The fatty acid composition and biosynthesis of fatty acids were studied during early embryogenesis of the toad Bufo arenarum Hensel. The ova and stages up to the 6½ day embryo have similar fatty acid compositions, with ca. 70% unsaturated acids. The eggs and embryo were permeable to acetate and impermeable to palmitic, linoleic, and eicosa-8,11,14-trienoic acid. Labeled acetate was incorporated by the eggs into the saturated acids—lauric, myristic, palmitic, stearic, arachidic, and behenic—and into the unsaturated acids—myristoleic, palmitoleic, oleic, and eicosapenoic acids. During segmentation and gastrulation, de novo biosynthesis of fatty acids increased, desaturation to myristoleic, palmitoleic, and oleic acids was enhanced; and fatty acids were esterified to triglycerides, phosphatidyl choline, and phosphatidyl ethanolamine. The feeding embryo (11 days) changed the pattern of incorporation to less incorporation into triglycerides.

INTRODUCTION

Several aspects of the biochemistry of lipids during the development of eggs in invertebrates have been published (1,2). However, very few studies have been done on the structure and biosynthesis of lipids during early development in vertebrates. Terner et al. (3) studied the incorporation of 1-14C acetic acid into different lipids of fish embryo. Jiamouyiannis and Din (4) and Pasternak (5) investigated the biosynthesis of gangliosides and phospholipids during development in amphibia. In the toad Bufo arenarum Hensel, Barassi and Bazan (6) examined the fatty acid distribution and 32P incorporation into phospholipids from unfertilized oocytes up to the blastula and neurula stages, respectively. The aim of the present work was to investigate the enzymatic activity of fatty acid synthesis during the early embryogenesis of Bufo arenarum Hensel.
of 84.6%. Expired CO₂ radioactivity was also measured by counting the KOH solution in the same apparatus. The lipids were esterified with 3 N HCl in methanol, and radioactivity distribution in the different fatty acids was measured by gas-liquid radiochromatography in a Pye apparatus with proportional counter (9). Fatty acids were identified by comparison with authentic samples.

**Embryo Homogenization**

Oocytes immediately after fertilization and embryos were homogenized in the cold in a Potter Elvehjem apparatus with a Teflon pestle. The homogenizing solution consisted of 5 mM MgCl₂, 1.5 mM reduced glutathione, 0.25 M sucrose, 62 mM phosphate buffer (pH 7), 0.1 M KCl, and 0.1 M ethylenediaminetetraacetic acid. Three ml of solution were used per mg of tissue.

The homogenate was used to measure fatty acid incorporation, desaturation, and elongation. Protein concentration was measured by the method of Lowry et al. (10). The homogenate was fractionated by differential centrifugation for the separation of a 105,000 x g pellet as already described (11).

**Assay for Oxidative Desaturation and Elongation of Fatty Acids**

Desaturation and elongation of 1-14C palmitic, 1-14C linoleic, and 1-14C eicosa-8,11,14-trienoic acids were measured by the procedures described elsewhere (11,12). Two nmol of each labeled acid were incubated 30 min at 35°C in a total volume of 1.5 ml in the presence of 10 mg protein of total homogenate or 2 mg protein of the 105,000 x g pellet suspension. The concentration of the labeled acid was reduced compared to other similar experiments (11,12) to increase the sensitivity of the detection. The reaction was terminated by addition of 2 ml of 10% alcoholic KOH. The methyl esters of the fatty acids were prepared by the procedure described previously (9) and were analyzed in a Pye gas liquid radiochromatograph provided with a proportional counter. The distribution of radioactivity between substrate and products was calculated.

**Incorporation of Labeled Fatty Acids in Lipid Classes**

After incubation of 2 nmol of labeled fatty acids with 10 mg embryo homogenates for 30 min under the conditions already described, the lipids were extracted by the procedure of Folch et al. (8). Lipids were fractionated by thin layer chromatography on Silica Gel G. Chloroform:methanol:water (65:25:1, v/v) was used for phospholipids and hexane:ethyl ether:acetic acid (80:20:1, v/v) for neutral lipids separation.