Br. J. clin. Pharmac. (1979), **8**, 425–432

STUDIES OF UPTAKE OF THE BRETYLIUM ANALOGUE, IODOBENZYLTRIMETHYLAMMONIUM IODIDE, BY NON-PRIMATE, MONKEY AND HUMAN HEARTS

E.A. CARR, Jr,[1,2,3,*], M. CARROLL[1,3], R.E. COUNSELL[4] & J.W. TYSON[5]

Departments of Pharmacology[1] and Medicine[2], University of Louisville and State University of New York at Buffalo, the Buffalo Veterans Administration Hospital[3], the Departments of Pharmacology[4] and Medicinal Chemistry[4], University of Michigan, and the Department of Radiology[5], Louisville Jewish Hospital.

1 Uptake of (±)-[^{3}H]-noradrenaline, [^{14}C]-bretylium and [^{125}I]-*o*-iodobenzyltrimethylammonium iodide (RIBA) by rat heart was studied by the Langendorff technique. All three compounds showed significant uptake.

2 Corticosterone and 17-β-oestradiol inhibited the uptake of all three compounds by rat heart, a finding consistent with extraneuronal uptake (uptake$_2$).

3 [^{131}I]-RIBA was injected intravenously into pigs and monkeys (*M. speciosus*). Myocardial samples taken from pigs killed 1 and 2 h after injection showed significant uptake. No significant uptake was found in myocardial samples of monkeys killed 10 min, 2 h and 24 h, respectively, after injection.

4 Four normal human volunteers received [^{125}I]-RIBA intravenously and the image of the precordial area was followed by means of a scintillation camera for the first 4 h after injection. In two of the subjects, the scintigrams were repeated at 22 and 23 h after injection, respectively. No evidence of myocardial uptake was observed.

5 These results suggest the possibility that man and at least one other primate species may differ from lower species with regard to uptake$_2$.

Introduction

A major advance in understanding the mechanism whereby catecholamines achieve high concentrations in certain tissues was made by Iversen (1963), who identified two different processes for catecholamine uptake in the rat isolated heart. It was clearly shown (Iversen, 1963; Burgen & Iversen, 1965) that the uptake process first revealed by high perfusing concentrations and subsequently termed uptake$_2$ (Iversen, 1965) differs in important respects from the previously described, neuronal uptake of catecholamines (uptake$_1$). Extraneuronal uptake of catecholamines (uptake$_2$) is inhibited by several steroids (Iversen & Salt, 1970; Salt & Iversen, 1972). Corticosterone and 17-β-oestradiol are among the most potent inhibitors. Whereas the majority of steroids tested, including corticosterone, show specificity in that they do not inhibit uptake$_1$, 17-β-oestradiol is also a weak inhibitor of uptake$_1$ (Iversen & Salt, 1970). Boura, Copp, Duncombe, Green & McCoubrey (1960) first demonstrated myocardial uptake of [^{14}C]-labelled bretylium, using cats. Brodie, Chang & Costa (1965) studied the process of uptake of labelled bretylium and guanethidine by rat heart *in vivo* and noted differences between the two drugs. Whereas uptake of tracer doses of labelled guanethidine was markedly decreased in the presence of large doses of stable guanethidine, no such saturation process was observed in analogous experiments with bretylium. Namm, Wang, El-Sayad, Copp & Maxwell (1975) showed that hearts of immunosympathectomized rats retain their ability to take up bretylium. These results suggest that uptake of bretylium by the heart is primarily mediated, even in intact animals, by the uptake$_2$ mechanism. Boura, Duncombe & McCoubrey (1961) showed that [^{14}C]-labelled ortho-bromobenzyltrimethylammonium iodide is taken up by cat myocardium. We have previously shown (Counsell, Yu, Ranade, Buswink, Carr & Carroll, 1974) that a radio-iodinated analogue of bretylium, ortho-iodobenzyltrimethyl-

* Present address: Department of Pharmacology and Therapeutics, School of Medicine, State University of New York at Buffalo, 127 Farber Hall, Buffalo, New York 14214, USA.

0306–5251/79/080425–08 $01.00

ammonium iodide, labelled in the ring position, is selectively taken up by the myocardium of the intact rat and dog. This radioactive compound permitted clear demonstration of the myocardium of living dogs through the intact chest wall by scintigraphy. The compound was thus considered a potential new radioactive imaging agent for the scintigraphic diagnosis of myocardial disease. Whereas the clinical use of scintigraphic agents in the diagnosis of heart disease has been possible for several years (Carr, Gleason, Shaw & Krontz, 1964; Parkey, Bonte, Meyer, Atkins, Curry & Willerson, 1974; Cooper, 1974; Wackers, Schoot, Sokole, Samson, Niftrik, Lie, Durrer & Wellens, 1975) the various classes of radioactive compounds used for this purpose have significant drawbacks (Carr, 1973; Thrall, Swanson & Wieland, 1978) and promising new compounds deserve investigation. Ortho-iodobenzyltrimethylammonium iodide, in its stable form, has been shown to possess anti-fibrillatory properties (Kniffen, Lomas, Counsell & Lucchesi, 1974) similar to those shown for bretylium by Leveque (1965).

In view of the interest attached to the uptake$_2$ mechanism, which has previously been studied chiefly in rats, the radio-iodinated bretylium analogue (hereafter termed RIBA) offered a unique advantage, for its possible use as a myocardial imaging agent presented the opportunity to study its uptake in intact human subjects. We therefore first studied the effect of steroids on the uptake of bretylium and RIBA by the rat isolated heart. Corticosterone, one of the most potent inhibitors of uptake$_2$ of catecholamines, was selected as the test steroid. For comparison, 17-β-oestradiol, a potent inhibitor of uptake$_2$ and a weak inhibitor of uptake$_1$, was also used. We then extended our previous studies of the myocardial uptake of RIBA by hearts of intact animals to include an additional non-primate species, the pig and two primates, the stump tail monkey (*Macaca speciosus*) and man.

Methods

In vitro *studies*

Male Sprague-Dawley rats, weighing 160–275 g, received i.p. sodium pentobarbitone, 60 mg/kg, and heparin, 1,000 u. Thirty minutes later the hearts were removed and placed in a beaker of isotonic ice-cold saline until beating ceased. The hearts were then perfused by the Langendorff technique, as described by Iversen (1963). During the 5 min of preliminary perfusion with Krebs-Henseleit solution (Perry, 1968) at 37°C, the heart established a steady rate of beating. After the preliminary perfusion the hearts were perfused for a further period with Krebs-Henseleit solution containing one of the following radioactive compounds: (±)-[^{3}H]-noradrenaline, 8.0 Ci/mmol, diluted with non-radioactive (±)-noradrenaline to a final specific activity of 100 μCi/mg, and used in a final concentration of 500 nCi/ml; [^{125}I]-RIBA (ring-labelled), 3.7 mCi/mmol, used in a final concentration of 25 nCi/ml; [^{14}C]-bretylium, 15 mCi/mmol, used in a final concentration of 50 nCi/ml. No non-radioactive o-iodobenzyltrimethylammonium iodide or bretylium was added. Perfusion was continued until 15 ml of solution had been collected and an additional perfusion period of 4 min had elapsed. When steroids were used, they were dissolved in 1 ml propylene glycol and added to the medium. Hearts were removed, blotted and weighed. When [^{125}I]-RIBA was used, the radioactivity of the intact hearts was determined in a gamma scintillation counter. When the other radioactive compounds were used, hearts were first homogenized in 2 ml 1% EDTA. Eight ml acid ethanol (0.3 mol/l HCl) was added. After mixing and centrifugation, the precipitate was resuspended in 8 ml acid ethanol and again centrifuged. Aliquots (0.1 ml) of both supernatants were placed in scintillation vials and dissolved in a tissue solubilizer ('Protosol'). Their radioactivity was counted in a Beckman liquid scintillation spectrometer, using PPO (0.5%) and POPOP (0.3%) in toluene as the scintillator. Efficiency of counting was determined with external standards. In calculating the content of labelled noradrenaline the results were corrected for the presence of [^{3}H]-noradrenaline in the extra-cellular space, as described by Lightman & Iversen (1969). Control hearts were studied in the same manner as experimental hearts, except that no steroid was added. Two control hearts were interspersed with experimental hearts (maximum of 10) each day; the mean value obtained from the two control hearts was used as a control value for that day.

In vivo *studies in animals*

Pigs of either sex, weighing 27–30 kg, received 20 μCi/kg of [^{131}I]-RIBA (16.1 mCi/mmol) intravenously. One or 2 h after injection, respectively, the pigs were killed by an excessive dose of barbiturate. Samples of left and right ventricles were blotted and weighed. Their radioactivity was measured in a gamma scintillation counter. Radioactivity of blood samples was similarly measured. Male stump tail monkeys, weighing 7.2–10 kg, were similarly studied except that the intervals between injection and death were 10 min, 2 h and 24 h, respectively.

Studies in man

Four healthy adult males, aged 24–30 years and weighing 58–75 kg, were studied. All were considered normal on the basis of history, physical examination

and laboratory examination. The latter included a complete blood count, urinalysis, and measurements of liver function by serum glutamic oxaloacetic transaminase, creatine phosphokinase and lactic dehydrogenase activities. Roentgenograms of the chest and 12-lead electrocardiograms were normal in all subjects. They received [^{131}I]-RIBA (71.3–1,370 mCi/mmol) in a rising dose study. The respective doses were 2, 5, 10 and 20 μCi/kg intravenously. Each subject received only one dose. The radioactive compound was supplied as a sterile solution for human use and diluted in sterile isotonic saline before injection. To decrease uptake of radioactive iodine by the thyroid gland, each subject received 10 drops of Strong Iodine Solution (Lugol's Solution) U.S.P. by mouth 1 h before receiving RIBA, and 10 drops/day for 10 days thereafter. Each subject received a written and verbal description of the procedure in advance and gave written informed consent. The investigational protocols and procedures for obtaining informed consent were approved by the University Committee on Human Studies and by the U.S. Food and Drug Administration before the studies began. The study of each subject was completed before beginning the study of the next subject, who received a higher dose than his predecessor.

Myocardial uptake of radioactivity was observed with a gamma camera (Pho/Gamma 4, Searle Radiographics) placed over the supine subjects' chest. The uptake was continuously displayed and also recorded on video tape for 2 h after injection of RIBA. During the third and fourth h post-injection the subjects walked about the room for brief periods but at frequent intervals during this time they were again placed supine under the camera and the precordial image was displayed; these displays were also recorded on video tape. At various times, ranging from 5 min to 4 h after injection, pictures of the precordial display were recorded on photographic film with a polaroid camera. Each picture represented 10 min of exposure, except in one instance where a 15 min exposure was used. Ten such pictures were taken of subject 3; four pictures were taken of each of the other three subjects. A Polaroid photograph of the left lateral thoracic scintigram of subject 4 was also obtained 4 h after injection of RIBA. The anterior chest area of subject 4 was additionally examined by rectilinear scanning; the area was scanned with an Ohio Nuclear Scanner [model 84, 5 inch (127 mm) crystal] from 65 to 106 min after injection, and from 160 to 180 min after injection. The precordial area of subjects 1 and 2 were again examined under the gamma scintillation camera at 23 and 22 h, respectively, after injection of RIBA. After absence of late uptake had been shown in these two subjects, and information on the half life of RIBA in human plasma had been obtained in them (see below), subjects 3 and 4 were not studied at these late times.

Serial venous blood samples (a mean of nine per subject) were obtained from all subjects at various intervals during the post-injection period. The radioactivity of aliquots of blood was measured in a gamma scintillation counter. Electrocardiographic (lead 2) tracings were taken at frequent intervals during the first 4 h after injection. A 12-lead electrocardiogram was taken 2–4 days after injection.

The doses of stable compound given to subjects 1 and 2 were 2 μg/kg and 1.6 μg/kg, respectively. As the doses given to subjects 3 and 4 were higher (111 μg/kg and 173 μg/kg, respectively), serial measurements of pulse and blood pressure were taken throughout the first 4 h after injection in these two subjects. In subjects 1 and 4, respectively, reliable complete urine collections were obtained at 12, 24, 48 and 72 h after injection, to permit measurements of radioactivity. The completeness of urine collections was less reliable in subjects 2 and 3, respectively, except for one early period of special interest (see below). The radioactivity of aliquots of urine was measured in a gamma scintillation counter and the total radioactivity of the urine collected for each period was calculated.

As an additional step of quality control in the RIBA used in human subjects and in monkeys, aliquots of each of the lots were injected intravenously into rats after receipt of the lot in our laboratory. Three samples were taken from each lot; each sample was injected i.v. (60 μCi/kg) into a rat, which was killed 2 h thereafter. Myocardial uptake of radioactivity and the ratio, radioactivity of myocardium/radioactivity of blood, was determined and compared with results previously obtained (Counsell *et al.*, 1974) in rats with lots of known high radiochemical purity.

Results

In vitro *studies*

The uptake of noradrenaline by rats hearts was inhibited, as expected, by both corticosterone and 17-β-oestradiol (Table 1). Both steroids also inhibited the myocardial uptake of bretylium and RIBA (Table 1).

In vivo *studies in animals*

Pigs showed significant myocardial uptake of RIBA (Table 2), similar to that previously observed in dogs and rats. Monkeys did not show significant myocardial uptake at any of the three times studied (Table 3). The maximum value for the ratio, radioactivity of left ventricle/radioactivity of blood, achieved with RIBA in monkeys was only 2.6. The rats used to verify the lot of RIBA used in monkeys showed a mean myocardial uptake of 0.236%

administered (kg) dose of radioactivity/g; the ratio, radioactivity of rat myocardium/radioactivity of rat blood, was 45.6. Thus, the failure of myocardial uptake in monkeys was not due to any defect in the lot of RIBA used.

Studies in man

No significant myocardial radioactivity was observed after injection of RIBA in any of the four subjects. Continuous observation of the precordial display during the first 2 h and frequent observations during the succeeding 2 h revealed no suggestion of myocardial uptake. A precordial scintigram obtained in subject 4 is shown in Figure 1, as an example. The scintigram shown here is representative of the entire series in that no other picture taken at any time in any subject showed more convincing evidence of myocardial uptake. Samples of −11 four lots of RIBA used in these subjects were also injected into rats as described previously and all four lots showed good uptake by rat myocardium. The respective mean values were 0.265, 0.168, 0.266 and 0.163% administered (kg) dose/g of myocardium. The respective mean ratios, radioactivity of rat myocardium/radioactivity of rat blood, for the four lots were 33.1, 9.3, 44.3 and 33.7.

Table 1 Effect of steroids on uptake of labelled compounds by rat myocardium

Labelled compound	*Steroid*	*Concentration of steroid (μg/ml)*	*n*	*Uptake in presence of steroid/ control uptake*
[^{14}C]-Bretylium	Corticosterone	1	12	0.75±0.055†
[^{14}C]-Bretylium	Corticosterone	5	12	0.60±0.067
[^{14}C]-Bretylium	Corticosterone	10	12	0.52±0.052
[^{14}C]-Bretylium	17-β-oestradiol	1	6	0.90±0.069
[^{14}C]-Bretylium	17-β-oestradiol	5	6	0.53±0.061
[^{14}C]-Bretylium	17-β-oestradiol	10	6	0.43±0.048
[^{125}I]-RIBA*	Corticosterone	0.5	20	0.76±0.042
[^{125}I]-RIBA*	Corticosterone	1	20	0.63±0.037
[^{125}I]-RIBA*	Corticosterone	2	20	0.47±0.036
[^{125}I]-RIBA*	Corticosterone	5	20	0.38±0.025
[^{125}I]-RIBA*	Corticosterone	10	20	0.33±0.026
[^{125}I]-RIBA*	Corticosterone	30	10	0.15±0.016
[^{125}I]-RIBA*	17-β-oestradiol	0.1	20	0.67±0.050
[^{125}I]-RIBA*	17-β-oestradiol	0.5	20	0.60±0.036
[^{125}I]-RIBA*	17-β-oestradiol	1	20	0.58±0.035
[^{125}I]-RIBA*	17-β-oestradiol	2	20	0.46±0.025
[^{125}I]-RIBA*	17-β-oestradiol	5	20	0.35±0.016
[^{125}I]-RIBA*	17-β-oestradiol	10	20	0.24±0.014
(±)-[^{3}H]-Noradrenaline	Corticosterone	1	6	0.72±0.059
(±)-[^{3}H]-Noradrenaline	Corticosterone	5	6	0.79±0.089
(±)-[^{3}H]-Noradrenaline	Corticosterone	10	6	0.41±0.086
(±)-[^{3}H]-Noradrenaline	17-β-oestradiol	1	6	0.76±0.015
(±)-[^{3}H]-Noradrenaline	17-β-oestradiol	5	6	0.44±0.057
(±)-[^{3}H]-Noradrenaline	17-β-oestradiol	10	6	0.13±0.064

*see text
†s.e. mean

Table 2 Uptake of [^{131}I]-RIBA* by tissues of pigs

				[^{131}I](% administered [kg] dose/g) in				*Ratios*	
Pig no.	*Weight (kg)*	*Sex*	*Time after injection (h)*	*Blood*	*Left ventricle*	*Right ventricle*	*Thyroid gland*	*Left ventricle: blood*	*Thyroid: blood*
1	30	F	1	0.023	0.372	0.299	0.039	16	1.7
2	30	F	1	0.022	0.446	0.316	0.040	20	1.8
3	30	F	2	0.034	0.369	0.240	0.031	11	0.9
4	27	M	2	0.011	0.265	0.218	0.021	24	1.9

*see text

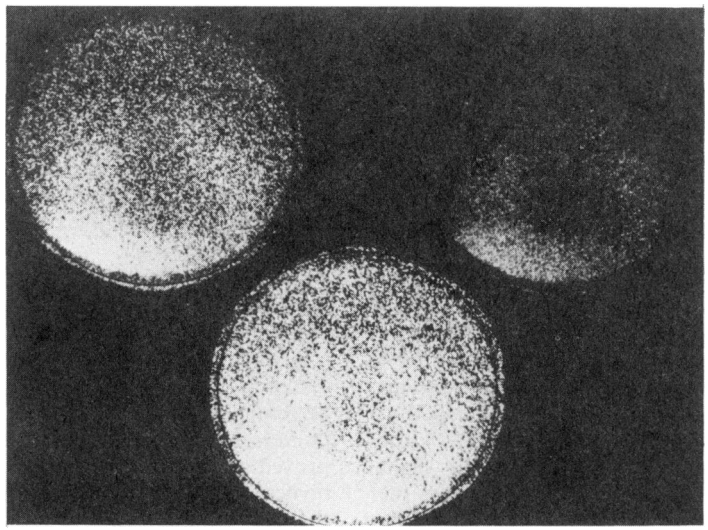

Figure 1 Precordial scintigram obtained in a normal man 227–237 min after injection of 20 μCi/kg of [^{131}I]-RIBA. In the recording system used here, the camera automatically displayed a trio of simultaneous images on all occasions; the images in each trio differed in intensity. In this man pulmonary densities are clearly seen on each side of the heart. The heart itself shows little density. The upper portion of the liver is also seen at the bottom.

The results obtained with the lots given to subjects 1, 3 and 4 were consistent with our previous experience in rats. The ratio obtained with the lot given to subject 2 was somewhat lower, but it still indicated definite myocardial uptake in the rat.

The disappearance of radioactivity from the blood showed a rapid, presumably distributional phase and a slower second phase. The half-time of disappearance, calculated from the second phase, was 85, 185, 144 and 192 min in the four subjects, respectively. Subjects 1 and 4, who made reliable urine collections, showed striking differences in urinary excretion of radioactivity. In subject 1, 95.9% of the administered dose was excreted in the first 12 h. In subject 4, only 5.8% was excreted in the first 12 h and a total of only 7.13% was excreted during the first 5 days. Although urine collections at later times in subject 3 were not considered reliable, his first urine collection, 1 h after injection, was reliable and showed that 75.3% of the injected radioactivity was excreted during this short period.

No electrocardiographic changes were observed at any time. Subject 3 had a blood pressure (right arm, subject reclining) of 106/72 at the time of initial screening of subjects. On the day of the study he showed an elevation of blood pressure, possibly due to anxiety, to 145/95 at 21 min and 140/90 at 12 min before injection. Four min after injection his blood

Table 3 Uptake of [^{131}I]-RIBA* by tissues of monkeys

			[^{131}I] (% administered [kg] dose/g) in				Ratios	
Monkey	Weight (kg)	Time after injection	Blood	Left ventricle	Right ventricle	Thyroid gland	Left ventricle: blood	Thyroid: blood
1	8.0	10 min	0.132	0.060	0.081	0.500	0.45	3.8
† 2	9.0	2 h	0.018	0.026	0.031	0.022	1.4	1.2
† 3	10.0	2 h	0.009	0.023	0.025	0.040	2.6	4.4
4	8.4	24 h	0.0005	0.0009	0.0014	0.107	1.8	214.0
5	7.2	24 h	0.0003	0.0007	0.0016	—	2.3	—
†For comparison, the mean values in 3 dogs 2 h after injection were:			0.011	0.329	0.381	0.174	29.9	15.8

*see text

pressure was 125/90 and no significant further changes were found in 18 subsequent determinations over the next 4 h. His pulse dropped slightly during the study but remained within normal limits at all times. Subject 4, whose blood pressure at the time of initial screening of subjects was 140/85, had blood pressures of 111/84, 111/86, and 116/88 at 6, 4, and 3 min, respectively before injection. His blood pressure changed only slightly during the study, reaching a nadir of 110/78 at 10 min after injection, and a maximum of 140/98 at the end of the period of observation (260 min). However this subject showed slight postural hypotension at 140 min after injection, when his blood pressure dropped from 130/94 (reclining) to 112/90 (standing). At 260 min, when his blood pressure was 140/98 (reclining), it changed to 130/98 (standing). He experienced no dizziness in the upright position on either occasion. The slight postural drop of blood pressure, observed with the highest dose given in those studied, was the only pharmacological effect observed at any time. Subject 4 showed no significant pulse changes at any time, the range being 65–79 beats/min throughout the study.

Subjects 1, 2 and 4 agreed to return 2–4 days after injection to permit complete blood counts, urine analysis and determinations of liver function. The 12-lead electrocardiograms were also taken again at this time. No significant change from control values were observed in any subjects.

Discussion

The inhibition of uptake of bretylium by the rat heart in the presence of corticosterone suggests that bretylium is taken up to an important degree by the uptake$_2$ mechanism. The results with 17-β-oestradiol support this view. This finding is consistent with the results obtained by Namm *et al.* (1975), using the immunosympathectomized rat heart. Our results with the bretylium analogue, RIBA, suggest that it also is taken up by the rat heart through the uptake$_2$ process. In view of these findings, the differences between uptake of RIBA by the hearts of sub-primate species and primates is of interest. We have previously shown (Counsell *et al.*, 1974) that the myocardium of rats and dogs concentrates RIBA to a high degree after its i.v. administration to intact animals. The present work shows that the pig heart also takes up RIBA avidly, but neither humans nor monkeys showed myocardial accumulation of RIBA after its i.v. administration.

Several possible explanations for this difference may be considered: the imaging technique may have had insufficient sensitivity to demonstrate myocardial uptake; the monkeys and humans may have been studied at times that missed the period of maximum uptake; differences in specific activity among the various lots of RIBA could be invoked to account for differences in uptake or the lots of RIBA prepared for primate use may have differed in some other, unknown way from the lots used in lower animals; differences between primates and other species in the biotransformation and excretion of RIBA may affect uptake of RIBA by the heart; primates may differ from other species, either quantitatively or qualitatively, with regard to uptake$_2$. We shall consider these possibilities in order.

It is unlikely that the imaging system used here would fail to detect significant myocardial uptake. Myocardial uptake has been extensively studied over a period of many years and the gamma camera system used here has proved to be very reliable for detecting myocardial uptake of radioactive compounds in many species, including man. The dog has proved to be a very useful model for predicting myocardial uptake of many radioactive substances by the human heart. Various radioisotopes of caesium, rubidium and potassium have been shown, by scintigrams and by counting of radioactivity of tissue specimens, to be taken up by the dog myocardium; these results have been confirmed by subsequent scintigrams in man (Cooper, 1974). It is of interest that radioactive caesium is taken up by the monkey heart also (unpublished experiment). The uptake of ^{99m}Tc-pyrophosphate and similar compounds by infarcted myocardium was first demonstrated in dogs and subsequently confirmed by scintigrams in man. The uptake of RIBA by the dog heart, demonstrated by counting of radioactivity of tissue specimens, was also confirmed by scintigrams obtained in the intact dog (Counsell *et al.*, 1974). Moreover, the lack of myocardial uptake in monkeys was clearly shown by direct determination of radioactivity of tissue specimens and thus could in no way be attributed to insensitivity of scintigraphic imaging systems.

The uptake of RIBA by the myocardium of rats and dogs has been shown to be high 1–2 h after injection and to decline subsequently. In cats, Boura *et al.* (1960, 1961) found higher myocardial uptake of [^{14}C]-labelled bretylium and *o*-bromobenzyltrimethylammonium iodide at 1–3 h after injection than at later times. Our present data also show high uptake by the pig myocardium after 1 and 2 h. Yet the continuous observation of the precordial display during the first 2 h after intravenous administration to human subjects and frequent observations during the next 2 h showed no myocardial uptake at any time. Although no Polaroid pictures were taken earlier than 5 min after injection of RIBA, the precordial area was displayed by the gamma camera at all times after intravenous injection in humans, except for the few seconds necessary for final adjustment of the camera over the subject after injection. Each subject was already supine on a stretcher at the time of injection and only a few

seconds elapsed between completion of the injection and beginning of observation of the precordial display. No suggestion of myocardial uptake was observed even at these early times. The monkey showed no significant uptake either at 10 min or 2 h after injection, as confirmed by counting of myocardial samples. Neither the monkey studied at 24 h nor the humans studied at 22 and 23 h after injection showed any myocardial uptake. In view of the more rapid elimination of RIBA by humans than by dogs, it is unlikely that the maximum myocardial uptake would appear at a later time in humans, in any event.

Differences in specific activities cannot account for the differences in uptake observed here. Although the RIBA prepared for human use had higher specific activity than the RIBA used *in vitro* in the present studies, we have in previous work consistently obtained high myocardial uptakes after administration of RIBA i.v. to intact rats and dogs, using preparations of RIBA with specific activities ranging from 15 mCi/mmol to 145 mCi/mmol. The specific activities of the RIBA used in subjects 3 and 4 were 71.3 mCi/mmol and 78.6 mCi/mmol, respectively. Chemical differences among lots of RIBA cannot have been significant, for the actual lots of RIBA used in man and monkey were all tested in rats and showed good uptake by rat myocardium.

Differences in excretion of radioactivity after administration of RIBA were observed among species. In a previous study we collected all urine and faeces from six dogs for 6 days after intravenous administration of RIBA. The mean urinary and faecal excretion was 89.7% and 9.2% of administered radioactivity, respectively, during the period of collection (unpublished observation). The lowest urinary excretion in any dog was 70% of radioactivity. Yet one of the four human subjects showed little urinary excretion of radioactivity during the first 5 days. The rapid disappearance of radioactivity from the blood as well as the high count rate we observed when a scintillation detector was placed over the liver area strongly suggest that the biliary route of excretion, with subsequent loss of radioactivity in the faeces, was important in this subject. The short half-life of radioactivity in the blood of the four human subjects, ranging from 85 to 192 min, was in contrast to the long half-life of radioactivity in the blood of 21 dogs (3 at each of 7 time periods post-injection) we previously studied. The mean in dogs was 17.3 h. The half-life in rat blood was probably about 4 h; as rats were studied only at 2 and 6 h after injection this is only a rough estimate.

It is therefore conceivable that rapid elimination of RIBA may have interfered with myocardial uptake in man. But, as noted above, no suggestion of uptake was observed even at very early times after injection. It would also be very difficult to explain the low *ratio,* radioactivity of myocardium/radioactivity of blood, in the tissue samples taken from monkeys simply on the basis of rapid elimination of the compound. Even if rapid elimination resulted in relatively low tissue counts, one would still expect a high ratio if the monkey had significant capacity for myocardial uptake of RIBA. The maximum value in monkeys (2.6) is not only very low in comparison with the value for the same ratio achieved with RIBA in other species, but also with the values reported for several other labelled compounds: 12 for radio-iodinated oleic acid in dogs (Evans, Gunton, Baker, Beanlands & Spears, 1965), 13 for radio-iodinated toluidine blue in dogs (Carr, Carroll, DiGiulio & Blair, 1973), 15 for [^{11}C]-labelled noradrenaline in mice (Ansari, 1974), 20 for 131-caesium in dogs (Carr *et al.,* 1964) and 22 for 201-thallium in rabbits (Hetzel, Westerman, Quinn, Meyers & Barresi, 1977). The difference between the low myocardial uptake of RIBA in monkeys and the high uptake in rats, dogs and pigs cannot be ascribed to more rapid deiodination of RIBA in monkeys, for the ratio, radioactivity of the thyroid gland/radioactivity of blood, 2 h after i.v. injection of RIBA was less in the monkey than in the dog or rat (Table 3 and Counsell *et al.,* 1974) and only slightly greater than in the pig (Table 2). The stable iodine given to the human subjects precluded an estimate of thyroidal iodide uptake by external counting over their thyroid glands.

We must therefore consider the possibility that uptake$_2$ may not be precisely the same in primates as in lower species. Although the present results cannot be deemed conclusive, they suggest the possibility that at least some primates may differ, to an important degree, from lower species with regard to uptake$_2$. In view of the clear demonstration of the existence of uptake$_2$ as a significant mechanism in lower species, the possibility that man and at least one species of monkey may differ from them in this respect deserves further consideration.

We wish to thank Professor Ivan Delalande, University of Adelaide, for his helpful suggestions. [^{14}C]-labelled bretylium was supplied through the courtesy of Drs D. H. Namm and F. C. Copp, Wellcome Research Laboratories. [^{125}I]-labelled RIBA was synthesized in the laboratory of Dr Counsell. [^{131}I]-labelled RIBA was supplied through the courtesy of Searle Laboratories and the University of Michigan Nuclear Pharmacy. This work was supported in part by U.S. Public Health Service Grants 2T11-GM-15559 and 04-D-001274, and by G. D. Searle Company.

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(Received December 24, 1978)