fertilization, on morphogenesis are irreversible, the total synthetic rate of RNA, even in anomalous embryos, may be restored. This further substantiates the hypothesis that a precise sequence of RNA syntheses is required during early stages for successful morphogenesis.

The relatively long life of certain messenger RNA molecules during oogenesis and embryonic cleavage stages appears to be well established. A number of explanations for the stability of these mRNAs may be offered, including binding with a protein, sequestering from cellular ribonuclease, or the absence of ribonuclease during early stages of embryogenesis. In order to narrow the field of possible explanations, the latter hypothesis was tested, i.e., the presence of ribonuclease was tested for at all stages of Fundulus development. The assay was a modification of that by Zimmerman and Sandeen (Anal. Biochem. 10:444-49, 1965). The enzyme was incubated with polycytidylic acid and the extent of release of $HClO_4$-soluble nucleotides was measured by observing the absorbance at 268 mμ. The embryo extract assayed was the 100,000 x g supernatant obtained from centrifuging an homogenate prepared in 0.005 M Tris buffer, pH 8.2. The results of these assays showed measurable quantities of ribonuclease present in all stages of Fundulus embryos, remaining at about the same level throughout development. Thus, the absence of ribonuclease from cleavage stages of Fundulus embryos would not serve as a reasonable explanation for the stability of mRNA.

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BRAIN BARRIER SYSTEMS: AGE AND SPECIES DIFFERENCES

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We have examined brain barrier systems in several species of Chondrichthyes and one species of Agnatha using ^{14}C-inulin. Results indicate differences both between species and between age groups of a single species.

Plasma ^{14}C-inulin concentration was maintained relatively constant in Chondrichthyes by repeated i.m. injections: Myxine received a single injection. Chromatographs of tissue samples showed that ^{14}C-inulin accounted for more than 90% of tissue radioactivity in plasma of Myxine and in plasma, CSF and brain of Squalus acanthias (mature and pups). Potassium concentration of Myxine plasma was higher than reported in the literature (12.2 versus 9.6 meq/kg H_2O), raising questions concerning the physiological condition of these specimens.

Distribution ratios for ^{14}C-inulin between brain and plasma (R_{Br}) and between CSF and plasma (R_{CSF}) are summarized in Table 1. Values for rats are included for comparison (J. Physiol. 169:816-50, 1963; Arch. Neurology 12:284-95, 1965). R_{Br} and R_{CSF} in mature dogfish (S. acanthias), nurse shark (G. cirratum), and hagfish (M. glutinosa) are higher than in adult rats. Increased inulin content of brain is not a feature common to all lower vertebrates, however, as shown by values for rays (D. sabina and D. sayi), significantly more inulin was found in CSF and brain of immature than adult dogfish suggesting that in this species, as in mammals, the ability to exclude substances from the central nervous system develops with age. This conclusion was further supported by comparisons of CSF and plasma protein concentrations (Lowry)

Table 1

SPECIES AND AGE DIFFERENCES IN BRAIN BARRIER SYSTEMS TO INULIN
(# of observations)

Class	Species	Age, weight, etc.	Δt, hrs	R_{Br} *	R_{CSF}
Mammalia	Rattus	Adult	24	.015	.02
"	"	8 day old	24	.22	-
Chondrichthyes	Squalus acanthias	Adult	24	.06 (6)	.09 (10)
"	" "	Pups with yolk sac attached	24	.121 (11)	.48 (7)
"	" "	"	48	.202 (5)	.75 (2)
"	Ginglymostoma cirratum†	3-10 kg	24	.07 (3)	-
"	Dasyatis sabina and sayi‡	.6 - .9 kg	24	.007 (3)	-
"	" " " "	.2 kg immature claspers	24	.034 (2)	-
Agnatha	Myxine glutinosa		24	.14 (10)	-

*In Chondrichthyes, R_{Br} corrected for residual blood within brain.
†From the Lerner Marine Laboratory, Bimini.
‡From the Mote Laboratory, Sarasota, Florida.

in mature and immature dogfish. CSF protein concentration was higher in pups (N = 4) than in adults (N = 5), both in terms of absolute concentration (105 mg% versus 40 mg%) and in terms of the ratio, [CSF] ÷ [plasma] (.104 versus .016). The limited data for rays also suggests variations in brain barrier systems with age.

Mechanisms underlying observed differences in R_{Br} and R_{CSF} for inulin are unclear. Possible explanations include variations in rate of inulin entry into or exit from the central nervous system, variable compartment sizes, or (for brain only) cellular uptake of inulin.

Modern anatomical studies have located brain barrier systems to the protein molecule, horseradish peroxidase (Brightman and Reese, J. Cell Biol. 40:648-77, 1969). Tight junctions between endothelial cells of parenchymal cerebral capillaries and between epithelial cells of the choroid plexuses are impermeable to horseradish peroxidase, thereby preventing the protein from moving from plasma into brain and CSF, respectively. In order to examine the possibility that observed differences in brain barrier systems to inulin may be associated with variations in the ultrastructure of cerebral membranes, samples of adult and immature dogfish brain were prepared for subsequent electron microscopic examination.