intensity of any point in the image could be determined from the number at the memory address of the point. The entire image could be displayed on a television monitor for inspection and photographing.

Preliminary results are shown in Figs. 1 and 2. Figure 1 is a comparison of the response of the electronic technique to that of the x-ray film as measured on our densitometer. The same ^{3}H- and scintillant-containing test strips were used for both curves. The improved linearity of the electronic response curve is evident. A comparison of the film image from a dried polyacrylamide gel containing electrophoretically separated ^{3}H-labeled proteins and PPO with the image generated from digital memory and photographed from the television screen is shown in Fig. 2. After an electronic exposure of 2 minutes, the images are comparable in visible detail and resolution, although the exposure time for the electronic method was over 1400 times less. Easily recognizable and measurable features were visible after 1 minute.

Because of the increased sensitivity at low radioactivity levels, the picture generated from our digital data shows larger spots than the film autofluorograph but more nearly represents the actual sizes of the spots. Film, because of its threshold, can give an exaggerated appearance of resolution by suppressing weak peripheral activity and emphasizing the peaks. Manipulation of the digital data in order to display the peaks of activity is easily done with our system and would have produced a picture more like that of the film. We chose to display all the data down to background level. Since the resolution of our camera and recording system was better than 0.5 mm for a point image, smaller spots than those in the photograph could have been seen. We conclude that the resolution (minimum spot size and separation) was determined by the electrophoretic technique.

We believe that these results point the way to a new instrument for rapid and improved quantitation of low-energy, beta-emitting isotopes used in chromatography and electrophoresis. Furthermore, isotopes such as ^{55}Fe, ^{125}I, or ^{51}Cr, which emit Auger electrons, or fluorescent x-rays should be amenable to this technique (*12*). Higher energy beta particles, such as those from ^{32}P and electron-capture gamma rays, could be detected with interposed phosphors and conversion layers. The greatly increased sensitivity should permit the use of much less radioactivity, which would lower the cost and ease the problem of radioactive waste disposal. In addition, as has been pointed out by O'Farrell (*13*), smaller initial amounts of protein should improve the resolution of closely spaced spots. Alternatively, the speed with which the distributions can be visualized suggests that one might be able to observe the movement of the macromolecules by taking a sequence of photographs constituting a time-lapse motion picture of the pattern development. Drift rates should be easily determined from such digital pictures. Since light is the agent that enables one to detect the particles, the system can be adapted for the quantitation of fluorescence and visible light absorption introduced by selective staining (*14*).

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11 August 1981

Human Liver MAO-A and MAO-B Separated by Immunoaffinity Chromatography with MAO-B–Specific Monoclonal Antibody

Abstract. *A monoclonal antibody was used to prepare immunoaffinity columns that efficiently bind monoamine oxidase B activity but not monoamine oxidase A activity from detergent extracts of human liver mitochondria. The only discrete polypeptide component that eluted from affinity columns with potassium thiocyanate migrated in sodium dodecyl sulfate–polyacrylamide gels with an apparent molecular weight of 59,000, as expected for human monoamine oxidase B. These results support the hypothesis that there is an intrinsic structural difference between monoamine oxidase A and B and demonstrate that immunoaffinity chromatography can physically resolve the two enzyme species in liver extracts.*

Monoamine oxidase (MAO) (E.C. 1.4.3.4) is an integral protein of the outer mitochondrial membrane (*1*) and plays an important role in the degradation of dopamine, norepinephrine, epinephrine, and serotonin (*2*). In most tissues, MAO consists of a mixture of two catalytically distinct forms, A and B, each expressed in characteristic proportions (*3*). MAO-A is inhibited by the irreversible active site inhibitor clorgyline and oxidizes serotonin (*4*), while MAO-B is inhibited by the related compounds deprenyl (*5*) and pargyline (*6*) and oxidizes phenylethylamine and benzylamine (*5*). These inhibitors mimic substrates of the respective MAO forms and bind covalently to a cysteinyl-flavin residue in the active site subunit of MAO (*7*). Studies with [^{3}H]pargyline-labeled enzyme have shown that the flavin-adenine dinucleotide–containing subunits of rat MAO-A and -B migrate in sodium dodecyl sulfate (SDS)–polyacrylamide gels with apparent molecular weights of 55,000 and 60,000, respectively (*8*). Similarly, human MAO-B from platelets and MAO-A from placenta are reported to have molecular weights of 64,000 and 67,000, respectively (*9*), or 60,000 and 64,000 (*10*).

One approach to studying molecular differences between the two MAO species is to investigate the ability of antibodies to distinguish them. Results of studies with conventional antiserums have been equivocal (*11–13*). Since pub-

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lication of the earlier immunological studies on MAO-A and -B, the hybridoma technique of Köhler and Milstein (*14*) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens (*15*). We now present evidence that an immunoaffinity column carrying a monoclonal antibody to human platelet MAO (primarily type B) binds MAO-B from human platelets and liver but fails to bind MAO-A from liver.

We identified antibody MAO-1C2 among the products of a polyethylene glycol–mediated fusion (*16*) of mouse myeloma P3/X63 Ag 8 (*14*) and spleen cells from a BALB/c mouse immunized with partially purified platelet protein in which 20 to 30 percent of the protein was human MAO-B. The enzyme used for injection and screening was purified by ammonium sulfate precipitation and DEAE-cellulose chromatography (*17*) and then labeled with [^{3}H]pargyline (New England Nuclear; specific activity, 15 Ci/mmole) to a specific activity of 800 count/min per nanomole of MAO protein (assuming a molecular weight of 120,000 for the native enzyme). The enzyme was further purified by isoelectric focusing or chromatofocusing. Figure 1A shows SDS–polyacrylamide gel profiles of the DEAE-cellulose–eluted and chromatofocused MAO preparations. The tritiated protein in both preparations migrated as a single peak corresponding to a molecular weight of 59,000 (not shown), which is close to the molecular weight of the flavin-adenine dinucleotide-containing subunit of MAO-B reported by Cawthon *et al.* (*10*). The band of stained protein indicated by the arrow in Fig. 1A comigrated with [^{3}H]pargyline, as demonstrated by excision of the band and estimation of its radioactivity (*18*). Hybridoma MAO-1C2 secretes an antibody that binds 97 percent of the radioactivity in [^{3}H]pargyline-labeled human platelet MAO (*19*). Details on the isolation and characterization of MAO-1C2 are given elsewhere (*19*).

The source of antibodies for these experiments was peritoneal fluid from a mouse bearing an ascites tumor of a subclone of MAO-1C2 (1C2 8). The fluid, which was capable of binding the equivalent of 14.1 mg of MAO per milliliter, was precipitated with ammonium sulfate, dialyzed, and linked to cyanogen bromide–activated Sepharose 4B or Affi-gel-10 (Bio-Rad), as described in the legends to Fig. 2 and Table 1. The [^{3}H]pargyline-labeled protein extracted from human platelets and purified by DEAE Sephacel chromatography (lanes 3 and 4 in Fig. 1A) was bound in a Sepharose-1C2 column, while the vast majority of protein (97 percent) was not (Fig. 2A). The [^{3}H]pargyline-labeled MAO did not elute from Sepharose-1C2 with 1*M* NaBr. However, 4*M* KSCN eluted 77 percent of the applied [^{3}H]pargyline-labeled protein and less than 2 percent of the total protein, indicating at least a 40-fold increase in the activity of the labeled MAO-B. Adding a large amount of an octylglucoside extract of human liver mitochondria to the [^{3}H]pargyline-labeled platelet extract before exposure to the column did not interfere with the binding of [^{3}H]pargyline-labeled platelet MAO (Fig. 2B).

To test the ability of an MAO-1C2 affinity column to discriminate human MAO-A and -B, we mixed 74 μg of protein containing [^{3}H]pargyline-labeled human liver MAO-B (legend to Table 1) with 13.8 mg of protein from the same extract containing unlabeled, catalytically active MAO-A and -B and circulated the mixture through the column, as described in the legend to Fig. 2. After 90 minutes, the radioactivity of the labeled MAO-B in the extract exposed to the column dropped from 29,000 to 100 count/min per milligram of protein, indicating 94.3 percent removal of labeled MAO-B. Binding of labeled MAO-B was highly selective, since at least 95 percent of the applied protein was not bound. Samples of the extract before and after column exposure were assayed for MAO catalytic activity by using as substrates phenylethylamine and serotonin, which, under the assay conditions, are highly specific for MAO-B and -A activities, respectively. The results of the activity assays revealed a large decrease in the specific activity of MAO when phenylethylamine was used as the substrate, but no corresponding reduction with serotonin (Table 1). These results suggest that the column efficiently discriminated between human liver MAO-A and -B. As expected, serotonin oxidation, both before and after column exposure, was virtually all due to MAO-A (sensitive to clorgyline but not deprenyl) (Table 1). Conversely, before column exposure, phenylethylamine oxidation was virtually all due to MAO-B (sensitive to deprenyl but not clorgyline) (Table 1). After column exposure a low level of phenylethylamine oxidation was detected, some of which, judging from its sensitivity to clorgyline, was catalyzed by

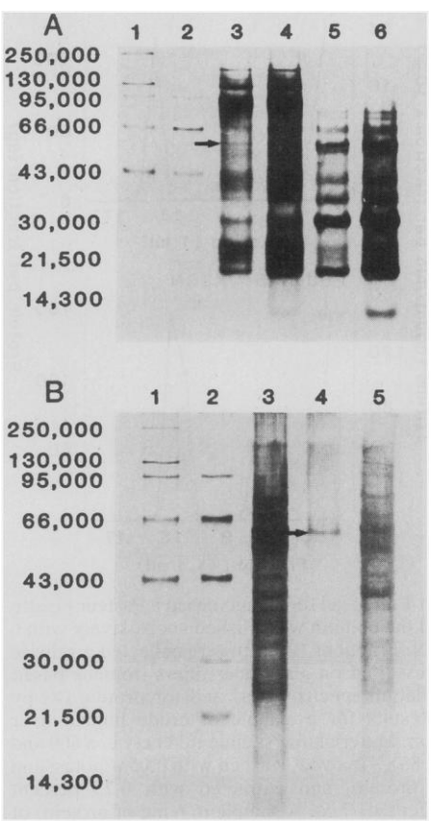

Fig. 1. Profiles of protein in selected samples containing human MAO run on SDS–polyacrylamide slab gels. Samples were run in the discontinuous system of Fairbanks *et al.* (*25*), with 2.5 percent stacking gel and 7.5 percent running gel. Gels were electrophoresed in a Bio-Rad vertical slab gel apparatus. Samples were denatured by treatment with 1 percent (weight to volume) SDS and 5 percent (by volume) β-mercaptoethanol at 100°C for 5 minutes before application to gels. (A) Protein profiles stained with silver (*26*). Lane 1 [high molecular weight standards (Bio-Rad), 0.1 μg of protein per band] shows (from top to bottom) myosin, 250,000 daltons; β-galactosidase, 130,000; phosphorylase B, 95,000; bovine serum albumin, 66,000; and ovalbumin, 43,000. Lane 2 [low molecular weight standards (Bio-Rad), 0.1 μg of protein per band] shows phosphorylase B, 95,000; bovine serum albumin, 66,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,338. Lane 3 shows the DEAE fraction from human platelets containing [^{3}H]pargyline-labeled MAO (arrow) (1.4 μg of total protein). Lane 4 is the same as lane 3 (2.8 μg of total protein). Lane 5 shows the [^{3}H]pargyline-labeled fraction from human platelets containing MAO purified by chromatofocusing (1.25 μg of total protein). Lane 6 is the same as lane 5 (2.5 μg of total protein). (B) Protein profiles of liver mitochondrial extract chromatographed in the column experiment illustrated in Fig. 2B. Lane 1 shows high molecular weight standards as in lane 1 in (A). Lane 2 shows low molecular weight standards as in lane 2 in (A). Lane 3 shows proteins of the liver mitochondrial extract which failed to bind to Sepharose-1C2 in the column experiment illustrated in Fig. 2B (1.65 μg of total protein from fraction 1, Fig. 2B). Lane 4 shows KSCN-eluted material (arrow) from the column experiment illustrated in Fig. 2B (0.3 μg of protein applied). Lane 5 shows liver mitochrondrial extract before exposure to the column (1.45 μg of protein applied).

Table 1. Selective removal of MAO-B by the MAO-1C2 immunoaffinity column. A sample of octylglucoside extract of human liver mitochondria (13.8 mg total protein) was mixed with 76 μg of protein from the same extract, which had been treated with $10^{-6}M$ clorgyline to block MAO-A and then exposed to $10^{-6}M$ [^{3}H]pargyline (specific activity, 15 Ci/mmole) to label MAO-B. The mixed sample was circulated through a 2-ml column containing 9 mg of MAO-1C2 antibody coupled to Affigel-10 (Bio-Rad). After 90 minutes the column flow was stopped. The mixed sample applied to the column was assayed for catalytic activity of MAO by using $10^{-6}M$ [^{14}C]phenylethylamine or $10^{-3}M$ [^{14}C]serotonin as substrates (*17, 24*). The substrate concentrations were chosen so that phenylethylamine and serotonin were oxidized preferentially by MAO-B and -A, respectively. Each value is the mean (the range for duplicate samples is shown in parentheses). One unit of MAO activity oxidizes 1 nmole of substrate per hour at 37°C. In addition, the concentration of [^{3}H]pargyline-labeled MAO-B in the extract before and after column exposure was determined by direct scintillation counting. The deprenyl-sensitive component was calculated as the total activity minus the activity for the same substrate not sensitive to $10^{-6}M$ deprenyl. The clorgyline-sensitive component was calculated as the total activity minus the activity for the same substrate not sensitive to $10^{-6}M$ clorgyline. A drop in the deprenyl-sensitive component of phenylethylamine activity is the best measure of the binding of MAO-B activity in the column, whereas a drop in the clorgyline-sensitive component of serotonin activity is the best measure of the binding of MAO-A activity.

Substrate	Activity component	Catalytic activity of MAO (U/mg protein)	
		Before column	After column
Phenylethylamine	Deprenyl-sensitive	73.6 (72.7 to 74.8)	4.7 (3.6 to 5.8)
	Clorgyline-sensitive	3.6 (1.3 to 5.9)	4.9 (3.9 to 5.9)
	Total	73.8 (72.8 to 74.8)	8.1 (7.2 to 9.0)
Serotonin	Deprenyl-sensitive	0 (−23 to 2)	18 (8 to 28)
	Clorgyline-sensitive	264 (256 to 272)	274 (269 to 277)
	Total	268.5 (261 to 276)	274 (270 to 278)

MAO-A. With the reduction in the deprenyl-sensitive component of phenylethylamine oxidation taken as the best measure of the MAO-B loss, exposure of the extract to the column reduced the MAO activity from 73.6 to 4.7 deprenyl-sensitive phenylethylamine units per milligram of protein (Table 1), or 93.8 percent. This result agrees with the removal of 94.3 percent of the [^{3}H]pargyline-labeled MAO-B by the column and suggests that the loss of MAO-B activity in the column is due to binding of MAO-B rather than to its inactivation. The results also confirm that monoclonal antibody MAO-1C2 does not distinguish between catalytically inactive, [^{3}H]pargyline-labeled human liver MAO-B and catalytically active, unlabeled human liver MAO-B. Using as a measure of MAO-A activity either the clorgyline-sensitive component of serotonin oxidation or the total serotonin oxidation, we found no detectable binding of MAO-A activity by the column (Table 1).

Although fractions eluted from the MAO-1C2 immunoaffinity columns with KSCN contained most of the [^{3}H]pargyline-labeled MAO-B applied, they were enzymatically inactive, as were fractions eluted with NaBr. Nevertheless, the protein in KSCN-eluted fractions derived from human liver mitochondria appeared to be virtually all MAO, judging from the presence in SDS-polyacrylamide gels of a discrete polypeptide with the apparent molecular weight expected of human MAO-B (lane 4 in Fig. 1B). The simplicity of the protein profile of the KSCN eluate should be contrasted with the complexity of the SDS–polyacrylamide gel profile of the liver extract before column exposure (lane 3 in Fig. 1B). It appears that very little protein other than the specific antibody-binding protein was bound by these columns, since the SDS–polyacrylamide gel profile of the proteins that were not bound (Fig. 2B) was similar in complexity to the profile of the applied sample (lane 5 in Fig. 1B).

From these data we conclude that Sepharose-1C2 columns bind MAO-B but not MAO-A from human liver. We have tested extracts of human placental mitochondria, which contain primarily MAO-A, and found that the serotonin-oxidizing activity in the extracts also does not bind to Sepharose-1C2 columns (*19*). Furthermore, immunoprecipitation experiments indicate that catalytically active MAO-B in extracts of mouse liver mitochondria does not bind to MAO-1C2, suggesting that the antibody discriminates between human and mouse MAO-B (*19*). The inability of the antibody to recognize mouse MAO-B is not surprising, since the antibody was elicit-

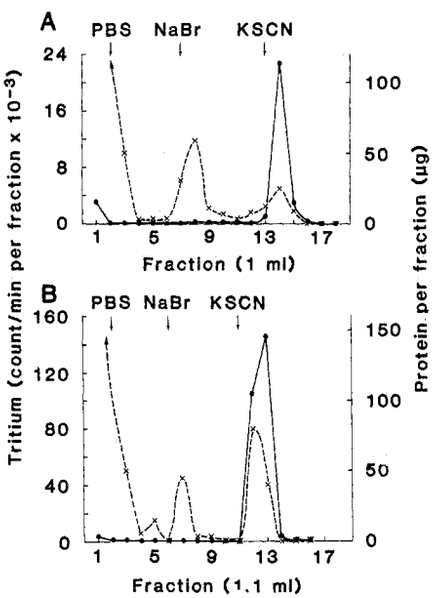

Fig. 2. Chromatography of extracts of human platelets and liver on Sepharose-1C2. Sepharose-1C2 was made by linking ammonium sulfate–precipitated, dialyzed ascites fluid from a BALB/c mouse bearing a tumor of hybridoma MAO-1C2 to cyanogen bromide–activated Sepharose. Sepharose 4B was activated for 5 minutes at *p*H 11 with 0.5 g of CNBr per 25 ml of settled gel and incubated overnight in 0.1*M* $NaHCO_3$ and 0.15*M* KCl (*p*H 8.6) with 10 mg of protein from ammonium sulfate–precipitated (40 percent saturation), dialyzed ascites fluid per 8.5 g of wet, activated gel. The gel was blocked with 1*M* 2-ethanolamine and washed extensively with phosphate-buffered saline (PBS) (0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 1.144 g of Na_2HPO_4 per liter), 2*M* NaBr, and 4*M* KSCN and equilibrated with PBS containing 0.75 percent (weight to volume) octylglucoside. Columns were run at 4°C in solutions containing 0.75 percent octylglucoside. (A) Results for a sample (0.3 ml, containing 1.08 mg of protein) of [^{3}H]pargyline-labeled MAO extracted from human platelets and purified on DEAE Sephacel (see lanes 3 and 4 in Fig. 1A). The sample was diluted to 1 ml and mixed with 1 ml of gel for 30 minutes in a Pasteur pipette plugged with glass wool. The flow was started and the column was washed successively with 6 ml of PBS, 6 ml of 1*M* NaBr, and 6 ml of 4*M* KSCN. Fractions (1.1 ml) were collected dropwise and assayed for ^{3}H (●) by counting 50-μl samples dried on glass fiber filters (toluene-based scintillation fluid, Packard Tri-Carb liquid scintillation spectrometer) and for protein (X) by mixing 50 μl with 1 ml of reagent (*27*). (B) Results for a sample of crude human liver mitochondria prepared by differential centrifugation. Material that sedimented between 600 and 6500*g* (crude mitochondrial pellet) was frozen at −80°C, thawed, washed with 0.05*M* potassium phosphate buffer (*p*H 7.5) to remove soluble protein, and extracted with 0.75 percent octylglucoside in 0.05*M* potassium phosphate buffer (*p*H 7.5). A sample (6.6 mg of protein) of this extract was mixed with a sample of a [^{3}H]pargyline-labeled DEAE fraction of human platelet MAO (0.36 mg of protein containing 3.6 ng of MAO; see lanes 3 and 4 in Fig. 1A), diluted to 7 ml with PBS, and adjusted to a final octylglucoside concentration of 0.75 percent. This mixture was circulated continuously for 90 minutes through a column containing 1 ml of Sepharose-1C2 (flow rate, 2.4 ml/min) by means of a peristaltic pump. The column was then eluted and analyzed in the same way as the column described in (A).

ed by injecting a mouse with the human enzyme.

Our identification of a monoclonal antibody that discriminates between MAO-A and -B is consistent with recent evidence of structural differences in the active site subunits of the two species (*8, 9, 20*). Our results also agree with McCauley and Racker's (*11*) finding that bovine liver MAO-B has at least one antigenic site not found on bovine liver MAO-A. Conversely, Powell and Craig (*12*) have prepared antiserums that react preferentially with human MAO-A. Our results do not support the conclusion of Dennick and Mayer (*13*) or Russell *et al.* (*21*) that human liver MAO-A and -B are immunologically indistinguishable. It should be noted, however, that these investigators raised antiserums against MAO purified from liver mitochondria, which contain both MAO-A and -B. It is possible that their purified MAO contained a mixture of inactive MAO-A and active MAO-B (*22*), and therefore elicited antiserums containing a mixture of antibodies to MAO-A and antibodies to MAO-B. Such antiserums could inhibit both MAO-A and -B even if no single antibody recognized both molecules. In view of these uncertainties, additional monoclonal antibodies should be evaluated for their ability to discriminate MAO-A and -B.

Correlations between the level of MAO-B activity in human platelets and a variety of psychiatric and neurological disorders have been reported by numerous investigators (*23*), but there has been no method for determining whether the low MAO-B activities observed in some individuals are due to low amounts of normal enzyme or normal amounts of altered enzyme. Preliminary experiments show that crude extracts of human liver and platelets inhibit the binding of [^{3}H]pargyline-labeled MAO-B to MAO-1C2 and suggest that this competition can form the basis for a highly sensitive quantitative assay for MAO-B protein in these extracts.

The ready availability and high specificity of antibody MAO-1C2 should prove valuable in a wide range of studies of human MAO-B, including measurement of MAO protein in platelets from patients with neurological and psychiatric disorders and preparation of human MAO-B suitable for structural studies.

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Brain Target Sites for 1,25-Dihydroxyvitamin D_3

Abstract. *Autoradiographic studies with ^{3}H-labeled 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] demonstrate, in certain neurons of rat forebrain, hindbrain, and spinal cord, a nuclear retention and concentration of radioactivity, which can be prevented by treatment with 1,25(OH)$_2$D$_3$, but not with 25-hydroxyvitamin D$_3$. These results indicate the presence of brain receptors in addition to pituitary receptors for 1,25(OH)$_2$D$_3$ and suggest a central modulation of calcium homeostasis and other central effects for this hormone. The existence of a brain-pituitary axis for certain 1,25(OH)$_2$D$_3$-mediated endocrine-autonomic effects is postulated.*

Calcium homeostasis was generally not considered to be centrally regulated until 1979, when evidence for a direct central regulation was provided by our autoradiographic studies. After radioactively labeled 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] was injected in rats fed a vitamin D–deficient diet, radioactivity was found to be concentrated in the nuclei of certain cells in the pituitary pars distalis and infundibular process (*1*). Target cells in the anterior pituitary were identified, through combined autoradiography and immunohistochemistry, as thyrotrophs (*2*). Subsequently, biochemical studies demonstrated binding proteins for 1,25(OH)$_2$D$_3$ in a pituitary tumor cell line and in homogenates of whole pituitaries, without consideration of the specific pituitary lobes or cell types (*3*). The biochemical studies did not provide evidence of receptors for 1,25(OH)$_2$D$_3$ in rat brain (*3, 4*); in contrast, our autoradiographic experiments revealed neuronal target sites for this steroid hormone in the brain and spinal cord (*5*), as well as 25 new target cell types in peripheral organs (*6*).

Nine 21-day-old male Holtzman Sprague-Dawley rats were fed a vitamin D–deficient diet (*7*) for 5 to 6 weeks and kept at a photoperiodic cycle of 12 hours of light and 12 hours of darkness. All of the animals were given intravenous injections (0.19 μg per 100 g of body weight) of 1,25(OH)$_2$[26,27-^{3}H]D$_3$ (specific activity 160 Ci/mmole), dissolved in 75 percent ethanol–isotonic saline. Three

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