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Atrophy of Rabbit Testes Associated with Production of Antiserum to Bovine Luteinizing Hormone.* (32174)

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The use of hormone-specific antibodies would provide a method of selective depletion of pituitary hormones superior to hypophysectomy, since the effects of single pituitary tropins could be eliminated without disturbing the independent activities of other pituitary factors. Experimental evidence(1-6) suggests specificity for antiserum to luteinizing hormone(LH), suggesting the use of this method to study the activity of endogenous LH.

Wakabayashi and Tamaoki(7) reported the effects of active immunization with ovine LH on the male reproductive organs and adrenal and pituitary glands of rabbits and rats. Similar results with ovine LH in rabbits were described by Quadri *et al*(8).

This report describes testicular and epididymal atrophy in rabbits actively immunized with bovine LH and cross reaction of the antiserum with rabbit anterior pituitary extract.

Materials and methods. Immunization. Four male albino rabbits which weighed 1,800 to 2,500 g were immunized with NIH-LH-B3 (BLH). Each rabbit received, on days 0, 10, and 20, an intramuscular injection of 2.0 mg

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BLH in 0.5 ml 0.85% physiologic saline solution (PSS) emulsified with 0.5 ml Freund's complete adjuvant (Difco). A series of 3 booster injections, each containing 2.0 mg BLH in 1 ml PSS, was begun on day 30 and again on day 62. The first inoculation of each series was subcutaneous, the second intraperitoneal, and the third intravenous. Additional booster injections of 5 mg BLH in 1 ml PSS were administered intraperitoneally on days 74, 94, and 104.

Collection of antisera. Blood was taken on day 0, prior to the first BLH injection, and on days 43, 53, and 85, with terminal bleeding on day 112. All bleedings were performed by cardiac puncture, and the antisera were stored at —20°C.

Detection of antibodies. Antisera were tested for precipitating antibodies using the microimmunodiffusion test described by Crowle(9). The agar concentration was 1.5% in phosphate buffered saline (PBS) at pH 7.4. Microimmunodiffusion was performed in a high humidity chamber at 5°C. Amido black and thiazine red R stains were used to differentiate precipitin bands.

Rabbit anterior pituitary extract (RP) was prepared from pituitary glands collected at an abattoir and stored for 36 hours in dry ice. The glands were thawed, the infundibular process was removed, and the remainder of the gland was ground in an ice cold Ten Broeck homogenizer. PBS was added, the homogenate was centrifuged at 6,000 R.P.M. for 30 minutes at 4°C, and the supernatant was used as RP.

Tissue specimens. After terminal bleeding, testes of the 4 immunized rabbits and those of an untreated rabbit (from the same stock, of similar weight (1850 g) and cared for like the immunized rabbits) were removed and fixed in Bouin's solution. Testes were trimmed and weighed after 6 days fixation. Sections were stained with hematoxylin and eosin.

Results. By day 112, the testes of immunized rabbits had ascended in the inguinal canal and could not be palpated. The scrotum and testes of the untreated rabbit were quite apparent. Paired testes weights of the 4 immunized and the untreated rabbit were 205, 247, 249, 262, and 2,700 mg, respectively.

Seminiferous tubules in the untreated rabbit gave the appearance of active spermatogenesis (Fig. 1A). Seminiferous tubules in all immunized rabbits were atrophic, with complete absence of spermatozoa, prominent Sertoli cells, a striking thickening of basement membranes, and there was an apparent proliferation of Leydig cells with intensely stained nuclei (Fig. 1C).

Epididymal tubules of the untreated rabbit have dense masses of spermatozoa in the lumens and were lined by tall columnar cells with abundant cytoplasm (Fig. 1B). Epididymal tubules of immunized rabbits were atrophic, separated by abundant intertubular stroma, contained no spermotozoa, and were lined by low cells with pyknotic nuclei and almost no cytoplasm (Fig. 1D).

Sera from all immunized rabbits contained antibodies which formed 4 precipitin bands with BLH (Fig. 2A). Two of the bands were produced by contaminating serum antigens: one was bovine gamma globulin; the other, not identified, was not bovine serum albumin (BSA).

Rabbit anti-bovine LH serum (RABLHS) cross reacted with crude rabbit anterior pituitary extract (RP), and RP showed reaction of partial identity when tested with BLH against RABLHS (Fig. 2B). The reaction of partial identity was not due to a normal bovine serum (NBS) contaminant of BLH, since reaction of non-identity was obtained when RP was tested with NBS against RABLHS (Fig. 2C).

Discussion. Our initial purpose was to characterize the antigens of NIH-LH-B3. Active immunization was accompanied by atrophy of the testes and epididymides. The basement membranes of seminiferous tubules of frogs respond to gonadotropins(10). Thickening of basement membranes of seminiferous tubules was marked in BLH-immunized rabbits and may reflect excessive LH stimulation. Such pronounced thickening would certainly interfere with nutrition and metabolism of the seminiferous epithelium. The apparent increase in Leydig cells might also indicate excessive gonadotropin stimulation. Atrophy of the epididymal tubules

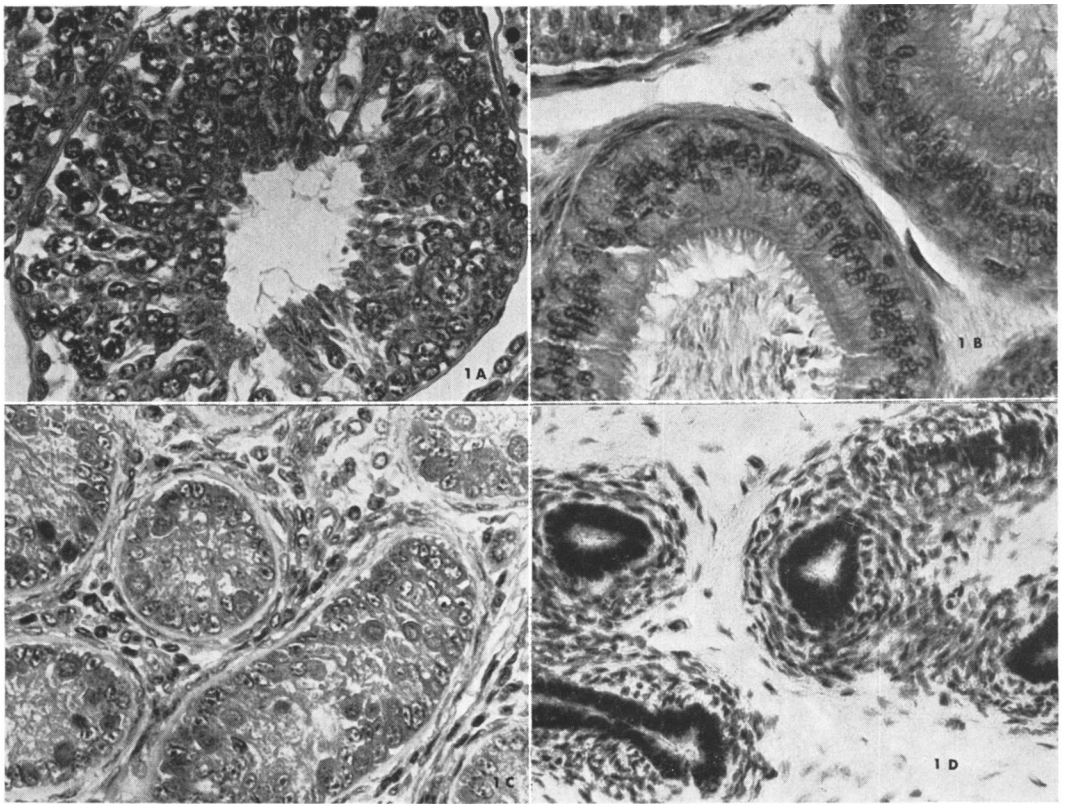

FIG. 1. Testes (A, C) and epididymides (B, D) of untreated (A, B) and immunized (C, D) rabbits. × 225. Hematoxylin and eosin.

could even be explained on the basis of exhaustion of the Leydig cells.

However, Wakabayashi and Tamaoki(7) and Quadri *et al*(8) established that the antisera of rats and rabbits actively immunized against LH were able to inhibit endogenous or exogenous gonadotropins. We conclude, therefore, that the atrophy of testes and epi-

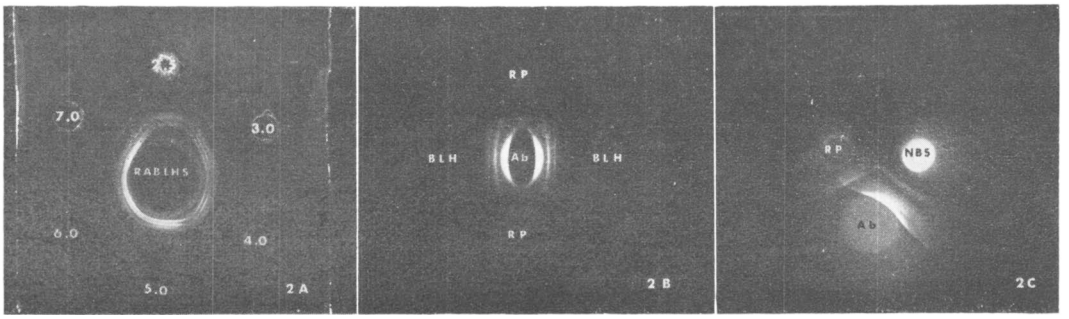

FIG. 2. Microimmunodiffusion. Direct projection enlargement, ×2. Thiazine Red R stained.
A. Antigenic components of bovine LH (NIH-LH-B3). LH in mg/ml PSS in peripheral wells. Rabbit anti-bovine LH serum (RABLHS) in central well. 72 hr of diffusion.
B. Cross reaction, showing partial identity, between rabbit pituitary extract (RP) containing 100 mg anterior pituitary tissue (wet wt.)/ml PBS and bovine LH (BLH = 3 mg/ml PSS), tested against rabbit anti-bovine LH serum, pooled terminal bleeding (AB). Diffused for 48 hr.
C. Two contaminating serum components of BLH are shown by precipitin bands formed between normal bovine serum (NBS) and rabbit anti-bovine LH serum (AB). The precipitin band formed between rabbit pituitary extact (RP) and AB shows reaction of non-identity with the contaminating serum components. Diffused for 48 hr.

didymides in this investigation was due to circulating antibodies to BLH which inhibited endogenous gonadotropin.

Convincing confirmation of this postulate is found in our cross reactivity studies. We found 4 antigenic components of NIH-LH-B3. One of these antigens was a gamma globulin contaminant, while BSA was not a contaminating antigen. A pituitary factor in RP extracts showed reaction of partial identity with one of the antigens of BLH, and the cross reaction was not due to an NBS contaminant of BLH. We cannot be certain that the cross reacting factor is LH, and it will not be possible to know this until contaminant-free gonadotropins are available.

To our knowledge, this is the first demonstration of an autoimmune phenomenon in which antibodies produced in response to heterologous gonadotropin were shown to cross react with homologous pituitary extract.

Summary. Injections of purified bovine LH (NIH-LH-B3) in rabbits produced circulating antibodies which were associated with atrophy of the testes and epididymides. Bovine LH and crude rabbit anterior pituitary extract contained a similar antigen which was not a component of normal bovine serum. Results were attributed to an autoimmune phenomenon with circulating antibodies common to NIH-LH-B3 and endogenous gonadotropin.

1. Bourdel, G., Gen. Comp. Endocrinol., 1961, v1, 375.
2. Bourdel, G., Li, C. H., Acta Endocrinol., 1963, v42, 473.
3. Hayashida, T., J. Endocrinol., 1963, v26, 75.
4. Henry, S. S., Van Dyke, H. B., ibid., 1958, v16, 310.
5. Kelly, W. A., Robertson, H. A., Stanfield, D. A., ibid., 1963, v27, 127.
6. Mougdal, N. R., Li, D. H., Arch. Biochem. Biophys., 1961, v95, 93.
7. Wakabayashi, K., Tamaoki, B-I., Endocrinol., 1966, v79, 477.
8. Quadri, S. K., Harbers, L. H., Spies, H. G., Proc. Soc. Exp. Biol. & Med., 1966, v123, 809.
9. Crowle, A. J., J. Clin. Med., 1958, v52, 784.
10. Mukherji, M., Experientia, 1966, v22, 466.

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Infectious Virus Particles.* (32175)

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Most animal viruses produce plaques representing not over 10% of the particles in the inoculum(1,2,3). Ten percent of well adapted vaccinia virus particles produce visible plaques by standard procedures on monolayers of L cells, but independent evidence indicates the ability of all the particles of these preparations to infect L cells(4). It seems likely, therefore, that some virus particles do infect monolayer cells in areas where no visible plaques appear. This note provides evidence that they do, as well as a measure of the extent to which virus grows in these cells.

Materials and methods. The vaccinia virus of WR (mouse neurotropic) strain as well as the L cells, culture media, plaque titration procedure and counting of virus particles by electron microscopy have been previously described(5). Virus of L cell passage No. 22 was chosen because its average plaque yield was 1 per 125 virus particles (VP), much better than earlier passage virus but still about 1/10th of that attainable on further passage.

Experiments and results. Experiment A. A sonic lysate of infected L cells was diluted with phosphate-buffered saline (PBS) to contain 200 VP per 0.1 ml. Several 6 cm plates containing monolayers of L cells were in-

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