glucose 6-phosphatase activity of liver slices and, because of the higher dosages needed, presumably the involvement of this enzyme is inconsequential to the mechanism of the action.[20] In regard to other enzymes, the insulinase levels of rat liver are depressed in the presence of sulfonylureas. However, this effect requires relatively large amounts of the agents, and also, tolbutamide does not influence the half-life of the injected ^{131}I-insulin.[21] A greater effect of the enzyme might be engendered in relation to regenerating liver, a point not investigated in the present study.

The structural configuration of phenformin may predispose to the observed inhibition of liver regeneration since the parent compound, guanidine, depresses liver regeneration in the rat, an effect which extends even to arginine.[22] Guanidine itself is hypoglycemic[23, 24] and, as it displays high toxicity, it has served as a model for the screening of a large number of allied derivatives.

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Studies of the chemical nature of the α-adrenergic receptor—II. Investigation of the labeling procedure

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TWO PROCEDURES have been described for labeling the α-adrenergic receptors of rabbit aorta with ^{3}H- or ^{14}C-dibenamine hydrochloride.[1–3] In procedure I the receptors were partially protected with

adrenaline, while other receptors were masked with unlabeled dibenamine hydrochloride; after washing, the receptors were labeled with ^{14}C-dibenamine hydrochloride. In procedure II the receptors were partially protected with adrenaline while other receptors were labeled with ^{14}C-dibenamine hydrochloride. As controls for both procedures, the experiments were repeated with the omission of adrenaline. Since different methods were used for washing the tissues after labeling, it was possible that loosely bound radioactive dibenamine hydrochloride was incompletely removed in some of the labeling procedures. It was therefore likely that loosely bound radioactive dibenamine hydrochloride contributed to the radioactivity in the lipid extracts of aortic strips and accounted for the variation observed in the percentage radioactivity of lipid fractions obtained in different labeling procedures. For this reason experiments have been carried out to establish an adequate procedure for removing loosely bound ^{14}C-dibenamine hydrochloride prior to chemical fractionation of aortic tissue.

The amount of radioactivity observed in control strips in labeling procedure I was surprisingly high and complicated our further work. It was apparent that exposure of the strips to unlabeled dibenamine hydrochloride in our previous experiments did not result in saturation of the tissue with the reagent, and the tissue retained the capacity to take up ^{14}C-dibenamine hydrochloride. It was therefore important to determine whether a method could be established to saturate the tissue with unlabeled dibenamine hydrochloride.

Four aortic strips (approximately 2 cm in length) were prepared from a single rabbit according to the procedure of Furchgott and Bhadrakom[4] and mounted as single strips in muscle chambers of 15 ml working volume with the aid of thread and steel hooks. In previous work[3] carried out in this laboratory the two ends of a single strip were hooked together and the hook attached by means of thread to a transducer. The loop thus formed was placed around a hook embedded in the muscle chamber. All other experimental procedures were carried out as described previously.[3] The points plotted in Figs. 1, 2, 3, and 4 represent the means of two independent determinations. The vertical bars in the figures represent the standard deviation of the mean. Probability values (P) were obtained by means of a one-tailed *t* test.

Removal of loosely bound ^{14}C-dibenamine hydrocyloride from rabbit aortic strips. The responses of four aortic strips to several concentrations of adrenaline were recorded and the strips then exposed to ^{14}C-dibenamine hydrochloride (3×10^{-6}) for 20 min. One strip was removed from a muscle chamber without washing, and the remaining three strips were washed at 15-min intervals. A second strip was removed from a muscle chamber after 1 hr, a third strip after 3 hr, and a fourth strip after 6 hr. The radioactivity in the lipid and lipid-free residues of the strips was determined (Fig. 1).

Uptake of ^{14}C-dibenamine hydrochloride by rabbit aortic strips. (a) Four rabbit aortic strips were exposed to ^{14}C-dibenamine hydrochloride (3×10^{-6}) for the following time periods: strip 1, 10 min; strip 2, 30 min; strip 3, 60 min; strip 4, 90 min. The strips were washed at 15-min intervals for 3 hr and the total radioactivity taken up by the strips determined (Fig. 2).

(b) Four rabbit aortic strips were exposed to the following concentrations of unlabeled dibenamine hydrochloride for 20 min: strip 1, 3×10^{-8}; strip 2, 3×10^{-7}; strip 3, 3×10^{-5}; and strip 4, 3×10^{-4}. After one wash, strips were exposed to ^{14}C-dibenamine hydrochloride (3×10^{-6}) for 20 min and washed at 15-min intervals for 3 hr. The total radioactivity taken up by the strips was then determined (Fig. 3).

(c) Four rabbit aortic strips were exposed to the following concentrations of ^{14}C-dibenamine hydrochloride for 20 min: strip 1, 3×10^{-8}; strip 2, 3×10^{-7}; strip 3, 3×10^{-5}; and strip 4, 3×10^{-4}. The strips were washed for 3 hr at 15-min intervals and the total radioactivity present in the tissues determined (Fig. 4).

Repetition of labeling procedures I and II. Labeling procedures I and II were repeated with the modification that the strips were washed for 3 hr at 15-min intervals after labeling with ^{14}C-dibenamine hydrochloride. The radioactivity present in the lipid and lipid-free residues of experimental and control strips was determined (Table 2).

Investigation of the lipid extracts of aortic strips labeled by procedure II. The purified lipid extracts obtained from four experimental strips were combined and concentrated to 0·2 ml. The lipid extracts of four control strips were treated in the same manner. Lipid components were identified by thin-layer chromatography and the radioactivity in each segment of the chromatogram determined[3] (Table 3).

After labeling the aortic strips with ^{14}C-dibenamine hydrochloride, approximately 50 per cent of the radioactivity taken up can be removed by washing for 3 hr at 15-min intervals (Fig. 1). Extending

the period of washing to 6 hr does not result in a further significant removal of radioactivity .The radioactivity removed by washing may be associated with a transformation product of dibenamine hydrochloride, and this question requires further investigation. Of the ^{14}C-dibenamine hydrochloride taken up by the lipid-free residue, 31 per cent is removable by washing, whereas 77 per cent of the radioactivity associated with the lipid fraction is removable by washing. It follows that if a tissue is not adequately washed after labeling with ^{14}C-dibenamine hydrochloride, a high percentage of radioactivity will be found in the lipid fraction. Differences in washing procedure might therefore account for failure to observe similar percentages of radioactivity associated with lipids in different labeling procedures.

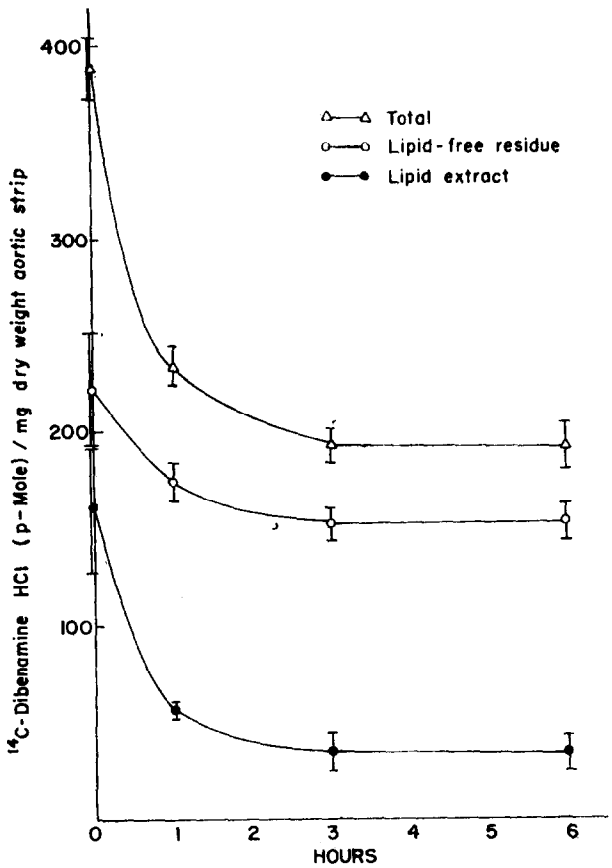

FIG. 1. Removal of loosely bound ^{14}C-dibenamine hydrochloride from aortic strips by washing for various time periods.

Rabbit aortic strips are apparently saturated with dibenamine hydrochloride after 90-min exposure to this reagent (Fig. 2). It therefore seemed desirable to extend the period of exposure of strips to unlabeled dibenamine hydrochloride from 20 to 90 min prior to labeling with ^{14}C-dibenamine hydrochloride in order to reduce the radioactivity associated with control strips in procedure I. Before doing so an experiment was carried out to check that the tissue was saturated with dibenamine hydrochloride after exposure to this reagent (3×10^{-6}) for a 90-min period. Strips were exposed to a freshly prepared solution of ^{14}C-dibenamine hydrochloride (3×10^{-6}) for 90 min after prior exposure to this reagent (3×10^{-6}) for the same period of time. The results (Table 1) show that the tissue was not saturated with dibenamine hydrochloride after exposure to this reagent for a 90-min period. The probable reason for the apparent saturation as indicated by the flattening of the curve (Fig. 2) was the inactivation of the ethylenimmonium ion of dibenamine hydrochloride by interaction with water.[5]

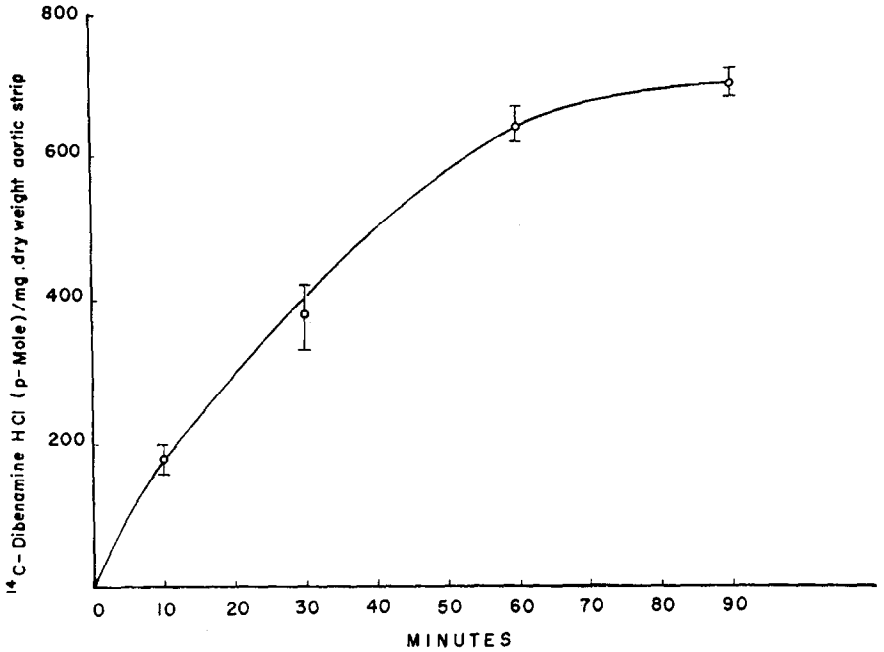

FIG. 2. The uptake of ^{14}C-dibenamine hydrochloride by aortic strips after exposure to this reagent (3×10^{-6}) for various time periods.

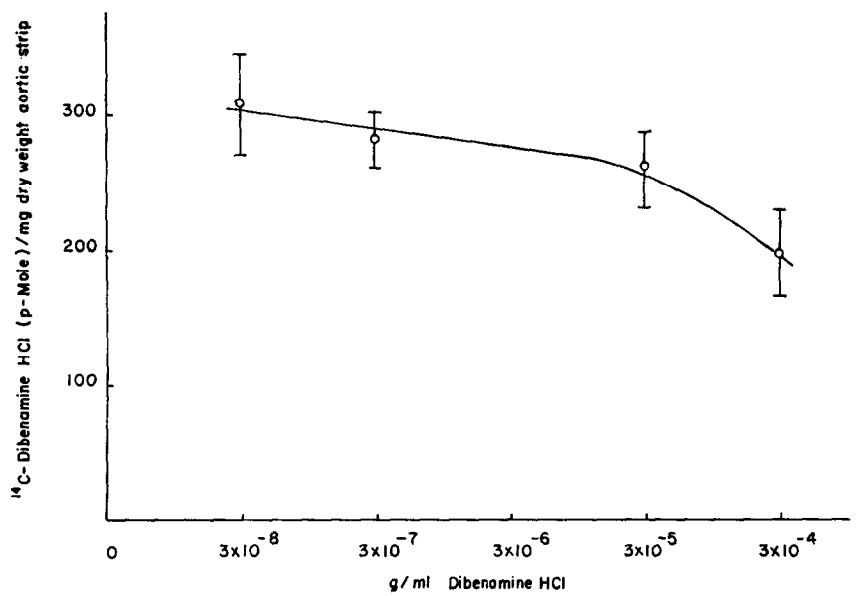

FIG. 3. Uptake of ^{14}C-dibenamine hydrochloride by aortic strips after exposure to different concentrations of unlabeled dibenamine hydrochloride.

Thus there does not appear to be an advantage in increasing the length of exposure of strips to unlabeled dibenamine hydrochloride in procedure 1.

The studies summarized in Fig. 4 show that it is not possible to saturate the tissue with dibenamine hydrochloride by increasing the concentration of this reagent. A sharp increase in uptake of firmly bound ^{14}C-dibenamine hydrochloride occurs when the concentration is raised beyond 3×10^{-6}.

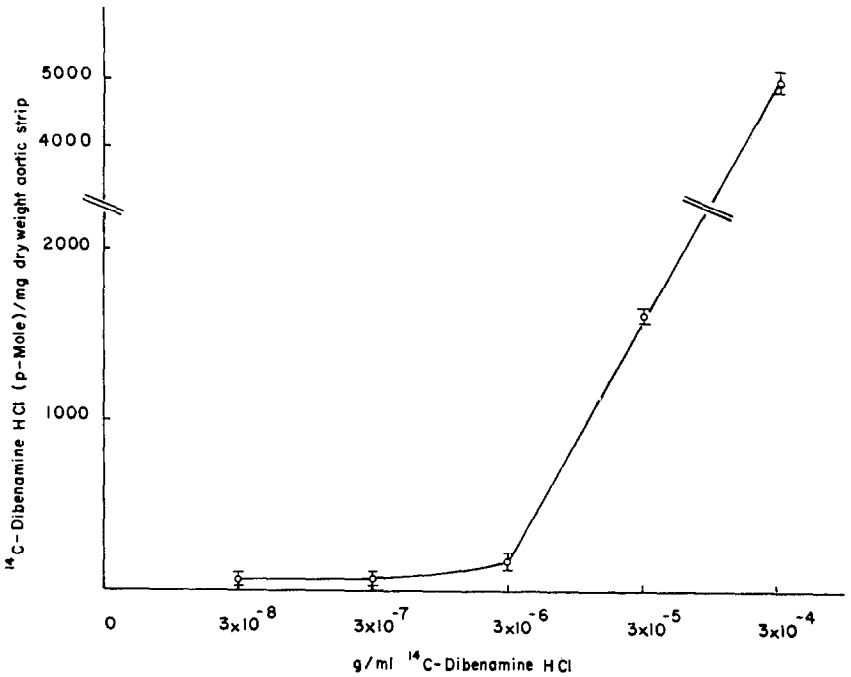

FIG. 4. Uptake of ^{14}C-dibenamine hydrochloride by aortic strips after exposure to different concentrations of this reagent.

TABLE 1. DISINTEGRATIONS DETECTED IN RABBIT AORTIC STRIPS

Disintegrations/min after (a) exposure to ^{14}C-dibenamine hydrochloride (3×10^{-6} g/ml) for a 90-min period; (b) after re-exposure for a further 90-min period to a freshly prepared solution of this reagent (3×10^{-6} g/ml).

Experiment no.	Total dpm/mg dry weight of strip	
	(a) First 90-min exposure	(b) Second 90-min exposure
1	746	1269
2	784	1421

which is the concentration required to completely block the α-adrenergic receptors.[6] Studies summarized in Fig. 3 show that raising the concentration of unlabeled dibenamine hydrochloride to which the tissue is first exposed cuts down only to a moderate extent on the subsequent uptake of ^{14}C-dibenamine hydrochloride.

On the basis of the experiments described above, labeling procedures I and II have been repeated with the modification that strips were washed for 3 hr at 15-min intervals after labeling with ^{14}C-dibenamine hydrochloride. The results are summarized in Table 2. Several differences were noted when these results were compared with those obtained previously:[3] (1) The amount of ^{14}C-dibenamine hydrochloride taken up is greater when tissues are mounted as single strips rather than as loops. Apparently there was inadequate access of ^{14}C-dibenamine hydrochloride in our previous experimental arrangement.[3] (2) The percentage radioactivity detected in the lipid fraction in labeling procedure I was the same as that detected in the lipid fraction in labeling procedure II (15–20%). This was not the case in our previous study.[3]

TABLE 2. THE DISINTEGRATIONS PER MINUTE DETECTED IN THE LIPID EXTRACTS AND LIPID-FREE RESIDUES OF EXPERIMENTAL AND CONTROL RABBIT AORTIC STRIPS

The total dpm/mg dry weight of strip is the sum of the dpm/mg of the lipid extract and lipid-free residue.

Aortic strip	Lipid free residue/mg dry weight of strip ± S.D. (dpm)		Lipid extract/mg dry weight of strip ± S.D. (dpm)		Total dpm/mg dry weight of strips ± S.D.	
	Labeling procedure I	Labeling procedure II	Labeling procedure I	Labeling procedure II	Labeling procedure I	Labeling procedure II
Control	186 ± 28 (10)*	302 ± 38 (8)	43 ± 6 (10)	58 ± 15 (8)	229 ± 33 (10)	360 ± 50 (8)
Experimental	204 ± 39 (10)	211 ± 38 (8)	52 ± 9 (10)	38 ± 10 (8)	256 ± 46 (10)	249 ± 47 (8)
P Value	<0·15	<0·01	<0·01	<0·01	<0·1	<0·01

* Numbers enclosed by parentheses indicate the number of experiments performed.

TABLE 3. THE DISINTEGRATIONS PER MINUTE DETECTED IN THE COMPONENTS OF LIPID EXTRACTS OF EXPERIMENTAL AND CONTROL RABBIT AORTIC STRIPS LABELED BY PROCEDURE II

R_f Value	10 μg ^{14}C-dibenamine HCl (dpm. per segment of chromatogram)	Control strip (dpm per segment of chromatogram/ 100 mg dry weight of aortic strip)	Experimental strip (dpm per segment of chromatogram/ 100 mg dry weight of aortic strip)
0–0·04	0	40	80
0·04–0·13*	4	30	50
0·13–0·29†	33	60	90
0·29–0·50‡	8	400	400
0·50–0·70	20	200	200
0·70–1·00	13,688	1500	1000
Total	13,753	2230	1820

* Range for sphingomyelin.
† Range for phosphatidylserine.
‡ Range for cephalin.

The results obtained in the present study (Table 2) indicate that procedure II is the procedure of choice. It is of interest to consider why this should be the case. In procedure II, exposure of experimental strips to adrenaline (1×10^{-4}) for 5-min prior to addition of ^{14}C-dibenamine hydrochloride cuts down on the amount of bound radioactivity by 111 dpm/mg dry weight of tissue. By

procedure I it was therefore anticipated that the amount of radioactivity bound by experimental strips would exceed that in control strips by a similar amount. The much smaller difference observed (27 dpm/mg dry weight of tissue) is probably due to the fact that approximately 20% of the adrenaline taken up by the experimental strips is not removed by washing in our experiments* and prevents the subsequent uptake of ^{14}C-dibenamine hydrochloride. A study of the distribution of radioactivity in the lipid components of the lipid extracts (Table 3) confirmed those previously obtained.[3] In view of the unsuspected complications with labeling procedure I, it is planned to use labeling procedure II in our further studies.

It is necessary to point out, however, that even with procedure II, complications arise in the interpretation of our results. The major complication is the fact that adrenaline undoubtedly masks sites of dibenamine hydrochloride uptake other than the α-adrenergic receptors.

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Amino acid composition of neurohypophysial secretory granules and Van Dyke protein*

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THE POSTERIOR pituitary is now known to secrete two major hormones, the octapeptides vasopressin and oxytocin. However, some early attempts at extracting the active principle from the gland resulted in the isolation of what appeared to be a single substance possessing both oxytocic and vasopressor activity.[1] Rosenfeld[2] submitted posterior lobe press juice to ultracentrifugation and found both hormone activities associated with material that sedimented as a single species. Van Dyke *et al.*[3] isolated a proteinaceous substance that was soluble in dilute acid and contained high activity of both hormones in a 1:1 ratio. The solubility, sedimentation, and electrophoretic properties of this material indicated that it was a single protein with a molecular weight of approximately 30,000. The Van Dyke protein was later shown to be a complex consisting of the two peptide hormones in non-covalent association with a protein component[4] subsequently named "neurophysin."[5] It was postulated

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