# THE SELECTIVE IN VIVO INCORPORATION AND METABOLISM OF RADIOACTIVE PUTRESCINE IN THE ADULT MALE RAT

Robert B. Clark and William R. Fair

*Stanford University School of Medicine, Stanford, California*

***Putrescine (1,4, diaminobutane), a known precursor of the polyamines, spermine, and spermidine, was studied as a possible vehicle for a radioisotope scan of the prostate and other tissues rich in polyamines. Male Sprague-Dawley rats received intravenous injections (2.3 μCi/100 gm) of $^3$H putrescine dihydrochloride (specific activity, 107 μCi/μM). One hour after injection the ventral prostate and pancreas showed uptake of radioactivity that was three and four times greater than that of the liver, respectively. The ratio of the amount of radioactivity in the ventral prostate compared with that of abdominal wall musculature was 8:1. The pancreas-to-muscle ratio was 10:1. At 1 hr the ventral prostate contained 0.6% (0.9%/gm wet wt) of the total injected radioactivity and the pancreas, 0.5% (1.2%/gm wet wt) of the injected dose. More than 90% of the radioactivity in the rat ventral prostate, 6 hr after intravenous injection of $^{14}$C-putrescine dihydrochloride, was found to be in the form of spermine and spermidine, thus confirming previous in vitro biosynthetic studies.***

The basic polyamines, spermine [$H_2N$–$(CH_2)_3$–NH–$(CH_2)_4$–NH–$(CH_2)_3$–$NH_2$] and spermidine [$H_2N$–$(CH_2)_3$–NH–$(CH_2)_4$–$NH_2$], are aliphatic amines present in varying quantities in microorganisms, plant, and animal tissues (*1*). The biologic functions and the intracellular location of the polyamines remain unclear, but recently observed in vitro effects including stabilization of nucleic acids and polyribosomes, partially attributable to the polycationic nature of the polyamines, may prove to be important (*2–5*). In this regard it is interesting that, in mammals, the highest polyamine concentrations are found in regions of highest ribonucleic acid (RNA) and protein synthesis, i.e., pancreas, prostate, and certain tumors (*1,2,6*). Human prostatic tissue contains abundant amounts of spermine (*1*). Similarly, the ventral lobe of the rat prostate contains relatively high concentrations of polyamines (*7,8*). [The rat prostate consists of two ventral lobes and two dorsolateral lobes (*7*).]

Putrescine [$H_2N$–$(CH_2)_4$–$NH_2$] has been shown to serve as a precursor of spermine and spermidine both in vitro (*9*) and in vivo (*10,11*) in some organs. In vitro experiments have demonstrated that putrescine can serve as a precursor for the rat ventral prostate polyamines (*9*) but until the present study in vivo confirmation of this observation was lacking.

We have demonstrated the selective in vivo incorporation of radioactively labeled putrescine by the rat ventral prostate. Adequate tissue uptake of this diamine tagged with a suitable isotope may provide a basis for developing a means of radioisotopic visualization of the human prostate or other tissues containing high polyamine concentrations.

## MATERIALS AND METHODS

**Putrescine distribution studies.** Sixteen-week-old male Sprague-Dawley rats weighing approximately 500 gm were housed at room temperature and fed food and water ad libitum. The animals were anesthetized with diethyl ether by inhalation, and a cutdown exposing the femoral vein was performed. Putrescine (2,3, $^3$H-putrescine dihydrochloride, New England Nuclear—specific activity, 107 μCi/μM) at a concentration of 78 μCi/ml in 0.15 *M* NaCl solution was injected into the femoral vein with a tuberculin syringe fitted with a 27-gage needle. The dose given was 0.03 ml (2.3 μCi)/100 gm wet wt (a total dosage of approximately 17 μg of putrescine dihydrochloride). The cutdown site was closed with

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For reprints contact: William R. Fair, Div. of Urology, Stanford University Medical Center, Stanford, Calif. 94305.

skin clips, and the animals were awakened, kept in cages at room temperature, and fed food (without water) ad libitum. At 30 min, 1 hr, 3 hr, 6 hr, and 24 hr after the injection of $^{3}$H-putrescine, the animals were again anesthetized with diethyl ether and sacrificed by exsanguination (three animals per time period). The abdomen was immediately opened through a midline vertical incision. Tissue samples, each approximating 0.3 gm in wet weight, were taken from the abdominal wall musculature, liver, pancreas, kidney, ventral prostate, and dorsolateral prostate. A sample of whole blood was taken during exsanguination and, when possible, the bladder urine was also sampled. The tissue samples were weighed on a Mettler balance in tared plastic vials to the nearest 0.1 mg. The samples were then stored in these vials at —20°C for no longer than 24 hr. The tissues were wrapped in filter paper and oxidized in a model 300 Packard tissue oxidizer. The radioactivity was collected in 10 ml of Bray's solution as tritiated water. (Bray's solution: 60 gm napthalene, 4 gm 2,5-diphenyloxazole, 0.2 gm 2,2-p-phenylenebis, 100 ml methanol, made to 1 liter with p-Dioxane.) The radioactivity was counted in a model 3000 Packard liquid scintillation spectrophotometer with an efficiency for $^{3}$H of 18%. The counts per minute were corrected for counter efficiency and water quenching by the use of a $H_2O$ quench curve. The $^{3}$H content of the tissues was recorded as percentage of injected dose taken up per total organ weight and percentage of injected dose per gram of tissue.

**Polyamine synthesis studies.** In those experiments done to determine the fate of the injected putrescine, $^{14}$C-labeled putrescine [1,4, $^{14}$C-putrescine dihydrochloride (New England Nuclear—specific activity, 0.25 mCi/1.9 mg)] was preferred over $^{3}$H-labeled putrescine because the $^{14}$C recovery from the electrophoresis paper was increased eight-fold over that obtained using $^{3}$H-putrescine. As mentioned previously, male Sprague-Dawley rats weighing 500 gm were anesthetized and femoral cutdowns were performed. Twenty microcuries of 1,4-$^{14}$C-putrescine in 0.2 ml of 0.15 *M* NaCl solution (a total of 0.15 mg putrescine dihydrochloride) was injected into each animal. The cutdown site was closed with skin staples and the animals kept at room temperature in a cage without food or water. Six hours after injection, the rats were sacrificed by exsanguination, the abdominal cavity opened with a midline incision, and the two ventral prostatic lobes were removed. Great care was taken not to injure the bladder or other surrounding organs. The two lobes of the ventral prostate were immediately weighed (wet wt) in a plastic vial, and kept at —20°C for not longer than 1 week. At the time of assay, the polyamines were extracted from the prostatic tissue using the technique described by Raina (*12*). The entire ventral prostate (both lobes) was homogenized in 2 ml of 10% trichloracetic acid (TCAA). One milliliter of this homogenate was then mixed with 3 ml of 10% TCAA, allowed to stand for 10 min, and centrifuged at 1,000 *g* for 20 min. The supernatant was removed and saved; 2 ml of 10% TCAA was added to the precipitate, mixed well, allowed to stand for 10 min and centrifuged as above. The supernatants were combined and TCAA removed by six equal volume extractions with diethyl ether. The ether was removed by suction, the resulting product saturated with a sodium sulfate-sodium phosphate salt mixture, and the pH adjusted to above 9.0 with NaOH. An equal volume of n-butanol was added and the solution mixed on a vortex mixer for 10 min. The butanol layer containing the polyamines was removed and the pH adjusted to 1.0 with 12 *N* HCl. The butanol extraction was done twice and the butanol fractions combined. The acidified mixture was evaporated overnight in an evaporating hood. The resulting precipitate was reconstituted in 0.5 ml of 0.1 *N* HCl. Ten microliters of each sample was then electrophoresed using the paper electrophoretic technique previously described (*13*). For each ventral prostate sample two duplicate electrophoretic strips were run side by side (10 $\mu$l on each), along with a strip containing spermine, spermidine, and putrescine standards. One prostate strip was stained with a ninhydrin solution (100 mg cadmium acetate, 10 ml $H_2O$, 5 ml glacial acetic acid, 100 ml acetone, and 1 gm ninhydrin); the other was left unstained. The unstained strip was then compared with the stained strip and with a stained strip containing known concentrations of spermine, spermidine, and putrescine standards. This comparison enabled us to divide the unstained strip into sections representing spermine, spermidine, and putrescine. These paper sections were placed in appropriately labeled scintillation vials. The remainder of the unstained electrophoretic strip was cut into eight arbitrary areas numbered one through eight, and each section was placed into appropriately marked scintillation vials. To each vial was added 10 ml of Aquasol (New England Nuclear). The vials were counted for 10 min in a Packard liquid scintillation spectrophotometer with an efficiency for $^{14}$C of 70%. In addition, appropriate controls were done to determine the recovery of the polyamines through the extraction and electrophoresis steps.

## RESULTS

**Prostate.** The average weight of the total ventral prostate (both lobes) in the rat weighing 500 gm

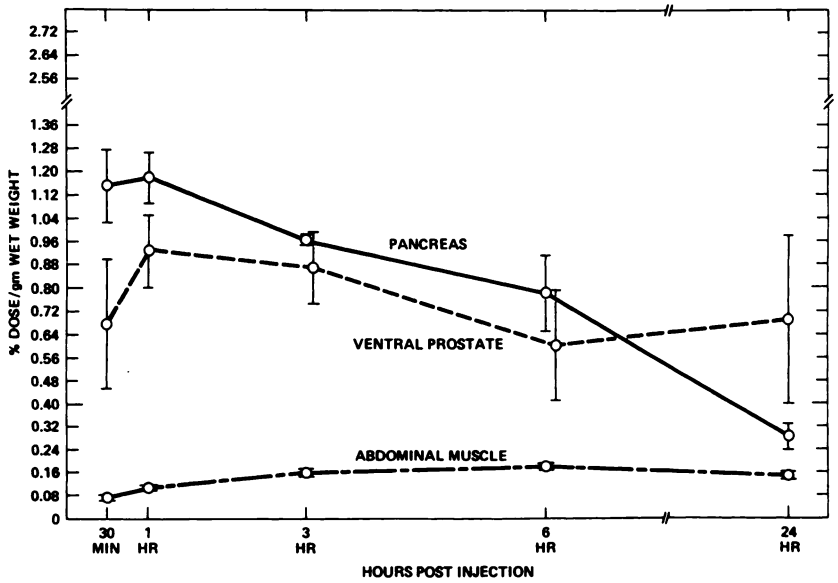


**FIG. 1.** In vivo uptake of $^{3}$H-putrescine by rat ventral prostate, pancreas, and abdominal muscle at various intervals after intravenous injection. Results are expressed as percentage of total injected dose per gram wet tissue weight.

was found to be approximately 650 mg. Of the time periods studied, the peak concentration of radioactivity in the ventral prostate was reached at 1 hr after intravenous injection (Fig. 1). Over the course of the 24 hr the animals were studied, the concentration in the ventral prostate did not vary greatly from the peak values. At 1 hr the ventral prostate-to-muscle ratio of $^{3}$H concentration was measured at 8.3:1 (Table 1). The 650 mg of ventral prostate contained 0.6% of the total injected dose of radioactivity at 1 hr following injection (0.9%/gm wet wt) (Table 1).

**TABLE 1. UPTAKE OF $^{3}$H-PUTRESCINE AT 1 HR AFTER INTRAVENOUS INJECTION**

| | Percent dose/gm tissue | Percent dose/total organ weight | Organ: abdominal wall muscle ratio |
|---|---|---|---|
| Abdominal wall muscle | 0.11 | 24.6 | 1:1 |
| Ventral prostate | 0.91 | 0.6 | 8.3:1 |
| Dorsal prostate | 0.36 | 0.1 | 3.3:1 |
| Pancreas | 1.16 | 0.5 | 10.5:1 |
| Liver | 0.31 | 5.0 | 2.8:1 |
| Kidney | 0.59 | 2.0 | 5.3:1 |
| Whole blood | 0.11 | 3.5 | 1:1 |

The average weight of the dorsolateral prostate in the 500-gm rat was approximately 330 mg. Of the time periods studied (the 30-min values were discarded for technical reasons), the concentration of radioactivity in the dorsolateral prostate was greatest at 1 hr (Fig. 2). The concentration of radioactivity in the dorsolateral prostate, as in the ventral pros-

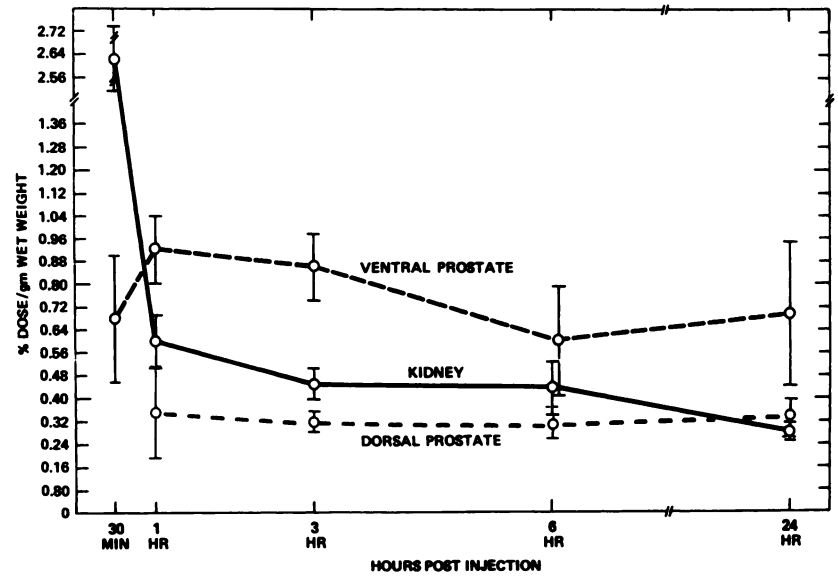


**FIG. 2.** In vivo uptake of $^{3}$H-putrescine by rat ventral prostate, kidney, and dorsal prostate at various intervals after intravenous injection. Results are expressed as percentage of total injected dose per gram wet tissue weight.

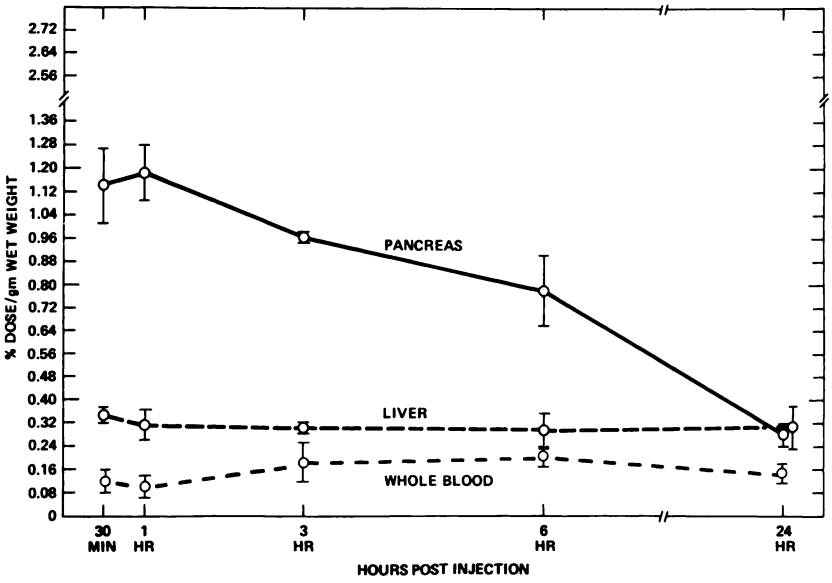


**FIG. 3.** In vivo uptake of $^{3}$H-putrescine by rat pancreas, liver, and whole blood at various intervals after intravenous injection. Results are expressed as percentage of total injected dose per gram wet tissue weight.

tate, did not vary greatly over 24 hr. The lowest values observed were at 6 hr, at which time the concentration of radioactivity was still approximately 85% of the peak value. At 1 hr the dorsolateral prostate-to-muscle ratio of $^{3}$H concentration was 3.3:1 and the 330 mg of dorsolateral prostate contained approximately 0.1% of the injected dose (0.3%/gm wet wt) (Table 1). Thus, approximately 0.7% of the total injected $^{3}$H dose was taken up by the dorsolateral and ventral lobes of the rat prostate under these experimental conditions (Table 1).

**Pancreas.** The average weight of the pancreas in a 500-gm rat was 430 mg. The pancreas like the ventral prostate was found to have the peak concentration of radioactivity at 1 hr. At this time the pancreas-to-muscle ratio of radioactivity concentration was 10.5:1 (Table 1). The concentration of radioactivity in the pancreas after peaking at 1 hr fell steadily over the next 24 hr (Fig. 1). At 24 hr, the pancreas contained about 25% of its peak concentration. At 1 hr the pancreas contained 0.5% of the total injected radioactivity (approximately 1.2%/gm wet wt) (Table 1).

**Liver.** The average weight of the liver in the 500-gm rat was approximately 15 gm. Peak values of $^{3}$H were found in the liver at 30 min after injection (Fig. 3). Over the next 24 hr, there was little change found in $^{3}$H concentration in the liver, the lowest levels being at 6 hr. The liver-to-muscle ratio approximated 2.8:1 for the entire study. The 15-gm liver contained approximately 5.0% of the injected dose at 1 hr (0.3%/gm wet wt) (Table 1).

**Kidney.** The average weight of the two kidneys in the 500-gm rat was 3.3 gm. Peak values of $^{3}$H in the kidney were found at 30 min, at which time the kidney contained 2.6% of the dose per gram wet weight (Fig. 2). At 1 hr the concentration of radioactivity in the kidney had fallen to 0.6% of the dose per gram wet weight and fell only slightly over the next 24 hr. At 30 min the kidney-to-muscle ratio was 36:1, but at 1 hr the ratio had fallen to 5.3:1. At 1 hr the 3,300 mg of kidney contained approximately 2% of the injected dose (Table 1).

**Urine.** The urine, when available, was obtained by bladder aspiration at the time the rodent was sacrificed. The urine radioactivity, while reflecting the accumulation of urine over varying periods of time, nevertheless demonstrated a similar time-concentration pattern as the kidney. Urine values were highest at 30 min (with values of 8% and 23% of the dose per milliliter), and fell quickly at 1 hr to 1.3% of the dose per milliliter, to plateau for the remaining 24-hr period at approximately 0.8% of the dose per milliliter.

**Muscle.** The average weight of muscle in a 500-gm rat is approximately 45.4% of body weight or 227 gm (*14*). The concentration of $^{3}$H-putrescine in the abdominal wall muscle rose slowly to a mean peak value of 0.2% dose/gm wet wt at 6 hr (Fig. 1). The 24-hr $^{3}$H concentration had decreased only slightly. At 1 hr the 227 gm of muscle contained approximately 24.6% of the injected dose (0.1%/gm wet wt) (Table 1).

**Blood.** A 500-gm rat has a whole blood volume of approximately 6–7% of body weight (32.5 gm) (*14*). In the time periods examined, the greatest concentration of radioactivity in whole blood was found at 6 hr with values of 0.2% dose per milliliter and the lowest at 1 hr (Fig. 3). At 1 hr the whole blood-to-muscle ratio of $^{3}$H concentration was 1:1. The approximately 32.5 gm of blood contained

3.5% of the injected dose 1 hr following administration (0.1%/ml) (Table 1).

Excluding urine, the organs studied at 1 hr account for approximately 36% of the total injected disintegrations per minute (Table 1).

### POLYAMINE SYNTHESIS STUDIES

These experiments were performed to determine the fate of the injected radioactive putrescine in the prostate, specifically to demonstrate the in vivo biosynthetic pathway for the polyamines in the prostate as has been shown in vitro (*9*) and in vivo using other organs (*11*). The results of these studies are seen in Fig. 4. The areas of the electrophoretic strip are expressed as putrescine, spermine, and spermidine and the "remainder of the strip." The latter category was used because there was no area on the strip that consistently contained as many radioactive counts as those found in the polyamine fractions. The "remainder of the strip" represents the total counts on the strip minus the counts found in the three amine areas. The values shown in Fig. 4 represent the average percentages obtained from the ventral prostates of three rats at 6 hr. More than 90% of the radioactivity recovered from the prostate 6 hr following injection of the isotope was in the form of spermine and spermidine rather than putrescine (approximately 4%); the total radioactivity in the other areas of the paper strip accounted for the remaining 5% of the isotope recovered.

When the $^{14}$C-putrescine that was used for injection was electrophoresed without having been run through the extraction procedure, 98.6% of the recovered radioactivity was found in the putrescine area; only 1.4% of the injected $^{14}$C-putrescine was recovered in the "remainder of the strip" and the spermine and spermidine areas.

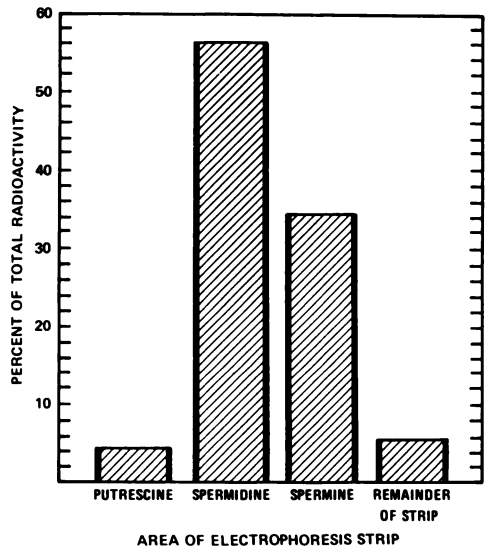


**FIG. 4.** Percentage distribution of total radioactivity recovered from rat ventral prostate 6 hr after intravenous injection of $^{14}$C-putrescine. Bars represent mean percentage of total electrophoretic strip radioactivity that was recovered from areas corresponding to migration of spermine, spermidine, putrescine, and radioactivity on remainder of strip.

### DISCUSSION

In this study we demonstrated that exogenously administered $^{3}$H-putrescine is selectively taken up by the rat ventral prostate in vivo and that the degree of incorporation was greater for the prostate than for many other organs. These experiments were based on previous work that had shown that the prostate contained large amounts of polyamines (*1*) and that putrescine could serve as a precursor for these polyamines in vivo in other organs (*11*) and in vitro for rat prostate homogenates (*9*).

Intravenously injected $^{3}$H-putrescine was selectively concentrated in the rat pancreas as well as the ventral prostate. As early as 30 min after the administration of $^{3}$H-putrescine, the concentration of radioactivity in the pancreas and prostate was many times greater than that found in most other organs examined. The concentration of radioactivity in these two organs was not surprising in that both contain high concentrations of polyamines compared with the other body organs (*1*). Excluding the 30-min kidney and urine values, the greatest amount of radioactivity was found in the pancreas, the activity peaking at 1 hr at a concentration ten times greater than abdominal musculature and more than three times greater than liver concentrations. The radioactivity in the ventral prostate also peaked at 1 hr after injection at concentrations slightly less than those found in the pancreas. At 1 hr the concentration of radioactivity in the ventral prostate was approximately eight times greater than that of the abdominal muscle. As would be expected considering the lower concentrations of polyamines reportedly found in these tissues (*1,7*), the liver, kidney (except for early time periods), abdominal muscle, dorsal prostate, and blood all contained significantly lower concentrations of radioactivity. In the liver the concentration of radioactivity was consistent over the time periods examined, the values being approximately 35% of those of ventral prostate at its peak.

Interestingly, Jänne (*11*) found that 2 hr following intraperitoneal injection of $^{3}$H-putrescine, normal liver contained 6% of the injected dose. In agreement, we found that at 1 and 3 hr following intravenous injection the liver contained approximately 5% of the injected dose. The highest concentration of radioactivity in the kidney was found at 30 min after injection. At this time the values were almost three times as great as the peak values in the pan-

creas and prostate, but the kidney values decreased rapidly by 1 hr after injection to 50% of peak values found in the prostate. The urine concentration of radioactivity, while not accurately reflecting timed excretion (due to collection through bladder aspiration), also showed a similar time concentration pattern. We believe that the high and early peaking of radioactivity in the kidneys most probably represents the very quick renal clearance of $^{3}$H-putrescine or its metabolites into the urine and this probably accounts for much of the 64% of the radioactivity not recovered.

Abdominal wall musculature contained concentrations of radioactivity that consistently ranged at levels about 15% of peak ventral prostate levels. The dorsal prostate contained concentrations that approximated 33% of peak ventral prostate levels even though these two organs are in anatomical contiguity. The concentration of radioactivity in whole blood was approximately equal to that of muscle and varied very little over the time period observed. At 24 hr after injection only the ventral prostate concentrations remained slightly elevated above the values found in abdominal muscle.

The finding that most of the radioactivity recovered from the ventral prostate was incorporated into spermidine and spermine was in agreement with the in vivo work of Jänne (*11*) with rat liver and confirmed the in vitro observation that the biosynthesis of the polyamines involves the formation of spermine and spermidine through putrescine (*9*).

The prostate in man as in the rat contains relatively high amounts of polyamines. The selective uptake of the labeled polyamine precursor by the rat prostate thus raises the possibility that putrescine might serve as a vehicle for a radioisotope scan of the prostate in humans if a suitable gamma-emitting isotope could be incorporated into the molecule without significantly altering its distribution characteristics in the body. Furthermore, the ratio of ventral prostate-to-abdominal muscle uptake (8:1) may make a scan feasible. While spermine has been shown to be somewhat nephrotoxic in mice, spermidine was found to be only one-twentieth as toxic and putrescine was found to have no nephrotoxicity (*15*).

The rat pancreas is also rich in polyamines, especially spermidine (*16*), and as expected, it incorporated the administered putrescine readily (1.2% of injected dose per gram at 1 hr). The human pancreas, however, does not contain the relatively high amounts of polyamines that are found in the rat pancreas (*1*). Therefore, using putrescine to visualize the pancreas in man may not prove to be as feasible as using the diamine to visualize the human prostate and other tissues rich in polyamines such as certain neoplasms (*6*).

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REFERENCES

*1.* TABOR H, TABOR CW: Spermidine, spermine, and related amines. *Pharmacol Rev* 16: 245–300, 1964

*2.* RAINA A, JÄNNE J, SIIMES M: Stimulation of polyamine synthesis in relation to nucleic acids in regenerating rat liver. *Biochim Biophys Acta* 123: 197–201, 1966

*3.* TABOR H: The protective effect of spermine and other polyamines against heat denaturation of deoxyribonucleic acid. *Biochemistry* 1: 496–501, 1962.

*4.* SILMAN N, ARTMAN M, ENGELBERG H: Effect of magnesium and spermine on the aggregation of bacterial and mammalian ribosomes. *Biochim Biophys Acta* 103: 231–240, 1965

*5.* RAINA A, TELARANTA T: Association of polyamines and RNA in isolated subcellular particles from rat liver. *Biochim Biophys Acta* 138: 200–203, 1967

*6.* RUSSELL DH, LEVY CC: Polyamine accumulation and biosynthesis in a mouse L1210 leukemia. *Cancer Res* 31: 248–251, 1971

*7.* WILLIAMS-ASHMAN HG, LOCKWOOD DH: Role of polyamines in reproductive physiology and sex hormone action. *Ann NY Acad Sci* 171: 882–894, 1970

*8.* PEGG AE, LOCKWOOD DH, WILLIAMS-ASHMAN HG: Concentration of putrescine and polyamines and their enzymic synthesis during androgen induced prostatic growth. *Biochem J* 117: 17–31, 1970

*9.* PEGG AE: Biosynthesis of putrescine and polyamines in mammalian tissues. *Ann NY Acad Sci* 171: 977–987, 1970

*10.* RUSSELL DH, MEDINA VJ, SNYDER SH: The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain. *J. Biol Chem* 245: 6732–6738, 1970

*11.* JÄNNE J: Studies on the biosynthetic pathway of polyamines in rat liver. *Acta Physiol Scand (Suppl)* 300: 7–71, 1967

*12.* RAINA A: Studies on the determination of spermidine and spermine and their metabolism in the developing chick embryo. *Acta Physiol Scand (Suppl)* 60: Suppl 218: 5–81, 1963

*13.* FAIR WR, CLARK RB, WEHNER N: A correlation of seminal polyamine levels and semen analysis in the human. *Fertil Steril* 23: 38–42, 1972

*14.* DONALDSON HH: *The Rat-Data and Reference Tables*, No 6, Memoirs of the Wistar Institute of Anatomy and Biology. Philadelphia, Wistar Institute, 1915, pp 77 and 132

*15.* TABOR CW, ROSENTHAL SM: Pharmacology of spermine and spermidine. Some effects on animals and bacteria. *J Pharmacol Exp Ther* 116: 139–155, 1956

*16.* ROSENTHAL SM, TABOR CW: The pharmacology of spermine and spermidine. Distribution and excretion. *J Pharmacol Exp Ther* 116: 131–138, 1956