incubation to either disappear (chick) or become a minor component (pheasant) by the 21st day of incubation. The pheasant embryo serum contains a stage-specific serum protein, alpha-3 globulin-E, which in addition is species-specific, *i.e.*, no similar staining protein is found in the chick embryo serum.

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Possible Role of Protein Binding in Failure of Antibody to Porcine Relaxin to React with Pregnant Rabbit Serum Relaxin. (31107)

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Noall and Frieden(1) reported that extended treatment of guinea pigs with porcine relaxin results in a decreased responsiveness to the hormone. Experiments designed to explain this phenomenon by immunological mechanisms were unsuccessful. Subsequent reexamination of the problem(2) led Frieden to postulate the probable formation of a species specific antibody to porcine relaxin since guinea pigs made resistant to porcine relaxin were capable of reacting to pregnant rabbit serum relaxin.

Cohen(3) described the preparation and biological properties of an antiserum to porcine relaxin and presented evidence indicating the absence of species specificity. Steinetz *et al*(4) extended and confirmed these observations but also noted the inability of antisera formed to porcine relaxin to neutralize the relaxin activity of pregnant rabbit serum.

Presented below are the results of our efforts to explain this anomolous behavior of circulating rabbit relaxin toward exogenous antibody to porcine relaxin.

Our experiments were based on the speculation that circulating rabbit relaxin is complexed with a serum component which does not inactivate the biological activity of the hormone but offers steric hindrance to the combination of the hormone with neutralizing antibody. If the foregoing conjecture is correct, the treatment of pregnant rabbit serum with agents which tend to dissociate such protein-protein combinations should free the serum relaxin from its conjugate and make it available for combination with antibody.

Materials and methods. 8 M urea and glacial acetic acid were used as dissociating agents.

132 ml of serum collected from female rabbits during the fourth week of proven pregnancy were lyophilized, yielding 7.5 g of dried powder.

a. 5 g of the above powder were suspended in 100 ml of glacial acetic acid, brought to 70°C and held at that temperature for 15 minutes. The solution was rapidly cooled, filtered and the hormone precipitated by addition of 4 volumes of acetone. The resulting precipitate was collected by filtration and dried *in vacuo* to yield 25 mg of powder

b. 2.5 g of the lyophilized pregnant rabbit serum was dissolved in 100 ml of 8 M urea at pH 7.8 and kept at room temperature for 5 hours. The solution was then precipitated with 10 volumes of acetone, the precipitate washed with acetone and dried *in vacuo*.

Antibody to porcine relaxin was prepared by the procedure described previously (Cohen. 3).

TABLE I. Neutralization of Biological Activity of Urea- and Glacial Acetic Acid-Treated Pregnant Rabbit Serum (PRS) by Antibody to Porcine Relaxin.

	Anti-serum	N	Ligament, mm + S.E.	% inhibition by antibody	P value
Controls	—	10	.68 ± .13	—	—
Porcine relaxin 1 u s.c.	—	20	2.62 ± .17	73	>.01
" "	+	10	1.21 ± .17		
*PRS (0.2 ml s.c.)	—	15	1.66 ± .15	none	n.s.
" "	+	15	1.42 ± .15		
Urea denat PRS	—	15	2.32 ± .15	34	>.02
" " "	+	15	1.77 ± .14		
Glacial acetic acid-treated PRS	—	15	1.60 ± .14	65	>.01
" " "	+	15	1.00 ± .13		

% inhibition calculated after first subtracting control ligament measurements from treated ligament values.

* PRS = Pregnant rabbit serum.

— = Assay animals not treated with antiserum.

+ = " " " given 0.2 ml antiserum intraperitoneally ½ hr prior to subcutaneous injection of either pregnant rabbit serum or porcine relaxin.

The bioassay procedures used to measure the relaxin activity of the treated pregnant rabbit serum and the antihormonal activity of the antisera have been described by Cohen (3) and Steinetz *et al*(4). Briefly, 20 g female mice were estrogen primed with 5 μg estradiol cyclopentylpropionate/0.2 ml oil s.c. One week later, groups of 10-20 primed mice each were injected with either porcine relaxin (R, 1 unit s.c.), pregnant rabbit serum relaxin (PRS, 0.2 ml s.c.) or antiserum to porcine relaxin (AS, 0.2 ml i.p.) ½ hour prior to injection of either R or PRS s.c. Twenty-four hours later, the mice were killed and interpubic ligaments measured directly.

The difference between the increase (over control value) in ligament length of the group receiving only R (or PRS) and the increase obtained with R (or PRS) in mice pretreated with antiserum was taken as the measure of inhibition.

Results and discussion. Table I demonstrates the capacity of antibody made to porcine relaxin to inhibit the biological activity of the antigen, whereas hormonal activity of untreated pregnant rabbit serum (PRS) is not affected. This is in accord with the data of Steinetz *et al*(4) and confirms the observations made by Frieden(2) in the guinea pig. However, when PRS is treated with either glacial acetic acid or 8 M urea, a significant amount of inhibition by antibody occurs. These experiments have been repeated several times with similar results.

Three of the immunized female rabbits were mated and at day 25 were bled. The sera were then assayed for relaxin activity and relaxin neutralizing antibody. In each

TABLE II. Effect of Previous Immunization to Porcine Relaxin on Serum Relaxin Content and Antibody Titer of Pregnant Rabbits (25 Days Post-Coitum).

Rabbit serum, 0.2 ml i.p.	Interpubic ligament, mm* No exogenous relaxin	Interpubic ligament, mm* 1 u relaxin s.c.	% Inhibition of pubic ligament
None	.80 ± .12	2.69 ± .11	—
Immunized rabbit #2 serum			
Before mating	.90 ± .13	1.12 ± .24	89
Pregnant	.87 ± .13	2.10 ± .23	40
Immunized rabbit #3 serum			
Before mating	.76 ± .18	.74 ± .12	100
Pregnant	.73 ± .17	2.13 ± .16	33
Immunized rabbit #4 serum			
Before mating	.89 ± .16	1.46 ± .14	73
Pregnant	.82 ± .14	2.41 ± .13	24
Pooled pregnant rabbit serum from unsensitized animals	1.66 ± .15 1.95 ± .18		

* 10-20 mice per group.

case the capacity of the serum to neutralize exogenous relaxin was markedly greater in the nonpregnant than in the pregnant state (Table II). Also, the relaxin content of the serum from the immunized pregnant female was low or not detectable in contrast to the high relaxin titers of non-immune pregnant animals.

These data lend credence to the hypothesis that circulating relaxin in pregnant rabbit serum is complexed with a serum component which hinders combination of the hormone with exogenous antibody, inasmuch as treatment designed to disrupt protein-protein combination tends to render the hormone susceptible to the action of antibody. An alternative possibility is that glacial acetic acid or urea treatment causes a change in the structure of the hormone such as an unfolding of or actual reduction in size of the molecule. In the pregnant rabbit immunized to porcine relaxin prior to mating, the circulating antibody appears to react with the native hormone, since both a decrease in relaxin activity and in antibody titer as measured by ability of the serum to inactivate exogenous relaxin are observed. This observation indicates successful competition for the hormone by the circulating antibody and is evidence that favors our first hypothesis.

Summary. Antibody to porcine relaxin fails to inactivate the biological activity of circulating pregnant rabbit serum relaxin. However, treatment of the serum with protein dissociating agents such as glacial acetic acid or 8 M urea makes the circulating hormone amenable to neutralization by the exogenous antibody. A possible explanation for this phenomenon is discussed.

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Food Intake and Activity of Rats with Rostral Hypothalamic Lesions.* (31108)

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When they are transferred to a warm environment, rats eat less food. Thus, a rise in air temperature from 24° to 32°C reduces food intake by 75% in the first 24-hour period. Rats with bilateral lesions of the anterior hypothalamic area (AHA) when similarly heat stressed do not reduce their food intake so much, and may not reduce it at all if the lesions are of proper size and position(1). We have now extended our study of animals with these lesions by confirming their responses to acute exposure to heat or cold, and then performing experiments to investigate the following: (a) their spontaneous activity levels during chronic exposure to heat or cold, and (b) their food intake during 24-hour exposure to 9 different environmental temperatures ranging from 6° to 32°C.

Methods. The *Ss* were 11 male Sprague-Dawley rats, 60 days old at the time of operation, and maintained on powdered Purina chow and water *ad libitum*. In 5 rats selected randomly, bilateral electrolytic lesions were placed stereotaxcially in the Horsley-Clark plane at 8½ mm anterior to the ear bars, ¾ mm from the base of the skull, and ¾ mm lateral to the midline. Later histology showed the lesions to be placed dorsal to the optic chiasma and ventral to the anterior commissure with some asymmetry. After operation, the animals were kept in individual cages at

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