TABLE III.

Name	Method	Result
Cartland and Kock(2)	Dye	6 to 7.9% B.W.
Jolly and Stini(3)	Welcker	4 to 5 % B.W.
Chisolm(4)	Welcker	5.3 to 7.3% B.W.
Scott and Barcroft(5)	CO	5.4 to 7.9% B.W.
Griffith and Campbell(6)	Dye	4.1 to 5.3% B.W.
Cutting and Cutter(7)	"	4 to 5 % B.W.
Went and Drinker(8)	"	6.9 to 7.9% B.W.

for the measurement of the blood volume of the normal rat may thus be considered to be a reliable one.

Conclusions. 1. A method for the determination of blood volume of the rat by radio-isotope dilution is described. 2. In 34 normal adult white rats, average blood volume was 6.3% of body weight, with a stand. dev. of 0.7. 3. In 7 rats, the determination was repeated with a maximum error of ±1% body weight. 4. These results are in agreement with those reported in the literature using different methods.

3. Jolly, J., and Stini, J., *Compt. rend. Soc. de biol.*, 1905, v58, 835.

4. Chisolm, R. A., *Quart. J. Exp. Physiol.*, 1911, v4, 207.

5. Scott, J. M. D., and Barcroft, J., *Biochem J.*, 1924, v18, 1.

6. Griffith, J. Q., Jr., and Campbell, R., PROC. SOC. EXP. BIOL. AND MED., 1937, v36, 38.

7. Cutting, W. C., and Cutter, R. D., *Am. J. Physiol.*, 1935, v113, 50.

8. Went, S., and Drinker, C. L., *Am. J. Physiol.*, 1929, v88, 468.

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Human Ovarian Grafts onto the Chorio-Allantoic Membrane of Developing Chick Embryos.* (18809)

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Murphy(1) was the first to demonstrate that mammalian tissue (Jensen rat sarcoma) could live and grow actively on the chick embryo. Minoura(3) grafted ovary and testis from chick embryo and adult chickens onto the chorio-allantois of chick embryos. His conclusions indicated that these ovarian and testicular grafts remained functional and caused, in general, modification of the chick reproductive system in the direction of the type of gonadal graft. Oakley(4), studied grafts of liver from chicks, hens, and fetal rats on the chorio-allantois of chick embryos.

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1. Murphy, J. B., *J.A.M.A.*, 1912, v59, 874.

3. Minoura, *J. Exp. Zool.*, 1921, v33, 1.

4. Oakley, C. L., *J. Path. and Bact.*, 1938, v46, 109.

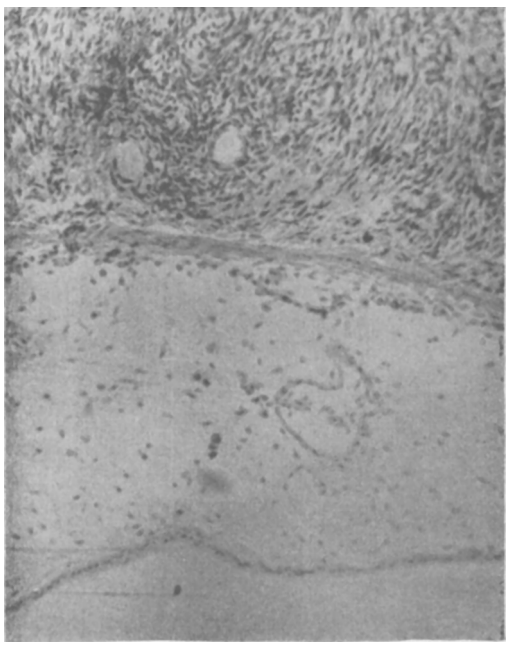

FIG. 1. Ovarian Graft, 4 Days, ×130. Follicular structures and ovarian stroma are identifiable. Contact is intimate. Vascularization absent.

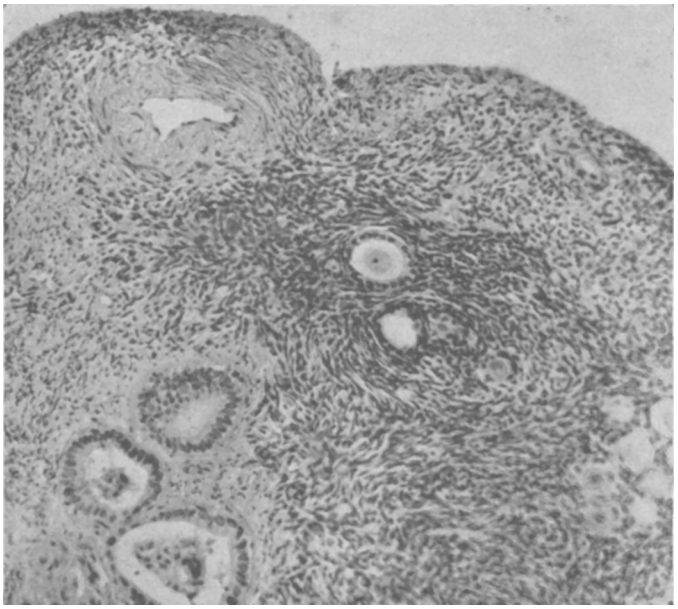

FIG. 2. Ovarian Graft, 5 Days, ×130. Primary follicles and acinar structures surrounded by ovarian stroma.

He concluded that the success of the graft and degree of growth is a function of the "individualization" (tissue differentiation? maturity?) of the transplanted material. He noted that the grafts began to fail on about the 20th day. Quoting Sandstrom, he felt that this phenomenon might be due to the progressive degeneration of the chorio-allantoic membrane as the chick approached hatching age. This is an alternate view of the one proposed by Murphy(2), who postulated that the chick embryo was incapable of a specific response to heterologous tissues until the chick was approaching hatching age.

Goodpasture, Douglas, and Anderson(5) reported the successful grafting of human skin on the chorio-allantois of the chick embryo. They describe 3 stages in the life of their grafts: 1. Stage of "plasmatic circulation". 2. Stage of vascularization. 3. Stage of regeneration and repair.

Hurst, Cook and McLennan(6) reported survival and growth of human and rabbit tissues on the chorio-allantois of the chick embryo. "Takes" were obtained with human carcinomatous tissue from the breast, gastric carcinoma metastasis to lymph glands and to omentum, inflamed salivary gland, normal lymph gland, and rabbit kidney. Goodpasture and Anderson(7) grafted human chorion and amnion onto chick chorio-allantois and succeeded in inoculating the grafts with herpes simplex, variola, and vaccinia virus. Blank, Coriell, and Scott(8) demonstrated that grafts of human skin on the chorio-allantois of developing chick embryos could be passed serially from egg to egg at weekly intervals and remain viable. Harris and Harris(9) grafted rabbit lymphatic tissue onto the chorioallantoic membrane.

Method. Seven to 10-day-old chick embryos were prepared by the "false air sac" method(10). Human ovarian tissue was ob-

2. Murphy, J. B., *J. Exp. Med.*, 1913, v17, 482.

5. Goodpasture, E. W., Douglas, B., Anderson, K., *J. Exp. Med.*, 1938, v68, 891.

6. Hurst, E., Cook, B., McLennan, G. C., *Austr. J. Exp. Biol. and Med. Sc.*, 1939, v7, part 2, 215.

7. Goodpasture, E. W., Anderson, K., *Am. J. Path.*, 1942, v18, 563.

8. Blank, H., Coriell, L. L., Scott, T. F., McNair, PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 341.

9. Harris, S., Harris, T. N., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 142.

10. American Public Health Association, *Diagnostic Procedures for Virus and Rickettsial Diseases*, 1948, 243.

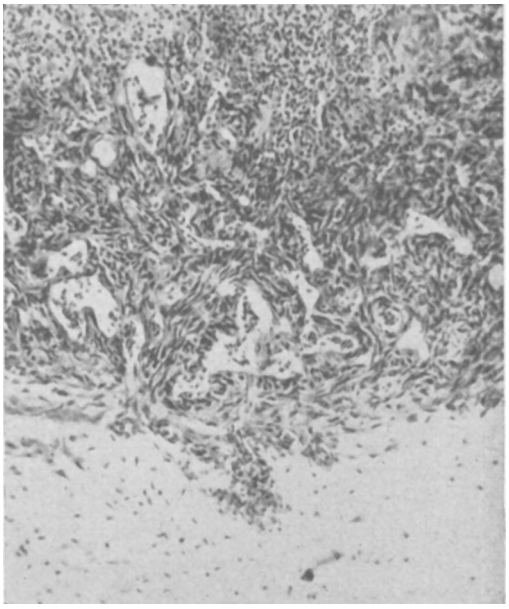

FIG. 3. Ovarian Graft, 7 Days, ×130. Vascularization of graft by capillary sprouts from chick chorio-allantoic membrane.

tained during operation. Pieces of tissue approximately 1 x 2 x 2 mm were placed in physiologic saline containing 250 units of penicillin and 250 units of streptomycin per cubic centimeter, and were introduced onto the chorio-allantoic membrane as soon as possible. The shell defects were closed either with "Scotch tape" or with sterile cover-glasses mounted on sterile vaseline. Daily observations were made with the dissecting microscope. As a rule, 7 to 10 eggs were "inoculated" at a time with the specimen of tissue to be studied. The first egg was usually sacrificed at 3 days and another egg daily thereafter. The tissues were fixed in 10% formalin, blocked in paraffin, and stained with hematoxylin and eosin. A few preparations were stained by Van Giesen and Giemsa technic.

Results. A. Ovarian Grafts. Four days after grafting, the ovarian stroma is well preserved. At the site of contact, fibroblasts are apparent. No chick erythrocytes are demonstrable within the graft. The visible follicular structures are isolated mononuclear elements, probably ova devoid of follicular epithelium, and occasional primary follicles.

After 5 days, the graft is closely apposed to the chorio-allantoic membrane. No chick erythrocytes are demonstrable within the vessels of the graft, but the capillary buds are luxuriant at the site of contact with the graft. At the graft periphery are noted ova and primary follicles in various stages of degeneration. (Fig. 1 and 2.)

Six days after grafting, the graft is en-

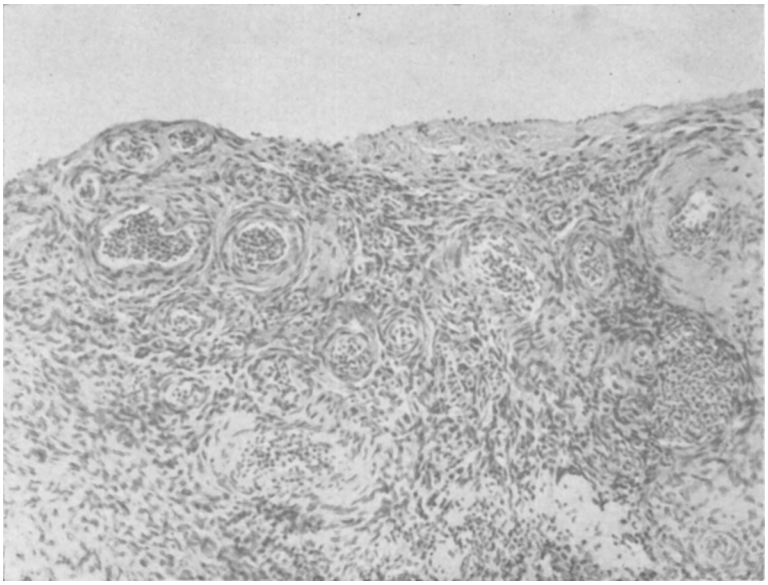

FIG. 4. Ovarian Graft, 7 Days, ×130. Intrinsic muscular walled blood vessels of graft containing chick erythrocytes.

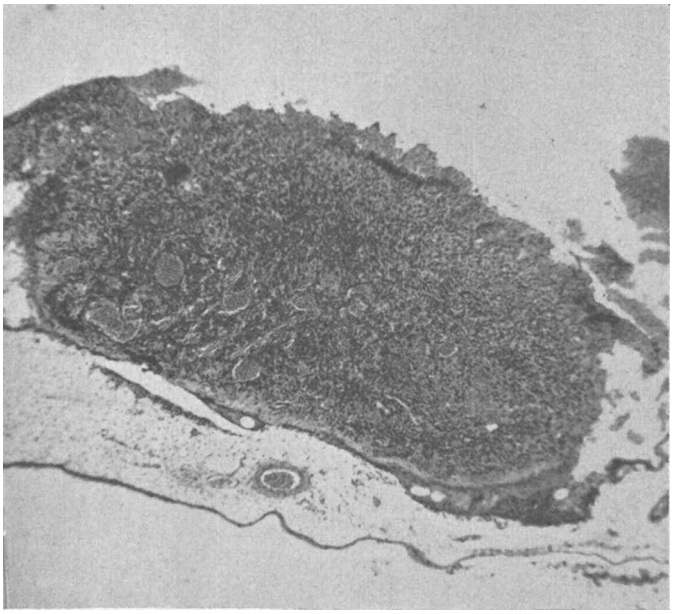

FIG. 5. Ovarian Graft, 13 Days, ×49. Extensive vascularization and early degeneration of graft.

veloped peripherally by a fine capillary network. That portion of the graft most distant from the point of contact with the chorio-allantois appears homogeneous, relatively avascular and acellular, and takes an eosinophilic stain, suggesting early hyalinization. The ovarian stroma is identifiable. Occasionally, an apparent ovum persists. Blood vessels containing chick erythrocytes extend through the graft. Many of these vessels have definite muscular walls, indicating that they are pre-existent vessels of the ovarian tissue (Fig. 3 and 4.)

Eight, 10 and 13 days after grafting, the ovarian stroma remains identifiable and is vascularized. No follicular elements are seen. (Fig. 5.)

B. Endometrial Grafts. Endometrium was obtained by curettage or suction biopsy from several patients of various ages and at different phases of the menstrual cycle. Initial efforts at grafting endometrium were unsuccessful, but finally "takes" were obtained when antibiotics were eliminated from the solution into which the endometrium was placed. The specimen is proliferating endometrium obtained by curettage. After 4 days of growth, there is vascularization of the chorio-allantoic membrane. Nucleated red blood cells are seen in the vessels of the graft, and there is an intimate connection of the graft with the chorio-allantoic membrane by spindle shaped cells, loose connective tissue, and blood vessels. Well preserved cuboidal epithelial elements in glandular formation are present. The graft surface most distal to the point of contact with the chorio-allantois showed fibrin, erythrocytes and nuclear detritus (Fig. 6).

Discussion. The general pattern of development of these grafts parallels the reports from the literature. At 4 days, survival of the graft is demonstrable, even though there is no vascular connection with the chorio-allantois established. The next 4 or 5 days after grafting reveals progressive vascularization of the graft, survival (growth?) of the less "specialized" elements and degeneration of the more "specialized" elements of the ovarian tissue. As the chicks approach hatching age, there is more tissue degeneration in the grafts, and there is progressive peripheral investment of the graft by capillary networks from the chorio-allantois. At this stage, the grafts appear definitely to act as foreign bodies and to provoke a destructive action by the chorio-

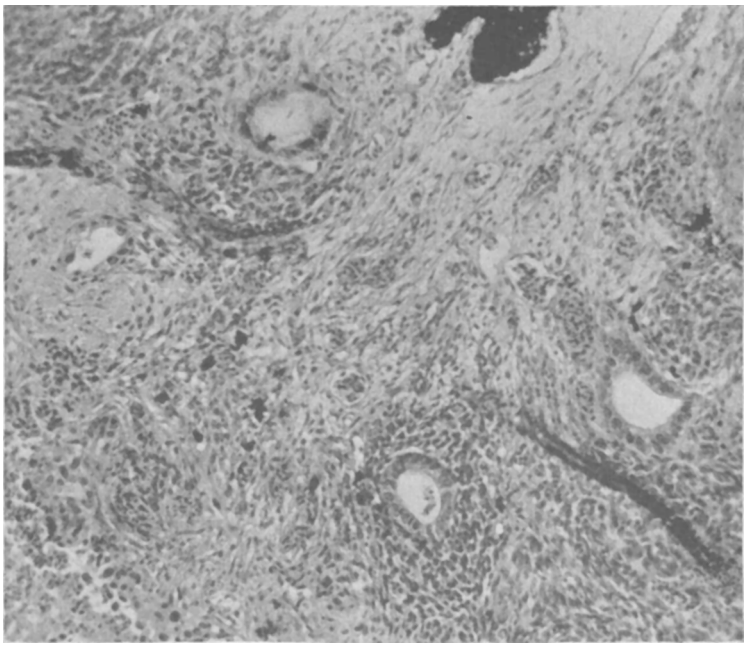

FIG. 6. Endometrial Graft, 4 Days, ×130. Carbon particles, injected into chorioallantoic membrane blood vessels, appear in vessels of graft. Meager glandular elements persist surrounded by endometrial stroma.

allantois of the chick.

Endometrial grafts follow the same general pattern of growth as ovarian grafts. However, the grafts were unsuccessful when they had been bathed in solutions containing antibiotics.

These preliminary data indicate that human ovarian and endometrial tissue will survive and grow when grafted onto the chorio-allantois of the developing chick embryo. To date, serial transfers of these grafts from egg to egg has not been attempted. The data suggests that the optimum time for serial transfer of the grafts would be from day 4 to day 8 after the initial grafting. The explanted tissue is apparently nourished by the "plasmatic circulation" prior to vascularization from the chorio-allantois. Serial transfer of the explants before the establishment of a "take" is worthy of investigation.

Conclusions. 1. Human ovarian and endometrial tissues have been grafted successfully onto the chorio-allantois of the developing chick embryo. 2. The active survival (growth?) span is short and the grafts provoke a foreign body reaction by the chorio-allantois as hatching age of the chick is approached. 3. Possible technics and investigative applications of the described methods are discussed.

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