CERTAIN ASPECTS OF THE PHARMACOLOGY OF SEROTONIN

by

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(Chairman, Graduate Council)

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INTRODUCTION

The recent identification of the "hormonal" substance serotonin (1) as 5-hydroxy, 3-(β ethylamine) indole provides opportunities to explain cause and effect of many types of cardiovascular disease. Moreover, because of its peculiar pharmacological properties it may prove to be a valuable therapeutic agent.

One may theorize that serotonin plays an important role in establishing and maintaining a myocardial infarct. It is known that serotonin is released from disintegrating platelets accompanying the clotting process; thus following thrombus formation in a coronary vessel the serotonin released causes vasoconstriction in the area surrounding the clot. The ischemia resulting from the vasoconstriction adds to that of the mechanical block of the thrombus and increases the area of the infarct.

If this theory be accepted, then an explanation for the effectiveness of heparin in the therapy of angina pectoris is also provided. Serotonin's coronary vasoconstriction causes the pain; serotonin can be released only when the platelets are clumped; and heparin prevents platelet clumping. The effectiveness of the nitrites in the therapy of angina also is consistent with the serotonin hypothesis as the nitrites can relax blood vessels constricted by serotonin.

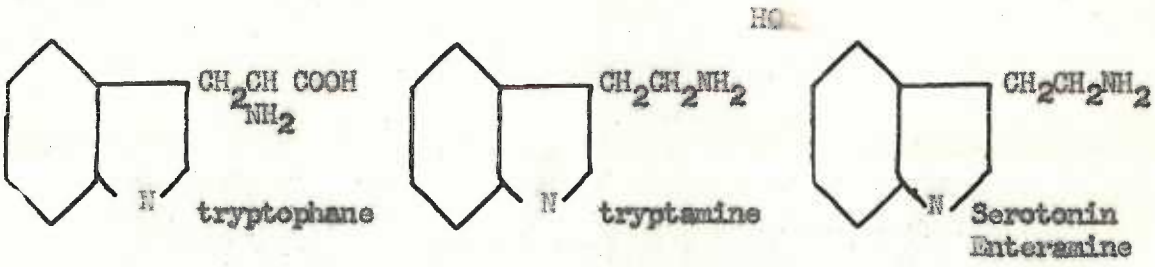
For many years an explanation for the cause of essential hypertension has been sought. With the identification of each new pressor substance found in blood, an attempt is made to explain the cause of hypertension in terms of the substance's action. Epinephrine (sympathin I)

( ) l-nor-epinephrine (sympathin E), renin-angiotonin, and VDM (vasodepressor mechanism) - VEM (vasoexcitor mechanism) all have been indicted. Serotonin may be added to this list. Taylor, Page, and Corcoran (2) stimulated the central end of the cut vagus and found that blood from the jugular circulation contained a substance which provoked hypertension in a recipient animal. The properties of this pressor substance are similar or identical to those of serotonin. This explanation, involving serotonin as the cause of essential hypertension, is attractive because of the clinically recognised overactivity of the central nervous system and the fact that the hypnotic drug, phenobarbital, decreases hypertension. As additional evidence, it is known that during sleep the blood pressure is normal in the early hypertensive patient.

One can predict that attempts will be made to fit serotonin into the scheme of explanations involving peripheral vasoconstriction, Raynaud's disease and Buerger's disease. Serotonin's presence in platelets will also focus attention on such blood disorders as thrombocytopenia purpura.

Serotonin is also to be found in the nonmammalian world. Under the name "enteramine" the same chemical substance has been isolated from the posterior salivary gland of Octopus vulgaris and in the skin of amphibian Discoglossus pictus (3).

If attention is directed to the structural formula of serotonin it can be seen that it may be formed by decarboxylization and oxidation of the amino acid tryptophane. The biochemical role of this observation has not been investigated.



Serotonin may well prove to be a useful therapeutic agent. Since it is a vasoconstrictor agent twice as potent as epinephrine it may well be used as a styptic in oozing wounds, as a nasal vasoconstrictor to shrink congested turbinates and to localize injected local anesthetics such as procaine.

It is obvious that before serotonin's role in the explanation of cardiovascular conditions or before it may be used in therapeutics, the pharmacology of serotonin must be thoroughly studied. As serotonin was not identified and synthesized until December of 1951 (4) (5), very little of the Pharmacology so far has been elucidated. IN THIS THESIS THE STUDY OF THE PHARMACOLOGY OF SEROTONIN HAS BEEN LIMITED TO AN INVESTIGATION OF ITS EFFECT ON THE AURICLES AND VENTRICLES OF THE HEART, AND IN PARTICULAR, ON CARDIAC RHYTHM.

be detected in blood from peripheral circulation. O'Connor (16), Stevens and Lee (12), and Brodie (13) all observed the relationship between coagulation and vasoconstriction. Vasoconstrictor action does not appear in blood samples or extracts until coagulation takes place. Confirmation of this relationship was noted by Trendelenburg (21), Schultz (22), Stewart and Harvey (23) and Janeway and Park (24). Further proof that the substance is not epinephrine is that supplied by Battelli (10) and Stewart and Harvey (23) who retarded (blocked) the vasoconstriction action of epinephrine by previous administration of apocodeine but found the serum effective to cause vasoconstriction. Evidence that the vasoconstrictor material was a product from formed elements in the blood was produced by Stewart and Zucker (25) who compared serum to plasma and with other body fluids. Their conclusion was that the vasoconstrictor property is developed during the process of coagulation and was not connected only with the change of fibrinogen to fibrin. Stewart and Zucker (25) found that extracts of platelets obtained from citrated blood, the plasma of which is totally inactive, exert a strong constricting effect on arterial rings. O'Connor (16) LeSourd and Pagniez (26), Janeway, Richardson, and Park (27), Reid and Bick (18) and Freund (20) present evidence to confirm this observation.

ISOLATION OF SEROTONIN

The isolation and purification of Serotonin was undertaken with bovine serum as the source (28). Fresh blood is placed in a cold room until the serum is freed from the retracted clot. The serum is then filtered through a gauze and wire screen. The collected serum is centrifuged in the cold and the supernatant layer acidified to a pH of 4.5 - 5.0 with 2 NHCl. Proteins of the serum are precipitated by 95% ethyl alcohol using a ratio of one part alcohol to 3 of serum. The mixture is stirred, cooled overnight, and the protein precipitate discarded. The hydro-alcoholic solution is concentrated by vacuum distillation under nitrogen at a temperature below 40° C. Phosphatides and other acetone insoluble substances are removed by adding an excess of acetone. Chilling in the cold room, 5° C., allows the separation of a clear light amber supernatant phase which is decanted off and further concentrated by vacuum distillation under nitrogen, below 40° C. There now remains a heavy, amber colored oil floating on a yellow emulsion which is transferred to a separatory funnel adding an equal volume of chloroform. Careful extraction with chloroform at 5° C. removes many of the impurities, leaving most of the desired vasoconstrictor substance in the aqueous phase. The aqueous extract is then adjusted to a pH 5.9 ± 0.3 using 5 N NaOH. The solution is saturated with ammonium sulfate (about 0.6 gm. per ml.) and the active principle is extracted three times with equal volumes of n-butanol. The butanol extracts are chilled to - 10° C. over night, then decanted from residual water and salt. The

active principle in butanol is now precipitated as a complex with a saturated solution of dilituric acid (5-nitrobarbituric acid) in absolute methanol.

Complete precipitation of serotonin is assured by addition of small amounts of the dilituric acid solution to the supernatant liquid, repeating this until a precipitate fails to form. The precipitate is separated by centrifugation and washed free of dilituric acid and butanol with small volumes of cold methanol. The final precipitate is dried in a vacuum desiccator and once dry is stable indefinitely. The dilituric acid-vasoconstrictor complex may be "broken" by precipitation and removal of the dilituric acid as the insoluble magnesium salt with $MgCl_2$ or $MgSO_4$ leaving pure serotonin.

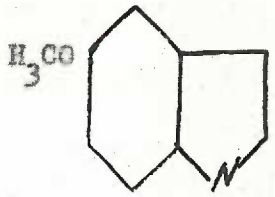
PHARMACOLOGICAL ASSAY

Until the time that serotonin could be identified and synthesised, a bioassay procedure was necessary during the process of its isolation from serum. The bioassay method usually employed is a modification by Page and Green (29) of the method described by Pissensky (30) as follows: using an unanesthetized rabbit, the base of an ear is clamped with a Wolfson intestinal clamp. The ear is severed distal to the clamp using a sharp scalpel. The skin is slit on either side of the central artery for a distance of about 2 cm. and the skin flap pulled back and cut off. The artery is dissected free and held steady with a small hemostat close to the severed end. A small V incision is made into the artery near the hemostat and a small glass cannula is inserted and tied. Warm Ringer's solution is used to wash out the remaining blood. The ear is then placed on an inclined draining plate and secured with adhesive tape. The cannula is connected with the perfusing solution which is allowed to flow at a constant rate. The initial perfusion pressure must be 40-50 mm. of mercury, but after a few minutes 10 mm. is satisfactory for drop rates of about 20 per minute. Injections of test substances are made into the perfusate flask or into the tubing connected to the cannula. The perfusate is maintained at 37° C. and the ear is warmed with electric lights or by an incubator.

CHEMICAL IDENTIFICATION

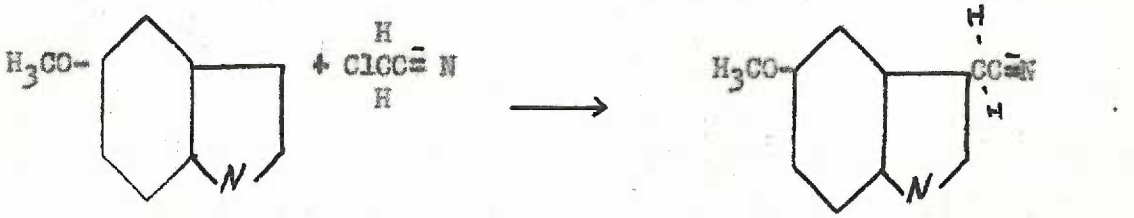
The identification of serotonin was facilitated by the observation that it formed a complex with 5 - nitrobarbituric acid, a common precipitating agent for nitrogenous substances.

Elementary analysis of serotonin on two different batches indicate: Carbon 41.4%, Hydrogen 6.0%, Nitrogen 17.0%, Sulfur 8.0%, Methylamine 3.0%. This corresponds to an approximate empiric formula of $C_{14} H_{23} O_7 N_5 S$ with a molecular weight of about 405. Ionic sulfate determination indicates 21 - 23% of sulfate. This observation suggests serotonin to be the sulfate salt of an organic base. Low solubility in water (8mg. per ml) substantiates this. Color reactions such as Hopkins - Cole, Ehrlich, Folin, and pine splinter give positive tests. Although serotonin reduces ammoniacal silver nitrate, the reaction is unlike that of typical aldehydes. It is precipitated by 70% mercuric sulfate in 2.5 N sulfuric acid. The compound reacts with iodine in an aqueous solution, but does not give the typical bromine-tryptophane reaction in water, though it does in methanol. Special color reactions of purified material to give an indication of its substituents are on table 1

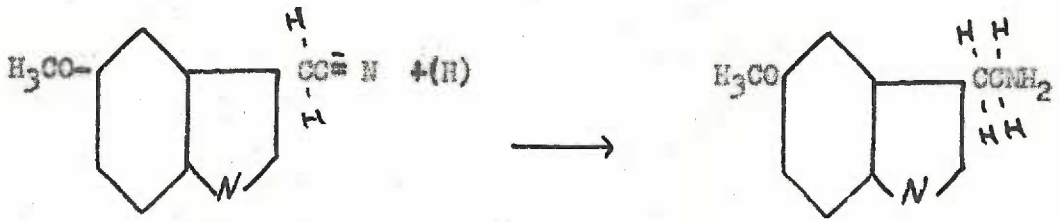


(5-methoxyindole)

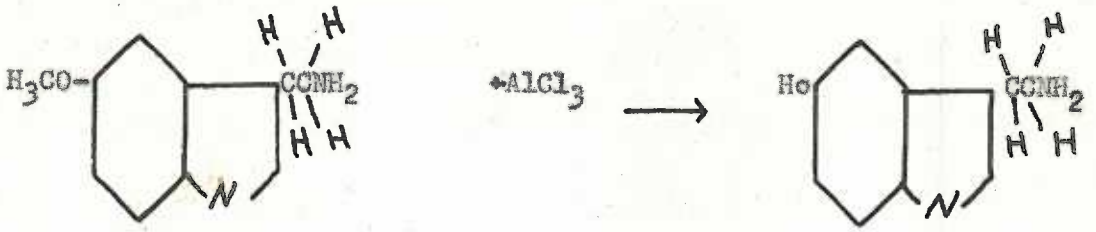
With this compound, the side chain is added by chloro-acetonitrile addition to form 5-methoxy, 3-acetonitrile indole. This compound is reduced by nascent hydrogen formed from metalic sodium and ethyl alcohol to form 5-methoxytryptamine which is demethylated with aluminum chloride to form "Serotonin".



(5-methoxyindole) (chloro-acetonitrile) (5-methoxy, 3-acetonitrile indole)



(5-methoxy, 3-acetonitrile indole) (nascent Hydrogen) 5-methoxy trypta-mine



(5-methoxy tryptamine) (Aluminum chloride) (SEROTONIN)

PHARMACOLOGY OF SEROTONIN: PRESENT STATUS

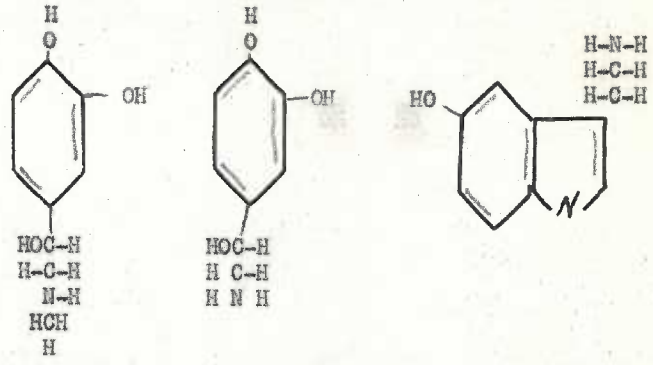
With the identification of serotonin as a tryptamine derivative, its pharmacologic properties can be predicted with reasonable certainty to fall into the class of sympathomimetic amines. It seems logical, therefore, to compare its properties with the most extensively studied pressor amines, l-epinephrine and l-nor-epinephrine (sympathins I and E). An outlined summary comparison of their properties is presented in table 2.

The primary pharmacologic action of serotonin is to stimulate (contract) smooth muscle; all blood vessels are therefore constricted. The vessels of the skin, (35) (24) (36) (11) mucosa (17), muscles (6), pulmonary vessels (6) and vessels of abdominal organs and mesentery (37) (23) (7) (21) have been studied. This action of serotonin differs from that of epinephrine which when given intravenously actively or passively dilates vessels through the action on the heart. Nor-epinephrine and serotonin both appear to cause only vasoconstriction. On the lung, specifically the bronchiolar smooth muscle and the trachea, epinephrine and nor-epinephrine act to dilate the lumen and constrict the musculature, while serotonin appears to evoke constriction of the lumen. On the coronary vessels, epinephrine and nor-epinephrine produce either active or passive dilation while serotonin constricts the coronary vessels as it does all other vessels. The smooth musculature of the urinary bladder, uterus, and gastrointestinal tract show striking differences to the three pressor amines. In response to epinephrine and nor-epinephrine, under most circumstances these

TABLE 2

A comparison of some common pressor amines (104).

l-epinephrine l-nor-epinephrine serotonin



β(3,4,dihydroxy phenyl)β hydroxy methyl-ethylamine

β(3,4 dihydroxy-phenyl)β hydroxy-ethylamine

5-hydroxy, 3(β ethylamine) indole

Source	Chromaffin tissue	Sympathetic nerve endings	platelets
Tissue	Action and comparison to l-epinephrine l-epinephrine = 1.0		
1. Arterial muscle	dilator	constrictor	constrictor
2. Coronary vessels	dilator 1.0	dilator 0.06 - 0.14	constrictor
3. Bronchiolar muscle	dilator 1.0	dilator 1.0	constrictor
4. Urinary Bladder	retention of urine	retention of urine	expulsion of
5. Uterus	relaxation 1.0	relaxation 0.1-0.4	constrictor
6. Intestine	relaxation 1.0	relaxation 1.0	constrictor
7. Sphincter pupuli	mydriasis	mydriasis	mydriasis
8. Cerebrum Hyperglycemia	stimulation 1.0 Yes	stimulation 0.5 Yes	Yes
9. Heart			
a. Blood Pressure	increase 1.0	increase 1.25-1.5	increase 0.03-0.1
b. Output	increase 1.0	increase 1.0	unchanged
c. Rate	tachycardia	bradycardia	tachycardia
d. Auricle	weak stimulation	strong stimulation	stimulation
e. Ventricle	stimulation	stimulation	unaffected

tissues relax, enabling greater urine retention, relaxation of the uterus (virgin guinea pig) and decreasing tonus and motility of the intestinal tract. Serotonin causes spasm of all viscera resulting in forcible urination, uterine constriction, and spasms of the gastro-intestinal tract as indicated by vomiting and defecation. The action of all three on the eye in producing mydriasis is qualitatively similar. (16) (19) (35) (37)

Among the miscellaneous effects exhibited by sympathomimetic amines, these three amines show less uniformity in action. The action on the central nervous system by epinephrine shows features of stimulation such as euphoria; with nor-epinephrine and serotonin in this respect there is no satisfactory data. With regard to hyperglycemia, which is caused by all sympathomimetic amines, in the case of serotonin, it appears to be a direct action on the liver and is not mediated through the adrenal medulla since there is no change in response before or after adrenal demedullation.(35)Serotonin, like epinephrine and nor-epinephrine, has no direct action on clotting processes of blood; hemostasis caused by serotonin being only due to vasoconstriction, although the epinephrine stimulated liver produces prothrombin. (The actions of serotonin on the heart and rhythm are the basis of this thesis and will be discussed elsewhere.)

There have been synthesized several hundred aralalkylamines having strong pharmacologic properties; these are roughly classified as pressor amines. A better terminology might be employed by using the word sympathocaricature since some of these derivatives actually cause a fall in blood pressure on injection, and other actions are a parody of sympathetic nerve action. To illustrate: ephedrine, desoxyephedrine and

amphetamine possesses strong central nervous system stimulating properties which are exaggerated when compared with epinephrine. Isopropyl nor-epinephrine, (Isuprel[R]), markedly demonstrates the vasodilator property of epinephrine. This compound and certain alkylamines, as e.g., 2-amino-heptane evoke sinus tachycardia and appear to lack an action of stimulating the ventricle as exhibited by epinephrine. These examples are but a few of the many types of comparisons of "pressor amines" that must be made before one single compound can be adequately characterized and evaluated as a possibly useful therapeutic agent. Although such comparisons of serotonin with the various synthetic pressor amines would be very profitable, it is not the purpose of this thesis to compare, exhaustively, serotonin with other sympathomimetic amines.

off and concentrated by vacuum distillation as described previously. Evaporation of the acetone and some of the remaining water leaves a residue of about 100 ml. of a yellowish-brown oil floating on a yellow emulsion. This residue was transferred to a separatory funnel adding an equal volume of chloroform and 10 ml. of ethanol. (After this point all operations were undertaken in the refrigerator room at 5° C.) Shaking of the two phases resulted in a stiff, tenacious emulsion which required 5 days, 25 grams of added ammonium sulfate, and 10 ml. of methanol to separate sufficiently so that the aqueous phase containing serotonin could be removed. (This was much different from Rapport, Green, and Page (28) who seemed to have little difficulty with the extraction of impurities by chloroform and separation of the aqueous-chloroform layers.) The aqueous extracts were adjusted to a pH of 5.9 (Beckman pH meter), with 5 N NaOH and the solution was saturated with 60 gm. ammonium sulfate (0.6 gm. per ml.). The active principle was extracted 3 times with 100 ml. of n-butanol. The butanol extracts were chilled in the ice compartment of a cabinet refrigerator and decanted from the aqueous phase. The active principle was now precipitated as a complex with 150 ml. of a saturated solution of dilituric acid (5-nitrobarbituric acid) in absolute methanol. (2 gm. per 150 ml.) The precipitate was separated from the solvents by centrifugation and washed free of butanol with 3-10 ml. portions of cold absolute methanol. Attempts to hydrolyse the serotonin-dilituric acid complex with water and water-alcohol mixtures resulted in a viscid yellow oil. Further purification was not undertaken. An aliquot of the preparation was dissolved in saline and perfused through the ear of a rabbit according to

the procedure of Reid and Bick (18). Strong vasoconstriction was seen to occur.

Repetition of this procedure on a larger scale was made unnecessary by the timely synthesis of serotonin. Serotonin Creatine Sulfate was generously supplied by Dr. R. K. Richards, Abbott Laboratories, North Chicago, Illinois and Dr. Marvin E. Speeters, The Upjohn Company, Kalamazoo, Michigan. This crystalline material was used in these experiments.

B. Pharmacologic Studies

In all of the early experiments the effect of Serotonin on blood pressure was also investigated. The potency, the dosage pressor response curve, and the phenomenon of tachyphylaxis were found in these experiments with Serotonin to confirm the data supplied by Dr. W. A. Freyburger, Research Laboratories, The Upjohn Company, Kalamazoo, Michigan. Thus the results of these experiments are not reported in detail. We found it necessary to preclude recording carotid blood pressures inasmuch as animals used only to record the electrocardiogram and not cannulated could be used again.

1. The Effect of Serotonin on Cardiac Rhythm.

Sinoauricular tachycardia. With the knowledge that Serotonin has an effect twice as potent as epinephrine on smooth muscle, it is necessary from a therapeutic and physiological standpoint to ascertain its action upon the heart. As we could find no mention in the meagre information available of the effects of Serotonin on the heart, it appeared that such a study was indicated. Effects of Serotonin on the heart were studied by the use of a recording electrocardiograph with standard lead II electrodes on pentobarbital anesthetized dogs. Serotonin Creatinine Sulfate with intravenous doses exceeding 5 γ/kg. (2.17 γ/kg. of Serotonin base) provoked a sinoauricular tachycardia. The magnitude of the tachycardia was not directly related to the dose of Serotonin employed. With an initial rate of 140/min., 5 γ/kg. Serotonin Creatinine Sulfate resulted in a rate of 220/min. Larger doses 100 - 1600 γ/kg. (43.5-696 γ/kg. of Serotonin base) also were followed by a tachycardia amounting to an increase of 80 - 120 beats/minute depending on the initial heart rate. The maximum heart rate

obtained thus appeared to correlate with the relative refractory period of myocardium and not with an incremental stimulation of the sino-auricular node.

There was, however, a dosage-response relationship between the dose of Serotonin used and duration of tachycardia. Serotonin at 5γ/kg., (2.17γ/kg. as the base) evoked a tachycardia lasting 17 seconds. Using doses of Serotonin Creatine Sulfate at 200 γ/kg. and 800 γ/kg. (86.9 γ/kg. and 347.8 γ/kg. of the base) the duration of tachycardia was 83 and 168 seconds respectively.

It was noted that some tachyphylaxis to the Serotonin tachycardia existed; thus a repetition of the same dose of Serotonin in 5 - 15 minutes was followed by a tachycardia of somewhat smaller magnitude and shorter duration than that of the first. This tachyphylaxis was never complete. This phenomenon was not further investigated but all results reported here are with animals to which no Serotonin had been administered for 24 - 48 hours.

It has been reported (39) that pentobarbital sodium anesthesia is accompanied with a sinus tachycardia, and that morphine sulfate can counteract this tachycardia. Measurements, using a stethoscope, of 14 dogs in the animal colony at the University of Oregon Medical School, the Winter of 1952, indicated resting heart rates of 90 - 110. Examination of the control electrocardiographic records of dogs anesthetized with 35 mg./kg. of pentobarbital sodium intra-peritoneally, indicated heart rate of 140 - 160/min. The administration of morphine sulfate 1 mg./kg. frequently provided heart rates of 90 - 100/min. although in many there was an irregular rate. Serotonin Creatinine Sulfate appeared to be more effective in producing a sinus tachycardia

2. The Mechanism of Action of Serotonin: Autonomic Effects.

This portion of the study of the action of Serotonin on the heart consisted of proved methods to block components of the autonomic nervous system. This was done by the following methods: The effect of the vagus on the auricle was blocked by the action of atropine, of which 1 mg. per kg. was injected intravenously in saline. There was no change in the action of Serotonin before or after the injection of atropine and the full effect of atropine had taken place as shown by effective blocking of intravenous doses of acetylcholine calculated to cause auricular block. Apresoline(R), (Ciba-5968,) an adrenergic blocking agent, was injected intravenously 1 mg. per kg. After a latent period to allow Apresoline(R) to become effective, Serotonin was observed to be in effective in raising the rate of the auricle. Priscoline(R), an adrenergic blocking agent, was injected intravenously in a dosage of 3 mg. per kg; it had little effect in blocking the action of Serotonin on the auricle. SY-28, (Parke-Davis and Co.), an improved Dibenamine-(R) like adrenergic blocking agent, was injected intravenously in a dosage of 1 mg./kg. This drug also had no effect on the auricular stimulating effect of Serotonin. (Fig. 1 and Fig. 2)

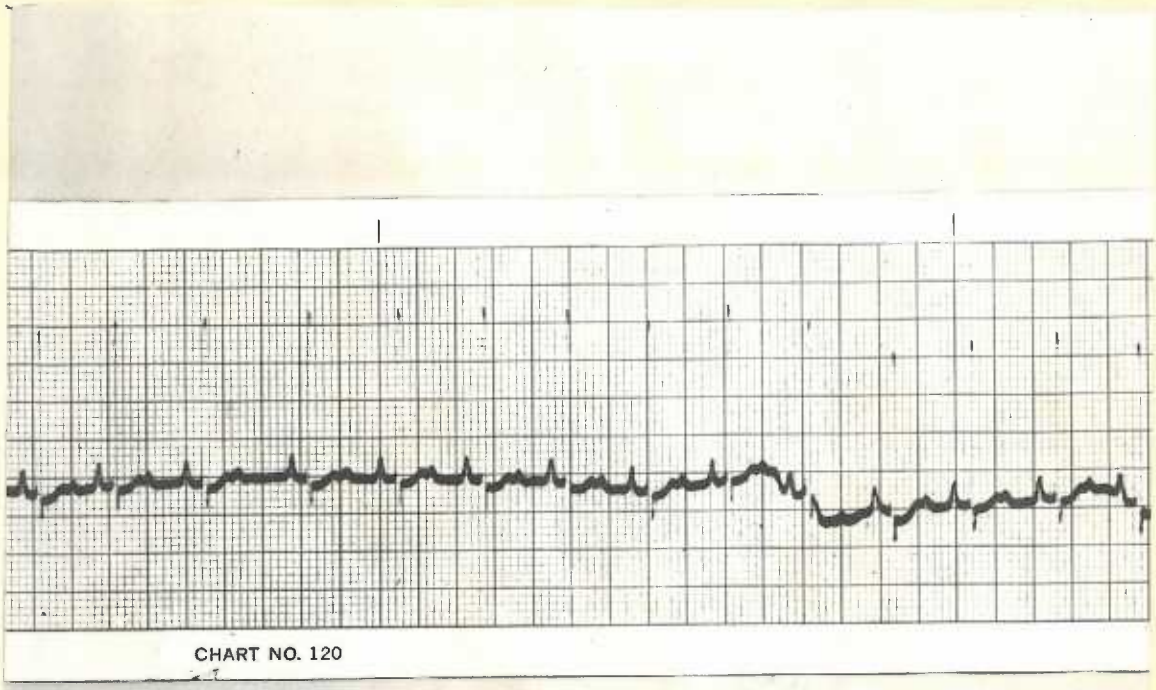
These experiments tend to demonstrate that the action of Serotonin Creatinine Sulfate on the auricle is not mediated through any nervous means and is solely a stimulating action on the sino-auricular node. In order to conclusively eliminate sympathetic influences, an experiment must be performed by which the effect of Serotonin on the auricle is obtained both before and after bilateral removal of the stellate ganglion. This particular experiment is planned to be a continuing portion of the research of the action of Serotonin on the heart.

FIGURE I

Effect of SY-28 Adrenergic Blockade on Serotonin and Epinephrine Tachycardia

A. Control lead II electrocardiogram

B. Post SY-28 1.0 mg./kg. record



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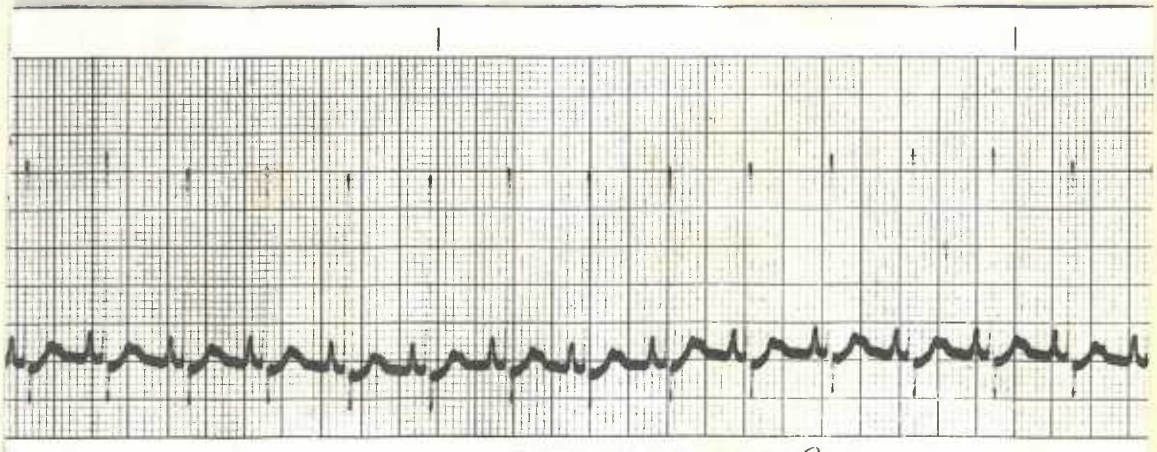


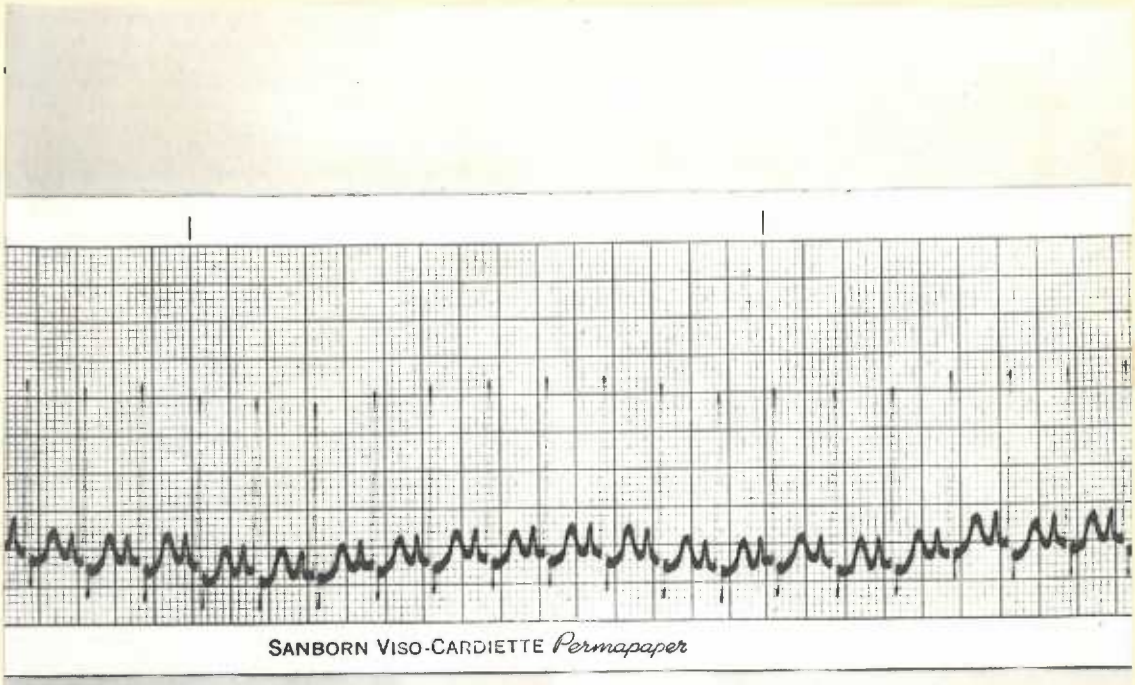
SANBORN VISO-CARDIETTE *Permapaper*

FIGURE 2

Effect of SY-26 Adrenergic Blockade on
Serotonin and Epinephrine Tachycardia
(continued)

C. Tachycardia produced by 0.8 mg./kg. Serotonin Creatine Sulfate 30 minutes following SY-26 injection. This sinus tachycardia began 20 seconds after the start of the injection and lasted 160 seconds.

D. Tachycardia produced by epinephrine hydrochloride USP 10 /kg. This sinus tachycardia began 12 seconds after the start of the injection and lasted 100 seconds.



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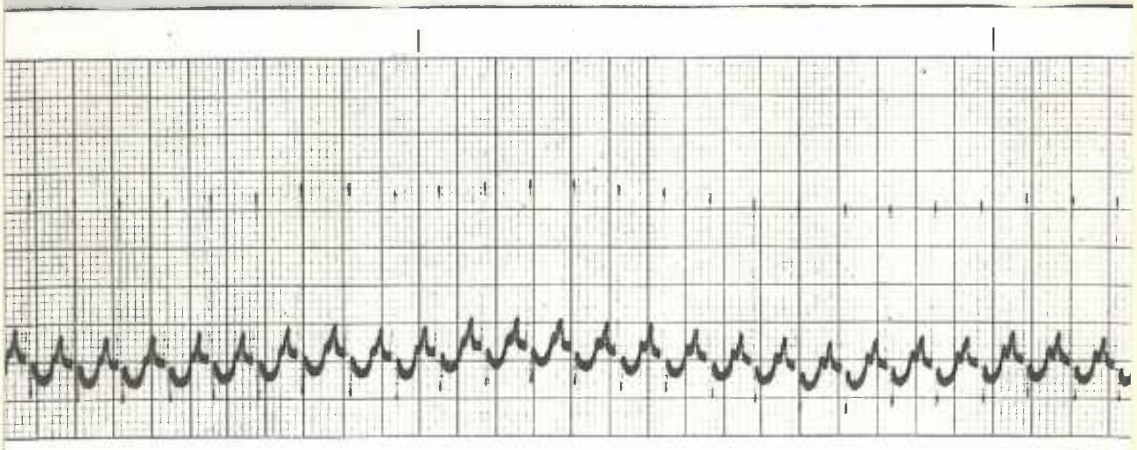


SANBO

3. Interaction of Serotonin and acetylcholine on the auricle (auricular fibrillation).

Vagal stimulation has long been known to suppress the activity of the sino auricular pacemaker. Following vagal stimulation there may be bradycardia or complete auricular block. This action may be duplicated by the injection of acetylcholine or other para-sympathomimetic drugs.

On occasion, following a sufficiently intense stimulation of the vagus or an appropriate dose of acetylcholine, auricular fibrillation will occur. Accordingly one explanation for the genesis of clinical auricular fibrillation consists of vagal hyperactivity supported or instigated by an as yet unknown E or excitant factor. Nahum and Hoff find that in thyrotoxic petients the injection of acetyl β methylcholine is followed by auricular fibrillation and propose that hyperthyroidism may produce one such E factor.

These observations were utilized to detect any possible arrhythmia producing quality of Serotonin.

Mongrel dogs were anesthetized with pentobarbital sodium 35 mg./kg. and the lead II electrocardiogram was taken. Acetylcholine chloride was injected using a peripheral saphenous vein. Acetylcholine was administered in graduated doses from 0.01 - 1.0 mg./kg. With small doses sinus bradycardia was observed. At approximately 0.02 mg./kg. there was minimal block; a single beat containing two p waves. Larger doses provoked a progressively longer block. With an occasional animal auricular fibrillation was evident. (Fig. 3 and Fig. 4)

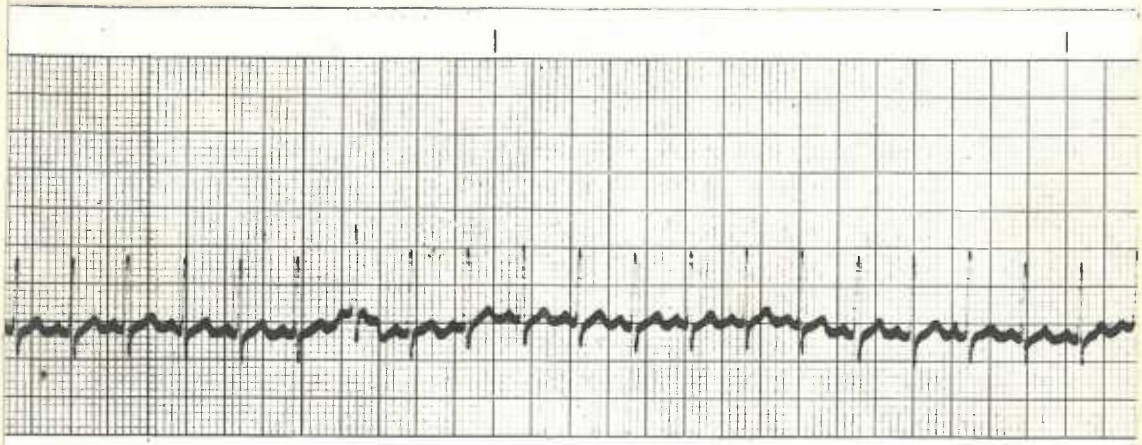
Serotonin Creatine Sulfate synergizes the action of acetyl choline on the auricles. For example in one animal acetyl choline chloride alone 0.6 mg./kg. was followed by a block (no QRS waves) lasting 24.5 seconds

FIGURE 3

Effect of acelylcholine on the heart

A. Control lead II electrocardiogram

B. Block produced by 0.8 mg./kg. acetyl choline chloride



SANBORN VISO-CARDIETTE *Permapaper*

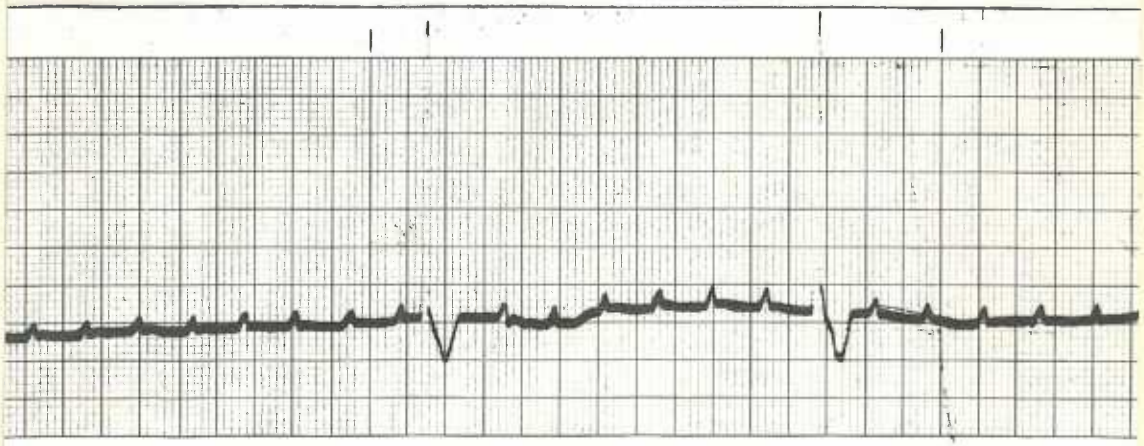


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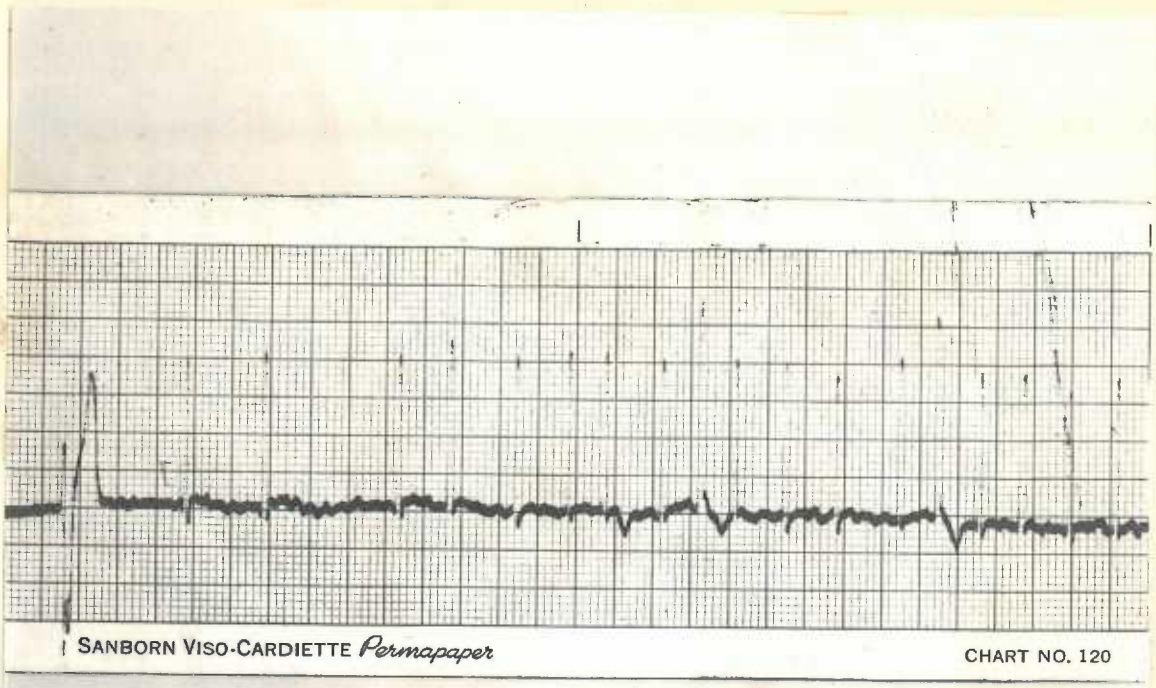
FIGURE 4

Effect of acelyclcholine on the heart

C. Coarse auricular fibrillation produced by 1.0 mg./kg. acetyl choline chloride

D. Auricular fibrillation from the combined action of 0.8 mg./kg. acetyl choline chloride and 0.8 mg./kg. Serotonin Creatine Sulfate



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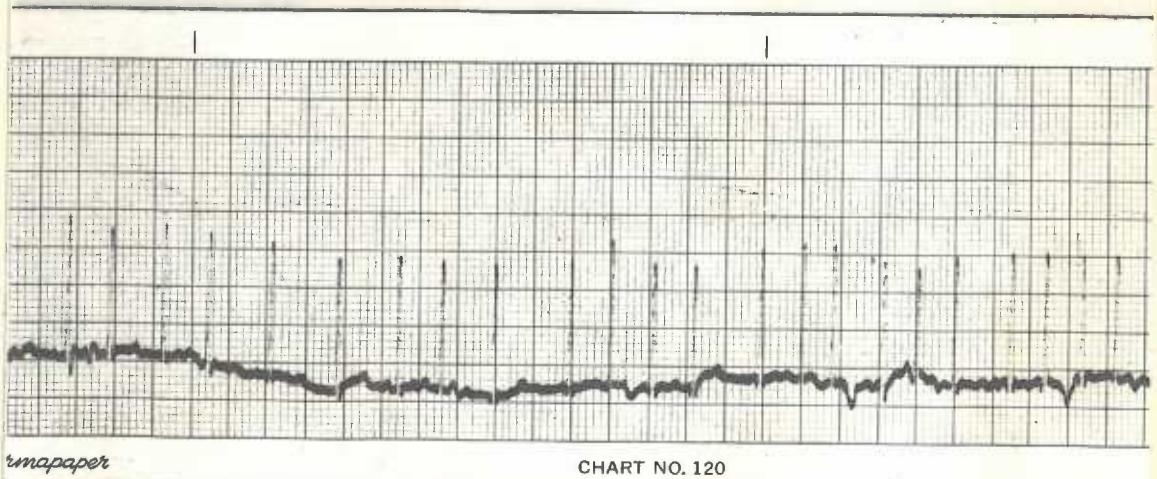

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while the Serotonin Creatine Sulfate, 0.8 mg./kg. added to the same dose of acetyl choline was followed by a block for 32.25 seconds.

In those animals which spontaneously fibrillated with acetyl choline, Serotonin could be shown to be synergistic in action. One animal could be shown to fibrillate to 1.0 mg. but not to 0.8 mg./kg. acetyl choline. This "Fibrillatory threshold" was found quite stable if suitable intervals of time were allowed between test doses. Serotonin added to a subthreshold dose of acetyl choline did provoke auricular fibrillation.

It is evident that Serotonin can hardly be an E factor in clinical auricular fibrillation even though it be present in humans. The dose required to synergize vagal action (acetyl choline) in these experiments is much above the physiologic range. It is probable that the synergistic action of Serotonin in this experimental auricular fibrillation can be explained quite simply. Acetyl choline does reach the sino auricular node by the circulation but it is likely that stimulation of the vagus nerve can provide an additional action. Through an increase of arterial pressure by Serotonin on the carotid body and arch of the aorta reflex stimulation of the vagus would occur.

4. Cardiac Effects of Serotonin During Cyclopropane Anesthesia in the Dog. (Action on ventricle)

The injection of epinephrine provokes the heart to respond with premature beats, AV blocks and slow ectopic rhythms. "In accordance with long accepted interpretation these are believed to be escape phenomena from lower centers. Their appearance is largely due to the fact that the sino-auricular pacemaker is reflexly inhibited because of the high arterial pressure, while the sub-auricular centers are under little or no vagal control. Under cyclopropane anesthesia the picture is essentially different. Premature beats of various kinds may appear as before and for the same reasons, but they last only for brief periods and are not followed by slow ectopic rhythms. On the contrary in every one of the 30 animals studied in the present series there appeared 30 to 40 seconds after injection a marked and long lasting tachycardia, with abnormal QRS complexes in the electrocardiogram" (40).

This phenomenon has been used to detect a latent power of many sympathomimetic amines to evoke ventricular tachycardia or ventricular fibrillation. Meek standardized the conditions of this cyclopropane sensitizing experiment so that it might be used as a pharmacologic assay (41). Chloroform (42), xylene, methane, dichlorodiphenyl trichloromethane or butane (43) also may be used to enhance epinephrine's ability to produce ventricular tachycardia or fibrillation. The method used in the experiments on serotonin reported here is a slight modification of Meek's procedure using cyclopropane (44).

Dogs without any form of premedication were anesthetized with Cyclopropane-oxygen using a mask. A cuffed Magill orotracheal tube was then inserted with the aid of a laryngoscope. The animals were then

maintained for 30 minutes on a concentration of gases obtained with a flow of 300 cc/min. cyclopropane and 700 cc/min oxygen; the excess gases are periodically released from the three-liter rebreathing bag thus maintaining a constant concentration (32% cyclopropane). This maintains the animals in partial intercostal paralysis -- (stage III, plane iij). A Heidbrink gas machine was employed for administration and maintenance of anesthesia.

In more than 100 dogs studied by this technique, we found that 10 γ/kg. epinephrine U.S.P. produces the following effects:

(1) Transitory ventricular escapes with predominate auricular stimulation in 26% of the animals.

(2) Ventricular tachycardia lasting 38 - 85 seconds in 65 percent of dogs, and fatal ventricular fibrillation in 13% of dogs used.

Serotonin Creatine Sulfate in doses of 400 - 1600 γ/kg. (1739-695.5 γ/kg. of base) did not produce any evidence whatsoever of any kind of ventricular arrhythmias. It is to be noted that 83.2 times the dose of epinephrine was used (10 γ/kg. epinephrine hydrochloride is 8.3 γ/kg. of epinephrine base). (Fig. 5)

Serotonin Creatine Sulfate thus does not exhibit the stimulation of ventricular pacemakers as shown by epinephrine and similar catechol amines, but rather resembles the phenylisopropylamines. Ephedrine, propadrine, amphetamine, desoxyephedrine and Methoxine(R) like Serotonin evoke only sinus tachycardia with cyclopropane anesthesia. (45)

FIGURE 5

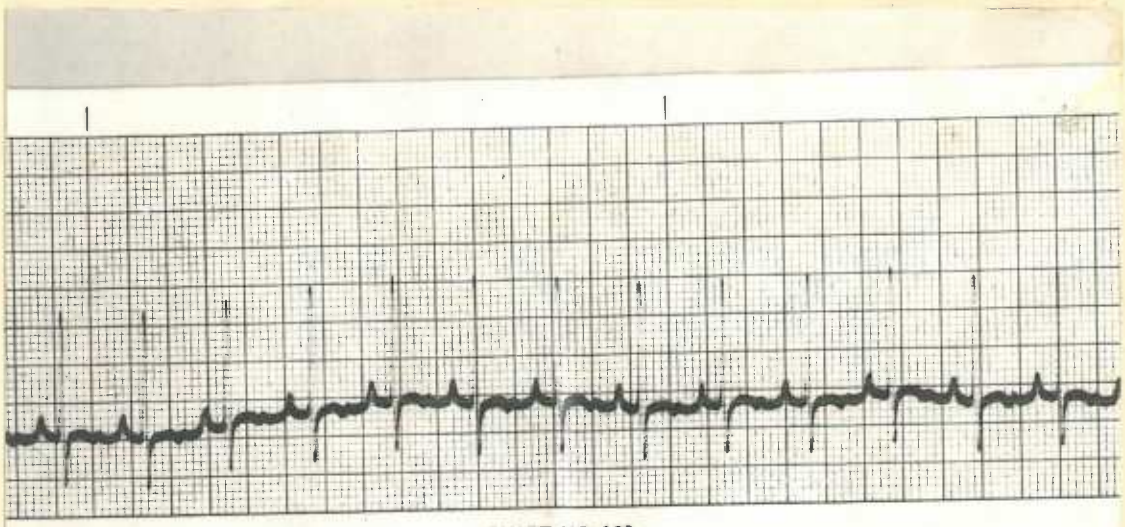
Action of Serotonin on Cardiac Rhythm

during Cyclopropane Anesthesia

A. Control record 30 minutes after induction of anesthesia with cyclopropane.

B. Epinephrine hydrochloride USP 10 γ/kg. Record taken 12 seconds after injection. This ventricular arrhythmia persisted 31 seconds and was followed by a regular sinus rhythm of 210/min. showing a depressed ST segment which lasted an additional 60 seconds.

C. Serotonin Creatinine Sulfate 1.6 mg./kg. Record taken 60 seconds after injection. This sinus tachycardia with ST depression and rate of 210/min. appeared 57 seconds after the start of injection and lasted 110 seconds; a gradually decreasing "normal" sinus tachycardia followed.



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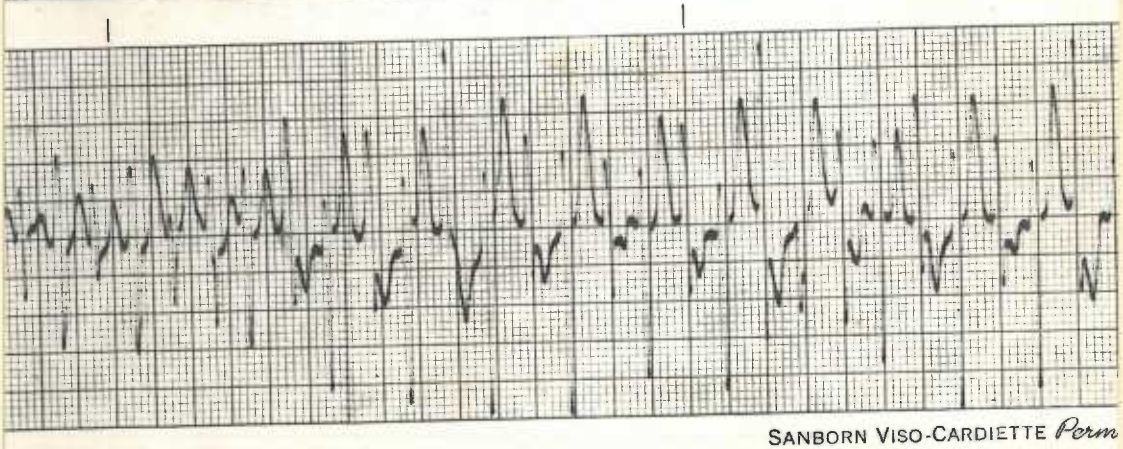

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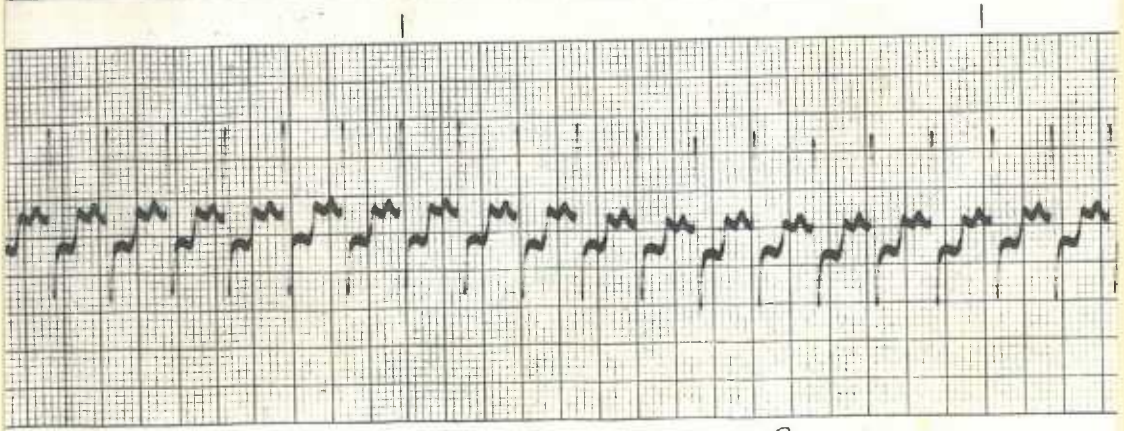

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5. Acute Toxicity of Serotonin.

As Serotonin may have promise as a therapeutic agent, it was deemed necessary to ascertain the acute toxicity by intravenous injection of "massive" doses. Previous work has been meager, and as far as is known, has only been done previously with rats and mice, (37) mainly because of the scarcity of the supply of Serotonin. Experimental work previously (37) has indicated powerful bronchiolar and tracheal constriction by Serotonin (37). An additional effect noted is that rats anesthetized with minimal quantities of barbiturates often succumb to as little as 0.1 mg. of Serotonin given intravenously. In an unanesthetized rat up to 50 mg. intravenously are tolerated. Studies have been carried out with a number of anesthetics and regardless of the anesthetic the qualitative result is the same to injected Serotonin; enhancement of respiratory depression of the hypnotic. (37)

The experimental procedure for determination of acute toxicity was to anesthetize a dog with pentobarbital sodium (25 mg. per kg. intraperitoneally). The animal was prepared for injection by means of a femoral cannula through which 5 mg./kg. of Serotonin Creatine Sulfate, 2.17 mg./kg. of the base, was injected in 10 ml. of isotonic saline. After a 2 - 3 minute period of increased respirations, respiration was arrested; at this time, a Magill oro-tracheal tube was passed by the use of a laryngoscope and the animal was bag-breathed with pure oxygen for 10 minutes. After 9 minutes the animal initiated respiratory movements and at 10 minutes he was able to breathewith no mechanical assistance. This effect was interpreted as a synergistic effect between the action of Serotonin and

pentobarbital on the respiratory center. (Fig. 6 and Fig. 7)

This experiment shows the pharmacologic complexity of even physiological materials: A substance which functions as a smooth muscle constricting agent also exhibiting synergistic action when given with hypnotics, at least in the action on the respiratory mechanism.

It is evident from an inspection of the electrocardiogram that even a lethal dose of Serotonin (for if no artificial respiration had been supplied death would have occurred) there is little abnormal ventricular activity. The ventricular extrasystoles may possibly be interpreted as being caused by hypoxia.

FIGURE 6

Effects of a "lethal" dose of Serotonin Creatine Sulfate on Cardiac Rhythm

A. Control lead II electrocardiogram

B. Record taken 31 seconds following 5.0 mg./kg. Serotonin Creatine Sulfate

C. Record 94 seconds after injection

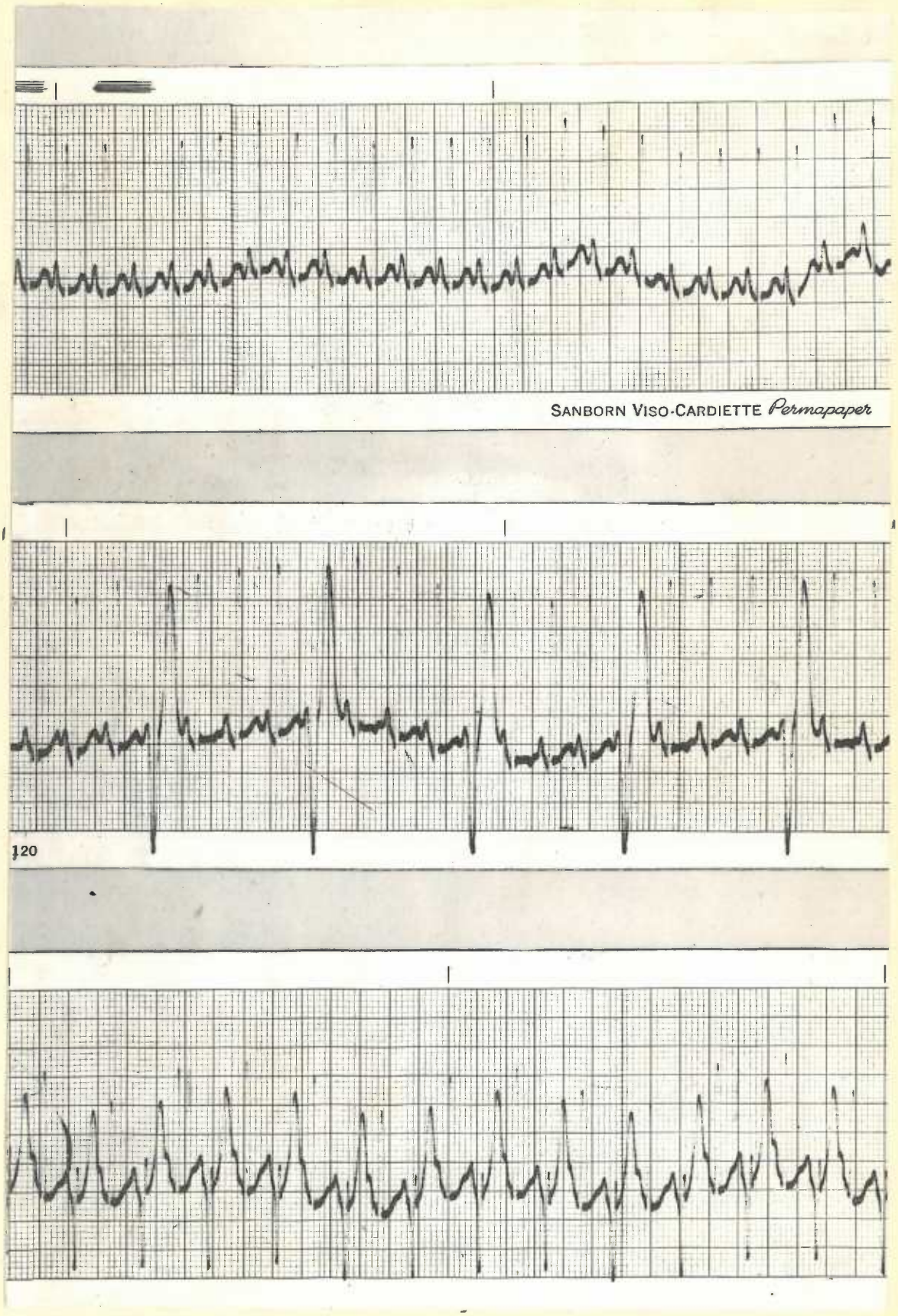
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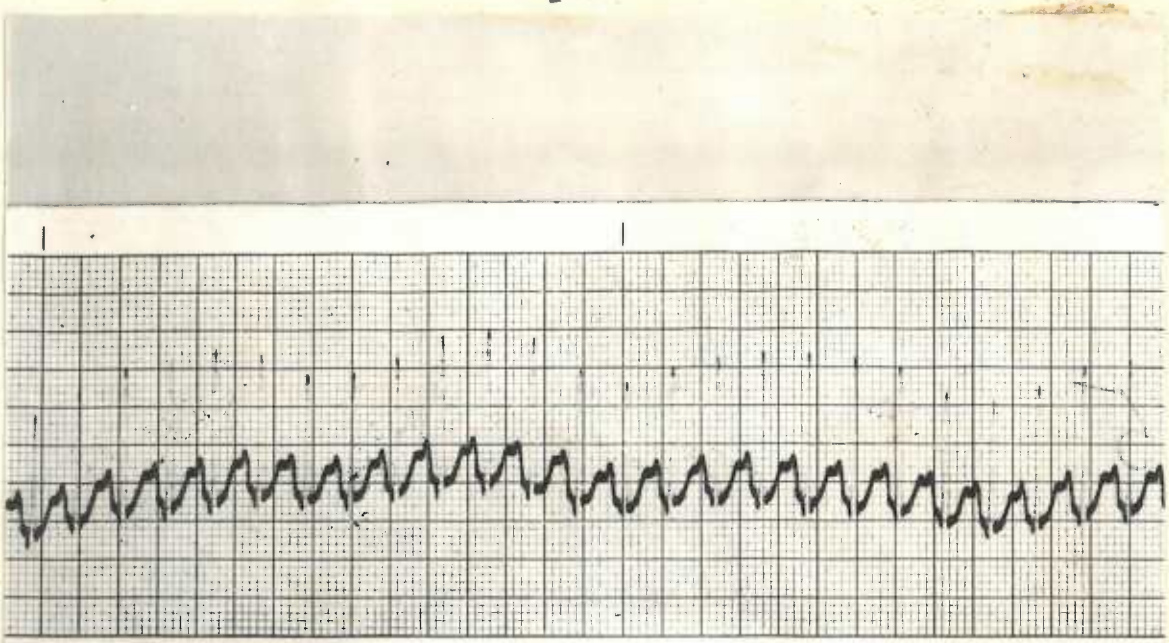
FIGURE 7

Effects of a "lethal" dose of Serotonin Creatine Sulfate on Cardiac Rhythm

(continued)

D. Record 167 seconds after injection

E. Record 720 seconds after injection



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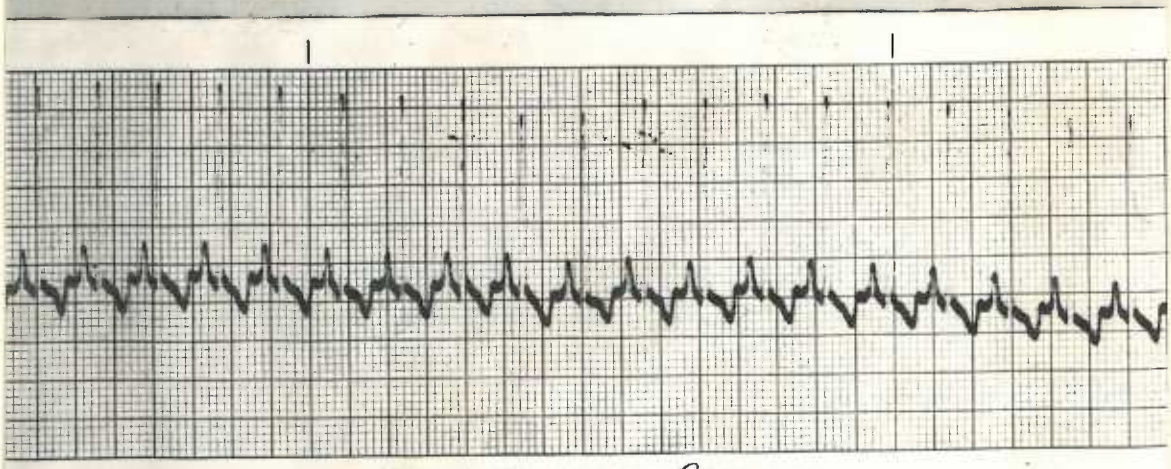

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SUMMARY

1. The historical background for the recognition, discovery and identification of the serum vasoconstrictor substance, Serotonin creatinine sulfate, is presented. In addition, the various routes of synthesis of Serotonin (5-hydroxytryptamine) are surveyed. The possible role of this substance in myocardial infarction is theorized, as well as its possible importance in essential hypertension. Therapeutic trial of Serotonin creatinine sulfate was isolated from human serum and identified by biologic assay.

3. The pressor potency and phenomenon of tachyphylaxis with Serotonin was confirmed.

4. The action of Serotonin on cardiac rhythm was investigated in detail. The sinoauricular tachycardia evoked by Serotonin was evaluated and an attempt made to elucidate its mechanism of action by the use of appropriate "blocking" agents. Serotonin though acting on the auricle was found <u>not</u> to be an "excitatory" factor initiating or maintaining auricular fibrillation. Serotonin did not provoke ventricular arrhythmias even though the ventricles were "sensitized" by cyclopropane.

5. A preliminary study of the acute toxicity of Serotonin in the dog maintained by artificial respiration was made and synergy with hypnotic drugs noted.

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