testinal lymph than the liver and other tissues, suggesting that most of the absorbed vitamin A was transported by the lymphatic route. In the absence of bile, however, when the absorption of vitamin A was grossly impaired, there was a tendency for more to be recovered from the liver than the lymph, suggesting that an alternative route of transport of the absorbed vitamin was used.

1. Johnston, J. M., "Advances in Lipid Research," Paoletti, R. and Kritchevsky, D., eds., p. 105. Academic Press, New York, 1963.

2. Senior, J. R., J. Lipid Res. 5, 495 (1964).

3. Borgstrom, B., Acta Physiol. Scand. 28, 279 (1963).

4. Saunders, D. R. and Dawson, A. M., Gut 4, 254 (1963).

5. Gallagher, N, Webb, J., and Dawson, A. M., Clin. Sci. 29, 73 (1965).

6. Ganguly, J., Krishnamurthy, S., and Mahaderan, S, Biochem. J. 71, 756 (1959).

7. Bollman, J. L., Cain, J. C., and Grindlay, J. H., J. Lab. Clin. Med. 33, 1349 (1948).

8. Bruno, G. A. and Christian, J. E., Anal. Chem. 33, 650 (1961).

9. Greaves, J. D. and Schmid, C. L. A., Am. J. Physiol. 111, 502 (1935).

10. Annegars, J. H. Arch. Internal Med. 93, 9 (1954).

11. Dawson, A. M., Gallagher, N., Saunders, D. R., and Webb, J., in "Metabolism and Physiological Significance of Lipids," Dawson, R. M. C. and Rrodes, D., eds. Wiley, New York, 1965.

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## Fatty Acids of RBC Ghosts, Liver Mitochondria and Microsomes of Cold-Acclimated Hamsters* (32631)

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Fawcett and Lyman (1) and later Kodama and Pace (2) showed that cold acclimation produces changes in the levels of unsaturated fatty acids in white adipose tissues of hamsters. Recently Williams and Platner (3) studying whole homogenates of livers of cold acclimated hamsters also found that there were changes in the fatty acid percentage composition. The obvious question is: does this change occur solely in the hamster liver depot fat or are there also changes in the liver lipoprotein membranes, both those already present and those newly formed?

Chaffee Hoch, and Lyman (4) have found that prolonged cold-exposure affects even the appearance of mitochondrial pellets of hamster livers. In addition to the change in appearance, mitochondrial counts per gram of liver rise about 18–20% by the 9th week of cold acclimation at 2 ± 1°C. Since normal mitochondrial replacement no doubt also takes place throughout acclimation, a considerable portion of the liver mitochondria of such cold-acclimated hamsters can be expected to be newly formed. Similarly, Reynafarje and Chaffee (5) found a noticably larger liver microsomal pellet which differed in appearance in cold-acclimated hamsters. Therefore, during cold-acclimation, it is reasonable to suppose that lipoprotein membrane synthesis occurs in the liver, where it is known that percentage levels of total fatty acids also change (3).

Brock (6) has reported that the half-life of the red cells (RBCs) of both awake cold-acclimated, and control hamsters is about 38 days. Our cold-exposure period was 5–9 weeks, which would allow for the genesis of at least some new RBCs. However, since these originate from preformed hemocytoblasts which may be stored, it is obvious that longer acclimation should yield a higher percentage of newly formed RBCs, and thus more newly formed lipoprotein plasma material.

Therefore, we have made comparative studies by means of gas chromatography of

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the fatty acids in liver mitochondria, microsomes, and RBC ghosts from cold acclimated and control hamsters *(Mesocricetus auratus)* and the results of these studies are herein reported.

*Methods and Materials.* Adult male hamsters were caged singly and cold-acclimated at 2 ± 1.5°C. Purina Laboratory Chow pellets and water were available *ad libitum*. The fatty acid analysis of the diet expressed in per cent was as follows: palmitic 20.70, palmitoleic 4.63; stearic 6.50, oleic 26.70, linoleic 32.50, linolenic 5.20, and arachidonic 3.77. This represented 4.9% of the diet.

Liver mitochondria were isolated in 0.25 *M* sucrose according to the standard methods of Hogeboom and Schneider (7) as outlined by Hoch and Lipmann (8). For the sake of comparison, crude mitochondrial membranes were prepared by freezing and thawing isolated mitochondria and homogenizing them with a loose fitting homogenizer. This was followed by centrifugation. The precipitate had no marked differences in percentages of fatty acids from those of whole mitochondria. Therefore, we have used whole mitochondria in this study. Liver microsomes were isolated by the method of Reynafarje and Potter (9).

The RBC ghosts were made from blood removed by cardiac puncture. The cells were cleaned in saline + citrate and ruptured by osmotic shock and freezing, and centrifuged.

Total fatty acids were extracted from mitochondria, microsomes, and ghosts according to the method of Lasker and Theilacker (10). The only modification in the method was acidifying pH 4.5 with HCl instead of $H_2SO_4$ and saponification overnight at room temperature, rather than heating at 75°C. Nonsaponifiables were extracted with five 10 ml washes of petroleum ether. The samples were then acidified with HCl and extracted with three 10 ml washes of petroleum ether. The combined washes were dried over anhydrous sodium sulfate and stored at 4°C until they were methylated.

Methylation of the fatty acids was accomplished by the boron trifluoride method of Metcalfe and Schmitz (11). After the final water wash the petroleum ether was evaporated and heptane was added to give a fatty acid concentration range between 0.5 and 0.75 mg/ml.

Gas chromatography was done with a Barber-Colman gas chromatograph, series 5000, equipped with dual hydrogen flame detectors and dual 8 foot by ⅛ inch diameter columns, packed with 60–80 mesh 20% ethylene glycol succinate, on GAS-PACK WAB, which was used to separate and identify the fatty acids. All analyses were done isothermically at 195°C using nitrogen as the carrier gas, at a flow rate of 12 ml/min. Air flow was 100 ml/min and hydrogen 35 ml/min. Each gas flow was carefully regulated with flow meters.

Qualitative analysis of the fatty acids was done by comparison to known methylated standards obtained from NIH or purchased from commercial sources. "Carbon number" was obtained by plotting the log of retention time against carbon number as described by Woodford and van Gent (12).

Detector response was plotted against concentration and found to be linear. All solvents were checked for contamination. Peak area was obtained with the model 205 Disc Chart integrator.

*Results and Discussion.* The results are expressed as percentage area of all the peaks considered. Only fatty acids from 16 to 20 carbons in chain length are included in this study, because most animal fatty acids fall in this group and previous work on liver fatty acids has indicated changes in these acids. The fatty acid designations used in this report give the number of carbon atoms and double bonds present. Thus palmitic acid is designated 16:0; palmitoleic, 16:1; stearic, 18:0; oleic, 18:1; linoleic, 18:2; linolenic, 18:3; and arachidonic, 20:4.

In the mitochondria, the only major change with cold-acclimation is an increase in the oleic acid (Table I). Unlike the results from studies on whole liver, stearic acid is unchanged. Thus cold induced fatty acid changes in mitochondria differ from those of whole liver.

In the microsomes, Table II shows a similar increase in oleic acid in cold-acclimation, but there is also a significant change in palmitic acid. Here again there is no change in stearic acid. Thus the pattern of change in micro-

somes is different from that in the mitochondria, and from that of the whole liver. In the RBC ghosts (Table III) none of the fatty acid percentages change during cold-acclimation.

Thus, comparing cold-acclimated and control animals, it appears that the fatty acid percentage composition of mitochondria and endoplasmic reticulum of the liver are different, and that neither of these organelle components show the pattern of fatty acid compositional change characteristic of whole liver fat. Obviously these findings, suggest the possibility that further fractionation of the various classes of lipid components such as cephalins, lecithins, etc. will better elucidate which membranous components undergo change.

Another question which must be pursued further concerns the possible functional effect of these changes in the organelle lipoprotein structure. In the case of mitochondria, for example, one might imagine changes in permeability as a possibility.

An interesting by-product of this research, not concerned with cold-acclimation, is the presence of striking differences in percentage fatty acid composition between the RBC

TABLE I. Percentage Fatty Acid Composition of Liver Mitochondria from Cold-Acclimated and Control Hamsters.

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 |
|---|---|---|---|---|---|---|---|
| Mitochondria, control (18)[a] | 19.59 ±0.77 | 2.99 | 14.02 | 17.11 ±0.50 | 29.83 ±1.04 | 1.42 | 13.38 |
| Mitochondria, cold (20) | 17.78 ±0.61 | 2.97 | 13.65 | 20.51 ±0.55 | 28.29 ±0.69 | 1.21 | 13.83 |
| Probability value | *[b] | * | * | $p \leq .001$ | * | * | * |

[a] ( ) No. of animals.
[b] No Significant difference.

TABLE II. Percentage Fatty Acid Composition of Liver Microsomes from Cold-Acclimated and Control Hamsters.

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 |
|---|---|---|---|---|---|---|---|
| Microsomes, control (20)[a] | 23.25 ±0.94 | 3.21 | 15.29 | 19.09 ±0.75 | 23.63 ±0.80 | 1.49 | 12.36 |
| Microsomes, cold (23) | 20.56 ±0.63 | 3.78 | 14.11 | 22.74 ±0.77 | 22.33 ±0.77 | 1.44 | 13.10 |
| Probability value | $p \leq .02$ | *[b] | * | $p \leq .001$ | * | * | * |

[a] ( ) No. of animals.
[b] No Significant difference.

TABLE III. Percentage Fatty Acid Composition of RBC Ghosts of Cold-Acclimated and Control Hamsters.

| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 18:3 | 20:4 |
|---|---|---|---|---|---|---|---|
| Ghosts, control (16)[a] | 19.03 | 4.89 | 14.69 | 22.59 | 18.06 | 1.13 | 18.96 |
| Ghosts, cold (13) | 19.73 | 4.83 | 16.18 | 22.37 | 18.13 | 1.38 | 16.10 |
| Probability value | *[b] | * | * | * | * | * | * |

[a] ( ) No. of animals.
[b] No Significant difference.

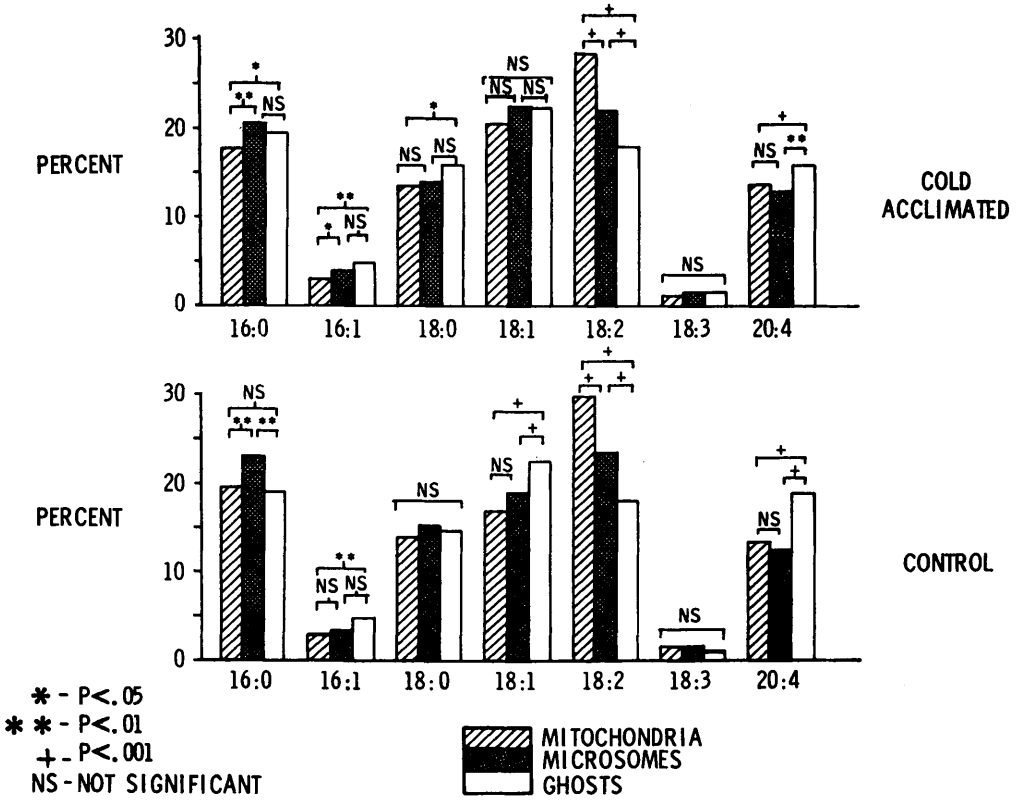


FIG. 1. Comparison of per-centage fatty acid composition of organelle fractions of cold-acclimated and control hamster livers and RBC ghost cells.

plasma membranes, liver mitochondrial membranes, and the endoplasmic (microsomal) membranes (Fig. 1).

There is clearly a higher percentage of palmitic acid in microsomes than in mitochondria, but a lower percentage of linoleic acid (Fig. 1). Thus these two hepatic organelles either have different membranous structure, or the mitochondria have absorbed more linoleic and other fatty acids as has been described by Fritz and others (13–15). However, arguing against this possibility of differential adsorption are data of Reshef and Shapiro (16) which show that in liver homogenates, mitochondria absorb and bind more palmitic acid than do microsomes. Thus the greater percentage of palmitic acid content in microsomes may indicate a true difference in structural composition in mitochondria and microsomes. In addition, there is a strikingly higher percentage of linoleic acid in mitochondria than in microsomes. However, since we are dealing with percentage content, maybe this is an inverse resultant of lower percentages of other fatty acids in mitochondria than in microsomes (Fig. 1).

In the RBC ghosts there is a strikingly low level of linoleic acid with a somewhat compensatory higher percentage of arachidonic acid.

In conclusion, the data in Fig. 1 support Korn's (17) contention that biochemical (structural) differences do exist among cellular organelle membranes. Our data suggest that changing the environmental temperature can modify the biochemical components of the organelle membranes.

*Summary.* When hamsters were exposed to cold, the relative percentage of various fatty acids in mitochondrial and microsomal membranes of liver cells changed. In mitochondria, the percentage of oleic acid increases, and in microsomes the percentages of oleic increased and palmitic acid decreased. These findings

are in contrast to results of earlier studies on whole livers, which showed that under similar conditions, the percentage of stearic acid decreases. There were no changes in fatty acid percentage composition in RBC ghost cells after 5–9 weeks of exposure. These studies also indicate that there were striking differences in the fatty acid composition of membranes of various organelles in the cell.

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1. Fawcett, D. and Lyman C., J. Physiol. **126**, 235 (1954).
2. Kodama, A. and Pace, N., Federation Proc. **22**, 761 (1963).
3. Williams, D. D. and Platner, W. S., Am. J. Physiol. **212**, 167 (1967).
4. Chaffee, R. R. J., Hoch, F. L., and Lyman, C. P., Am. J. Physiol. **201**, 29 (1961).
5. Reynafarje, B. and Chaffee, R. R. J., Proc. Soc. Exptl. Biol. Med. **103**, 225 (1960).
6. Brock, M. A., Ann. Acad. Sci. Finnicae, Ser. A. IV 71(4), 53 (1964).
7. Hogeboom, G. H. and Schneider, W. C., J. Biol. Chem. **186**, 417 (1950).
8. Hoch, F. L. and Lipmann, F., Proc. Natl. Acad. Sci. U. S. **40**, 909 (1954).
9. Reynafarje, B. and Potter, V. R., Cancer Res. **17**, 1112 (1957).
10. Lasker, R. and Theilacker, G., J. Lipid Res. **3**, 60 (1962).
11. Metcalfe, L. and Schmitz, A., Anal. Chem. **33**, 363 (1961).
12. Woodford, F. and van Gent, C., J. Lipid Res. **1**, 188 (1960).
13. Fritz, I. B., Physiol. Rev. **41**, 52 (1961).
14. Fritz, I. B. and McEwen, B., Science **129**, 334 (1959).
15. Fritz, I. B. and Kaplan, E., in "Protides of the Biological Fluids," Peeters, H., ed. p. 252. Elsevier, Amsterdam, 1960.
16. Reshef, L. and Shapiro, B., Biochim. Biophys. Acta **64**, 578 (1962).
17. Korn, E. D., Science **153**, 1491 (1966).

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## Mammary Gland Fatty Acids in Rats of Different Susceptibility to Mammary Carcinoma Induction* (32632)

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Shay *et al.* first demonstrated that oral (intragastric) administration of 3-methylcholanthrene to Wistar rats induced mammary carcinomas (1). Huggins and associates (2) later showed the exquisite and invariable sensitivity of the young female Sprague-Dawley rat to mammary carcinoma induction by optimum doses of either 3-methylcholanthrene or 7,12-dimethylbenz(*a*)anthracene; and Sydnor *et al.* (3) subsequently demonstrated the relative resistance of the female Long-Evans rat.

Since the bulk of rat mammary gland is adipose tissue and since the polycyclic hydrocarbon carcinogens are strongly lipophilic and hydrophobic, the lipid composition of mammary gland may be important in mammary carcinogenesis. The lipid composition of the induced carcinomas and of the mammary tissue of the Sprague-Dawley (Holtzman) rat has been published (4). Data on the mammary fat composition of some additional strains of rats are presented below.

*Methods.* All rats, except those of the Sprague-Dawley strain, used in these experiments were bred in this institution by brother–sister mating, and were maintained under identical environmental conditions. Osborne-Mendel, Fischer, and Marshall strains can be considered to be genetically homogeneous. The Long-Evans strain was derived from a single male and two females obtained from the Diablo farms. The Sprague-Dawley rats were purchased from the Holtzman Co., Madison, Wisconsin. The animals re-

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