aqueous liver extract are also required. We have now found that the protein hydrolysate may be replaced by a mixture of pure amino acids mixed in such proportions as to simulate the composition of casein. The liver extract[2] was purified by adsorption on and elution from lead sulfide, followed by adsorption on and elution from fuller's earth. The resulting concentrate was active at a level of 1 microgram per cc. The properties of the active material revealed by these concentration procedures led us to try various basic growth factors, and it was found that crystalline vitamin B_6[3] was effective.

Representative data illustrating the response obtained on our chemically defined media are shown in Table 1. The amino acid mixture supplied the following amounts of material per cc of medium: glycine 0.1 mg, dl-alanine 0.4 mg, dl-valine 1.6 mg, dl-leucine 1.0 mg, d-isoleucine 0.5 mg, dl-serine 0.2 mg, l-proline 1.0 mg, l-hydroxyproline 0.1 mg, dl-phenylalanine 1.0 mg, l-tyrosine 0.7 mg, l-cystine 0.1 mg, d-arginine hydrochloride 0.8 mg, l-histidine hydrochloride 0.4 mg, d-lysine hydrochloride 1.0 mg, l-tryptophane 0.3 mg, dl-methionine 0.8 mg, l-aspartic acid 0.5 mg, d-glutamic acid 2.5 mg, dl-threonine 0.6 mg. Growth was measured by quantitative determination of turbidity, as previously described. The figures in Table 1 represent the per cent. of the incident light which was transmitted by the culture. The pantothenic acid was supplied as a highly purified concentrate, but a synthetic product derived from di-hydroxy caproic acid was previously shown to replace it.

TABLE 1

Additions to glucose-salts medium	Photometer reading
(1) Riboflavin + reduced Fe + pantothenic acid	100
(2) (1) + amino acids	74
(3) (2) + vitamin B_6 (0.5γ/cc.)	10

It was noted that slight growth usually occurred when the amino acids, but not vitamin B_6, were added. This may possibly be due to contamination of some of the amino acids with the vitamin. It was not possible to supply every amino acid as a synthetic product, and while those that were not synthesized were carefully recrystallized, it may be that they were still contaminated.

The amino acid requirements for *S. zymogenes* have been determined on our medium, and glutamic acid and tryptophane were found to be essential. Details of the work, together with observations on a variety of species, will be published elsewhere.

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2 We wish to thank Dr. David Klein of Wilson Laboratories for generous gift of this material.
3 We wish to thank Dr. S. Lepkovsky for a sample of crystalline vitamin B_6.

THE PSEUDO-AMNION, PSEUDO-CHORION, PSEUDO-PLACENTA AND OTHER FOETAL STRUCTURES IN VIVIPAROUS CYPRINODONT FISHES

OVOVIVIPARITY or viviparity occurs in all species of four families of the cyprinodont fishes. Most of the specializations for viviparity in embryos and ovaries found in other viviparous fishes occur in this single order, and in addition there are unique features not found elsewhere. Within two of the families (Poeciliidae and Goodeidae) different species show degrees of specialization varying from simple conditions similar to those of oviparous fishes to extremes involving the partial degeneration of the yolk sac, and the development of new structures (pseudo-amnion, pseudo-chorion, pseudo-follicular placenta, trophotaeniae and gut modifications). Simple adaptations occur in the early embryos of extremely specialized forms, but these are ephemeral and are replaced in later stages of gestation by newer and more specialized structures. In the families Jenynsiidae and Anablepidae the living species have become highly specialized and no species retains simple modifications, except in early stages of ontogeny. There are numerous parallel developments in the four families.

The development, function and history of these adaptational structures is parallel roughly to that of the foetal membranes and yolk sac in the amniotes as a whole in the following respects:

(1) The yolk supply is adequate for nutrition up to the time of birth or hatching in some cases, but the yolk sac becomes degenerate in more specialized groups and the function of nutrition is taken over by new structures.

(2) Membranes or specialized organs functioning as respiratory, nutritive and excretory devices are developed from the somatopleure of the pericardial or peritoneal spaces and from the gut. They become highly vascular.

(3) In cases of advanced viviparity the uterus (ovary in fishes) becomes greatly modified.

(4) Foetal membranes or specialized structures are temporary and are discarded at birth.

Poeciliidae. The embryos are retained in the ovarian follicles for the whole period of gestation. In some genera (Mollienesia and others) the yolk sac is large and furnishes an adequate nutritional supply for the whole period of gestation. The somatopleure of the extra-embryonic pericardial cavity becomes vascularized, thus augmenting the respiratory surface, and lateral extensions fuse dorsal to the posterior head region to form a continuous band, a unique feature in this family. Specializations within the family are of two types. (1) *Heterandria formosa* and others have very small yolk sacs. The lateral extensions of the somatopleure of the extra-embryonic pericardial cavity completely envelop the head, forming an outer vascular membrane (*pseudo-chorion*) and an inner non-vascular layer (*pseudo-amnion*) which is adherent to the embryo. The vascular membrane with its blood vessels derived from the old vitelline circulation is used as an organ for respiration and nutrition throughout gestation. The surrounding follicle becomes highly vascular and develops a layer of high, irregular columnar cells. (2) In Aulophallus and some species of Poeciliopsis which have small yolk sacs the lateral portions of the pericardial somatopleure are poorly developed, but the ventral portion becomes enlarged into an extensive vascular belly sac. The surrounding follicle develops on the internal surface an extensive system of anastomosing villi which are vascular and are covered with a secretory epithelium. The wall of the belly sac, together with the wall of the follicle with its villi, form a *follicular pseudo-placenta.*

Anablepidae. A more complicated and apparently more effective *follicular pseudo-placenta* has been developed in at least two of the three existing species of Anableps. The belly sac becomes very large and at an early stage takes over and expands the vitelline circulation. The blood vessels of the sac become expanded into rows of connected and projecting vascular bulbs. The surrounding follicle is covered on its inner surface with a mat of long vascular villi which are covered with a low epithelium.

The looped small intestine is everted into the cavity of the belly sac in *Anableps anableps.* In *A. dowei* the large intestine becomes a balloon-like sac which nearly fills the cavity of the sac. In both species there is a metamorphosis of the gut at birth involving shortening and reorganization. Follicular fluid is imbibed by the embryo and absorption takes place by means of specialized villi of the gut. The concentrated unabsorbed material is retained in the gut.

Goodeidae. In this family and in the Jenynsiidae the embryos are evacuated from the ovarian follicles into the ovarian cavity at early stages and develop in the ovarian cavity.

Goodeid species have various degrees of specialization in foetal structures. (1) In the primitive Ataeniobius the yolk sac is fairly large, and there is no expansion of the pericardial membrane. In late gestation the embryos imbibe ovarian fluid through an enlarged opercular opening and the mouth. (2) *Goodea luitpoldii* has a smaller yolk sac. The extra-embryonic pericardial sac is somewhat enlarged and vascular in early stages but recedes with the development of the trophotaeniae, ribbon-shaped outgrowths from the rectal lip which increase the absorbing surface. (3) Lermichthys, Neotoca and others have very small yolk sacs. Extensions of the pericardial somatopleure arise early but begin to recede at the thirty-five somite stage. A very extensive system of vascular trophotaeniae begins to develop at the twenty somite stage and with the recession of the extra-embryonic pericardial membrane takes over the functions of respiration and nutrition.

Jenynsiidae. In *Jenynsia* (*Fitzroya*) *lineata* the embryo augments the respiratory surface by expanding the vascular pericardial sac. The sac reaches the maximal size at the 6 mm stage at the point at which the yolk becomes exhausted. The pericardial sac begins to recede at this point, but in the meantime club-shaped vascular flaps grow out from the ovarian wall and extend into the branchial and mouth cavities through an enlarged opercular opening. Respiratory exchanges are accomplished by this device during the remainder of the gestational period, but nutritional materials are obtained by absorption from ovarian fluid imbibed through the mouth or opercular openings.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A THORACIC WINDOW FOR OBSERVATION OF THE LUNG IN A LIVING ANIMAL

DURING my investigation of the presence of fluid in the pulmonary air sacs a number of techniques have been tried to make these chambers accessible for observation in the living animal. Whereas this is a simple matter in the case of amphibians, it is not so in dealing with mammals, as every investigator who has approached the provinces of structure or physiology of the terminal spaces of the lung has realized. In attempts to overcome the difficulty of maintaining the lung under natural physiological conditions and at the same time having it available for observation and experiment, an instrument has been devised which is simple in construction, easy to adjust and which has proved successful for observations of the superficial air sacs and alveoli in the cat's lung. The principle of the method seems to be sound and practicable; some changes in the construction of accessory parts and in the means of illumination may be found desirable.

The instrument, made of bronze, consists of a short hollow cylinder, at one end of which is mounted a small cover glass window and at its opposite or internal end is formed a quadrilateral plate or flange. When adjusted in the thoracic wall, the cylinder projects through an opening made in an intercostal space, the plate fitting against the internal surface of the wall and the instrument fixed in place by means presently to be explained. When the air has been drawn out of the pleural cavity by an exhaust tube, the surface of the lung comes in contact with the window and remains there in view during respiration. Details of