TISSUE LOCALIZATION STUDIES OF A DDD ANALOG

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1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichloroethane (*o,p'*-DDD) has a specific cytotoxic action on the adrenal cortex of dogs (*1,2*). When an adequate dose is administered, the *o,p'*-isomer can cause adrenal necrosis and suppression of adrenal function in humans (*3*) while the *p,p'*-isomer appears to be relatively inactive (*4,5*). The mechanism of action is not understood. In addition to a direct adrenal effect, an effect on peripheral metabolism (*6,7*) of corticosteroids has been implicated. Some reports indicate that DDD or its metabolites concentrate in the adrenal (*8*). Because of these findings we have studied the tissue distribution of an ^{131}I analog of *p,p'*-DDD as part of a program aimed at developing an effective radiopharmaceutical for adrenal gland photoscanning. This report presents the distribution of radioactive iodine in rabbit tissues 5 min to 24 hr after the intravenous administration of ^{131}I-1,1-(*p*-iodophenyl)-2,2-dichloroethane-(^{131}I-diiodo-DD) and preliminary results of attempts to visualize the adrenals in dogs by photoscanning.

MATERIALS AND METHODS

Radiolabeled compounds. ^{131}I-1,1-(*p*-iodophenyl)-2,2-dichloroethane (Fig. 1) was obtained through the courtesy of Howard Glenn at Abbott Laboratories. The specific activity was between 0.64 and 2.6 μCi/mg.

Rabbit distribution studies. Twenty-nine rabbits weighing between 2.5 and 3.2 kg were given ^{131}I-diiodo-DD intravenously. In 16 animals the dose was 0.4 mg/kg, and ethanol was used as a solvent. In the remaining 13 rabbits dimethylsulfoxide was used as a solvent, and the dose was 5 mg/kg. There was no apparent dose effect on the distribution of radioactivity so the animals are presented as a single group. The rabbits were sacrificed by a blow on the head at intervals between 5 min to 24 hr after injection. Samples of whole adrenal gland, omental fat, perirenal fat, liver, muscle, kidney, lung (base and apex), thymus, ventricle, auricle, pancreas, spleen and thyroid were removed, blotted, weighed and then counted in a Nuclear-Chicago scintillation well counter. Blood was obtained by heart puncture and urine samples were collected from the bladder at the time of sacrifice.

Dog scans and distribution studies. Fifty-five to 468 μCi of ^{131}I-diiodo-DD dissolved in dimethylsulfoxide were given intravenously to three mongrel dogs weighing 25–52 lb. Scans were performed using a Picker Magnascanner with a 2 × 3-in. NaI(Tl) crystal and a 19-hole collimator. Scans were started immediately after injection and repeated at frequent intervals. X-rays were taken at appropriate intervals and radio-opaque markers were used for anatomic localization. A ½-in. lead plate was placed over the liver to reduce background. Dogs were lightly anesthetized with nembutal during scanning and were sacrificed with a concentrated solution of sodium pentobarbital. The radioiodine content was measured in tissue specimens. Three additional dogs were sacrificed 24 hr after the intravenous administration of 24–28 mg of ^{131}I-diiodo-DD and tissue specimens were measured in the same way.

RESULTS

Concentrations of radioiodine in rabbit tissues. The radioiodine content of rabbit tissues expressed as micrograms of ^{131}I-diiodo-DD per gram of tissue 5 min–24 hr after the intravenous administration of ^{131}I-diiodo-DD is given in Table 1. At 2–4 hr the adrenal concentration of radioiodine was higher than that of any other organ studied. By 24 hr the adrenal concentration had declined to ⅙th of its peak value. The liver concentration of radioactivity paralled that in the adrenals but was generally lower by a factor of 2. Large quantities of radioactivity were noted in the bile. The fat content of radioactivity increased progressively, becoming 3–4 times higher than the adrenal content by 24 hr. At that time all other tissues contained less radioactivity than the fat did. Moderate concentrations of ^{131}I were noted in the kidney during the first 4 hr, and the concentrations in random urine samples taken from the bladder at the time of sacrifice suggested that significant quantities of the radionuclide were eliminated in the urine. The thyroid content of radioiodine was lower than would be expected if significant deiodination of the ^{131}I-diiodo-DD had occurred. High and variable levels of label were noted in the lung during the first

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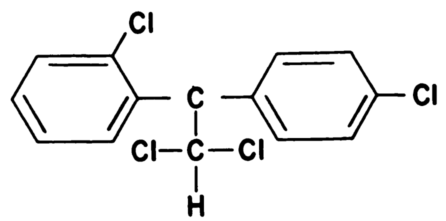
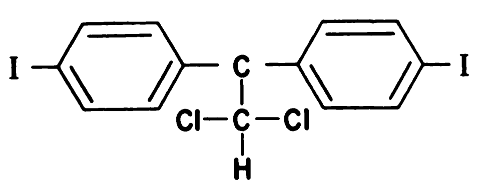
TABLE 1. RADIOIODINE CONTENT OF RABBIT TISSUES AFTER INTRAVENOUS ^{131}I-DIIODO-DD*

	5 min	30 min	1 hr	2 hr	4 hr	24 hr
Adrenal	1.28 ± 0.41	2.98 ± 1.15	5.34 ± 2.81	6.38 ± 3.24	5.15 ± 2.26	1.02 ± 0.52
Omental fat	0.12 ± 0.06	0.53 ± 0.42	0.60 ± 0.58	0.82 ± 0.60	1.29 ± 0.60	2.99 ± 1.52
Perirenal fat	0.29 ± 0.13	0.32 ± 0.17	0.44 ± 0.45	0.81 ± 0.51	1.71 ± 0.72	4.01 ± 1.54
Liver	0.37 ± 2.07	2.19 ± 1.36	3.39 ± 1.80	3.01 ± 0.78	3.45 ± 1.27	0.38 ± 0.27
Muscle	0.44 ± 0.14	0.52 ± 0.23	0.64 ± 0.34	0.62 ± 0.29	0.54 ± 0.20	0.23 ± 0.18
Kidney	0.77 ± 0.46	0.91 ± 0.31	0.95 ± 0.41	0.97 ± 0.54	1.40 ± 0.87	0.13 ± 0.05
Serum	0.57 ± 0.30	0.30 ± 0.25	0.35 ± 0.22	0.46 ± 0.49	0.36 ± 0.16	0.04 ± 0.02
Lung base	11.86 ± 9.06	19.02 ± 12.10	16.23 ± 13.67	5.92 ± 5.29	2.07 ± 0.48	0.51 ± 0.30
Lung apex	8.24 ± 6.11	7.76 ± 3.72	11.68 ± 11.73	5.30 ± 3.46	2.08 ± 1.43	0.57 ± 0.63
Thymus	0.19 ± 0.08	0.36 ± 0.24	0.45 ± 0.24	0.44 ± 0.25	0.60 ± 0.10	0.35 ± 0.18
Ventricle	1.50 ± 0.34	1.14 ± 0.59	1.47 ± 0.47	1.29 ± 0.55	1.10 ± 0.33	0.34 ± 0.36
Auricle	0.68 ± 0.28	0.59 ± 0.51	0.79 ± 0.64	0.59 ± 0.52	0.60 ± 0.10	0.24 ± 0.07
Pancreas	0.47 ± 0.11	0.54 ± 0.14	0.92 ± 0.43	0.69 ± 0.27	0.75 ± 0.07	0.53 ± 0.12
Spleen	1.21 ± 0.58	0.32 ± 0.24	0.54 ± 0.20	0.88 ± 1.11	0.39 ± 0.21	0.07 ± 0.02
Thyroid	0.29 ± 0.03	0.42 ± 0.14	0.64 ± 0.41	0.56 ± 0.40	1.04 ± 0.52	0.63 ± 0.92
Urine	0.18	0.30 ± 0.23	0.53 ± 0.09	0.66 ± 0.57	6.33 ± 5.36	1.56 ± 0.93

* Calculated from radioiodine content of tissue and specific activity of injected ^{131}I-diiodo-DD; expressed as μg of ^{131}I-diiodo-DD per gm of tissue.



1-(O-Chlorophenyl)-1-(P-Chlorophenyl)-2,2-Dichloroethane (O,P' - DDD)



1,1-(P-Iodophenyl)-2,2-Dichloroethane (P,P' -DI IODO DD)

FIG. 1. Structure of o,p'-DDD and p,p'-diiodo-DD.

hour after injection, but the concentrations declined throughout the 24-hr period. ^{131}I-diiodo-DD is highly insoluble in aqueous media, and these lung concentrations may reflect trapping of insoluble precipitates which form when the injection is made. The concentration of radioiodine in the brain was negligible throughout the period of study. There was no consistent difference between the content of radioactivity in gray matter and white matter. Skeletal and cardiac muscles followed parallel patterns of uptake; uptake was lower at 24 hr than at earlier intervals, but the concentration of ^{131}I in cardiac muscles was consistently higher than in skeletal muscle, perhaps because of differences in blood supply. The serum radioactivity was consistently greater than that in whole blood and was quite stationary between ½ and 4 hr. By 24 hr the serum radioactivity was reduced by a factor of 10. Thymus, pancreas and spleen were generally low in radioactivity content throughout the period of study.

Photoscans. Figures 2 and 3 are photoscans of Dog 1 taken at ½ and 4 hr after injection of ^{131}I-diiodo-DD. Two areas of high counting rate are indicated by arrows. After sacrificing the animal, the adrenals were marked with metal clips and an x-ray was taken. The position of the metal clips on the x-ray corresponded to the two areas of high counting rate noted on the scans. The adrenals were also vis-

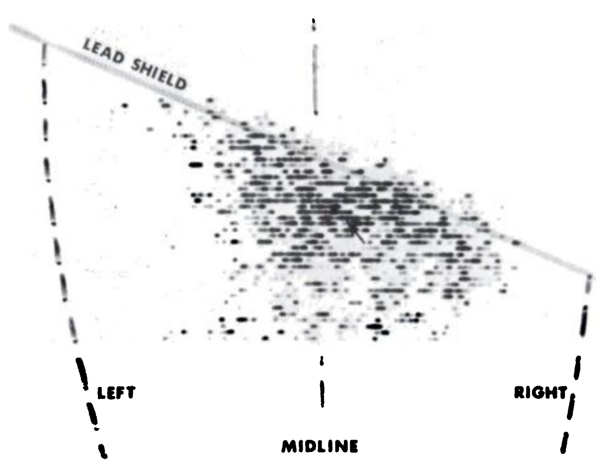


FIG. 2. Scan of Dog 1 30 min after injection of ^{131}I-diiodo-DD. Uptake is seen in liver. Arrow indicates area of higher counting rate which may represent ^{131}I concentration in right adrenal.

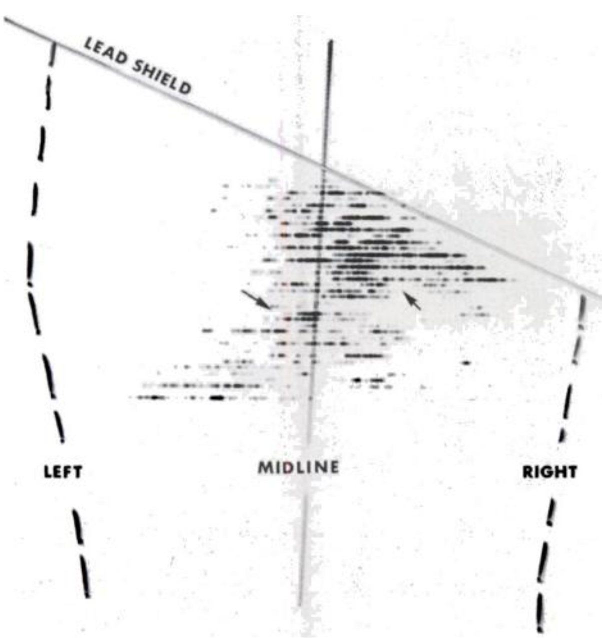


FIG. 3. Scan of Dog 1 4 hr after intravenous ^{131}I-diiodo-DD. Arrows point to 2 areas which are believed to reflect concentration of radioiodine in adrenals.

ualized by scanning after they were removed from the carcass (Fig. 4).

In the second dog uptake of ^{131}I in the liver was clearly seen in the first and second scans. The third to fifth scans taken at 2–7 hr after injection showed concentration of radioactivity along the midline and in the perirenal areas. Two areas of increased uptake (Fig. 5) were noted near the midline in the area overlying the adrenals. Long spinal needles inserted into the body of the dog in positions corresponding to the prominent areas on the scan, penetrated the right adrenal and missed the left adrenal by 1 cm.

Seven scans were performed on Dog 3 from 30 min to 10 hr after injecting 270 μCi of ^{131}I-diiodo-DD. The first scan showed diffuse distribution of radioactivity in the abdomen and high liver uptake.

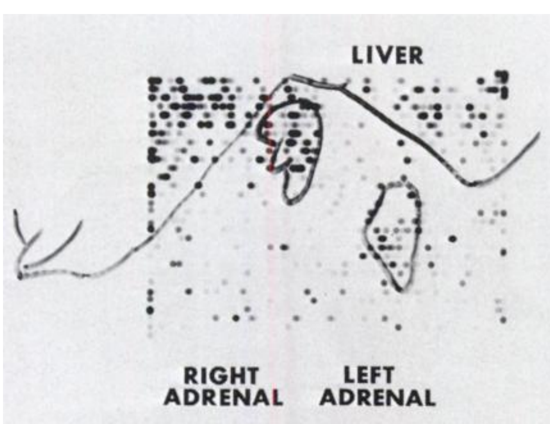


FIG. 4. Scan of lower edge of liver and of two adrenals. Organs were removed from dog 6 hr 20 min after injection.

Scans 2 through 6 taken at 1–7 hr after injection showed uptake in the midline which became progressively more prominent. Scans 4 through 6 (4–7 hr) showed uptake appearing in both perirenal areas as well. The adrenals were not localized in these scans.

Concentrations of radioiodine in dog tissues. The concentration of ^{131}I-diiodo-DD (or metabolites) in the adrenals of the 5 dogs is given in Table 2. The lack of success in obtaining an adrenal localization by scanning in Dog 3 may have been related to the comparatively small weight and ^{131}I content of the adrenals from that dog. Dog 1, however, with a higher adrenal weight and adrenal ^{131}I content, had a positive localization on the scans.

The distribution of radioactivity in dog tissues expressed as ratios of cpm/mg in adrenals to cpm/mg in the tissues is also given in Table 2. Comparison of these ratios with similar ratios calculated from the rabbit distribution studies (Fig. 6) suggests that the adrenals of dogs may concentrate ^{131}I-diiodo-DD (or metabolites) more avidly than rabbit adrenals.

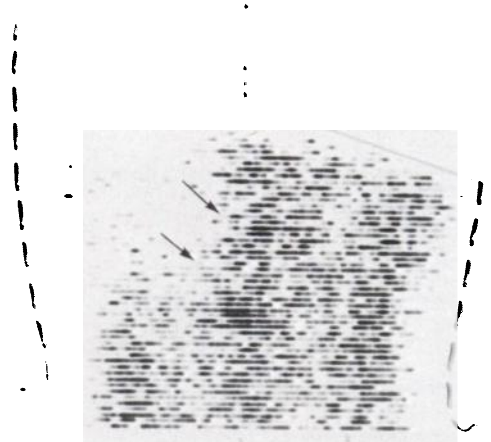


FIG. 5. Scan done 4 hr after injection of ^{131}I-diiodo-DD in Dog 2 shows two discrete areas of increased uptake of radioiodine (arrows).

DISCUSSION

The pattern of uptake of radioiodine by rabbit tissues following the intravenous administration of ^{131}I-diiodo-DD suggests that the optimum period for scanning is 1–4 hr after intravenous injection of ^{131}I-diiodo-DD when the liver is the only organ with sufficient activity to pose a problem. At 4–24 hr the adrenal-to-liver ratio of radioactivity declines. The concentration of activity in the adrenal also declines while radioactivity concentration in fat increases steadily. The limited tissue distribution data available in dogs suggests that a similar pattern of uptake occurs in the dog. However, it should be noted that adrenal-to-liver ratios observed in dogs were higher than in rabbits at comparable time intervals after injection.

TABLE 2. DISTRIBUTION OF ^{131}I-DIIODO-DD IN DOG TISSUES

Dog	Wt (lb)	^{131}I-diiodo-DD (mg)	Time (hr)	μg diiodo-DD in adrenals*	Weight of adrenals (mg)	Ratio concentration adrenals to tissue: liver	fat	serum	muscle	bile
1	24.5	192	6.5	366.0	1,902	4.8	3.8	35.6	10.5	—
3	41.5	190	11.0	46.0	620	7.2	0.6	46.2	12.0	0.16
4	26.5	24	24.0	5.4	635	1.5	0.2	17.9	4.0	—
5	26.5	24	24.0	4.1	430	2.9	0.5	20.7	4.9	—
6	24.0	28	24.0	2.5	334	2.2	0.9	9.8	2.6	—

* Calculated from radioiodine content of adrenals and specific activity of injected ^{131}I-diiodo-DD.

It may be possible to overcome the problem of high uptake in the liver by developing appropriate shielding and positioning techniques and by using electronic devices such as those described by Kaplan (*9*) for pancreas scanning.

The dose of ^{131}I required will also be an important consideration in developing a technique for scanning adrenals in humans. Approximately 0.1–0.2% of the administered radioiodine was concentrated in rabbit adrenals at 1–4 hr after injection of ^{131}I-diiodo-DD. If similar concentrations occur in humans, doses of radioiodine in excess of 500 μCi would be required to obtain adequate scans.

The toxicity of structurally related DDD analogs is well known, and oral doses of 10 gm/day are commonly used to treat patients with adrenal cortical carcinoma. Smith *et al* (*10*) have reported an extensive study of the relative toxicity of analogs of DDT. Substitution of bromine for chlorine in the aromatic positions of DDT did not alter its toxicity. Thus it seems unlikely that the iodinated analogs of DDD will be more toxic than *o,p'*-DDD.

The radiopharmaceutical used in the present study may not be the most effective DDD analog for human adrenal scanning. Cueto *et al* (*4*) reported that *o,p'*-DDD was stored at a significantly greater level than *p,p'*-DDD in dogs. If differences in the distribution of DDD isomers do occur in humans, *o,p'*-iodinated analogs of DDD may distribute more favorably for adrenal scanning than the present compound. We are investigating this possibility.

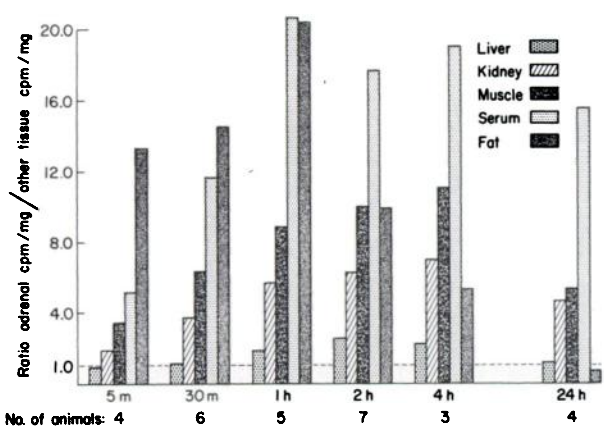


FIG. 6. Distribution of radioiodine label after IV ^{131}I-diiodo-DD (in rabbits).

Our successful preliminary attempt to scan adrenals in dogs is encouraging and suggests that further studies with iodinated DDD analogs may lead to a technique for scanning human adrenal tumors.

ACKNOWLEDGMENT

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