

sodium, when administered intraperitoneally and intramuscularly, occurred at similar time intervals, intramuscular plasma concentration was approximately twice intraperitoneal concentration, and renal accumulation following intramuscular administration was 4 times that of the latter. This may indicate that renal tissue possesses a tremendous avidity or binding capacity for mercurial compounds.

Summary. 1. Using Hg^{203} labeled mercaptomerin sodium and studying radioautographs and renal distribution, 91.7% of an intramuscularly injected dose of 1 mg Hg/kg was found in the kidney within the first hour, and maximum concentration occurred in the cortical area. 2. Radioactivity was detected for 28 days in plasma and in renal tissue. 3. Intravenous administration of radioactive mercaptomerin sodium resulted in a rapid decline in plasma concentration during the first 5 min-

utes. Rate of urinary excretion appears to be a function of arterial plasma-renal vein plasma difference.

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In vitro* Response of Mouse Testis to Human Chorionic Gonadotropin. (25400)

ARDIS J. LOSTROH[†] (Introduced by M. E. Kahl)

Lab. D'Embryologie Exp'tl. du Collège de France, Paris

A number of investigators have cultured the mammalian testis successfully, either as entire organ or as fragments thereof (1-5). Irrespective of the medium employed or duration of culture, it was concluded that testicular explants had continued to develop in an essentially normal manner. These results are in contrast to those which I have obtained with ovarian explants from young post-natal mice in which an atrophy of the follicular and thecal cells occurs leading to a general loss of organization of the tissue; maintenance of ovarian structure *in vitro* was dependent upon incorporation of proper hormones into the basic medium (6). The following studies with isolated testis were therefore undertaken, using

the same medium as that employed for the post-natal ovary, to determine response of the testis under these conditions. Human chorionic gonadotropin (HCG) and lactogenic hormone were the hormones selected for study.

Procedure. Testes from mice of the C57 Black strain, 3 days of age, were cultured on a medium made of 2 parts agar,[‡] 1 part Tyrode's solution enriched with equal quantity of synthetic medium 199 (7), and 1 part chick embryo extract. Details of the method have been discussed (8), and preparation of hormone-enriched medium has been described (6). Explants were cultured at $31 \pm 1^\circ\text{C}$; every other day they were displaced to new sites on the media. In earlier experiments, the whole testis was cultivated 7 days on a medium enriched with lactogenic hormone or with HCG. In later experiments, each testis

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[†] Fellow of National Foundation '57-58. Present address: Dept. of Physiology, Univ. of Chicago, Ill.

[‡] Agar preparation containing 1 g agar (Difco)/100 cc Gey's solution.

was cut into 4 pieces and arranged around a fragment of ventral prostate; the explants were transferred to new media after 4 days and the experiments were terminated after 14 days. Tissues were fixed in Bouin's solution, embedded in paraffin and sectioned at $6\ \mu$. The lactogenic hormone (30-35 I.U./mg) was prepared by Dr. C. H. Li(9); the HCG (1000 I.U./mg) was a product of Leo Pharmaceutical Co. Hormones were dissolved in Tyrode-medium 199 solution and incorporated into the basic medium in concentration indicated; 0.10 cc of the hormone-enriched medium was then added to each embryological watch glass in which a mixture of 6 drops agar, 3 drops Tyrode's solution and 3 drops extract had already congealed.

Results. The testis of the mouse, 3 days

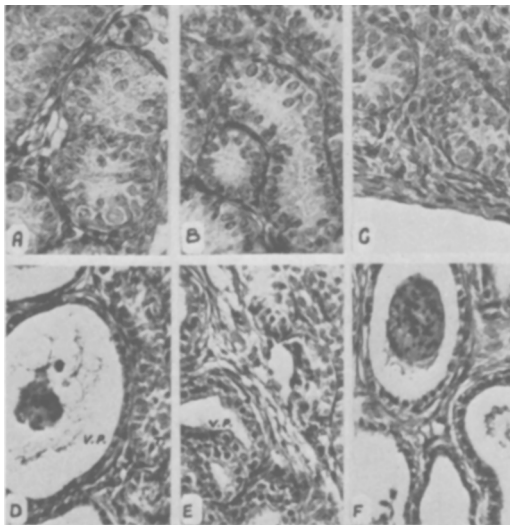


FIG. 1. A-C. Sections of gonads taken from male mice 3rd day after birth and cultured on hormone-enriched media for 7 days. $192\times$. D-F. Sections of ventral prostate taken from mice 27 days of age and cultured on hormone-enriched media for 14 days. $176\times$. Stain, hematoxylin-eosin; thickness, $6\ \mu$.

A. Normal onset control. B. Testis cultured on medium enriched with lactogenic hormone, 1.25 mg/cc. C. Testis cultured on medium enriched with HCG, 250 I.U./cc. Cytoplasm of interstitial body distinguishable and eosinophilic. D. Explant cultured in association with testis on medium enriched with lactogenic hormone, 1.25 mg/cc. No evidence of androgen production by testicular fragment. T = testis, VP = ventral prostate. E. Explant cultured in association with testis on medium enriched with HCG, 1250 I.U./cc. Note stimulation of prostatic epithelium. F. Explant cultured on medium enriched with HCG, 1250 I.U./cc.

after birth, was too large to be cultured as a whole organ without occurrence of necrosis at its center; tissue at the periphery survived. On the other hand, fragments of testis were maintained without necrosis but there was a tendency toward tubular disorganization; a similar phenomenon was observed by Champy (1) with testicular explants from rabbits. At this stage of development the spermatogonia were distributed at the periphery of the tubules among future sertoli cells, and interstitial cells with a well developed cytoplasmic body were abundant (Fig. 1A). Representatives of each cell type persisted under *in vitro* conditions. Nonetheless, in 7 days of culture tubular size decreased, tubules came to lie in close proximity to one another, and interstitial cell cytoplasm was reduced (Fig. 1B). In 4 experiments it was then demonstrated that the inclusion of HCG in the basic medium ameliorated consistently the degenerative changes; however, it did not maintain the testicular morphology evident prior to culture (Fig. 1C). Especially relevant is the fact that, although the interstitial cells could be distinguished by their eosinophilic cytoplasm, the cytoplasmic body of these cells was not maintained.

Finally, to establish whether or not an enhancement in production of androgen could be effected by HCG under *in vitro* conditions, a target organ specific for the male hormone was cultured with the testis; fragments of one gonad were associated with a fragment of ventral prostate taken from a male mouse, 27 days of age. Figs. 1D and 1E demonstrate that testicular explants continued to secrete androgen for a period of 14 days when cultured on a medium enriched with HCG but not when cultured on a medium enriched with lactogenic hormone. In the absence of the testis, HCG was ineffective (Fig. 1F).

Discussion. Degenerative changes observed in the testis after 7 days of culture are modest. These results are in contrast to those previously obtained with ovarian explants taken from mice of the same age(6) and suggest that the testis is not as intimately dependent upon pituitary hormones as is the ovary during this period of development. The fact that embryonic testes can continue to secrete

androgen in an isolated system lends support to this interpretation(10).

Previous investigations have shown that anterior pituitary explants have a morphological influence on the isolated testis(5,11). The present study demonstrates that HCG has a similar effect on testicular explants and can stimulate production of androgenic hormone under *in vitro* conditions. Thus, as in the case of the ovary, the results obtained with the isolated testis are similar to those previously observed in experiments with the whole animal(12).

Summary. Testes from mice, 3 days of age, were cultured in an *in vitro* system for 7 or 14 days on a medium composed of agar, Tyrode's solution, synthetic medium 199 and chick embryo extract. Degenerative changes in control explants included a decrease in size of tubules and a reduction in cytoplasm of interstitial cells. Inclusion of HCG in the medium ameliorated these changes. Moreover, measurable amounts of androgen were produced by testes cultured on media enriched

with HCG whereas no detectable amount was produced by those cultured on media enriched with lactogenic hormone.

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Studies on the Small Particle Complement-Fixing Antigen of Foot-and-Mouth Disease Virus. (25401)

JEROME POLATNICK (Introduced by M. S. Shahan)

Plum Island Animal Disease Lab., Agric. Research Service, U. S. Dept. of Agriculture, Greenport, N. Y.

It has been well established that foot-and-mouth disease is associated with at least 2 specific particles(1,2). The larger particle, reported to be 23 $m\mu$ in diameter and having a sedimentation coefficient (s-rate) of 140, possesses all the viral properties of the infection together with about half of the complement-fixing activity; the smaller particle, 8-10 $m\mu$ in diameter and having an s-rate of 14 displays complement-fixing (CF) activity only (3,4). Preliminary chemical investigation of the smaller particle, hereafter to be called the CF-antigen, indicated a globulin nature for the material(5). This report presents the results of biochemical studies on the CF-antigen and purification procedures.

Materials and methods. Virus infectivity

was measured by a plaque assay using primary bovine-kidney cell layers in 4-oz prescription bottles(6). *Complement-fixing activity* was measured by modification of the Traub and Möhlmann procedure(7). As used in this laboratory,* the test is a direct semi-quantitative one, using guinea pig complement with overnight fixation at 4°C. The endpoint was read visually from 0 to +4 on 2-fold serially diluted samples. Readings from 0 to +2 were counted as belonging to the next lower dilution; readings of +3 or +4 were counted as belonging to the dilution used. Thus, a sample having a reading of +4 at a dilution of 1:256 and a reading of +1 at a

* Savan, M., Edward, A. G., Martinsen, J., Pop-pensiek, G. C., unpublished.