

No attempts were made to test the antigenicity of ultraviolet-killed BCG or heat killed virulent tubercle bacilli vaccines because of the ineffectiveness of such vaccines previously reported by others(9,10).

Summary. Several lots of ultraviolet-killed virulent human type tubercle bacilli (H37Rv) vaccine were prepared which were equal or

slightly superior in antigenic potency to a standard BCG vaccine on the basis of dba mouse vaccination tests. The irradiated vaccine preserved with 1:10,000 merthiolate was stable and retained its immunogenicity for as long as 12 months after preparation.

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Amount of Luteal Tissue Required for the Maintenance of Pregnancy in the Rat.* (18324)

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Gustav Born first suggested that the corpora lutea maintain the uterus in a condition compatible for pregnancy(1). Fraenkel and Cohn(2), Fraenkel(3,4) and Corner(5) confirmed this hypothesis in the rabbit. Harris(6) and Parkes(7) found that ovariectomy always produced resorption or abortion in mice. Loeb(8) showed that pregnancy in guinea pigs was terminated if the corpora were removed before the end of the second day but was uninterrupted if removed after the end of the second day. That ovariectomy causes abortion in a high percentage of guinea pigs was demonstrated by Herrick(9), but ovarian transplants reduced the possibility

of abortion. Courier(10) suggested that endocrine secretions of the placenta may prevent some abortions which would normally be expected. Hartmann(11) suggested that man, the guinea pig, the horse, and the monkey are exceptions to the dependence of successful gestation on the presence of luteal secretions. Similar work on the rat by Harris and Pfiffner(12), Nelson and Haterius(13), Johnson and Challans(14,15), Pencharz and Long(16), Hain(17) and Zeiner(18) demonstrated that in a majority of the cases pregnancy was terminated within 48 hours after bilateral ovariectomy. Preliminary studies of lutein tissue required to maintain pregnancy in the rat done in our laboratories, indicate that the rat is able to parturite having only

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TABLE I. Effects of Corpora Lutea Ablation on Maintenance of Pregnancy.

Group	No. of animals	Corpora wt, avg range, mg	Adrenal wt, avg range, mg	No. of fetuses avg range	No. of placental sites avg range
Normal	8*†	4.4(4.0-5.3)	63.1(56.8-77.0)	10(5-12)	10+(5-12)
2 c. 1, 8th day	10*‡	5.1(3.5-6.8)	61.3(52.0-78.5)	9(3-15)	10 (5-15)
2 c. 1, 8th day	3†	2.4(2.3-2.5)	52.3(49.0-58.5)	—	9 (7-12)
1 c. 1, 8th day	9‡	2.5(1.0-3.0)	56.8(51.0-69.0)	—	9 (4-11)
1.5 c. 1, 8th day	6‡	3.3(2.0-4.3)	58.6(49.5-67.0)	—	11 (9-13)
1 c. 1, 15th day					
Group A	6‡	4.7(4.5-5.0)	73.3(51.5-112.0)	—	11 (7-14)
Group B	4*‡	5.1(4.5-6.0)	56.9(52.5-61.0)	3(1-5)	8 (5-10)
Group C	5*‡	5.3(5.0-6.0)	65.8(50.0-92.0)	4(1-7)	10 (8-13)
2 c. 1, 8th day	5*†	4.7(4.0-5.0)	37.6(33.0-44.0)	8(5-9)	9 (8-10)
A/P 12					

* Animals in these groups parturated or had living young in uteri at autopsy.

† Autopsied on 20th day of pregnancy.

‡ Autopsied post partum.

two corpora lutea (unpublished data).

The purpose of this paper is to present more extensive data and to show the importance of the corpora lutea of pregnancy in the rat.

Methods and materials. The rats used were 90- to 120-day-old females of the Holtzman strain. Day one of pregnancy was considered the day following insemination. The experiment was divided into 6 sections: (1) normal pregnant rats; (2) rats from which all corpora except 2 were removed on the eighth day of pregnancy; (3) rats from which all corpora except one were removed on the eighth day of pregnancy; (4) rats from which all corpora except one and part of another were removed on the eighth day of pregnancy; (5) rats from which all corpora except one were removed on the 15th day of pregnancy; and (6) rats from which all corpora except 2 were removed on the eighth day of pregnancy and the pituitary on the 12th day. The eighth day of pregnancy was chosen as the day for the removal of the corpora in the first sections of the study because by that time implantation of the fetuses is underway.

The hypophysectomized group having 2 corpora was autopsied on the 20th day of pregnancy and all other groups as soon as possible after parturition or at the time of resorption as indicated by vaginal bleeding. The majority of the data was collected on the 21st and 22nd day of pregnancy. At this time very little involution of the corpora of pregnancy has taken place as shown by Bid-

dulph, Meyer and McShan(19). The pituitaries were removed by means of the parapharyngeal approach and immediate post-operative care was limited to the feeding of dextrose and bread. Ablation of the corpora lutea was performed by baring the ovaries one at a time and removing the corpora with sharp pointed forceps after first rupturing the bursa. Bleeding was kept at a minimum by occluding the ovarian circulation with a hemostat fixed on the mesovarium and swabbing the surface of the ovary from time to time with Thromboplastin.†

Results and discussion. Data collected from 8 intact rats on the 20th day of pregnancy provide normal weight values for corpora lutea and adrenal glands, and for the number of fetuses and placental sites (Table I).

The first experimental data were from rats in which all but 2 corpora lutea were removed on the eighth day of pregnancy. Ten of the 13 rats used in this group parturated and in the remaining 3 the fetuses were resorbed by the 12th day. The reabsorption may have been due to an interference with the blood supply to the corpora lutea at the time of operation as the corpora did not increase in size.

Nine animals were in the group in which all but one corpus were removed on the eighth

19. Biddulph, C., Meyer, R. K. and McShan, W. H., *Endocrinology*, 1946, v38, 358.

† E. R. Squibb and Sons, New York.

day of pregnancy (Table I). Pregnancy failed in all of these. Resorption was indicated by vaginal bleeding which occurred 24 to 36 hours after treatment and was observed to last only a few hours.

In the work just described, one rat parturated which had only one corpus and part of another present at autopsy. Since 2 corpora will maintain pregnancy, although one will not, it was considered possible that the progesterone level required to maintain pregnancy might be produced by one corpus and part of another. A group of 6 rats had all corpora but one and part of another removed on the eighth day of pregnancy, but in none did pregnancy continue to beyond the 13th or 14th day of gestation, at which time the majority of these rats were autopsied. Considering the data presented thus far, it is clear that to ensure a successful pregnancy in the rat at least 2 corpora must be present.

Fraenkel(2-4) working with rabbits, demonstrated that the removal of all corpora lutea on or after the 20th day of pregnancy did not terminate gestation. Assuming that the 15th day would be an equivalent time in the gestation period of the rat, it was thought advisable to secure data from rats in which all corpora but one were removed on the 15th day. Fifteen rats were so treated and on the basis of the results the data have been placed in 3 categories. The first one consists of those in which pregnancy was interrupted soon after the operation; the second, those which gave birth to some of the fetuses but had others present in the uterine horns in a partially resorbed condition; and the third category includes those rats which gave birth to all of their young. The size of the litters for the last 2 categories as given in Table I are small because of cannibalism on the part of some of the rats.

The first group included 6 rats in which the average weight of the adrenals was 73.3 mg per pair as compared to the normal value of 63.1 mg. This increase in weight may reflect the metabolic stress caused by the resorption of the fetuses. The data in the second group were gathered from 4 rats while data from 5 rats made up the third group.

It is interesting to note that although the first group had a greater average number of placental sites than the other 2 groups, the placentae, as possible supplementary sources of progesterone, were not capable of maintaining pregnancy (Table I). Selye, Collip and Thomson(20) have shown that in pregnant rats which are castrated and the embryos removed the placentae can produce enough progesterone to maintain the endometrium in a state which will support the placentae. They, however, cannot secrete enough progesterone to maintain the endometrium in a condition which will support both the placentae and the embryos. Therefore, the secretory capacity of the single corpus may be the determining factor whether or not pregnancy will continue uninterrupted.

It has been shown by Pencharz and Long (21), Selye, Collip and Thomson(22) and Meyer and Shipley(23) that rats which are hypophysectomized on the 12th day of pregnancy will go to the end of the gestation period without difficulty, but they may not parturate. Pregnancy is terminated in those hypophysectomized before the 10th day of gestation. This points to the placenta as a source of luteotrophin for the maintenance of the corpora lutea. That the placenta in the rat is a source of luteotrophin was demonstrated by Astwood and Greep(24) who maintained the corpora of pseudopregnancy in hypophysectomized rats by injecting saline suspensions of fresh rat placental tissue twice daily.

It was not known whether this placental-corpora lutea relationship would be effective in rats where all except 2 corpora were removed on the eighth day and hypophysectomized on the 12th day of pregnancy. To test this hypothesis, data from 5 animals treated

20. Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinology*, 1935, v19, 151.

21. Pencharz, R. E., and Long, S. A., *Am. J. Anat.*, 1933, v53, 117.

22. Selye, H., Collip, J. B., and Thomson, D. L., *Proc. Soc. Exp. Biol. and Med.*, 1933, v30, 589.

23. Meyer, R. K., and Shipley, Elva G., unpublished data, 1948.

24. Astwood, E. B., and Greep, R. O., *Proc. Soc. Exp. Biol. and Med.*, 1938, v38, 713.

in this manner were collected; in all pregnancy continued to the time of autopsy.

The average weights of the corpora of the hypophysectomized animals are much like those of the normal animals which indicate that the corpora are not grossly affected by the removal of the pituitary. Also, the average weights of the corpora in this group were of the same order as the average weights of those in the group having only 2 corpora from the eighth day of pregnancy. The corpora in this group were maintained by the luteotrophin produced by the placentae. However, the adrenals were greatly reduced in size which demonstrates the effect of the complete lack of a trophic hormone on its end organ (Table I).

The experimental design included procedures for studying the correlation between adrenal weight and the maintenance of pregnancy. There is evidence from several sources, among them Beall(25,26), Beall and Reichstein(27), Riddle and Lahr(28), VanHeuverswyn, Collins, Williams and Gardner(29) and Zarrow, Hisaw and Bryans(30), that there are adrenocortical compounds having a progesterone-mimetic effect. On the basis of this evidence it was thought possible that in pregnant rats where the corpora lutea are reduced in number the adrenals might respond by hypertrophy and hypersecretion and thus

maintain pregnancy. This hypothesis is not supported by the data as the adrenals do not show any generalized increase in weight over the normal (Table I). However, this does not discount the possibility of a hypersecretion without an accompanying hypertrophy which could not be detected by the methods employed in this study.

Conclusions. The albino rat of the Holtzman strain was used to determine the minimum number of corpora lutea required to maintain pregnancy. It was found 2 corpora will maintain pregnancy from the eighth day of gestation, but one corpus will not, nor will one corpus and part of another. In animals where all corpora but one were removed on the 15th day of pregnancy, two-thirds either had litters of normal fetuses or some of the fetuses were normal while others were being resorbed. All of the fetuses of the other one-third of the animals were being resorbed at the time of autopsy. Pregnancy was maintained in a group of animals in which all but 2 corpora were removed on the eighth day and hypophysectomized on the 12th day of pregnancy.

There is no evidence that the placentae are adequate supplementary sources of progesterone in those groups where the corpora were reduced below two at the eighth day, since pregnancy was terminated in all cases. However, when the corpora were reduced to one on the 15th day of pregnancy, pregnancy was terminated in only one-third of the group which may indicate that the placenta is a more effective supplementary source of progesterone in the latter third of pregnancy. Reduction in the number of corpora to two per rat does not cause a hypertrophy of the adrenal glands.

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