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Radioautography Related to Amounts of [^{35}S]Sulfate Taken up by Prostate Glands of 2-Year-Old Mice (39540)

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The dry weight of the solvent-extracted ventrolateral prostate gland of the 2-year-old is 1.5 times heavier than that of the 1-year-old strain A mouse (5.76 ± 1.84 and 3.78 ± 0.92 mg, respectively; $P = 0.0024$). This increase in the prostate gland occurs without any significant increase in the body weight of the mouse (33.8 ± 3.8 g for the 2-year-old and 33.4 ± 2.8 g for the 1-year-old; $P = 0.80$) (1). In addition, since old strain A mice could produce spontaneous adenocarcinoma of the prostate gland (2) and a suitable animal model is desirable, ^{35}S-uptake studies were performed (i) to determine whether the ventral and lateral prostatic lobes of the 2-year-old mice could take up ^{35}S injected intraperitoneally, and, if they could do so, (ii) to measure the amounts of ^{35}S in the lobes, (iii) to demonstrate radioautographically the ^{35}S localization in the prostatic tissue at predetermined times after ^{35}S injection, and (iv) to relate the relative ^{35}S distribution in the prostatic tissue with the amounts taken up by the prostate gland.

The studies above were based on nonsoluble ^{35}S, the ^{35}S retained by formalin-washed prostate glands. In order to obtain some relationship between the nonsoluble ^{35}S and the total prostate ^{35}S (nonsoluble plus soluble ^{35}S), the amounts of ^{35}S in the glands were measured before and after washing with formalin solution. Moreover, to obtain the relative amounts of ^{35}S in the blood of the prostate gland itself, the amounts of ^{35}S in the prostate glands were measured before and after perfusion with saline.

Materials and methods. Two-year-old, male, strain A mice (purchased from the Kirschbaum Memorial Lab, Baylor College of Medicine, Houston, Tex.) were injected intraperitoneally with dilute aqueous $H_2{}^{35}SO_4$ (carrier-free and HCl-free, purchased from New England Nuclear, Boston, Mass.) at a dose of 5 μCi/g body weight. The mice were individually anesthetized with diethyl ether and at 1, 5, 25, 50, and 125 hr after the injection, they were bled by cutting the inferior vena cava. Moistened with a few drops of saline, the lateral and ventral lobes of the prostate gland were freed from the prostatic capsule and excised (1). A dissecting microscope with a zoom attachment was used during the prostatectomy. The lobes were soaked immediately in 10% formalin solution containing 0.1% each of Na_2SO_4 and hexadecyltrimethylammonium bromide (Eastman Kodak Co.; practical grade was twice crystallized from acetone solution) and transferred into fresh solutions at regular intervals to remove soluble ^{35}S. Washing with the formalin solution was continued for 1 week, at which time the radioactivity of the wash was at background level. After blotting with gauze sponges, the washed prostate glands were dried to constant weight at 0.001 mm Hg, oxidized to a clear solution with a mixture of 0.1 ml of 70% $HClO_4$ and 0.2 ml of 30% H_2O_2 at 60°, cooled to room temperature, and then measured for radioactivity in a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.) (3).

For radioautography, the prostate glands from four mice were excised at 10, 20, and 30 min and 1, 2, 5, 25, 50, and 125 hr after ^{35}S injection. After washing with the formalin solution described above, the glands from three of the four mice at each time point were dehydrated with ethanol, embedded in Epon-Araldite (4), cut at a thickness of 1 μm, and mounted on glass slides.

The prostate gland of the fourth mouse at each time point was embedded in paraffin, cut serially at a thickness of 10 μm, and placed on consecutively numbered glass slides. After deparaffinizing thoroughly with xylene and drying under reduced pressure, the even-numbered sections were kept

in methanolic HCl and the odd-numbered sections in absolute methanol at 37° for 16 hr (5, 6). Both groups were then washed with five changes of methanol and dried under reduced pressure.

The 1- and 10-μm sections were coated with nuclear track emulsion NTB2 (Eastman Kodak Co.) by the dipping method, equilibrated at 40° and 80% humidity, dried for 1 hr at room temperature, sealed in light-proof boxes containing 8-mesh Drierite, and stored at 4°. At regular intervals of time, a set of these coated sections was developed in Dektol (7) and stained with hematoxylin and eosin (8) for microscopic studies.

Results. Both the ventral and lateral prostatic lobes of the 2-year-old mice took up intraperitoneally injected $^{35}SO_4$. The amounts of nonsoluble ^{35}S (not removable by washing with formalin solution) were the same for both lobes, at the various time points (Table I). Moreover, there was a significant "peak" in the amounts of nonsoluble ^{35}S at 5 hr after the injection. At 25 hr, however, the amounts of ^{35}S had decreased to half the peak value; at 50 hr, to the 1-hr value; and at 125 hr, to less than the 1-hr value.

TABLE I. SPECIFIC RADIOACTIVITIES OF FORMALIN-WASHED VENTRAL AND LATERAL LOBES OF THE MOUSE PROSTATE GLANDS AT THE SPECIFIED TIMES AFTER $^{35}SO_4$ INJECTION.

Time (hr)	Ventral lobe[a]	*P* value	Laberal lobe[a]	*P* value
1	169 ± 81		175 ± 112	
5	436 ± 68	0.069	484 ± 86	0.089
25	241 ± 58	0.016	242 ± 62	0.015
50	167 ± 49	0.15	213 ± 34	0.53
125	105 ± 51	0.23	98 ± 31	0.018

[a] Mean ± SD. Each group consisted of five mice. Values are reported as counts per minute per milligram of dry weight.

Table II shows that the injected ^{35}S appeared rapidly in the blood: The 1-hr blood samples had almost twice as much ^{35}S as the 5-hr samples. Once in the blood, the ^{35}S decreased relatively rapidly, from 26,400 cpm/mg at 1 hr to 59 cpm/mg at 125 hr. Similarly the ^{35}S of the nonperfused and saline-perfused prostate glands decreased from 1 to 125 hr. Nevertheless, there was a slight difference in the amounts of ^{35}S between the nonperfused and perfused glands, indicating the presence of small amounts of ^{35}S in the blood and large amounts in the tissue proper. In contrast to the unwashed glands (nonperfused and perfused), the formalin-washed glands showed a more gradual loss of ^{35}S. Consequently, the nonsoluble ^{35}S (represented by the formalin-washed glands) amounted to 1% of the total prostate ^{35}S (represented by the unwashed nonperfused and perfused glands) at 1 hr and to about 100% at 125 hr. Moreover, unlike the total prostate ^{35}S, the nonsoluble ^{35}S was greater in the 5-hr than in the 1-hr samples.

Radioautography showed that, at 10 min after its injection, ^{35}S was present in the epithelial cells and lumen of the acinus (Fig. 1A). At 1 hr, there was a relatively high concentration of ^{35}S in the epithelial cells with small amounts in the lumen (Fig. 1B). At 5 hr, there was a massive concentration

TABLE II. SPECIFIC RADIOACTIVITIES OF BLOOD AND NONPERFUSED, SALINE-PERFUSED, AND FORMALIN-WASHED VENTROLATERAL PROSTATE GLANDS OF MICE AT THE SPECIFIED TIMES AFTER $^{35}SO_4$ INJECTION.

		Prostate glands[b]		
Time (hr)	Blood[a]	Nonperfused[c]	Saline-perfused[d]	Formalin-washed[e]
1	26,400 ± 7,530	24,500	19,000	232
5	15,200 ± 5,910	13,000	11,000	480
25	238 ± 61	805	740	281
50	193 ± 53	550	384	161
125	59 ± 26	126	175	131

[a] Mean ± SD. Each group consisted of six mice. Values are reported as counts per minute per milligram of dry weight.

[b] Average. Each group consisted of two mice.

[c] Prostate glands excised from bled mice.

[d] Prostate glands excised after perfusing with saline through the descending aorta.

[e] Prostate glands (excised as nonperfused) thoroughly washed with formalin solution as described in the text.

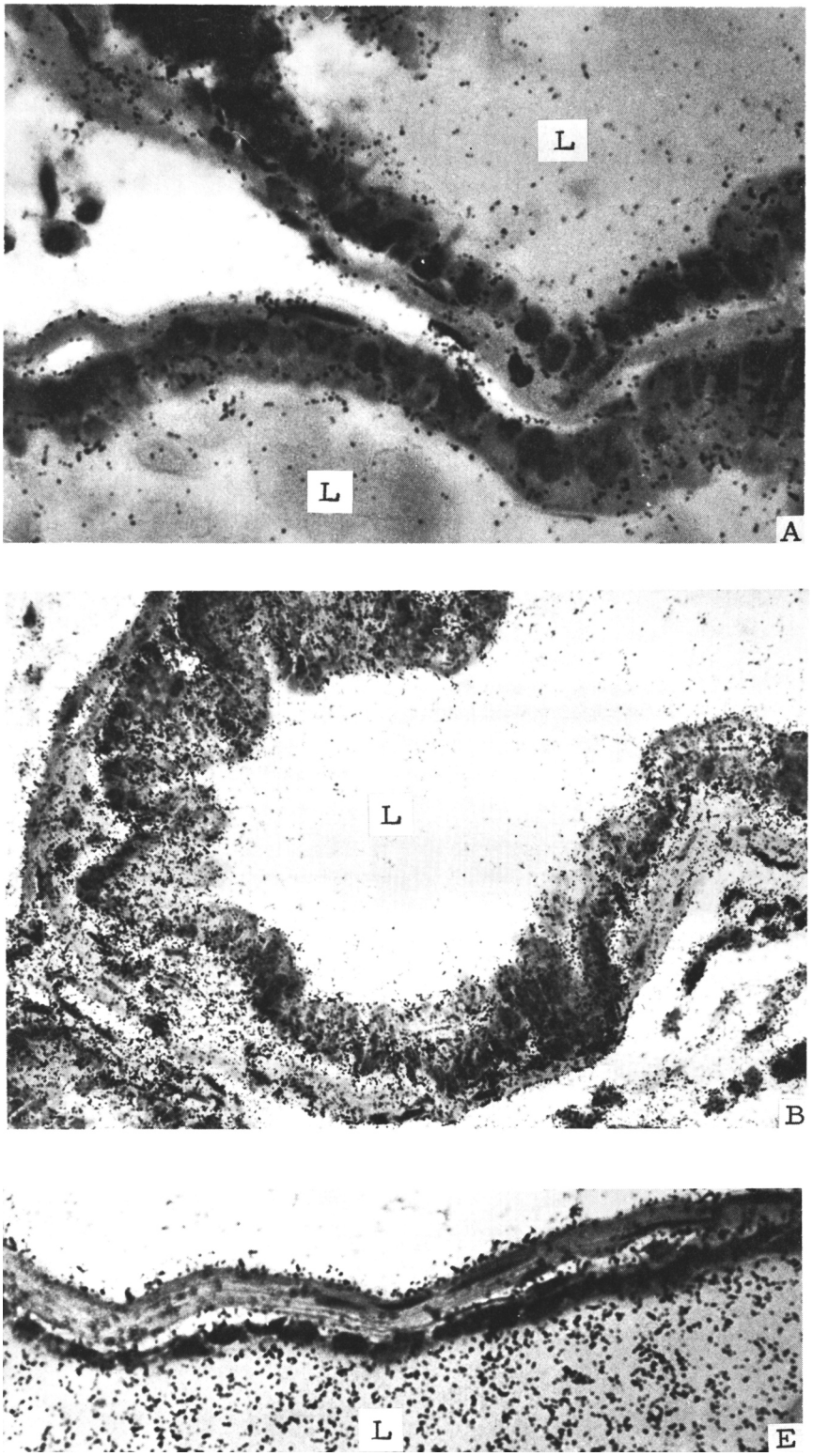
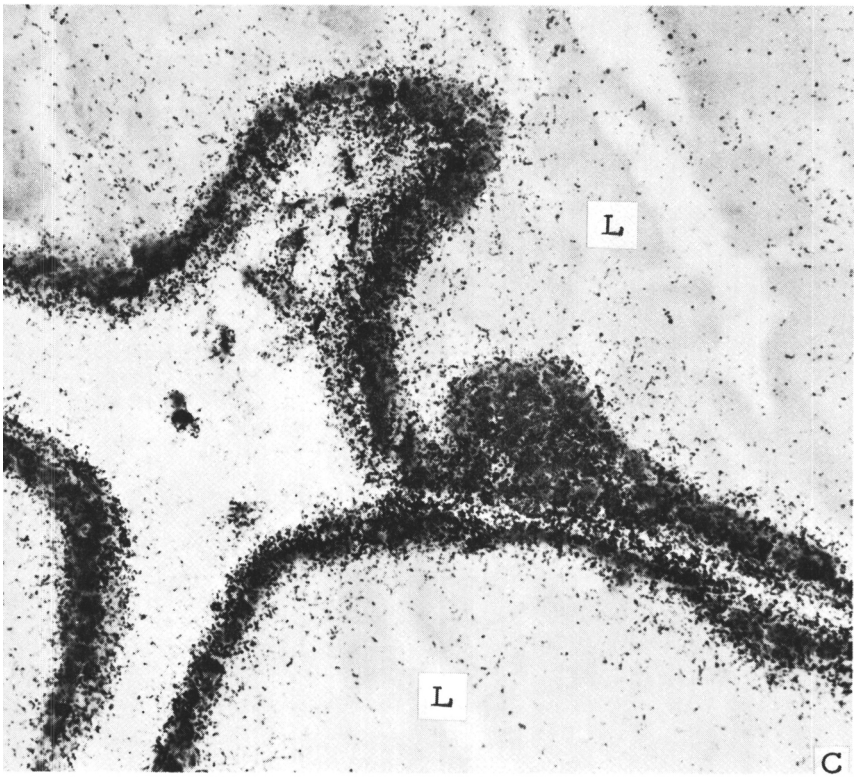


FIG. 1. Radioautographs of 1-μm Epon–Araldite sections of ^{35}S-labeled mouse prostate glands developed simultaneously at 3 months of exposure and stained as described in the text. The glands were excised at 10 min (A), 1 hr (B), 5 hr (C), and 50 hr (D and E) after $^{35}SO_4$ injection. Photographed at 400 × (A, B, and E), 250 × (C), and 160 × (D). L, lumen.



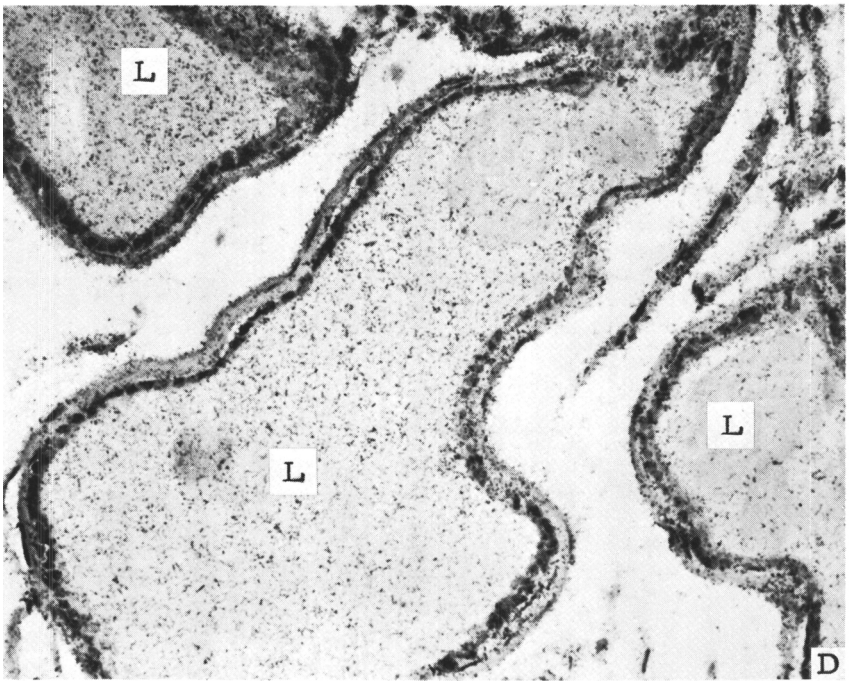


FIG. 1. (*Continued*)

of ^{35}S in the epithelial cells (Fig. 1C); however, at 50 hr, the amounts in the epithelial cells had decreased to the 10-min level while the amounts in the lumina had greatly increased (Fig. 1D). Marked differences in the amounts and distribution of ^{35}S were not observed between the 50- and 125-hr samples.

The radioautographs also showed that ^{35}S was taken up by connective tissue interstitial cells at the base of the prostatic acinar cells. However, the amounts of connective-tissue ^{35}S appear to be relatively small in comparison to those in the acinar cells at 10 min (Fig. 1A) and the acinar lumen at 50 hr (Fig. 1D).

Comparing Fig. 2A and 2B, it can be seen that the treatment of the prostatic tissues with methanolic HCl reduced the number of silver grains.

Discussion. The radioautographs showed that prostate glands of the 2-year-old mice took up intraperitoneally injected ^{35}S (Fig. 1). The nonsoluble ^{35}S was initially concentrated in the epithelial cells of the glands and then moved into the lumina of the acini. This interpretation is based on the early appearance of ^{35}S in the epithelial cells (10 min after its injection, Fig. 1A), its relative increase in concentration from high (at 1 hr, Fig. 1B) to massive amounts (at 5 hr, Fig. 1C), and then to its decrease in the epithelial cells with a concomitant increase in the lumina (at 50 hr, Figs. 1D and E).

For comparative purposes, the ^{35}S-labeled prostate glands were processed for specific

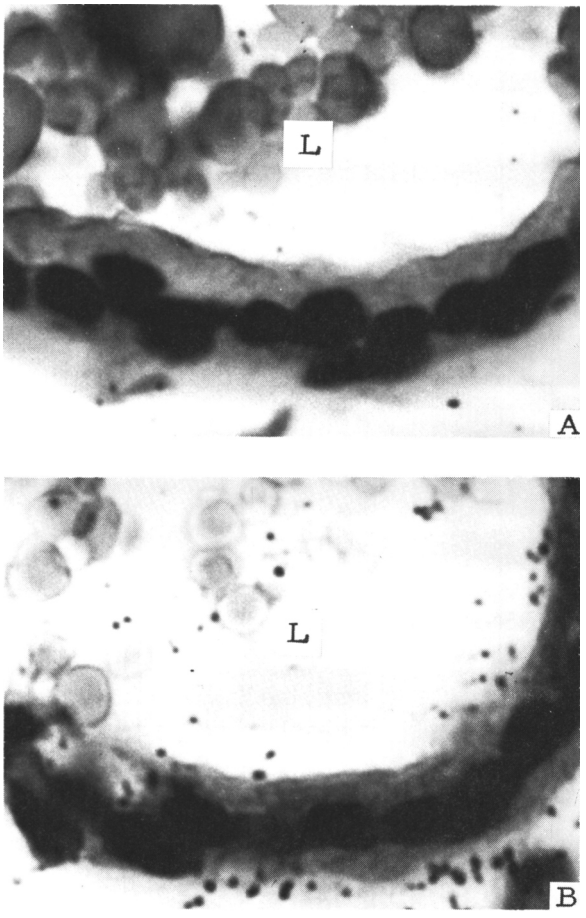


FIG. 2. Radioautographs of adjacent 10-μm paraffin sections of ^{35}S-labeled mouse prostate gland. (A), Treated with absolute methanol; (B), with methanolic HCl. Photographed at 400 ×. L, lumen.

radioactivity determination (counts per minute per milligram of dried glands; Table I) in the same way as for radioautography. *P* values, calculated from the specific radioactivities of the prostate glands, showed a significant peak at 5 hr after ^{35}S injection. However, this peak appears not to be a true peak since one would expect the true peak to be just beyond the 5-hr time point (the experiment was not designed to determine the peak). At 25 hr, the specific radioactivity decreased to 55% of the peak; at 50 hr, to 38%, which was the same as the 1-hr time point. At 125 hr, the specific radioactivity had decreased to 24% of the peak.

The radioautographs and specific radioactivities taken together led us to the following conclusion: The massive concentration of ^{35}S in the epithelial cells observed in the radioautograph (Fig. 1C) corresponded to the peak specific radioactivity of the 5-hr time point. After the 5-hr time point, the decrease in the specific radioactivities corresponded to the ^{35}S decrease in the epithelial cells and increase in the lumina (Fig. 1D). However, since the specific radioactivities of the 1-hr (^{35}S predominantly in the epithelial cells, Fig. 1B) and the 50-hr (^{35}S predominantly in the lumina, Fig. 1D) time points were the same and amounted to only 38% of the peak, this discrepancy in the specific radioactivity of the 50-hr time point could be accounted for by normal loss through excretion of the prostate glands. These results suggest that the prostate gland of the 2-year-old mouse is physiologically active and capable of metabolizing $^{35}SO_4$.

It has been reported that only negligible amounts of administered $^{35}SO_4$ are incorporated into sulfur-containing amino acids (9). Further, the nonsoluble ^{35}S in rat rib cartilage (9), rat bone cartilage (10), and rabbit ear cartilage (11) is incorporated almost exclusively into chondroitin sulfate. Moreover, direct exhange of $^{35}SO_4$ with the sulfate of glycosaminoglycans (GAGs) appears not to occur (12). We have shown that the nonsoluble ^{35}S in formalin-washed prostatic tissues are removable by treatment with methanolic HCl but not with absolute methanol (Fig. 2), just as the ester sulfate of GAGs are (6). Presumably, the water-, ethanol-, xylene-, and methanol-insoluble ^{35}S (which is demonstrable radioautographically to be localized in specific areas of the prostatic tissues at specified times after ^{35}S injection) is an integral component of GAGs. If this is so, the secretion of the normal mouse prostate gland should contain sulfated GAGs (Fig. 1). However, it may be difficult to demonstrate their presence histochemically. Although the presence of GAGs in the luminal contents of carcinomatous prostate glands could be demonstrated, GAGs in normal human prostate glands could not be demonstrated with GAG stains (13, 14). Possibly, the GAGs of the normal gland are bound in such a way as to render them nonstainable with the usual methods (15). Nevertheless, various sulfated GAGs have been extracted from normal human prostate glands and those GAGs have been characterized and quantitated (16).

Summary. Intraperitoneally injected $^{35}SO_4$ appeared rapidly in the blood and in the prostate glands of the 2-year-old mouse. Most of the prostate ^{35}S was in the tissue proper with small amounts in the blood. The amounts of soluble ^{35}S of prostate glands were greatest at 1 hr and least at 125 hr after ^{35}S injection. The amounts of nonsoluble ^{35}S (not removable by washing with formalin solution) decreased much more gradually than the soluble ^{35}S. Consequently, the nonsoluble ^{35}S amounted to 1% of the total prostate ^{35}S at 1 hr and to about 100% at 125 hr.

The amounts of nonsoluble ^{35}S were the same for both the ventral and lateral prostatic lobes at the various time points after the injection. The nonsoluble ^{35}S increased to a peak at 5 hr, decreased to half the peak at 25 hr, and it continued to decrease with time.

At 10 min after its injection, ^{35}S was detectable in the epithelial cells and lumen of the acinus by radioautography. The amounts of ^{35}S in the epithelial cells increased from relatively large at 1 hr to massive amounts at 5 hr. However, at 50 hr, the ^{35}S in the epithelial cells had decreased to the 10-min level while the amounts in the lumina had greatly increased.

Results of this investigation suggest that the nonsoluble prostate ^{35}S was initially concentrated in the acinar cells, moved into the

lumen, and then was excreted by the prostate gland. Thus, the prostate gland of the 2-year-old mouse is physiologically active and capable of metabolizing $^{35}SO_4$.

The water-, ethanol-, xylene-, and methanol-insoluble ^{35}S of the prostate gland appears to be an integral component of GAGs.

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