of generalized disease embracing carcinoma, leukemia, lymphosarcoma and Hodgkin's disease. All enzymes are discussed under the newly accepted terminology and classification of the Commission on Enzymes of the International Union of Biochemistry with the principle behavior of these enzyme activities referred to as oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Evidence is offered to support the belief that the elevated amount of lactate dehydrogenase activities are uncharacteristic of the malignant cell itself and probably are reflections of its rapid growth rate as well as more active metabolism. Also, summarizing the numerous reports in the literature on this enzyme, there is much data to indicate that the clinical chemist will soon supply the cancer diagnostician with the cellular source from which originating. Isocitrate dehydrogenase can be an asset when it is noted to have a marked rise in the serum accompanying the enlargement of the liver, which would be consistent with rapid metastatic tumor growth in the liver during the terminal period of the patients life. The catechol oxidase reaction provides a new approach to the diagnosis of malignancy in pigment-cell neoplasms, such as functional nevi *versus* malignant melanoma, and to the differentiation of amelanotic malignant melanoma from other highly undifferentiated malignant lesions which it simulates, such as fibrosarcoma, lymphoma, and squamous-cell carcinoma. The non-specific aspartate aminotransferase and alanine aminotransferase as currently viewed appear to be measures of cell damage rather than cell function and can be regarded as normal intracellular enzymes. Ribonuclease, although only indirectly related to cancer, has been shown to exist as several isoenzymes differing either in pH optima, their tissue origin or their nucleotide end-products. Stress in recent years on acid phosphatase activity has been not only to detect the occurrence of metastases in prostatic disease, but to assess the progress of therapy in such cases. The relationship of over thirty peptide hydrolases has positive value in cancer involving pancreas, small intestine, gastric mucosa and gastric muscle, but need further clarification. No attempt is made to depict clinical enzymology as the singularly significant approach to cancer, but to point out correlations which are illustrative of the larger problems to face in cancer definition.

The Biosynthesis of Palmitylcarnitine in Cell Subfractions

Jon Bremer*

Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Oslo, Norway

Carnitine is known to stimulate the oxidation of long chain fatty acids in tissue homogenates [1]. Recently we have shown that palmitylcarnitine and other fatty acid esters of carnitine are metabolized by mitochondria from several tissues of the rat [2], indicating that these esters are intermediates in the carnitine stimulated oxidation of fatty acids.

Table 1. The biosynthesis of palmitylcarnitine by rat liver microsomes. Rat liver was homogenized in 10 volumes of 10 % sucrose containing 10^{-2}M glycylglycine buffer, pH 7.5. Cell debris, nuclei and mitochondria were removed by centrifugation at 20 000 × *g* for 10 min. The microsomes were isolated from the supernatant by centrifugation at 150 000 × *g* for 60 min.

The complete reaction mixture contained in a total volume of 1 ml: Microsomes corresponding to 70 mg fresh liver in 0.5 ml of homogenizing medium; 0.5 μmole of palmitate in complex with 10 mg of crystalline bovine albumin; 10 μmoles of ATP; 0.1 μmole of CoA; 5 μmoles of magnesium chloride; 10 μmoles of GSH; 25 μmoles of sodium fluoride; and 0.27 μmole of L-carnitine-1-^{14}C = 37 000 counts/min. The incubation was performed for 15 min at 30°. The formation of palmitylcarnitine was assayed as butanol-extractable radioactivity with a method previously described [3]. Palmitylcarnitine is quantitatively extracted by butanol, while free carnitine is not extracted.

Factor *omitted* from the reaction mixture	Counts/min extracted with butanol	
None	15 500	15 400
ATP	50	20
Co A	520	460
Albumin palmitate	3 000	2 900
$MgCl_2$	13 100	12 950
NaF	13 100	12 900
GSH	14 900	14 200
Microsomes	0	

* Fellow of the Norwegian Cancer Society, Oslo, Norway.

In the present communication we report results which show that rat liver homogenate subfractions can catalyze the biosynthesis of palmitylcarnitine from free palmitate and carnitine. Table 1 shows that rat liver microsomes contain the enzyme system catalyzing this reaction. There is an absolute requirement for ATP and CoA, while the addition of albumin-complexed palmitate gives a 5-fold stimulation. The addition of magnesium chloride, glutathione and of sodium fluoride all have small, but significant stimulating effects. These results indicate that the following reactions take place:

(1) Palmitate + CoA + ATP $\xrightarrow{Mg^{2+}}$ Palmityl-CoA + AMP + pyrophosphate

(2) Palmityl-CoA + carnitine → palmityl-carnitine + CoA

Isolated mitochondria have also been found to catalyze the formation of palmitylcarnitine from carnitine.

These results confirm that fatty acid esters of carnitine are intermediates in the carnitine-stimulated oxidation of fatty acids. The results are also in agreement with the idea that carnitine functions as a carrier of activated fatty acids between metabolic compartments within the cell, as previously suggested [2].

1. Fritz, I. B., Kaplan, E. and Yue, K.T.N. *Am. J. Physiol.* **202** (1962) 117.
2. Bremer, J. *J. Biol. Chem.* **237** (1962) 3628.
3. Bremer, J. and Greenberg, D. M. *Biochim. Biophys. Acta* **46** (1961) 205.

Studies on Sphingosines. The Isolation and Preliminary Identification of Some Preparative Byproducts of C_{18}-Sphingosine

Karl-Anders Karlsson

Department of Medical Biochemistry, University of Göteborg, Sweden

With an earlier described method for the dinitrophenylderivatives of sphingosines [1] in combination with silicic acid column and thin layer chromatography several hitherto unknown byproducts of the acid isolation of sphingosine have been prepared. Two seem to be results of an isomerisation of the allyl group in sphingosine and at least two seem to be dehydration products of sphingosine and of its allylic isomers.

1. Karlsson, K.-A. *Nature* **188** (1960) 312.

The Mechanism of Ethionine Antilipotropic Action

Pål Björnstad and Jon Bremer

Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Oslo, Norway

Administration of ethionine to female rats results in a rapid and marked accumulation of triglycerides in the liver [1]. Concomitant with this accumulation, a pronounced drop in plasma lipoproteins, including the phospholipid lecithin, occurs. Thus, the accumulation of triglycerides in the liver may be due to an ethionine induced inhibition of plasma lipoprotein synthesis in the liver.

Two pathways for the biosynthesis of lecithin are known: (1) By incorporation of preformed, free choline *via* cytidinediphosphocholine [2], (2) By methylation of phosphatidylethanolamine [3].

In vitro experiments have shown that ethionine is an antimetabilite to methionine in protein biosynthesis [4], and to methionine as a methyl donor in lecithin biosynthesis from phosphatidylethanolamine [3].

Thus there are two possible mechanisms for the ethionine induced inhibition of plasma lipoprotein biosynthesis: (1) Interference with the incorporation of methionine into the protein of plasma lipoproteins, (2) Interference with the incorporation of the methionine-methyl group into the lecithin of plasma lipoproteins.

To test the relative importance of the two pathways of lecithin biosynthesis for plasma lipoprotein synthesis, and to test the relative importance of the two possible mechanisms of ethionine inhibition of plasma lipoprotein synthesis, the experiments shown in Table 1 have been performed.

The following points may be made: (1) The specific activity of blood lecithin compared to the specific activity of liver lecithin is nearly the same both when methyl-^{14}C-methionine and when hydroxyethyl-^{14}C-choline is injected, indicating that lecithin synthesized by both pathways enters a common pool in equilibrium with the plasma lecithin. (2) The inhibiting effect of ethionine on the incorporation of radioactivity into liver lecithin is more pronounced with methionine as the precursor. This may in part be due to an increased pool of free, unlabelled methionine in the liver of ethionine-treated rats, and in part to an inhibition of transmethylation [3]. (3) The specific activity of blood lecithin compared to the specific activity of liver lecithin in the ethionine-treated rats