TABLE IV. Effect of Rescinnamine on Pentobarbital-Induced Sleeping Time of Mice.*

Dose, mg/kg	No. of groups (10 mice ea.)	Sleeping time, % of controls
2.5	2	126
5.0	4	144 ± 17†
10.0	4	167 ± 3†
20.0	2	213 †

* Rescinnamine given intraperitoneally 2 hr before standard dose of pentobarbital. See text.
† Significant increase ($p = .05$ or less).

carotid occlusion, and blockade or reversal of primary blood pressure rise elicited by faradization of afferent vagus. 3. The sedative effects of rescinnamine were demonstrated by gross observation in dogs, rats and mice and by prolongation of pentobarbital-induced sleeping time in mice. 4. Rescinnamine produced marked eyelid ptosis in mice and a copious nasal discharge in rats. 5. On a weight basis, rescinnamine appeared to be several times as potent as Rauwiloid® and similar to reserpine. 6. Rescinnamine is the second highly potent alkaloid derived from *Rauwolfia serpentina.*

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Received April 23, 1954. P.S.E.B.M., 1954, v86.

Cultivation of Human Tissues in Media Containing Bovine Allantoic and Amniotic Fluids. (21028)

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Allantoic and amniotic fluids have been used in tissue cultures by a number of workers. Moppett(1), Wendrowsky, and Zapolska(2), and Grossfeld(3) employed amniotic fluid from the embryonated egg in drop cultures of chick tissues. Fedorow(4) used the fluid surrounding the embryo of the cephalopod Rossia as the medium in which to culture nervous tissue from this organism. Thomas and his coworkers(5) described the use of deproteinated bovine amniotic fluid for cultivation of the virus of foot and mouth disease in suspended cell cultures of foetal calf skin; and Enders(6) used whole bovine amniotic fluid in roller tube cultures of various human tissues, propagating a number of viruses in these cultures. Moppett(7) also advocated the use of chick embryo allantoic fluid in cultures of chick tissues. Since the healing of wounds appeared to be stimulated by extracts of embryonic tissues from species possessing a well-developed allantois, Robinson(8) considered that solutions of allantoin might be of benefit in the treatment of wounds, and clinical trials with commercially-prepared allantoin confirmed his view. As a result of his observation, Shipp and Hetherington(9) studied the effect of allantoin on drop cultures of chick heart tissue, but found no significant stimulation of fibroblast growth. Chu(10) used allantoin in greater concentration than these workers, and concluded that it had a slightly retarding action on the growth of chick epithelial cells and chondroblasts in culture.

During the collection of bovine embryonic fluid in this laboratory, it was frequently noted that blind puncture of the gravid uterus by means of a hollow trocar might result in the withdrawal of 2 kinds of fluid, differing in

* Work conducted during tenure of a fellowship in the Division of Medicine and Public Health, Rockefeller Foundation.

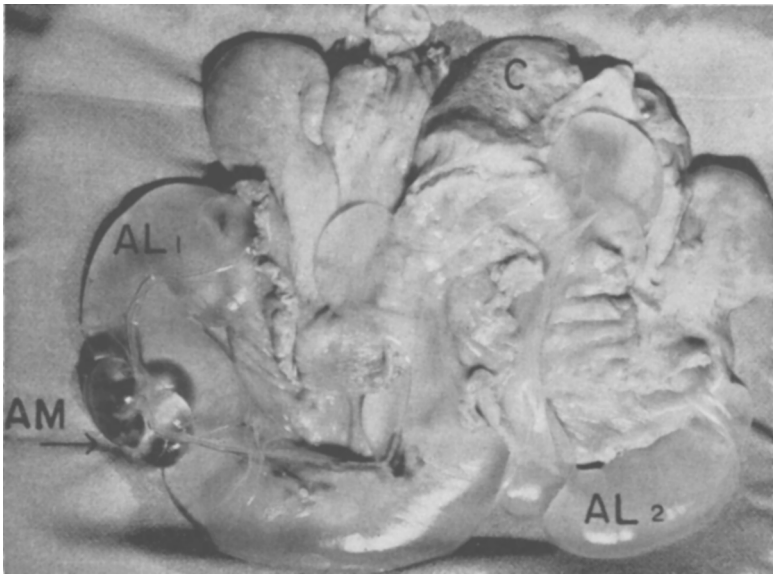

FIG. 1. Anatomy of gravid bovine uterus. C, cervix; AM, small amniotic sac—embryo 2 in. long; AL1, allantoic sac extension in pregnant horn; AL2, allantoic sac extension in non-pregnant horn.

appearance. Dissection confirmed that either allantoic or amniotic fluid, or both, could be obtained, depending on which sac had been penetrated. The present study was made to determine whether both fluids would support tissue growth; and the ability of Type 2 poliomyelitis virus to multiply in tissues cultured in either of these fluids was tested. Because of the usefulness of bovine amniotic fluid in the tissue culture field, a brief description of the anatomy of the gravid uterus is also presented.

Methods. Bovine uteri. These were used on the day of slaughter. A number were dissected to determine their anatomy; and fluids for the experiments were removed under direct vision from the allantoic and amniotic sacs. The embryos in the uteri examined measured up to 8 inches crown to rump. *Tissues.* Adult human uterus was used in Exp. 1. Skin and muscle from a 3-month human embryo were used in Exp. 2. *Tissue cultures.* Roller tube cultures were prepared by the method of Robbins, Weller, and Enders(11), and the medium was made up as follows:

Unfiltered allantoic or amniotic fluid	90%
Normal horse serum	5%
Bovine embryo extract	5%
Penicillin	100 units/ml
Streptomycin	100 μg/ml

0.1 ml of a stock 1% solution of soybean trypsin inhibitor was added for every 10 ml of medium. 2.0 ml of medium were put into each tube. The cultures were rotated at 37°C, and the medium was changed at intervals of 4 to 7 days, depending on the rate of metabolism of the tissues. *Growth measurement.* Tubes were examined daily under the microscope, using a 10× objective and a 5× ocular. The extent of outgrowth in each tube was estimated in millimeters, taking the average distance cells had spread radially from the edges of at least 6 explants. Very little difference was observed between the measurements for individual tubes in each group, and the results are therefore expressed as the mean for all tubes in each group.

Results. Anatomy of gravid uterus. The bovine uterus is bicornate, both horns participating in the development of the embryo (Fig. 1). *The chorion* lines both cavities, being attached to the uterus at a number of placental sites, or cotyledons; and umbilical vessels run along the lesser curvature of the embryonic sacs, with branches to the placentae. The chorion invests both the allantoic and the amniotic sacs, from which it can be separated by blunt dissection. *The allantoic sac* has 2 extensions connected by a narrower

TABLE I. Growth of Human Uterus Tissue in Media Containing Bovine Allantoic or Amniotic Fluid (Exp. 1).

Medium	No. of tubes	Mean outgrowth in mm on stated day after culture 3	5	8	10
Allantoic	5	0	.15	.35	.9
Amniotic	4	.1	.35	1.0	1.6

channel where it passes the amniotic sac. The longer extension may reach at least two-thirds of the way up the non-pregnant horn, and it is frequently possible to obtain a considerable volume of allantoic fluid from this horn, although it may occasionally be found that the contents of the sac in this situation are of a thinly gelatinous consistency and will not flow through a trocar. The uterine vessels accompany the sac into this horn. The second extension is in the tapering portion of the pregnant horn. Allantoic fluid may be clear in small embryos, but in larger specimens it tends to be opalescent or turbid, and on standing may form an amorphous deposit. Within the allantoic sac may be found detached bodies of a rubbery consistency, conforming to the description of hippomanes(12). As a rule, *amniotic fluid* is quite clear, and in older embryos it is darker than allantoic fluid, being of a yellow or yellow-green color. In the case of embryos under approximately 4 inches in crown-rump length, the amniotic sac is considerably smaller than the allantoic sac, and does not extend into the non-pregnant horn. There is thus a greater chance that allantoic fluid will be withdrawn from embryos of this size when blind puncture of the uterus is performed. In older embryos, distension of the amniotic sac obliterates any clear distinction between the broader parts of the 2 horns, and the allantoic sac is relatively much smaller.

Tissue growth. A. *Adult human uterus.* Table I summarizes the results of an experiment which demonstrated that while a medium containing allantoic fluid supported the growth of uterine tissue, the outgrowth was less extensive than that resulting from cultivation in amniotic fluid medium. In the tubes containing allantoic fluid the cells were somewhat granular, and outgrowth was less dense than in those containing amniotic fluid. It was also noted that in all the tubes with allantoic fluid the supporting plasma coagulum developed dark wrinkles or folds (Fig. 2) which tended to obscure the outgrowth. The outgrowths appearing in Exp. 1 on the 8th day after cultivation in the 2 kinds of fluid are shown in Fig. 2. The fluids used in this experiment were obtained from a single embryo measuring 7 inches crown to rump. Allantoic fluid was taken from the extension of the sac in the narrow portion of the pregnant horn, which contained about 200 ml of opalescent liquid. On standing, a white precipitate formed, and the material used consisted of the supernatant fluid after centrifugation at 1000 rpm for 5 minutes. The amniotic sac contained approximately one liter of clear yellow-green fluid from which after

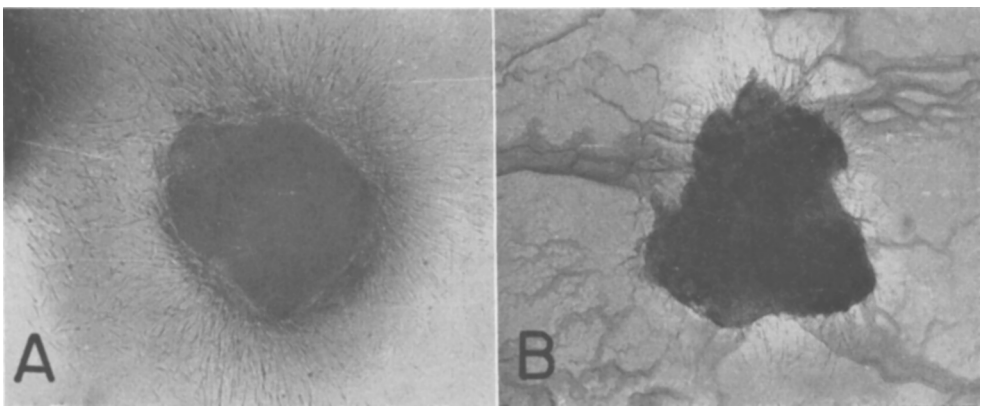

FIG. 2. Cultures of human uterus tissue in media containing bovine amniotic and allantoic fluids (Exp. 1). Outgrowths on 8th day after culturing. 25×.
A—amniotic fluid medium. B—allantoic fluid medium.

TABLE II. Growth of Human Embryonic Skin and Muscle in Media Containing Bovine Allantoic or Amniotic Fluid (Exp. 2).

Medium	No. of tubes	Mean outgrowth in mm on stated day after culture 2	3	4	5
Allantoic	7	.26	.53	1.0	1.4
Amniotic	7	.35	.7	1.4	2.1

withdrawal no solid material separated.

B. *Human embryonic skin-muscle.* In Table II are given the results of Exp. 2 in which tissue from a human embryo was grown in allantoic or amniotic fluid obtained from 2 bovine embryos measuring 6 inches crown to rump. The allantoic fluid was taken from the extension of the sac in the narrow end of the pregnant horn; it was opalescent but was not centrifuged prior to use. In the allantoic fluid tubes it was again noted that wrinkling of the plasma coagulum occurred, while the cells were granular and less numerous than in the amniotic fluid tubes. The amniotic fluid was transparent and yellow-green in color; and it supported the growth of clear and abundant cells while the coagulated plasma was not affected.

Cultivation of Type 2 poliomyelitis virus. Embryonic skin and muscle cultures from Exp. 2 were inoculated with 100 ID_{50} of Type 2 (Lansing) poliomyelitis virus. The media were replaced by allantoic and amniotic fluids without the addition of horse serum or embryo extract. Within 72 hours after inoculation approximately one-third of the cells in the inoculated tubes were destroyed. Further incubation resulted in progressive degeneration, while uninoculated control tubes remained unaffected.

Discussion. The above experiments indicate that, as a culture medium, bovine amniotic fluid is preferable to allantoic fluid. From a practical standpoint, however, it is not possible to be certain that fluid obtained by blind puncture of a gravid uterus does not contain some allantoic fluid, and it is probable that a small proportion will not significantly influence tissue growth. Relatively large amounts of allantoic fluid are associated with embryos up to a crown-rump length of about 4 inches, which, according to the data provided by Swett, Matthews, and Fohrman(13) would indicate an age of approximately 60-90 days.

Some information concerning the composition of bovine amniotic fluid is available(14). There is evidence(15) that in the later stages of gestation, fetal urinary excretion occurs into the amniotic sac; but the direct connection of the allantoic sac with the urachus suggests that excretory products will be found at an earlier period in allantoic fluid. These products may account for the difference in growth observed in the above experiments with human tissues cultured in allantoic and amniotic fluids.

Summary. 1. Growth of adult human uterus and human embryonic skin and muscle tissues in roller tube cultures is supported by media containing 90% of either bovine allantoic or bovine amniotic fluids. 2. Amniotic fluid medium produces a profuse outgrowth of clear cells, and does not affect the plasma clot; whereas allantoic fluid medium tends to produce wrinkling and darkening of the clot, and induces granularity in a less profuse outgrowth of cells. 3. Either fluid, used alone as the medium, permits the cultivation of Type 2 poliomyelitis virus.

Appreciation is expressed to Dr. J. F. Enders and Dr. T. H. Weller for their advice and guidance; to Dr. W. J. Cheatham, Mr. F. R. Harding and Mr. L. B. Brown for the photographs; and to Dr. K. McEntee of Cornell University for bibliographic assistance.

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Received April 26, 1954. P.S.E.B.M., 1954, v86.

Use of Tissue Culture Mediums Sterilized with Gamma Radiation from Cobalt-60.* (21029)

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Recent interest in tissue cell cultivation, resulting primarily from its successful application to problems in virology, has stimulated considerable effort towards simplification and improvement of methods and materials. In spite of the increased use, however, the greatest difficulty confronting the tissue culture worker continues to be the lack of a simple medium which can be easily prepared and handled. A synthetic medium, capable of sustaining cell proliferation, has not yet been developed and almost all tissue culture mediums contain serum and embryonic extract as major components. While these materials may be sterilized by filtration the procedure is both tedious and uncertain.

The present paper is a preliminary report of studies designed to test the feasibility of using tissue culture mediums sterilized with gamma radiation from Cobalt-60. Though ample evidence is available that such radiations will sterilize a variety of biological materials under appropriate conditions(1-3) it was necessary to establish the fact that the particular materials in question here, namely serum and embryonic extract, could be sterilized in the form in which they are commonly used for tissue culture work. A second and more critical point was to determine the suitability of such irradiated mediums for cell cultivation and was concerned with: a) possible destruction of growth promoting properties, b) production of toxic substances or materials capable of stimulating abnormal growth, c) alteration of other significant physical or chemical properties. Since plasma is frequently used in tissue culture work to provide an imbedding matrix, it was substituted for serum in these initial studies. In this way it was possible to determine the effect of irradiation on the clot forming ability as well as on growth promoting properties. The effect of gamma radiation on the ability of embryonic extract to induce clot formation was also studied.

Materials and methods. Irradiation for this study was carried out with the large Cobalt-60 source of the Michigan Memorial Phoenix Project(4). This source develops 2.2 x 10^5 rep/hr in the center well: samples were irradiated in the center well and received total dosages varying from 1 x 10^5 to 1.2 x 10^6 rep. In all experiments, control samples were kept at approximately 15°C, the temperature of the irradiation chamber, for the duration of the exposure. Tests were made with *chicken plasma* and *embryonic extract*: they were used in the form of commercially dehydrated products (Difco) and as freshly prepared fluid samples. The former were irradiated both in the dehydrated state and following

* This work was supported by the Difco Laboratories, Detroit, Mich., and by the Michigan Memorial Phoenix Project.

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