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DISCLAIMER

Technetium-99m Labelled Stannous-Ethane-1-Hydroxy-1,1-Diphosphonate: A New Bone Scanning Agent

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Ethane-1-hydroxy-1,1-diphosphonate (EHDP*) has been reported to be biochemically active by chemisorption (1) to hydroxyapatite and the subsequent inhibition of bone crystal formation and resorption (2). It has also been reported to be an effective agent for the treatment of Paget's disease of the bone (3). Because these properties of EHDP indicate bone specificity, we have investigated the usefulness of ^{99m}Tc labelled Sn-EHDP as a bone scanning agent. Various methods of labelling EHDP with ^{99m}Tc were investigated to provide a bone specific radiopharmaceutical which makes available the superior scanning characteristics of ^{99m}Tc, i.e. high yield of 140 keV photons and low radiation dose.

Although initial efforts to label EHDP with ^{99m}Tc by the zirconium electrolytic method were unsuccessful, a method using stannous tin is effective in labelling EHDP with ^{99m}Tc.

The chemical mechanism involved is uncertain, but it is probable that an electron transfer process takes place between divalent tin and Tc (a transition metal ion) and a chelating ligand such as tartrate or citrate (3,4), and in this case EHDP.

Materials and Methods

The EHDP used for this study was made available through the courtesy of M. D. Francis, A. J. Tofe, and the Procter and Gamble Co.

Reagent grade $SnCl_2 \cdot 2H_2O$ is used and freshly prepared in 0.01 N HCl acid just prior to the labelling procedure.

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* EHDP; trade name, Procter and Gamble Co.

Commercially available MEK extracted $^{99m}TcO_4$ (20 mCi/ml) in isotonic saline is used for the preparation of ^{99m}Tc-Sn-EHDP.

The method for making ^{99m}Tc-Sn-EHDP is as follows:

1. Dissolve 10 mg of reagent grade $SnCl_2 \cdot 2H_2O$ in 100 ml of 0.01 N HCl acid and filter 1 ml of this solution through a 0.22μ Millipore filter into an evacuated serum vial.

2. Dissolve 5 mg of EHDP in 1 ml of sterile O_2-free H_2O and add to the 1-ml Sn(II) and mix for 1-2 minutes.

3. Add the desired activity of $^{99m}TcO_4$ in 3 ml of saline and mix for 1-2 minutes.

4. Adjust the preparation to pH 6 with dilute NaOH solution.

5. Finally filter the ^{99m}Tc-Sn-EHDP through a 0.22μ Millipore filter to insure sterility of the preparation.

The ^{99m}Tc-Sn-EHDP preparation is Tc "carrier free" and contains 1 mg EHDP/ml, 0.02 mg $SnCl_2 \cdot 2H_2O$/ml and 10-15 mCi ^{99m}Tc/ml. The chemical labelling efficiency is about 95% of the $^{99m}TcO_4$ added to the preparation.

The binding efficiency of the chemical labelling procedure and the stability of the preparation were determined by ascending paper chromatography using Whatman #1 paper strips in 85% methanol solvent.

To extend the shelf life of ^{99m}Tc-Sn-EHDP, the reagent solutions should be made free of dissolved oxygen by saturating the solution with nitrogen and performing the chemical preparation in an evacuated and sealed serum vial. The reagents and glassware used for the preparation are sterile and pyrogen free.

Evaluation of ^{99m}Tc-Sn-EHDP in Animals

The ^{99m}Tc-Sn-EHDP compound was evaluated in Sprague-Dawley rats weighing 200-250 grams and in beagle dogs weighing about 10 kg. In rats 0.5 ml of the material was injected intravenously by tail vein, scintillation camera pictures were obtained to visualize the distribution of ^{99m}Tc-Sn-EHDP at different times after intravenous injection. In rats both the multichannel and 1/8" pinhole collimators were used. In dogs the whole body scanner (5) and/or the tomographic scanner (6) were also used.

Tissue distribution studies were done in rats by administering 0.5-1.0 ml of ^{99m}Tc-Sn-EHDP and sacrificing the animals at 3 hours and 20 hours.

Blood disappearance half-times were determined in dogs by taking 1-ml blood samples from 1 min to 180 min after administrationof the ^{99m}Tc-Sn-EHDP. The blood samples were counted in a well counter using an NaI(Tl) crystal and the data were plotted on semilog paper to obtain the blood disappearance half-times. A known dilution of the injected dose of ^{99m}Tc was counted to determine the blood activity at zero time after injection.

Results and Discussion

Table I shows the percent uptake of ^{99m}Tc from ^{99m}Tc-Sn-EHDP for whole organs of rat (except skeleton which is represented by femur uptake and blood which is shown as uptake per ml) for 10:1, 50:1, and 100:1 molar ratios of EHDP to $SnCl_2 \cdot 2H_2O$ three hr after intravenous injection. The uptake of ^{99m}Tc in the carcass is primarily skeletal uptake and appears to be optimal at the 50:1 molar ratio of

EHDP:$SnCl_2 \cdot 2H_2O$. The percent uptake in the marrow was determined by difference from femur + marrow counts/min less the counts/min of femur alone.

Figure 1 is a composite scintillation camera picture made with the 1/8" diameter pinhole collimator. It shows the ^{99m}Tc-Sn-EHDP uptake to be primarily in the skeleton of a rat 3 hr after intravenous injection. There is no visible uptake in liver, spleen or stomach. The uptake in the pelvic region is urinary excretion accumulating in the bladder.

Figure 2 shows the uptake in a dog 20 hr after injection of 5 mCi of ^{99m}Tc-Sn-EHDP. On the left is the posterior view and on the right a lateral view taken with the scintillation camera using the multi-channel collimator.

A number of preparations of ^{99m}Tc-Sn-EHDP were given to rats and dogs. In each case, when the labelling method described in this paper was followed, good bone imaging was obtained.

Good bone imaging is also obtained in rats when the method of preparation is modified by substituting H_2O in place of the 0.01 N HCl acid. This modification simplifies the procedure by eliminating the neutralization step. ^{99m}Tc-Sn-EHDP prepared by the water method and using ^{14}C labelled EHDP is being investigated by A. J. Tofe (8).

Variations in the method of preparation of the compound such as increased concentrations of Sn(II) and EHDP resulted in some preparations that localized primarily in the kidneys of rats with retention of the activity in the kidneys even after 24 hr. One of the results of increased concentrations of EHDP (10 mg/ml) and SnII (0.2 mg/ml) is shown in Figure 3.

The high uptake and prolonged retention of ^{99m}Tc-Sn-EHDP within the kidneys with the ten times greater concentration of EHDP and Sn than used in the standard preparations, indicates an overload factor of EHDP which causes a failure of the renal excretion mechanism. On the other hand, when the described method of preparation was followed, approximately 60-70% of the radioactivity was excreted by way of the kidneys. This excretion was nearly complete at 3 hr. There was little retention of activity within the kidneys which were only faintly visualized by scintillation camera pictures as shown in Figure 1.

Table II shows the distribution of ^{99m}Tc-Sn-EHDP in the whole organ of rats for variations in the order of addition of the chemical reagents; first by EHDP, Sn(II), $^{99m}TcO_4$ and second by Sn(II), $^{99m}TcO_4$, EHDP. The tissue distribution was determined at 3 and 20 hr after administration of ^{99m}Tc-Sn-EHDP. By the first method the uptake in blood, liver, kidneys and gut is relatively low at 3 hr, while the bone uptake is high. However when the second method is used the bone uptake decreases while the blood, liver and kidney uptake increase.

These data indicate that EHDP and SnII form a chelate which has a greater reducing potential than Sn(II) alone and that the Sn(II)-EHDP chelate will combine with the reduced ^{99m}Tc. On the other hand, when the Sn(II) and $^{99m}TcO_4$ are combined first there is an insoluble Sn-^{99m}Tc probably as the ^{99m}Tc coprecipitated with SnO (9). This coprecipitate is not as readily chelated by the EHDP. This is reflected in the increased uptake in liver.

Table III shows the results of diluting the standard ^{99m}Tc-Sn-EHDP preparation 100-fold. The data indicates a breakdown of the compound in the diluted preparation with a decrease in bone uptake

and an increase in soft tissue uptake.

The blood disappearance curves can be approximated with at least two components with $T_{1/2}$ 120 min and $T_{1/2}$ 17 min.

The method of using Sn(II) for labelling EHDP with ^{99m}Tc is dependent upon the ability of chelating agents to stabilize Sn(II) and increase its usefulness as a reducing agent for $^{99m}TcO_4^-$. Further the stability of the ^{99m}Tc-Sn-EHDP preparation *in vivo* minimizes Sn colloid formation at neutral pH and also minimizes the binding of Sn-^{99m}Tc to blood proteins.

The stability of the ^{99m}Tc-Sn-EHDP *in vitro* is dependent upon removing air, i.e. oxygen, from the preparation. The initial chemical binding efficiency ranges from 90-96% and the amount of ^{99m}Tc still bound to Sn-EHDP after 6 hr is 85-90% in an O_2-free system. When the O_2 is not excluded, the preparation breaks down within 2-4 hr to about 65-75% ^{99m}Tc bound to Sn-EHDP.

The estimated radiation dose delivered to a 70 kg patient from 10 mCi of ^{99m}Tc-Sn-EHDP for the standard acid method of preparation is:

$D_{\beta,\gamma}$ (whole body) = 0.148 rad

$D_{\beta,\gamma}$ (skeleton) = 0.267 rad

$D_{\beta,\gamma}$ (kidneys = 3.68 rad

$D_{\beta,\gamma}$ (bladder) = 7.36 rad

The bladder is the major target organ. The skeletal uptake is estimated to be 30% of the injected dose 3 hr after intravenous administration and the urinary excretion is about 70%.

The amounts of Sn(II) as $SnCl_2 \cdot 2H_2O$ and EHDP administered to a 70 kg patient for 10 mCi of ^{99m}Tc-Sn-EHDP are well below the toxic

levels for Sn(II) and therapeutic levels for EHDP.

Summary

A method has been described for labelling Sn-EHDP with ^{99m}Tc to provide a bone specific radiopharmaceutical which makes available the superior scanning characteristics of ^{99m}Tc. Preliminary results in animal studies indicate that ^{99m}Tc-Sn-EHDP will be extremely useful as a bone scanning agent because of its high uptake in bone, rapid disappearance from blood and soft tissues, and low radiation dose to the patient.

The concentrations of Sn(II) and EHDP as well as the order of addition of the reagents are important factors in determining the in vivo distribution of the radiopharmaceutical. In vitro stability is dependent upon an O_2-free environment for the preparation. Further studies are being done on the chemistry, stability, and biological distribution of ^{99m}Tc-Sn-EHDP (10).

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TABLE I

Percent Uptake of ^{99m}Tc from ^{99m}Tc-Sn-EHDP Per Whole Organ of Rat for Varying Molar Ratios of EHDP:$SnCl_2 \cdot 2H_2O$ Three Hours After Intravenous Injection.

Organ	Molar Ratio EHDP:$SnCl_2 \cdot 2H_2O$		
	10:1+	50:1*	100:1‡
Blood	0.023/ml	0.054/ml	0.022/ml
Heart	0.009	0.020	0.012
Lungs	0.044	0.109	0.205
Liver	0.173	0.273	0.420
Kidneys	1.30	0.996	0.876
Spleen	0.012	0.016	0.036
Gut	1.40	0.798	0.842
Muscle of femur			0.008
Marrow + femur	1.08	1.88	1.10
Marrow (by diff.)	0.07	0.03	
Femur	1.01	1.85	
Carcass	35.7	39.9	34.4

+ mean of 2 rats, 0.5 mg EHDP/kg rat.

* mean of 4 rats, 5 mg EHDP/kg rat.

‡ mean of 2 rats, 5 mg EHDP/kg rat.

TABLE II

Percent Uptake of ^{99m}Tc from ^{99m}Tc-Sn-EHDP Per Whole Organ of Rat for EHDP-Sn(II)-^{99m}Tc and for Sn(II)-^{99m}Tc-EHDP Preparations in Order of Reagent Addition.

	3 Hours		20 Hours	
Organ	EHDP-Sn(II)-^{99m}Tc+	Sn(II)-^{99m}Tc-EHDP+	EHDP-Sn(II)-^{99m}Tc*	Sn(II)-^{99m}Tc-EHDP‡
Blood	0.047/ml	0.430/ml	0.008/ml	0.087/ml
Heart	0.015	0.125	0.004	0.030
Lungs	0.104	0.409	0.019	0.137
Liver	0.273	11.41	0.223	10.3
Kidneys	1.04	2.77	0.910	2.70
Spleen	0.020	0.280	0.015	0.304
Gut	0.698	1.72	0.672	1.41
Marrow + femur	2.11	1.22	1.63	1.13
Marrow (by diff.)			0.13	0.06
Femur			1.49	1.07
Carcass	33.5	33.3	30.4	26.8

+ mean of 2 rats, 5.0 mg EHDP/kg rat.

* mean of 2 rats, 5.0 mg EHDP/kg rat.

‡ one rat, 5.0 mg EHDP/kg rat.

TABLE III

Percent Uptake of ^{99m}Tc from ^{99m}Tc-Sn-EHDP Per Whole Organ of Rat for Standard 50:1, EHDP:SnII, for Undiluted and 100 Times Dilution of Preparation at 3 Hrs.

Organ	Undiluted*	100X Dilution+
Blood	0.062	0.201
Heart	0.025	0.069
Lungs	0.115	0.262
Liver	0.272	2.74
Kidneys	0.954	9.27
Spleen	0.013	0.055
Gut	0.897	26.88
Marrow + Femur	1.65	0.551
Carcass	46.4	

* 5 mg EHDP/kg rat.

+ 0.05 mg EHDP/kg rat.

Figure Captions

Figure 1. Scintillation camera picture of a rat 3 hr after administration of ^{99m}Tc-Sn-EHDP. The rat received 1 mg of EHDP. A 1/8" pinhole collimator was used.

Figure 2. Scintillation camera picture of a dog from posterior and lateral views 20 hr after intravenous administration of 5 mCi of ^{99m}Tc-Sn-EHDP. The dog received 1 mg of EHDP.

Figure 3. Distribution of non-standard ^{99m}Tc-Sn-EHDP preparation in a rat 24 hr after administration. A composite of 1/8" pinhole collimator pictures. Note retention in kidneys even after 24 hr. The rat received 10 mg of EHDP and 0.2 mg Sn(II).

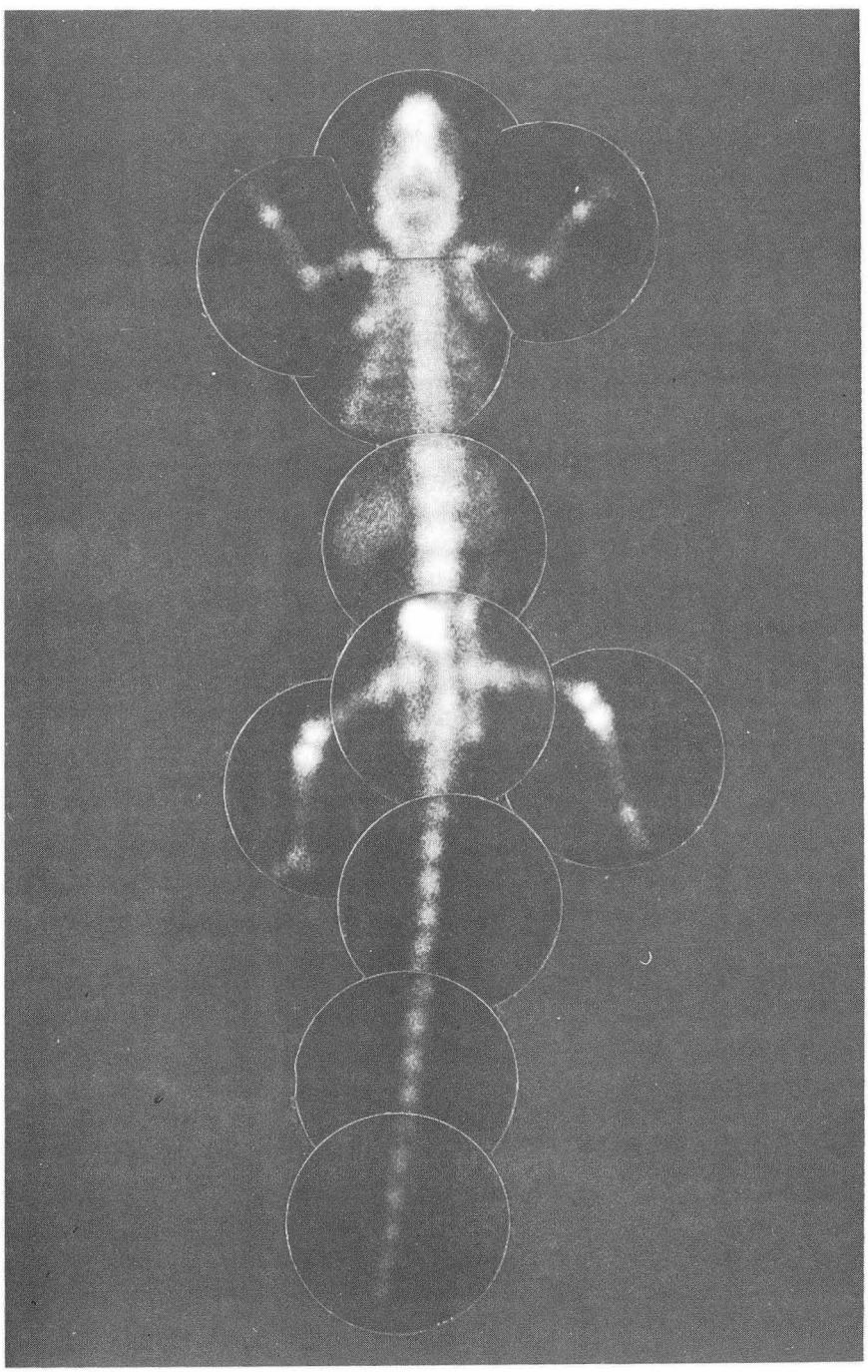

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Fig. 1

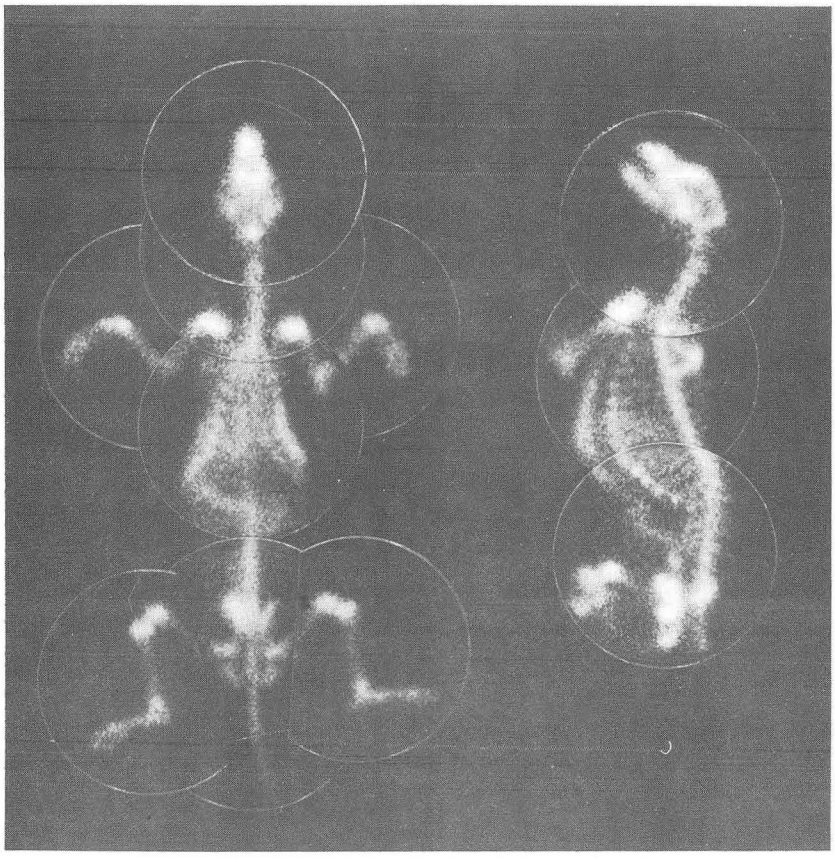

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Fig. 2

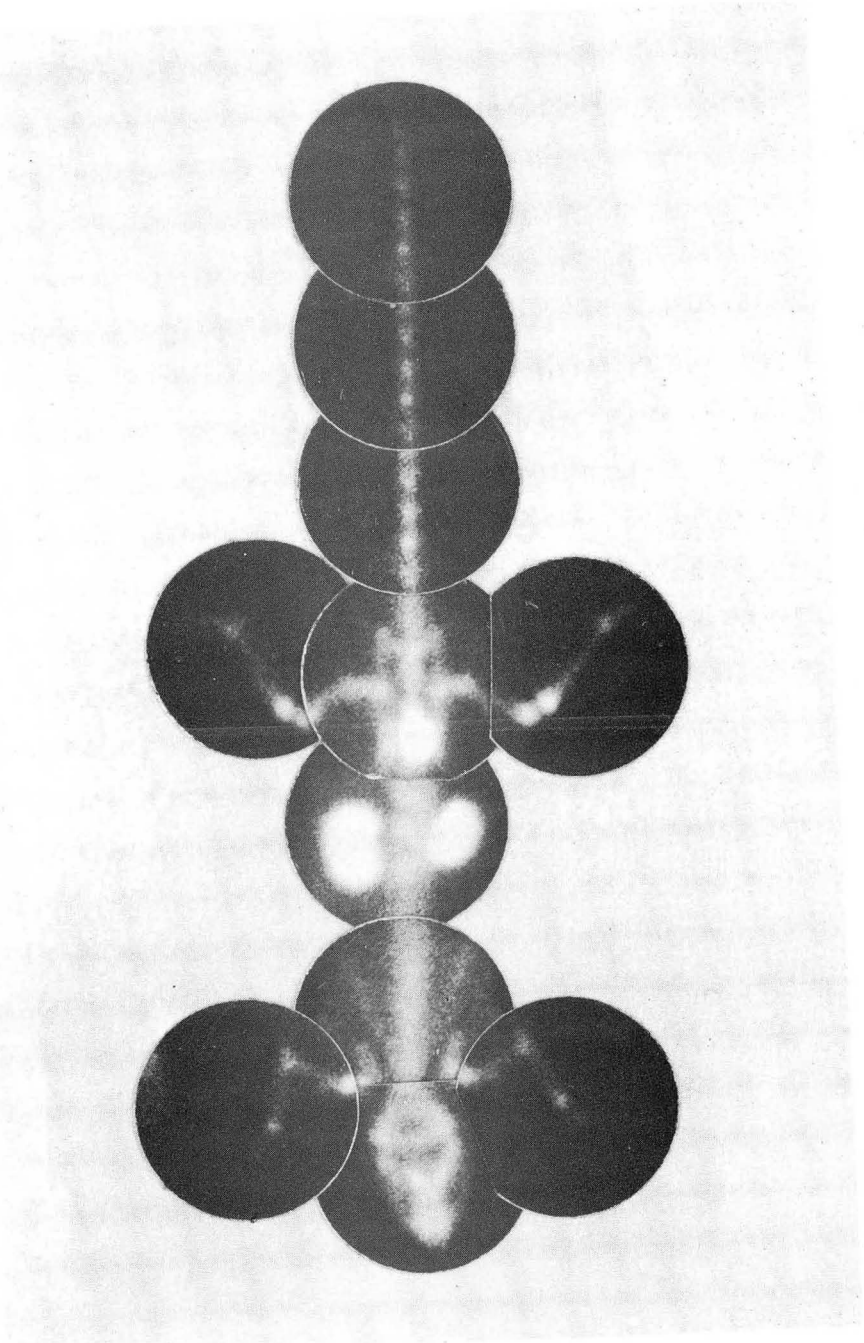

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Fig. 3

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