the implantation sites is not the same enzyme as that which is increased by estrogens.

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1. Hafez, E. S. E., Acta Endocrinol., 1964, v46, 217.
2. Manning, J. P., Meli, A., Steinetz, B. G., J. Endocrinol., 1966, v35, 385.
3. Atkinson, W. B., Elftman, H., Endocrinology, 1947, v40, 30.
4. Finn, C. A., Hinchliffe, J. R., J. Reprod. Fertil., 1964, v8, 331.
5. Leathem, J. H., Ann. N. Y. Acad. Sci., 1959, v75, 463.
6. Giering, J. E., Zarrow, M. X., Acta Endocrinol., 1958, v29, 449.
7. Watenabe, K., Fishman, W. H., J. Histochem. Cytochem., 1964, v12, 908.
8. Hayashi, M., Fishman, W. H., Acta Endocrinol., 1961, v38, 107.
9. Prahlad, K. U., ibid., 1964, v39, 407.
10. Manning, J. P., Steinetz, B. G., Babson, A. L., Butler, M. C., Enzymologia, 1966, v30, 309.
11. Talalay, P., Fishman, W. H., Huggins. C., J. Biol. Chem., 1946, v166, 757.
12. Manning, J. P., Babson, A. L., Butler, M. C., Priester, S. F., Can. J. Biochem., 1966, v44, 755.
13. Lobel, B. L., Tic, L., Shelesnyak, M. C., Acta Endocrinol., 1965, v50, 452.
14. ———, ibid., 1965, v50, 469.
15. Psychoyos, A., J. Endocrinol., 1963, v27, 337.
16. Shelesnyak, M. C., Kraicer, P. F., Zeilmaker, G. H., Acta Endocrinol., 1963, v42, 225.
17. Fishman, W. H., J. Biol. Chem., 1940, v136, 229.
18. ———, ibid., 1947, v169, 7.
19. Dallenbach-Hellweg, G., Battista, J. V., Dallenbach, F. D., Am. J. Anat., 1965, v117, 433.
20. Dallenbach-Hellweg, G., Dawson, A. B., Hisaw, F. L., ibid., 1966, v119, 61.
21. Manning, J. P., Hisaw, F. L., Steinetz, B. G., Kroc, R. L., Anat. Rec., 1967, v157, 465.

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Selenium-75: An Autoradiographic Study of its Disposition in Cartilage And Bone.* (32134)

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Both *in vitro* and *in vivo*, L-phenylalanine-C^{14} and L-leucine-C^{14} are incorporated into the protein moieties of the proteinpolysaccharides and collagen of cartilage(1,2). Through the use of these radioactive compounds it was possible to study autoradiographically the turnover of the protein portion of the epiphyseal plate of the rat concurrently with a study of the turnover of the S^{35}-labeled chondroitin sulfate(2). Since Se^{75} derived from $^{75}SeO_4^=$ is also fixed to the protein moieties of the proteinpolysaccharides of cartilage rather than to the chondroitin sulfate(3), it was of interest to study autoradiographically the fate of Se^{75} in rat epiphyseal cartilage and bone and to compare its disposition to that of $^{35}SO_4^=$ and the C^{14}-labeled amino acids.

Materials and methods. $Na_2{}^{75}SeO_4$ was prepared from $Na_2{}^{75}SeO_3$ (Oak Ridge National Laboratory, Oak Ridge, Tenn.) as described elsewhere(3). Sixteen 10-day old rats were injected intraperitoneally with 50 μc of $Na_2{}^{75}SeO_4$. After 4, 24, 48, and 72 hours, the rats were killed, and their tibiae and humeri removed. In another type of experiment, slices of costal cartilage from freshly killed calves were incubated for 2 hours at 37°C in a solution of salts which contained 50 μc of $Na_2{}^{75}SeO_4$(3). After incubation, the slices of cartilage were rinsed with water, a 0.5% solution of Na_2SeO_4, and

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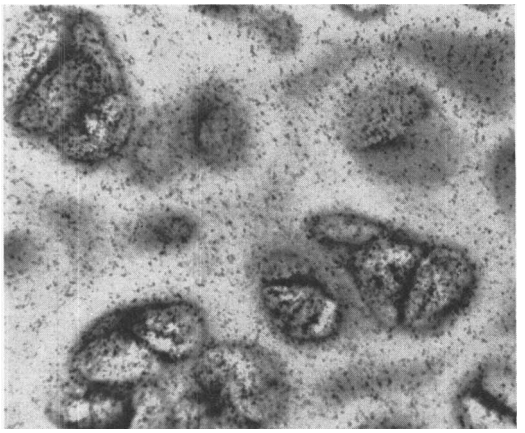

FIG. 1. Coated autoradiogram of a section of a slice of cartilage which had been incubated for 2 hr in a solution which contained $Na_2Se^{75}O_4$. The tissue section is stained with toluidine blue. Radioactivity, although apparent over the extracellular matrix, is more concentrated over the chondrocytes. ×533.

by 2 more rinses with water. These slices of cartilage and the rat bones were fixed in formalin, dehydrated in solutions of increasing concentrations of ethanol, cleared in xylene, and embedded in paraffin (64°C, mp). Sections of 7 μ thickness were cut, mounted and used to prepare coated autoradiograms with Nuclear Track Emulsion NTB-3(4) and contact autoradiograms with Contrast Process Ortho Film(5) (Eastman Kodak Co., Inc., Rochester, N. Y.).

Experiments were done in parallel using $Na_2{}^{35}SO_4$.

Before preparing the autoradiograms, representative sections were immersed in a decalcifying solution of dilute acetic acid for 15 minutes. Some of these, as well as other sections of costal cartilage were incubated in a solution of testicular hyaluronidase(6) to remove chondroitin sulfate.

The work described herein is representative of 4 replicate experiments.

Results. Fig. 1 is an autoradiogram from a cartilage slice which had been incubated in a solution of salts which contained $Na_2{}^{75}SeO_4$. There is much extracellular radioactivity evident and a greater concentration of radioactivity is seen over chondrocytes. When the labeled tissue sections are incubated with testicular hyaluronidase there is no greater loss of Se^{75} than after incubation in NaCl solution alone. $^{35}SO_4^{=}$, on the other hand, is almost completely removed following hyaluronidase digestion of cartilage sections prepared from slices which had been incubated in the presence of $Na_2{}^{35}SO_4$.

The intracellular concentration of the Se^{75} is seen also in epiphyseal plate cartilage 4 hours after injection into rats. At later times after injection of $Na_2{}^{75}SeO_4$, more of the radioactivity is seen in the extracellular cartilage matrix than in cells, however, the distribution of radioactivity between extracellular matrix and cells is never as clearly defined as with $^{35}SO_4^{=}$.

The greatest deposition of Se^{75} is in the metaphysis (arrows, Fig. 2, a-d) and is displaced towards the medullary cavity as a result of endochondral growth (Fig. 2, a-d).

In epiphyseal cartilage, the greatest concentration of Se^{75} is observed as a band overlying the proliferating and maturing

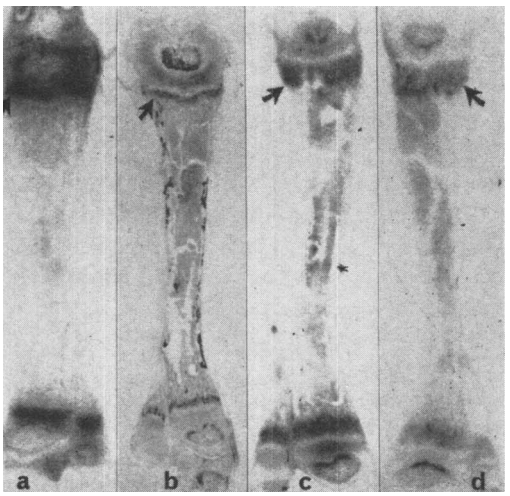

FIG. 2. Autoradiograms prepared from the tibiae of rats which were sacrificed 4 hrs (a), 24 hrs (b), 48 hrs (c), and 72 hrs (d) after they were injected with $Na_2Se^{75}O_4$. Lantern slide negatives were made from coated autoradiograms (b) and contact autoradiograms (a, c, d) and the prints made from these. ×15. At all times after injection, the majority of the radioactivity in the epiphyseal plates is in a band overlying the proliferating and mature chondrocytes. There is a relatively non-radioactive region interposed between this and the highly radioactive metaphysis (arrows). The metaphyseal radioactive reaction increases in length with increasing times after injection. The dark regions in the shaft bone of (b) are caused by refractile portions of bone in the histological section and do not represent radioactivity.

chondrocytes (Fig. 2, b). While this localization was not so obvious 4 hours after injection, it became more apparent by 24 hours. There is a relatively non-radioactive region between this radioactive band and the metaphysis which persisted for the duration of the experiment 72 hours (Fig. 2, a-d).

Decalcification of sections of bone resulted in a slight overall diminution of Se^{75}-radioactivity.

Mast cells show a marked uptake of S^{35} but they take up little Se^{75}.

The mass of selenium used in these experiments was extremely small, about 0.0025 mg Se/kg body weight and no toxic effects were noted. In other experiments Se^{75} of low specific activity was used, and as a result of the greater mass of selenium administered (7 mg/kg), all of the rats died within 24 hours. When non-radioactive selenate was given to rats in non-lethal doses (0.76-1.25 mg/kg/day) over a period of 3 days there resulted pathological alteration of the epiphyseal plate characterized by the following features: 1) widening of the epiphyseal plate, 2) disruption of chondroblast columniation, 3) apparent decrease in the number of chondroblasts, 4) decrease in the basophilia of the matrix, and 5) disturbed uptake and distribution of $^{35}SO_4^{=}$. These toxic effects were most pronounced in the proximal epiphyses of humeri and tibiae; these ends of the bones also take up the greatest amounts of Se^{75} (Fig. 2).

Discussion. The failure to remove significant amounts of radioactivity of Se^{75} from slices of bovine costal cartilage following digestion with hyaluronidase supports the observation that Se^{75} is not incorporated into chondroitin sulfate(3). From slices such as those shown in Fig. 1, proteinpolysaccharides were isolated in which it was found that the Se^{75} was affixed to the protein moiety(3). Similarly, in experiments done in conjunction with those reported herein, high levels of Se^{75} were detected in neutral salt and acid extracted collagen from rat tail tendons, but less than 1% of the whole rat liver radioactivity was accounted for in isolated liver glycogen and lipid fractions. On the basis of these observations, it can be concluded that the Se^{75} becomes associated with the protein components of the cartilage matrix. Since Se^{75} was not significantly removed from sections of bones which had been decalcified, it is probable that the isotope is also affixed to the protein of bone matrix, and not to the mineral phase.

The increased concentration of grains seen over chondrocytes relative to that over cartilage matrix suggests that Se^{75}-fixation may be dependent upon cellular activity. This suggestion is compatible with previous observations that proteinpolysaccharides isolated from boiled cartilage slices which were incubated in a solution of $^{75}SeO_4^{=}$, had specific activities which were only about 14% of those from viable cartilage(3).

The distribution of Se^{75} in epiphyseal plates of rats resembles that seen following injection of L-phenylalanine-C^{14} and L-leucine-C^{14}(2). Most of the activity is observed over the zones of proliferation and early maturation. The failure of the relatively non-radioactive region of the epiphyseal plate to acquire radioactivity was also seen with the C^{14}-amino acids(2). This possibly indicates a loss of protein coincidental with the calcification of cartilage in the zone of provisional calcification. The reduction in concentration of $^{35}SO_4^{=}$ in this region is less pronounced (2).

The distribution of Se^{75} radioactivity in the metaphysis is also reminiscent of that seen with L-phenylalanine-C^{14} and may reflect the deposition by osteoblasts of collagenous bone matrix on the calcified cartilage trabeculae (2). Ca^{45} is also deposited in this region of bone(2) but it is completely removed by decalcification procedures; C^{14}-amino acids and Se^{75} are not.

Summary. Following incubation of cartilage slices in a solution with $Na_2{}^{75}SeO_4$, or injection of $Na_2{}^{75}SeO_4$ into suckling rats, radioactivity is detected autoradiographically in long bones and cartilages. Histochemical examination revealed that the Se^{75} is associated with the protein rather than the polysaccharide or mineral components of these tissues. The disposition of Se^{75} in long bones resembles that seen after the injection of C^{14}-labeled amino acids.

1. Campo, R. D., Dziewiatkowski, D. D., J. Biol. Chem., 1962, v237, 2729.
2. ———, J. Cell. Biol., 1963, v18, 19.
3. Campo, R. D., Wengert, P. A., Jr., Tourtellotte, C. D., Kirsch, M. A., Biochim. Biophys. Acta, 1966, v124, 101.
4. Joftes, D. L., Lab. Invest., 1959, v8, 131.
5. Dziewiatkowski, D. D., J. Exp. Med., 1951, v93, 451.
6. Pearse, A. G. E., Histochemistry, Little, Brown & Co., 1960, p917.

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Renal Function Studies in the Early Stage of Salt Hypertension in Rats.* (32135)

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The evidence for a genetic component in the pathogenesis of human essential hypertension suggests that a prehypertensive state must exist and if so, that it may be possible to recognize the potentially hypertensive individual prior to the development of clinical hypertension. In experimental hypertension a genetic factor is manifested by the fact that we have developed two strains of rats, one of which is susceptible to, whereas the other is resistant to, manipulations commonly used to provoke experimental hypertension(1,2,3). We are exploring physiological parameters that may differentiate these two strains of rats before the onset of hypertension, in the hope that such differences will also be applicable in establishing the diagnosis of a "prehypertensive state" in man.

Previous studies have shown that rats of the strain prone to hypertension (the S strain) manifested an increased vascular reactivity in response to vasopressor agents(4) and had a relative aversion for NaCl when offered a free choice between water and saline(5) compared to those of the other (the R strain). On the other hand, rats of both strains responded similarly to acute salt loading(6) and had comparable values for total body sodium and exchangeable sodium(7).

The present report of renal function studies in the Sensitive strain was undertaken as part of a more general investigation of the role of the kidney in experimental hypertension. This study showed no evidence of gross renal dysfunction either before or during the early development of experimental hypertension from salt ingestion.

Material and methods. Thirty-one female weanling rats of the Sensitive (S) strain were maintained on a low salt chow (0.38% NaCl) and tap water until the age of 6 weeks, when they were randomly divided into 2 groups. At this time, half of the animals, the "high salt" group, were fed a diet containing 8% NaCl whereas the other half remained on the low salt chow, and served as controls. The first renal function studies were performed on 14 animals (7 from each group) after 2 weeks on this regimen; a second set of tests was performed on the remaining 17 animals (9 "high salt" and 8 controls)—after 6 weeks on the regimen.

The studies were carried out in unanesthetized, undisturbed rats, by a modification of the method of Kleinman *et al*‡(8). Two to three days before the clearance studies, polyethylene cannulae were introduced under ether anesthesia in the jugular vein, the abdominal aorta, and the dome of the bladder. The tubes were led subcutaneously to the back of the neck, exteriorized and shielded by a plastic tube attached to the skin. Thereafter the jugular and aortic cannulae were

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