EFFECTS OF PARATHYROID EXTRACT ON SERUM AND KIDNEY: I. Effect on Sulfur-35 Incorporation Into Components of Rat Serum and Kidney

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INTRODUCTION

Thomson and Collip (1932) and Selye (1932) postulated that parathyroid extract (PTE) has its major effects directly on the bone. Evidence for direct action of PTE on bone was presented by Barnicot (1948). Engel (1951) observed an increase in seromucoid following the administration of PTE, and proposed that this elevation resulted from depolymerization of bone matrix glycoprotein, which was then liberated into the blood. An increase in both serum total glycoprotein and seromucoid has been observed in this laboratory in PTE-treated rats (Shetlar, et al., 1956b). Increased amounts of "acid" and "neutral" polysaccharide have been demonstrated by histochemical techniques in the kidneys of PTE-treated rats, particularly associated with calcified lesions (Shetlar, et al., 1956b; 1958). The origin of these components of renal calcification is not known. The present study was undertaken to investigate the possibility that some of these components may be derived from the bone matrix.

Dziewiatkowski and co-workers (1949; 1951) have demonstrated the preferential incorporation of sodium sulfate-S^{35} into the sulfated mucopolysaccharides of the connective tissue. This paper describes preliminary results obtained on administering PTE to rats which had previously been given a tracer dose of sodium sulfate-S^{35}.

EXPERIMENTAL

Fifty male Holtzman rats, 223-304 gms (approximately 10 weeks of age), were

each given daily intraperitoneal injections of 1.0 ml of $Na_2S^{35}O_4$ (approximately 0.95 mc/ml) for 2 successive days. Two groups of ten rats were given no further treatment and served as controls; two groups of fifteen were given PTE beginning the second day after the last isotope injection. A total of 2.60 ml (260 units) of PTE was administered subcutaneously in divided doses to each rat over a 3-day period. Seventy-two hours after the first hormone injection, each rat was anesthetized with ether, the peritoneal cavity opened, blood drawn from the abdominal aorta, and the kidneys removed.

Serum samples obtained from animals of the respective groups were pooled. Serum radioactivity, before and after dialysis, was determined on aliquots from each group. Aliquots of the pooled sera of each group were separated by continuous paper curtain electrophoresis, using 0.050 M barbital buffer, pH 8.6 (modified from Shetlar, et al., 1956a), and radioactivity determinations made on the resulting fractions after dialysis.

One kidney from each group was placed in 10% buffered formalin immediately after removal. Tissue sections for autoradiography were prepared according to the method of Dziewiatkowski (1956). After a one-month exposure, these autoradiograms were developed, and sections studied both unstained and after hematoxylin staining. The remainder of the kidneys from each group were separated chemically following a scheme for the isolation of mucopolysaccharides. The kidneys were homogenized in isotonic saline, yielding a "Saline Extract" and a precipitate, which was extracted with 95% ethyl alcohol. The alcohol-insoluble material was suspended in 0.5 N NaOH; the alkali-soluble fraction was then neutralized and alcohol added to a concentration of 64%. The resulting precipitate ("64% Alcohol Ppt.") was removed and alcohol added to a concentration of 84%. A precipitate ("84% Alcohol Ppt.") was removed from the alcoholic solution (84% Alcohol Soluble"). The resulting fractions were separated by paper electrophoresis, then the paper strips were treated with dyes to demonstrate specific components, as follows: protein demonstrated by bromphenol blue; glycoprotein by periodic acid-Schiff (PAS); acid mucopolysaccharide by Alcian Blue. In addi-

tion, radioactivity determinations were made on each fraction before and after dialysis. Each fraction was then treated with 0.66 N perchloric acid (PCA). The PCA-precipitate of each fraction was re-dissolved, and aliquots taken for radioactivity determinations and paper electrophoresis. The PCA-supernatant of each fraction was dialyzed and studied similarly.

RESULTS

The data in Table 1 show that the S^{35}-radioactivity in the serum of the PTE-treated rats is approximately twice that of the control group, and that only a portion of this activity is dialyzable. Fig. 1 indicates that this increased amount of non-dialyzable radioactivity in the serum of the PTE-group distributes in two major areas relative to serum protein, i.e., (1) the slow-moving albumin or fast α-globulin region, and (2) the slow β- or fast γ-globulin region. Only the first radioactivity peak corresponds with a peak found in the control serum.

TABLE 1

SERUM RADIOACTIVITY

	BEFORE DIALYSIS		AFTER DIALYSIS	
SAMPLE	counts/min/ml	Ratio	counts/min/ml	Ratio
PTE	25482	1.9	13110	1.3
Control	13299		9982	

The whole homogenates of the kidneys from the control and the PTE-treated rats were found to contain substantial radioactivity; the homogenate from the PTE-treated group had approximately 1.3 times the radioactivity found in the homogenate from the control group. Each fraction resulting from these two homogenates had radioactivity, only a portion of which was dialyzable. The Saline Extract contained the majority of the total radioactivity, both before and after dialysis. The kidneys of the PTE-treated rats had increased amounts of S^{35}-incorporating components in the fractions precipitated from the alkaline extract by both 64% and 84% alcohol concentrations, as shown in Table 2.

Paper electrophoretic analysis of the kidney fractions revealed increased

TABLE 2

RADIOACTIVITY DISTRIBUTION IN KIDNEY FRACTIONS
(After Dialysis)

KIDNEY FRACTION	counts/min/kidney CONTROL	PTE	RATIO: PTE/CONTROL
64% Alcohol Ppt.	10300	21000	2.04
84% Alcohol Ppt.	1600	2100	1.31

amounts of PAS-positive and Alcian Blue-positive components in the 64% Alcohol Ppt. from the PTE-treated group. When the sub-fractions obtained with PCA were separated by paper electrophoresis, increased amounts of PAS-positive and Alcian Blue-positive components were seen in the Saline Extract-PCA soluble sub-fraction of the PTE-treated group. This latter Alcian Blue-positive component coincided well with a radioactivity band demonstrated on the paper strip. A more pronounced increase in PAS-positive and Alcian Blue-positive components was found in the 64% Alcohol Ppt.-PCA insoluble sub-fraction of the PTE group. Unstained autoradiograms prepared from a kidney of the PTE-treated group revealed discrete radioactive areas, which corresponded with the hematoxylin-positive calcified areas observed in the stained autoradiograms. No corresponding radioactive areas were seen in the kidney of the control animals.

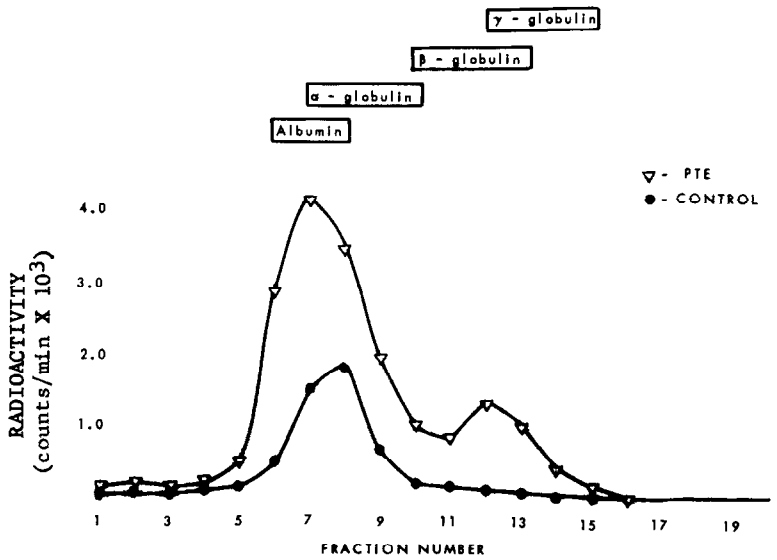

FIGURE 1. ELECTROPHORETIC DISTRIBUTION OF NON-DIALYZABLE SERUM RADIOACTIVITY

DISCUSSION

An increase in sulfur-35 radioactivity was observed in the serum of the PTE-treated rats, confirming the observations of Bronner (1957). The increase in non-dialyzable activity of this serum appears in two electrophoretic bands, one which may represent an increased amount of a normal constituent, and the other a new constituent not found in the control serum. The observed increase in S^{35}-radioactivity in the serum of the PTE-treated rats is consistent with the results which would be expected if the increased sulfomucopolysaccharide or sulfur-containing glycoprotein found in the kidney of the PTE-treated rat were released from the bone matrix and carried to the kidney by the blood. Characterization of the constituents of these two bands will aid in further evaluation of these observations.

The finding that those kidney fractions which should contain much of the acid mucopolysaccharides from the PTE-treated rats have relatively more S^{35}-incorporating components than the corresponding fractions from the controls would appear to indicate that these components originate, at least in part, outside the kidney. The increase in the total radioactivity of the kidney several days after sulfur-35 administration, together with the increase in the S^{35}-activity of the serum, tends to support the hypothesis that bone matrix or other connective tissue is the ultimate source of the material found in the kidney lesions after PTE administration.

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REFERENCES

1. Barnicot, N. A., J. Anat. 82, 233 (1948).
2. Bronner, F., Fed. Proc. 16, 158 (1957).
3. Dziewiatkowski, D. D., Benesch, R. E., and Benesch, R., J. Biol. Chem. 178, 931 (1949).

4. Dziewiatkowski, D. D., J. Biol. Chem. 189, 187 (1951).

5. Dziewiatkowski, D. D., private communication (1956).

6. Engel, M. B., A.M.A. Arch. Path. 53, 339 (1951).

7. Selye, H., Endocrinology 16, 547 (1932).

8. Shetlar, M. R., Cahill, C., Stidworthy, G., and Shetlar, C. L., Proc. Soc. Exper. Biol. & Med. 93, 44 (1956a).

9. Shetlar, M. R., Howard, R. P., Joel, W., Courtright, C. L. and Reifenstein, E. C., Jr., Endocrinology 59, 532 (1956b).

10. Shetlar, M. R., Howard, R. P., Joel, W., Bowling, D. C., and Shetlar, C. L., Am. J. Physiol. 195, 535 (1958).

11. Thomson, D. L. and Collip, J. B., Physiol. Rev. 12, 309 (1932).