

DISTURBED REPRODUCTIVE FUNCTION FOLLOWING SECTION OF THE PORTAL VEIN*

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It will be shown in this paper that integrity of the portal vein is a condition for normal function of the reproductive tract of female rats.

The work originated with the observation that there was a very high incidence of abortion in rats subjected earlier to ligation and section of the portal vein (PVS) and which subsequently had become pregnant. Yet the livers of these animals appeared normal in the gross. The experiments, now to be described, demonstrated that elimination of blood flow through the liver *via* the portal vein always led to abnormal endocrine changes in female rats, most pronounced in disturbed sexual function, and that the changes were often drastic in magnitude.

The blood flow through the portal vein of dogs greatly exceeds the amount passing through the hepatic artery (1); the ratio is of the order of 4 to 1. In both the dog (2) and the rat (3) constriction of the portal vein prevents, to some extent, hepatic restoration that follows partial removal of the liver. We have been unable to find previous observations indicating any relationship of the portal vein to sexual function.

In 1937 it was found in 3 laboratories that the ovaries synthesize steroids with androgenic activity (4-6). Among these androgens are testosterone and 4-androstene-3,17-dione (7).

Biskind and Biskind (8) discovered that transplantation of an ovary (in the absence of its mate) to the spleen abolished estrus, due to inactivation of ovarian steroids in the liver. The liver inactivates testosterone (9), methyl testosterone (10), and estrone (11).

Methods

Biological.—Rats, age 60 to 65 days at the start of the experiment, were used throughout; unless mentioned otherwise, rats of the Sprague-Dawley strain were studied. PVS was per-

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formed through a midline incision in 2 stages by the method of L. R. Dragstedt (12) under ether anesthesia. The operations were done with aseptic precautions and every rat was injected with procaine-penicillin (100,000 units) after each operation. At the first stage the portal vein was constricted with a silk ligature ligated snugly around the vein and including a wire, 1 mm. in diameter (No. 20 gauge) which was removed immediately. At the second stage, 48 hours later, the portal vein was doubly ligated and sectioned.

Section of the ovarian veins (OVS) was achieved after isolating them near their junction with the inferior vena cava; both ovarian veins were doubly ligated and sectioned in one sitting.

Vaginal smears were obtained for 5 days before PVS and OVS and daily thereafter using a pipette and saline.

In one of the experiments stilbestrol, dissolved in sesame oil, 1 cc., was administered by stomach tube; the amount refers to the dose given each day for 10 days.

At necropsy, appropriate organs were weighed on a torsion balance and paraffin sections, stained with hematoxylin and eosin, were prepared. Whole mounts of the abdominal mammary glands were prepared to demonstrate the site of alkaline phosphatase (13, 14).

The statistical probability, p , of significance was derived from Fisher's table (15) of t values.

The portal vein was injected at necropsy with a thin solution of BaSO₄ (about 1 per cent) and radiographs were taken to study the distribution of the portal vein and its tributaries.

Chemical.—The levels of certain soluble enzymes were determined on a small piece of liver, about 100 mg., excised at stage 1 of PVS; similar assays were conducted at necropsy. The sample was weighed rapidly and homogenized for 3 minutes in an ice-cold solution of 0.15 M NaCl containing 0.003 M NaHCO₃; the enzymes were kept in an ice bath until the assays were made. The homogenates were centrifuged at 11,000 g for 15 minutes and assays were done on undialyzed supernatant solutions. β -glucuronidase was determined by the method of Talalay *et al.* (16); acid, and alkaline phosphatase were assayed by the method of King and Armstrong (17); the units are those of the respective authors.

The activity of soluble dehydrogenases at pH 7.38 was also determined. Glucose-6-phosphate,¹ and 6-phosphogluconic dehydrogenases were assayed by the method of Glock and McLean (18); lactic dehydrogenases by the method of Kubowitz and Ott (19); isocitric (20) and malic dehydrogenase (21) were also determined. All of the assays were conducted under conditions (22) which yielded zero order kinetics at 25°. One unit of 6-PGD, G-6-PD, or ICD is defined as the enzyme activity which reduced 1 μ mole of TPN/1 minute under the stated conditions. One unit of LAD or MDH is defined as the enzyme activity which oxidized 1 μ mole of DPNH/1 minute under the stated conditions. The units are expressed in terms of 1 gm. of fresh liver (wet weight).

Lipid determinations were determined on liver of intact controls and PVS rats at necropsy. Fresh liver, about 1 gm., was weighed and then dehydrated in an oven at 100° for 24 hours; the moisture content was determined from the difference in weight. The dehydrated liver was then extracted continuously in ethyl ether followed by petroleum ether each for 24 hours. The extracted lipids were determined by difference in weight.

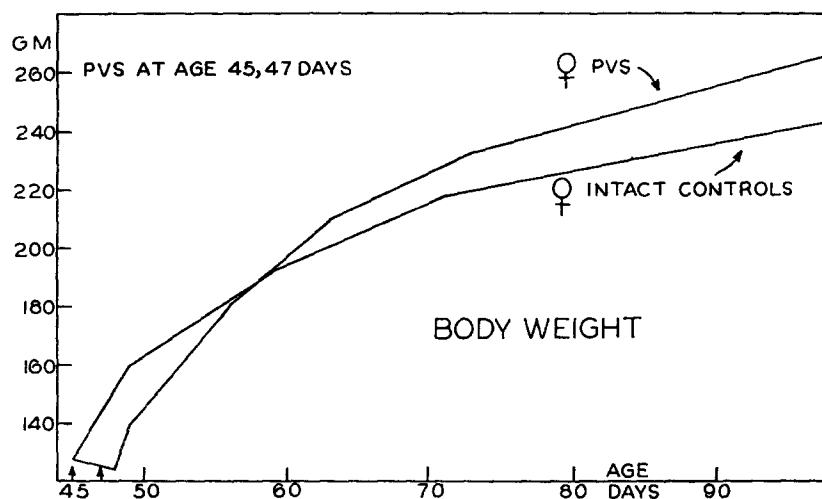
RESULTS

Vaginal smears were obtained each day from every rat but there was no evidence that this procedure disturbed the sexual cycles. The sexual rhythm of

¹ The following abbreviations are used; G-6-PD, glucose-6-phosphate dehydrogenase; 6-PGD, 6-phosphogluconic dehydrogenase; ICD, isocitric dehydrogenase; LAD, lactic dehydrogenase; MDH, malic dehydrogenase; DPNH, dihydrodiphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

normal rats will be described below. No normal pregnant rat aborted despite daily vaginal smears and all delivered between day 21 to 22 of pregnancy.

Following PVS, dilated veins were seen in the anterior abdominal wall and were found around the esophagus and in the retroperitoneal space. Ascites was not present. Dense adhesions were always found between stomach and liver in the vicinity of the site of PVS. Injection of the portal vein of normal rats (Fig. 1) with BaSO₄ demonstrated passage of barium to liver, spleen, pancreas, and the entire gastrointestinal tract. Similar injection after PVS always demon-



TEXT-FIG. 1. Following PVS, at age 45 and 47 days, there was an initial decline in body weight followed by a gain which exceeded the growth of normal sisters.

strated barium passing to the liver through adhesions; neither in normal rats nor in those subjected to PVS did barium reach the ovary or uterus.

Following PVS, there was an initial decline in body weight, followed by growth at a more rapid rate (Text-fig. 1) than took place in intact control sisters.

Sexual Cycle.—In our colony of Sprague-Dawley rats, the average duration of the sexual cycle is 4.15 days. The regularity is impressive. Daily vaginal smears were obtained from 11 intact control rats between 63 and 108 days; estrus recurred 79 times in 328 rat-days. The distribution of the 79 estrus periods was: 3-day cycles, 3; 4-day cycles, 75; 5-day cycles, 1. The mean ovarian weight was 79 ± 13 mg. (Table I).

Daily vaginal smears were obtained from 23 rats subjected to PVS. None had periodic cycles. We classified them in 3 groups: (a) persistent estrus, 6 rats; (b) anestrus, 6; (c) aperiodic estrus, 11.

Six rats were in persistent estrus for 19 to 60 consecutive days. At necropsy

TABLE I

Comparison of Weights of Organs of Rats Subjected to PVS with Those of Normal Controls

	Normal controls	PVS	<i>p</i>
	<i>mg.</i>	<i>mg.</i>	
2 Preputial glands	135 ± 30 (92 - 176)	177 ± 53 (91 - 246)	<0.01
2 Ovaries	79 ± 13 (66 - 104)	46 ± 18 (28 - 86)	<0.01
2 Adrenals	73 ± 4 (66 - 78)	65 ± 8 (51 - 82)	<0.03
Pituitary	12.8 ± 1.1 (11.8 - 14.8)	11.2 ± 2 (7.2 - 13.4)	<0.09
Spleen	588 ± 82 (438 - 687)	795 ± 143 (581 - 1030)	<0.01

There were 14 control rats and 23 rats subjected to PVS 37 to 84 days earlier.
±, standard deviation of mean.

TABLE II

Weight of Endocrine Glands and Targets of Normal Female Rats and of Males Subjected to PVS

No. of rats	Ovaries	Adrenals	Pituitary	Uterus	Liver	Prostate	
						No.	Weight
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>per cent*</i>		<i>mg.</i>
Normal controls							
14	79 ± 13 (66-104)	73 ± 4 (66-78)	12.8 ± 1.1 (11.8-14.8)	504 (401-633)	2.93 (2.9-3.2)	2	5.6, 8.6
PVS: Aperiodic estrus							
11	63 ± 16 (41-86)	66 ± 7 (50-82)	12.4 ± 1.2 (10.2-13.0)	551 (363-666)	3.54 (2.9-4.9)	5	20-137
PVS: Persistent estrus							
6	38 ± 7 (29-50)	56 ± 4 (50-63)	11.1 ± 2.4 (7.2-12.0)	506 (375-694)	2.92 (2.8-3.3)	0	—
PVS: Anestrus							
6	31 ± 4 (28-39)	59 ± 5 (51-65)	9.4 ± 1.3 (8.6-10.9)	270 (197-398)	2.89 (2.8-3.1)	1	40.4

* Per cent of body weight.

PVS performed 37 to 84 days before necropsy.

the ovaries were small (Table II) and the uteri were large and ballooned with fluid. Prostate glands were not observed in these rats.

Six rats never were in estrus after PVS. Irregular cycles culminating in proestrus were observed in 3 rats but vaginal cornification was not observed in these animals; 3 rats had sustained metestrus. One rat had prostate glands of large size (Table II); all of the ovaries were small.

Eleven rats had irregular estrus and the normal 4.15-day cycle was not observed. In this group, estrus was detected 46 times in 328 rat-days; the number in intact controls was 79. Estrus appeared at irregular intervals of 3 to 17 days. For example, in 1 rat the intervals of estrus were 5, 5, 6, 3, 7 days. In rats with aperiodic estrus the ovaries were larger than in other PVS rats and enlarged prostate glands were observed in 5 rats with irregular estrus (Table II).

Necropsy Findings.—In the PVS series of 23 rats, prostates of large size were found in 6 animals (Table II) and the prostatic epithelium was secretory (Fig. 3); extensive epithelial mucification (Figs. 4, 5) of the vagina was encountered in 8 rats; pronounced hyperplasia of the mammary gland was demonstrated in 10 rats. In PVS rats in persistent estrus, squamous cells were found in the vagina and its epithelium was keratinized. In 14 normal females, vestigial prostates (Fig. 2) were encountered in 2 rats (Table II).

Statistically, in comparison with intact sisters, the group of rats subjected to PVS had a significant decrease in the weight of ovaries and an increase in weight both of the preputial glands and spleen (Table I). Yet in certain rats (always in the group with aperiodic estrus) the weights of ovaries and preputial glands of the experimental rats were within the range of the normal animals. The weight of the pituitary and of the adrenals of the PVS series did not differ significantly from the control series.

Chemistry of Liver.—The livers of 14 rats subjected to PVS were compared with those of normal females of the same age. The weight of the liver was similar in both groups (Table III); ether-extracted lipids of livers of the PVS rats were approximately one-half of the controls. No cytologic abnormalities were detected in the livers of any of the rats, experimental or normal controls.

No significant differences between the normal and the PVS series were apparent (Table III) with respect to the content in the liver of 5 soluble dehydrogenases (G-6-PD; 6-PGD; ICD; MDH; LAD). Likewise no essential differences were found in the content of acid or alkaline phosphatases or of β -glucuronidase.

Reproduction.—In the PVS series, 14 Sprague-Dawley females were caged each night with fertile males but only 1 rat subsequently underwent normal pregnancy and parturition.

In 4 rats spermatozoa were never detected in the vaginal smears and pregnancy never ensued; 1 of these rats had sustained metestrus and a prostate of large size was found at necropsy; 3 of these rats had sustained estrus.

Ten PVS rats accepted the male (Table IV) and all had aperiodic estrus. On day 7 after detection of spermatozoa, the 10 rats were subjected to laparotomy to ascertain the presence of fetus; 5 of these rats were not pregnant. In *normal* rats, the vaginal smear within 24 hours after coitus becomes metestrus

TABLE III
Enzymes and Lipids in Liver of Females Subjected to PVS and Normal Control Rats

Liver	Normal controls	PVS
	<i>gm./100 gm.</i>	<i>gm./100 gm.</i>
Percentage of body weight.....	2.90 ± 0.2	3.03 ± 0.5
Per cent, water.....	72.8 ± 1.1	72.7 ± 1.2
Per cent, lipids.....	3.67 ± 0.2	1.54 ± 0.74
	<i>Units*</i>	<i>Units</i>
G-6-PD.....	3.84 ± 0.9	3.7 ± 1.6
6-PGD.....	6.53 ± 0.9	5.7 ± 1.8
ICD.....	30.4 ± 2.7	30.1 ± 4.0
MDH.....	207.4 ± 31	180.5 ± 31
LAD.....	193.7 ± 33	212.6 ± 29
β-Glucuronidase.....	84.7 ± 25.1	97.9 ± 26.7
Acid phosphatase.....	8.8 ± 1.0	8.2 ± 8.2
Alkaline phosphatase.....	0.8 ± 0.1	0.8 ± 0.1

There were 14 control rats and 14 sisters subjected to PVS.

* The enzymes were obtained on liver obtained by biopsy at the time of the first stage operation of portal vein section; the values for the liver of rats with PVS were obtained from the same rats 38 to 42 days later.

TABLE IV
Pregnancy, Abortion, and Parturition in PVS Rats

No. of rats	14
Accepted the male	10
Pregnant	5
Abortion*	4
Normal delivery	1

Pregnancy was determined at laparotomy 7 days after detecting spermatozoa in the vagina.

* Abortion occurred on day 10, 13, 14, and 18 of pregnancy.

(with mucified cells present) and this state persists throughout pregnancy. In the 5 non-pregnant rats which had accepted the male, estrus did not disappear in 3 after coitus and in the 2 remaining rats it reappeared on day 5 and 6 respectively.

In 5 PVS rats, fetuses were found at laparotomy on day 7. Abortion occurred in 4 of these between day 10 to 18 of pregnancy (Table IV). Normal delivery took place in 1 rat on day 22; at autopsy of this rat injection of the portal vein with BaSO₄ demonstrated that much barium entered the liver.

Ovarian Vein Section.—Is the disturbed ovarian function after PVS due to increased venous pressure? The ovarian veins were ligated bilaterally near their junction with inferior vena cava in 4 rats which were observed for 62 to 77 days subsequently. Ovarian function was not disturbed profoundly by this procedure. Four-day estrus cycles were frequent in all of the animals. In 1 rat subjected to OVS, there was persistent metestrus for 12 days, followed by regular estrus cycles of 4-day duration. In another rat in the OVS series, 4-day cycles of estrus persisted continuously after interruption of the ovarian veins. At necropsy, the ovaries were normal in appearance (mean weight 95 mg.) and the uteri were of normal size (mean weight 513 mg.); sizable prostates were not observed.

PVS and Effectiveness of Stilbestrol Administered by Mouth.—Ovariectomy was performed in rats and one-half of this group was subjected, in addition, to PVS. Three weeks later stilbestrol (DES), dissolved in sesame oil 1 cc., was administered by stomach tube daily to rats of both classes; 5 rats of each class were treated at each dose level for 10 days. Vaginal smears were obtained daily.

In rats subjected to ovariectomy alone, DES, 0.75 μ g. did not induce vaginal cornification in any rat; DES, 1 μ g., induced estrus in all rats between 72 to 96 hours.

In rats subjected to ovariectomy combined with PVS, DES, 0.1 μ g., was ineffective in producing vaginal cornification while DES, 0.25 μ g., induced estrus in 4/5 rats in 72 hours and DES, 0.5 μ g. was effective in this regard in all rats.

PVS in the Long-Evans Strain.—The effects of PVS were investigated in 12 Long-Evans rats and profound disturbances of sexual function were observed; these were very reminiscent of the abnormalities in Sprague-Dawley rats. No PVS rat had periodic sexual rhythm. In 226 rat-days, 4-day cycles were observed twice; the expected number is 57. Five rats had persistent estrus.

Six Long-Evans rats in the PVS series were placed in contact with fertile males. Two rats did not accept the male. Spermatozoa were found in the vagina of each of 4 rats; 3 of these rats aborted and 1 rat had normal delivery on day 22 of pregnancy.

DISCUSSION

After PVS no female rat of either Sprague-Dawley or Long-Evans strain had normal sexual function. Disturbances were always seen in the rhythm of

the sexual cycle and, in most rats, in the ability to support normal pregnancy. After PVS many rats were flooded with amounts of steroid hormones which were excessive for the normal physiological economy. In some rats the predominating hormones had androgenic properties; in other animals excessive amounts of estrogens prevailed; in a third group large amounts of several classes of steroids seemed to be present.

The administration of testosterone or related compounds (23) to female rats induces characteristic changes. Among these are: (a) increased growth rate; (b) pigmentation of skin; (c) growth of nipples; (d) hyperplasia of mammary glands; (e) growth of the vestigial prostate; (f) enlargement of preputial glands; (g) persistent diestrus; (h) mucification of the vaginal epithelium. Many rats subjected to PVS manifested all of these effects.

Other rats had persistent estrus which was sustained continuously for many days. They refused to accept the male. The vaginal epithelium was keratinized and the uterus was large and ballooned with fluid. These are manifestations which follow the administration of effective amounts of phenolic estrogens to rats.

The design of the experiments did not permit an analysis of the reasons for the predominance of androgenic effects in some rats while estrogens prevailed in other animals. It might be due to differences between individual rats in the rate of production of different classes of steroids by the ovary. More probably, it is related to differences in the rate of blood flow through the liver *via* portal vein through adhesions of the stomach to the liver; adhesions of this kind always were present after PVS but were not constant in their magnitude, being extensive in some rats and inconsiderable in others.

Disturbed ovarian function is not due to deleterious venous congestion with back pressure in the ovary caused by ligation of the portal vein. For the ovarian veins drain into the inferior vena cava rather than the portal system. Secondly, ligation of the ovarian veins caused no significant changes in ovarian function.

Following PVS, infarcts in the liver were not observed and there were no remarkable histologic differences from normal liver. Indeed, no great changes were detected in the liver except that the content of ether-extracted lipids was diminished about one-half. After PVS there was no significant difference from normal hepatic values of the soluble enzymes which were studied. These included dehydrogenases in the Krebs cycle (ICD:MDH), in the Warburg pentose pathway (G-6-PD; 6-PGD), or in the Embden-Meyerhof-Parnas pathway (LAD). Also, there were no significant changes in the concentrations of hepatic hydrolytic enzymes (β -glucuronidase; acid and alkaline phosphatases). Certainly the liver was not profoundly damaged following section of the portal vein.

The threshold dosage of estrogens required to exert a physiological effect was lowered in the PVS series. Significantly smaller doses of stilbestrol, ad-

ministered by mouth, were required in the PVS series of ovariectomized rats than were needed to produce estrus in mates with an intact portal vein.

The evidence points to the conclusion that rapid passage of blood through the liver is necessary to conjugate excessive amounts of ovarian steroids and that the normal portal vein is an essential unit in this mechanism. It is a newly recognized principle of endocrinology that a gland (ovary) is forced to secrete excessive amounts of hormones to compensate for their partial inactivation by a filter (liver) before reaching the targets in which they promote growth.

The results of experiments, reported in this paper, demonstrating disturbances in reproduction function after interruption of the portal vein are the converse of those in which the ovary is implanted in the spleen. In the experiments of the Biskind's (8-11), function of the ovary is abolished after its intrasplenic implantation because the transport of ovarian steroids through the portal vein causes their rapid inactivation in the liver. In the present work, lack of integrity of the portal vein fails to permit the transport of growth-promoting steroids to the liver with sufficient rapidity to inactivate excessive amounts so that disturbed function results in the reproductive tract.

Section of the portal vein of the rat provides a novel, simple, and useful method for the experimental study of abortion. The animals are healthy, are not unusually prone to infection or other intercurrent illnesses, and the difficulties in conception or in maintenance of pregnancy are due to changes of several sorts in the hormonal status of the female rats.

CONCLUSIONS

Integrity of the portal vein is a condition for normal function of the reproductive tract in female rats. Section of the portal vein (PVS) always abolished periodic sexual function. In a series of 23 animals, 6 developed continuous estrus and other signs of the presence of excessive amounts of estrogenic hormones; yet the ovaries of these rats were small. In 6 other rats there was growth of the prostate and additional evidence of increased activity of androgenic hormones. Many rats failed to become pregnant and nearly all pregnant rats aborted. Except for a decrease in lipid content, the composition of the liver of PVS rats resembled that of intact mates in the criteria which were applied.

The rapid passage of blood through the liver is necessary to conjugate the excessive amounts of steroids which the rat ovary normally produces and an intact portal vein is an essential unit in the mechanism necessary for their partial inactivation.

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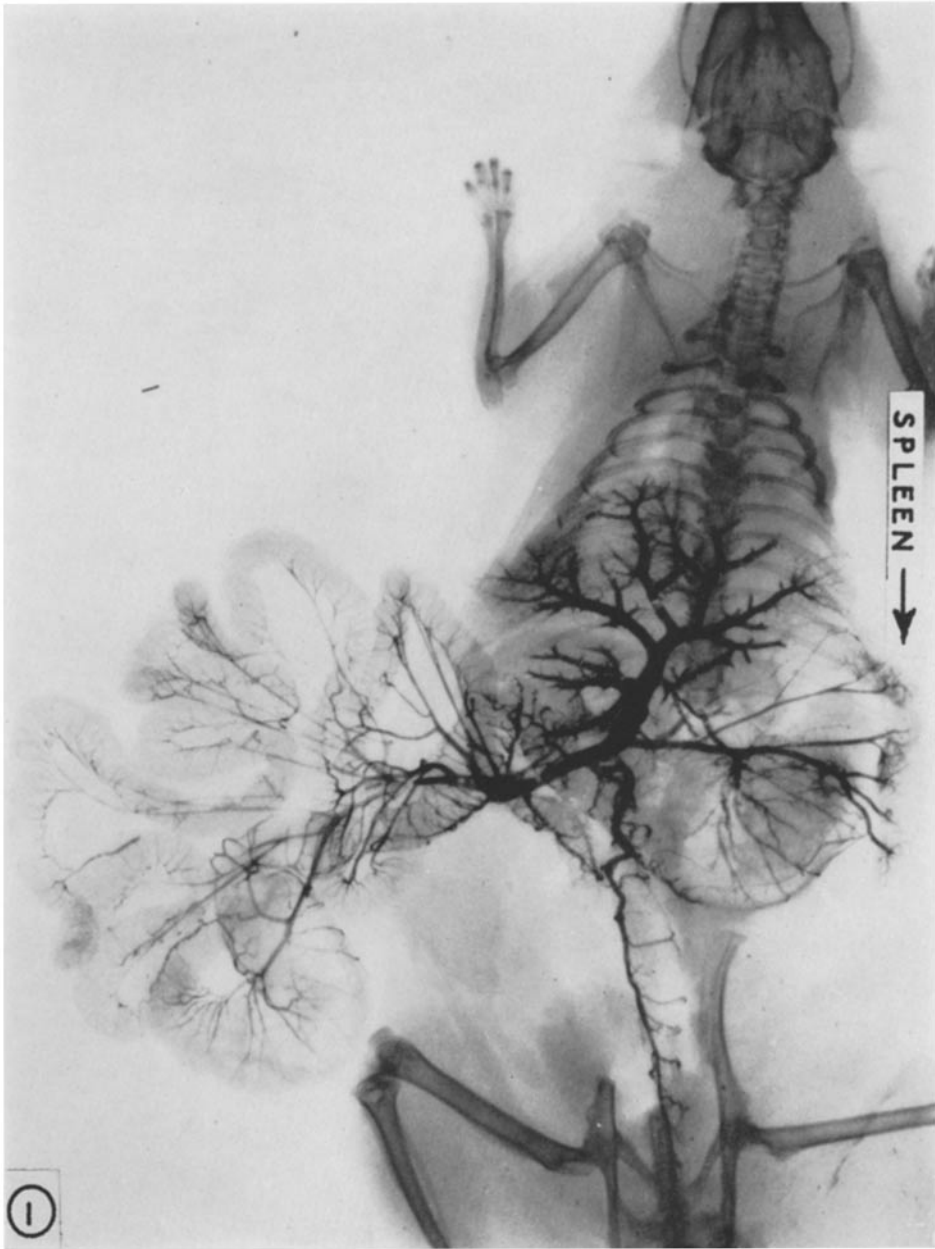
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EXPLANATION OF PLATES

PLATE 73

FIG. 1. Following the injection of BaSO_4 (1 per cent) into the portal vein at necropsy, barium passed to the liver, spleen, pancreas, lower esophagus, and the entire gastrointestinal tract but not to the uterus or ovary. Natural size.



(Huggins and Okamoto: Portal vein section)

PLATE 74

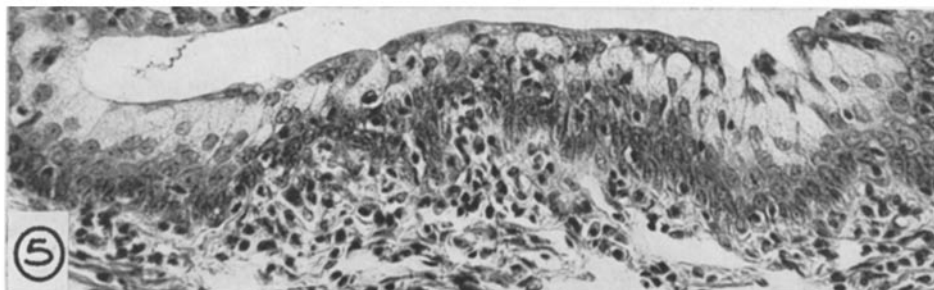
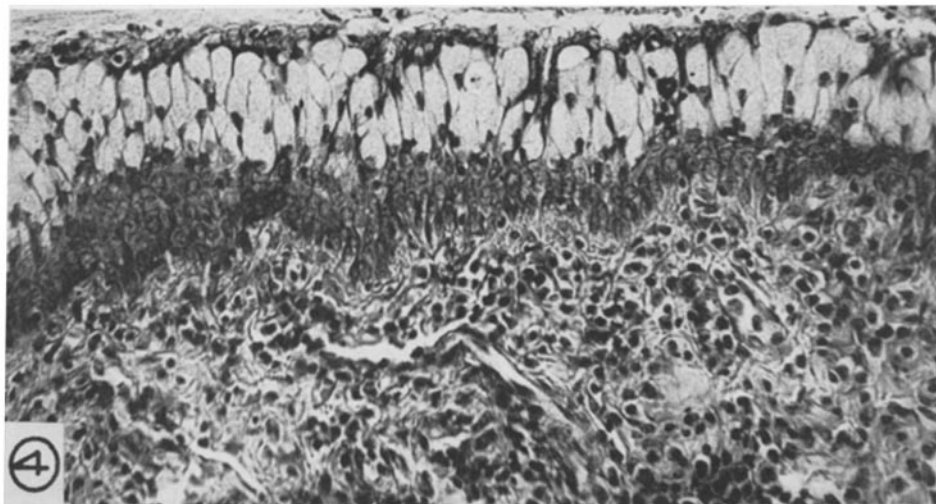
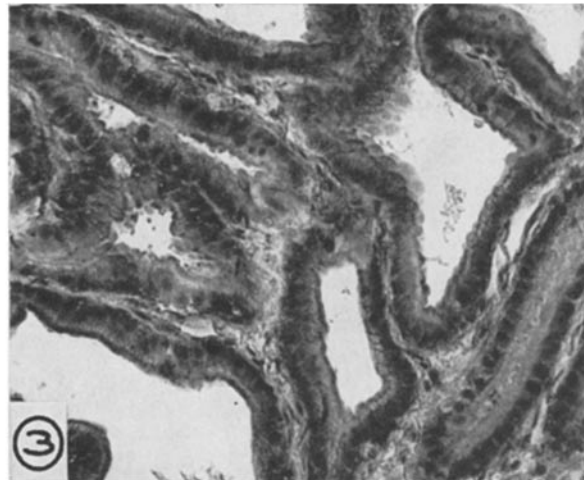
The photomicrographs are of paraffin sections stained with hematoxylin and eosin.

FIG. 2. Vestigial prostate of an intact female rat showing flat epithelium. $\times 270$.

FIG. 3. Prostate of a female rat 8 days after PVS; the epithelium is cylindrical and pale "secretory" areas border on the lumina. $\times 270$.

FIG. 4. Vagina of a masculinized female rat, 8 days after PVS, showing mucification of the epithelium. $\times 270$.

FIG. 5. Vagina of a masculinized female rat, 67 days after PVS showing mucification of the epithelium. $\times 270$.



(Huggins and Okamoto: Portal vein section)