A CONTRIBUTION TO THE PHARMACOLOGY OF A SYNTHETIC ERGOT-LIKE COMPOUND

by

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A THESIS

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INTRODUCTION

The scarcity of enough crude ergot high in alkaloidal content, even in normal times, makes any synthetic preparation of this nature worth investigation. Today, with the War, the problems of transport and the devastation of the rye fields of Poland, Russia and Spain, as well as the depletion of manpower in these countries, make it imperative that other sources of supply of this important medicinal be found. Sollmann (1) quotes Stoll, without citing the reference, that "the richest source, Spanish ergot, never yields more than 60 mg. of this alkaloid (ergonovine) per Kg. Portuguese ergot yields less, and none can be obtained from Polish and Russian ergot. On the other hand, good ergots yield 2 Gm. of the ergotamine and ergotoxine group per Kg. The small yield and limited source of ergonovine would be inadequate for medical use, if this employed ergonovine exclusively". In view of the limited supply of ergot compounds, the attention of clinicians and investigators is turned hopefully to the promise of a solution of this problem by the synthetic chemist and pharmacologist. From a biochemorphologic (2) standpoint many profitable possibilities of such synthesis are offered. Once the chemistry and pharmacology of the active ergot alkaloids is understood, then the development of more ideal ergot compounds may follow, the toxic and therapeutic actions of these drugs further separated and the various desirable and specific actions more readily differentiated.

In this investigation the amount of material available for study has been limited. Because of this, it has been considered preferable to cover a large field of experimental work by doing a few each of several different experiments in order to obtain a general picture of the pharmacology of this new ergot drug. When more drug is available for the addition of a few more trials, several of these separate studies will each be suitable for publication at a later date. The small amounts of the drug so far received here have come from Switzerland, but it is encouraging to know that supplies will soon be available from a manufacturing laboratory in this country. Also further quantities of related synthetic ergot compounds, which have been only partly investigated, are available now. Thus an opportunity is offered for an investigation on a broader scope which may reveal, by comparison of the pharmacologic actions of the various side chains of lysergic acid, what combinations are responsible for the diverse actions of the natural alkaloids of ergot.

HISTORY

Ergot and its pharmacologic actions in man have long been known. Since ancient times, the gangrenous, convulsive and oxytocic actions of this phytochemically active substance have been recognized and more recently other actions have become apparent. Few other botanicals have served as a source of more pharmacologically active components. It is interesting to note that several drugs now well known and generally obtained from other sources were first discovered through chemical investigations on ergot. Ergosterol owes its name to the fact that it was originally found in ergot; ergothioniene, now a subject of biochemical interest, and potent pharmacologic agents such as histamine, tyramine, and acetylcholine were either discovered or early identified through researches on its chemistry. While considerable work has been done on the isolation (3), identification and determination of the exact chemical composition of all the constituents of this fungus, its chemical investigation remains incomplete. Even the exact chemical formula of a single one of the several alkaloids of ergot is undetermined with complete certainty. Today, research in this field is being actively pursued in the hope of obtaining either synthetic ergot alkaloids or more ideal ergot-like substitutes (4). So far, some progress has been made in synthesizing certain active compounds. Search has revealed that some ergot-like alkaloids such as yohimbine and sensibamine

(8), while Stoll and Burckhart (9) discovered ergotamine in 1920. With the isolation of these alkaloids and other non-oxytocic components of ergot, it was thought at this time that all the specific utero-active components of ergot had been identified. Based on usual pharmacologic precepts, it was therefore expected that these purified active principles should provide some advantages over crude ergot for obstetrical use. However, with the increasing clinical use of ergotoxine, and, particularly, ergotamine in the next few years certain workers began to question the efficacy of the pure alkaloids over the fluid extract of crude ergot and concluded that the latter was more effective. As Rothlin (10) so aptly states, "the entire situation with respect to the ergot alkaloids was made somewhat questionable in 1932 when Moir published his work". Moir (11) compared the effects of various preparations of ergot on the puerperal human uterus by means of inserting a balloon and studying the activity over a period of time. He found that the ordinary fluidextract of ergot, or watery extracts remaining after the active alkaloids ergotoxine and ergotamine had been removed produced an immediate, intensive and prolonged uterine motor action which was not the case even after large doses of ergotoxine and ergotamine. He thus concluded that the classical uterine response to ergot could not be ascribed wholly to the alkaloids such as ergotoxine and ergotamine thus far discovered. In a classical description by Dudley and Moir (12) in 1935 it was demonstrated

that there was another hitherto undiscovered principle present in ergot which was more utero-active than ergotamine. These workers named this substance "Ergometrine". The intensive search that followed Moir's report in 1932 for this other active component of ergot resulted in the simultaneous announcement of its isolation in four different medical centers in 1935 (13). While credit for this discovery of this new alkaloid should properly go to Moir, the other workers had some justifiable claims to priority of discovery of the actual compound as well. However, in order to clarify the unfortunate condition which had arisen from the fact that each of the four different discoverers had given this drug a distinct name, it was decided in this country by the Council on Pharmacy and Chemistry of the American Medical Association to give the product the non-therapeutically suggestive and non-proprietary name of Ergonovine. Ergonovine is now included in the latest revision of the United States Pharmacopoeia.

Since its discovery in 1935, ergonovine has been the subject of a few further pharmacologic studies, but, on the other hand general interest in this drug has resulted in a voluminous literature dealing with its clinical usefulness, methods of assay, methods of isolation from ergot, the elucidation of its chemical structure, and attempts to synthesize compounds of this general structure. Jacobs and Craig (3) of the Rockefeller Foundation have been particularly active in the chemical research on ergonovine and

the other ergot alkaloids. They have presented a series of experiments to show that lysergic acid, a compound found common to all the active ergot alkaloids, may be looked upon as the common denominator, so to speak, of the utero-active alkaloids. In fact, these workers state that "Lysergic acid is unquestionably the component of the ergot alkaloids to which they owe their pharmacodynamic action". While the formula for lysergic acid is now considered almost certain (14), no more than an empirical formula for any of the ergot alkaloids has been proposed. Most investigators are in agreement with Jacobs and Craig that ergonovine is hydroxy isopropylamide lysergic acid, though a doubt as to linkage may exist. Although several of the simpler lysergic acid compounds have been synthesized thus far, there are no reports to date that this writer can find describing the successful synthesis of ergonovine or an ergonovine substitute other than the one concerned in this paper. Stoll (15) reports the synthesis of ergobasinine, an isomer of ergobasine (ergonovine) and claims he can obtain ergobasine from this synthetic product but this work awaits substantiation. Greenhill in his recent revision of De Lee's "Textbook of Obstetrics" (16) states that ergonovine is now synthesized but he admits that this is an error*. Some of the other compounds of lysergic acid recently investigated such as ergine and lysergine are

* Personal Communication

apparently lacking in oxytocic effect (17).

Perhaps the nearest approach to providing a synthetic compound possessing active oxytocic effects is found in the recent work of Stoll and Hoffman of Basel, Switzerland. As a result of their exhaustive investigations of synthetic lysergic acid derivatives they have prepared two of promise --- d lysergic acid 1-3 dihydroxy 2 trimethylene amide and d lysergic acid d 1 hydroxybutylamide 2 (18). The latter compound was sent to this laboratory for pharmacologic study and, so far as is known, this is the only non-clinical investigation on this new synthetic ergot-like drug now being carried out. This compound differs from most of the active alkaloids of ergot in that it is not laevo-rotatory and from ergonovine in that it possesses a hydroxy butylamide radical in place of the iso propylamide group. No other information with respect to its chemistry or physical properties has been forthcoming from Basel. Because of the unwieldly chemical name, hereafter the term methergine, by which this drug will probably be known, will be used to designate d lysergic acid d 1 hydroxybutylamide 2.

This thesis deals largely with the pharmacology of this new synthetic drug. Since it has a definite chemical relationship to the ergot alkaloids, it is considered important to compare it from certain aspects similar to those which have been followed in investigating ergotamine and, especially, ergonovine.

EXPERIMENTAL

I. Chemical Studies. The proposed structural formulae for lysergic acid, ergonovine and methergine are shown in Figure 1.

Stoll and Hoffman describe this compound as being obtained by condensing isolysergic acid azide with d 2 amino 1 butanol and subjecting the product thus formed to a transposition treatment as by the use of acetic acid, phosphoric acid, sodium hydroxide or potassium hydroxide. The product is difficultly soluble in ethyl alcohol and acetone and gives Keller's and Van Urk's color reaction (a colorimetric method for estimation of ergot alkaloids). The tartrate salts and other salts are easily soluble.

The only material available for studying the physical and chemical properties of methergine was the solution, contained in sealed ampules, 0.2 mgm/cc, and a small amount of bulk solution in the same concentration. This solution showed the following properties: The reaction was faintly acid. Under ultraviolet light a distinct blue fluorescence was noted. No precipitates were observed with the following alkaloidal reagents --- Mayer's, Mercuric chloride, tannic acid, alkalis, gold chloride, gold bromide and potassium platinum bromide. With Lugol's an amorphous precipitate was obtained. The alkalinized solution was not soluble in the following --- ether, ethyl acetate, and isoamyl alcohol. The alkalinized material was soluble in hot benzene and appeared as a white crystalline residue which started to

melt at 168° C. It also is soluble in acetone and ethyl alcohol. In general, the material does not follow the usual alkaloidal reactions. Table I is presented to show the comparison of the so far determined physical and chemical properties of methergine with ergonovine.

Since this is not a chemical investigation primarily, the work on this phase is brief. It may be that the chemical reaction will be further elucidated in the near future. With ergonovine, the true alkaloidal nature as well as its definite chemical reactions was not at first apparent. It too was not considered alkaloidal, but later was found to precipitate alkaloidal reagents when a a very high concentration of test material was used.

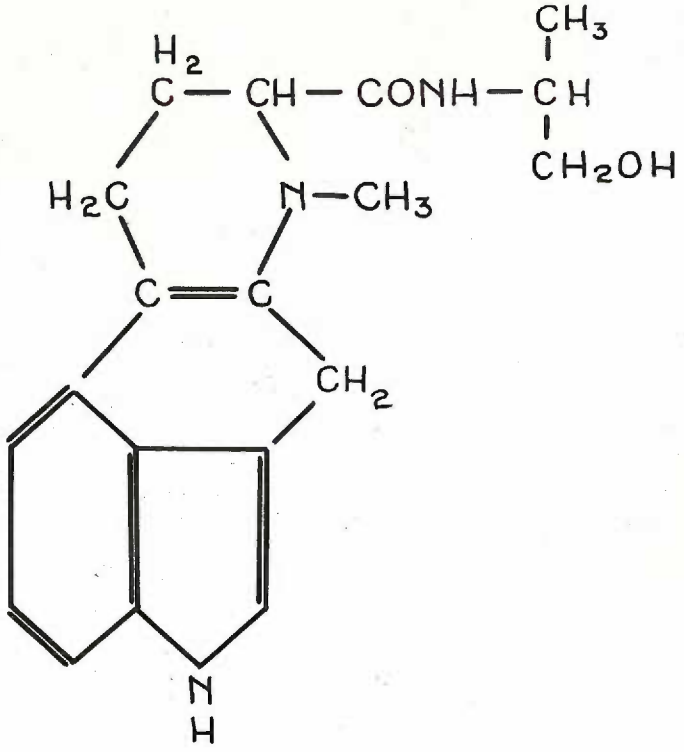
TABLE 1.

A COMPARISON OF THE PHYSICAL AND CHEMICAL CHARACTERISTICS

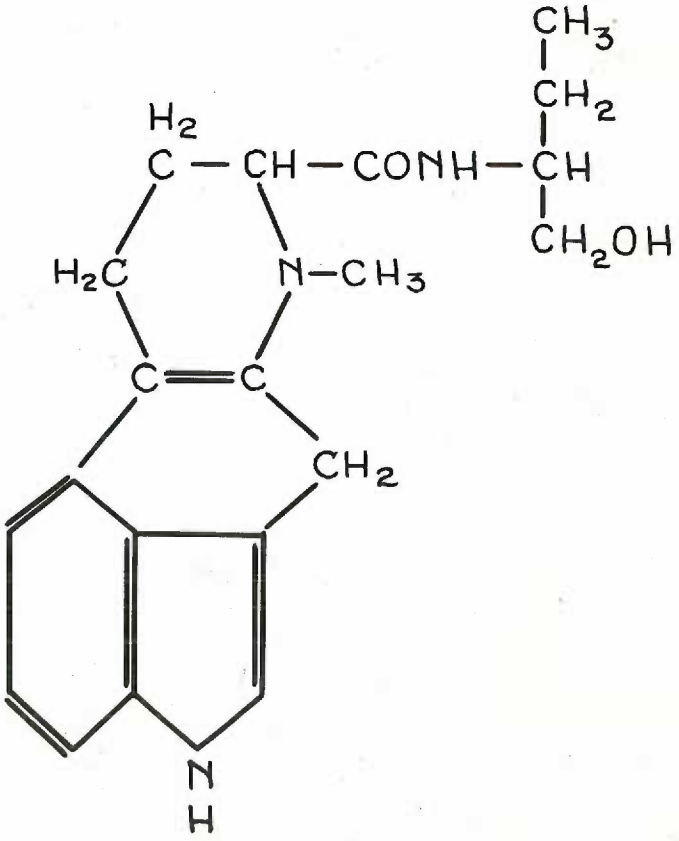
OF METHERGINE AND ERGONOVINE

Physical and Chemical Characteristics	Ergonovine P Base*	Methergine Base
Empirical Formula	$C_{19}H_{23}N_3O_2$	$C_{20}H_{25}O_2N_3$
Chemical Constituents	lysergic acid d-2-amino propanol 1	d lysergic acid d-1 hydroxybutyl amide 2
Optical Rotation	laevo-rotatory (salt is dextro)	
Alkaloid base	colorless, tasteless, odorless, crystalline substance	
Water Solubility	Appreciably sol. (1: 200-300 at 20°C.)	diff. sol. in H_2O
Reaction	definitely alkaline	
Fluorescence	bluish	bluish under ultra-violet
Solubility in organic solvents	dil. aqueous solutions, solubility:	dil. alk. sols:
	ethyl acetate - ##	insol.
	ethyl alcoh. - ##	soluble
	acetone - ##	soluble
	ether - #	insol.
	benzene - #	sol. in hot
	chloroform - s.sol.	insol.
Crystals	long fine needles from chloroform and benzene	crystals
Melting Point	with decomposition at 159-163° C.	liquefies at 168°C.
Alkaloidal Reagents	PPt. by Mayer's in dilutions less than 1:5,000	not ppt. in dilutions used
Color Tests	Keller's - #	#
	Van Urk's - #	#

* For the alkaloidal base of Ergonovine. From R.Smith , The Present Status of Ergonovine, J.A.M.A., 1938.



ERGONOVINE



METHERGINE

II. Pharmacologic Studies. Active ergot compounds possess two outstanding pharmacologic actions on the animal body; one, a motor effect on the uterus and the other a general stimulation of smooth muscle, particularly that of the circulatory system. Other less important pharmacologic actions are the stimulatory or depressant effects, depending on the test animal used, on such structures as the intestinal strip or the iris of the eye, and on the body temperature. Most of the ergot alkaloids cause discoloration of the cock's comb. In general, because some of these effects are peculiar to ergot, the pharmacology of ergot and its alkaloids may be appropriately considered in the following order:

1. Toxicity --- animals and man.
2. Gangrene producing effects --- rat's tail and cock's comb.
3. Systemic effects on circulatory system; effect on sympathetic nervous system.
4. Systemic effects on other organs --- respiration, heat production, the iris.
5. Oxytocic effects in various animals and man.

1. Toxicity. With respect to the toxicity of ergonovine in small animals, relatively little information is available. Rothlin (10), Smith (19), Nelson and Calvery (20) and others present some data. From this information, Sollmann and Hanzlik (21) give the following toxicity figures:

M. F. D. --- Mouse, 250 mgm. per kilo intravenously using a 1% solution; Guinea Pig, 80 mgm. per kilo intravenously using a 2% solution; and Rooster, a dose less than 10 mgm. per kilo given I. M. Smith (19) states that ergonovine appears to be from three to four times less toxic in mice, rats and rabbits than ergotamine and ergotoxine. On comparison with ergotamine it is ten times less toxic in the cock, given intramuscularly, but of approximately equal toxicity in the cat subcutaneously.

In this study the investigation of the toxicity of methergine was not only limited by the small amount of the solution available but also because the powdered drug was not supplied. Methergine was given subcutaneously in doses of 0.25 mgm. per 10 grams (the full therapeutic dose used for humans is 0.25mgms.) to three white rats, each weighing 50 grams. No toxic symptoms were noted although the animals appeared uncomfortable. Control rats with saline also were dejected and rumpled, so the effect was considered due to the quantity of fluid injected which was 10 per cent of the body weight.

In rabbits the intravenous injection of increasing doses of 0.25 mgm.; 0.5 mgm.; 0.75 mgm.; and 1.0 mgm of methergine to two animals averaging 2.2 kilograms was without apparent ill effect, although it was noted that the respiratory rate was increased. Brown and Dale (22) state that ergometrine (ergonovine) injected intravenously in the rabbit in a dosage of 1.8 to 2.8 mgm. per kilo caused

mydriasis, elevation of temperature, tachypnea, crawling instead of hopping, in many ways resembling beta tetrahydro naphthylamine. De Beer and Tullar (23) have proposed pyrexia as a supplementary assay procedure for ergonovine inasmuch as this particular response occurs regularly. In order to check these findings for methergine, a rabbit was given 2 mgm. per kilo of this drug intravenously. No excitement, no crawling and an increase of only 0.4° F. (rectal) was observed, but tachypnea and mydriasis were definite. No untoward effects were observed. Table 2 shows the temperature and pupillary changes following an injection of methergine.

Table 2

Time	Interval mins.	Room temp. °F.	Temp. Rectal.	Remarks
10:40	-	68	102.2	Normal
10:45	-	68	102.4	Normal
10:50	-	68	102.4	Normal
10:55	-	68	102.4	Normal
10:58	Methergine, 2 mgm./kg. i.v.			immediately increased resp.
11:12	14	68	102.4	pupils dilated.
11:25	27	68	102.8	" "
11:43	45	68	102.6	" "
12:05	67	68	102.4	less dilation.

While De Beer and Tullar obtained an average temperature increase rectally of 4.59°F., in four rabbits given a dose of 1.6 mgm. per kilogram, it is evident from the above

table that the hyperthermy with 2 mgm. per kilogram of methergine is much less.

In humans no untoward or toxic symptoms have been noted in over one hundred administrations of methergine to women post-partum when doses of 0.25 mgm. (1 ampule) have been given intravenously. The largest total daily dose used for these patients was 0.75 mgm. This compares favorably with ergonovine which has been regularly administered in similar doses in many thousands of cases. Recently oral administration was tried, and no toxic effects or side actions were noted.

2. Production of Gangrene. Another important factor to be considered in evaluating the toxicity of ergot compounds is whether or not they possess the ability to produce gangrene. Rothlin (24) of Basel first pointed out in 1923 that ergotamine given subcutaneously in several divided doses to white rats would produce regularly gangrene of the tail within a week or two. Loewe and Lenke (25) of New York repeated this work and demonstrated that by administration of a total dose of 50 mgm. per kilo of a saline solution of the powdered ergotamine given in three divided doses daily over a period of three days, rat tail gangrene could be produced regularly. McGrath (26) later substantiated these findings. This action is important in that a correlation exists in ergotamine action on human peripheral structures, gangrene of the toes and feet being not only of historical interest, but also has been

recently reported in therapeutic uses (27). In this laboratory, ergonovine solution and methergine solution were tried on groups of five rats each to determine if these two drugs could produce tail gangrene similarly to ergotamine. A dose of 20 mgm. per kilogram divided into two doses daily and given for a period of seven days to make a total of 140 mgm. per kilogram for each of the two drugs was used. The drugs were administered subcutaneously to the rats. Although this total dose is over twice that used by Loewe and Lenke for ergotamine, gangrene was not produced in any of the rats by ergonovine or methergine.

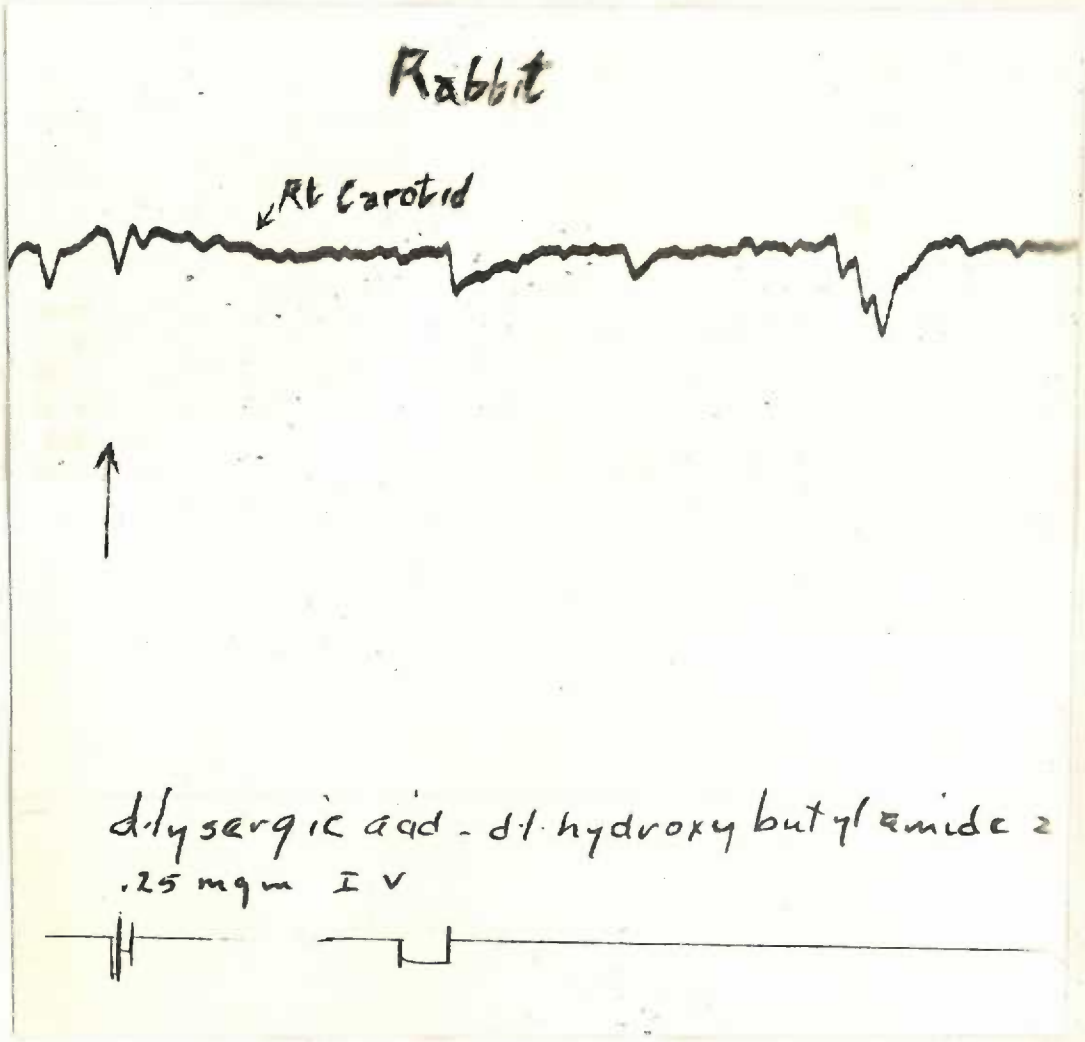
3. Circulatory Effects. One of the most interesting, if not clinically applicable effects of ergot is its stimulatory effect on sympathetic innervated structures, particularly on the blood vessels. Dale (28) in 1906 first showed that ergotoxine causes a primary stimulation of plain muscular tissue, especially the arteries, the uterus and the sphincter of the pupil. This primary stimulation was short lasting and followed by a secondary selective paralysis of the effector or motor elements innervated by the true sympathetics and stimulated by adrenalin. Dale demonstrated the vasoconstrictor and the later paralyzing effects of ergotoxine on cats with the brain destroyed and kept alive under artificial respiration. Later work, mainly by Stoll (29) showed ergotamine to have identically the same effects as ergotoxine. Ergonovine, however, while possessing some

vasoconstrictor effect on the circulation does not, however, lead to a secondary paralysis of the motor elements to the blood vessels. Thus, following ergonovine, there is no inhibition of the typical rise in blood pressure caused by the administration of adrenalin. Rather, as Rothlin (10) points out, ergonovine may lead to a slight but appreciable enhancement of the adrenalin effect (i.e., adrenergic) when the latter drug is given immediately afterwards.

Two rabbits under light sodium pentobarbital anesthesia were prepared for recording blood pressure changes on a kymograph drum by means of a carotid cannula connected to a mercury manometer. A membrane tambour connected to the trachea recorded the changes in the respiratory rate and excursions. When methergine was administered in doses of 0.25 mgm. and 0.5 mgm. to the animals the changes noted in the blood pressure were insignificant but there was some increase in the respiratory rate and volume. Similar to ergonovine, the administration of adrenalin after methergine showed a definite increase, more noticeable with succeeding doses, of the rise in blood pressure normally produced by a given dose of adrenalin. Effects on the adrenalin curve is better illustrated by the work on dogs.

Using the same experimental procedure, six dogs were given doses of methergine, ergonovine, ergotamine, and dihydroergotamine (another synthetic with ergotamine-like action). Figure 3 demonstrates the effects on the blood pressure elicited by methergine and, particularly, the

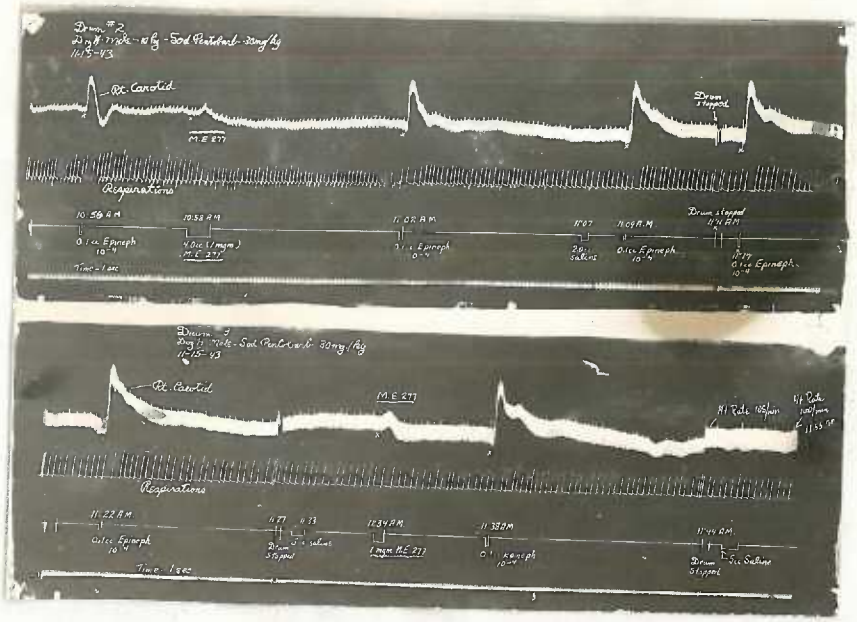
Fig. 2. Contact print from original smoked kymograph record. Rabbit, Sodium Pentobarbital anesthesia. Blood pressure record taken from right carotid. An intravenous injection of Methergine, 0.25 mgm., was given at arrow, with indefinite effects. Indentations shown on bottom line do not indicate injections of drug but time when respirations were being counted.

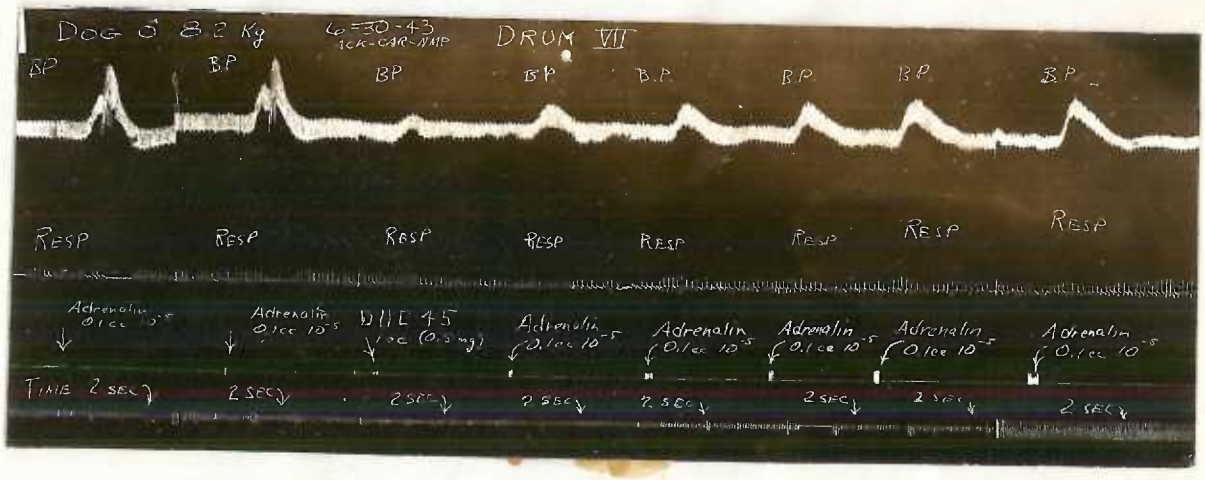

Rabbit
Rt Carotid
d-lysergic acid - d-l-hydroxybutylamide 2
.25 mgm IV

enhancement of the adrenalin blood pressure rise. When repeated injections of the same amount of adrenalin are given previous to methergine and then following it, it is clearly evident that methergine is not adrenolytic, i.e., it does not inhibit the response to adrenalin, but, rather, often increases it. This corresponds with the circulatory effects seen when adrenalin follows ergonovine but not with the adrenolytic action of ergotoxine and ergotamine. In the animal used for procuring the record shown in Figure 3, the protocol provides added information not included in this figure. Previous injections of 0.1 cc. of adrenalin in 1:10,000 dilution gave the following maximal increases in blood pressure over the normal as follows: after the first injection, a rise of 36 mms. of mercury; a second injection after 7 minutes interval gave a rise of 36 mms. of mercury; the third injection 4 minutes later showed a rise of 30 mms. of mercury; the fourth after 4 minutes, a rise of 34 mms. of mercury; the fifth, 6 minutes later, a rise of 36 mms. of mercury. Methergine in a dose of 1 mgm. was then injected two minutes after the last adrenalin administration. Only a slight rise of 6 mms. of mercury followed by a drop of 10 mms. of mercury, as shown in Figure 3, was produced by the methergine. Adrenalin was given again 4 minutes later, this time producing a rise in the blood pressure to 50 mms. of mercury. An injection of 2.0 cc. of physiological saline after the adrenalin was without effect, indicating that no adrenalin

21. Fig. 3. Photograph of smoked kymograph recording. Dog H, Sodium Pentobarbital anesthesia, 30 mgms. per kilogram. Blood pressure record from right carotid. Respiratory changes recorded by tracheal cannula and tambour. Showing enhancement of adrenalin effect after intravenous injection of 1 mgm. of Methergine (M.E. 277)

Fig. 5. Photograph of smoked kymograph recording. Dog 4, Sodium Pentobarbital anesthesia, 30 mgms. per kilogram. Blood pressure record from right carotid. Respiratory changes recorded by tracheal cannula and tambour. Showing adrenolytic effect when epinephrine is injected after dihydroergotamine (D.H.E. #45).


Rt. Carotid
M.E 277
Respirations
Time - 1 sec
Rt. Carotid
M.E 277
Respirations
Time - 1 sec

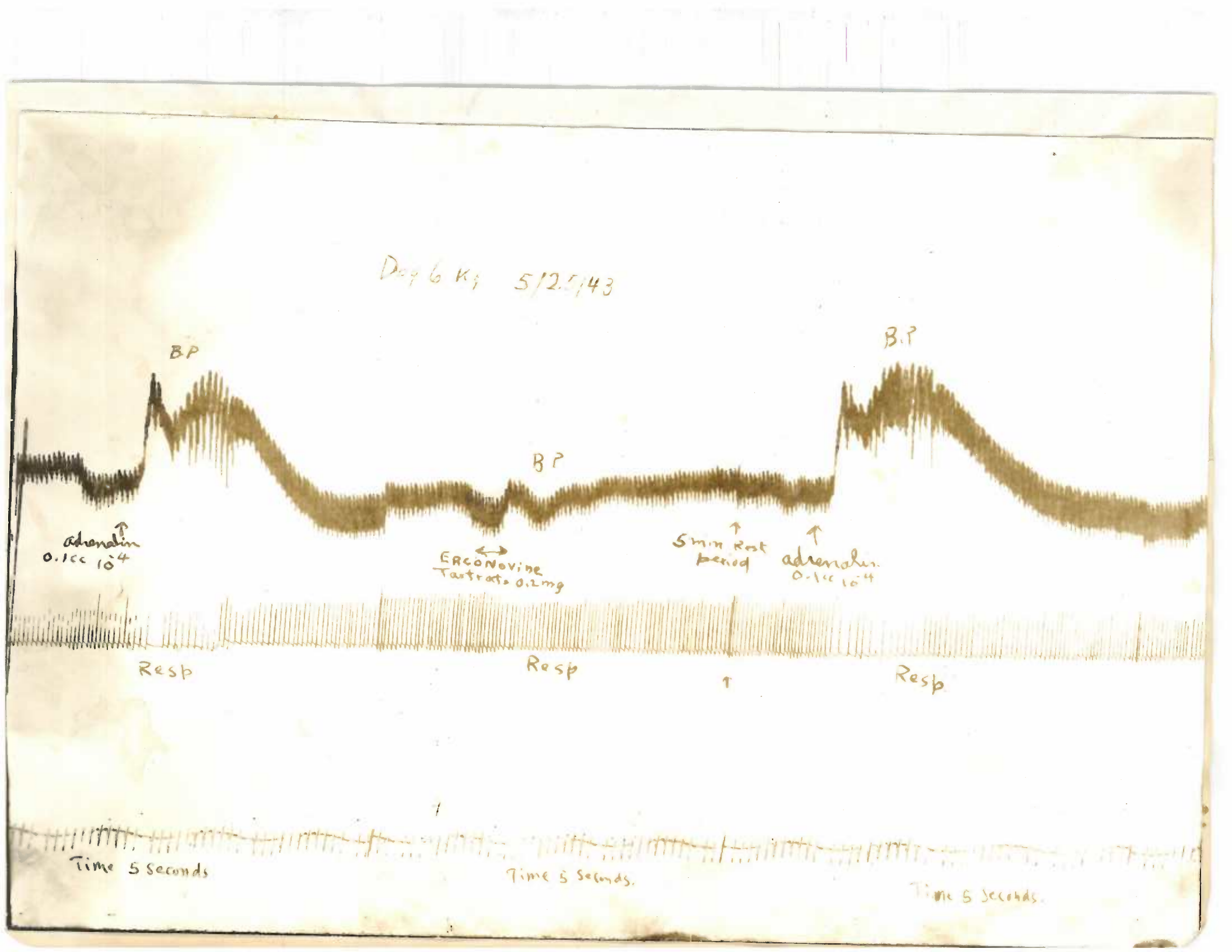

DOG ♂ 8.2 Kg
DRUM VII
BP
RESP
Adrenalin 0.1cc 10⁻⁵
DHE 45 1cc (0.5 mg)
TIME 2 SEC

had remained at the injection site to be washed into the circulation by subsequent administrations. A second dose of the same amount of adrenalin given 15 minutes after the previous one increased the blood pressure to 56 mms. of mercury while the third injection 5 minutes later increased it to 60 mms. of mercury. Similar results were obtained with methergine in experiments on the other dogs.

The circulatory changes produced by the intravenous injection of ergonovine into the dog corresponded to those seen with methergine. Figure 4 is a record of the blood pressure changes following injection of ergonovine. It is seen that there is little direct effect on the blood pressure which shows only a very slight rise followed by a drop of about 8 mms. of mercury. When adrenalin was given before and after ergonovine, the rise in the blood pressure before ergonovine was 40 mms. of mercury, while that 10 minutes after this drug and caused by adrenalin was 52 mms. of mercury, and again, 6 minutes later it was 60 mms. of mercury.

On the other hand, ergotamine injection resulted in a definite sustained rise in the blood pressure and was followed by the typical adrenolytic or epinephrine inhibition when this latter drug was given afterwards. This is well shown in Figure 5 where dihydroergotamine (D. H. E. #45) was used. Injection of adrenalin previous to dihydroergotamine gave the typical adrenalin response in the rise of blood pressure. Following this, the injection of 0.5 mgm. of dihydroergotamine (D. H. E. #45) gave a slight, transient

Fig. 4 Contact print from original smoked kymograph record. Dog 1, Sodium Pentobarbital anesthesia, 30 mgms. per kilogram. Blood pressure record from right carotid. Respiratory changes by tracheal cannula and tambour. Showing indefinite effect on blood pressure by injection of Ergonovine Tartrate, 0.2 mgms., intravenously. The second adrenalin injection curve shows a definite enhancement of the adrenalin effect after ergonovine.


Dog 6 Kg 5/12.5/43
BP
BP
B.P
adrenalin
0.1cc 10⁻⁴
ERGONovine
Tartrate 0.2mg
5min Rest
Period
adrenalin
0.1cc 10⁻⁴
Resp
Resp
Resp
Time 5 Seconds
Time 5 Seconds.
Time 5 Seconds.

rise in the blood pressure and produced a definite adrenolytic effect, since subsequent injections of adrenalin showed considerable decrease in the rise of blood pressure compared with that obtained before dihydroergotamine. Five more injections of adrenalin given in succession at about 2 minute intervals all failed to give a blood pressure response as great as the original injection.

5. Respiratory Effects. In previous sections of this thesis, mention is made that the intravenous injection of methergine into rabbits resulted in an increase in the respiratory rate and volume. While the respiratory changes were recorded when the circulatory effects of methergine, ergonovine and ergotamine were studied in the dog, the depression caused by the sodium pentobarbital anesthesia prevented marked changes. In other studies on the intact, unanesthetized rabbit (reported later under uterine effects) it was noted also that injection of methergine was attended by considerable increase in the respiratory rate and thoracic excursions. Since the rabbit appeared to be a suitable test animal for demonstrating the respiratory effects of these drugs, it was decided to carry out further experiments on this animal. The Dreser technic was used for this purpose on rabbits under light sodium pentobarbital anesthesia. This technic consists in counting the number of expirations per minute and in measuring the volume of water displaced from a tall glass cylinder per minute of time. Table II

TABLE III

RESPIRATORY EFFECTS IN RABBITS FOLLOWING INTRAVENOUS INJECTION OF 0.5 MILLIGRAMS OF METHERGINE USING THE DRESER APPARATUS.

TRIAL NO.	RESPIRATORY RATE	RESPIRATORY VOLUME
	min.	ccs.
Normal #1	42	950
#2	44	900
#3	44	900
#4	46	900
Methergine, 0.5 mgm. injected at 4:25 P.M.		
4:27 P.M.	54	1000
4:30 P.M.	60	1100
4:33 P.M.	57	1110
4:35 P.M.	57	1200
4:42 P.M.	42	960

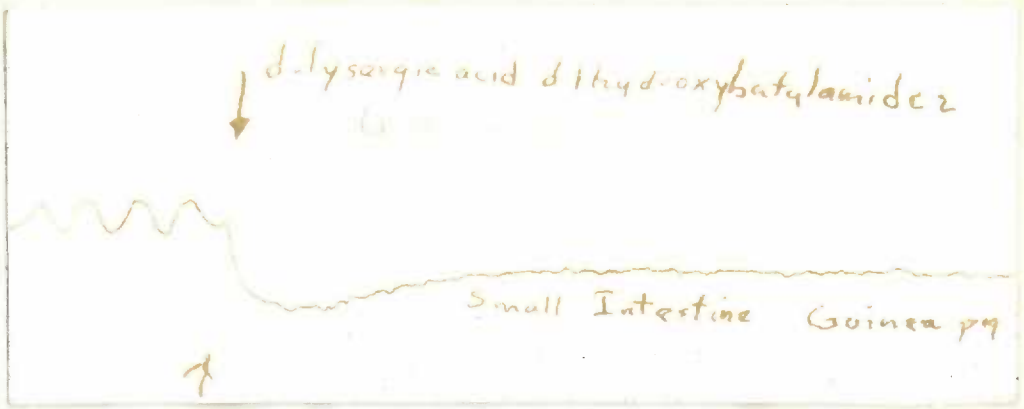
shows a graph of the results obtained for the normal respiratory rate and volume of a rabbit and after administering successive doses of methergine. It is apparent that methergine has a definite stimulatory effect on both the respiratory rate and volume.

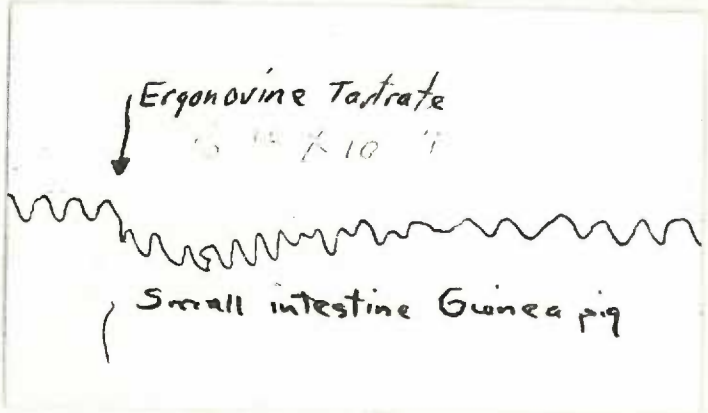
5. Isolated Guinea Pig Intestine. Adrenalin causes an inhibition of contractions and a relaxation in tone. It was thought worth while to test a few strips of the isolated guinea pig intestine for this adrenergic effect. For this purpose isolated strips of jejunum were prepared in a muscle bath as described later, for the isolated uterine strips. The addition of either ergonovine or methergine to the Locke's solution were found to cause relaxation as shown in Figure 6. This shows the same type of action asthat produced by adrenalin and agrees with other findings indicating that methergine has a slightly adrenergic effect.

6. Oxytocic Effects --- Uterine Activity. Several different methods may be used for studying the effects of these drugs on the uterus. Isolated strips suspended in Locke's solution and attached to a lever writing on a smoked drum may be used. The motility of the intact uterus may be studied under anesthesia by the Barbour (29) technic or in the normal, unanesthetized rabbit by the uterine fistula technic of Reynolds (30) as recently modified by Kirchhof and David (31). The Lorand tocо-

Fig. 6 Contact print from original smoked kymograph record. Guinea pig intestinal strip in 30 cc. Locke's solution. Upper recording shows depression of normal intestinal activity when Methergine is added to muscle bath solution.

Lower recording shows similar depression of normal intestinal activity when Ergonovine tartrate is added to muscle bath solution.


d-lysergic acid dihydroxybutylamide 2
Small Intestine Guinea pig


Ergonovine Tartrate
Small intestine Guinea pig

graph may be used for recording uterine contractions in the human during labor. Another method for studying uterine activity and used practically by the obstetrician is the estimation of the blood loss occuring from the time of the delivery of the baby to the birth of the placenta in humans; this is an indirect method but provides important information as to the tone and contractility of the uterus.

The effects of methergine on uterine motility have been studied by the above various methods.

a. Isolated uterine strips. Because of the relationship of methergine to ergonovine and other ergot alkaloids through its lysergic acid fraction, it was expected that the study of the uterine activity of methergine should prove of considerable importance. With ergonovine, as well as the other alkaloids ergotoxine and ergotamine, it has been shown that marked differences in sensitivity and response to the drug exist when uterine strips from various species are tested, as well as from animal to animal in the same species. The rabbit and the guinea pig are best suited for demonstrating the oxytocic effect on isolated strips. Of these two, the isolated guinea pig uterus is the most sensitive, but it is less reliable, generally speaking, than the rabbit uterus for comparative assay of oxytocic activity.

In this study numerous strips from a number of different animals were used. The method of testing consisted of

placing a thin, longitudinal strip in 30 cc. of Locke's solution (32) contained in a glass muscle bath tube. The solution was kept at 37.5° C. by immersing the glass container in a large thermostatically controlled water bath. The free end of the uterine strip was attached by a thread to a long lever writing on a smoked drum. The drug solution to be tested was added to the 30 cc. of Locke's in various amounts to give desired dilutions. After testing and studying the effects of one drug on the strip, the solution was washed out of the glass container, fresh warm Locke's added and allowed to stand in contact with the strip for a few minutes and then again washed out. After this, Locke's was again added and then another drug tested on the strip in order to have a comparison with the action of the first drug used. It was found that the response to both drugs decreased with the age of the strip after removal from the animal, although unused strips were immersed in iced Locke's for preservation.

(1). Guinea Pig. Comparable effects were obtained for ergonovine and methergine when tested on the same strip from a virgin guinea pig, as shown in Figures 7 and 8. It will be noted for both drugs that the action comes on promptly and that the type of action elicited is very similar in degree. In the dilution of drug used, 1:150,000, the result was usually a prolonged tetanic contraction. When older strips were used, it was found

Fig. 7 Contact print from original smoked kymograph record. Uterine strip from virgin guinea pig in 30 ccs. Locke's solution. Showing response to 1: 150,000 dilution of Methergine. This same uterine strip was used in making the record shown in Figure 8.

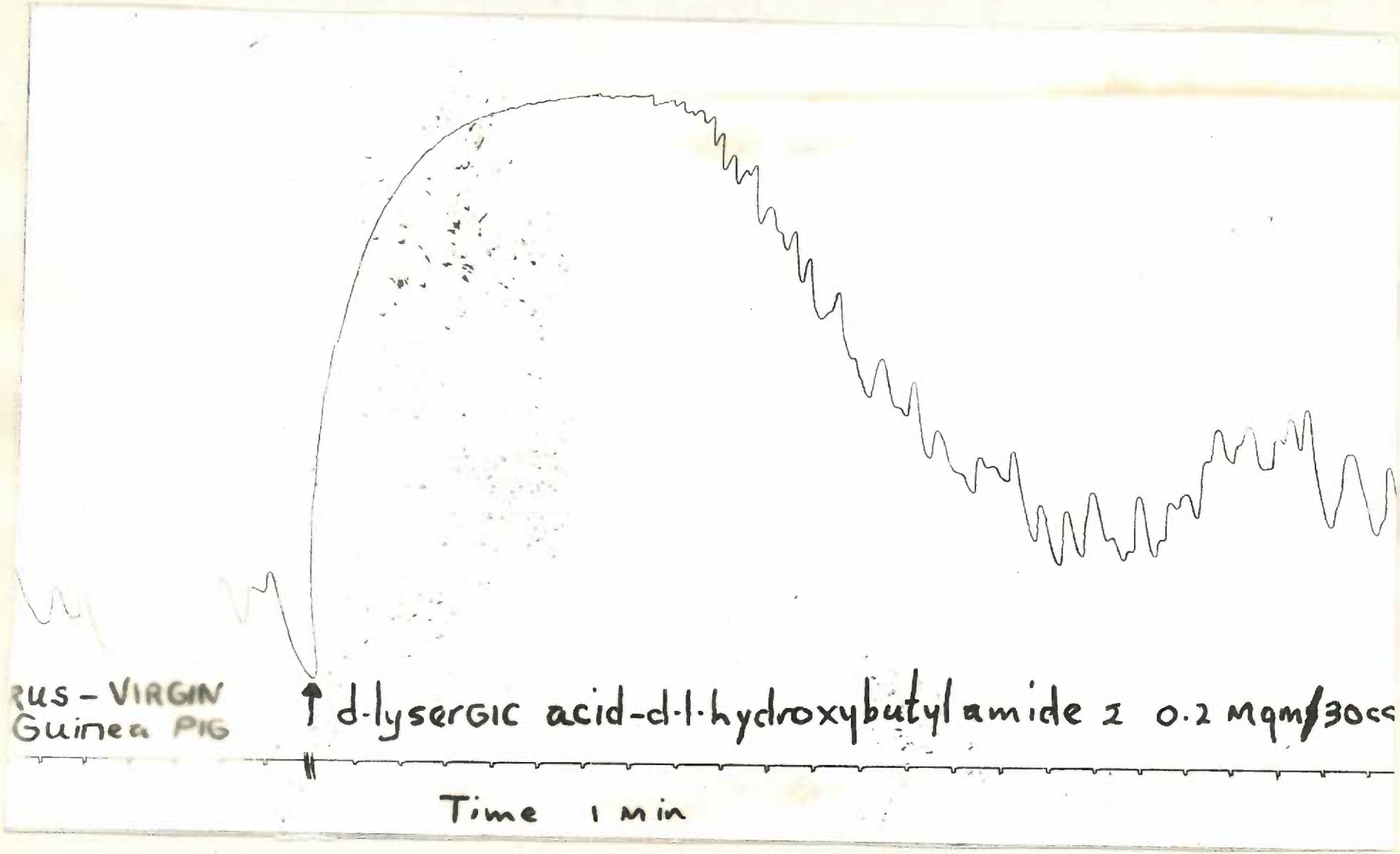
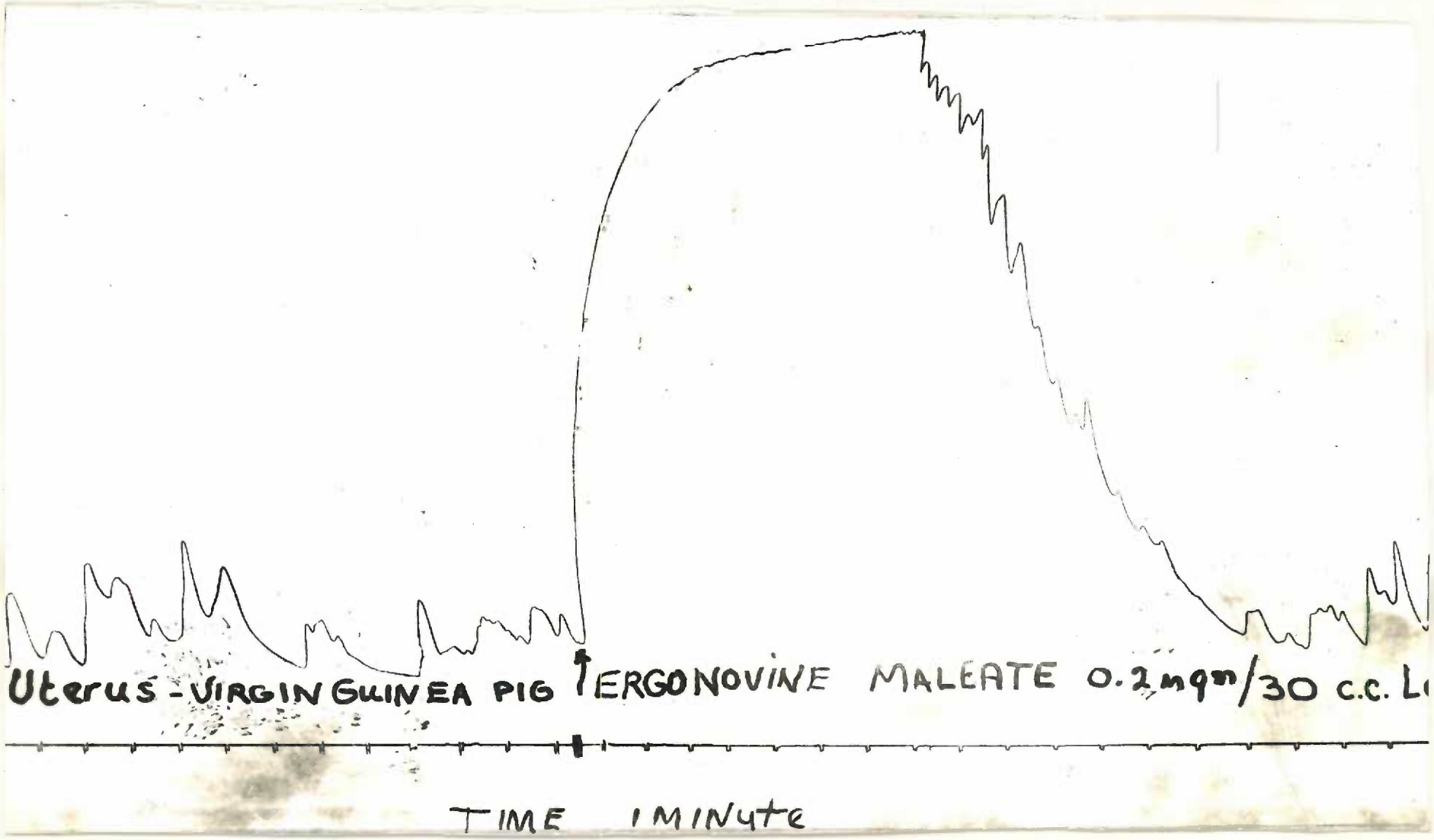

RUS – VIRGIN
Guinea PIG
d-lysergic acid-d-l-hydroxybutylamide 2 0.2 Mgm/30cc
Time 1 min

Fig. 8 Contact print from original smoked kymograph record. Uterine strip from virgin guinea pig in 30 ccs. Locke's solution. Showing response to Ergonovine malleate (Ergotrate-Lilly) in 1: 150,000 dilution. This same uterine strip was used for making the record shown in Fig. 7.


Uterus - VIRGIN GUINEA PIG
ERGONOVINE MALEATE 0.2 mgm/30 c.c. L
TIME 1 MINUTE

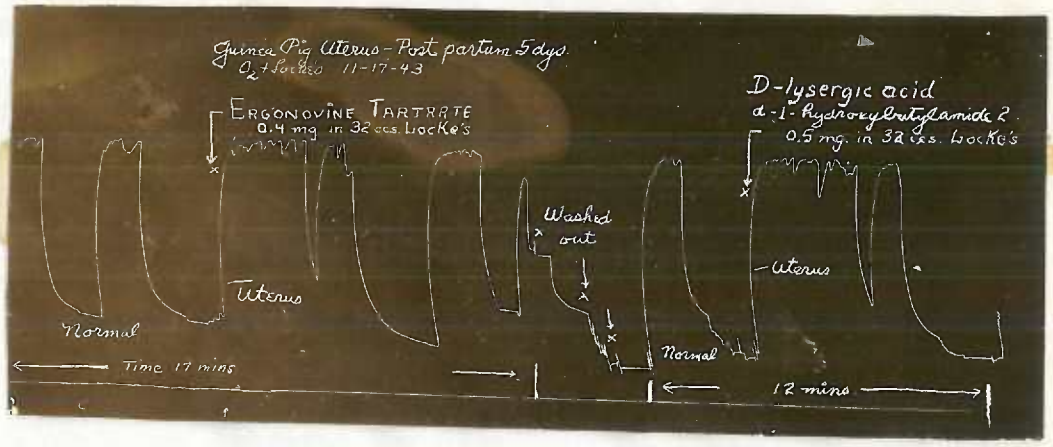
that the drugs occasionally increased the rhythmicity and excursion of the contractions with a less pronounced tetanic effect. When used in higher dilutions, both drugs were found to be lacking in the tetanic effect noted with the lower dilutions but, however, caused an increase in the height of the contractions as well as their frequency with fresh strips, and to show little response when older strips were studied.

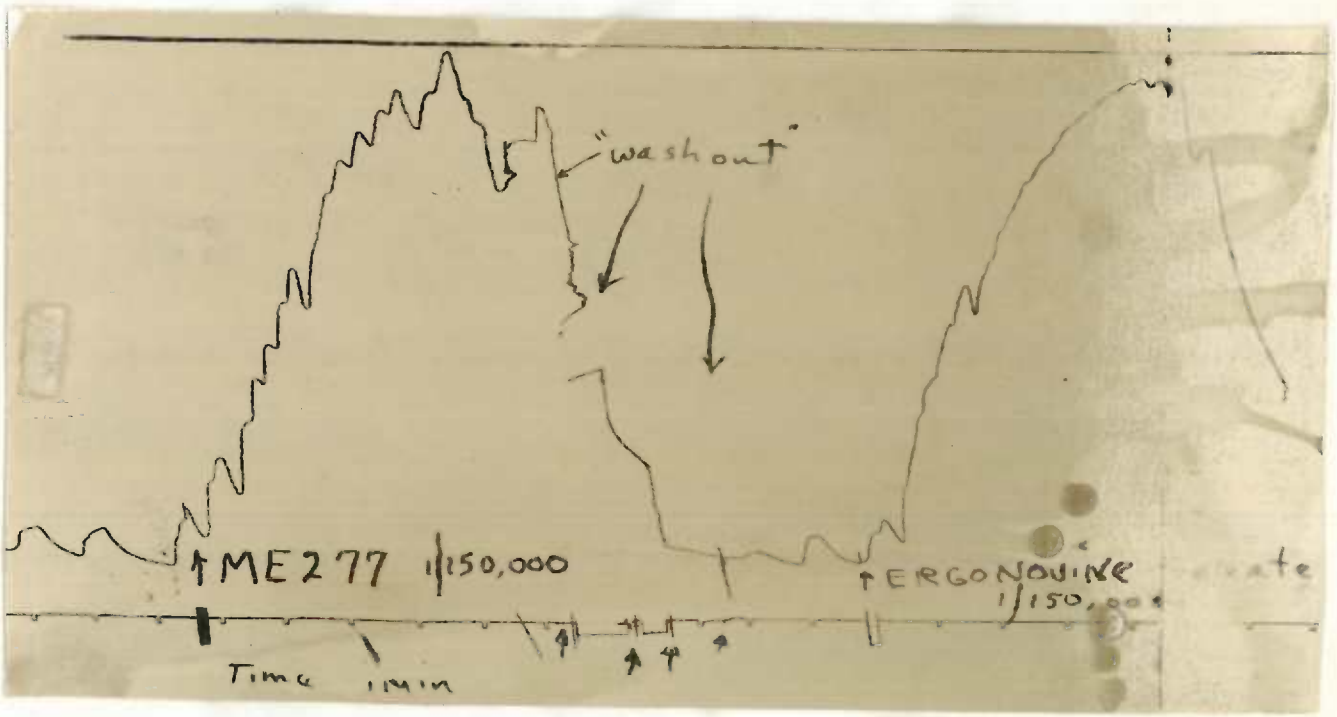
Similar comparisons between the two drugs, as shown in Figures 9 and 10, were obtained when strips from a post-partum guinea pig were used.

It has been shown that sympathetic stimulants (adrenergic) such as adrenalin inhibit the motility of the guinea pig uterus but, in the rabbit and cat elicit a typical stimulatory effect on the uterus. Furthermore, the variable responses - inhibition or stimulation of the uterus - produced in different animals by adrenalin may be prevented after ergotamine or ergotoxine. The same response, i.e., inhibition of the adrenalin effect after ergotamine or ergotoxine, is noted for the blood pressure changes as previously mentioned. However, it was found in this work that addition of epinephrine to the Locke's solution in the bath caused relaxation of the strip of a guinea pig's uterus both before and after methergine. Since systemically ergonovine and methergine have an adrenergic effect, and this effect produced by adrenalin on the strips of guinea pig uterus results in relaxation, the contraction of

Fig. 9 Photograph of smoked kymograph record. Uterine strips post partum (5 days) guinea pig in 32 ccs. Locke's solution showing comparative effects of Ergonovine Tartrate (Basergin-Sandoz) and Methergine. Two ccs. containing 0.4 mgm of the drug were used in each case and added to 30 ccs. Locke's, making the total amount of solution in the muscle bath 32 ccs. The amount indicated for Methergine, 0.5 mgm., is in error.

Fig. 10 Contact print of original smoked kymograph record. Uterine strip guinea pig, post partum, showing comparative effects of Methergine (M.E. 277) and Ergonovine when used in dilutions of 1: 150,000.


Guinea Pig Uterus - Post partum 5 dys.
Locke 11-17-43
ERGONOVINE TARTRATE
0.4 mg. in 32 ccs. Locke's
D-lysergic acid
d-1-Hydroxybutylamide 2
0.5 mg. in 32 ccs. Locke's
Washed out
Uterus
Normal
Time 17 mins
Normal
12 mins


"washout"
ME277 1/150,000
ERGONOVINE 1/150,000
Time 1 MIN

the strip by methergine can only be explained as being due to the action of the drug directly on the musculature itself. Moreover, the epinephrine mediated relaxation after methergine further demonstrates the difference between this drug and the ergotamine-ergotoxine group which paralyze the adrenalin response.

(2). Rabbit. The isolated rabbit uterus has become a standard method of assay for the ergot alkaloids. Swanson, Hargreaves, and Chen (33) have compared several methods for assay of ergot alkaloids and have concluded that the isolated rabbit uterus is the best for practical use. Brown and Dale (22) find that strips from the rabbit uterus regularly give a motor response to adrenalin and also show a consistent excitatory responsiveness to ergonovine. These workers record activity in an isolated rabbit strip with dilutions of ergonovine as high as 1: 750,000. While Thompson (34) got contractions in the most sensitive uteri with dilutions of 1:3,000,000 of ergonovine, he gives 1:500,000 as the amount of ergonovine needed to get an appreciable effect in most strips. Some difficulty, however, is occasionally encountered in the use of rabbit uterine strips since some are insensitive and do not give the usual normal rhythmic contractions in Locke's solution. This objection may be overcome by using the method of Wiek and Powell (35) who claim they can sensitize the uterus by giving small amounts of estrogenic material to rabbits for

several days preceding testing. Using this technic, with Theelin as the estrogenic substance, it was found that the strips used in this study usually gave good activity.

Since both ergonovine and methergine do not have an inhibitory effect on the adrenalin motor response, the assay method of Broom and Clark (36) for ergotoxine and ergotamine on the rabbit uterus would not be applicable here.

Dilutions of methergine up to 1: 3,100,000 were definitely stimulant to the rabbit uterine strip. Figure 11 shows an assay procedure using ergonovine and methergine in this dilution on the same strip. It was also found that repeated administration of methergine did not inhibit the contraction caused by adrenalin (Figure 12). It is seen on comparing the activity of ergonovine and methergine in the same dilutions on the same strip that the results are approximately equal (Figure 11), or superior to ergonovine, and this was noted for all the experiments performed. A total of 10 strips each were used from 5 different rabbits for this comparison. It was found that the response elicited is in proportion to the dilution of the drug in Locke's, weak dilutions producing less effect than stronger concentrations. Depending on the sensitivity and age of the strip, weak dilutions gave some increase in the frequency of the rhythmic contractions and a slight increase in the amplitude or height of contractions. Dilutions of greater concentration (1:150,000) always produced an

Fig. 11 Contact print from original smoked kymograph record. Isolated uterine strip of rabbit in Locke's solution, showing a comparison of the actions of Methergine and Ergonovine when used in dilutions of 1: 3.1×10^6. The increased activity of the uterine strip is slightly greater when Methergine is used.

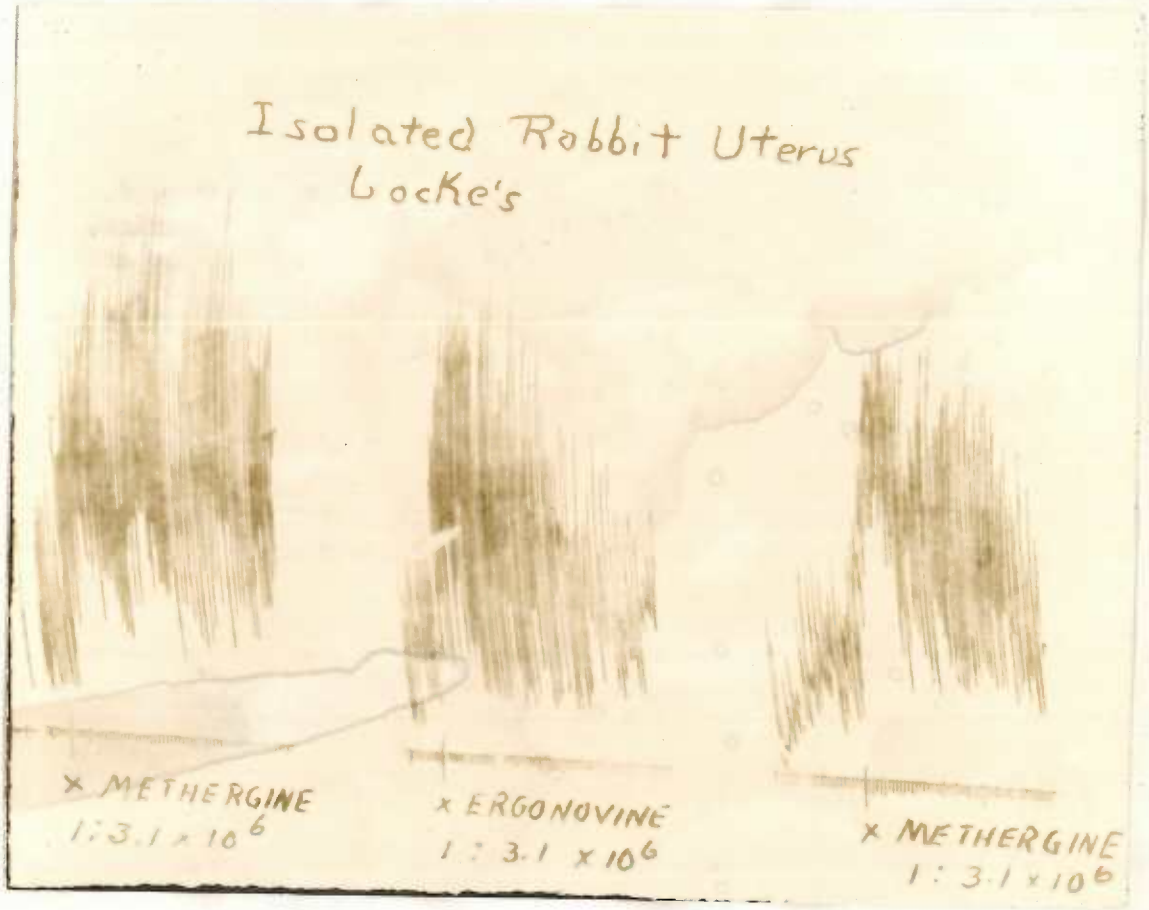
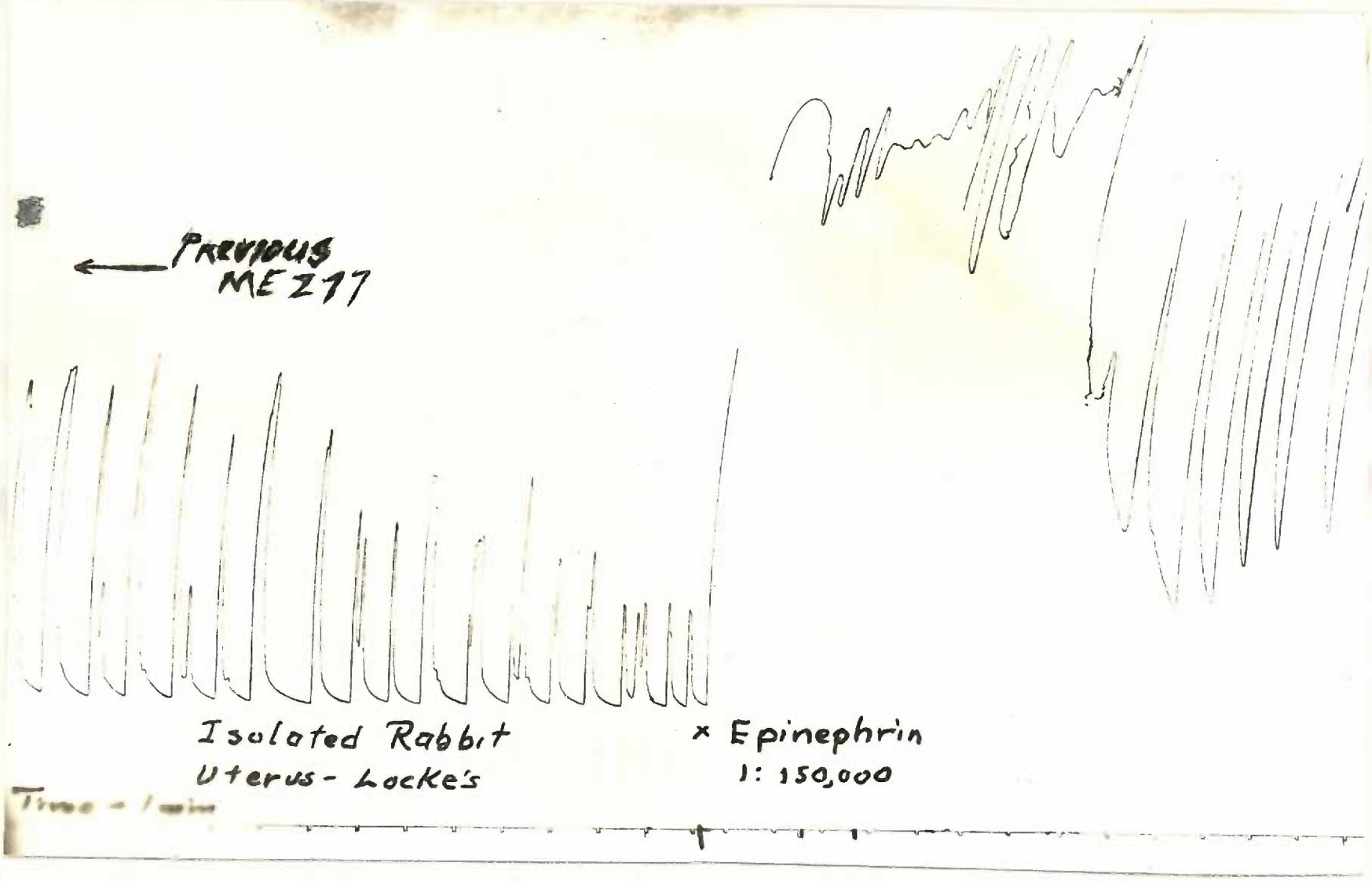

Isolated Rabbit Uterus
Locke's
x METHERGINE
1 : 3.1 x 10^6
x ERGONOVINE
1 : 3.1 x 10^6
x METHERGINE
1 : 3.1 x 10^6

Fig. 12 Contact print from original smoked kymograph record. Uterine strip from rabbit in Locke's solution. The previous solution in the muscle bath contained Methergine 1: 150,000 and the first part of the record shows the response obtained. Addition of epinephrin to the muscle bath in 1: 150,000, without washing out, gave a marked response showing that Methergine does not inhibit adrenalin effect on the rabbit uterine strip.


← PREVIOUS
MEZ77
Isolated Rabbit
Uterus - Locke's
× Epinephrin
1: 150,000
Time - 1 min

increase in tone although the amplitude or extent of the contractions was less due to the increased tonicity. Figures 13 and 14 show typical responses of the rabbit uterus to varying dilutions. The range of effective dilution for methergine is higher than that given by Thompson (34) for ergonovine. He found dilutions as high as 1:3,000,000, which approximately coincides with the results obtained in this laboratory, as the least effective concentration for producing a response to ergonovine. Results were occasionally obtained using a solution somewhat more dilute than Thompson's figure for ergonovine, which can perhaps be explained on the basis of improved methods of purification since his work in 1935.

Isolated Pregnant Rabbit Uterus. When tested on the isolated pregnant rabbit uterus taken on the twentieth day of gestation, methergine was found to be effective in a dilution higher than has been reported for ergonovine. The highest dilution given for that substance is 1: 3×10^6 by Thompson (34). With methergine it was found that a dilution of 1: 4.1×10^6 was definitely stimulant when strips from the pregnant rabbit were used. This is shown in Figure 15. In spite of the high dilution used, the effect is clearly discernible for the two hour period beginning from the time the drug was added until the recording was stopped, a very slow moving drum being used.

Fig. 13 Contact print from original smoked kymograph record. Rabbit uterine strip in Locke's solution from Rabbit No. 3, strip being used first day after isolation. Addition of Methergine in dilution of 1: 300,000 increased motility of strip.

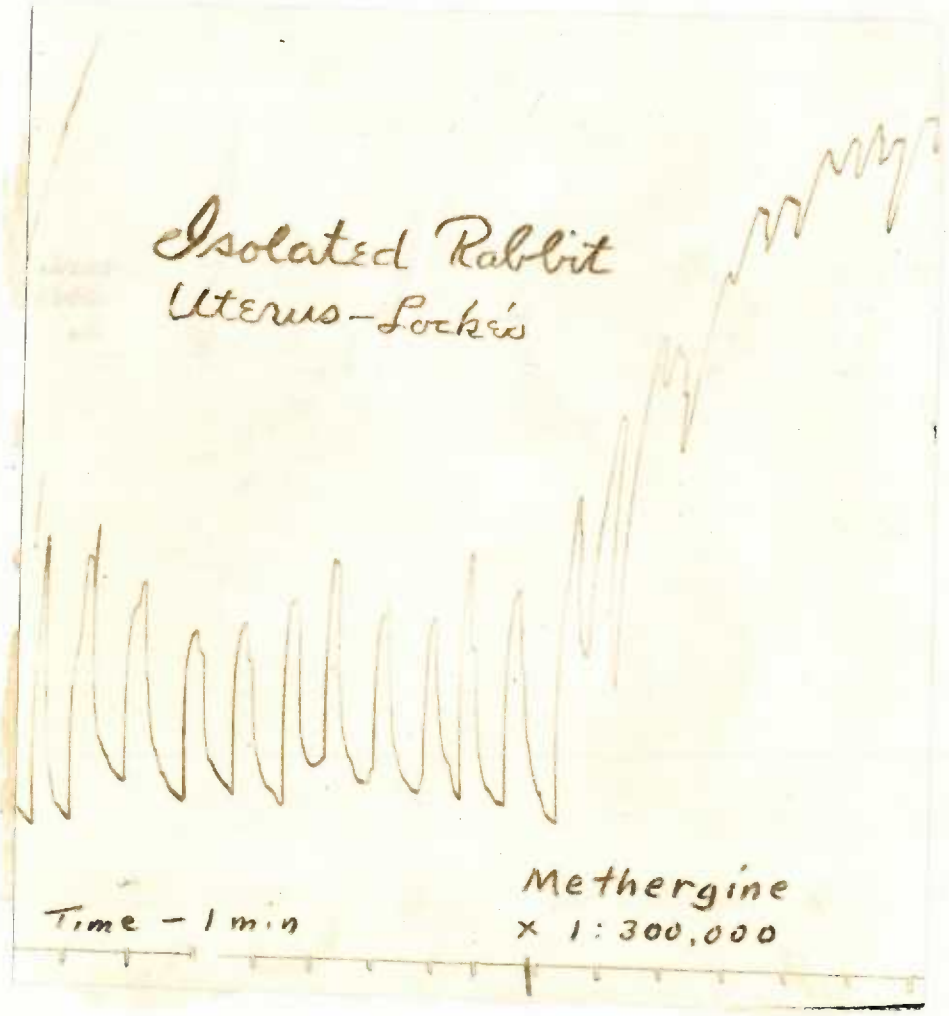
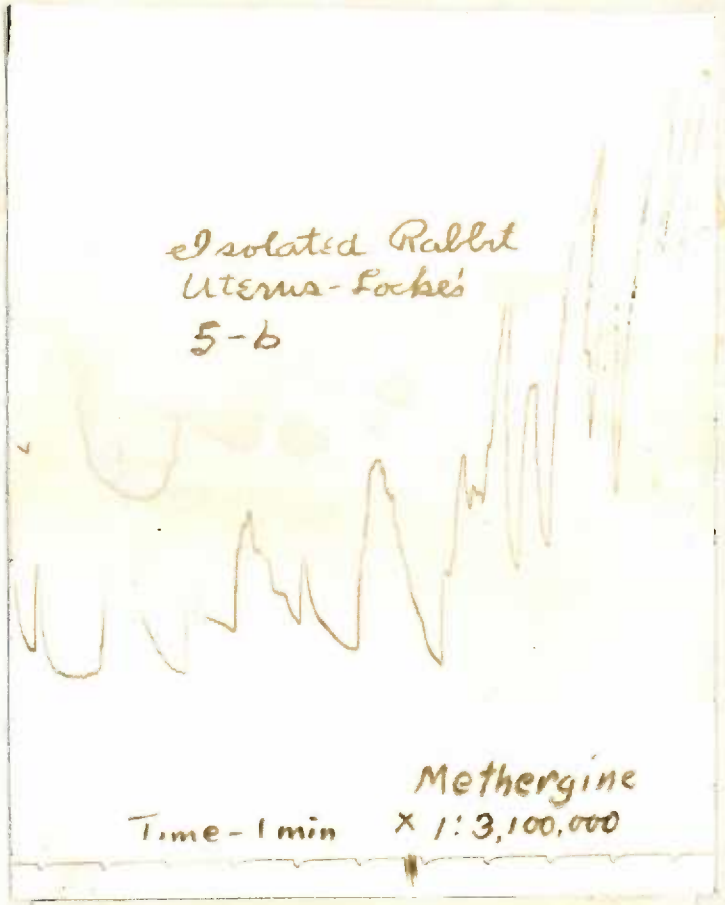

Isolated Rabbit
Uterus – Locke's
Methergine
× 1: 300,000
Time – 1 min

Fig. 14 Contact print from original smoked kymograph record. Isolated strip from rabbit uterus in Locke's solution. For purposes of comparison, the effects of Methergine and Ergonovine used in the same dilution, 1:3,100,000, and on the same strip are shown


Isolated Rabbit
Uterus - Locke's
5-b
Methergine
Time - 1 min
x 1: 3,100,000

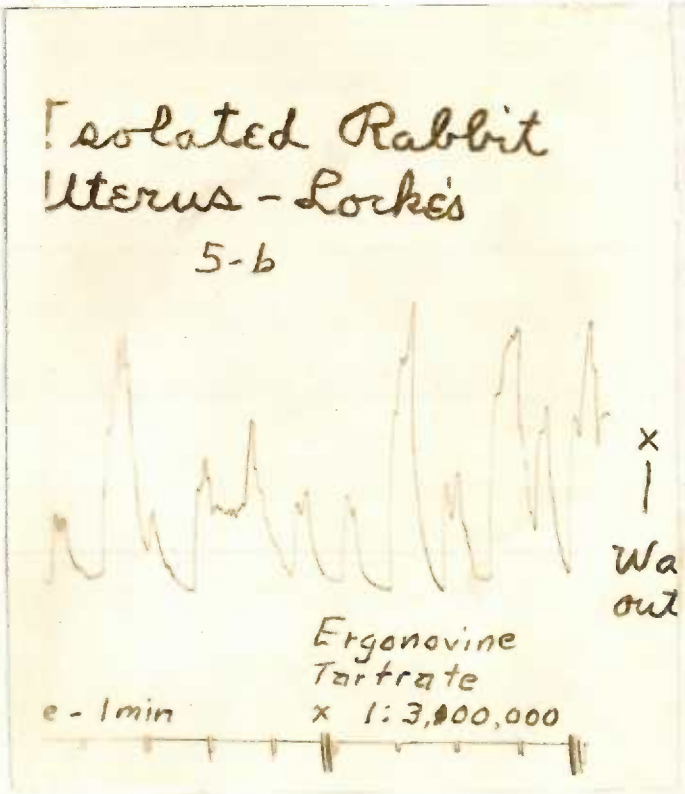
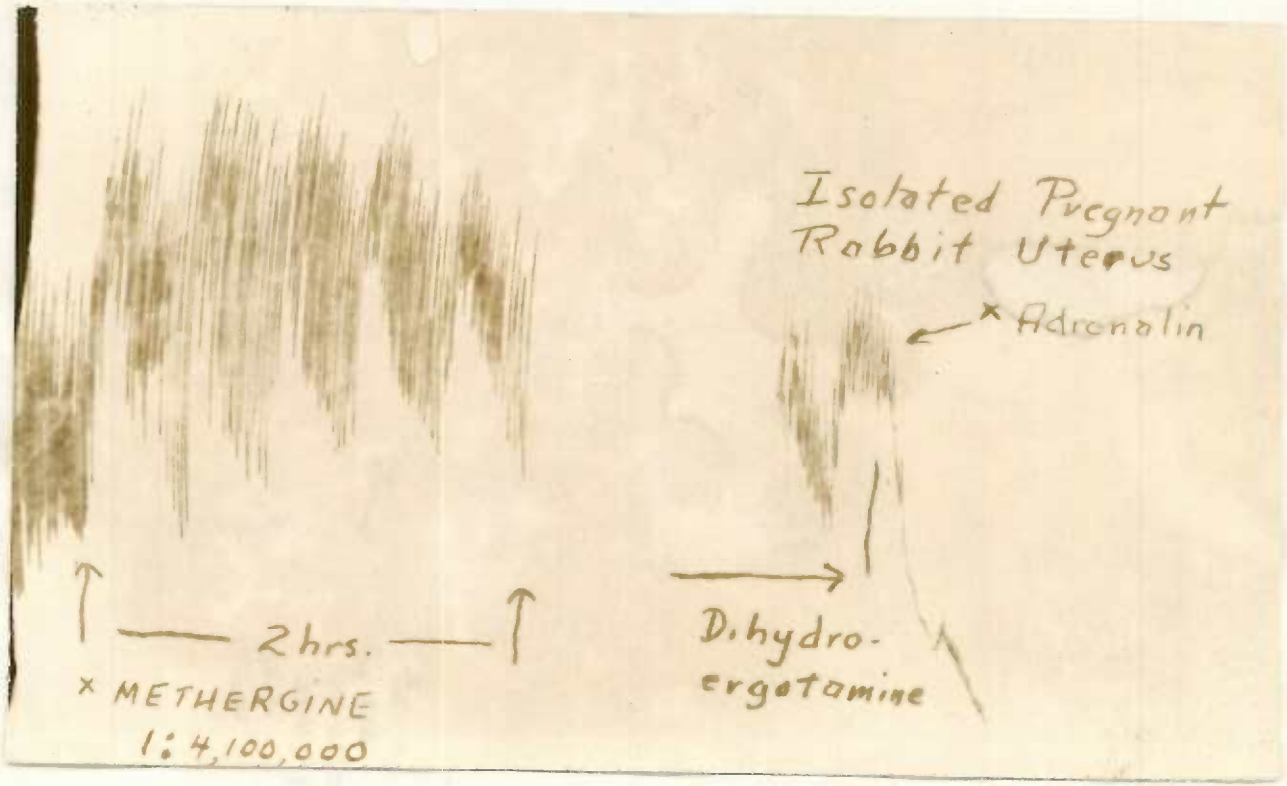

Isolated Rabbit
Uterus - Locke's
5-b
x
1
Wa
out
Ergonovine
Tartrate
e - 1 min
x 1: 3,200,000

Fig. 15 Contact print from original smoked kymograph record. Isolated strip from pregnant rabbit uterus (20 days gestation). The first record (left hand) shows stimulation of strip when a dilution of 1:4,100,000 of Methergine is used. The second record shows the reversal of the adrenalin response by previous treatment with dihydroergotamine.

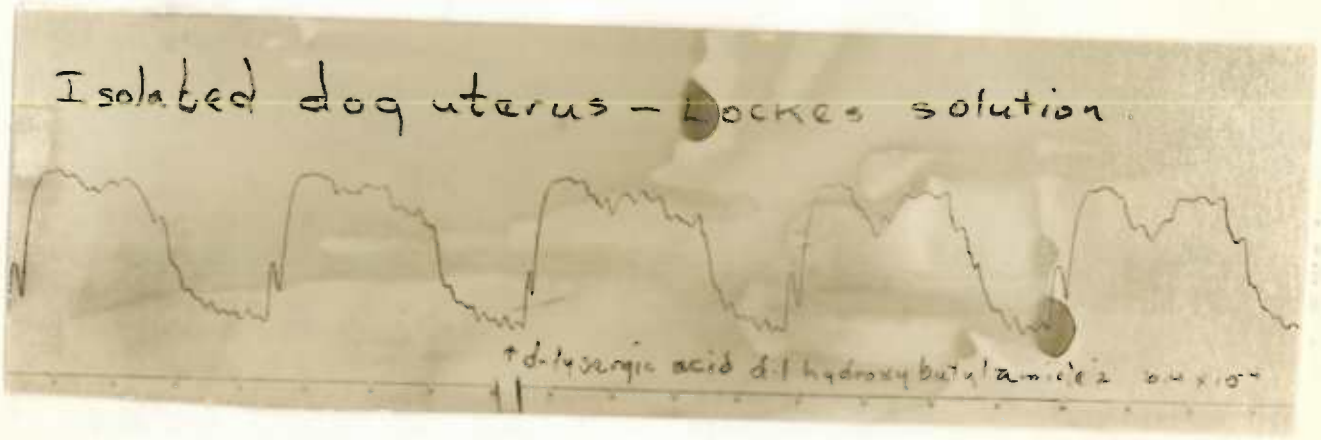

Isolated Pregnant Rabbit Uterus
× Adrenalin
2 hrs.
× METHERGINE 1:4,100,000
Dihydro-ergotamine

(3). Isolated Dog Uterus. The puerperal dog's uterus in situ has been found sensitive to ergonovine, but the virgin dog's uterus does not give a reliable response. An isolated strip of virgin dog uterus showed no effect to the administration of methergine (Figure 16). The same was true for ergonovine.

(4). Isolated Rat Uterus. It was found that the isolated rat's uterus was unresponsive also to methergine and to ergonovine even when used in high concentrations. It appeared that the administration of ergonovine and methergine relaxed the uteri somewhat, although this was not as marked as that occuring after adrenalin or trasentin. On the other hand, a parasympathetic stimulant, furfuryl tri methyl ammonium iodide caused contractile responses in the isolated rat's uterus.

b. Intact Rabbit Uterus., (1). Reynolds Preparation. Five multiparous rabbits were prepared by the Reynolds method (30) which consists of transecting the vagina, closing the distal end, and grafting the upper part just below the cervices to the abdominal wall. The ovaries were removed at the same operation and estrogenic substance (Theelin in oil, P.D. & Co.) administered daily. On the fifth post operative day the skin stitches were removed and on the seventh day the animal was ready to use for uterine recordings without anesthesia. Records were made by the Kirchhof and David (31) technic for photo-

Fig. 16 Contact print from original smoked kymograph record. Isolated uterine strip from dog's uterus in Locke's solution showing lack of response to addition of Methergine.

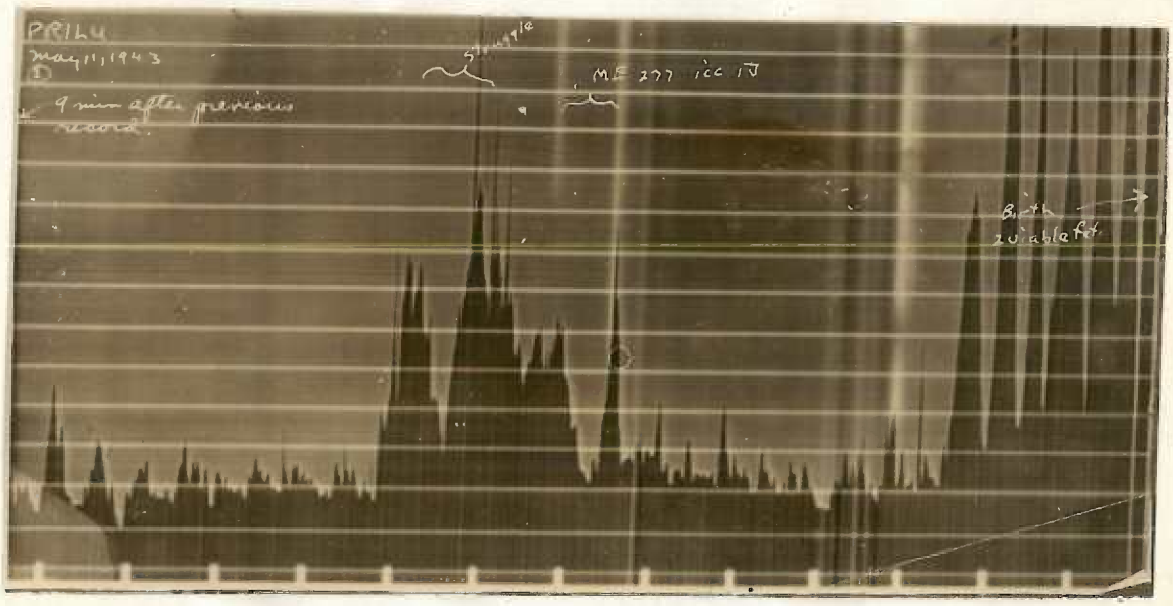

Isolated dog uterus – Lockes solution
+ d-lysergic acid diethylamide 2 x 10⁻⁷

graphic measurement. With this method, the manometer levels fluctuating in a fine glass tube record uterine volume changes of the degree of 0.1 cc. as a level change of 2 cms., a magnification of 20.

In each of the 5 animals studied, methergine produced an increased tone of the uterus but the contractions were decreased in amplitude and not of a periodic or regular frequency. The increased tone, which lasted for about 5 minutes after an intravenous injection of 0.05 to 0.25 mgm. of methergine, and the type of contractions produced were similar to those seen after ergonovine. The average displacement from the intra-uterine balloon in one horn was 0.5 cc. of water, the pressure in the balloon in all instances being maintained at 15 cms. of water.

(2). Pregnant Rabbit Uterus. The method of Oettel and Bachman (36) was modified for use on three pregnant rabbits. One week after breeding, the uterus was grafted to the abdominal wall as described above. Recordings were then made on the date of expected delivery. Figure 18 shows such a recording wherein it is seen that 4 minutes after administration of methergine contractions were initiated which resulted in the birth of 2 viable feti, a period of two minutes being taken from the start to the delivery of the first through the fistulous cervix. All of the three rabbits so prepared delivered in a short time after the administration of the drug, although no spon-

Fig. 18 Photograph (reduced) of photographic record obtained from pressure changes in an intrauterine balloon. Left uterus, pregnant rabbit at term. Four minutes following injection of Methergine (M.E. 277) two viable foti were born and intrauterine balloon expelled.


PRILU
May 11, 1943
D
9 min after previous record.
ME 277 icc IV
Birth
2 viable fet.

taneous labor contractions were present before the drug administration.

c. Studies on Humans., (1) Tocograph. A tocographic record was made on a primipara at term, but not in labor, in order to study the effects of oral administration of methergine on the uterus*. A solution of 0.2 mgm. per cc. was used, the dosage used being the same as the amount and schedule recommended for ergobasine (38). This was an oral dose of one minim followed in one-half hour by two minims, then after another one-half hour 3 minims, and then 3 minims not oftener than at one-half hour intervals for 5 more doses. The tocographic recording showed that with the 3 minim doses there was an increase of motility and tone starting from 5 to 10 minutes after administration of methergine and returning to normal in from 15 minutes to 30 minutes from the first observed activity. That this effect was not due to the onset of spontaneous labor contractions was shown by the fact that the patient did not go into true labor until 2 days later.

(2). Effects of drugs used at delivery. Another method used to estimate the effect of oxytocic drugs is the indirect one where measurement of the blood loss from the time of the delivery of the baby until the time

*This trial was made through the courtesy of Dr. Wm. Wilson and St. Vincent's Hospital.

of delivery of the placenta is made. Normally, without the contractile effect of oxytocic drugs on the uterus, about 300 to 400 cc. of blood is lost during this third stage of labor. The ability of an oxytocic drug to immediately contract down the partially emptied uterus results in a hemostatic effect inasmuch as this contraction of the uterus closes off many opened sinuses and bleeding spaces. The decreased loss of blood seen when an oxytocic is used may be measured or estimated. Estimated blood losses are not as reliable as measured blood losses, but, at present, facilities in hospitals are insufficient to allow for the extra work required to measure the blood loss and to separate the blood from the amniotic fluids by the several described methods, (39). However, where the person making the estimate of blood loss is a regular operator doing many deliveries each week, the estimate may show only a small error from the true measure. In this study, the blood loss from the time of delivery of the child to the birthof the placenta was estimated, and these estimates were made by Doctor William Wilson, who has had considerable experience in this work.

Methergine was given intravenously with the birth of the anterior shoulder of the baby in a dose of 0.2 mgm. Table 3 shows the average time from the administration of the drug to the time of the birth of the placenta. The cases presented here were consecutive with the exception of 4 which were deleted, 3 because the drug was given

intramuscularly instead of intravenously which would lead to confusion, and 1 because of post-partum hemorrhage from placenta accreta, later proven by pathological section and study. Since this latter case could not be considered due to uterine inertia, it was not included.

The usual time given by most authorities (40) for the birth of the placenta varies from 5 to 20 minutes as the average. From the time of injection of methergine with presentation of the anterior shoulder to the birth of the placenta, an average interval of 3.22 minutes was noted in the 22 cases. In this series of cases no patient showed delayed hemorrhage. Patients who were given methergine three times daily for three days post-partum showed no increase in temperature over that usually seen post-partum.

TABLE 4

DURATION OF THIRD STAGE OF LABOR AND BLOOD LOSS FOLLOWING ADMINISTRATION OF 0.2 MGMS. METHERGINE*

	AVERAGE	HIGHEST	LOWEST
DURATION OF THIRD STAGE	3.22 mins.	6 mins.	1 min.
BLOOD LOSS	150 cc.	350 cc.	75 cc.

*The drug was given intravenously at the time of the birth of the anterior shoulder. This series of patients were all delivered at Emanuel Hospital.

DISCUSSION

In this investigation on a new synthetic ergot-like drug, the most attention has been given to those aspects which appeared of the greatest practical interest. Consequently, emphasis has been placed on the action of methergine, in comparison with the ergot alkaloids, on the uterus and on the sympathetic nervous system. The guinea pig and rabbit uteri are the best experimental organs for demonstrating oxytocic activity since most oxytocic drugs effective in the human likewise produce a definite stimulatory action on the uterus of these two animals. Other animals are less suitable and dependable. Standard laboratory procedures were used in studying the uterine responses to these drugs with the exception of the technic developed in this laboratory for the photographic measurement of uterine contractions. This method is an improvement on the usual in situ methods for uterine study and is considerably more sensitive.

Methergine has been shown by these methods to be equal or superior in activity to ergonovine when studied in the guinea pig or rabbit. With further trial on humans, it was effective in a limited series of cases in reducing the time of delivery of the placenta and to minimize post-partum bleeding. Fatal toxicity could not be demonstrated in small animals in the range of doses used and administration to humans has been without untoward incident.

The side actions of methergine resemble those of ergonovine. There is a slightly stimulant action on the sympathetics in which respect this drug differs greatly from ergotamine. Because of this, its field of clinical usefulness would be limited to use in obstetrics. Sympathico-tonic diseases, such as migraine, could not be expected to be benefited by this drug. The lack of sympathicolytic action of methergine was demonstrated in the experiments described above on the circulatory studies on dogs, the failure of methergine to inhibit the adrenalin action on isolated uteri, and the ability of adrenalin to inhibit the contractions of the guinea pig intestine after methergine.

In studying the various actions of methergine many points of interest, some of less import than others, have been investigated. In this way a general overall picture of the more obscure actions along with the more important ones elicited by the drug has been provided to allow a better evaluation of this new oxytocic. With the information at hand, it is thus possible to draw certain conclusions as to the activity and potency of methergine in comparison with its congeners and to deduct from this information its possible usefulness clinically.

In addition, certain biochemorphologic conclusions may be arrived at as a result of these investigations. Lysergic acid, which, in itself, is not utero-active, may be,

in combination with an alkyl amide of low molecular weight, made utero-active and sympathico-inactive as is the case for ergonovine and methergine. Or, lysergic acid may be combined with more complex side chains to provide compounds such as ergotoxine and ergotamine which are both utero-active and sympathico-active. Further, the third possibility exists where by alteration of the ergotamine molecule a compound may be obtained which has lost its utero-activity but retained its sympathico-activity. Such a compound as this latter one would provide pharmacodynamically reverse actions to methergine. A compound of this type, possessing little or no uterine stimulation and good sympathico-activity has been provided this laboratory and is now being studied. This compound, dihydroergotamine (D. H. E. #45) has been used for its sympatholytic effects in several of the investigations reported in this thesis.

With compounds of this type, the two main therapeutic indications for the use of ergot alkaloids --- the oxytocic effect and the sympathicolytic --- could be made separate in their treatment. Post-partum hemorrhage and uterine atony could thus be treated with a drug more or less specific in its action on the uterus and having a minimal amount of side action. On the other hand, sympathico-tonic disorders such as migraine could be treated more effectively and without uterine side effects. That promise of realization of obtaining this selective action with an ergot-like

compound, insofar as it applies to treatment of sympathicotonic diseases is born out by Horton (41) who has made a brief report on the clinical trial of dihydroergotamine.

The final objective of ergot research was thought to have been achieved with the introduction of ergotamine in 1920. Yet this conception and complacency was completely upset in 1935 when ergonovine was discovered. Since then no great advance in the field of ergot research has been made. The recent literature has consisted mainly of elucidation of minor points and reviews of the literature.

It is entirely possible that with the demonstration of the pharmacodynamics of a synthetic ergot-like compound that another new era in ergot research and study will be opened up. If the biochemorphologic possibilities are not exploited, then, at least it is possible to conclude from this investigation that the production of one compound that appears as effective and non-toxic as ergonovine for post partum use has now been provided medicine by synthetic chemistry. It remains for the obstetrician, who is to use this compound practically, to be the final judge and make the final decision on this new synthetic after more clinical trial.

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