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Gonadal Effects of Vasectomy and Vasoligation

Abstract. *During a 28-week study, vasectomy and vasoligation of immature male Wistar rats revealed that there was a significant decrease in urinary 17-ketosteroid in the vasectomized group at week 15; at week 28 there were significant decreases in the weights of the testes of the test groups, as compared to those receiving sham operations, with maximum alterations in the vasectomized rats. Small, soft discolored testes with cysts in the cauda epididymis and vas deferens regions occurred frequently in the test groups. The output of 17-ketosteroid in the urine and the findings in the testes indicate significant alterations in the morphology and function of the testes and suggest the need for caution and extensive investigations in man before recommending vasectomy as a simple, innocuous, "physiologic" means to ensure conception control.*

Valid social ends do not justify invalid, unscientific means. It has been reported that in the United States "last year 750,000 of the operations [vasectomies] were performed, and 1 million are expected in 1972" (*1*). Reports similar to this one led us to investigate what scientific data existed to justify vasectomy as demonstrably safe without sacrifice of normal physiologic patterns. The desirability of zero population growth cannot excuse the routine resort to irreversible, controversial procedures, be they drug or surgical.

Since Steinach's report in 1920 (*2*) that vasectomy caused a temporary decline in spermatogenesis of rabbits, analyses of the effects of vasectomy and vasoligation surgical procedures have been contradictory (*3–5*).

In man, other than as a contraceptive procedure, vasectomy has few proponents even though it has been proposed for alleviating senile, prostatic hypertrophy (*6*) and has been used in conjunction with prostatectomy to prevent nonspecific epididymitis and for disputed "regenerative" effects in males (*7*). Psychic disturbances have been recorded after vasectomy (*8, 9*), and unexplained thrombophlebitis and systemic disorders have been reported (*9*). Autoantibodies against spermatozoa have been found in the blood of a man with obstructed vas deferens (*10*) and in the blood of unilaterally vasectomized or vasoligated rats (*11*).

In our study, we sought to define some of the effects of vasectomy and vasoligation on testicular morphology and function. Immature albino male Wistar rats averaging 82 g were matched by weight and divided into three groups (vasectomy, group A; vasoligation, group B; and sham-operated controls, group C). The operations were performed under ether anesthesia, and the suprapubic incisions were performed under clean but not necessarily aseptic procedures (*12*). In the vasectomized rats, a minimum of 1 cm of the duct was removed. In the vasoligated rats (group B) a sterile black braided silk (Ethicon) tie was placed in the mid-region of the vas deferens. In group C (sham-operated rats) the testes and vas deferens were handled but without resection or ligation.

At week 15, the various test and control rats were placed singly in metabolism cages for collection of urine specimens for 17-ketosteroid analyses (*13*). The assay of urinary androgens has frequently been used as an index of gonadal output (*4, 14*) even though it represents the combined total of the predominant gonadal and smaller urinary 17-ketosteroid contributions of the adrenal glands (*15*).

All rats were decapitated at week 28. The sites of vasectomy, vasoligation, and other cavities of the body were examined, and the testes, seminal vesicles, adrenals, spleen, thymus, liver, and kidneys were dissected free of fat and connective tissue and weighed. Heparinized blood specimens were collected for biochemical investigations.

Urinary 17-ketosteroid values at week 15 after operation were reduced in both the vasectomized and vasoligated groups, but only group A vasectomized rats revealed statistically significant decreases compared to the controls (Table 1). Other investigators have reported no changes in the output of 17-ketosteroids in the urine of vasectomized rats (*4*).

Statistically significant reductions in the absolute weights of the testes of group A (vasectomized) and group B (vasoligated) rats were found (Table 1). In accord with the previous results, the decreases in testes weight were less in the vasoligated animals.

Gross examination of the testes at autopsy indicated that only 10 of the 31 animals in the group A rats had testes that appeared normal. In 17 animals, the pairs of testes were small, less turgid or soft, and had an abnormal purplish coloration (Fig. 1).

Table 1. Effects of vasectomy and vasoligation on urinary 17-ketosteroid output at week 15, testes weights at week 28, and white blood cell counts (WBC) at week 20 of immature Wistar rats (*N*, number of rats). Group A, vasectomy; group B, vasoligation; group C, sham control.

Group	Total urinary 17-ketosteroid		Blood count		Testes weight		Final body weight (g)
	N	μg	*N*	WBC/mm³	*N*	g	
A	30	86.85 ± 4.75	31	16876.6 ± 612.5	31	2.2014 ± 0.1829	544.7 ± 8.1
B	31	98.71 ± 3.89	31	16372.6 ± 479.6	31	2.5005 ± 0.1603	562.8 ± 8.6
C	27	106.48 ± 7.85	30	14314.2 ± 373.2	29	3.1532 ± 0.1134	540.8 ± 10.5
				Differences (%)			
A vs. C		−18.4		+17.9		−30.2	+0.7
P		.04		< .001		< .001	.77
B vs. C		−7.3		+14.4		−20.7	+4.1
P		.38		< .01		< .01	.11
A vs. B		+13.7		−3.0		+13.6	+3.3
P		.06		.52		.23	.14

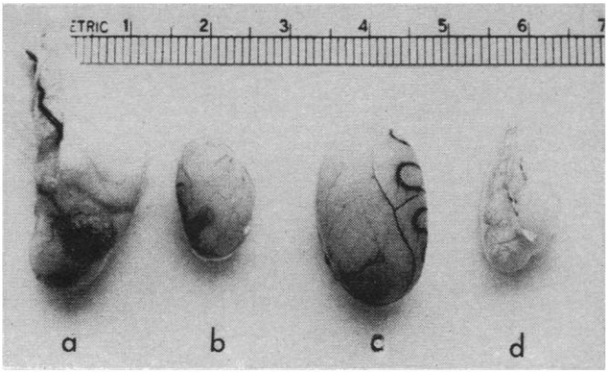

Fig. 1. Testes of vasectomized rat. (a) Enlarged cyst in epididymis; (b) small testis of a vasectomized rat; (c) normal-sized testis is shown for comparison; (d) the normal epididymis of a sham-operated rat. Tissues were fixed in a 10 percent formaldehyde solution.

Four animals displayed one normal testis and one that was either atretic or softer and smaller.

In the vasoligated group, more rats (17 of 31) had testes pairs showing normal size and appearance. In 11 animals, the pairs of testes were small, soft, and purplish in color, and three rats had one normal testis and one of reduced size. The more frequent occurrence of normal testes in the vasoligated group would coincide with the intermediate range of the testes weights in that experimental group. In the sham-operated controls, only three animals had smaller than normal testes, and 26 rats had normal appearing testes.

Other unusual findings were the occurrence of numerous small-to-large yellowish cysts located in the cauda epididymis and vas deferens regions of both the vasectomized (Fig. 1) and vasoligated animals. The cysts ranged in size from minute to a length of 2.5 cm in the cauda epididymis of the experimental groups. Few of the animals in either group A or group B failed to exhibit cysts in either the cauda epididymis or vas deferens regions. Whereas fewer of the vasectomized rats had cysts in the vas deferens, more of the vasoligated rats showed cysts at or near the site of the ligature. Four of the group A animals presented cysts at other sites; single or a few small yellowish cysts were observed in the fatty tissue near the spleen, liver, and lungs. Similarly, three of the group B rats also revealed single or a few small cysts in similar regions of the abdominal cavity. In unilateral vasectomy of rats (*16*) cysts found in the vas deferens of the cut end were reported to consist of a dense suspension of spermatozoa. Inflammatory lesions and granulomas of the epididymis due to interstitial invasion by spermatozoa have been reported in man (*17*), and spermatic granulomas have been cited as postoperative complications of vasectomy (*18*). In our study, at week 28, cysts of large size containing hard, yellow, and cheese-like debris were found. Although complete aseptic techniques were not used during the various operative procedures on all three groups, nevertheless in the sham-operated group no cysts were observed in the cauda epididymis or vas deferens regions and only one small cyst in the abdominal cavity of one rat.

It is evident from the output of urinary 17-ketosteroids and the testes findings that vasectomy and vasoligation after week 28 produced damaging effects on the morphology and perhaps function and hormonal activity of the testes and closely related structures. Whether the vasoligation procedure induces less damage than vasectomy must be the subject of further investigation. It is possible that blockage of the vas deferens associated with consequent increase in hydrostatic pressure produces damage and atrophy to the testicular elements. The high frequency of cyst formation in accessory sex organs might relate to an inability of elements in the cauda epididymis and vas deferens to competently phagocytize and remove the cellular debris arising from the accumulation and entrapment of fluid, live and dead spermatozoa, and degenerated materials. The caput epididymis is known to be the site of large quantities of testicular fluid resorption and the cauda epididymis for spermatozoa resorption (*5*). It remains to be determined whether a comparable reduction in weight of testes or of the urinary 17-ketosteroids (or both) would be reproducible in mature rats. At the very least, the occurrence of cysts in the reproductive organs and occasional cysts in the abdominal cavity (and the like), accompanied by leukocytosis data in the experimental animals, would strongly suggest that Roberts' previous observation (*9*) of systemic disorders and the reports of granulomas (*18*) following vasectomy warrants both additional investigation and caution.

In the absence of adequate, valid scientific evidence to support its "safety" or justify it as a "physiologic" measure, vasectomy for contraceptive control has become one of the commonest elective surgical procedures. Despite general ignorance of its effects and early warnings of potentially undesirable modifications, governmental, medical, and mass media of communications are promoting a procedure that may have significant social and somatic consequences. Moreover, little attention is given the "certain significant failure rate [as evidenced by semen tests] even though both ends of the vas are tied and a segment 4 to 5 cm long is resected in between" (*19*).

Our study demonstrates experimentally in rats that vasectomy causes statistically significant decreases in both the amounts of androgen excreted in the urine and in the weights of the testes, and is associated with the development of cysts in the cauda epididymis and vas deferens. It suggests that pending extensive study of the endocrine and somatic effects of vasectomy in man greater caution be observed in the use of vasectomies as a routine contraceptive procedure.

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Collagen Synthesis in vitro by Embryonic Spinal Cord Epithelium

Abstract. *Isolated spinal cords from chick embryos (stages 12 to 15) were incubated in vitro with radioactive proline. The proteins synthesized were fractionated by coprecipitation with added carrier collagen, followed by molecular-sieve and ion-exchange chromatography. A portion of the isotopically labeled proteins were found to be collagen molecules consisting only of α1 chains.*

There is evidence that extracellular materials, such as collagen and the acid mucopolysaccharides, which are present between interacting tissues, might be causally related to interactions between developing tissues (*1*). Independent support for this function of the extracellular matrix comes from the fact that myoblast differentiation in vitro is influenced by a macromolecule (or macromolecules) which is excreted by fibroblasts, and for which pure collagen can substitute (*2*). Similar studies (*3*) have indicated that collagen and other stromal macromolecules may be, in some as yet undefined manner, involved in inductive interactions.

Mesenchyme is the source of the connective tissue macromolecules that have been causally implicated in tissue interactions (*4*). However, epithelial cells, especially in the embryo, can also synthesize collagen (*5*). The spinal cord of the chick embryo, which is an epithelial tissue and an inducer of cartilage differentiation in adjacent somites (*6*), produces cross-striated collagen fibrils in vitro at the stage of this induction (*7*). There are at least four distinct molecular species of collagen in vertebrates (*8–11*), which differ in the primary structure of their component α chains; we studied and characterized the collagen produced by the embryonic spinal cord in vitro. Because of the small amounts of collagen produced by this tissue, we analyzed its structure by molecular-sieve and ion-exchange chromatography of radioactively labeled proteins mixed with carrier collagen.

Spinal cords were dissected from the posterior regions of chick embryos at stages 12 to 15, during which stages the posterior portion is composed of pseudostratified epithelium. Adjacent somites were removed by a brief treatment with 1 percent trypsin in Hanks balanced salt solution, at 5°C, and the isolated neuroepithelium was explanted onto a lens killed by freezing (*7*). The tissue and substratum were grown on rayon rafts and on F-10 nutrient medium supplemented with both chick embryo extract and fetal calf serum as previously described (*7*). β-Aminopropionitrile (β-APN) was added to the culture medium (50 μg/ml) in order to inhibit cross-linking in the newly synthesized collagen, thereby facilitating its solubilization for subsequent biochemical analyses. The proteins synthesized by the culture were labeled during a 16-hour incubation of the culture, from the time of explantation, with L-[2,3-^{3}H]proline (New England Nuclear, NET-323) at a concentration of 200 μc/ml.

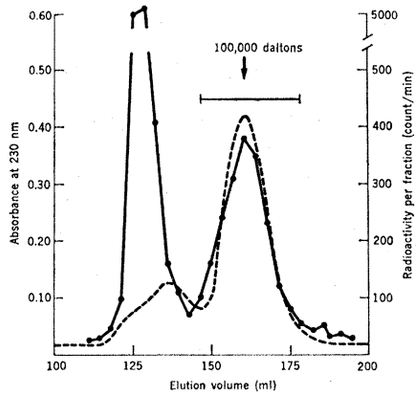

Fig. 1. Chromatogram, from a calibrated molecular sieve column (Agarose), of unlabeled α chains from collagen and of proteins, labeled with [^{3}H]proline, produced in vitro by spinal cords from stage 12 to 15 chick embryos. Absorbance, broken line; radioactivity, solid line. A portion of the labeled proteins elute with the α chains at 100,000 daltons. The bar indicates the material used for subsequent ion-exchange chromatography (Fig. 2).

After being labeled, the medium and culture were extracted overnight with 0.16*M* phosphate buffer, *p*H 7.6, at 4°C, and the resultant extract was clarified by centrifugation at 100,000*g* for 1 hour. The solubilized radioactive proteins were then mixed with a neutral solution of carrier collagen purified from whole lathyritic 13-day embryos by standard methods (*10*). The collagen and labeled proteins were precipitated twice from neutral solution by dialysis against 0.01*M* Na_2HPO_4, and were then denatured into component α chains by warming them to 45°C for 20 minutes. The denatured α chains were dialyzed to remove salt, then lyophilized, and dissolved in 1*M* $CaCl_2$ and 0.05*M* tris(hydroxymethyl)aminomethane-HCl, *p*H 7.5, and further purified by chromatography on a 2 cm by 110 cm column of Agarose (BioGel A 1.5). An aliquot of each fraction from the Agarose column was assayed for radioactivity by liquid scintillation spectrometry with Aquasol (New England Nuclear).

A typical elution pattern (Fig. 1) indicates the presence of radioactively labeled material migrating with the α chains of the carrier collagen. Approximately 15 percent of the labeled material applied to the column eluted with the α chains. The radioactive proteins from the α-chain fraction were pooled, excess salts were removed by dialysis, and the solution was then further fractionated on columns of carboxymethyl cellulose (*12*).

About 85 percent of the radioactive material applied to this column was eluted with the carrier α1 chain, whereas no significant amounts of radioactivity were present in the region of elution of the α2 chain. The remaining 15 percent of the radioactive material was equally distributed in the void volume, and in material eluted from the column with a solution of 1.0*M* NaCl and 0.05*M* NaOH (Fig. 2). The elution profile of the radioactive proteins in the α1-chain region indicates some heterogeneity of the chain, with one peak slightly preceeding that of the carrier α1 chain, another migrating precisely with the carrier, and a small shoulder slightly trailing the carrier. The presence of β-APN in the culture medium, and the prior chromatography