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In vitro Cultivation of Porcine Kidney. (22323)

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In early tissue culture experiments, choice of a menstruum for proliferation of a virus was generally restricted to host and tissue normally parasitized by the given virus. It is now apparent that these restrictions are not so clearly defined. Enders and his co-workers (1) for instance, propagated poliomyelitis virus in non-neural tissues (kidney, skin-muscle and intestine) and Maitland and Laing (2) grew vaccinia virus on rabbit kidney.

Recent observations that epithelial cells of renal origin support multiplication of a number of viruses beyond the confines of host or tissue specificity prompted us to study the nutritive factors involved in culturing of kidney tissue. Kidney of porcine origin was used because of favorable economic considerations and its availability.

Materials. A. *Porcine Constituents.* 1. *Hog Embryo Extract (HEE).* Embryos (10-20 cm long) were removed from the gravid uteri of sacrificed sows and washed with cold physiological saline. The tails, hooves, heads, ribs, and intestinal tracts were removed and the remaining skin-musculature and organs were cut into small pieces and placed in the deep freeze. The dismembered embryos were thawed 24 hours later and homogenized in a Waring Blendor for 30 seconds. An equal volume of Hanks Balanced Salt Solution containing antibiotics was added and the mixture rehomogenized an additional 15 seconds. The homogenized embryo extract was clarified by cheese cloth filtration and by centrifugation (4,000 rpm for 45 minutes at 1°C). The HEE was sterilized by filtration through an EO (Ertel) pad and stored in the deep freeze in 50 ml amounts. 2. *Hog Serum (HS)* was Seitz filtered and stored in deep freeze in 10 ml amounts until used. 3. *Hog Serum Ultrafiltrate (HSU)* was prepared by forcing hog serum through Visking casing. The pH of HSU was adjusted to 7.3 by means of 0.1 M NaH_2PO_4. The HSU was then Seitz filtered and stored in deep freeze in 10 ml amounts. (Selection of kidney tissue and hog constituents should be made from healthy non-immune stock, otherwise, humoral antibody or endogenous viruses may interfere with virus proliferation.) B. *Bovine Constituents.* Beef embryo extract (BEE), serum (BS), and serum ultrafiltrate (BSU) were obtained from Microbiological Associates, Bethesda, Md. C. *Balanced Salt Solution (BSS)* used in preparation of the media was prepared in the manner specified by Hanks and Wallace(3). Just previous to use, 2.5 ml of a 1.4% solution of sterile $NaHCO_3$ was added/100 ml of BSS. The pH of BSS was approximately 7.4. D. *Synthetic Mixture #199 (SM #199),* com-

posed of inorganic salts, amino acids, vitamins, nucleic acid derivatives and lipids, was prepared in accordance with procedure recommended by Morgan, Morton and Parker(4). SM #199 was refrigerated until used. E. *Nutritive Media* for initiation of cellular growth consisted of isotonic saline solution (either BSS or SM #199) together with varying amounts of serum, serum ultrafiltrate and embryonic extract. The media were compounded and used within 24 hours. F. *Maintenance Media* were used for cellular maintenance: Hanks-Simms medium was prepared by combining one part of sterile serum ultrafiltrate with 3 parts of BSS. Tenth molar NaH_2PO_4 was used to adjust the pH to 7.5. SM #199 was used without further adjustment. G. *Antibiotics.* Crystalline penicillin G (the potassium salt) and streptomycin (calcium chloride complex) were employed routinely in all media at levels of 250 units and μg/ml respectively. H. *Chick Embryo Extract (CEE)* was prepared in accordance with directions of Syverton *et al.*(5). I. *Hog Serum Fractions* II, IV, V and IV + V were prepared according to methods described by Cohn *et al.*(6) or Cammarata and Deutsch (7).

Methods. An entire kidney was removed from young hog (40-60 lbs. weight) using aseptic technic. The kidney was washed with BSS containing antibiotics. Capsule and macroscopic vasculature were removed and a pie shaped portion consisting of cortical and medullary substance was minced in a Petri dish containing several ml of BSS or SM #199. Tissue fragments were reduced to approximately 1 mm and washed several times with BSS or SM #199 until supernatant was clear. A. *Plasma Clot Technic.* The lower two-thirds of each culture tube (16 x 150 mm) was coated with 0.05 ml chicken plasma (Microbiological Associates). Using a platinum wire inoculating loop, 6-8 fragments of tissue were planted in a single vertical row at 10-15 mm intervals in the plasma coated area. Five hundreths ml of CEE was added and distributed over the tissue embedded in the plasma to induce coagulation. Excess CEE was allowed to drain. Following complete coagulation, 1 ml of nutritive medium was added to each tube. The culture tubes were then capped with white "non-toxic" rubber stoppers (The West Co.) and incubated at 36°C in a roller drum revolving at rate of 12 revolutions/hour. B. *Dry Tube Technic.* Minced tissue suspended in small amount of maintenance medium was introduced into lower half of prewarmed (45°C) test tubes by Wright pipettes. The tubes were inverted to effect drainage and stored at 4°C for 30 minutes. The tubes were then reinverted and 1 ml of nutritive medium was added to each tube. Tubes were stoppered and incubated at 36°C in a roller drum. The dry tube technic was used almost exclusively throughout the investigation when preliminary studies revealed that growth exceeded that of the plasma clot technic while granulation was almost nonexistent. C. *Fluid Change.* When growth was well established (usually within 5 to 7 days) the nutritive medium was removed. Each culture was rinsed twice with 1 ml aliquots of BSS or SM #199. One ml of maintenance medium was added and cultures were reincubated for 5 additional days. The maintenance fluid was changed at intervals of from 4 to 7 days depending upon pH of the medium.

Results. The nutritive media recommended for proliferation of cells in tissue culture are numerous, and vary as to their constituents and percentages in accordance with the organ and species cultured(8,9,10,11,12). To determine the influence of homologous and heterologous constituents of nutritive media on propagation of hog kidney epithelial cells, cultures were grown in media consisting of both beef and hog serum, serum, serum ultrafiltrate and embryo extract. The composition of the various media as well as their influence on growth of hog kidney epithelial cells are shown in Table I. The data derived from this initial study indicated that an all hog nutritive medium was superior to an all beef system and the introduction of any hog constituent into a nutritive medium significantly enhanced rate and extent of growth of hog kidney epithelial cells. Furthermore, growth in a medium composed of hog serum (10%) and SM #199 (90%) excelled that of any

TABLE I. Composition of Nutritive Media Used for Proliferation of Hog Kidney Epithelial Cells in Tissue Culture and Their Influence on Rate and Extent of Growth.

Medium constituent	% composition	Medium No. 1	2	3	4	5	6	7
Serum ultrafiltrate	17.6	B*	H*	H	B	B	—	—
Serum	20.0	B	H	B	H	B	—	—
Embryo extract	10.0	B	H	B	B	H	—	—
Balanced salt sol.	52.4	+	+	+	+	+		
Serum	10.0	—	—	—	—	—	B	H
Synthetic mixture #199	90.0	—	—	—	—	—	+	+
		pH 7.4					pH 7.6	
% of cultures growing†		26	82	75	78	74	56	88
Extent of growth‡		1+	2+	2+	2+	2+	2+	4+

* B—Beef origin; H—Hog origin.
† Within 7 days.
‡ Extent of cellular outgrowth based upon No. of layers of cells radiating from the plant and was evaluated in terms of 1 to 4+.

other media tested.

In accordance with these findings, a more definitive experiment was designed to determine optimum combinations and concentrations of hog constituents to be used in conjunction with SM #199 for growth of kidney cells. The results of this study, summarized in Table II, confirm and stress the growth stimulating role of hog serum in the cultivation of epithelial cells. Cultures grown in a medium containing hog serum were consistently more proliferative than cultures lacking that constituent. HEE and HSU, on the other hand, either alone or in combination with HS did little to influence growth. The data also indicate that a minimum of 7.5% hog serum in SM #199 was necessary to insure good growth.

It is interesting to note that none of the media itemized in Tables I or II adequately supported growth of either porcine splenic or testicular fibroblasts.

Having ascertained that porcine serum was a necessary medium constituent, attempts were made to further reduce the complexity of the nutritive medium by substitution of various porcine serum fractions for whole serum. Epithelial cells were consequently cultured in SM #199 containing serum fractions (II, IV, V, or IV + V) in concentrations equivalent to those found in whole serum (8,9). Findings (Table III) indicate that Fraction II did not initiate or support epithelial growth, and that Fractions IV and V, when used independently, produced growth inferior to that of whole serum. However, when Fraction IV + V was used, growth superior to that of whole serum resulted, suggesting a complementary action. The results also indicate the presence of an inhibitor in serum which is inactivated or removed during fractionation procedures.

It is interesting to note that bovine plasma Fractions IV and/or V have been found to enhance growth of monkey kidney cells in tissue culture(13).

Summary and Conclusions. 1. Some of the nutritional requirements for the *in vitro* culti-

TABLE II. Influence of Different Concentrations of Hog Constituents on Growth of Hog Kidney Epithelial Cells in Tissue Culture.

Medium constituent*	Medium No. 1	2	3	4	5	6	7	8	9	10	11	12
Serum ultrafiltrate	10	5	0	0	0	0	0	0	5	5	0	0
Serum	0	0	10	7.5	5	2.5	0	0	5	0	5	0
Embryo extract	0	0	0	0	0	0	10	5	0	5	5	0
Synthetic mixture #199	90	95	90	92.5	95	97.5	90	95	90	90	90	100
% of cultures growing†	50	35	90	100	70	75	0	0	75	15	78	30
Extent of growth	0	0	4+	4+	3+	3+	0	0	3+	0	2+	0

* In %. † Within 7 days.

TABLE III. Influence of Hog Serum Fractions II, IV, V and IV + V on Growth of Hog Kidney Epithelial Cells in Tissue Culture.

Hog serum fractions*	% of cultures exhibiting various degrees of growth				
	0	1+	2+	3+	4+
II	100	0	0	0	0
IV	11	44	45	0	0
V	43	28	29	0	0
IV + V	0	0	0	0	100
Whole serum	12	13	37	38	0

* In synthetic mixture #199.

vation of hog kidney epithelial cells were determined. The data indicate that cultures grown in a "simple" medium containing Synthetic Mixture #199 (92.5%) and hog serum (7.5%) were consistently more proliferative than cultures cultivated in more complex media containing embryo extract, serum ultrafiltrate and serum of homologous and heterologous origin. 2. None of the media tested adequately supported growth of either splenic or testicular fibroblasts. 3. The influence of various Porcine Serum Fractions (II, IV, V and IV + V) on growth of hog kidney epithelial cells was also investigated. When Fraction IV + V was used, excellent growth was achieved. When Fraction II, IV or V was used independently, growth inferior to that of whole serum resulted.

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Some Metabolic Effects of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione (Meticorten) Administered Orally to Dogs. (22324)

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The discovery of the glucocorticoid and anti-inflammatory activities of Meticorten ($\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione) in experimental animals(1,2) and of its anti-rheumatic activity in human subjects(3) prompted studies on the metabolic actions of this steroid. Most experimental animal studies with Meticorten have utilized mice or rats. There are, to our knowledge, no published studies of the effects in dogs of large doses of Meticorten on water and nitrogen balance, or of the effects on blood sugar level or on the glucose tolerance curve before and during prolonged oral treatment. The production of a marked polyuria and polydipsia by daily *subcutaneous* treatment of dogs with 10 mg/kg of cortisone has been reported(4). Dogs given the same dose orally did not exhibit these effects. Also, it has been noted(5) that daily intramuscular injections of cortisone (50-300 mg/day) to dogs produced polydipsia and polyuria but there were no changes in fasting blood sugar levels or on the glucose tolerance curves. More recent studies with