Phosphorus–Nitrogen Compounds VI

Some Phenethylamine Derivatives

By L. A. CATES, W. H. LAWRENCE*, and R. J. MCCLAIN†

Eight diphenyl or diethyl phosphoramidates and one phenyl phosphorodiamidate containing phenethylamine moieties were synthesized for pharmacological testing. The derivatives were screened for their effect on the rabbit eye and isolated intestine, on dog blood pressure, and locomotor and anorexigenic activity in mice. The anorexigenic properties exhibited by some of these compounds may be related to their sedative effects. Three of the compounds inhibited spontaneous duodenal contractions at concentrations of 10 p.p.m. followed by tissue recovery. This antispasmodic effect was not reversed by acetylcholine or barium chloride.

THE EFFECT of *N*-substitution on the pharmacological activities of adrenergic amines has been extensively investigated and much of the work in this area has been directed toward determining the influence of various *N*-alkyl modifications on the cardiovascular and central nervous systems. Some attention has, however, been given to acyl derivatives of phenethylamines, principally in efforts to reduce cardiovascular side effects of the parent compound and to prolong the duration of adrenergic activity. One extensive synthetic program involving amides of dextroamphetamine and phenylpropanolamine resulted in the testing of an orally effective locomotor stimulant without significant cardiovascular effects (1). An attempt in protracting sympathomimetic activity is exemplified by the acetylation of epinephrine (2).

In view of these previous studies, the effect of phosphorylation on the pharmacological properties of phenethylamines was considered worthy of investigation and amphetamine, methamphetamine, ephedrine, phenylpropanolamine, and homoveratrylamine were selected as representative adrenergic agents which readily lend themselves to phosphorylation. Testing of compounds which have been previously reported in this series indicates that conversion of various amines to phosphoramidates reduces the biological activities of the parent compounds in that most of the derivatives possess low toxicities. The majority of *p*-toluidine (3), 2-aminopyridine (4), and guanidine and 2-aminopyrimidine (5) derivatives displayed little or no toxicity when administered to mice for 7 to 11 days at dosage levels of 100 to 500 mg./Kg./day.[1] These derivatives may be excreted unchanged and not undergo the desired *in vivo* enzymatic hydrolysis to liberate the physiologically active amines. To test this supposition, potent central nervous system stimulants were phosphorylated and the resulting phosphoramidates screened for their ability to increase locomotor activity in mice. Acute toxicity studies were made on all but one of the derivatives and most of the compounds were also screened for anorexigenic activity and their effects on the eye and isolated intestine. In addition, five of the derivatives were tested for their acute effects on blood pressure in the anesthetized dog.

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EXPERIMENTAL

Chemical Synthesis

Phenethylphosphoramidates (Table I, Compounds I–VIII).—Compounds I–VI were prepared by refluxing the appropriate amine (2 moles) and diethyl or diphenyl phosphorochloridate (1 mole) in anhydrous benzene for 1–4 hr.

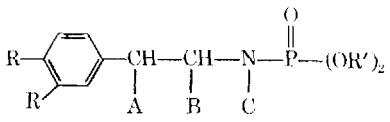
In compounds II–VI the resulting amine hydrochloride was removed by filtration and the filtrate spin-evaporated over a steam bath to yield an oil. The oil was washed with petroleum ether to give a white solid which was washed with dilute hydrochloric acid and water. The product was crystallized from ethanol (compound II) or petroleum ether (compounds III–VI).

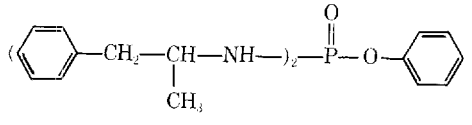
Compound I was isolated from the precipitate formed in the reaction mixture which was washed with water and crystallized from ethanol–water as the monohydrate. The ephedrine required for this synthesis was dried over alumina prior to its use in the reaction.

Reagent chloroform was employed as the reaction solvent for compounds VII and VIII. Following a reflux period of 8 hr. the phenylpropanolamine hydrochloride was removed by filtration and the filtrate was spin-evaporated to yield an oil. The oil was converted to a white solid when crystallized from ethanol–dilute hydrochloric acid. Hemihydrolysis occurred with the more labile aryl ester

[1] Testing results furnished by the Cancer Chemotherapy National Service Center, Bethesda, Md.

TABLE I.—PHENETHYLPHOSPHORAMIDATES



Compd.	R	A	B	C	R′	M.p.° C.[a]	Formula	Anal., % Calcd.	Found
I	H	OH	CH_3	CH_3	Phenyl	134–135	$C_{22}H_{24}NO_4P \cdot H_2O$	C, 63.6 H, 6.3 N, 3.4	63.8 6.3 3.4
II	H	H	CH_3	H	Phenyl	101–102	$C_{21}H_{22}NO_3P$	C, 68.6 H, 6.0 N, 3.8	68.3 5.9 3.7
III	H	H	CH_3	H	Ethyl	57–58	$C_{13}H_{22}NO_3P$	C, 57.5 H, 8.2 N, 5.2	57.0 8.6 4.9
IV	H	H	CH_3	CH_3	Phenyl	63–65	$C_{22}H_{24}NO_3P$	C, 69.3 H, 6.3 N, 3.7	69.4 6.4 3.5
V	CH_3O	H	H	H	Ethyl	63–64	$C_{14}H_{24}NO_5P$	C, 53.0 H, 7.6 N, 4.4	52.9 7.9 4.4
VI	CH_3O	H	H	H	Phenyl	88–89	$C_{22}H_{24}NO_5P$	C, 63.9 H, 5.8 N, 3.4	63.6 5.9 3.4
VII	H	OH	CH_3	H	Ethyl	81–83	$C_{13}H_{22}NO_4P$	C, 54.3 H, 7.7 N, 4.9	54.4 7.4 4.8
VIII	H	OH	CH_3	H	Phenyl hydroxyl	243–245 dec.	$C_{15}H_{18}NO_4P$	C, 58.6 H, 5.9 N, 4.6	58.1 6.1 4.6
IX						76–77	$C_{24}H_{29}N_2O_2P$	C, 70.6 H, 7.2 N, 6.9	70.3 7.2 6.7

[a] All melting points are uncorrected.

product (compound VIII) during this purification step as shown by its solubility in sodium hydroxide solution, high melting point, infrared spectra, and microanalysis. The phenylpropanolamine required in this synthesis was prepared by treatment of the hydrochloride salt with an equal molar amount of sodium hydroxide.

Phenethylphosphorodiamidate (Table I, Compound IX).—Dextroamphetamine (0.1 mole) and phenyl phosphorodichloridate (0.025 mole) were refluxed 12 hr. in anhydrous benzene. The reaction mixture was filtered and the filtrate spin-evaporated over a steam bath to yield a viscous oil. The oil was dissolved in ether and dried over anhydrous sodium sulfate. Spin-evaporation of the ethereal solution gave an oil which developed crystals after 3 days. Washing of the crystals with petroleum ether yielded phenyl-*N*,*N*′-bis-(1-methyl-2-phenylethyl)-phosphorodiamidate as a white, crystalline solid.

Infrared Spectra

All starting materials and products were examined by means of a Beckman IR-8 spectrophotometer using a Nujol mull. The new derivatives showed the following characteristic absorptions, γ in cm.$^{-1}$: 1238–1250 (P=O in compounds I–VIII), 1220 (P=O in compound IX), 1140–1160 (POEt in compounds III, V, and VII), and 1070–1100 and 1200 (POCaryl in compounds I, II, IV, VI, VIII, and IX).

Derivatives of primary amines showed the N–H stretching vibration of the intermolecularly hydrogen-bonded form at 3210–3260 cm.$^{-1}$ (6), while compounds I and IV gave no bands in this region. Compound VIII showed a weak absorption at 2550 cm.$^{-1}$ which can be assigned to P–OH (7).

Pharmacological Screening

The compounds were subjected to screening tests for anorexigenic properties; central nervous system effects, as indicated by spontaneous locomotor activity; toxicity; stimulation or inhibition of isolated rabbit intestine; and pupillary dilation or constriction, topical anesthesia, and irritating effects in the rabbit's eye. Five of the nine compounds were also tested for their acute effect on blood pressure in anesthetized dogs, when administered intravenously.

Anorexigenic Screening Test.—Food was withheld from mice for 20–24 hr. prior to the test but water was

provided *ad libitum*. Each test compound was administered in three dose levels, 4, 8, and 16 mg./Kg., and each dose level was given to a group of three mice of approximately equal weight. Appropriate dilutions were prepared so a consistent volume of solution was injected into each mouse (0.01 ml./Gm.).

Each mouse was weighed and injected intraperitoneally (i.p.) with the appropriate solution and placed back in its individual cage for 30 min., after which it was permitted free access to a food pellet for exactly 10 min. The food pellet was accurately weighed to one-tenth of a milligram (0.1 mg.) using a model H-6 Mettler balance, before and after the feeding period. Control mice were employed which received a similar volume of saline and others which received 0.5, 1, and 2 mg./Kg. of dextroamphetamine.

CNS Activity. Spontaneous Locomotor Activity, 24 hr.—Each mouse was injected i.p. with 0.01 ml./Gm. of 1% propylene glycol in saline and placed in an actophotometer (Metro Scientific) for 24 hr. The instrument was located in a windowless room free of external noise and a dim light was maintained during the test period. Each breaking of the beams of infrared light by the mouse was recorded by means of a digital counter. The mouse had free access to food and water during this time. The count obtained was used as the control for this particular mouse and each animal was used at 7-day intervals. The mouse received 5 mg./Kg. of the test compound i.p. and the activity was determined for 24 hr. under the same environmental conditions.

CNS Activity. Spontaneous Locomotor Activity, 5-min. Test.—The method used was essentially that of Dews (8) as modified by Moffett and Seay (9). Five mice of approximately equal weight comprised an experimental group. Each mouse was weighed, administered 4 mg./Kg. i.p. of the compound, and returned to his cage for 30 min. The animal was then placed in an actophotometer for exactly 5 min. Solutions were prepared so each mouse received 0.01 ml./Gm.

Irritancy, Local Anesthesia, and Pupillary Reactions in the Rabbit Eye.—These three tests were run simultaneously. All compounds tested were 1% solutions (w/v) in propylene glycol. Healthy rabbits were selected and, after their careful examination, 2 drops of the test solution were placed in one eye, and 2 drops of propylene glycol in the other eye as a control. The animals were observed at 10-min. intervals during the next hour for evidence of corneal anesthesia, pupillary dilation, or constriction and irritation.

Isolated Rabbit Intestine.—A piece of rabbit small intestine, about 2 to 3 cm. long, was suspended in aerated Tyrode's solution in a constant-temperature bath. The tissue was anchored at the bottom, and the top was attached *via* a lever system to a Grass force-displacement transducer. Contractions of the isolated intestine were recorded with a Grass model 7, ink-writing polygraph. Quantities of compounds administered are all expressed in terms of final concentrations of the derivative in Tyrode's solution.

Toxicity Studies.—Acute toxicity tests were conducted according to the method of Thompson and Weil (10, 11) for estimating the LD_{50}. Four mice were used for each dose level, and four dose levels were employed for each compound. The dose levels selected represent a geometric progression in that the logarithm of adjacent doses differed by a factor of 0.176. Solutions of the compounds were prepared to give the desired quantity when 0.01 ml./Gm. was injected. The mice were weighed, injected i.p., and observed for 1–4 hr. Following this they were returned to the animal room where another check was made at the end of 24 hr.

Our definition of "acute toxicity" includes any deaths which occur within 24 hr. after administration of the compound. Since few compounds exhibited significant toxicity within the 24-hr. period, observation of these animals was continued for an additional 6 days. No further administration was given. Any deaths which occurred during these additional 6 days is reported as subacute toxicity (7-day toxicity).

Blood Pressure Effects in Dogs.—Mongrel dogs were anesthetized with sodium pentobarbital, 30 mg./Kg., intravenously, and supplemented as needed to maintain the desired level of anesthesia. The carotid artery was cannulated and attached to a Statham pressure transducer, the femoral vein was cannulated for intravenous injections, and the trachea was cannulated to permit free air flow. Arterial pressure responses were recorded using a Grass model 7 polygraph.

RESULTS AND DISCUSSION

Anorexigenic Activity.—Since some of these compounds are derivatives of potent anorexigenic drugs, amphetamine and methamphetamine, it was deemed advisable to see if these agents would exhibit any effect on food consumption of mice. As noted (Table II), most of these compounds produced some decrease in food consumption during the feeding period. Food consumption was determined for each mouse during its 10-min. feeding period; this was converted to grams of food consumed per kilogram of body weight of the mouse for uniformity in comparing one animal, or group of animals, with another. Saline-treated animals served as controls in determining relative food consumption for the various treatments. Each value presented in Table II represents the mean response of three animals.

The inconsistency of these data is probably a reflection of the variability of response as seen in the small size of animal groups (three mice). The decreased food consumption by many of these animals was apparently due to varying degrees of hypoactivity and sedation of the animals which was noted

Table II.—Anorexigenic Activity

Compd.	Food Consumption, as % of Control (Control = 100%) 4 mg./Kg.	8 mg./Kg.	16 mg./Kg.
I	107.4	40.1	102.3
II	22.5	16.5	36.9
III	12.2	14.2	21.0
IV	3.2	3.8	3.3
V	63.7	95.4	78.0
VI	23.3	29.0	48.9
VII	104.4	120.1	24.7
VIII	64.6	69.2	77.1
IX	63.7	15.7	20.2
Dextroamphetamine[a]	51.9	54.0	17.9

[a] Dose levels of dextroamphetamine were 0.5, 1, and 2 mg./Kg., respectively.

TABLE III.—SPONTANEOUS LOCOMOTOR ACTIVITY

Compd.	5 min. Test[a] Drug, 4 mg./Kg.	Control (Saline)	% of Control[c]	t Values	24 hr. Test[b] Drug, 5 mg./Kg.	Control	% of Control[d]
I	75.6	142.2	53.2	3.437[e]	4908	9101	53.9
II	99.4	105.2	94.5	0.132	8507	17,382	48.9
III	41.8	105.2	39.7	2.065[f]	15,824	18,867	83.9
IV	112.4	105.2	106.8	0.226	8286	10,852	76.4
V	5.8	142.2	4.1	10.10[e]	12,866	10,513	122.4
VI	53.0	105.2	50.4	1.259	3963	6503	60.9
VII	106.0	105.2	100.8	0.040	16,877	18,867	89.5
VIII	110.0	105.2	104.6	0.141	...	...	...
IX	101.6	105.2	96.1	0.118	15,522	15,817	98.1

[a] Mean value of five animals. [b] Value for one animal. [c] Drug counts/control counts ×100. [d] Drug counts/control of same animal (1% propylene glycol in saline) ×100. [e] Significant at 5% level. [f] Significant at 10% level.

TABLE IV.—EFFECT ON ISOLATED RABBIT INTESTINE

Compd.	Concn.	Effect	Response to Acetylcholine (1:100,000)	Response to Barium Chloride (1:10,000)	Response to Adrenaline (1:1,000,000)
I	1:5000	Inhibition	NSR[a]	NSR	...
II	1:100,000	Inhibition	NSR	NSR	...
IV	1:100,000	Inhibition	NSR	NSR	...
V	1:10,000	Stimulation	...	...	Inhibition
VI	1:25,000	Inhibition	NSR	NSR	...
VII	1:10,000	NSR	Stimulation[b]	...	...
VIII	1:10,000	NSR	Stimulation[b]	...	...
IX	1:100,000	Inhibition	NSR	NSR	...

[a] No significant response. [b] Acetylcholine concentration = 1:1,000,000.

during the period of the experiment. In a few instances this was sufficient to cause the animal to lose its righting reflex. It was thought that the 5-min. locomotor activity tests would clarify this aspect, but the results of the actophotometer tests do not correlate well with the decreased food consumption.

Spontaneous Locomotor Activity.—Initially it was thought these compounds would probably increase locomotor activity, with a slow onset and long duration of effect. Consequently, spontaneous motor activity determinations were made over a 24-hr. observation period (Table III). As testing proceeded, it became obvious that at least some of the compounds exerted their action rather promptly; therefore, spontaneous motor activity was again determined 30 min. after intraperitoneal injection. This time the observation period was reduced to 5 min., thus minimizing acclimatization of the mouse to the environment of the actophotometer.

Since the 24-hr. test was discontinued in favor of a 5-min. test, the results presented represent the response of a mouse after receiving the designated compound and comparing this response to that obtained from the same mouse after receiving 0.01 ml./Gm. of 1% propylene glycol in saline.

The 5-min. locomotor activity test utilized groups of five animals for each compound and for the saline controls. The mean for each five-animal group was used to compare the counts for the experimental animals with the controls and to compute the per cent of control values. A comparison between the means of each series of five animals and its control was made by calculating Student t values. Two compounds (I and V) showed a decrease in locomotor activity which was significant at the 5% level and compound III gave a t value which would be significant at the 10% level. None of the compounds produced any statistically significant increase in such activity, which was somewhat unexpected, particularly for the amphetamine and methamphetamine derivatives.

Fig. 1.—Typical response of isolated small intestine to compounds I, II, IV, VI, and IX. Key: A, compound (in this case, VI, 1:25,000); B, acetylcholine br, 1:1,000,000; C, acetylcholine br, 1:250,000; D, acetylcholine br, 1:100,000.

Topical Anesthesia, Pupillary Reaction, and Irritation in the Rabbit's Eye.—No local anesthesia, pupillary dilation, or constriction was noted during the period of observation (1 hr.). The solvent used (propylene glycol) irritated the eyes, as indicated by redness of the cornea. The solutions of the compounds did not appear to produce any greater irritation than the solvent, although this was difficult to assess.

Isolated Rabbit Intestine.—Three of the eight compounds tested produced a marked decrease or complete inhibition of spontaneous activity and tonus of the isolated small intestine at a concentration of 1:100,000 (compounds II, IV, and IX). While a 1:1,000,000 concentration of acetylcholine was sufficient to produce spasm of the normal intestinal strip, following administration of each of these three compounds, concentrations of acetylcholine as great as 1:100,000 failed to produce any response (Table IV). A typical response of this type is shown

TABLE V.—ACUTE AND SUBACUTE TOXICITIES[a]

Compd.	Dose,[b] mg./Kg.	24 hr. 100	150	225	337	7 Day 100	150	225	337
I		0	2	3	2	2	2	3	2
II		0	0	0	0	1	2	1	2
IV		0	0	0	0	0	2	3	0
V		0	1	0	0	0	2	4	3
VI[c]		0	0	0	0	2	3	4	4
VII		1	0	0	0	1	0	0	0
VIII[d]		0	0	2	3	0	0	3	4
IX		1	0	0	0	1	0	0	0
Propylene glycol, 0.01 ml./Gm.		0	0	0	0	0	0	0	0

[a] Number of deaths out of four animals. [b] mg./Kg. in propylene glycol given i.p. [c] Dose levels = 66.7, 100, 150, and 225 mg./Kg. [d] In propylene glycol/NaOH. Test of this solvent system without compound VI resulted in three deaths in 24 hr. and four deaths in 7 days.

in Fig. 1. In addition, barium chloride in concentrations up to 1:10,000 failed to contract the isolated intestine following these drugs. Thus, these compounds relaxed the intestinal strip and prevented it from responding to the neurogenic stimulation of acetylcholine and the myogenic stimulation of barium chloride.

Two other compounds (I and VI) produced a similar response but a higher concentration of the compound was required for this effect (1:25,000 for VI; 1:5000 for I).

One compound (V) produced stimulation of the isolated gut at a concentration of 1:10,000. This stimulation was counteracted by 1:1,000,000 adrenaline, and prevented by prior addition of 1:1,000,000 atropine.

The other two compounds (VII and VIII) did not produce any significant response upon the intestine in concentrations up to 1:10,000.

Three diethyl phosphoramidates were synthesized in order to give an indication of what different effect, if any, an alkyl ester produces compared to an aryl ester. One obvious difference in properties is the much greater water solubility exhibited by the ethyl esters. The pharmacological activity most affected by this difference in solubility appears to be that on the isolated gut. It is noted that the four compounds (II, IV, VI, and IX) most active as antispasmodics were insoluble in water, while only one water-soluble compound (I) exhibited this activity, and quantitatively it was much weaker than the others, and another water-soluble compound (V) produced intestinal stimulation. It is also interesting to note that compound VI, which produced inhibition of the intestine is the diphenyl phosphate derivative of homoveratrylamine, while compound V, which produced stimulation of the intestine, is the diethyl phosphate derivative of homoveratrylamine.

Toxicity Studies.—These tests indicate that most of the compounds are much less toxic when considered in terms of acute toxicity (24 hr. or less) than when considered in terms of subacute toxicity (7 days or less). Thus, the data suggest that most of the compounds exert their toxic effect indirectly by adversely affecting a vital organ or function (*e.g.*, possibly renal or hepatic function) or else they are retained in the body and slowly converted into some more toxic substance. No attempt was made to determine the cause of death for the mice that succumbed to these compounds. This pattern of delayed death is readily illustrated by compounds V and VI (Table V). Only one of the 16 mice which received V died within 24 hr., however, by the end of the 7th day, nine of the 16 mice were dead; compound VI produced no deaths during the first 24 hr., however, by the end of the 7th day, 13 of the 16 mice which had received the drug were dead.

Fig. 2.—Effect on arterial blood pressure of anesthetized dog from slow intravenous injection of 1.6 mg./Kg. of compound I.

Some of the other compounds did not exhibit significant toxicity at the doses utilized. Compounds VII and IX produced only one fatality each during the 7-day period, and the rapidity of death in both cases (less than 4 hr.), and the fact that both deaths were from the lowest dose, would lead one to suspect something, other than drug toxicity, as a possible cause of these deaths. Two groups of four mice each were administered an equivalent volume of propylene glycol; none of these eight mice died within the 7-day observation period.

Compound VIII presents a different problem. It is not soluble in water or propylene glycol but does form the water-soluble sodium salt. Sodium hydroxide was used to solubilize the compound; the result was seven deaths out of the 16 injected. The same quantity of sodium hydroxide as used to solubilize VIII was added to propylene glycol and an equivalent volume of this sodium hydroxide-propylene glycol solution was injected into four mice; three of these died within 24 hr. and the other died before the 7th day. This would represent the sodium hydroxide concentration in the strongest solution (337 mg./Kg.), but since the solutions for the lower doses were prepared by diluting some of this solution with propylene glycol, the lower dose levels contained correspondingly less caustic.

Blood Pressure in Anesthetized Dogs.—Five of

the nine compounds were administered intravenously to anesthetized dogs to determine their effect on systemic arterial pressure. Compounds I, III, IV, V, and VII were administered in various dosage schedules ranging from 0.5 to 10.0 mg./Kg. Only compound I produced a marked change in blood pressure. Figure 2 shows the alteration in carotid arterial pressure produced by 1.6 mg./Kg. of compound I. It will be noted that there is an increase in both systolic and diastolic pressure, however, systolic pressure increased more than diastolic, thus increasing the pulse pressure too.

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A Potent α-Receptor Blocking Agent, SU-14542

By T. F. BURKS* and J. P. LONG

SU-14542 was studied in isolated cat and rabbit hearts and in *in situ* dog hearts for possible β-adrenergic receptor stimulating activity. It was determined that this compound has no demonstrable action on β receptors. Investigation of its effects on isolated mesenteric arteries demonstrated powerful α-adrenergic receptor blockade; SU-14542 was found to be 2–7 times more potent than phentolamine as an α-receptor blocking agent. The ID_{50} for SU-14542 in blocking epinephrine was determined to be $6.6 \times 10^{-9} M$.

IT HAS BEEN reported by Barrett *et al.* (1) that SU-14542, which is 3′-methoxy-4′ [(4-phenyl-1-piperazinyl)-butoxy]-acetophenone monohydrochloride, decreases arterial blood pressure in both anesthetized normotensive dogs and unanesthetized renal hypertensive dogs. Decreases in pressor responses produced by epinephrine, norepinephrine, and amphetamine were observed following oral administration of 0.20 and 1.80 mg./Kg. of SU-14542. These workers reported that the experimental compound does not possess ganglionic blocking activity but, since tachycardia was observed following administration of SU-14542, that it does possess β-adrenergic receptor stimulating activity.

Povalski *et al.* (2) reported that while 5.0 mg./Kg. SU-14542 given orally to anesthetized dogs produced a decrease in mean arterial blood pressure, cardiac output was not significantly altered. Rutledge *et al.* (3) found that SU-14542 increased femoral arterial blood flow in dogs but did not significantly increase renal blood flow.

In this communication evidence will be presented demonstrating that SU-14542 is a potent α-adrenergic receptor blocking agent that has no β-adrenergic receptor stimulating properties on dog, cat, or rabbit hearts.

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EXPERIMENTAL

Cat and Rabbit Hearts.—Dutch rabbits of either sex, weighing from 1.5–2 Kg. were sacrificed by cervical dislocation, and the beating hearts were removed and flushed through the aorta with a heparin–saline solution. An aortic cannula was tied into place and the hearts perfused in the usual Langendorf preparation (4) with Locke Ringer solution for isolated hearts warmed to 35–37° and aerated by bubbling 95% O_2–5% CO_2. Aortic pressure was maintained at 40–50 mm. Hg to ensure adequate coronary perfusion. Drugs were injected in 0.5–1 ml. vol. into the aortic cannula. Force of contraction was measured from a Grass Instrument Co. force - displacement transducer (FTO3C) and recorded on a Gilson (GME) polygraph. Heart rates were obtained by direct observation of recorder pen movement.

Cat hearts were prepared in a similar manner from cats of either sex weighing 1.5–3 Kg., anesthetized by intrathoracic administration of 30 mg./Kg. sodium pentobarbital.

Dog Hearts.—Mongrel dogs of either sex, weighing 10–12 Kg., were anesthesized by 15 mg./Kg. sodium thiopental and 250 mg./Kg. sodium barbital administered intravenously. Systemic blood pressure was measured from a carotid artery cannula by a Statham pressure transducer (P23AA) and recorded by an Offner type RS Dynograph. Drugs, in volumes of 0.1–1 ml., were injected through a cannula placed in a femoral vein. Both vagus nerves were sectioned in the cervical region and each animal was administered 20 mg./Kg. hexamethon-