od of Wachstein and Meisel can be used to demonstrate presumptive ATP hydrolysis by isolated nuclei from embryonic myocardium. Characteristics of the reaction are in agreement with those previously found using unfixed (or fixed) cryostat sections and with enzymatic studies on the isolated nuclei(1). There can be no appreciable diffusion artifacts resulting from inorganic phosphate liberation by other cell components in the present study. Intense reactions occur at the nuclear surface as if the enzyme is located at least partly in the membrane; less intense reactions are associated with components of the nucleoplasm. On the basis of present experiments, it cannot be ascertained whether Na^+ stimulation or Mersalyl inhibition occurs more at one nuclear locus than another. ATPase activity has also been reported to be associated both with the nuclear membrane and the nucleoplasm of liver nuclei (4). Certain properties of the myocardial enzyme have been compared(1) to those of isolated kidney(5,6) and liver(4) nuclei.

Under carefully controlled conditions, the present method using isolated nuclei may be suitable for quantitative and statistical analysis through dry mass determination in terms of electron opacity(7).

Summary. A histochemical method was modified to demonstrate ATP hydrolysis by nuclei isolated from embryonic myocardium. Under a variety of experimental conditions, the results are in agreement with previous histochemical studies using cryostat sections and with enzymatic studies using isolated nuclei. The data give further support to the localization of ATP phosphohydrolase activity both at the nuclear surface and in the nucleoplasm, under conditions which eliminate possible diffusion artifacts resulting from enzymatic activity by other cell components and non-enzymatic hydrolysis of ATP by Pb^{++}.

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Incorporation of Selenium into Spermatogenic Pathway in Mice.* (31981)

SAMUEL A. GUNN, THELMA CLARK GOULD, AND W. A. D. ANDERSON
Department of Pathology, University of Miami School of Medicine, Miami, Fla.

During preliminary studies in male mice on tissue distribution of a single subcutaneous injection of high specific activity Se-75, it was noteworthy that, whereas most tissues acquired and then lost the radioisotope quickly, the testis continued to cumulate selenium. Little information was available on distribution of an actual tracer amount of selenium to male reproductive organs of mammals. But the fact that in fowl Se-75 was retained in testis(1,2) and found in the protein fraction of spermatozoa(2), suggested that the question of possible selenium incorporation into the mammalian spermatogenic pathway merited further investigation.

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Materials. A total of 115 male mice of

TABLE I. Se-75 Uptake in Various Tissues of CD-1 Male Mice Following Subcutaneous Administration of Tracer Dose (1 μc Se-75; .03 μg Se).*

Tissue	Se-75 uptake in % admin. dose/g tissue Time interval after injection 1 Hr	4 Hr	1 Day	2 Days	7 Days
Kidney	11.19†	11.03	7.34	5.98	2.86
Liver	7.52†	7.28	5.55	5.23	3.61
GI tract	7.41†	4.97	2.79	1.86	.63
Injected leg	3.32†	1.08	.95	.82	.31
Blood	1.84	3.80†	1.68	1.27	.74
Lungs	1.13	2.32†	1.50	1.35	.76
Pancreas	.95	1.16†	1.10	.79	.40
Spleen	.82	1.34	1.76†	1.60	.91
Heart	.66	1.82†	.88	.83	.51
Testis	.56	.67	1.37	1.58	1.97†
Non-inj leg	.50	.30	.67†	.56	.32
Skeletal muscle	.28	.49†	.37	.31	.19

* Mean values from 5 mice are shown.
† Indicates time period in which highest concentration observed.

CD-1 strain (Charles River Breeding Laboratories, Inc.), 20 weeks of age, weighing 40 g, were used in these studies. High specific activity Se-75 (33 mc/mg) was purchased from Nuclear Science and Engineering Corp.

Methods. Each mouse received, as a single 0.2 ml subcutaneous injection (thigh), either a tracer dose of Se-75 (1 μc) or this same amount of Se-75 in the presence of a subtoxic dose of carrier selenium dioxide (equivalent to 72 μg of selenium). At varying times after administration of Se-75, mice were sacrificed in groups of 5, specific tissues were removed, weighed (wet) and placed in 20-ml counting tubes for determination of radioactivity (large well-type scintillation detector). Standards (containing 1% of the administered dose) were always counted simultaneously with tissue samples. Calculations of Se-75 concentration in tissues (based on % of dose administered as indicated by the standard) were thereby automatically corrected for decay.

Experiments and results. Tracer Se-75 uptake in various tissues. A comparison of Se-75 uptake in various tissues is shown in Table I. Tissues are listed in descending order of their radioisotope concentration observed at 1 hour. With the exception of testis, all tissues had attained maximal Se-75 concentrations between 1 hour and 1 day and had lost 50 to 90% of their selenium levels by 7 days. In contrast, testis, which was one of the lowest ranking tissues in Se-75 uptake at 1 hour, continued to cumulate this element. By 7 days, selenium concentration in testis was 3.5 times greater than that observed at 1 hour and the male gonad was now the third ranking tissue, after liver and kidney, in Se-75 concentration.

Tracer Se-75 in testis-epididymis complex. Fig. 1 shows the temporal distribution pattern of Se-75 in testis and epididymis (dissected as entire epididymal tract, including caput, cauda and corpus). Se-75 attained peak concentrations in testis at 1 week, and as testicular levels fell, Se-75 content of epididymis rose, reaching maximal concentration levels between 2 and 3 weeks following injection.

Testicular uptake of Se-75 in subtoxic carrier selenium. McConnell(3) reported that selenium levels in testis were extremely low in comparison with other tissues when Se-75 was used as a tag for a single subtoxic injection of sodium selenate to rats. We questioned then, whether in the presence of carrier selenium, the mouse testis would still cumulate the element. Fig. 2 compares the Se-75 distribution in testis between carrier and tracer amounts of selenium. With the carrier, peak levels of Se-75 were attained in the male gonad by 30 minutes following injection. Although at first there was a flushing out of the initial high levels of selenium in testis,

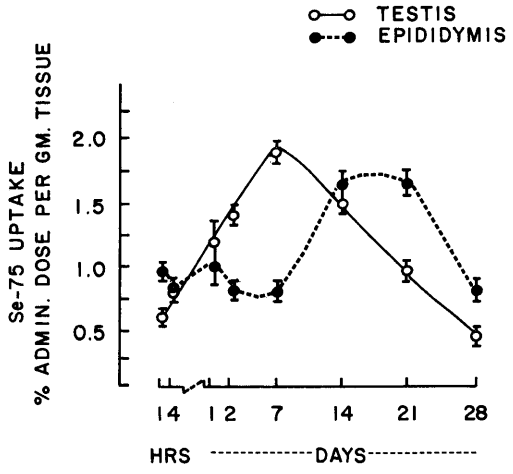

FIG. 1. Temporal cumulation pattern of Se-75 in testis and epididymis of CD-1 mice following subcutaneous administration of tracer dose (1 μc Se-75; .03 μg Se). Mean values and S.E.M. are shown.

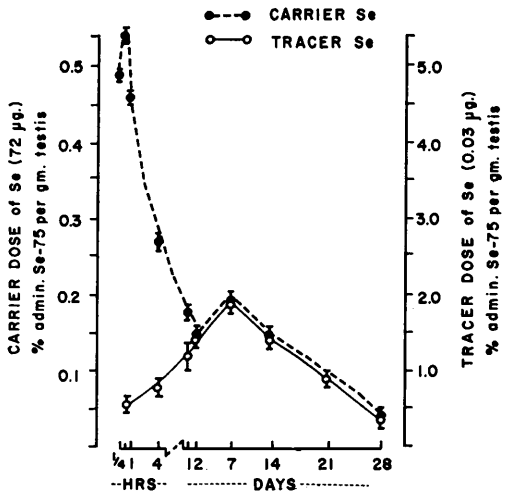

FIG. 2. Temporal cumulation pattern of Se-75 in testis of CD-1 mice; comparison between Se-75 (1 μc) administered as tracer (.03 μg Se) and with carrier (72 μg Se). Mean values (5 mice) and S.E.M. are shown.

as has been noted for most tissues(4), there was, as with the tracer dose, a concentration pattern from the 2nd day to the 1st week time-period and a subsequent fall by the 2nd week (differences which were statistically significant at the $P<0.0001$ level). The fact that McConnell(3) did not note higher levels of Se-75 in rat testis following a single injection may have been because his observations did not extend beyond 96 hours. Following repeated administration of tracer doses (7.5 μg per day) of Se-75 to rats for 21 days, Rosenfeld(5) reported that after the kidney, testis retained the highest concentration of selenium.

Discussion. The increase of selenium in testis followed by a cumulation in epididymis as testicular levels wane bears a startling resemblance to the distribution pattern of zinc(6,7), an element known to be essential for spermatogenesis(8). Wetterdal(6) showed that the spermatozoa in the seminiferous tubules were primarily responsible for Zn-65 taken up by the testis and that as spermatozoa were transported into epididymis they carried a large amount of zinc with them. The similarity of distribution patterns of Zn-65 and Se-75 leads us to conjecture a possible zinc-selenium complex. This suggestion is reinforced by the knowledge that zinc is commonly bound to sulfhydryl groups(9) and that selenium enters many metabolic reactions by substitution for sulfur(10).

Biologically selenium is a diversified element. It not only displays a variety of toxic manifestations but plays the role of an essential nutrient as well(10). That selenium interferes with reproduction by causing decreased fertility and developmental malformations has been noted by many investigators in different species of animals(10). But, whereas toxic and subtoxic amounts of selenium interfere with reproduction and fertility in the female, studies in rats show that fertility in the male was not impaired(11). Patrick *et al*(2) suspected that in the fowl selenium replaced the sulfur in sulfur amino acids synthesized by the testis for sperm formation. Most selenium analogues are fully active as substrates for enzymes of sulfur metabolism and proteins altered by substitution of seleno-amino acids for sulfur amino acids can still function(12). The seeming lack of toxicity of selenium to male fertility, in doses which interfere drastically with reproduction in the female, and the distinct cumulation of Se-75 in the testis-epididymis complex, in the face of its depletion in other tissues, suggests that this element is capable of entering some established metabolic pathway in the male reproductive system with no apparent adverse effects.

Summary. Studies were conducted on tissue distribution of high specific activity Se-75 in CD-1 male mice. As a tracer (1 μc Se-75; 0.03 μg Se) and in the presence of carrier (72 μg Se) Se-75 showed a cumulation pattern in the testis-epididymis complex indicative of association with the wave of spermatogenesis.

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Electrolyte and Water Composition of Renal Tissue in Common Laboratory Animals.* (31982)

L. J. CIZEK AND M. R. NOCENTI
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

While studying rabbit renal tissue slices under a variety of conditions(1), it was observed that the medulla consistently had a significantly higher water content than the cortex. Although no published comment pertaining to this difference can be found, it would appear from data scattered in the literature(2,3,4,5,6,7) that this difference is a very frequent, if not constant, finding. In order to substantiate this impression, the water and electrolyte contents of renal tissue were determined in several mammalian species. In addition, studies were made to ascertain what changes, if any, occurred in renal tissue water content during alterations in body hydration.

Methods. Renal tissue from the following species was used: male dogs, cats, rats (Sherman), rabbits (New Zealand) and male and female guinea pigs (English). Each group consisted of 3 normal adult animals. To study altered hydration, adult male rats were placed in the following groups: (1) normal control, (2) water deprived for 5 days (food *ad libitum)* and (3) hydrated with 5 ml water per 100 g body weight by gavage. In the latter group, the kidneys were removed 1 and 2 hours following the gavage; the fraction of the water load which had been excreted at these times was 55 and 72%, respectively.

The dogs, cats and rabbits were sacrificed with an overdose of intravenous Nembutal, and the rats and guinea pigs by an overdose of ether. Both kidneys were quickly removed, stripped of their capsule, and each sliced longitudinally to obtain a median sagittal section. From this section, a small sample was obtained from the cortical area and from the apical papilla. The sample was placed in a weighed 10 ml volumetric flask, and the wet weight obtained. The samples ware dried to constant weight at 100°C. The dried tissues were prepared for electrolyte analyses according to the method of Malvin and Wilde (8).

Results. In all species studied, the water content and the sodium concentration, expressed both as milliequivalents per gram of dry weight or of tissue water were significantly ($p<.01$) higher in the medulla than in the cortex (Table I). The medullary potassium concentration on a dry weight basis was significantly increased in the rabbit and rat. When expressed in terms of the tissue water, potassium concentration was decreased only in the cat and rabbit (Table I).

Regardless of the state of hydration, the medullary water content in the rat was significantly higher than that of the cortex (Table II). While a 5-day water deprivation period caused a reduction in the water content of both the cortex and medulla, the percentage of water in the medulla remained significantly greater than in the cortex.

Discussion. The present experiments dem-

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