simplex reported by Morgan, *et al.*(4), influenza virus also observed by Morgan and co-workers(5), the salivary gland viruses reported by Luse and Smith(6), and the virus-like structures seen by deHarven and Friend in mouse leukemia material(7), and by Fawcett in frog adenocarcinoma(8). The EAV herein reported conforms very closely to the pattern. These examples of correlation in structure of different types of animal viruses serve to accent the importance of the questions raised by Morgan(9) concerning the relationship between structure and function.

The subject of intranuclear inclusion bodies associated with viral lesions has been of sustained interest to virologists for many years. Until recently no incontrovertible evidence for the existence of viral inclusion bodies in infected cells had been demonstrated by electron microscopy. It may be, at least in some instances, that the substances comprising the inclusion bodies which are readily seen by light microscope are too similar in electron density to the surrounding nuclear material to be visualized by the electron microscope. However, conclusive electron microscopic evidence of intranuclear inclusion bodies has been presented recently by Fawcett, *op. cit.*, and by Luse and Smith, *op. cit.* It is of considerable interest that margination of chromatin was prominent in the renal tumor cells studied by Fawcett, but was not a feature of the infected salivary gland cells employed by Luse and Smith. In contradistinction the EAV material, although revealing no intranuclear inclusion bodies did show conspicuous margination of chromatin. Whether these contrasting characteristics are features of the different viruses or of the different host cells constitutes a problem which might be explored.

Summary. 1) Young Syrian golden hamsters were inoculated with equine abortion virus (EAV). Hepatic cells from these animals, in late stages of infection, were studied with the electron microscope, and compared with similar material from uninfected (control) animals. 2) Various forms in which virus particles were seen, are described, and their similarity to other viruses is discussed. 3) Virus particles were observed predominantly in the nuclei. 4) "Nests" of virus particles were seen, but these were not considered to be equivalent to the intranuclear inclusion bodies characteristically seen by light microscopy. No structures identifiable as inclusion bodies were seen. 5) Other cytopathogenic effects are described.

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Maintenance of Gonads of Frog Larvae on Synthetic Media.* (24172)

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Recent studies on gonads of adult and larval amphibians have shown that these organs can be grown and maintained *in vitro* on media composed of chick embryo extract and cock plasma, and on chick embryo extract and agar(1). To determine what factors influence differentiation of the amphibian gonad,

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synthetic media were employed as nutrients, eliminating possible effects of unidentified substances in media obtained from natural sources. The object of our study was to learn which of several chemically defined media could best maintain *in vitro* the sexually differentiated gonads of larval anurans.

Materials and methods. Ovaries and testes of second-year tadpoles of *Rana catesbeiana* were placed in watch glass cultures(2). Nutrient media used were T.C. 199(3), T.C. 1066 (communication from Connaught Medical Lab.) White's Nutrient Solution(4) an amino acid base medium containing purines and pyrimidines of the nucleic acids, which we developed and designated AAB, and Tyrode's solution. Larvae were made bacteriologically sterile by use of antibiotics and sulfa drugs before removal of gonads. Testes cultured were usually whole organs, while each ovary was cut into 3 parts before being placed in culture. Explants of gonads were placed on strips of cellulose acetate fabric and these laid on solid substrate containing the nutrients(5). A total of 56 testes and 320 ovarian explants were kept in cultures for periods varying 4 to 28 days. Numbers of explants on various media and lengths of culture periods are given in Table I. Culture media were prepared by putting 9 parts nutrient solution and 1 part penicillin solution (200-300 μg) in the watch glass, adding 10 parts 1% agar solution with phenol red indicator, stirring and allowing the mixture to solidify. Cultures were kept at 21°C and pH 7.2. Organs were transferred to fresh media each fifth day.

Results. Criteria for determining effectiveness of each medium were degree of maintenance of large ovocytes (auxocytes) and young germ cells, and maintenance and proliferation of non-germinal cells. Chick embryo extract and cock plasma proved more satisfactory for growth of ovaries and testes than the other media used. Characteristic changes in cellular pattern were associated with each medium and outgrowths of fibroblasts from explants were noted in all.

On T.C. 199 most auxocytes degenerated during the first 2 weeks in culture and few young germ cells were observed, but there was an increase in number of non-germinal cells which filled in the areas vacated by the germ cells. Immature ovarian germ cells appeared essentially normal when grown on T.C. 1066, even after 28 days in culture; but auxocytes underwent cytolysis. The same increase in number and size of follicular cells occurred on both of these media. White's nutrient solution maintained all cell types in apparently healthy condition during the first 2 weeks, but the organs then became necrotic. The AAB medium was distinct from the 3 previously described media, poorly supporting growth of even the non-germinal elements of the ovary. Few characteristic cells of any type were apparent after 2 weeks on this medium. Tyrode's solution alone failed to maintain cells of the ovary, although slight proliferation of fibroblasts occurred.

Cells of cultured testes were in better condition than those of the ovaries, and appeared essentially normal when grown on T.C. 199 or T.C. 1066, and also during the first 2 weeks on White's solution. The only group of testes cultured on AAB medium became necrotic prior to 28th day.

For maintenance of auxocytes 2 weeks or less, White's solution is most adequate: the other nutrient solutions in order of effectiveness were T.C. 1066, T.C. 199, AAB, and Tyrode's. For maintenance of young germ cells and non-germinal cells for longer times, media in order of decreasing effectiveness were T.C. 1066, T.C. 199, White's, AAB, and Tyrode's solution (Table I).

Discussion. These results indicate that media for growing mammalian and avian tissues will support amphibian gonads as organs for brief periods of time and maintain certain types of cells and tissues for a much longer time. However, none of the nutrient solutions was entirely adequate for maintenance of amphibian ovary or testis. The fact that ovaries, in general, were not as well maintained as testes might be due to inhibitory effects by products of cytolysis from the auxocytes. It appears that for the study of gonad differentiation which in amphibians may cover a relatively long period of time, of the used

TABLE I. Cellular Changes in Gonads on Synthetic Media for Varying Periods of Time.

Media	Days in culture	Explants of ovaries	Germ cells: Auxocytes	Germ cells: Immature	Non-germinal cells	Testes	Germ cells	Non-germinal cells
T.C. 199	4	12	Early stages of cytolysis	Normal	Increase in No. of follicular cells	1	Normal	Increase in No.
	15	42	Much cytolysis	Few	*Idem*	3	Present in only 1 gonad	*Idem*
	20	7	Remnants only	Very few	"	0		
T.C. 1066	5	17	Cytolysis of few cells	Normal	Slight increase in No.	4	Normal	Many mitoses
	10	48	25% cytolysis	"	Increase in No. and size of cells	11	"	*Idem*
	20	84	50-75% cytolysis	"	*Idem*	18	"	"
	23	29	*Idem*	"	Granulosa cells fill area around degenerating auxocytes	0		
White's	10	3	Normal	"	Slight increase in No.	5	Normal	Slight increase in No.
	13	2	Cytolysis of few cells	"	Few mitoses	6	"	Normal
	15	3	Cytolysis of peripheral cells	Necrotic	No increase in No.	3	Few apparent	Some necrosis
	25	9	Cells necrotic	"	Necrotic	2	Necrotic	Necrotic
AAB	6	6	50% cytolysis	Normal	Normal	0		
	16	17	Marked cytolysis	Necrotic	Some normal	0		
	28	24	Necrotic	"	Normal cells rare	3	Necrotic	Necrotic
Tyrode's	16	17	Marked cytolysis	"	*Idem*	0		

media. T.C. 1066 is most nearly adequate, while White's solution may serve for brief studies. It seems, however, that additions to or modifications of these media will be necessary for complete maintenance and differentiation of amphibian gonads *in vitro*.

Summary. Ovaries and testes of second-year larvae of *Rana catesbeiana* were cultured on synthetic media for varying periods of time up to 28 days. The organs were not as well maintained as on media from natural sources, but proliferation of non-germinal cells and young germ cells occurred on some of the tested media.

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Effect of Growth Hormone on Plasma Unesterified Fatty Acid Levels of Hypophysectomized Rats. (24173)

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Studies by Greenbaum *et al.*(1,2,3), Weil (4) and Curry(5) and others have shown that growth hormone effects mobilization of lipids from depots and probable utilization of these lipids as a primary source of energy for the body. The studies of Greenbaum(1) suggest that lipid mobilization and utilization are the probable mechanisms by which growth hormone effects positive nitrogen balance and hence growth. Russell(6) suggests that the "protein sparing" effect of growth hormone might be enhanced by furnishing large amounts of fat to the diet. Gordon(7,8) and Dole(9) postulate that unesterified fatty acid (UFA) is liberated from fat depots when other sources of calories are decreasing or are not available. This study relates the effect of growth hormone on plasma unesterified fatty acid levels of hypophysectomized rats. By using hypophysectomized animals it is possible to obviate the effect of endogenous growth hormone.

Methods. Female Sprague-Dawley rats hypophysectomized at 150 g (Exp. I and II) and 250 g (Exp. III) were obtained from Hormone Assay Inc., Chicago, Ill. Before the studies were undertaken the animals were kept on rat-chow plus meat supplement diet for 10-14 days post hypophysectomy. Animals whose body weight had plateaued were considered adequately hypophysectomized. On the morning the experimental animals were injected intraperitoneally with 4 mg of growth hormone [Somar-A (Armour R-50109) courtesy Endocrinology Study Section, USPHS] in 0.5 ml of distilled water adjusted to pH 9.5. Control rats in Exp. I were injected with 0.5 ml of distilled water adjusted to pH 9.5. In Exp. II and III one group of animals was injected with 4 mg of inactivated growth hormone in 0.5 ml distilled water adjusted to pH 9.5. The growth hormone was inactivated by autoclaving the solution at 18 lb, 123°C for 1 hour. The other group in these experiments was injected with an active growth hormone preparation which was from the same lot as the inactivated preparation. After injection, animals were placed in individual cages and provided *ad libitum* with drinking solution containing 10% dextrose in 0.5 normal saline. The animals were sacrificed by decapitation 8 hours after injection. This time interval was demonstrated by preliminary studies with fasted rats to be the time of most significant difference of UFA levels between experimental and control animals.