TABLE VI. Effect of pH on 83% Ethanol Precipitation of Glycogen-Gelatin Complexes in the Presence and Absence of Cupric Chloride.*

	$CuCl_2$ ($\times 10^{-3}$M)	1% NaOH A	1% NaOH B	pH 7.0 C	pH 7.0 D
Glycogen only	.0	107	103	99.0	98.2
Gelatin "	.0	2.3	.0	2.9	.0
Glycogen & gelatin	.0	99.2	6.1	8.8	1.2
	.6	96.2	35.2	2.3	.2
	1.2	93.1	53.8	3.6	.2

* Expressed as % precipitated (free) glycogen from reaction mixtures containing 0.5 mg glycogen and/or gelatin. A: precipitated from alkaline solution; B: reprecipitation of "A" from 0.83% TCA—83% ethanol; C: precipitated at neutrality; D: reprecipitation of "C" from 0.83% TCA —83% ethanol.

ities suggested that the polysaccharide binding sites of gelatins may be analogous to the intramolecular binding by the gelatin polypeptide chains presumably responsible for the gelation of gelatin (Harrington and Von Hippel(2).

According to Bello and Vinograd(1), formation of the biuret complex of gelatin with as little as 0.03 M $CuCl_2$ in 5% gelatin at pH 10-12 caused complete suppression of the gelation of gelatin. Partial inhibition of the binding capacity of gelatin was evident in alkaline solutions only. This similarity between inhibition of gelation and inhibition of glycogen-binding capacity of gelatins in alkaline solutions only further suggests that the intramolecular sites responsible for gelation and polysaccharide binding of gelatins may be similar.

Summary. Gelatin is capable of binding glycogen and dextran in a ratio of 1:1 on a dry weight basis and various other polysaccharides with α-1, 4 and/or α-1, 6 glucose linkages to a diminished degree. Gammaglobulins also display this property, although to a lesser extent. The glycogen-gelatin complex is stable in 0.5 N HCl, 0.5 N NaOH and 6 M urea for one hour, and to boiling water for 10 minutes. The glycogen-gelatin complex is salted out of TCA-ethanol solutions at a concentration of 3% NaCl. Dilute alkaline $CuCl_2$ solutions and autoclaving of gelatin solutions result in inhibition of glycogen-gelatin complex formation. Autoclaved gelatins exhibit decreased viscosities, with concomitant destruction of the gelatin phenomenon.

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PMS-Induced Ovulation in the Immature Rat Following Unilateral Castration. (30171)

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Unilateral ovariectomy in the mouse or rat results in a compensatory hypertrophy of the remaining ovary(1,2,3,4) and does not appear to affect litter size(1,2,3,5). Evidence indicates that the remaining ovary compensates for loss of follicles resulting from removal of the contralateral gonad by forming a greater number of mature follicles(3,5) and corpora lutea(6). This is confirmed by the observation that a 2-fold increase in ova released from the remaining ovary occurs following unilateral castration(7,8,9,10). It has also been shown that compensatory hypertrophy of the remaining ovary of hemicastrated rats can be blocked by administration of certain steroids. Indeed the unilaterally ovariectomized rat has been suggested as the animal of choice for determination of gonadotropin-inhibiting potency of such hormones (6).

The current investigation was undertaken to examine the effect of unilateral castration on PMS-induced ovulation in the immature rat and to determine whether a constant

amount of PMS could effect a doubling of ova released in the unilaterally castrated, immature rat.

Materials and methods. Twenty-eight-day-old rats of the Purdue-Wistar strain were used in this study since rats of this age show a maximum ovulatory response to PMS(11). All rats were weaned at 21 days of age and injected with PMS on the 28th day of age. The hormone was dissolved in physiological saline and each animal received a single subcutaneous injection of 15 or 30 I.U. in a volume of 0.1 ml. Unilateral castration was performed *via* the dorsolateral approach under ether anesthesia. The ovaries were removed either just prior to injection of PMS (this was designated the zero hour group) or at 48 hours after injection of PMS (designated the 48-hour group). The side from which the ovary was removed was alternated with each successive rat within each group. A similar procedure was followed for the manipulated control group in which the rats were anesthetized, an incision made and the ovary manipulated without being removed. A second group of controls received only the PMS treatment.

All animals were killed 74 to 78 hours after the PMS injection, at which time the ovaries were removed and weighed to the nearest 0.1 mg. The Fallopian tubes were also removed, the swollen segment (if present) isolated under the dissecting microscope, and incised to permit the release and counting of the ova(12,13).

During the study the rats were caged in groups of 3 or 4, supplied with food and water *ad lib* and maintained at an ambient temperature of 70-72°F and a light cycle of 13 hours light (7 A.M. to 8 P.M.) and 11 hours dark.

Results and discussion. The mean ovarian response of the various groups of rats receiving 30 I.U PMS is presented in Table I. The number of ova shed by the control group averaged 63.2 per rat which is comparable to the 61.1 ova shed per rat in the 48-hour manipulated controls. The average number of ova shed by the unilaterally ovariectomized rats treated with 30 I.U. PMS was approximately half that observed in the control and manipulated groups. Since the difference between the 27.6 ova shed by the group castrated at zero time and the 36.5 ova shed by those castrated at 48 hours was not statistically significant, time of removal of one ovary relative to PMS treatment apparently was without effect. The ova count of both the zero group ($p<.001$) and 48-hour castrated groups ($p<.005$) was significantly decreased when compared with the controls or the 48-hour manipulated group.

The response of the variously treated groups of rats to 15 I.U. PMS is different from that seen in rats receiving 30 I.U. PMS. In the control group, 15 I.U. PMS caused a mean response of 17.0 ova per rat (Table I). Irrespective of time of ovariectomy the mean number of ova shed by the unilaterally ovariectomized rats was not significantly different from that of the control rats. Manipulation at zero hour had no influence on ovulation response. Manipulation at 48 hours caused an apparent increase in the mean number of ova shed. This increased response, however, was not statistically sig-

TABLE I. Effect of Unilateral Castration on PMS-Induced Ovulation in 28-Day-Old Rats.

Dose of PMS	Treatment	No. of animals	Avg body wt at autopsy (g)	Avg ova count per rat ± S.E.	Avg wt of single ovary ± S.E.
30 I.U.	Control	19	76.4	63.2 ± 5.2	46.8 ± 1.7
	C 0 hr	21	76.8	27.6 ± 3.6[a, b]	43.2 ± 2.9
	C 48 "	22	76.5	36.5 ± 3.5[a, b]	53.4 ± 2.0[c]
	M 48 "	10	73.8	61.1 ± 9.1	42.3 ± 1.8
15 I.U.	Control	15	73.6	17.0 ± 4.8	24.5 ± 1.1
	C 0 hr	20	73.1	14.6 ± 3.2	26.8 ± 1.3
	C 48 "	20	95.5	17.9 ± 2.6	27.8 ± 2.1
	M 0 "	10	98.9	18.2 ± 2.4	25.7 ± .9
	M 48 "	15	88.5	28.6 ± 5.4	26.8 ± 1.4

[a] Significantly different from controls $p < .001$.
[b] Significantly different from manipulated group $p < .005$.
[c] Significantly different from controls $p < .05$.
C = Castrated (unilateral).
M = Manipulated.

nificant when compared with the response of the control or 48-hour castrated group.

Among the groups receiving 30 I.U. PMS, the mean ovarian weight of the 48-hour castrated group was significantly greater than that of the others ($p<.05$). No explanation is available for the increased ovarian weight seen in this group. No significant differences were noted in ovarian weights of the various groups receiving 15 I.U. PMS.

The present results indicate that when the ovaries of 28-day-old rats are not responding maximally to injected PMS, *i.e.*, a dose of 15 I.U., unilateral ovariectomy causes a 2-fold increase in the number of ova shed by the remaining ovary. Zarrow and Quinn(11) obtained a maximum response of 70.8 ova in the 28-day-old rat treated with 30 I.U. PMS. A comparable response was obtained in the present experiment. After unilateral ovariectomy the number of ova released was approximately halved indicating that at 30 I.U. PMS, both ovaries of the intact rats may have been responding maximally to PMS and hence additional ova were not available for release after removal of one of the ovaries. However, when the dose of PMS was reduced to 15 I.U., the number of ova released in the unilaterally castrated rats was comparable to the number shed by both ovaries of the intact, control rats. It is apparent from these data that under proper conditions, a single ovary can compensate for the lack of the contralateral ovary with regard to ova production and that this occurs in the presence of a constant amount of injected gonadotropin. This would imply that the ovary is binding or removing gonadotropin from the circulation so that following unilateral castration, the remaining ovary is exposed to an increased amount of hormone as compared with the intact animal where both ovaries are present.

The lack of statistical differences in the mean ovarian weights of the rats treated with 15 I.U. PMS suggests that the compensatory response in the phenomenon of augmented ovulation by the surviving ovary of the unilaterally ovariectomized rats is probably due to an increase in number of mature follicles and does not necessarily involve the entire ovary.

The failure to obtain increased ova release (compensatory ovulation) after unilateral ovariectomy in rats injected with 30 I.U. PMS could be due to the fact that each ovary is already at maximum stimulation for ova release. Such a failure of increased ova release was reported in the mouse following unilateral castration(10) and could also be due to the dose of PMS. Once the maximum ovulatory response is obtained, further increase in dosage of PMS could lead either to no change or to an inhibition of ovulation (12,14). Excessive doses of PMS have been shown to result in development of cystic anovulatory follicles in the rat. In addition the sensitivity of the animal to PMS changes with age(15) so that proper dosage would have to be established with each age group.

This experiment indicates that the compensatory ovulatory response is independent of whether the ovary is removed at the time of PMS injection or 48 hours later. Similarly in the hamster compensatory ovulation occurs regardless of whether unilateral ovariectomy is performed on the 1st or 2nd day of the estrous cycle(7,8,9).

The present results confirm previous findings that ether anesthesia will not block ovulation unless given at the time of endogenous LH release which occurs approximately 56 hours after PMS injection(16,17). As such these results are not in conflict with the findings that ether anesthesia blocks ovulation in the rat(18). In these experiments ether anesthesia was induced no closer than 8 hours prior to the time of LH release and was of less than 15 minutes duration.

Conclusion. Treatment of immature rats with 15 I.U. PMS causes the remaining ovary of unilateral castrates to release as many ova as both ovaries of intact rats. Failure to obtain compensatory ovulation in the unilateral castrates with 30 I.U. PMS is probably due to the fact that ovulation per ovary was already at a maximum.

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Effect of Thyrotrophin on *in vivo* Thyroid Uptake of Labelled Tyrosine, Arginine and Iodine in the Chick.* (30172)

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Injection of thyrotropic hormone into normal guinea pigs has been shown to increase the *in vitro* incorporation of labelled amino acids into thyroid protein. An *in vivo* study was reported at the same time from this laboratory showing an increased thyroid uptake of C^{14} tyrosine in TSH-treated chicks (3).

Thyrotropic hormone has been shown to increase *in vitro* incorporation of labelled leucine, valine, tyrosine, arginine, aspartic acid, phenylalanine, and proline into protein of thyroid slices(1). It was also demonstrated that the incorporation of labelled leucine into protein is depressed in surviving thyroid slices from hypophysectomized rats and enhanced in thyroid tissue from TSH-treated rats. A part of the carbon skeleton of the thyronines has been recently shown to come from tyrosine H^3(4).

To gain additional information regarding the effect of TSH on *in vivo* incorporation of amino acids into thyroid protein, the present time-response study was done relating the *in vivo* effect of thyrotrophin on tyrosine and arginine uptakes by the thyroid gland.

Methods and materials. Newly hatched cockerel chicks were used because of their high sensitivity to thyrotrophin(5). A Remington iodine deficient diet (Nutritional Biochemicals Corp.) was fed *ad lib* prior to study and daily requirement for iodine was satisfied by addition of 0.01 μg/ml of NaI to the drinking water.

I. *Thyroid uptake of NaI^{125} in response to TSH.* Thirty chicks 2 days old received subcutaneous injections of TSH (thytropar-Armour) 0.8 I.U. once a day for 2 days. This dose level of TSH was used since preliminary experiments indicated that thyroid concentration of I^{125} was not significantly elevated by doses in excess of 0.8 I.U. In addition, it was determined that the maximum TSH effect on I^{125} uptake occurred between 24 and 48 hours after a single TSH injection and that uptakes were not significantly altered by more than 2 doses on consecutive days.

Twenty-four hours after the last TSH injection these animals and an equal number of untreated control chicks were injected I.P. with 0.25 μc of I^{125} as sodium iodide.

Five animals from each of the 2 groups were sacrificed using ether at 1, 2, 4, 8, 24 and 48 hours after the isotope injection.

The thyroid glands were rapidly excised and weighed on a microbalance. Thyroid radioactivity of I^{125} injected animals was determined by placing the glands in plastic test tubes and counting in a crystal scintillation detector.

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