formed which was filtered, rinsed with water until neutrality, and dried in an oven at 110° for 24 hr, yield 35 g (96%). *Anal.* ($C_4H_3NO_2$) C, H, N, O. The polymer must be quantitatively dried before analysis to obtain good analytical values.

Method B. Polymerization in Thin Layer. Aspartic acid (100 g) was mixed with 50 g of phosphoric acid and the resulting pasty mass was spread on a Teflon-covered tray of about 1000 cm^2. The tray was placed in a vacuum oven and heated to 180° for 2.5 hr under reduced pressure. The polymer was isolated as previously described; the yield and analytical characteristics were the same.

Condensation with Dicyclohexylcarbodiimide. Poly-DL-succinimide (1 g) was dissolved in 5 ml of DMF; DCC (50 mg) was added to the solution and the mixture stirred for 24 hr at room temperature. The solution was then filtered in order to eliminate the dicyclohexylurea and the poly-DL-succinimide precipitated by the addition of water. The precipitate was rinsed with water and ethanol and dried at 110° for 24 hr, yield quantitative. The product has the same analytical characteristics as the original poly-DL-succinimide. Similar experiments were carried out using different amounts of DCC to establish the amount to be used to obtain the highest molecular weight (see Results and Discussion).

Reaction with Ethanolamine. Poly-DL-succinimide (30 g) was dissolved in 150 ml of DMF. Ethanolamine (45 ml) was then added drop by drop and the solution cooled in an ice bath to keep the temperature at 25–30°. The mixture was stirred for 2 hr and then neutralized with glacial acetic acid (about 30 ml), diluted with water, dialyzed, and lyophylized, yield 42 g (86%). *Anal.* ($C_6H_{10}N_2O_3$) C, H, N, O. The product obtained by this method had an average intrinsic viscosity of 25 ml/g; the molecular weight, determined in the ultracentrifuge, was about 50,000.

The reaction was also carried out using polysuccinimide treated with DCC. The product obtained had the same analytical characteristics as the previous one. The intrinsic viscosity was about 40 ml/g and the molecular weight about 80,000.

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Long-Acting Delivery Systems for Narcotic Antagonists. 1†,‡

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The relationship between the release rate of cyclazocine from composites with poly(lactic acid) and (a) the molecular weight of the polymer and (b) the form of the composite, as a film sealed in an envelope or as discrete small particles, has been investigated *in vivo* and *in vitro*. The release rate is not very sensitive to variations in the molecular weight of the polymer within the values investigated. As may be expected, the lower molecular weight polymer is absorbed faster than the higher molecular weight polymer. The use of the composite as a film sealed in an envelope of pure polymer permits control of the release rate. Desirable delivery rates have been obtained by injecting suspensions of small particles of the composite thereby eliminating the necessity of surgery. In experiments with films, the release rate of cyclazocine *in vivo* is faster than *in vitro*, whereas in experiments with small particles a reverse effect is observed.

In the past few years we have been interested in developing a method of delivering narcotic antagonists to a patient at a constant rate over a prolonged period, perhaps as long as several months. The migration of drugs through waxes, ointments, or polymers has been the object of various investigations.[1-3]

Composites of radioactive cyclazocine (2-cyclopropylmethyl-2′-hydroxy-5,9-dimethyl-6,7-benzomorphan) with polymeric materials in film or very small particle form were selected for this study. The delivery rate of the antagonist was determined by surgically implanting or hypodermically injecting these composites into rats and measuring the radioactivity of urinary excretion. A control experiment

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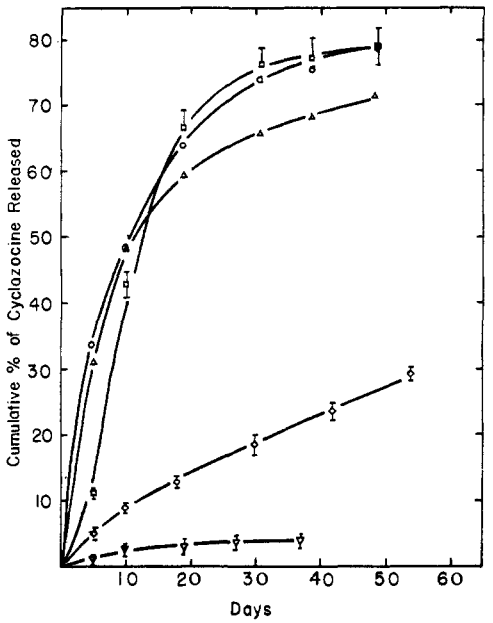

Figure 1. Cumulative amounts of cyclazocine excreted from composites of poly(lactic acid) implanted or injected in rats, expressed as per cent of maximum dose released. Each point represents the mean observation on three or four animals ± S.E. The S.E.'s for samples C and D are similar to that of sample B and are not reported for the sake of clarity. In this work S.E. is equal to the standard deviation divided by the square root of the number of animals tested (S.E. = $\sigma/\sqrt{N}$): (□) sample B; (○) sample C; (△) sample D; (▽) sample E; (◇) sample F.

performed by injecting subcutaneously a solution of tritiated cyclazocine into rats showed that urinary excretion of radioactivity is a useful measure for estimating the cyclazocine released. A total recovery of 72% of the dose was obtained. In the supposition that the remaining 28% consisted of material released through other ways and of experimental errors, the results of the *in vivo* tests reported below have been corrected by this factor.

Previously reported experiments[4-6] conducted by implanting composites of cyclazocine with polyethylene as the polymer in film form showed the feasibility of this method and suggested that the mechanism is by diffusion. However, the use of polyethylene has the disadvantage of requiring surgical removal of the polymeric matrix after the antagonist has been delivered. In order to circumvent this problem, biodegradable poly(lactic acid) was used in place of polyethylene.[7] It was found that 62 days after the implantation the polymeric matrix had practically disappeared, only tiny white specks having been observed at the implantation site.

The present investigation was designed to determine the influence of the following factors on the release of cyclazocine from composites containing poly(lactic acid): (a) molecular weight of the polymer, (b) use of the composite as a film sealed in an envelope of pure poly(lactic acid), and (c) form of the composite as discrete small particles.

In regard to the influence of the molecular weight of the polymer, films of composites containing poly(lactic acid) of molecular weight 70,000, 45,000 and a 50:50 mixture of both polymers were prepared and implanted into rats. The delivery rate of the excreted cyclazocine as a function of time was then measured.

The cumulative amount of cyclazocine released within 49 days, expressed as per cent of the maximum dose released, was approximately the same in all cases, within experimental error (Figure 1, samples B, C, and D). The time at which

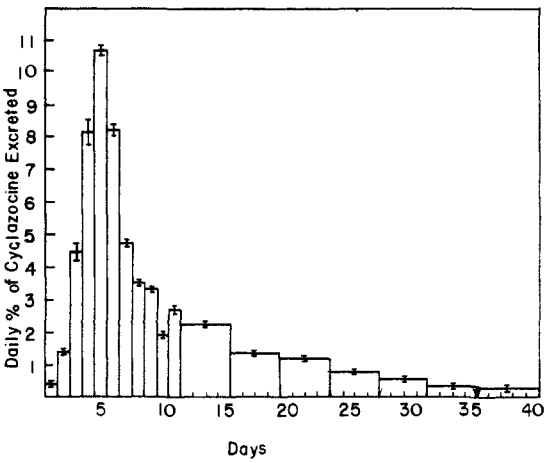

Figure 2. Daily amounts of cyclazocine excreted, expressed as per cent of the maximum dose released. Each point represents the mean observation on nine animals ± S.E. (Average of the results obtained in the experiments *in vivo* with samples B, C, and D).

half of the maximum dose had been released ($t_{1/2}$) was respectively 13.0, 11.0, and 11.5 days.

The daily amount of cyclazocine excreted, expressed as per cent of the maximum dose released, also showed no significant differences in all three cases. The release rate, after reaching a maximum during the first 3-5 days (see Figure 2), decreased rapidly in the following 4 days and then seemed to remain relatively constant for the duration of the tests at an average rate of 300 μg/kg/day which is approximately ten times the human dose.

Visual examination of the insertion area of the sacrificed animals showed that, as expected, the molecular weight of the polymer used in the composites exerted a significant influence in the rate of biodegradability with the lower molecular weight polymer apparently being metabolized faster.

It appears from these tests that the use of poly(lactic acid of molecular weights ranging between 45,000 and 70,000 gives composites capable of releasing cyclazocine upward of 2 months.

Delivery rates from films of the composite sealed in envelopes were then investigated. It was thought that enveloping the composite would reduce the initial delivery rate of cyclazocine. Experiments were performed by enveloping a 6.2-cm^2 film of cyclazocine-poly(lactic acid) composite in a poly(lactic acid) film containing no cyclazocine, heat sealing the edges of the obtained envelope, implanting the envelope into rats, and measuring the excreted cyclazocine. An average total release of only 3.6% of cyclazocine in a 37-day interval was found (Figure 1, sample E). In contrast, a 50% total release of cyclazocine in 11 days was obtained from a nonenveloped composite of the same composition (Figure 1, sample C). These results indicate that the initial delivery of the drug can be considerably reduced by enveloping the composite and that a constant release of cyclazocine can be obtained over a period of more than a month.

A considerable advantage in a delivery system for narcotic antagonists would be obtained if the active composite in the form of very small particles could be hypodermically injected as a suspension into the body tissue instead of implanted in film form. A preliminary experiment (Table II, sample A) performed by injecting into rats a suspension of small particles of cyclazocine-poly (lactic acid) composite in an aqueous solution of carboxymethylcellulose showed

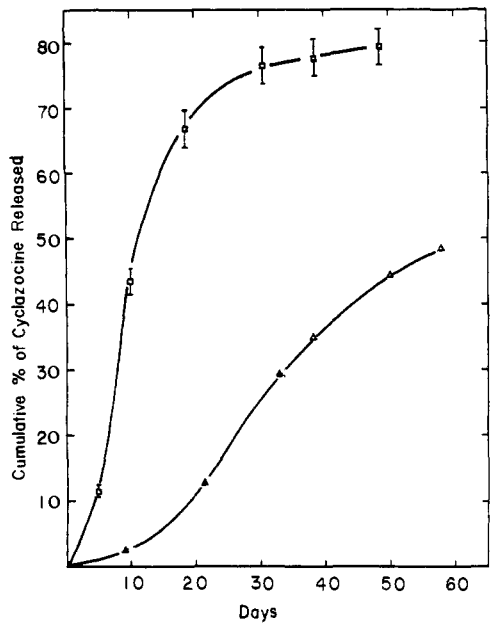

Figure 3. Cumulative amounts of cyclazocine released from composites in film form (sample B). (□) Experiment *in vivo* (implanted). Each point represents the mean observation on three animals ± S.E. (△) Experiment *in vitro*.

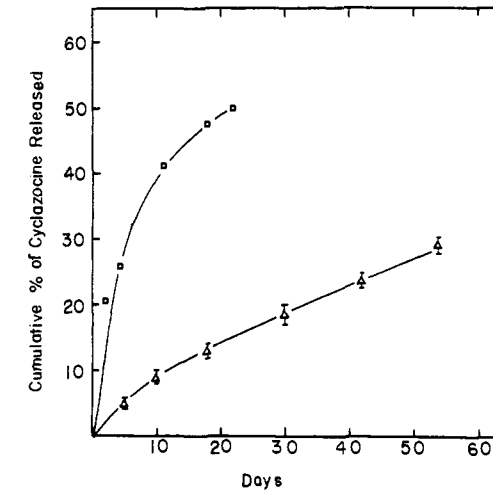

Figure 4. Cumulative amounts of cyclazocine released from composites as small particles (sample F). (□) Experiment *in vitro*. (△) Experiment *in vivo* (injected). Each point represents the mean observation on three animals ± S.E.

the feasibility of this system. A recent experiment supports quantitatively this finding (Figure 1, sample F). Comparison of these results with those reported for sample C (Figure 1) shows that films release cyclazocine at a surprisingly faster rate than particles. However, this is not inconsistent if consideration is given to the fact that after sacrificing the rats, a larger amount of fluid was observed in the compartments around the film than around the particles. Many factors are involved in this inflammatory process. Films may have produced a larger irritation than the particles, possibly causing an increase in temperature surrounding the composite and variation in the chemical composition of the body fluid or even in the mechanism by which the drug is transported. It is known that these variations accelerate the release rate of drugs.[8]

Paralleling the *in vivo* investigation thus far reported, a series of experiments *in vitro* have been conducted. The method under test consisted of extracting samples of radioactive cyclazocine-poly(lactic acid) composites with tepid water (29 ± 3°) and measuring the radioactivity of the extracted aqueous solution.

Composites of cyclazocine-poly(lactic acid) in film form (sample B) and as small chips (sample F) were prepared and the amount of cyclazocine extracted at fixed intervals of time was measured. The preliminary results are reported in Figures 3 and 4 in comparison with those obtained in the corresponding *in vivo* tests (samples B and F). In the experiments with films (Figure 3), we find that the delivery rate *in vivo* is faster than *in vitro*, whereas, due to the excess of water always available in the *in vitro* tests, we would expect the *in vitro* release to be faster than *in vivo*. The results of this experiment give further support to the interpretation given above for the release rates of films (sample C) *vs.* particles (sample F). When particles are used in the *in vitro* experiments (Figure 4), the delivery rate is faster than *in vivo*, as might be expected. It is postulated that the inflammatory process is considerably reduced for small particles.

Poor correlation between *in vivo* and *in vitro* results has been previously observed[9,10] and some of the factors discussed above may be the cause. To further improve the correlation between *in vivo* and *in vitro* results, we are continuing this investigation with other drugs in a poly(lactic acid) matrix.

Experimental Section

All countings were performed with a Packard Tri-Carb Model 3003 liquid scintillation spectrometer in the *in vivo* experiments and with a Beckman LS-100 spectrometer in the *in vitro* experiments. The counting solution consisted of a mixture of 2,5-diphenyloxazole (PPO, 22.0 g), 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene (dimethyl POPOP, 0.4 g), and Triton X-100 (1000 ml) diluted to 4000 ml with toluene in the *in vivo* experiments. Aquasol solution (New England Nuclear Corp., Boston, Mass.) was used in the *in vitro* experiments.

Preparation of Poly(lactic acid). A 1000-ml resin kettle, oven-dried overnight and cooled to room temperature under nitrogen, was charged under nitrogen with 400 g of L(−)-lactide (C. H. Boehringer und Sohn, Ingelheim am Rhein, West Germany). The kettle was then placed in an oil bath at 135° and stirred until the lactide melted. Diethyl zinc (16 ml of 26% heptane solution) was rapidly introduced into the stirred melt. The light yellow solid which formed within 2 min was taken up in dichloromethane (2000 ml) after cooling to room temperature and reprecipitated in a blender by adding hexane (v/v ratio, 5:1.5 hexane–dichloromethane). The white fine powder which formed was then taken up again in dichloromethane (1000 ml), precipitated as above, and dried.

Samples of poly(lactic acid) of 2 mol wt were prepared, *i.e.*, 45,000 and 70,000, the values being calculated from viscosimetric measurements. The results obtained from C and H analyses were within ±0.4% of the theoretical values. The COOH group content, expressed as milligrams of KOH per gram of polymer, varied between 2 and 10. The metal content was less than 0.5%. The physical properties of the samples of poly(lactic acid) used in these tests are listed in Table I.

Preparation of Cyclazocine–Poly(lactic acid) Composites. General Method. A benzene solution of tritiated cyclazocine (New England Nuclear Corp., Boston, Mass.) of specific activity 82.36 × 10^6 dpm/ml (1.8 ml) was evaporated to dryness. To the residue was first added a solution of unlabeled cyclazocine (Sterling Winthrop Co., 1.0 g) and tributyl citrate (0.25 g) in dichloromethane (50 ml) and then poly(lactic acid) (3.75 g). The solvent was flashed off under reduced pressure and the residue, wrapped in aluminum foil, was melt-pressed (Carver Laboratory Press, Mod C) at 165° under total load of one metric ton for 10 sec (shims 0.45 mm thick were used) to produce films of uniform thickness in which no imperfection due to air or gas was observed. The samples of composites used in the tests were obtained as follows.

(a) Samples in Film Form Coded B, C, and D. The film of composite, prepared as above, was cut into pieces having the dimensions listed in Table II.

(b) Sample as Enveloped Film Coded E. The film of the composite, prepared as in the general method with the exception of pressing at 140°, instead of at 165°, was cut into 6.2-cm² pieces. Each piece was enveloped in a film, obtained by pressing a mixture of 95% poly(lactic acid) of 45,000 mol wt and 5% tributyl citrate under the conditions of the standard method. The edges of the obtained envelope were heat-sealed as they were cut in order to have envelopes of the size indicated in Table II after final pressing under the above conditions using shims 0.91-mm thick.

(c) Samples in Particle Form Coded A and F. The film, prepared as in the general method with the exception of pressing at 145° instead of at 165° for sample F (*in vivo* and *in vitro*) and of using shims 0.91 mm thick for sample F and 0.81 mm thick for sample A, was ground in a blender. The particles obtained were screened and the fraction falling within No. 25/35 sieves was collected.

The specific radioactivities of these samples were determined by combustion of the polymer matrices and measurement of the radioactivities in the water trapped in a Tricarb oxidizer system. The characteristics of these samples are listed in Table II.

Experiments *in Vivo*. These experiments were conducted on groups of three or four male Sprague–Dawley rats weighing between 550 and 600 g.

A. Implantation Method. Rats were lightly anesthetized with halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) and a small incision was made through the skin on the dorsal surface of the rats; the composite (samples B, C, D, or E) was inserted subcutaneously and pushed away from the incision area. The incisions were sutured and the animals immediately placed into an individual metabolism cage designed for the collection of urine. The collected urine and cage washings were combined and counted on a daily basis over the first 10 days and then every 4 days for the duration of the experiment. The urine samples were diluted to 100 ml with water. Samples of 1 ml were pipetted into 15 ml of scintillation solution and radioassayed by liquid scintillation counting techniques. Internal standardization was used for the calculation of counting efficiency. The values of the cyclazocine delivered in each interval of time are reported as per cent of maximum dose released calculated on the basis of disintegration per min.

B. Injection Method. The opening at the tip of a 5-ml plastic syringe was enlarged with a 0.081-in. drill. With the tip of the syringe plugged, 210 mg of CM-cellulose-7LF was mixed with the composite (sample A or F) until uniform. Normal saline (0.5 ml) was added and stirred with a small spatula. Portions of normal saline (0.5 ml) were added until the total volume was 3 ml. The time of mixing and stirring was about 1 min. An intimate mixture of particles in nearly clear, viscous CM-cellulose resulted. The plug at the tip of the barrel was replaced with a 12 gauge, thin wall needle. The injection site on the rat was shaved, the area having been selected by measuring the length of the needle from the nape of the neck to a lower section of the back. The animal was lightly anesthetized to avoid excessive movement. The time required for injection was approximately 15 sec. On removing the needle the opening was pressed and supported with the finger, painted with antiseptic, and immediately closed with a clip or adhesive tape. The animals were immediately placed into an individual metabolism cage designed for the collection of urine and feces. The urine samples were collected and radioassayed as above described.

C. Control Studies on Urinary Excretion of Radioactivity. In order to determine the relationship of urinary excretion of radioactivity to actual release of cyclazocine from implanted or injected composites, a control experiment was performed. A solution of 2 mg of [^{3}H]cyclazocine hydrochloride in normal saline was injected subcutaneously into three rats and radioactivity of urinary excretion monitored daily for 3 days. As can be seen in Table III, an average of 60% of the injected radioactivity was collected during the first day and an additional 12% on the next 2 days for a total recovery of 72%. Considering the relatively small intraanimal variation in these results, it would appear that urinary excretion of radioactivity is a useful measure for estimating the subcutaneous release of cyclazocine from polymer composites. These urine samples were lyophilized and reassayed to check for possible presence

Table I. Physical Properties of Poly(lactic acid)

Mol wt	45,000	70,000
Visual melting range, °C	165–170	185–190
Intrinsic viscosity	1.0	1.8
Optical polarization, αD	−137	
Differential thermal analysis endotherm, °C	165	185
No. av mol wt[a]	24,200	
Wt av mol wt[a]	65,900	

[a] Obtained by gel permeation chromatography (Waters Association, Framingham, Mass. 01701).

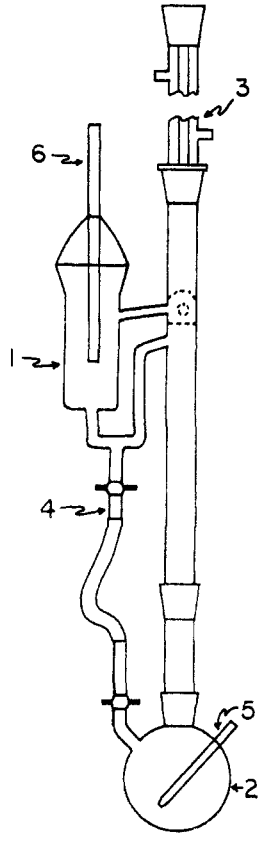

Figure 5. Modified Raab extractor: 1, sample holder; 2, boiler; 3, condenser; 4, drain; 5, bubbler; 6, thermometer.

Table II. Characteristics of the Samples of Cyclazocine Poly(lactic acid) Composites

Sample	Poly(lactic acid), mol wt	Size, cm²	Thickness, mm	Wt, mg	Specific radioactivity, dpm/g
		Experiments *in Vivo*			
A	45,000	*a*	0.70–0.80	40	22.8 × 10⁶
B	70,000	4	0.40–0.42	137–161	24.0 × 10⁶
C	45,000	4	0.37–0.42	119–129	24.59 × 10⁶
D	50% 70,000, 50% 45,000	4	0.37–0.42	99–130	25.65 × 10⁶
E	45,000	6.6	0.48–0.55[b]	450[c]	49.1 × 10⁶
F	45,000	*a*	0.91–0.92	400	18.4 × 10⁶
		Experiments *in Vitro*			
B	70,000	6.4	0.48–0.65	438	24.0 × 10⁶
F	45,000	*a*	0.91–0.92	1040	18.4 × 10⁶

[a] Particles falling within No. 25/35 sieves. [b] After final pressing. [c] Active composite 80 mg, envelope 370 mg.

Table III. Urinary Excretion of Radioactivity from Rats[a] after Subcutaneous Administration of [^{3}H]Cyclazocine

Hours after injection	% of dose excreted (mean ± S.E.)
0–24	59.6 ± 0.3
24–48	9.0 ± 2.2
48–72	3.0 ± 0.8

[a]Three rats each received 2 mg of [^{3}H]cyclazocine hydrochloride dissolved in saline.

of tritiated water. Since no significant radioactivity was lost during lyophilization, it can be concluded that the tritium tag is metabolically stable.

Experiments *in Vitro*. A sample of the composite (sample F) (0.5–1.0 g) was sewn into a cheese-cloth sack and anchored under the water level of the sample holder 1 (Figure 5) of a 300-ml modified Raab extractor. [When the composite was in film form (sample B) the sample was placed as such in holder 1.] The cyclazocine was extracted with tepid (29 ± 3°) water as the solvent. At intervals of 6, 24, and 48 hr and then every 6 days the aqueous solution (av 150 ml) in the boiler (2) was collected and the volume recorded. The collected solutions were replaced at every sampling with distilled water. A sample of aqueous solutions (1 ml) was pipetted into 15 ml of scintillation solution and the radioactivity measured. The values of cyclazocine extracted in each interval of time are reported as per cent of dose initially present in the sample, calculated on the basis of disintegrations per minute.

At the end of the experiment the sample of composite left in the extractor was dissolved in dichloromethane and the radioactivity of the obtained solution measured. The total radioactivity of the extracted aqueous solution plus the radioactivity found in the sample after extraction checked with that present in the sample before extraction.

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Antihypertensive Agents. Synthesis and Biological Properties of 2-Amino-4-aryl-2-imidazolines†

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The synthesis and antihypertensive activity of a series of 2-amino-4-aryl-2-imidazolines are described. Although halogenated aryls and primary 2-amino or 2-methylamino groups are particularly suitable for good antihypertensive activity, an obvious correlation of physical parameters of the aryl substituents or amine groups with antihypertensive effects is not observed. Members of this series are potent adrenergic neuronal blocking agents; they also affect uptake and release of heart norepinephrine and prevent reserpine-induced ptosis. However, none of these biological effects correlate with antihypertensive activity in DOCA rats. One compound, **19**, is particularly effective, after oral or subcutaneous administration, in several hypertensive models.

We have synthesized a series of 2-amino-4-aryl-2-imidazolines (V and VI) which incorporates the structural features of benzyl- and phenethylguanidines. These types of guanidines are believed to affect blood pressure by their actions at peripheral sympathetic nerve terminals.[1-3] This series of imidazolines is also chemically related to 2-(2,6-dichloroanilino)-2-imidazoline (catapres), a potent hypotensive agent, which appears to act mainly by a central mechanism.[4]

Many of the imidazolines (V and VI) are potent antihypertensive agents in hypertensive rat models, and we have attempted to relate some relevant biological actions and physical properties of these agents to their antihypertensive effects.

Chemistry. The 2-amino-4-aryl-2-imidazolines (Table I) were synthesized from β-aminophenethylamines III as shown in Scheme I. Primary amine derivatives V were obtained by cyclizing the β-aminophenethylamines with cyanogen bromide (method 10). The 2-substituted amino compounds VI were obtained by cyclizing the primary β-aminophenethylamines with carbon disulfide, followed by S-methylation with methyl iodide and displacement of methyl mercaptan with various amines (method 13). The hydrazones **37-40** were prepared by condensation of the hydrazine **36** with aldehydes or acetone (method 14).

Infrared spectra of the 2-amino-4-aryl-2-imidazolines are consistent with a delocalized guanidinium system. They exhibit strong NH absorption in the 3100-3400-cm^{-1} region rather than in the ammonium region. Their strong C=N absorption at 1680 and 1595 cm^{-1} is characteristic of the disubstituted guanidinium ion.[5]

The β-aminophenethylamine intermediates III (Table II) were prepared by several routes (A, B, and C) as shown in Scheme I. The unsubstituted diamine **51** and the 4-MeO de-

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