activities. Less than 10% of the microsomal fraction was mitochondrial and less than 20% of the mitochondrial fraction was microsomal.

Acetyl-CoA was utilized by mitochondria and microsomal fraction equally well for the synthesis of fatty acids. Under the optimum incubation conditions, the rates of fatty acid synthesis (means $\pm$ S.E.M.) were 8.23 ± 1.33 (10) and 10.8 ± 2.14 (10) nmol/h per mg of protein for mitochondria and microsomal fraction respectively.

Cofactor requirements were similar in both particles except that various bivalent cations, especially Mg^{2+} , specifically inhibited microsomal fatty acid synthesis. From cofactor requirements and the determination of radioactivity in the carboxyl end of synthesized fatty acids it was shown that fatty acid synthesis in both particles was principally due to elongation. Among the fatty acids synthesized under aerobic conditions 45 and 35% of the total radioactivity was found in C_{18} and C_{20} fatty acids respectively, and in each group the monounsaturated species was a major component.

This labelling pattern of fatty acids was more marked in microsomal fraction. To minimize the effects of desaturase activity, which is localized mainly in adipose-tissue microsomal fraction (Gellhorn & Benjamin, 1964), incubations were also performed under nitrogen. These experiments suggested that synthesis of palmitoleic acid and oleic acid involved desaturase activity, whereas eicosaenoic acid and eicosadienoic acid were the products of elongation of endogenous unsaturated fatty acids.

By adding, at the end of incubations, 5mm-magnesium chloride, 6mm-DL- α -glycerophosphate and 80 μ M-CoA, the fatty acids synthesized by elongation were utilized to form glycerides. Fatty acid synthesis was strongly suppressed in both particles when these cofactors for esterification were added at the beginning of incubation. The significance of this inability to carry out elongation simultaneously with esterification remains to be established.

We acknowledge advice by Dr R. M. C. Dawson. H. K. thanks the British Council for a Scholarship.

Angel, A. & Roncari, D. A. K. (1967). *Biochim. biophys. Acta*, **137**, 464.

Gellhorn, A. & Benjamin, W. (1964). *Biochim. biophys. Acta*, **84**, 167.

Roncari, D. A. K. & Hollenberg, C. H. (1967). *Biochim. biophys. Acta*, **137**, 446.

Incorporation of Acetate into β -Hydroxy- β -methylglutaryl-Coenzyme A by Rat Liver Microsomal Fraction: the Effect of Feeding with Cholesterol

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β -Hydroxy- β -methylglutaryl-CoA is the precursor of mevalonate during the biosynthesis of cholesterol from acetate. Dietary cholesterol is well known to inhibit the biosynthesis of cholesterol in the liver, and the enzyme β -hydroxy- β -methylglutaryl-CoA reductase (EC 1.1.1.34) is depressed in the liver microsomal fraction of cholesterol-fed rats (Linn, 1967). This may not be the only site of action of dietary cholesterol.

During the studies described here, livers of female rats of the Sprague-Dawley strain were homogenized in a loose-fitting Teflon-and-glass homogenizer in a medium described by Chappell & Hansford (1969). The homogenate was centrifuged at 350g for 10min and the supernatant was centrifuged three times at 8500g for 7min each time to remove mitochondria. Microsomal fraction was obtained from this postmitochondrial supernatant by centrifugation at 100000g for 1h. The object of this procedure was to remove mitochondria as completely as possible, because they contain interfering enzymes.

Microsomal fraction was incubated with [$1-^{14}C$]-acetate and an acetyl-CoA-generating system consisting of acetate kinase (EC 2.7.2.1), phosphotransacetylase (EC 2.3.1.8), ATP and CoA. The conditions of incubation were similar to those used by Williamson, Bates & Krebs (1968) in assaying β -hydroxy- β -methylglutaryl-CoA synthase (EC 4.1.3.5). After incubation, carrier β -hydroxy- β -methylglutaric acid was added and the mixture made alkaline to cleave thiol ester bonds. The carrier acid was then isolated from the reaction mixture and its specific radioactivity measured. During this procedure the generating system was present in excess; the formation of β -hydroxy- β -methylglutaryl-CoA was therefore dependent on the activities of acetoacetyl-CoA thiolase (EC 2.3.1.9) and β -hydroxy- β -methylglutaryl-CoA synthase.

Test animals were given, for 4 days before the experiment, a standard cube diet to which 2% by weight of cholesterol had been added. Control rats received the cube diet alone. When assayed as described above, incorporation of substrate into product was fourfold lower in the microsomal fraction of test rats than in those of controls. This

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is consistent with the view, but does not prove, that feeding with cholesterol inhibits the biosynthesis of β -hydroxy- β -methylglutaryl-CoA by liver microsomal fraction. In addition to its action on β -hydroxy- β -methylglutaryl-CoA reductase, dietary cholesterol may therefore affect other enzymes concerned in cholesterol biosynthesis. Essentially similar results have been reported by White & Rudney (1970). The significance of these observations will be discussed.

This work was supported by The Wellcome Trust and by the Scottish Hospitals Endowments Research Trust.

- Chappell, J. B. & Hansford, R. G. (1969). In *Subcellular Components*, pp. 43–56. Ed. by Birnie, G. D. & Fox, S. M. London: Butterworths.
- Linn, T. C. (1967). *J. biol. Chem.* **242**, 990.
- White, L. W. & Rudney, H. (1970). *Biochemistry, Easton*, **9**, 2725.
- Williamson, D. H., Bates, M. W. & Krebs, H. A. (1968). *Biochem. J.* **108**, 353.

The Effect of Development on the Gangliosides of Rat Brain

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Suzuki (1965) found that the concentration of total gangliosides in the whole brain of the rat rose rapidly from 6 days after birth to an almost adult value by 16 days. Changes in the amounts of the four major gangliosides, G1, G2, G3 and G4 (Korey & Gonatas, 1963), were also reported.

In the present investigation the developmental pattern in three parts of the brain (forebrain, cerebellum and brain stem) has been studied at 11 stages of growth between 1 and 810 days of age.

Wistar albino rats were reared from birth in litters of eight pups and weaned at 21 days. Animals were killed by decapitation and the brains dissected as described by Dickerson & Jarvis (1970). Gangliosides were extracted by Suzuki's (1964) modification of the Folch, Lees & Sloane-Stanley (1957) procedure, and fractionated by t.l.c. as described by Suzuki (1964). Total gangliosides and the gangliosides in the four major fractions were determined as *N*-acetylneuraminic acid by the method of Svennerholm (1957).

The concentration of gangliosides in the forebrain rose rapidly between 1 and 21 days after birth, fell to 90 days and subsequently rose to a mature value at 180 days. The concentration in the cerebellum rose to a peak value at 60 days and subsequently decreased to a lower adult value by 270 days. The concentration in the brain stem rose more slowly

and had a broad peak from 15 to 60 days and subsequently fell to an almost adult value by 180 days. In absolute terms the amounts per forebrain followed a similar pattern to the concentration and reached the adult value by 270 days, whereas the amounts in the cerebellum and in the brain stem showed a sharp peak at 90 days and fell to a mature value by 180 days.

In the forebrain the percentage of the total gangliosides present as ganglioside G3 (Korey & Gonatas, 1963) rose rapidly between 1 and 7 days and fell after 21 days to a mature value at 90 days. Reciprocal changes were found in the concentration of ganglioside G1.

In the cerebellum and brain stem the proportions of the individual gangliosides changed somewhat irregularly with time, with the exception of ganglioside G2 in the cerebellum, which accounted for about 20% of the total at all ages. In the forebrain ganglioside G3 accounted for the largest percentage of the total gangliosides, whereas in the mature cerebellum and brain stem the largest percentage was accounted for by gangliosides G1 and G2 respectively.

Possible explanations of some of these findings will be discussed.

A. M. is a British Council Scholar.

- Dickerson, J. W. T. & Jarvis, J. (1970). *Proc. Nutr. Soc.* **29**, 4A.
- Folch, J., Lees, M. & Sloane-Stanley, C. H. (1957). *J. biol. Chem.* **226**, 497.
- Korey, S. R. & Gonatas, J. (1963). *Life Sci.* **2**, 296.
- Suzuki, K. (1964). *Life Sci.* **3**, 1227.
- Suzuki, K. (1965). *J. Neurochem.* **12**, 969.
- Svennerholm, L. (1957). *Biochim., biophys. Acta*, **24**, 604.

The Effect of Development on the Gangliosides of Pig Brain

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This study is an extension of a previous investigation of the changes in chemical composition of the brain of the growing pig (Dickerson & Dobbing, 1966). Brains were obtained from foetuses of 93 and 104 days' gestation, newborn piglets and at 2, 5, 7, 9, 20, 52, 64 and 120 weeks after birth. The brains were dissected as described by Dickerson & Dobbing (1966) and gangliosides were extracted, separated and determined by the same method as those used in the parallel study on rat brain (Dickerson & Merat, 1971).