tensity of the tuberculin reactions and the severity of the tuberculous infections, and that the tuberculin reaction appears after a variable latent period, rises sharply in intensity, levels off, and then diminishes, perhaps disappearing at the time of death of the animal; therefore, we are unable to attribute the absence of the reaction in the one animal (which died 40 hours after the test) to the administration of ACTH.

Summary. (1) ACTH and Cortisone when given in proportionately large doses modified but did not abolish cutaneous tuberculin reaction in guinea pigs infected with virulent tubercle organisms. (2) Following administration of ACTH and Cortisone the histopathologic changes consisted in a quantitative diminution in the inflammatory exudate rather than a qualitative change in its cytologic structure.

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Development of Embryonic Guinea Pig Mullerian Ducts in Anterior Eye Chamber Grafts.* (18219)

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The problem of the relation of sex hormones to sexual differentiation in mammals has received the attention of many investigators who have utilized fundamentally different types of approach in their studies. The injection of sex hormones into the pregnant female(1-5), the treatment of developing pouch young in marsupials(6-9), gonadectomy during development(10-13), and the transplantation of embryonic tissues to postnatal hosts in different sexual conditions(14, 15) have all contributed new information on the subject. However, unanimity of opinion regarding the necessity for sex hormone action for proper sex differentiation does not yet exist. In 1945 I began a study of the relation of sex hormones to uterine gland development in the guinea pig by comparing the conditions found in normal newborn and later stages with those subjected to ovariectomy at birth, and with others treated by estrogen injections(16). Although the uterus of the newborn guinea pig readily responded to treatment with estrogens, it became clear that critical stages occurred earlier in development since uterine glands were already present in the guinea pig at birth.

In order to examine the possibilities for the differentiation of the uterus, and especially of uterine glands, in the absence of all hormones secreted by sex glands, portions of the embryonic reproductive duct system were removed from embryos of timed development

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1. Turner, C. D., *J. Morphol.*, 1939, v65, 353.

2. Greene, R. R., Burrill, M. W., and Ivy, A. C., *Am. J. Anat.*, 1940, v67, 305.

3. Raynaud, A., 1942, *Modification expérimentale, de la différenciation sexuelle des embryons de souris, par action des hormones androgènes et oestrogènes.* Paris: Hermann and Cie.

4. Bruner, J. A., and Witschi, E., *Am. J. Anat.*, 1946, v79, 293.

5. White, M. R., *J. Exp. Zool.*, 1949, v110, 153.

6. Burns, R. K., Jr., *J. Morphol.*, 1939, v65, 497.

7. Burns, R. K., Jr., *Biol. Symp.*, 1942, v9, 125.

8. Moore, C. R., *Physiol. Zool.*, 1941, v14, 1.

9. Moore, C. R., *Am. J. Anat.*, 1945, v76, 1.

10. Moore, C. R., *Anat. Rec.*, 1941, v80, 315.

11. Moore, C. R., *J. Exp. Zool.*, 1943, v94, 415.

12. Jost, A., *Compt. rend. Soc. biol.*, 1947, v141, 126.

13. Jost, A., *Arch. d'Anat. Micr. et de Morph. Exp.*, 1946-47, v36, 271.

14. Buyse, A., *J. Exp. Zool.*, 1935, v70, 1.

15. Moore, C. R., and Price, D., *J. Exp. Zool.*, 1942, v90, 229.

16. Bronski, M., Master's Thesis, U. of C. Library, 1946.

TABLE I. Data of Results from 23 Anterior Chamber Grafts.

Series	Embryo age	Graft duration, days	Age equiv., days	Donor embryo	Host	Observations		
						M.D.	Ut. gl.†	W.D.
2A	50 d.	30	12	♀	♂*	×	?	
					♀	×	++	
					♂	×	++	
2B	50 d.	30	12	♀	♀*	×	+++	
					♀	×	+++	
					♂	×	+++	
3A	50 d.	25	7	♀	♀*	×	++	
					♀‡	×	×	
					♂	×	0	
3B	50 d.	25	7	♀	♀*	×	++	
					♂*	×	0	
					♀‡	×	×	
					♂	×	0	
5A	38 d. 8 hr	29	0	♀	♀*	×	+	
					♂*	×	0	
4A	33 d. 10 hr	35	0	♂	♀*	×	0	
4B	33 d. 10 hr	35	0	♂	♀	×	0	×
6A	31 d. 4 hr	37	0	♀	♀*	×	+	
					♂*	×	0	
					♂	×	+	
6B	31 d. 4 hr	37	0	♂	♀*	×	++	
					♂*	×	0	×
					♂	×	0	

* Castrated or spayed.
† Symbol: + development equal to 0 day normal ♀.
‡ Female became pregnant. Evaluation of gland development impossible because of overgrowth of uterine submucosa.

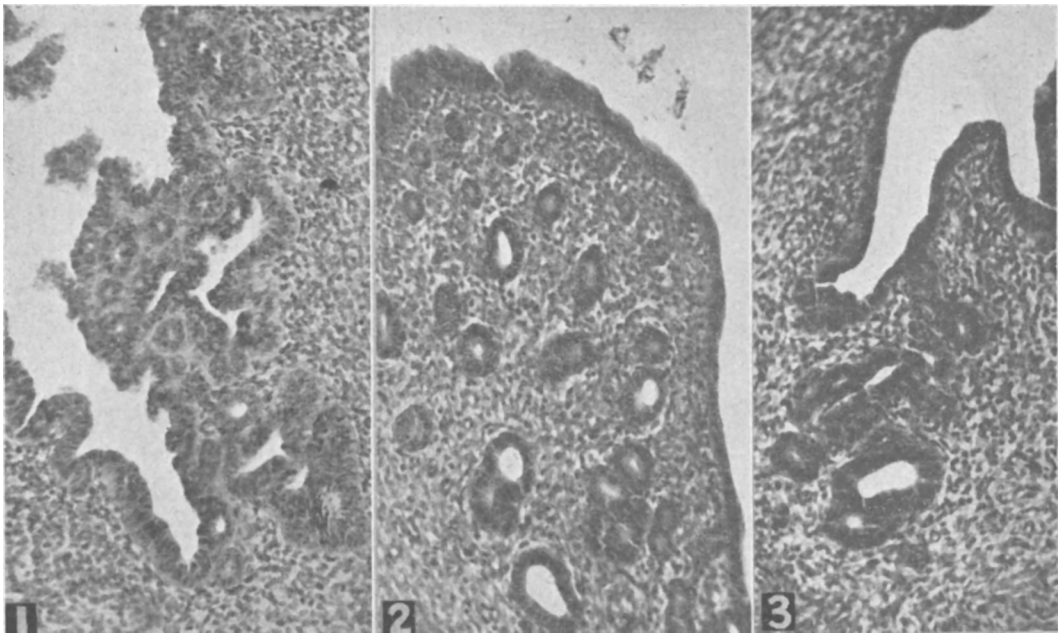
and transplanted into adult hosts in different hormonal conditions. Using a modification of the technic employed by Greene(17), transplants of portions of sex ducts from males or females were placed in the anterior chamber of the eye of previously castrated male and spayed female hosts. For comparison transplants were also made in the anterior chamber of both normal males and females.

Methods and materials. Tissues for transplantation were obtained from embryos removed from the uterus of a pregnant female guinea pig. Embryos were dissected in cold sterile saline within an hour after their removal, and tissues transferred to the anterior chamber of a host guinea pig through a corneal slit external to the iris. Castrated hosts had been operated at least 30 days previously.

Sixty-six grafts have been recovered from 94 transplants. Of these 29 have been studied. Tissues used for transplants have been removed from male or female embryos of 25-days to 50-days postcopulation age. Tissues studied have been obtained from embryos of 31-days to 50-days, and recovered as grafts from the anterior chamber 25 days to 37 days later. According to Bookhout(18), the gonad in the guinea pig is indifferent in structure at 22 days of embryonic life; the testis becomes recognisable about the 25th day. I have been able to distinguish the sexes of dissected embryos by day 38. When tissues for transplantation were removed from embryos of earlier ages, it was necessary to transplant the embryonic gonad to a separate host for determination of the sex of the embryo from which the transplant originated. The age of the recovered graft tissue is in terms of the equivalent expected date of birth on the basis of a 68-day gestation period. Thus, transplants from a 31-day embryo, persisting as a graft for 37 days are rated

17. Greene, H. S. N., and Saxton, J. A., Jr., *J. Exp. Med.*, 1938, v67, 691.

18. Bookhout, C. G., *Z. f. Zellforsch.*, 1937, v25, 728.



Sections through Mullerian duct graft tissue showing glandular development.
FIG. 1. Female tissue (31-day embryo) existing as graft in spayed female host for 37 days.
FIG. 2. Female tissue (50-day embryo) existing as graft in normal male host for 30 days.
FIG. 3. Male tissue (31-day embryo) existing as graft in spayed female host for 37 days.

as the equivalent of normal tissues at birth, or zero days; while transplants from a 50-day embryo, persisting as a graft for 30 days, are rated as equivalent to that of a 12-day postnatal guinea pig.

Results. Data covering 23 grafts of reproductive ducts studied thus far are listed in Table I. The study of normal embryos reveals that the Mullerian duct has not formed in a 24-day guinea pig. Both ducts are present and well developed in embryos of both sexes of ages 31 days and 33 days. By 37 days, in a normal female, the Wolffian duct has degenerated to a mere outline of tissue. In 60-day female embryos the uterus is not completely formed, but gland development is beginning at the extreme anterior portion.

Mullerian duct persistence. Sex duct tissues obtained from female embryos of 31 days to 50 days of age and transplanted to different hosts were removed as grafts from the anterior chamber of the eye at ages equivalent to birth or 12 days postnatal. Eighteen grafts contained recognisable uterine tissue and among these, 12 grafts had definitely formed uterine glands. These grafts were removed from normal males, normal females and spayed female hosts. Fig. 1 shows a section of uterine tissue of a 31-day female embryo recovered as a graft from a spayed female host 37 days later. Fig. 2 shows a section of uterine tissue of a 50-day female embryo that was recovered as a graft from a normal male host 30 days later. Thus far definite uterine glands have not been identified in grafts removed from castrated male hosts. Sex duct tissues obtained from male embryos yielded 5 grafts in which uterine tissue was recognisable and among these, one graft contained uterine glands. This graft had persisted in the eye of a spayed female host for 37 days (age equivalent—birth) after its transplantation from a male embryo 31 days of age. A section of this graft is illustrated in Fig. 3.

Wolffian duct persistence. Developed Wolffian duct tissue was present in the 4 youngest embryos only, ages 31 days and 33 days. The 3 grafts from the female contained no

Wolffian tissue. In the 5 grafts of transplanted embryonic male tissue, only 2 grafts contained recognisable Wolffian duct. One of these grafts was recovered from a normal female host; the other, from a castrate male host. In the latter graft a small piece of testis was present that had been inadvertently carried over with the original transplant.

Discussion. The results show that the Mullerian duct of 31-day to 50-day guinea pig donors transplanted to the anterior chamber will persist and continue to differentiate. Mullerian duct tissues from female donors differentiated into a recognisable uterus in all types of hosts. The differentiation of the uterus seems to be independent of the influence of host hormones since uterine glands, equal in development to that of normal females, were found in grafts from normal males, normal females and spayed females. Of even greater interest is the fact that tissue from a 31-day male donor in a spayed female host differentiated into a uterus with well developed glands. The results indicate that ovarian hormones are not necessary for the development and differentiation of the uterus, and that testicular hormone does not inhibit it.

Summary. (a) Anterior chamber grafts of embryonic guinea pig female reproductive ducts developed into uterus in all types of hosts. Definite uterine glands were found in grafts from all hosts except the castrate male. (b) Similar grafts of embryonic male reproductive ducts developed as Mullerian tissue, and in one case definite glands were present; in only 2 of these grafts was Wolffian tissue present also.

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Enhancement of Spreading Cortical Depression by a Dialyzing Technic (18220)

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Leão first described a response to stimulation of the exposed cerebral cortex of rabbits which he called spreading cortical depression (1). This phenomenon follows electrical or mechanical stimulation and manifests itself as a slowly migrating temporary reduction in the normal electrical activity of the cerebral cortex. Another manifestation of the depression wave is an associated transient reduction (7-15 Mv.) in the normal "D. C" potential of the cerebral cortex(2).

Although the rabbit is peculiarly susceptible to this reaction, protecting the cerebral cortex of cats and monkeys from exposure to room air by means of mineral oil effectively opposes the appearance of this response(3). However, it has been demonstrated in this laboratory that cooling the mineral oil covering the cerebral cortex makes it possible to elicit spreading depression with a high degree of regularity in rabbits, cats, and monkeys (4). In addition, the sequential phases of the migratory depression have been characterized and identified on the basis of the associated reduction in cortical "D. C." potential (4). The same designations have been used in identifying the "D. C." voltage changes (see Fig. 1) obtained in the experiments reported herein. The first important negative "D. C." potential change occurs at the proximal electrode E_1 (solid line) situated on the

1. Leão, A. A. P., *J. Neurophysiol.*, 1944, v7, 359.
2. Leão, A. A. P., *J. Neurophysiol.*, 1947, v10, 409.
3. Marshall, W. H., *E. E. G. and Clin. Neurophysiol.*, 1950, v2, 177.
4. Marshall, W. H., Essig, C. F., and Dubroff, S. V., *Am. J. Physiol.*, 1949, v159, 579.