appeared, moreover, from the iodine value (65.7 for the methyl esters) that the triglycerides of rat milk contain about 40 % oleic acid.

Experimental. The milk was collected 15–18 days after parturition from 15 rats which had been fed on the diets listed in Table 2. The milk donors were separated from the litter in the afternoon preceding milk collection the following morning. The rats were anesthetized with 0.1–0.2 ml of a 6 % Nembutal sodium solution (Abbott), given subcutaneously. The milk was expressed by the thumb and forefinger, and the droplets were collected in a pipette. About 1 ml was collected from each rat.

To a 10–12 ml sample of the collected milk was added 1 ml 10 % ammonium hydroxide and 10 ml ethanol (96 %), and the mixture was shaken vigorously. Now 25 ml ethyl ether were added and the mixture again shaken; the treatment was repeated with 25 ml petroleum ether, whereupon the mixture was allowed to stand over night for complete separation. The ether phase was separated and evaporated to dryness — finally in vacuum. The residue was dissolved in 20 ml methanol, and 20 mg sodium methoxide were added in the form of 2 ml of a methanolic solution. The reaction mixture was refluxed for a couple of hours and then set aside over night. After addition of 20 ml ethyl ether, just sufficient distilled water was added dropwise to cause the solution to separate into two phases (*i. e.* about 20 ml distilled water). The ether phase with the methyl esters was separated, and the aqueous phase was extracted once more with 25 ml ethyl ether. The two ether phases were combined, and an equal

Table 2. Percentage composition of the diets.

Diet No. 1 *		Diet No. 2 *	
Oat	42	Oatmeal	77.7
Wheat	42	Wheat	8.0
Pulverized sea shells	8	Hempseed	8.0
Gritted charcoal	2	Linseed	4.0
Cod-liver oil	6	Calcium carbonate	1.3
		Sodium chloride	1.0

* Diet No. 1 was given on Tuesday, Thursday, Saturday and Sunday. Diet No. 2 was given on Monday, Wednesday and Friday. — Water was given during the day and whole milk during the night *ad lib.*

volume of petroleum ether (30–35 ml) was added to cause water to separate out. The ether solution was finally dried by means of anhydrous sodium sulphate, and the ether was removed by evaporation. The last traces of volatile foreign matter were removed from the methyl esters by treatment for 1 hour at 60°C in vacuum produced by a water pump.

1. Hallanger, L. E. and Schultze, M. O. *Proc. Soc. Exptl. Biol. Med.* **96** (1957) 473.
2. Jart, Aa. *Acta Chem. Scand.* **13** (1959) 1723.
3. Lucky, T. D., Mende, T. J. and Pleasants, J. J. *Nutrition* **54** (1954) 345.
4. American Oil Chemists' Society. *Official and Tentative Methods of Analysis*, 2nd Ed. Rev. to 1958. Chicago 1946–58. Cd 7–58.

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