66–1872

HURST, Max Wayne, 1937–

EFFECT OF PARATHYROID EXTRACT ON THE KIDNEY MUCOPOLYSACCHARIDES AND SERUM S^{35}–CONSTITUENTS OF DOGS GIVEN SODIUM SULFATE–S^{35}.

The University of Oklahoma, Ph.D., 1966
Chemistry, biological

University Microfilms, Inc., Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

EFFECT OF PARATHYROID EXTRACT ON THE KIDNEY MUCOPOLYSACCHARIDES AND SERUM S^{35}-CONSTITUENTS OF DOGS GIVEN SODIUM SULFATE-S^{35}

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

MAX WAYNE HURST

Oklahoma City, Oklahoma

1966

EFFECTS OF PARATHYROID EXTRACT ON THE KIDNEY MUCOPOLYSACCHARIDES AND SERUM S^{35}-CONSTITUENTS OF DOGS GIVEN SODIUM SULFATE-S^{35}

APPROVED BY

Reagan H. Bradford

Mark R. Everett

Wallace Friedberg

Paul B. McCay

A. Kurt Weigl

DISSERTATION COMMITTEE

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to members of the Biochemistry Department of the University of Oklahoma Medical School and to the members of the Cardiovascular Section of the Oklahoma Medical Research Foundation for their support and advice during the course of this investigation. Special gratitude is expressed to Dr. R. H. Bradford and Dr. M. R. Shetlar who directed this research. Thanks are also due the members of the Dissertation Committee, Dr. R. H. Bradford, Dr. M. R. Everett, Dr. W. Friedberg, Dr. P. B. McCay and Dr. A. K. Weiss, for their advice during the completion of this report. Special gratitude is expressed to my wife without whose aid the completion of this work would not have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

EFFECT OF PARATHYROID EXTRACT ON THE KIDNEY MUCOPOLYSACCHARIDES AND SERUM S^{35}-CONSTITUENTS OF DOGS GIVEN SODIUM SULFATE-S^{35}

CHAPTER I

INTRODUCTION

The effects of parathyroid hormone on calcium and phosphate metabolism have been known for a number of years. The ability of this hormone to produce calcification in several tissues, including the kidney, lung, heart and gastrointestinal tract, has also been recognized for some time.

Parathyroid extract has been shown more recently to affect the metabolism of the glycoproteins and mucopolysaccharides of serum and tissue. The administration of parathyroid extract for a period of several days increased the serum glycoprotein level and the glycoprotein and mucopolysaccharide components of kidney, as demonstrated by histochemical techniques. In rats given sodium sulfate-S^{35}, parathyroid extract produced an increase in the S^{35}-containing constituents of serum and kidney. Radioautographic study of kidney sections demonstrated that the sulfur-35 was concentrated in the calcification lesions, presumably as sulfated mucopolysaccharides.

The objectives of the study reported here were to characterize the mucopolysaccharides of the kidney and the sulfur-35 constituents of the serum of dogs given sodium sulfate-S^{35} and to evaluate the effect of parathyroid extract on these substances.

CHAPTER II

HISTORICAL REVIEW

Isolation and Purification of Parathyroid Hormone

Remak (1) first observed the parathyroid glands in 1851 while dissecting a cat. Owen (2) provided in 1862 a gross anatomical description of these glands after dissection of a rhinoceros, but attributed no physiological function to them. Sandstrom (3), who assigned the name parathyroid to these glands, confirmed the description provided by Owen. The physiological function of the parathyroids in regulating calcium and phosphate metabolism was established in the early twentieth century by several investigators, including MacCallum and Voegtlin (4), Greenwald (5, 6) and Collip (7).

Hanson (8, 9) reported in 1924 that a hot dilute solution of hydrochloric acid would extract material capable of raising serum calcium in hypocalcemic human subjects. Another crude parathyroid extract with physiological activity was prepared in 1925 by Collip (7, 10), who showed that this extract prevented tetany in parathyroidectomized dogs. The hormonal principle has been extracted using several solvents, including hot dilute hydrochloric acid (7), 80 percent acetic acid (11) and phenol (12). More recently, Rasmussen (13) has prepared a stable and uniform product using an extraction mixture containing urea, cysteine and hydrochloric acid.

Purification of this hormone has been achieved by several procedures, including salt or solvent fractionation, dialysis or ultrafiltration, zone electrophoresis, countercurrent distribution, column chromatography and gel filtration. One of the most successful methods for purifying the hormonally active polypeptide has utilized countercurrent distribution, as described by Rasmussen and Craig (14 - 16). More recently, Rasmussen and co-workers (13) applied successfully gel filtration on Sephadex for purifying the parathyroid polypeptide. This procedure is simpler and requires less expensive equipment than the countercurrent distribution method.

Biological assay of the calcemic and phosphaturic activities of parathyroid hormone has been developed by Davies et al. (17, 18) and Munson et al. (19, 20). These much improved assay systems permitted an accentuation of interest in this field during the last decade.

Isolation and Purification of Calcitonin

Copp and co-workers (21) first postulated that the parathyroid glands had a second hormone whose physiological properties differed from those of parathyroid hormone. Copp (22, 23) obtained further evidence for the existance of this hormone, which he designated calcitonin, by demonstrating that hypocalcemia was produced when he perfused the parathyroid gland with hypercalcemic solutions or when he administered extracts of beef parathyroid glands. Hirsch (24) isolated a substance from thyroid with properties similar to those described by Copp. This was purified 50 to 100 fold by centrifugation and gel filtration. Baghdiantz et al. (25) confirmed the presence of a hypocalcemic factor in thyroid gland. Care (26) obtained evidence for the presence of a

hypocalcemic factor in thyroid by perfusing pig thyroid glands which had been shown to be devoid of parathyroid tissue. Arnaud (27) described a procedure for isolating the hypocalcemic factor from pork thyroid glands. A purified polypeptide with a molecular weight of 8500 was obtained by extraction with a mixture containing urea, cysteine and hydrochloric acid, solvent and salt fractionation, trichloroacetic acid precipitation and gel filtration.

Effects of Parathyroid Hormone and Calcitonin

An equilibrium apparently exists between parathyroid hormone and calcitonin production. Thus, hypocalcemia seems to stimulate parathyroid hormone production, thereby increasing the serum calcium. Hypercalcemia conversely seems to stimulate calcitonin production, thereby decreasing the serum calcium. The possibility that other actions attributed to parathyroid hormone are also antagonized by calcitonin should be considered.

Two principle sites of action of parathyroid hormone have been proposed. Albright and Reifenstein (28) postulated in 1948 that the phosphaturia following parathyroid hormone administration resulted from a direct effect on phosphate resorption from the renal tubule. Direct evidence supporting this hypothesis was obtained by Lavender *et al.* (29, 30) and subsequently confirmed by Horwith *et al.* (31).

Thomson and Collip (32) and Selye (33) proposed that parathyroid hormone exerted a direct action on bone to explain the increased number of osteoclasts observed in bone after administering parathyroid hormone (33) and the hypocalcemia observed following parathyroidectomy (32). Barnicot (34) and, subsequently, Chang (35) presented evidence supporting

this hypothesis by showing that transplanting the parathyroid glands to a position adjacent to the bone surface resulted in localized bone resorption. Gaillard (36 - 38) has provided additional support for this concept by studies *in vitro* on bone slices.

A third site of action of parathyroid hormone is the stimulation of intestinal calcium absorption. This phenomenon has been studied *in vivo* in isolated intestinal loops by Talmage (39) and *in vitro* in small intestinal sacs by Schachter (40 - 42).

These studies suggest that parathyroid hormone may alter calcium and phosphate metabolism as a result of its action on several tissues. The effects observed *in vivo* are apparently the composite of its actions at these and possibly other sites.

Engel (43) reported that parathyroid extract increased the seromucoid levels in rats. Shetlar *et al.* (44) confirmed this observation and found also that the serum glycoprotein was increased. Shetlar *et al.* (45) also demonstrated by histochemical techniques that parathyroid extract altered neutral and acid mucopolysaccharide constituents of rat kidney. Bradford *et al.* (46) reported that parathyroid extract increased the sulfur-35 content of serum and kidney in rats given sodium sulfate-S^{35}. These investigators proposed that the increased quantities of kidney glycoprotein and mucopolysaccharide may have been derived from some extrarenal source, probably the bone matrix, and transported to the kidney in blood.

Acid Mucopolysaccharide Chemistry

The development of mucopolysaccharide chemistry began with the isolation in 1861 of chondroitin sulfate A by Fischer and Boedeker (47).

Chondroitin sulfate C was first isolated by Meyer and Palmer (48) and chondroitin sulfate B by Meyer and Chaffee (49). Keratosulfate was isolated and characterized by Meyer (50). McLean (51) isolated heparin and described its anticoagulant properties. Heparin monsulfate was isolated by Jorpes and Gardell (52) and by Linker et al. (53). Meyer and Palmer (54) isolated and characterized hyaluronic acid.

Stacey (55, 56) demonstrated that chondroitin sulfate contained glucuronic acid and galactosamine. The monosaccharide constitutents of the acid mucpolysaccharides as tabulated by Walker (57), are presented in Table 1.

The use of sodium sulfate-S^{35} as a precursor of the sulfated acid mucopolysaccharides was demonstrated by Dziewiatkowski (58, 59) who also showed that the majority of the administered radioisotope appeared as inorganic sulfate in the urine and feces within a few days. Friberg (60) reported that the radioactive sulfate which is retained in the animal is associated with the tissue components which fail to stain using the periodic acid-Shiff procedure. Curran and Kennedy (61) reported that the sulfur-35 is associated with substances which react metachromatically with toluidine blue and stain with Alcian Blue. It has also been demonstrated that sulfate exchange does not occur with previously synthesized sulfated acid mucopolysaccharides. Schiller et al (62) showed that viable cells are required for sulfate incorporation into acid mucopolysaccharide which occurs at a very early stage of polysaccharide synthesis.

Acid Mucopolysaccharide Purification

The resolution of crude mucopolysaccharide mixtures has constituted a major problem. Meyer et al. (63) attempted to separate mucopolysac-

charides by ethanol fractionation of the calcium salts. A similar procedure using the barium salts was utilized by Jorpes and Gardell (52) and Smith and Gallop (64). Gardell (65) modified the ethanol fractionation method by using a cellulose column saturated with 80 percent ethanol and 3 percent barium acetate. The polysaccharide was dissolved in water and added to the column. After precipitation on the upper portion of the column, the polysaccharide was eluted from the column by altering the concentration of ethanol applied.

Electrophoresis on kieselguhr (66) and on paper (67) have been used for the separation of acid mucopolysaccharides.

The lack of suitable solvent systems has made impossible the application of countercurrent distribution or partition chromatography techniques, although Wolfrom et al. (68) purified commercial heparin using the Craig procedure and Berenson et al. (69) separated hyaluronic acid from chondroitin sulfate by reverse phase chromatography on kieselguhr.

The use of quaternary ammonium compounds for precipitating acid mucopolysaccharides was introduced by Scott (70) and Stacey (71). Scott (72) showed that different concentrations of various salt solutions were required to redissolve the precipitated acid mucopolysaccharides. Although hyaluronic acid, chondroitin sulfate and heparin have been separated using this procedure, it is difficult to separate the individual chondroitin sulfates from each other or from heparin monosulfate. Gardell (73) observed that keratosulfate behaved abnormally with quaternary ammonium compounds and tended to redissolve at low salt concentrations in the presence of high concentrations of cetyl pyridinium chloride. Gardell et al. (73) utilized column chromatography of the mucopolysac-

charide mixture on cellulose of kieselguhr saturated with quaternary ammonium compound. Following application of the sample in water solution, the acid mucopolysaccharides were eluted with increasing salt concentrations.

Ringertz and Reichard (74) separated acid mucopolysaccharides on Ecteola columns by elution with sodium chloride at low pH.

Davidson and Meyer (75) first used Dowex-1 for separating mucopolysaccharide fractions from bovine cornea. Schiller et al. (76) modified this method to separate known mixtures of hyaluronic acid, heparin monosulfate, chondroitin sulfate and heparin by eluting the column with increasing concentrations of sodium chloride. Keratosulfate was eluted from the column over a range of salt concentrations. This method was used to isolate heparin from rat skin after previous cetyl pyridinium chloride precipitation.

Berman (77) reported that DEAE-Sephadex could be used to prepare hyaluronic acid from bovine vitreous humor. The hyaluronic acid was eluted with sodium chloride gradient at neutral pH. Schmidt (78) applied this procedure successfully to the separation of hyaluronic acid, heparin monosulfate, chondroitin sulfate and heparin.

Acid Mucopolysaccharides of Kidney

Baker and Sison (79), Ginetzinsky (80) and Bradford et al. (81) demonstrated by histochemical techniques the presence of mucopolysaccharides in the normal kidney. Marx et al. (82) and Monkhouse et al. (83) found anticoagulant activity, presumably due to mucopolysaccharides, in the kidney. Allalouf (84) isolated a heparin-like compound from rat kidney.

CHAPTER III

EXPERIMENTAL PROCEDURES

Animal Treatment Protocol

Male mongrel dogs, 18 - 26 kg, were fed a standard animal diet (Superior Dog Food) and given water ad libitum. The dosage of sodium sulfate-S^{35} in each instance was 1 mc per kg of body weight, administered in a single subcutaneous injection. The radioisotope was injected in an aqueous solution containing 5 mc per ml. Parathyroid extract (commercial preparation containing approximately 100 units per ml, obtained from Eli Lilly Company), 8 units per kg per day, was injected subcutaneously in equal doses twice daily beginning 13 days after administration of the sodium sulfate-S^{35}. The quantity of parathyroid extract was increased after 10 days to 16 units per kg of body weight per day in two equal doses.

Four dogs (Dogs A, B, F and G) served as their own controls and were studied before and during treatment with the parathyroid extract. Each was given sodium sulfate-S^{35} at the beginning of the control period. Approximately 60 days later, a second injection of sodium sulfate-S^{35} was administered and the dogs were subsequently treated with parathyroid extract. One group of two dogs (Dogs H and O) was given only sodium sulfate-S^{35} and served as controls. A second group of dogs (Dogs I, K, L and M) was given sodium sulfate-S^{35} and, subsequently, treated with

parathyroid extract.

Blood was drawn each day for calcium and radioactivity determinations. The dogs were sacrificed 25 days after radioisotope administration by exsanguianation via the carotid artery. The blood and the kidneys were obtained. The capsule was removed from the kidney and the remainder frozen.

Separation of S^{35}-Containing Serum Components

The serum was separated using Sephadex G-200 column chromatography, following the procedure of Flodin (85). This was accomplished by applying 25-ml aliquots of serum onto a 4 by 47 cm column of the gel. The column was maintained at 4^{o} C and eluted with 0.1 M TRIS-HCl buffer, pH 8.0. The eluting solvent was maintained at a height sufficient to obtain a flow rate of 35 to 40 ml per hour. The eluate was collected in portions of 4.5 to 5.0 ml with the aid of an automatic fraction collector.

Radioactivity determinations demonstrated that the sulfur-35 had been eluted in two regions of the chromatogram. The eluates were combined to obtain these two fractions for further study. Aliquots of these fractions were dialyzed against distilled water for 48 hours. The radioactivity of the fractions was determined before and after dialysis. Aliquots were also applied to a Dowex-50 column, eluted sequentially with water, 0.3 N HCl and 4.0 N HCl and the radioactivity of each eluate determined.

Isolation of Mucopolysaccharide Fractions From Kidney

Crude Mucopolysaccharide Fraction

The kidney was cut into pieces measuring approximately 1 cm^3 and

lyophilized. The dried tissue was then subjected to exhaustive delipidization with a mixture of chloroform and methanol (2:1, v/v) in a Soxhlet extractor for 12 hours. After removing the excess of Folch reagent *in vacuo*, the dry, delipidized material was ground in a Wiley mill. Following the procedure of Allalouf (84), 5-g aliquots of this material were stirred for 4 hours at room temperature in 200 ml of 0.5 N NaOH. The extract was dialyzed against running tap water for 48 hours at 4°C. Ethanol was added to a final concentration of 5 percent and calcium added to 0.01 M. Fifty milligrams of Pronase (purchased from California Corporation for Biochemical Research) was added and the mixture incubated in a dialysis bag at 37°C for 24 hours. The mixture was dialyzed continuously against 0.05 M TRIS buffer with ethanol added to 5 percent and calcium acetate added to 0.01 M. After 24 hours, the buffer was changed and 50 mg of Pronase was added. Twenty-four hours later, the contents of the dialysis bag were removed and trichloroacetic acid was added to a final concentration of 10 percent. The precipitate obtained by centrifugation at 10,000 g was discarded. The supernatant portion was dialyzed against distilled water for 72 hours at 4°C. This solution was then reduced to approximately one-tenth volume and sufficient water was added to bring the volume to 80 ml. Sodium acetate was added to a final concentration of 5 percent (w/v) and ethanol added to 80 percent. The mixture was allowed to settle overnight at 4°C before the precipitate was collected by centrifugation. The precipitate was dissolved in water and the solution was passed through a column of Dowex-50. After eluting the column with three column volumes of water, the combined water eluates were lyophilized. The resulting light cream colored powder was then

designated the crude mucopolysaccharide fraction. This fraction was analyzed and aliquots taken for further purification on DEAE-Sephadex.

Purified Mucopolysaccharide Fractions

The crude mucopolysaccharide fraction was purified further by DEAE-Sephadex A-25 column chromatography. This was accomplished by a modification of the procedure of Schmidt (78). Three-milliliter aliquots containing 15 mg of the crude mucopolysaccharide fraction were applied to 0.9 by 40.0 cm columns of DEAE-Sephadex A-25. After the sample was placed on the column, an additional 5 ml of water was added. The uronic acid positive material was eluted by stepwise increases of a salt concentration gradient, using sequential addition of six 5-ml aliquots of 0.1 N NaCl through 1.0 N NaCl (0.1 N steps). All eluting solutions were made up in 0.01 N HCl. This 300 ml of eluting solvent was followed by 60 ml. of 2.0 N NaCl in 0.01 N HCl. The separation was carried out at room temperature and required 3 to 4 hours. The eluates were collected in 5-ml portions, which were combined subsequently to obtain the four fractions described in Figure 8. Purified mucopolysaccharide fractions were dialyzed for 24 hours against distilled water and lyophilized. Analyses for radioactivity, protein and monosaccharide constituents were obtained.

Radioisotope Analysis

The radioactivity of the serum and crude mucopolysaccharide fraction from the kidney was determined using a Nuclear-Chicago gas flow counter. Appropriate corrections were made for background, self-absorption and half-time decay. The radioactivity of the purified mucopolysaccharide

fractions from kidney was determined using a Nuclear-Chicago liquid scintillation unit. The quench curve for sulfur-35 was established and all determinations were corrected by the "channels ratio" method. Appropriate corrections were also made for background and half-time decay.

Analytical Methods

Electrophoresis

The acid mucopolysaccharides were separated by paper electrophoresis in a Durrum cell, using lithium sulfate buffer and a potential of 60 volts for 16 hours, as described by Foster and co-workers (86).

Paper Chromatography

Solvent mixtures containing ethyl acetate, pyridine and water (12:5:4) and ethyl acetate, pyridine, acetic acid and water (5:5:1:3) were utilized for paper chromatography, following the method of Fischer et al. (87).

Chemical Methods

Calcium was analyzed by the method of Kingsley and Robnett (88), sulfate by a modification of the method of Antonopoulos (89), total hexose by a modification of the tryptophane method of Shetlar et al. (90), protein by the method of Lowry et al. (91) and hexosamine by the Elson-Morgan technique, as modified by Boas (92).

Hydrolysis of the tissue for uronic acid determination was performed by the method of Shetlar et al. (93). A purified resin, Dowex-50 x 12, 100 to 200 mesh, was obtained from California Biochemical Corporation and treated before use by adding 4 N HCl and mixing overnight on a

magnetic stirrer. The acid was decanted and the resin washed with water until free from chloride. Twenty-five milligrams of tissue and 10 ml of 0.1 N H_2SO_4 were mixed with 4 g of resin and hydrolyzed with continuous agitation in an oven at 80°C for 72 hours. The resin was then removed by filtration and the clear solution analyzed for uronic acid by the carbazole method of Bitter and Muir (94).

CHAPTER IV

RESULTS

Response of Dogs to Parathyroid Extract

Figure 1 illustrates the hypercalcemia produced by administering parathyroid extract to dogs over a period of several days. Each dog served as his own control. The serum calcium level increased within 24 hours and subsequently remained above control values. When the dosage of parathyroid extract was increased, and additional increment in serum calcium was observed. The response illustrated her is typical of each dog given parathyroid extract.

Figure 2 illustrates the increase in urine volume observed during parathyroid extract administration. This increase in urine volume occurred within 72 hours after the administration of parathyroid extract was begun. The volume remained at elevated levels and was increased further when larger doses of parathyroid extract were subsequently begun.

Figure 3 demonstrates the effect of parathyroid extract on the radioactivity excreted in the urine of dogs previously given sodium sulfate-S^{35}. The urine radioactivity increased within 72 hours after treatment with parathyroid extract was begun and remained above control levels until the experiment was terminated. An additional increment in urine radioactivity was observed when the parathyroid extract dosage was increased.

Serum Constituents Containing Sulfur-35

Bradford et al. (46) observed that the serum sulfur-35 radioactivity increased within a few hours after treatment of rats with large doses of parathyroid extract was begun. The serum radioactivity was determined periodically, therefore, through these experiments. Figure 4 illustrates the disappearance curves for serum S^{35}-radioactivity in dogs before and during treatment with parathyroid extract. Within 24 hours after treatment with parathyroid extract was begun, the serum radioactivity increased and remained elevated for an interval of approximately 48 hours. A second increase in serum radioactivity occurred when the dosage of parathyroid extract was increased. The latter elevation of serum radioactivity was maintained until the animals were sacrificed 48 hours later.

Aliquots of these serum samples were separated using Sephadex G-200 column chromatography and the eluates collected into two fractions, as shown in Figure 5. Fraction 1 was the first eluted from the column and contained radioactivity and protein. Fraction 2, which subsequently was eluted from the column, contained radioactivity but no protein. No significant difference in the relative amounts of sulfur-35 in Fractions 1 and 2 was observed between control and parathyroid treated dogs. These two fractions were dialyzed and the percent of nondialyzable radioactivity was determined (Table 2). Essentially, none of the sulfur-35 in Fraction 1 and almost all of that in Fraction 2 was dialyzable.and consequently different molecular weight.

The chemical nature of the S^{35}-constituents of Fractions 1 and 2 was evaluated by separating aliquots of these fractions using Dowex-50

column chromatography (Table 3). The majority of the S^{35}-constituents of both Fractions 1 and 2 of the control and parathyroid extract treated dogs was eluted with water. Less than 10 percent of the radioactivity was eluted with 4 N HCl suggesting that sulfur containing amino acids did not account for an appreciable proportion of the total serum sulfur-35.

Kidney Tissue Studies

There was no change in kidney weight observed following parathyroid extract treatment.

The analyses of the monosaccharide components of the delipidized kidney powder are shown in Table 4. The average of the uronic acid contents of the kidney from the parathyroid extract treated dogs was approximately 30 percent greater than that of the controls. In each instance, the ratio of hexosamine to uronic acid exceeded two and one half.

The uronic acid, sulfate and radioactivity analyses of the crude mucopolysaccharide fraction from the kidney of each dog are presented in Table 5. Radioactivity values for Dogs F and C were omitted because these dogs had received an additional dose of sodium sulfate-S^{35} in the period immediately preceding this study. Although the serum radioactivity in these two dogs had fallen to background values, it was considered probable that detectable amounts of sulfur-35 were retained in tissue components, thus explaining the observation that the specific radioactivity values of the crude mucopolysaccharide fraction from the kidney of each of these dogs were appreciably higher than those of the other parathyroid extract treated or control dogs. Table 5 demonstrates that

the average mucopolysaccharide content of the kidney, based on average uronic acid concentration of the crude mucopolysaccharide fraction, is increased approximately 30 percent in the dogs given parathyroid extract. These dogs also had an increased proportion of uronic acid in the crude mucopolysaccharide fraction. These results indicate a quantitative and a qualitative change in the mucopolysaccharide fraction. The average amount of sulfate in this fraction also appeared slightly higher in the parathyroid extract treated dogs, but a considerable overlap of individual values between groups was noted. These results suggest that the material present in increased amounts following parathyroid extract treatment might be a nonsulfated acid mucopolysaccharide. The total and specific radioactivities, conversely, appeared to be reduced substantially (to approximately 50 percent of control value) in the parathyroid extract treated group.

Figure 6 illustrates the results obtained when the hydrolyzate of the crude mucopolysaccharide fraction was separated by paper chromatography. This fraction from the kidney of each dog contained mannose, glucose, galactose, glucosamine and galactosamine. The increased migration of the hexosamines was apparently due to the salt content of the crude mucopolysaccharide fraction, since this effect was not observed with the partially purified mucopolysaccharide fractions. A second aliquot of the crude mucopolysaccharide fraction was hydrolyzed at 80°C for 48 hours using Dowex-50 (hydrogen form). Paper chromatographic analysis demonstrated glucuronic acid. No other compound with a similar mobility to the glucuronic acid or galacturonic acid standards was observed, although no iduronic acid was available for comparison. Electro-

phoretic separation and Alcian Blue staining of this material demonstrated three bands which corresponded to hyaluronic acid, chondroitin sulfate and an unknown fraction of intermediate electrophoretic mobility (Figure 7).

The protein and monosaccharide contents and radioactivity of the aliquots of the crude mucopolysaccharide fraction applied to the DEAE-Sephadex columns are shown in Table 6. These data demonostrate that the hexosamine to uronic acid ratio of this material exceeded one in all instances, indicating contamination of the acid mucopolysaccharide fraction by a significant amount of glycoprotein.

Figure 8 illustrates the separation of the crude mucopolysaccharide fractions by DEAE-Sephadex column chromatography. The majority of the uronic acid-containing material was eluted in Fractions 2, 3 and 4. (Table 7). Paper electrophoresis of these four fractions demonstrated that a relatively complete separation of the individual mucopolysaccharide components had been obtained, with the exception that Fraction 4 contained a small amount of an Alcian Blue positive material with the electrophoretic mobility of that in Fraction 3 (Figure 9). These fractions were analyzed for protein, radioactivity and individual monosaccharides (Table 7 and 8). An increased proportion of the total uronic acid was found in Fraction 2 in the group given parathyroid extract. The hexosamine to uronic acid ratios of Fractions 3 and 4 were below one in all cases, indicating the absence of glycoprotein contamination.. These four mucopolysaccharide fractions were hydrolyzed and their monosaccharide constituents identified by paper chromatographic analysis (Figure 10). Glucosamine was found in Fractions 1, 2 and 3, galactosamine in Fraction

4 and galactose in Fraction 1. Separation of these hydrolyzates in a second solvent system yielded similar results.

Electrophoretic separation of these partially purified mucopolysaccharide fractions indicated the presence of three Alcian Blue positive materials in the kidney (Figure 9). Fraction 1 had no Alcian Blue positive material. This fraction contained relatively appreciable amounts of hexosamine (glucosamine), hexose (galactose), and protein, but little uronic acid or sulfur-35. It is presumed, therefore, that most of the carbohydrate in this fraction was present as glycoprotein. Fraction 2 had the lowest electrophoretic mobility, corresponding with hyaluronic acid. This fraction contained glucosamine, uronic acid (glucuronic acid was the only uronic acid observed in the crude mucopolysaccharide fraction) and relatively little sulfur-35 and thus had a composition consistent with that expected for hyaluronic acid (Table 1). Fraction 3 contained a diffuse mucopolysaccharide band of intermediate electrophoretic mobility which did not correspond with any available standards (hyaluronic acid, chondroitin sulfate or heparin). This fraction contained glucosamine, uronic acid (glucuronic acid was the only uronic acid demonstrated in the crude mucopolysaccharide fraction) and sulfur-35 and had an electrophoretic mobility which was greater than hyaluronic acid and less than chondroitin sulfate. The composition and the electrophoretic mobility of this mucopolysaccharide are similar to those previously reported for heparin monosulfate. Fraction 4 had the highest electrophoretic mobility, similar to that of chondroitin sulfate. This fraction contained galactosamine, uronic acid (glucuronic acid was the only uronic acid observed in the crude mucopolysaccharide fraction) and

a relatively large proportion of the sulfur-35 and thus had a composition similar to chondroitin sulfate (Table 1).

CHAPTER V

DISCUSSION

The daily administration of 8 to 16 units of parathyroid extract per kg of body weight produced a pronounced hyperparathyroidism in dogs, as evidenced by marked and sustained hypercalcemia, diuresis and lethargy. Increased levels of serum sulfur-35 were observed following the administration of parathyroid extract to dogs previously given sodium sulfate-S^{35}. This effect on the serum S^{35}-containing constituents is similar to that previously reported in parathyroid extract treated rats (46).

The serum radioactivity was eluted from Sephadex G-200 chromatography columns in two fractions suggesting that the sulfur-35 was present in at least two distinct molecular species. The sulfur-35 in the fraction eluted with the serum protein (Fraction 1) was nondialyzable, whereas that in the second fraction (Fraction 2), which contained no protein, was almost completely dialyzable. Essentially none of the sulfur-35 in either fraction was retained on Dowex-50 columns following elution with water. This observation suggests that the majority of the sulfur-35 must have been present in the serum components other than amino acids or proteins. The S^{35}-containing components in Fraction 1 either were bound to serum protein or were of sufficiently high molecular weight to be nondialyzable and be eluted from Sephadex G-200 with protein. These properties are

similar to those of the sulfated mucopolysaccharides. The sulfur-35 in Fraction 2 was present in low molecular weight compounds, possibly inorganic sulfate, sulfated carbohydrates, or sulfated detoxification products. The relative percentages of these two fractions did not appear to change upon parathyroid extract treatment.

Relatively low apparent ratios, in respect to the previously reported values for rat kidney, of hexosamine to uronic acid (approximately 4:1) were observed in analyses of the delipidized kidney tissue of dogs. One possible explanation for these low values is the fact that no correction for hexose was made in the uronic acid values. The interference of hexose in the carbazole procedure for uronic acid varies with different hexoses. When uronic acid was anlayzed in the presence of a hexose mixture containing equal portions of galactose and mannose, the apparent uronic acid value was increased by approximately 7 percent of the total hexose. Considerably higher apparent values were obtained when the same amount of uronic acid was analyzed in the presence of glucose. Since the analysis of the mucopolysaccharide fraction of the kidney demonstrated all three hexoses, it would be necessary to determine the relative percentages of each hexose present before reliable correction factor could be calculated. Nonetheless, the observation that the ratio of hexosamine to uronic acid exceeds one, suggests that the kidney contained an appreciable amount of glycoprotein. This conclusion is based on the fact that hexosamine is a constituent of both the acid mucopolysaccharides and the glycoproteins while uronic acid is found in acid mucopolysaccharides but not in glycoproteins. The absence of a change in hexosamine concentration of the kidney following parathyroid

extract treatment indicates that this agent had no appreciable effect on the total glycoprotein content.

Parathyroid extract apparently increased the concentration of kidney mucopolysaccharides, as indicated by the total uronic acid content of this tissue. The relative proportion of uronic acid in the crude mucopolysaccharide fraction also appeared increased, while that of the sulfate was essentially unchanged. These observations and the results obtained from the analyses of the partially purified mucopolysaccharide fractions suggest that parathyroid extract produced a preferential increase in hyaluronic acid, a nonsulfated mucopolysaccharide. The observed increase in the amount of uronic acid in the kidney without appreciable change in the sulfate content of the crude mucopolysaccharide fraction indicates that the total amount of sulfated mucopolysaccharides was essentially unchanged following parathyroid extract treatment. When the crude mucopolysaccharide fraction from these kidneys was analyzed for radioactivity, a marked reduction was found in the total radioactivity and the specific radioactivity of the sulfur-35, based on tissue weight and on the uronic acid and sulfate content of the crude mucopolysaccharide fraction.

These apparent changes in the metabolism of sulfated mucopolysaccharides could result from two possible mechanisms. Parathyroid extract may alter the kidney directly to stimulate the rate of turnover of these mucopolysaccharides. This increased turnover rate could lead to a decrease in the specific radioactivity of the sulfur-35 in the kidney, since the specific radioactivity of the sulfur-35 pool from which the mucopolysaccharides were being biosynthesized was decreasing. The

observed increase in hyaluronic acid concentration might also result from direct stimulation of its biosynthesis within the kidney.

The observed alterations in kidney mucopolysaccharides might also result from effects of parathyroid extract on extrarenal sites, such as the bone. Thus, mucopolysaccharides or closely related substances of low specific radioactivity would be released from these tissues and deposited in the kidney. The preferential increase in hyaluronic acid might result from morphological damage produced in the kidney by parathyroid extract. This damage could permit filtration of the lower molecular weight substances, including the sulfated mucopolysaccharides. The higher molecular weight mucopolysaccharides, such as hyaluronic acid, would thereby be retained preferentially. Although the latter mechanism seems less attractive, further investigation, including a study of the effect of parathyroid extract on the kidney maintained in vitro by organ perfusion techniques, will be necessary to determine the mechanism whereby parathroid extract alters mucopolysaccharide metabolism.

CHAPTER VI

SUMMARY

1. The mucopolysaccharides from the kidney of untreated control dogs and those administered parathyroid extract were isolated and partially purified. Hyaluronic acid, chondroitin sulfate and a third mucopolysaccharide, possibly heparin monosulfate, were demonstrated in each by electrophoretic analysis and characterization of the monosaccharide constituents of the partially purified mucopolysaccharide fractions.
2. The administration of parathyroid extract to a group of dogs for 12 days produced an increase of approximately 30 percent in the uronic acid concentration of the kidney, primarily due to an increased amount of hyaluronic acid.
3. Each dog was given sodium sulfate-S^{35} 25 days prior to sacrifice. The total radioactivity and specific radioactivity, based on uronic acid and sulfate content, of the crude mucopolysaccharide fraction isolated from the kidney of the group treated with parathyroid extract were reduced to approximately 50 percent that of the controls. Since no appreciable change in the total sulfated mucopolysaccharide content of the kidney was observed, these results suggest that parathyroid extract altered the turnover rate of these substances.
4. Two S^{35}-containing fractions were obtained when the serum from these

dogs was separated by Sephadex column chromatography. Little, if any, of the sulfur-35 in either fraction appeared to be incorporated into amino acids or proteins. No significant effect of parathyroid extract on the total or relative amount of these serum fractions was observed. The S^{35}-containing components of one fraction were either bound to the serum protein or were of sufficiently high molecular weight to be nondialyzable and be eluted from Sephadex G-200 with the protein. These properties are similar to those of the sulfated mucopolysaccharides. The S^{35}-containing components of second fraction were low molecular weight compounds, possibly inorganic sulfate, sulfated carbohydrates, or sulfated detoxification products.

5. The possibility that parathyroid extract may exert direct effects on mucopolysaccharide metabolism within the kidney has been discussed. The alternate possibility cannot be excluded, however, that parathyroid extract might stimulate the release of mucopolysaccharides or related substances from extrarenal sites and produce morphological damage to the kidney which would favor the observed retention in the kidney of the higher molecular weight mucopolysaccharide hyaluronic acid.

LIST OF REFERENCES

1. Remak, R., Reimer, Berlin, 25, 194 (1851).
2. Owen, R., Trans. Zool. Soc. London, 4, 31 (1862).
3. Sandstrom, I., Upsala Larkenforen Forh., 15, 441 (1880).
4. MacCullum, W. G. and Voegtlin, C., J. Exptl. Med., 11, 118 (1909).
5. Greenwald, I., Am. J. Physiol., 28, 103 (1911).
6. Greenwald, I. and Gross, J., J. Biol. Chem., 68, 325 (1926).
7. Collip, J. B., J. Biol. Chem., 63, 395 (1925).
8. Hanson, A. M., Mil. Surgeon, 54, 76 (1924).
9. Hanson, A. M., Mil. Surgeon, 54, 554 (1924).
10. Collip, J. B. and Clark, E. P., J. Biol. Chem., 64, 485 (1925).
11. Davies, B. M. A. and Gordon, A. H., Biochem. J., 61, 64 (1955).
12. Aurbach, G. D., Arch. Biochem. Biophys., 80, 466 (1959).
13. Rasmussen, H., Sze, Yi-Ling and Young, R., J. Biol. Chem., 239, 2852 (1964).
14. Rasmussen, H. and Craig, L. C., J. Biol. Chem. 236, 759 (1961).
15. Rasmussen, H., J. Biol. Chem. 235, 3442 (1960).
16. Rasmussen, H. and Craig, L. C., Biochem. Biophys. Acta, 56, 325 (1962).
17. Davies, B. M. A. and Gordon, A. H., J. Endocrinol., 9, 252 (1953).
18. Davies, B. M. A. and Gordon, A. H., J. Physiol. (London), 125, 383 (1954).

19. Kenny, A. D. and Munson, P. L., Endocrinology, 64, 513 (1969).

20. Munson, P. L., Ann. N. Y. Acad. Sci. 60, 776 (1955).

21. Copp, D. H. and Cameron, E. C., Science, 134, 2038 (1961).

22. Copp, D. H., Cameron, E. C., Cheney, B. A., Davidson, A. G. F. and Henze, K. G., Endocrinology, 70, 638 (1962).

23. Copp, D. H., Recent Prog. in Hormone Res., 20, 59 (1964).

24. Hirsch, P. F., Volkel, E. F., Savery, A. and Munson, P. L., Federation Proc., 23, 204 (1964).

25. Baghdiantz, A., Foster, G. V., Edwards, A., Jumar, M. A., Stack, E., Soliman, H. A. and MacIntyre, I., Nature, 203, 1027 (1964).

26. Care, A. D., Nature, 205, 1289 (1965).

27. Arnaud, C. D., Tennenhouse, A. and Rasmussen, H., Federation Proc., 24, 322 (1965).

28. Albright, F. and Reifenstein, E. F., The Parathyroid Glands and Metabolic Bone Disease. Baltimore: Williams & Williams, 1948.

29. Lavender, A. R., Aho, I., Rasmussen, H. and Pullman, T., J. Lab. Clin. Med., 54, 916 (1959).

30. Pullman, T. N., Lavender, A. R., Aho, I. and Rasmussen, H., Endocrinology, 67, 570 (1960).

31. Horwith, M., Rich, C., Thompson, D. D. and Rasmussen, H., The Parathyroids. Springfield, Illinois; C. C. Thomas, 1961.

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

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

34. Barnicot, N. A., J. Anat., 82, 233 (1948).

35. Chang, H., Anat. Record, 111, 23 (1951).

36. Gaillard, P. J., Acta Physiol. Pharmacol. Neerl., 8, 287 (1959).

37. Gaillard, P. J., Koninkl. Med. Akad. Wetenschap. Proc., c63, 25 (1960).

38. Gaillard, P. J., Koninkl. Med. Akad. Wetenschap. Proc., c64, 119 (1961).

39. Talmage, R. V. and Elliott, J. R., Federation Proc., 17, 160 (1958).

40. Schachter, D., Dowdle, E. B. and Schenker, H., Am. J. Physiol., 198, 263 (1960).

41. Schachter, D., Dowdle, E. B. and Schenker, H., Am. J. Physiol., 198, 275 (1960).

42. Schachter, D. and Rosen, S. M., Am. J. Physiol., 196, 357 (1959).

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

44. Shetlar, M. R., Howard, R. P., Joel, W., Courtwright, C. M. and Reifenstein, E. C. Jr., Endocrinology, 59, 532 (1956).

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

46. Bradford, R. H., Howard, R. P., Joel, W. and Shetlar, M. R., Biochem. Biophys. Res. Commun., 1, 350 (1959).

47. Fischer, C. and Boedeker, C., Liebigs Ann. Chem., 117, 111 (1861).

48. Meyer, K. and Palmer, J. W., J. Biol. Chem., 114, 689 (1936).

49. Meyer, K. and Chaffee, E., J. Biol. Chem., 138, 491 (1941).

50. Meyer, K., Linker, A., Davidson, E. A. and Weissman, B., J. Biol. Chem., 205, 611 (1953).

51. McLean, J., Am. J. Physiol., 41, 250 (1916).

52. Jorpes, J. E. and Gardell, S., J. Biol. Chem., 176, 267 (1948).

53. Linker, A., Hoffman, P. and Meyer, K., Federation Proc., 17, 264 (1958).

54. Meyer, K. and Palmer, J. W., J. Biol. Chem., 107, 629 (1934).

55. James, S. P., Smith, F., Stacey, M. and Wiggins, L., Nature (London), 156, 308 (1945).

56. Bray, H. G., Gregory, J. E. and Stacey, M., Biochem. J., 38, 142 (1944).

57. Walker, P. G., Biochem. Soc. Symp. 20, 109 (1960).

58. Dziewiatkowski, D. D., J. Biol. Chem., 178, 197 (1949).

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

60. Friberg, U., Arkiv. Kemi. 12, 481 (1958).

61. Curran, K. C. and Kennedy, J. S., J. Pathol. Bacteriol., 70, 499 (1955).

62. Schiller, S., Mathews, M. B., Cifonelli, J. A. and Dorfman, A., J. Biol. Chem., 218, 139 (1956).

63. Meyer, K., Davidson, E., Linker, A. and Hoffman, P., Biochim. Biophys. Acta., 21, 506 (1956).

64. Smith, H. and Gallop, R. C., Biochem. J., 53, 666 (1953).

65. Gardell, S., Acta Chem. Scand., 11, 668 (1957).

66. Gardell, S., Gordon, A. H., and Aqvist, S., Acta Chem. Scand., 4, 907 (1950).

67. Rienits, K. G., Biochem. J., 53, 79 (1953).

68. Wolfrom, M. L., Montgomery, R., Karabinos, J. V., and Rathgeb, P. J., Am. Chem. Soc., 72, 5796 (1950).

69. Berenson, G. S., Roseman, S. and Dorfman, A., Biochim. Biophys. Acta, 17, 75 (1955).

70. Scott, J. E., Chem. and Ind., p. 1568 (1955).

71. Stacey, M. W., Proc. Soc. Exp. Biol. N. Y., 80, 646, (1952).

72. Scott, J. E., The Assay of Acidic Polysaccharides from Tissue. Ph.D. Thesis, Manchester Univ. 1956.

73. Gardell, S., Biochem. Soc. Symp., 20, 39 (1960).

74. Ringertz, N. and Richard, P., Acta Chem. Scand., 14, 303 (1960).

75. Davidson, E. A. and Meyer, K., J. Biol. Chem., 211, 605 (1954).

76. Schiller, S., Slover, G. A., and Dorfman, A., J. Biol. Chem., 236, 938 (1961).

77. Berman, E. R., Biochim. Biophys. Acta, 58, 120 (1962).

78. Schmidt, M., Biochim. Biophys. Acta, 63, 346 (1962).

79. Baker, R. and Sison, F., J. Urol., 72, 1032 (1954).

80. Ginetzinsky, A. G., Nature, 182, 1218 (1958).

81. Bradford, R. H., Howard, R. P., Joel, W., Puls, J., and Shetlar, M. R., Am. J. Physiol., 203, 676 (1962).

82. Marx, W., Marx, L., Rucker, P., Ruggeri, L., and Freeman, L., Proc. Soc. Exptl. Biol. Med., 94, 217 (1957).

83. Monkhouse, F. C., MacKneson, R. G., and Bambers, G., Proc. Soc. Exptl. Biol. Med., 95, 489 (1957).

84. Allalouf, D., Ber, A., Sharon, N., Biochim. Biophys. Acta, 78, 278 (1964).

85. Gelotte, B., Flodin, P., and Killander, J., Arch. Biochem. Biophys., Suppl. 1, 319 (1962).

86. Foster, T. S., Pearce, R. H., Can. J. Biochem. Physiol., 39, 1773 (1961).

87. Fischer, F. G. and Dorfel, H., Hoppe-Selyer Z. Physiol. Chem., 301, 224 (1955).

88. Kingsley, G. R. and Robnett, O., Am. J. of Clin. Path., 29, 171 (1958).

89. Antonopoulos, C. A., Acta Chem. Scand., 16, 1521 (1962).

90. Shetlar, M. R., Foster, J. V. and Everett, M. R., Proc. Soc. Exptl. Biol. Med., 67, 125 (1948).

91. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951).

92. Boas, N. R., J. Biol. Chem., 204, 553 (1954).

93. By private communication with the laboratory of M. R. Shetlar.

94. Bitter, T. and Muir, H. M., Anal. Biochem., 4, 330 (1962).

Note:

References 1, 2, 3, 36, 37, 38, 47, 60, 71, and 72 were not available for review.

APPENDIX

TABLE 1

COMPOSITION OF CONNECTIVE TISSUE MUCOPOLYSACCHARIDES

Mucopolysaccharide	Composition
Hyaluronic Acid	N-Acetylglucosamine Glucuronic Acid
Chondroitin Sulfate A	N-Acetylgalactosamine Glucuronic Acid Sulfate
Chondroitin Sulfate B	N-Acetylgalactosamine Iduronic Acid Sulfate
Chondroitin Sulfate C	N-Acetylgalactosamine Glucuronic Acid Sulfate
Heparin	Glucosamine Glucuronic Acid Sulfate
Heparitin Sulfate (Heparin Monosulfate)	Glucosamine Glucuronic Acid Sulfate Acetate
Keratosulfate	N-Acetylglucosamine Galactose Sulfate

TABLE 2

NONDIALYZABLE RADIOACTIVITY IN FRACTIONS OBTAINED FROM SEPHADEX COLUMN CHROMATOGRAPHY OF SERUM OF DOGS ADMINISTERED SODIUM SULFATE-S^{35}

Dog	Treatment	Fraction 1	Fraction 2
0	Control	100	6
L	PTE	100	14
M	PTE	72	21

Fractions 1 and 2 were obtained by Sephadex G-200 column chromatography of serum as described in Figure 5. All values are expressed as the percent of the total radioactivity in each fraction which failed to pass through a dialysis membrane.

TABLE 3

RADIOACTIVITY IN SERUM FRACTIONS SEPARATED BY DOWEX-50 CHROMATOGRAPHY

	Dog 0 - (Control)		Dog L - (PTE)	
Eluting Solvent	Fraction 1	Fraction 2	Fraction 1	Fraction 2
H_2O	88.7	79.2	87.5	86.0
0.3 N HCl	4.8	16.7	3.1	8.0
4.0 N HCl	6.5	4.1	9.4	6.0

Fractions 1 and 2 obtained by Sephadex G-200 column chromatography of serum (cf. Figure 5), were separated by Dowex-50 chromatography. All values are expressed as the percentage of the total radioactivity recovered in the eluate from the Dowex-50 columns.

TABLE 4

EFFECT OF PARATHYROID EXTRACT ON THE MONOSACCHARIDE COMPOSITION OF THE DOG KIDNEY

Dog	Treatment	Hexose (ug/mg of tissue)[a]	Hexosamine (ug/mg of tissue)[a]	Uronic Acid (ug/mg of tissue)[a]	Ratio of Hexosamine to Uronic Acid
H	Control	18.2	6.34	1.64	3.86
0	Control	18.2	7.16	1.25	5.74
F	PTE	18.8	5.88	1.51	3.90
G	PTE	19.4	7.30	1.48	4.93
I	PTE	18.3	7.92	1.84	4.30
K	PTE	17.6	5.60	2.19	2.56
L	PTE	18.8	6.77	1.99	3.41
M	PTE	22.5	8.09	2.35	3.43

[a]Values are based on dry weight of the delipidized kidney. Glucuronic acid served as the standard for uronic acid determinations, glucosamine for hexosamine, and a mixture of mannose and galactose (1:1) for hexose.

TABLE 5

URONIC ACID, SULFATE AND RADIOACTIVITY ANALYSES OF THE CRUDE MUCOPOLYSACCHARIDE FRACTION OF DOG KIDNEY

Dog	Treatment	Uronic Acid (mg/5 g of tissue)	Uronic Acid (ug/mg of fraction)[a]	Sulfate (ug/mg of fraction)[a]	Specific Radioactivity		
					cpm/mg of fraction[a]	cpm/mg of uronic acid	cpm/mg of sulfate
H	Control	7.40	131.7	32.9	2,103	15,968	63,843
O	Control	5.64	112.2	34.1	1,663	14,822	48,740
F	PTE	7.70	164.9	44.8			
G	PTE	7.98	153.1	33.7			
I	PTE	9.52	174.8	32.7	1,086	6,213	33,252
K	PTE	8.95	167.2	18.7	761	4,551	40,350
L	PTE	8.57	109.7	58.6	973	8,870	16,604
M	PTE	8.27	118.4	34.1	856	7,230	25,103

[a]Values are based on dry weight of the crude mucopolysaccharide fraction. Glucuronic acid was used as the standard for uronic acid analyses.

TABLE 6

MONOSACCHARIDE, PROTEIN AND RADIOACTIVITY ANALYSES OF THE ALIQUOTS OF THE CRUDE MUCOPOLYSACCHARIDE FRACTIONS APPLIED TO DEAE-SEPHADEX COLUMNS

Dog	Treatment	Dry Weight (mg)[a]	Uronic Acid (mg)[a]	Hexosamine (mg)[a]	Hexose (mg)[a]	Protein (mg)[a]	Specific Radioactivity	
							cpm/mg of fraction[b]	cpm/mg of uronic acid
H	Control	15.40	2.12	3.26	2.84	2.90	38,291	180,788
O	Control	15.37	1.82	3.10	2.68	3.72	33,939	186,069
L	PTE	15.29	1.93	3.10	2.72	4.36	20,998	108,685
M	PTE	15.73	2.14	3.76	2.74	4.73	17,347	81,212

[a]Values are total material applied to the column. Glucuronic acid served as the standard for uronic acid determinations, glucosamine for hexosamine, and a mixture of mannose and galactose (1:1) for hexose.

[b]Values based on dry weight of the crude mucopolysaccharide fraction from kidney.

TABLE 7

MONOSACCHARIDE AND PROTEIN ANALYSES OF MUCOPOLYSACCHARIDE FRACTIONS FROM DOG KIDNEY AFTER SEPARATION BY DEAE-SEPHADEX COLUMN CHROMATOGRAPHY

Dog-Fraction[a]	Treatment	Uronic Acid[b]	Hexose[b]	Hexosamine[b]	Protein[b]	% of total uronic acid[c]
H-1	Control	121	516	595	513	10.4
H-2	Control	326	357	406	550	28.1
H-3	Control	413	281	366	176	35.6
H-4	Control	300	236	239	177	25.9
0-1	Control	176	749	798	792	17.0
0-2	Control	271	323	438	601	26.1
0-3	Control	320	257	221	162	30.8
0-4	Control	271	229	163	176	26.1
L-1	PTE	139	348	302	484	11.8
L-2	PTE	581	714	954	1305	49.3
L-3	PTE	277	174	246	213	23.5
L-4	PTE	181	42	116	132	15.4
M-1	PTE	138	472	382	565	10.5
M-2	PTE	640	796	1216	1393	48.6
M-3	PTE	189	45	126	103	14.3
M-4	PTE	351	211	273	88	26.6

[a]Fraction numbers are those of the combined eluates as seen on Figure 8.

[b]Values are the total amount (ug) in each fraction isolated from the column. Standards for the chemical determinations were as follows: Glucuronic acid for uronic acid, glucosamine for hexosamine and a mixture of mannose and galactose (1:1) for hexose.

[c]Values are based on percentage of total uronic acid from the column.

TABLE 8

RADIOACTIVITY OF MUCOPOLYSACCHARIDE FRACTIONS FROM DOG KIDNEY AFTER SEPARATION BY DEAE-SEPHADEX COLUMN CHROMATOGRAPHY

Dog-Fraction	Treatment	Radioactivity	
		total cpm	cpm/mg of uronic acid
H-1	Control	120	992
H-2	Control	360	1104
H-3	Control	980	2373
H-4	Control	1500	5000
O-1	Control	290	1649
O-2	Control	480	1771
O-3	Control	1300	4063
O-4	Control	2010	7417
L-1	PTE	50	360
L-2	PTE	640	1102
L-3	PTE	790	2852
L-4	PTE	610	3370
M-1	PTE	50	362
M-2	PTE	500	781
M-3	PTE	490	2593
M-4	PTE	1470	4188

The eluates obtained during DEAE-Sephadex A-25 column chromatography were combined to provide these fractions (cf. Figure 8). Glucuronic acid was used as the standard.

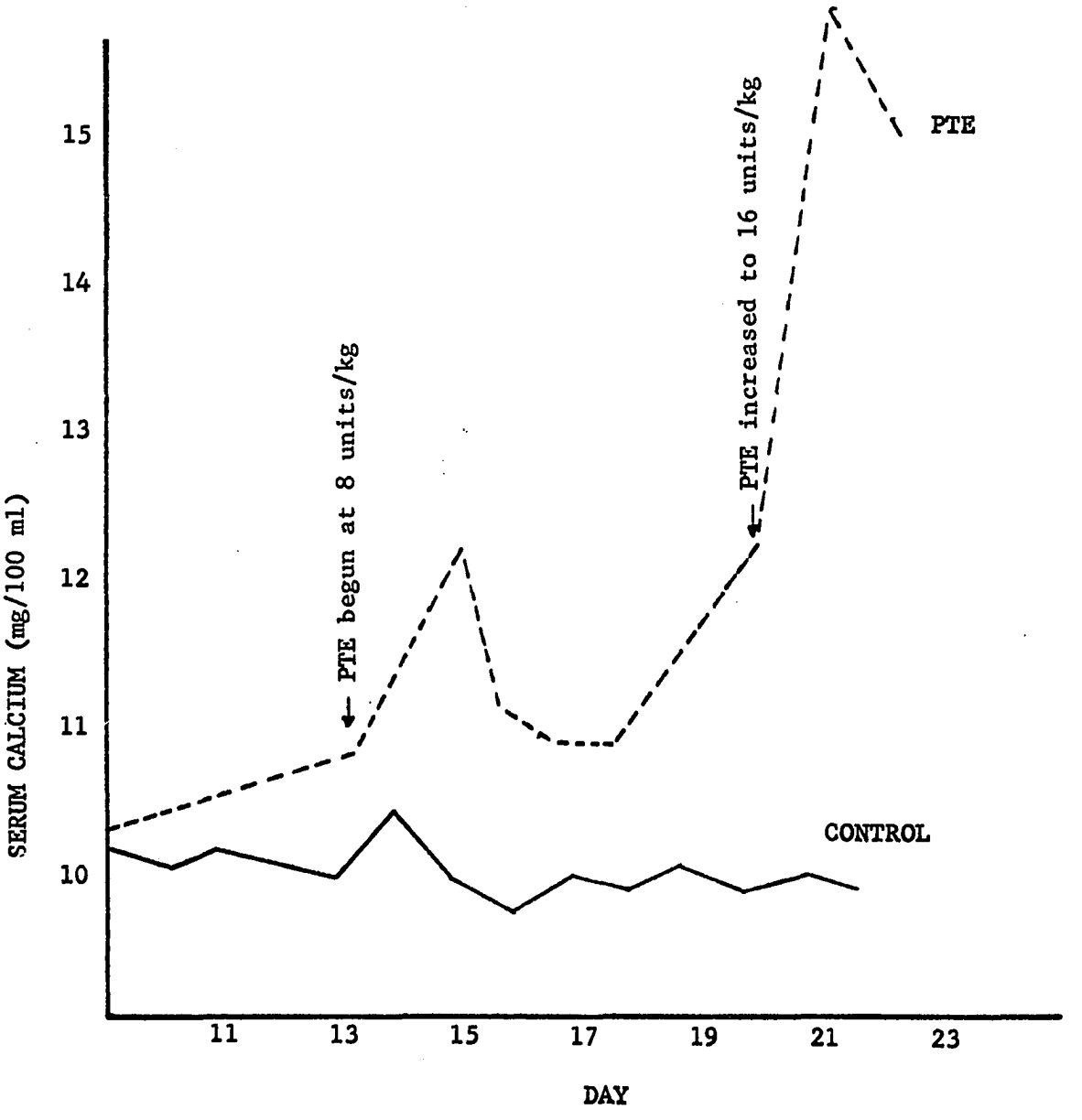


Figure 1. Serum calcium response in a dog before and during parathyroid extract (PTE) treatment. The number of days following administration of sodium sulfate-S^{35} is plotted along the abscissa.

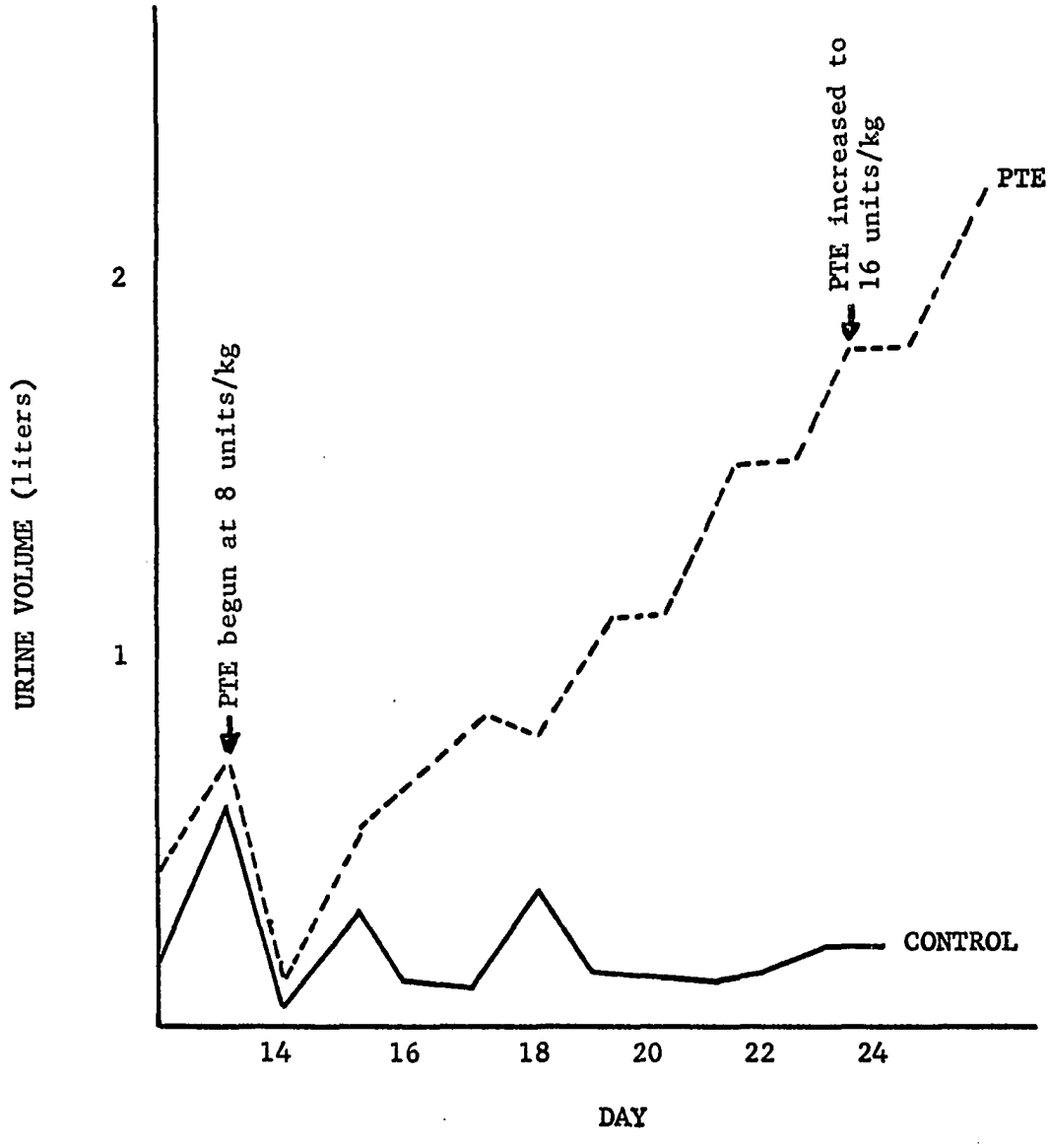


Figure 2. Urine volume response in a dog before and during parathyroid extract (PTE) treatment. The number of days following administration of sodium sulfate-S^{35} is plotted along the abscissa.

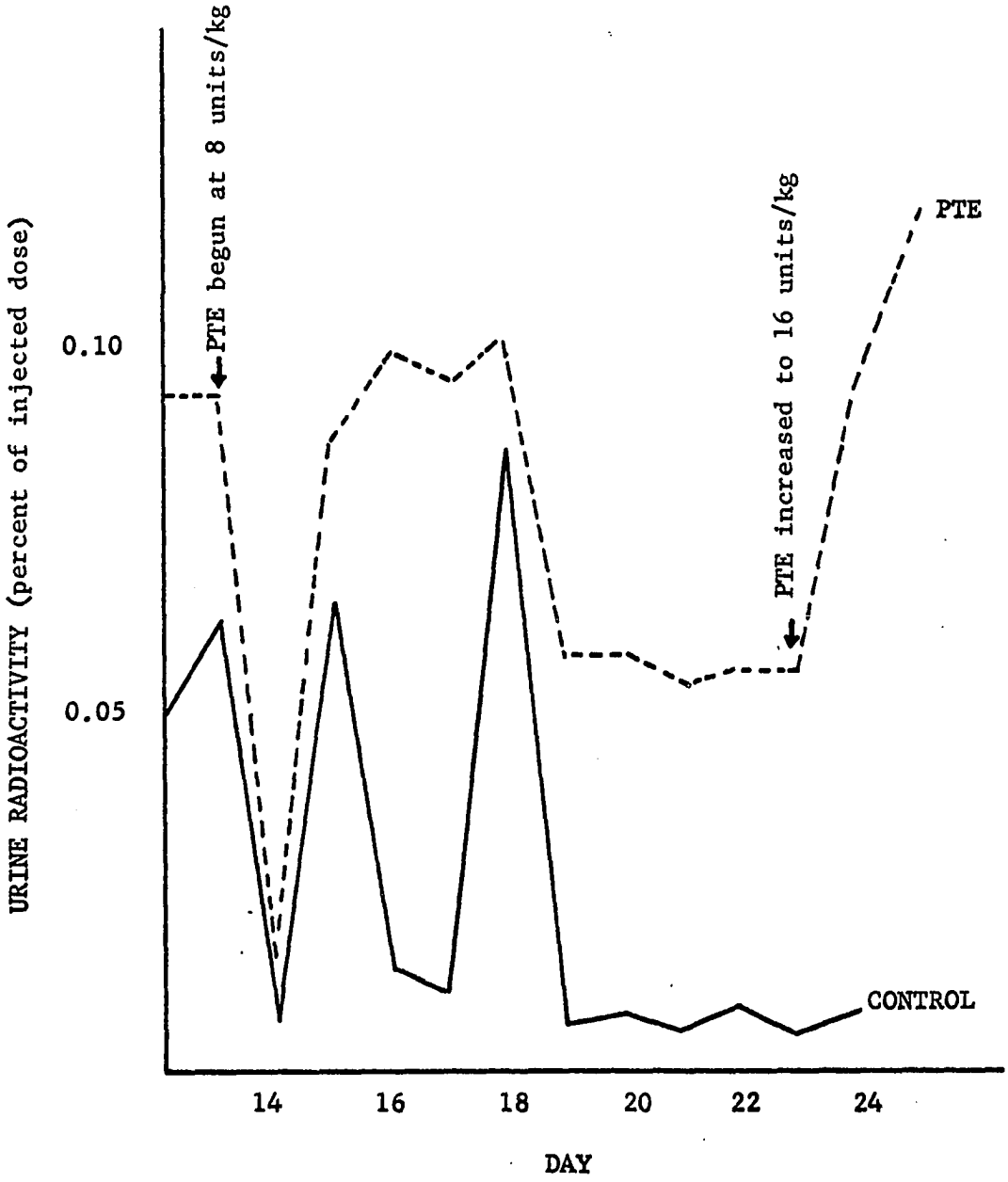


Figure 3. Urinary excretion of radioactivity before and during parathyroid extract (PTE) treatment in a dog administered sodium sulfate-S^{35}. The number of days following administration of sodium sulfate-S^{35} is plotted along the abscissa.

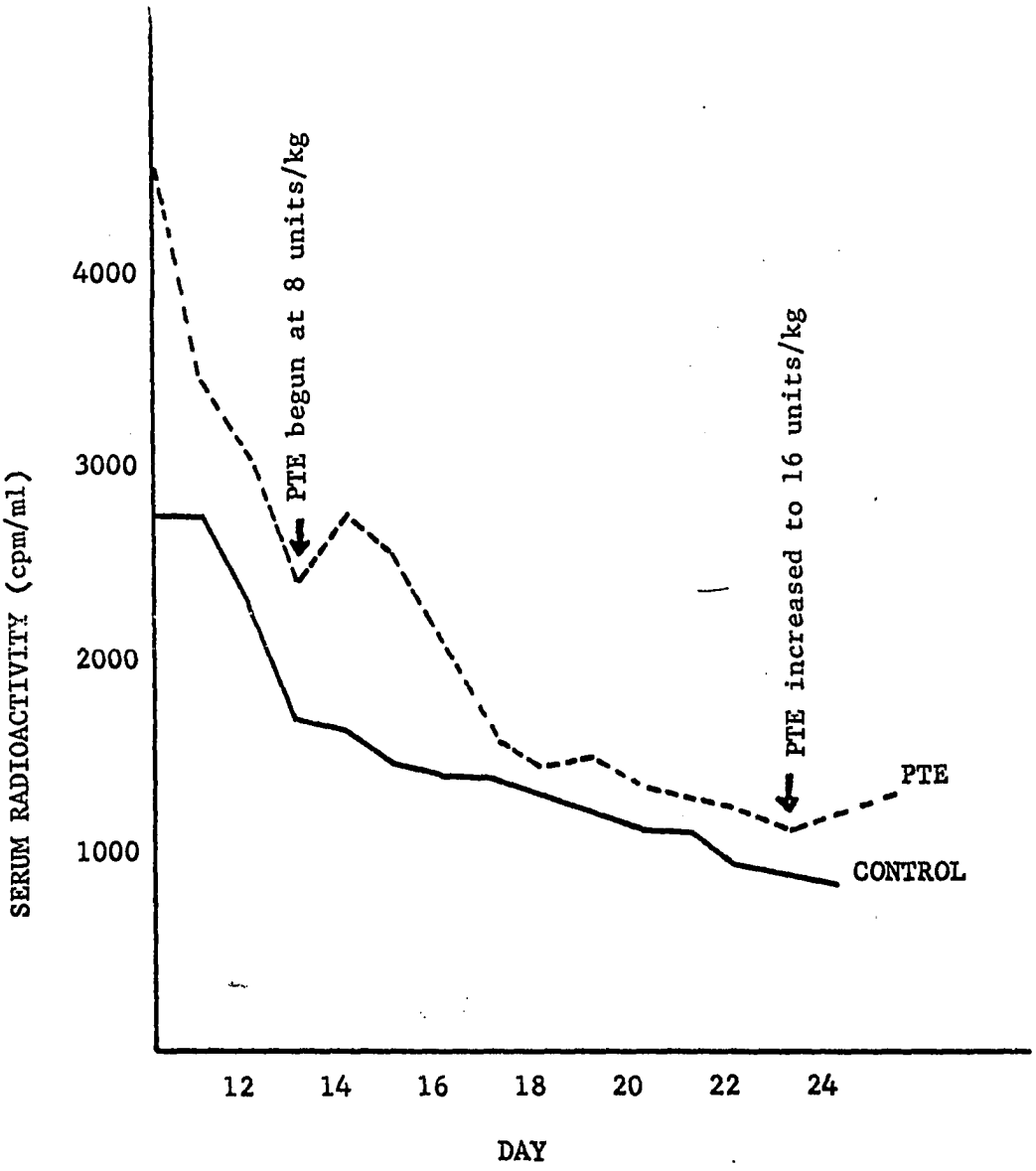


Figure 4. Serum radioactivity before and during parathyroid extract (PTE) treatment in a dog administered sodium sulfate-S^{35}. The number of days following administration of sodium sulfate-S^{35} is plotted along the abscissa.

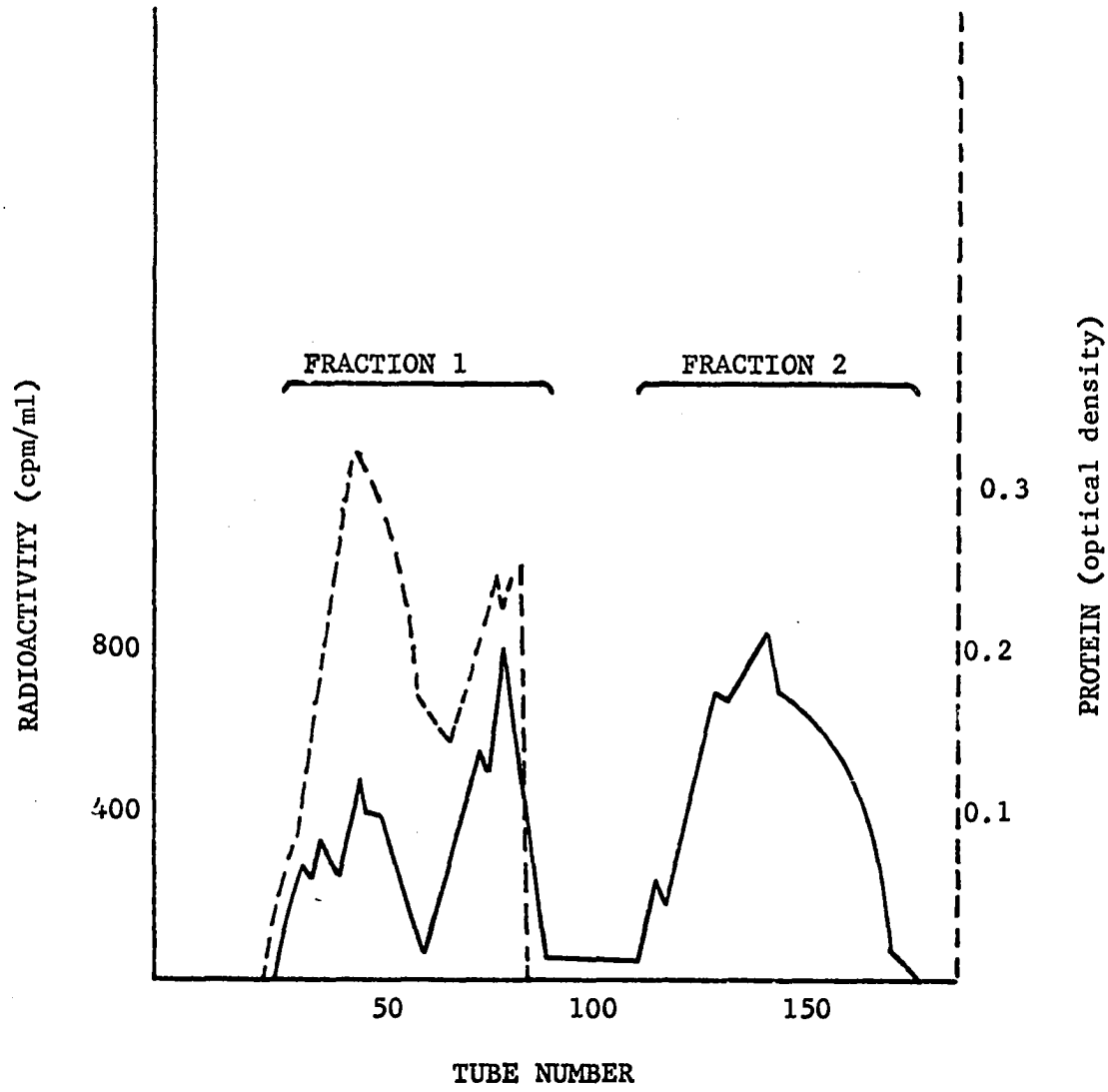


Figure 5. Radioactivity and protein content of eluates from Sephadex column chromatography of serum from dogs administered sodium sulfate-S^{35}. Protein values are expressed as the absorption (optical density at 500 mu) after adding biuret reagent to a 0.5-ml aliquot from each eluate tube. Tube numbers of the 5-ml fractions eluted from the Sephadex G-200 column are shown along the abscissa. The fractions were combined as indicated.

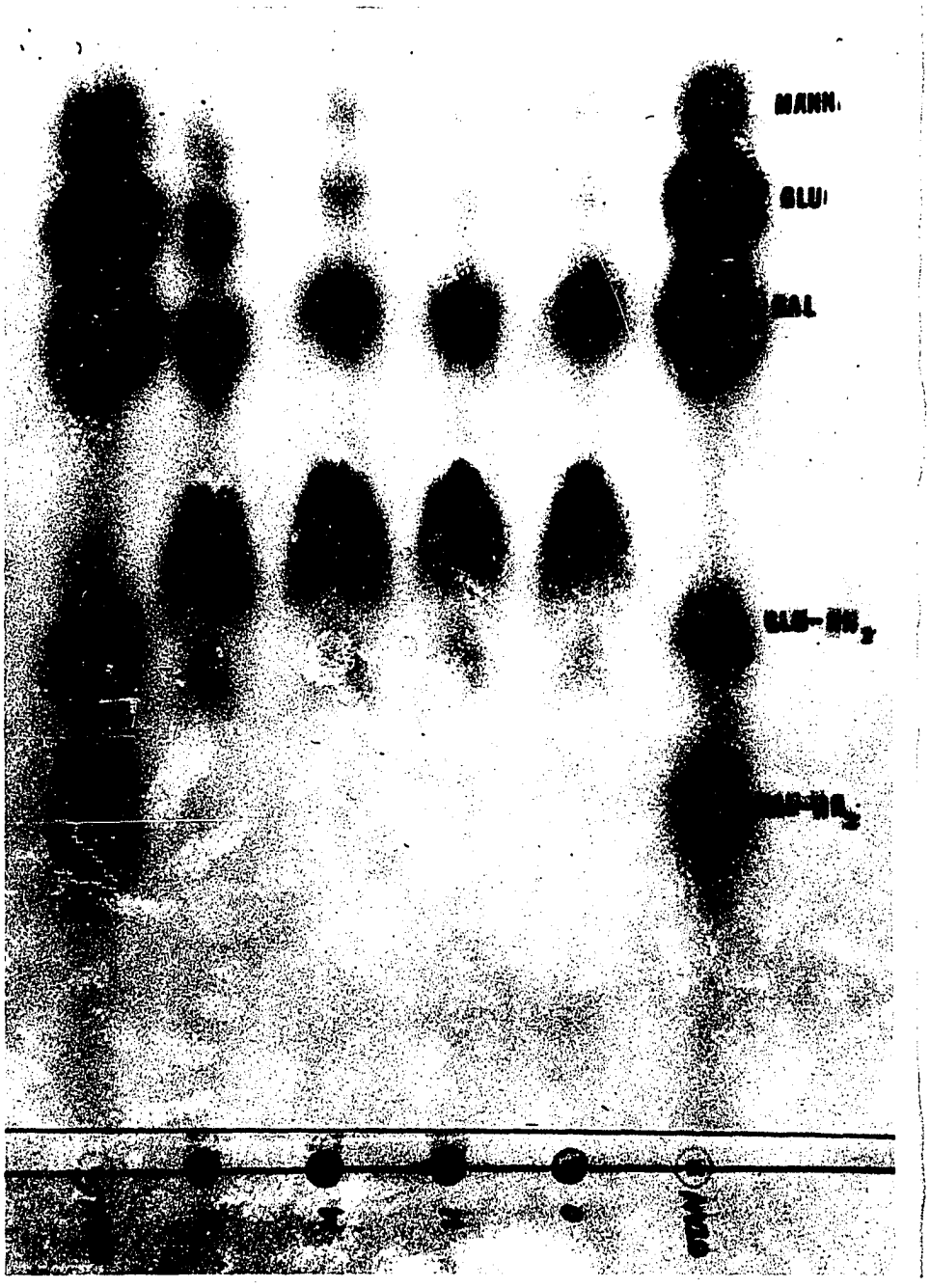


Figure 6. Separation by paper chromatography of hexoses and hexosamines in hydrolyzates of the crude mucopolysaccharide fractions from dog kidney. The crude mucopolysaccharide fractions obtained from Dogs L, M, H and O were hydrolyzed for 8 hours at 100°C in 4 N HCl. The hydrolyzates were taken to dryness, water was added and taken to dryness again. The residue was dissolved in 10 percent isopropanol before application to the paper chromatogram. The solvent system contained ethyl acetate, pyridine and water (12:5:4) and the spots were detected with silver nitrate.

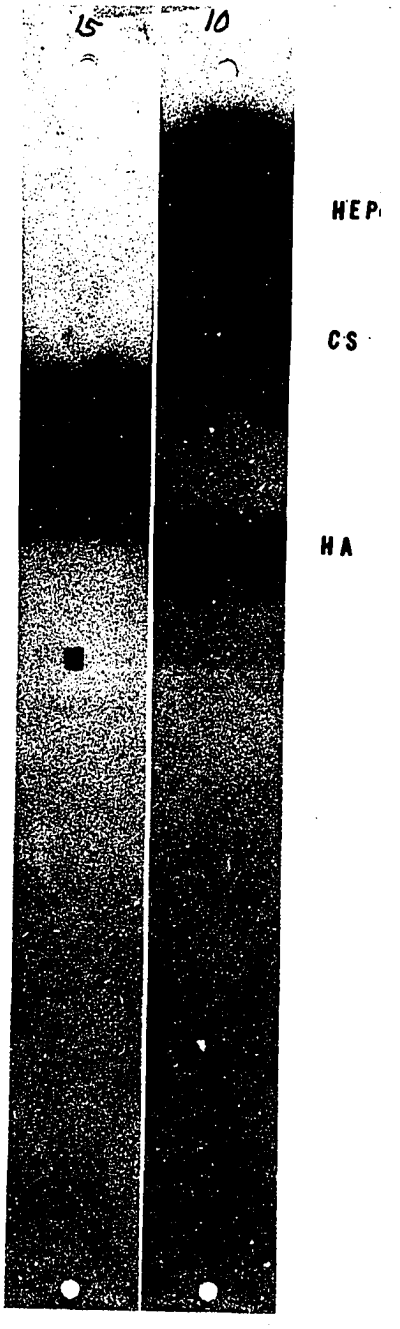
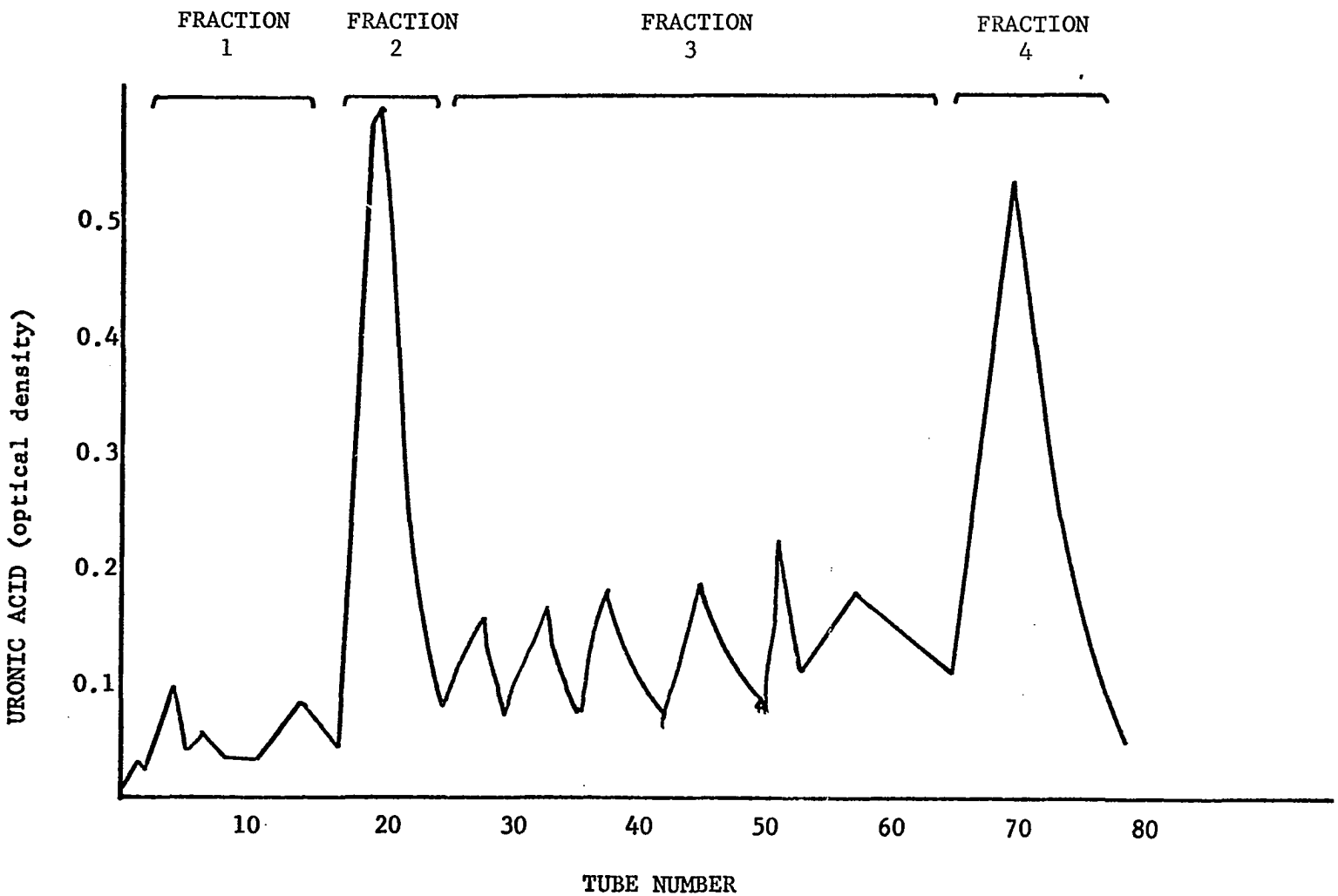


Figure 7. Paper electrophoretic separation of the crude mucopolysaccharide fraction from dog kidney. An aliquot of the crude mucopolysaccharide fraction from Dog M was applied to the paper strip on the left. Standards of hyaluronic acid, chondroitin sulfate and heparin, in order of increasing mobility, were applied to the paper strip on the right. The electrophoretic separation was performed in a Durrum cell using lithium sulfate buffer with a potential of 60 volts applied for 16 hours. The mucopolysaccharides were stained with Alcian Blue.

Figure 8. Elution of uronic acid-containing constituents during separation of the crude mucopolysaccharide fraction by DEAE-Sephadex column chromatography. The uronic acid values are expressed as the absorption (optical density at 530 mu) in the carbazole reaction with 1-ml aliquots of each fraction. Tube numbers of the 5-ml fractions eluted from the DEAE-Sephadex (A-25) column are shown along the abscissa. The fractions were combined as indicated.


FRACTION 1
FRACTION 2
FRACTION 3
FRACTION 4
URONIC ACID (optical density)
0.5
0.4
0.3
0.2
0.1
10
20
30
40
50
60
70
80
TUBE NUMBER

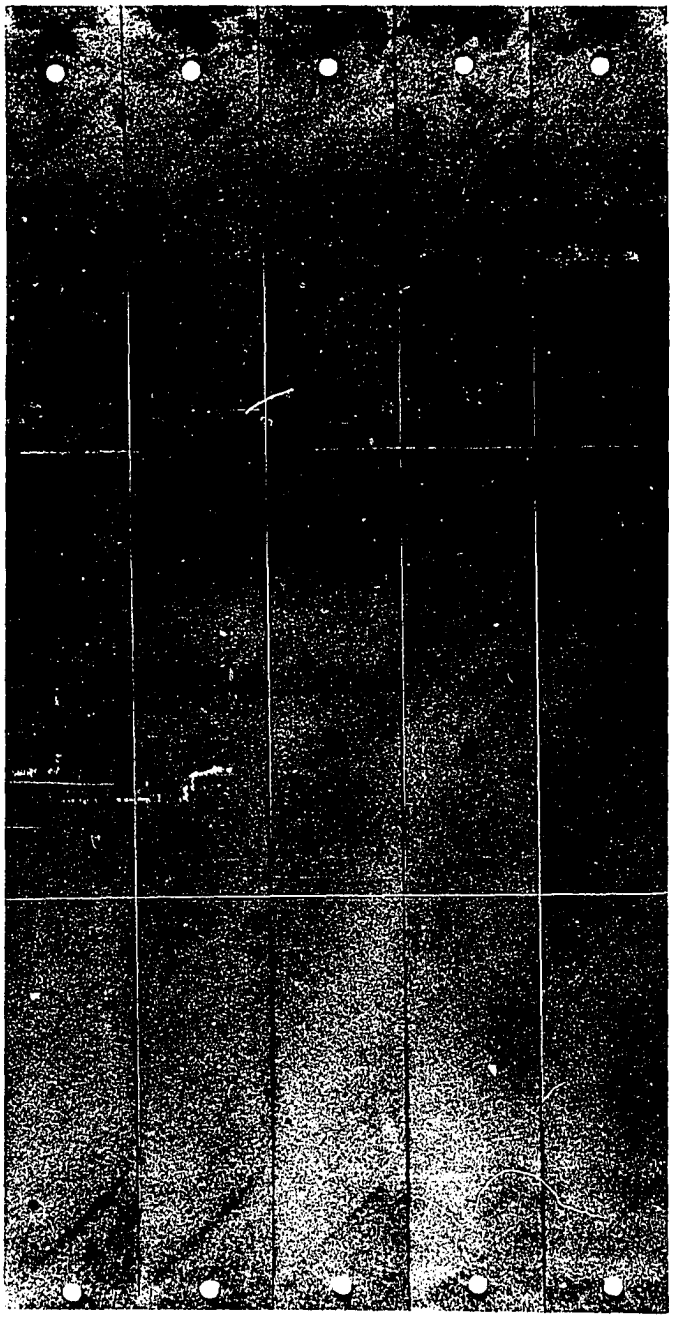


Figure 9. Paper electrophoretic separation of the mucopolysaccharide fractions obtained by DEAE-Sephadex column chromatography of the crude mucopolysaccharide fraction from dog kidney. Aliquots of Fractions 1 through 4 (Figure 8) were individually applied to paper strips 1 through 4, respectively. An aliquot of the crude mucopolysaccharide fraction was applied to paper strip M. The separation was performed in a Durrum cell using lithium sulfate buffer and a potential of 60 volts for 16 hours. The mucopolysaccharides were stained with Alcian Blue.

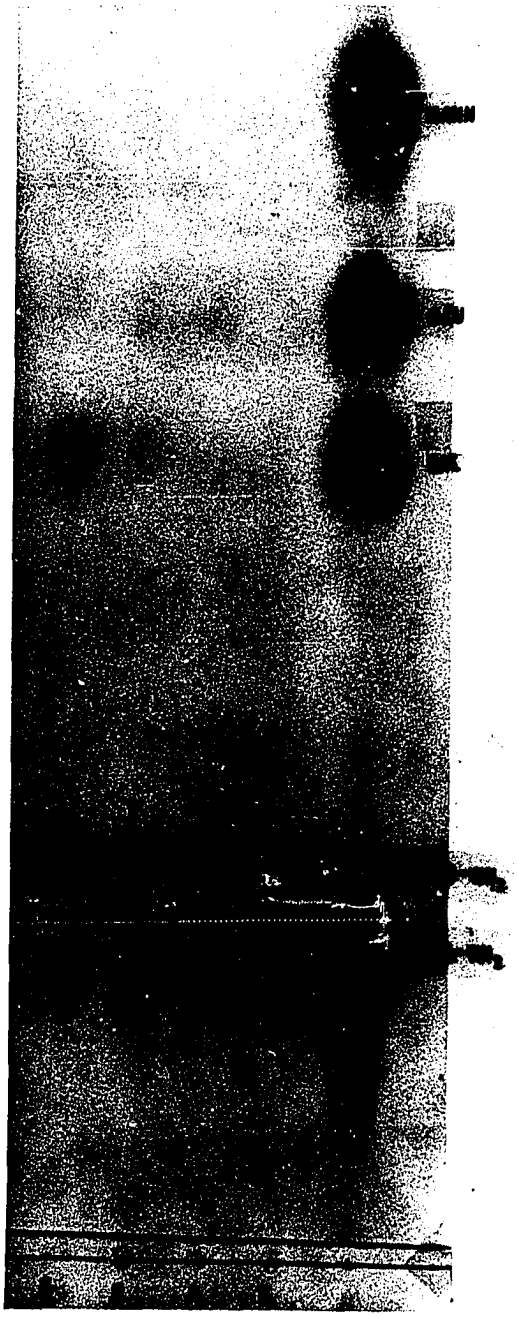
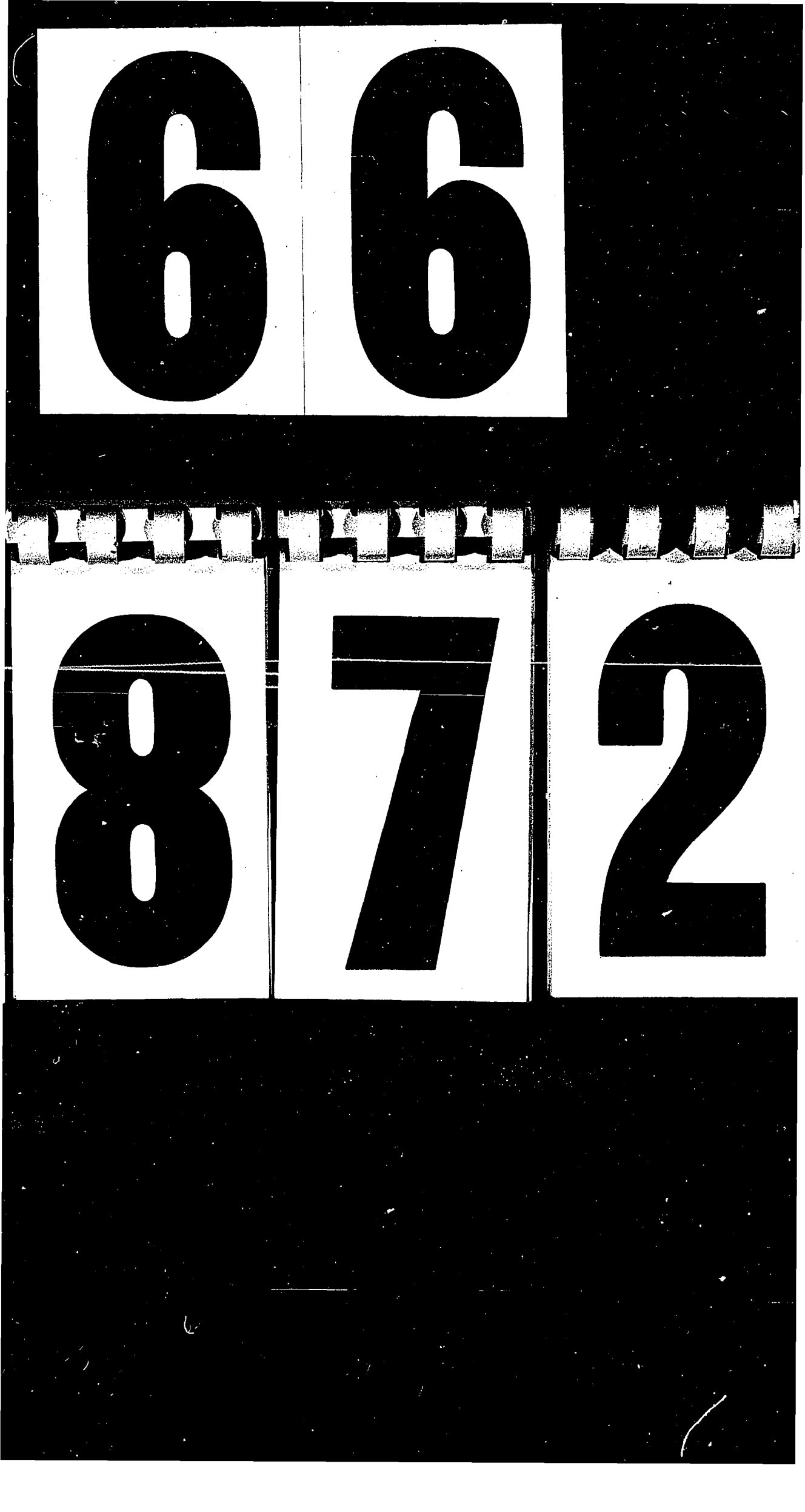


Figure 10. Separation by paper chromatography of the hexoses and hexosamines in hydrolyzates of Fraction 1 through 4 obtained by DEAE-Sephadex column chromatography. Fractions 1 through 4 (Figure 8) were applied onto the chromatogram at positions 1 through 4, respectively. A mixture of monosaccharides was applied beside Fraction 4. Fractions 1 through 4 were hydrolyzed individually for 8 hours at 100°C in 4 N HCl. The hydrolyzate was taken to dryness, water was added and taken to dryness again. The residue was dissolved in 10 percent isopropanol before application to the chromatogram. The solvent system contained ethyl acetate, pyridine and water (12:5:4) and the spots were detected with silver nitrate.


66
8872