medium of rat liver but not by other tissue extracts. This effect appeared whether or not circulating precipitating antibody against kidney antigens could be deomonstrated. The specificity of this reaction was further demonstrated in aminonucleoside nephrosis which involves no known immunologic mechanism; splenic monocytes from these animals, as well as from control animals, failed to show any inhibition of migration in the presence of these same antigens. There was no correlation between the degree of inhibition and the severity of disease. The role, if any, played by this specific cellular sensitivity in the pathogenesis of autoimmune nephrosis remains to be elucidated.

1. Grupe, W. E. and Kaplan, M. H., Federation Proc. **26**, 573 (1967).

2. Hess, E. V., Ashworth, C. T., and Ziff, M., J. Exptl. Med. **115**, 421 (1962).

3. Heymann, W., Hunter, J. L. P., Hackel, D. B., and Cuppage, F., Proc. Soc. Exptl. Biol. Med. **111**, 568 (1962).

4. Dixon, F. J., Unanue, E. R., and Watson, J. I., in "Immuno-Pathology. International Symposium on Immunopathology, 4th" Grabar, P., and Miescher, P., eds., Grune and Stratton, New York, 1965.

5. Heymann, W., Hackel, D. B., Horwood, S., Wilson, S. G. F., and Hunter, J. L. P., Proc. Soc. Exptl. Biol. Med. **100**, 660 (1959).

6. Wilson, S. G. F., Hackel, D. B., Horwood, S., Nash, G., and Heymann, W., Pediatrics **22**, 963 (1958).

7. George, M. and Vaughan, J. H., Proc. Soc. Exptl. Biol. Med. **111**, 514 (1962).

8. Janowsky, D. S., Rosenau, W., and Moon, H. D., Proc. Soc. Exptl. Biol. Med. **115**, 77 (1964).

9. Ouchterlony, O., Progr. Allergy **5**, 1 (1958).

10. David, J. R., Al-Askari, S., Lawrence, H. S., and Thomas, L., J. Immunol. **93**, 264 (1964).

11. David, J. R., Lawrence, H. S., and Thomas, L., J. Immunol. **93**, 279 (1964).

12. Holm, G., Clin. Exp. Immunol. **1**, 45 (1966).

13. Hunter, J. L. P., Hackel, D. B., and Heymann, W., J. Immunol. **85**, 319 (1960).

14. Perlmann, P. and Morgan, W. S., in "Biological Structure and Function," Goodwin, T. W. and Lindberg, O., eds., Academic Press, New York, 1961.

15. Alexander, C. S. and Hunt, V. R., J. Lab. Clin. Med. **62**, 103 (1963).

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Ovulation in the Rabbit Related to Dosage of Human Chorionic Gonadotropin and Pregnant Mare's Serum* (32915)

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Since the rabbit is an induced ovulator, it is very useful for the study of chemical stimuli for ovulation. Hill (1) reported that ovulation in the rabbit is due to the release of a neurosecretory substance, the chemical nature of which was not elucidated. Saxton and Greene (2) provided evidence that the female rabbit hypophysis releases follicle-stimulating hormone (FSH), luteinizing hormone (LH), and probably adrenal stimulating hormones as a result of mating. White and Leonard (3) demonstrated a rise in threshold of ovulatory response to anterior pituitary hormone and prolan (extract of pregnancy urine) gonadotropin in a rabbit hypophysectomized while in heat. A wide range of dosages of LH and human chorionic gonadotropin (HCG) used as a single stimulus (i.v.) for the induction of ovulation has produced ovulatory responses similar in type and timing to those obtained with natural mating (4–6). Unlike LH and HCG preparations, pregnant mare's serum (PMS) has been used primarily as an ovulatory priming substance as a consequence of

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its high FSH-activity (7,8). The importance of FSH as an early component of the ovulatory process is emphasized in the review by Hisaw (9). More recent evidence by Desjardins *et al.* (10) clearly shows that in the female rabbit the pituitary stores of LH, adrenocorticotropic hormone, and prolactin are depleted during the 11 hours following copulation, but that FSH levels remain unchanged. Since Lamond (11) has indicated that PMS probably contains both FSH and LH the present study was made to determine the ovulation threshold dosages of HCG and PMS administered singly and the relative ovulation-inducing effects of these hormonal preparations on mature rabbits of different strains. The possibility of delayed responses to those dosages regarded as subthreshold on the basis of their inability to induce ovulation about 10 hours postinjection (considered the normal response time postcoitus (12) is discussed.

Materials and Methods. Three hundred and eighty mature female rabbits including nine different strains and one F_1 hybrid were used. The does, of mean age 12.4 $\pm$ 0.4 (range 4–41) months, were maintained at The Jackson Laboratory colony at Hamilton Station and each doe was caged individually prior to treatment. Animals were grouped by age, strain, and reproductive history and assigned to treatment using a randomized block design. The HCG (Follutein, Squibb) was administered at dosages of 1, 2, 4, 8, and 16 IU and PMS (Equinex, supplied in part through the courtesy of Dr. J. B. Jewell, Ayerst Laboratories, New York, New York) at dosages of 10, 20, 40, 80, and 160 IU. Treatment consisted of a single intravenous injection into the marginal vein of the ear. Killing was done by intravenous injection of 2 ml of Euthanol 24 hours after stimulation. An incision was made and the ovaries, oviducts, and uteri were excised. The ovaries were separated from the oviducts and examined for number, type, and size of follicles at 6$\times$ under a dissecting microscope with an ocular micrometer.

The criteria used for the classification of follicles were the following: I. *Clear Follicles* —small to medium size (1.0–1.6 mm diameter) perfectly clear follicles lacking any trace of red or pink. Appear to glisten "silvery-white" under bright light. Expel only follicular fluid when punctured. II. *Hemorrhagic (Blood) Follicles*—small to large (1.0–2.5 mm) dark red to black follicles having follicular antrum completely infused with blood. No trace of follicular fluid present. When punctured, only blood runs out. III. *Stimulated Follicles* (a) Slightly stimulated—generally medium size (1.6–2.0 mm) appearing clear or pinkish under low power (6$\times$) due to increased vascularity. Observations under higher power reveal light red streaks which appear to be on the surface of the follicle. (b) Moderately stimulated—generally large (>1.8 mm) follicles characterized by a streak or small patch of bright red. Bulk of material inside is follicular fluid, but puncture results in outflow of clear fluid streaked with red (blood). (c) Extremely stimulated—generally large (>1.8 mm). Bulk of follicle bright red, infusion of blood into follicular antrum may be almost 100%. Unbroken appearance—vibrant red. Puncture results in outflow of blood slightly diluted with follicular fluid.

If ovulation had occurred, as evidenced by the presence of ruptured follicles, each oviduct was trimmed of adipose tissue and severed from the uterus slightly below the uterotubal junction. Each oviduct was then flushed with 10 ml of 0.85% NaCl into a 100-mm watch glass, using a 20-gauge blunt-nosed needle and syringe. The ova were counted under 12$\times$ magnification. A watchmaker's forceps was used to locate ova caught in debris. If no ova were recovered, flushing was repeated until another ovum-free washing of 10 ml was obtained.

A second experiment involving 40 strain III females 5 months of age was conducted to see if threshold dosages of the two hormone preparations affected time of ovulation. All procedures were similar to the first experiment, except that here the animals were killed at either 9, 10, 11, 12, or 13 hours after injection. Also the dosages used were 4 or 32 IU for HCG and 20 or 160 IU for PMS.

Results. The results of stimulation in terms of dose response to HCG are given in Table I. In all strains, a graded increase with dosage in the average number of ruptured follicles accompanied by a graded decrease in the average number of stimulated follicles was ob-

TABLE I. Effect of Different Levels of HCG on Ovulation in the Adult Rabbit.

Rabbit strain	Females per dosage	Criterion	HCG (IU)[a] 1	2	4	8	16
III	5	Ruptures	0	0.2	4.8	9.6	9.6
		Stimulated follicles	9.8	7.6	7.0	2.6	5.8
		Hemorrhagic follicles	9.0	1.2	8.0	3.0	2.0
III_c	5	Ruptures	0	0	4.0	11.0	9.2
		Stimulated follicles	7.6	10.2	5.2	1.8	2.2
		Hemorrhagic follicles	0.6	4.4	3.8	2.0	2.8
AX	5	Ruptures	0	2.0	3.8	8.8	7.2
		Stimulated follicles	8.0	8.4	9.4	1.4	2.6
		Hemorrhagic follicles	0.2	0	0	4.6	0.2
ACCR(Y)	5	Ruptures	0	0	1.8	9.0	10.6
		Stimulated follicles	11.0	9.6	10.0	4.2	1.2
		Hemorrhagic follicles	1.4	1.0	4.2	3.6	3.6
ACCR(B)	5	Ruptures	0	0.6	6.0	6.2	8.2
		Stimulated follicles	5.2	5.0	1.6	1.8	1.0
		Hemorrhagic follicles	0	0.2	0.2	0.4	1.6
AC	5	Ruptures	0	0.4	4.0	6.0	8.4
		Stimulated follicles	11.4	10.2	7.4	3.6	1.2
		Hemorrhagic follicles	0	0	0.2	0	0.4
OS	5	Ruptures	0	0	4.8	7.8	7.6
		Stimulated follicles	8.4	7.8	4.8	1.4	4.2
		Hemorrhagic follicles	1.0	6.0	2.4	1.6	0.8
WH	5	Ruptures	0	0	2.4	4.8	8.2
		Stimulated follicles	8.8	10.0	7.0	3.8	3.4
		Hemorrhagic follicles	0.8	0.4	3.2	5.6	5.2
X	5	Ruptures	0	0	0.8	4.6	8.2
		Stimulated follicles	9.6	9.4	7.8	5.2	0.8
		Hemorrhagic follicles	0	0	0	0.2	0.2
F_1 (III and X)	2	Ruptures	0	0	5.0	7.0	7.0
		Stimulated follicles	12.0	17.0	5.0	6.5	4.0
		Hemorrhagic follicles	0	0	0.5	1.5	0.5

[a] The arithmetic mean based on the number of females per dosage level.

served. The threshold for initiation of ovulatory response was found to be between 2 and 4 IU HCG.

The results of stimulation with PMS, given in Table II, also indicate a graded positive dose–response in terms of the average number of ruptured follicles, accompanied by a decline in the average number of stimulated follicles observed at each dosage. There was more variability with PMS than with HCG due in part to smaller numbers of animals used in some strains. The average numbers of ruptured and stimulated follicles resulting from HCG and PMS stimulation of all strains tested are given in Fig. 1 for comparison of the relative ovulation-inducing effects of the two preparations. Figure 1 also shows that HCG has about five times the ovulation-inducing ability of PMS based on the international unit (IU) hormone standard. Direct comparisons within any one specific strain made using Tables I and II reflect the same ratio but with greater variability as would be expected with smaller numbers.

Figure 2, where the means are based just on the number of females ovulating at each

TABLE II. Effect of Different Levels of PMS on Ovulation in the Adult Rabbit.

Rabbit strain	Females per dosage	Criterion	PMS (IU)[a] 10	20	40	80	160
III	5	Ruptures	0	5.8	9.0	9.6	11.0
		Stimulated follicles	11.6	9.2	9.0	6.8	5.4
		Hemorrhagic follicles	0.6	0	2.4	1.2	1.4
III_c	5	Ruptures	0	3.2	7.4	9.2	13.0
		Stimulated follicles	11.0	10.6	6.4	4.6	5.2
		Hemorrhagic follicles	3.8	0	0	0.4	1.2
AX	2	Ruptures	0	0.5	10.0	6.0	9.5
		Stimulated follicles	13.0	14.0	0.5	2.5	2.5
		Hemorrhagic follicles	0	1.5	0	0.5	8.0
ACCR(Y)	5	Ruptures	0	6.8	8.8	10.4	11.2
		Stimulated follicles	6.2	2.8	6.2	2.4	3.6
		Hemorrhagic follicles	0	0.8	2.0	4.0	1.2
ACCR(B)	5	Ruptures	1.2	5.0	5.4	6.0	4.6
		Stimulated follicles	8.2	7.6	4.8	3.2	8.0
		Hemorrhagic follicles	0	0	0	0	0
AC	2	Ruptures	0	3.5	7.5	8.0	7.5
		Stimulated follicles	12.5	7.0	2.5	2.5	4.5
		Hemorrhagic follicles	0	0.5	0	0	0
OS	2	Ruptures	7.0	1.0	5.5	6.0	8.5
		Stimulated follicles	3.0	7.5	5.5	3.0	2.0
		Hemorrhagic follicles	11.0	0	4.5	2.5	0
X	2	Ruptures	1.0	4.5	7.5	7.0	11.0
		Stimulated follicles	8.0	5.0	2.0	2.5	3.0
		Hemorrhagic follicles	0	0	0	0	0
F_1 (III and X)	1	Ruptures	0	0	11.0	9.0	9.0
		Stimulated follicles	8.0	10.0	5.0	2.0	6.0
		Hemorrhagic follicles	0	0	0	0	5.0

[a] The arithmetic mean based on the number of females per dosage level.

dosage, illustrates that the positively graded dose–response is a function of the mean number of ruptured follicles observed after HCG and PMS stimulation and not merely a trend resulting from a differential in the number of animals ovulating at each dosage.

The incidence of hemorrhagic follicles appears randomly distributed by strain, treatment, and dosage (Tables I and II and Fig. 1). With few exceptions ova recovery paralleled the ruptured follicles very closely.

Table III gives some indication of the time required for the occurrence of ovulation following stimulation with high and low dosages of HCG and PMS. A threshold dose of HCG requires a slightly longer time to induce ovulation than does a high dose. With PMS the picture is not clear.

Discussion. If one assumes that an "all-or-none" initiation of ovulatory response is present (5,6) and that ovulation has been completed within 9–14 hours after injection as the studies of Harper (5), Walton and Hammond (12) indicate, then the increase in number of ruptured follicles with increasing dosage of HCG and PMS (Fig. 2) observed 24 hours after injection can be explained only on the basis of a graded response to dosages above the threshold level of stimulation. This is in accord with the data of Parkes (4) who used unprimed Dutch rabbits, given 5, 10, 20, and 50 IU of chorionic gonadotropin, and observed

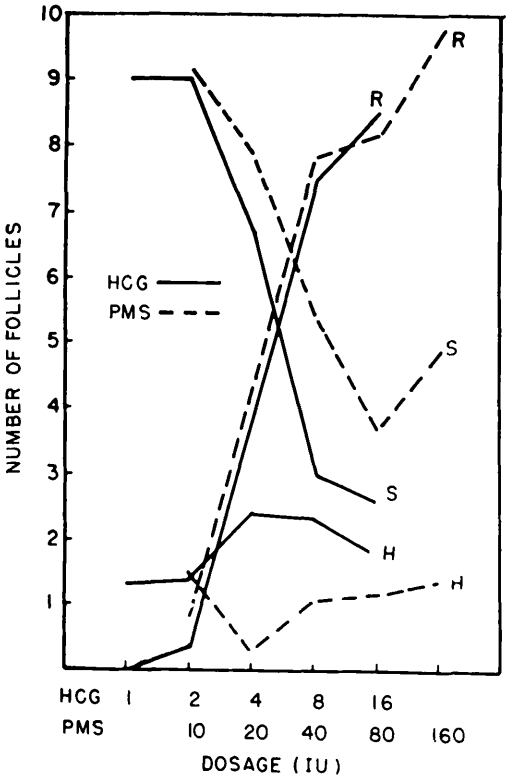

FIG. 1. The average numbers of ruptured (R), stimulated (S) and hemorrhagic (H) follicles at various dosages of HCG and PMS. The pooled *n* is 47 for each HCG dosage and 29 for each PMS dosage.

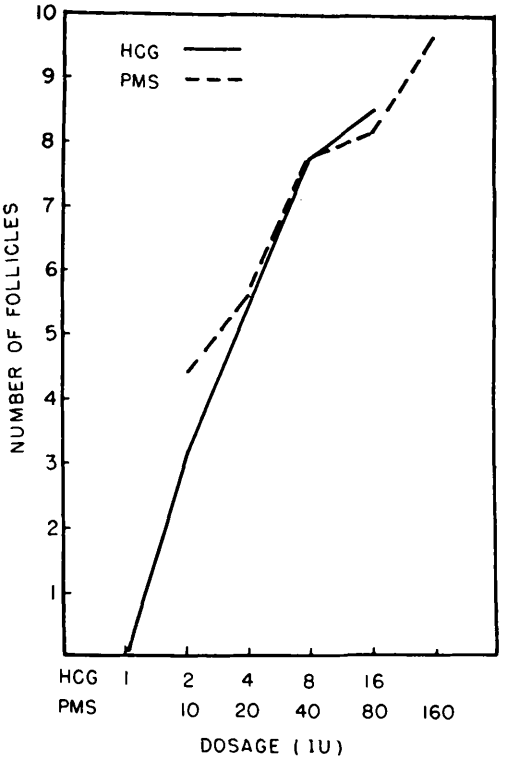

FIG. 2. The mean numbers of ruptured follicles at each dosage of HCG and PMS. These means are based on only those females which ovulated (i.e., no zero values are included). The pooled *n*'s for increasing dosage of HCG are 0, 5, 32, 45, and 47 and of PMS are 5, 22, 29, 29, and 29.

TABLE III. Effect of a Threshold Versus an Adequate Dosage of HCG or PMS on the Timing of Ovulation in the Adult Strain III Rabbit.

Treatment	Dosage (IU)	Criterion	Time of killing (hours after injection) 9	10	11	12	13
HCG	4	Mean no. of ruptures		0	0	4.67	10
		No. of females injected		3	3	3	1
		No. of females that ovulated		0	0	3	1
	32	Mean no. of ruptures	0	5.0	7.33	9.0	11.0
		No. of females injected	3	3	3	2	1
		No. of females that ovulated	0	3	3	2	1
PMS	20	Mean no. of ruptures		0.67	2.67	2.0	
		No. of females injected		3	3	3	
		No. of females that ovulated		1	1	2	
	160	Mean no. of ruptures		0	3.33	6.67	
		No. of females injected		3	3	3	
		No. of females that ovulated		0	2	3	

in the four females given 5 IU only about one-half the normal number of ruptures. The use of a randomized block design would, theoretically, eliminate the possibility that the differences in number of ruptured follicles are merely due to variation in number of potential rupture sites from one individual to another. A comparison of the mean ovulatory responses to HCG and PMS (Fig. 1) indicates that HCG is about five times as potent as PMS in its ovulation-inducing ability. If one assumes that LH is the primary factor in the induction of ovulation (10) and that HCG contains only LH, while PMS contains FSH plus LH, the relative ovulation-inducing effects of PMS and HCG mentioned above would indicate that the relative amounts of LH in PMS and HCG are 1:5. This ratio is consistent with the 5:1 ratio of FSH:LH in PMS which has been assayed for FSH activity alone (11).

Threshold dosages of HCG appear to induce ovulation but at a slightly longer time interval. With PMS the picture is not clear due to the fact that ovulation did occur with 20 IU of PMS at both 10 and 11 hours in one of three does. The observation that 160 IU of PMS stimulated the same degree of ovulation 11–12 hours after injection (Table III), as compared to 10–11 hours after injection observed with an equivalent dosage of HCG (i.e., 32 IU) is consistent with Zarrow and Quinn's suggestion that PMS may induce ovulation by stimulating endogenous release of LH from the pituitary (13). More precise information regarding the duration of the lag in response as well as results of timing experiments on hormonally stimulated hypophysectomized animals are needed before any conclusions may be reached.

The PMS induced a larger number of stimulated follicles than HCG at each dosage (Fig. 1) when the preparations were administered at dosages equivalent to each other in terms of ovulation-inducing effect (i.e., 5:1). If FSH is considered a priming factor influencing the maturation of the follicle in preparation for ovulation induction by LH, then the increased response to PMS at each dosage level could be attributed to the presence of a relatively large quantity of FSH accompanied by an insufficient quantity of LH to induce the rupture of these follicles. Wilson and Zarrow (14) have shown that in immature mice, high dosages of PMS result in overstimulation of follicles which will not rupture. Parkes (4) also showed in rabbits that a high priming dose resulted in gross overstimulation of the ovary, a large number of cystic and hemorrhagic follicles, and low ovulation.

Summary. A positively graded ovulatory response to human chorionic gonadotropin and pregnant mare's serum exists. Ovulation can be induced using much smaller dosages of HCG than have been commonly used heretofore. The relative ovulation-inducing abilities of HCG:PMS are approximately 5:1.

1. Hill, R. T., J. Physiol. (London) 83, 129 (1934).
2. Saxton, J. A., Jr. and Greene, H. S. N., Endocrinology 30, 395 (1942).
3. White, W. and Leonard, S. L., Am. J. Physiol. 104, 44 (1933).
4. Parkes, A. S., J. Endocrinol. 3, 268 (1943).
5. Harper, M. J. K., J. Endocrinol. 22, 147 (1961).
6. Foote, R. H., Hafs, H. D., Staples, R. E., Gregoire, A. T., and Bratton, R. W., J. Reprod. Fertility 5, 59 (1963).
7. Fox, R. R., Cavanaugh, J. L., Jr., and Zarrow, M. X., Endocrinology 75, 411 (1964).
8. Kennelly, J. J. and Foote, R. H., J. Reprod. Fertility 9, 177 (1965).
9. Hisaw, F. L., Physiol. Rev. 27, 95 (1947).
10. Desjardins, C., Kirton, K. T., and Hafs, H. D., Proc. Soc. Exptl. Biol. Med. 126, 23 (1967).
11. Lamond, D. R., J. Endocrinol. 20, 277 (1960).
12. Walton, A. and Hammond, J., J. Exptl. Biol. 6, 190 (1928).
13. Zarrow, M. X. and Quinn, D. L., J. Endocrinol. 26, 181 (1963).
14. Wilson, E. D. and Zarrow, M. X., J. Reprod. Fertility 3, 148 (1962).

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