A PHARMACOLOGICAL STUDY OF USTILAGO

By

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A PHARMACOLOGICAL STUDY OF USTILAGO.

INTRODUCTION

Ustilago was defined in the United States Pharmacopoeia of 1880 (1) as the spores of Ustilago maydis, which are parasitic on various parts of the corn plant. It appears as irregular, globose masses, sometimes 15 centimeters thick, consisting of a blackish membrane inclosing innumerable brown-black, globular and nodular spores and possessing an unpleasant odor and taste.

This parasitic growth upon corn, commonly known in the United States as "smut", has doubtless appeared with the cultivation of maize from a very remote period, though the precise date of its earliest notice is difficult to ascertain from the literature.

Among the first botanists who studied this growth were Jussieu and Linnaeus (2). These investigators, in opposition to the views that had hitherto prevailed, recognized in the morbid growth a living organism of a fungoid nature. However, little was known concerning the structure and life cycle of the growth until Leveille (2), in 1837, presented a memoire to the Societe Philomatique of Paris, in which, in addition to the numerous observations on other species of Ustilago he gave a brief and clear account of "Uredo du Mais" or Ustilago maydis.

After Leveille (2) little was contributed towards a complete knowledge of these fungi until, in 1846, the Tulasne brothers presented an illustrated monograph embodying all the information then available as well as a complete account of the literature of the subject.

Since the time of the Tulasne brothers, various observers, among whom may be especially mentioned Fischer, de Waldheim, Wolf, Winter,

Farlow and Prillieux (2), have contributed largely to a more exact knowledge of this extensive and interesting group. Spalding (2) states that corn smut is of common occurence in the United States and its distinctive characters can hardly have escaped the observation of anyone who has ever passed through a field of corn affected by it. It appears in the middle or latter part of the summer, when the stalks of maize are making a vigorous growth and the young ears are beginning to form. It occasions irregular swellings, chiefly upon the young ears but also on the staminate flowers ("tassels") and stalks. The leaves which contain far less succulent tissue than the other parts of the plant are usually exempt from its attacks. These swellings are of various sizes, some no larger than a pea while others are "of the size of a child's head". The parts affected change in color and assume at first a glistening silvery hue, which becomes darker as the transformation proceeds. Finally, at maturity, the whole mass breaks up like a great puff-ball, discharging a cloud of dark colored dust, which when collected has the appearance of a brownish-black, impalpable powder and gives off a peculiar, narcotic odor.

The exact time of the original chemical investigation of Ustilago maydis is difficult to ascertain from the literature, but among the earlier investigators Cressler's (3) work appears to be the first.

Cressler (3), after a careful investigation, deduced that this drug contained secalin (referred to as propylamine), combined with an acid, a thick viscid fixed oil which precipitates a heavy deposit on standing, a light yellow resin, soluble in ether but insoluble in alcohol, a large amount of pectin, gluten, and a sugar which crystallizes in tufts of needle-shaped crystals from an aqueous solution and behaves like cane

sugar under the influence of Trömmer's test.

Parsons (4), after a very detailed analysis of Ustilago maydis concluded that this substance should contain interesting material from the medical point of view; these substances being a fixed oil, a volatile substance resembling an amine, and sclerotic acid.

Rademaker (5) reported that, since the acid he found contained no nitrogen it therefore differed from sclerotic acid and he called it maydisic acid, to which he gave the formula C_4H_7O. Upon microscopic examination its crystalline structure was that of prisms.

Rademaker and Fischer (6) found two per cent. of sclerotic acid, and stated that it is soluble in alcohol, ether, and water. No method of preparation was given. Parsons' (4) sclerotic acid was found to be insoluble in alcohol and ether. Rademaker and Fischer also obtained by dialysis of an alcoholic extract, from which alcohol had been removed and to which sulfuric acid had been added, a crystalline alkaloidal substance, ustilagine, the analysis of which was not made. Balch (7) could not duplicate this experiment.

Testoni (8) stated that he did not exclude in an absolute manner the possibility that Ustilago contained principles of an alkaloidal nature. He was certain, however, that with the currently employed methods they can not be isolated.

Recently, Langecker (9) conducted numerous experiments in the hope of isolating the active principle of Ustilago maydis, but he was not able to isolate any alkaloid.

A review of the literature indicates that the use of this drug was very extensive during the period of 1875 to 1890. It was considered to

be of sufficient merit to be included in the United States Pharmacopoeia, Sixth Decennial Revision. However, it was deleted in the Seventh Decennial Revision. The deletion may be attributed to the conflicting reports which arose following numerous clinical, chemical, and pharmacological investigations, and a consequent lack of acceptable proof of value.

In 1887, Leonard (10) reported that he had used the fluidextract of Ustilago maydis with success as a uterine tonic and in cases of amenorrhea and after labor. He (11) also reported that dogs and cows aborted when fed on infested corn. Crist (12) and Cooperiden (13) also reported the successful use of the fluidextract in dysmenorrhea. Goss (14) reported that as a parturient Ustilago institutes an action resembling natural labor, producing paroxysmal pains of the natural type. Vaillant (15) used the fluidextract in the treatment of uterine and ovarian tumors with more than usual success. Hale (16) reported that the fluidextract seemed to be quite an efficient parturifacient, giving a steady prolonged tonic contraction of the uterus instead of the "fitful grinding, tearing, cutting, irritating contractile pains so frequently observed after the administration of ergot of rye." He claims that it appears to stimulate more directly the longitudinal fibers - causing contraction of its long diameter - than it does the circular fibers.

Mitchell (17) reported that fluidextract of Ustilago maydis when injected into the posterior lymph sac of normal frogs produced abolition of cerebral consciousness and that the sensory portion of the spinal cord was paralyzed first, the reflex paralysis being due to this cause. The motor portion of the cord as well as the motor nerves were also depressed and ultimately paralyzed. He considered it is also probable that the

sensory nerves shared in the general paralysis.

Balch (7) found by feeding 3000 grams of Ustilago maydis in the form of an alcoholic extract to cockerels, that the color produced in the wattels was never persistent, and further, that this darkening was also seen during the following twenty-one days when no extract was given. From this experiment and like experiments he concluded that this drug was inert.

Bogdanovitch (18) reported that fluidextract of Jugoslavian Ustilago maydis possessed the following properties when administered intravenously: upon the dog, prolonged hypotension, fleeting depressive cardiac action, transitory paralysis of the pneumogastric, more durable paralysis of the renal vasoconstrictors, a short apnea followed by an accelerated respiratory rhythm and finally an inhibitory intestinal action. By perfusion of a preparation of a posterior motor vessel of the guinea pig, he found intense vasodilation. It produced augmentation of the tonus of isolated guinea pig intestine and with isolated rabbit intestine produced augmentation of the amplitude and tonus. It produced intense contraction upon the isolated guinea pig uterus. He also found that the pharmacodynamic effects of epinephrine were modified by the fluidextract of Ustilago maydis. The hypertensive action of epinephrine upon the dog was slightly diminished and its vasoconstrictor action upon the kidney was almost completely inhibited. The epinephrine inhibitory action upon the intestine of the dog in situ was more often inhibited and was occasionally transformed into an excitatory action. Likewise, the inhibitory action of epinephrine upon the isolated intestine of the guinea pig and rabbit was more often transformed into an excitatory action. Final-

ly, the vasoconstrictor action exercised by epinephrine upon the posterior motor vessels of the guinea pig is diminished. After his investigation he believed that Ustilago maydis should be closely related to ergot of rye, in that it exhibited certain similar pharmacodynamic actions.

Dragisic and Varicak (19) made a comparative study of aqueous extracts of Ustilago maydis (Jugoslavian) with commercial ergot preparations upon mice. The commercial preparations used were ergotamine tartrate and ergotin, an aqueous extract. They found that Ustilago maydis possessed great toxicity when compared with commercial ergotamine tartrate or ergotin. However, results were obtained with ergotin which were readily comparable to the aqueous extracts of Ustilago maydis. Finally, they believed that the similarity of the symptoms obtained indicated that Jugoslavian Ustilago maydis contained a large quantity of an ergotamine-like principle.

Since the literature reveals many conflicting reports concerning the activity of Ustilago maydis, a qualitative pharmacodynamic investigation of the drug was initiated. It was decided that investigation upon certain functions and organs of the intact animal, as well as upon isolated organs, such as blood pressure, uterine experiments in vivo and in vitro, isolated intestine, perfusion of blood vessels, etc., would be helpful in obtaining the desired information concerning the activity of the drug.

THE EFFECT OF USTILAGO ON THE BLOOD PRESSURE OF THE CAT

Two hundred grams of ground, mixed, air-dried ripe and unripe drug were first macerated for eight hours and then percolated with 1000 cc. of fifty per cent. alcohol until the drug appeared to be exhausted. The per-

colate at all times was kept in the refrigerator to prevent possible deterioration by light or heat. This percolate was finally adjusted by evaporation in a current of air, without heat, so that 1 cc. was equivalent to 1 gm. of drug.

A non-pregnant female cat, weighing 2.7 Kg. was anesthetized by an intraperitoneal injection of sodium amytal, and prepared in the conventional manner for a blood pressure determination. The right femoral vein was exposed and canualized for the purpose of injecting the drug. As may be seen in Fig. 1 A a control tracing was obtained before the administration of the drug to be tested. After obtaining the control, 0.3 cc. of the percolate was injected into the femoral vein. The results may be seen in Fig. 1 A. Inasmuch as light anesthesia was maintained throughout the experiment, slight movement of the animal was to be expected. Since the above dose produced an apparent transitory fall of the pressure, it was decided to section the right vagus nerve. The effect produced by this procedure may be seen in the accompanying figure.

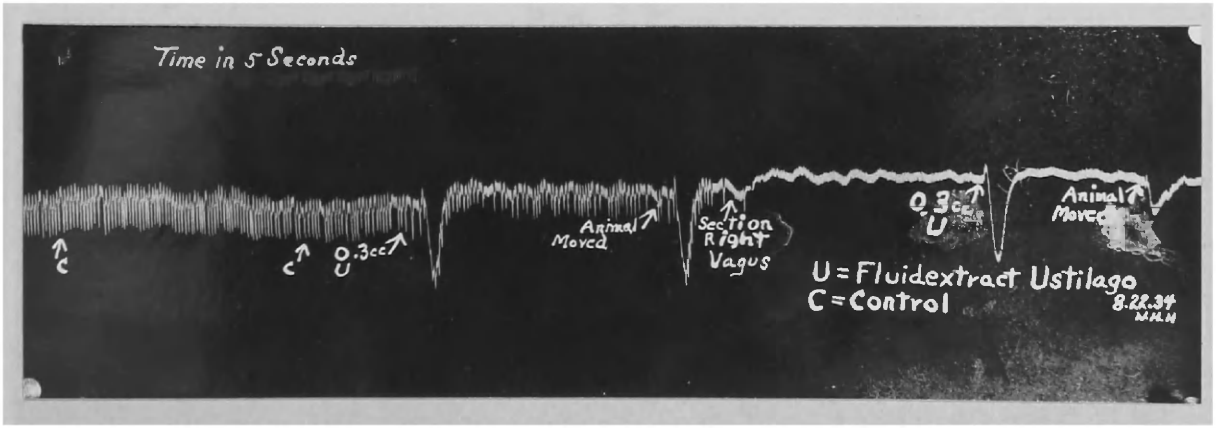

Fig. 1 A

Again a similar dose of the drug was given, which in turn duplicated the result obtained before section of the right vagus. The left vagus was then sectioned, the results of which may be seen in Fig. 1 B. Following this procedure o.3 cc. of drug was administered intravenously, producing a transitory effect similar to that noted before the section of the vagi. The dose was repeated to confirm the preceding result. Increased doses of the drug gave a correspondingly greater depressor effect. This is illustrated in Fig. 1 C.

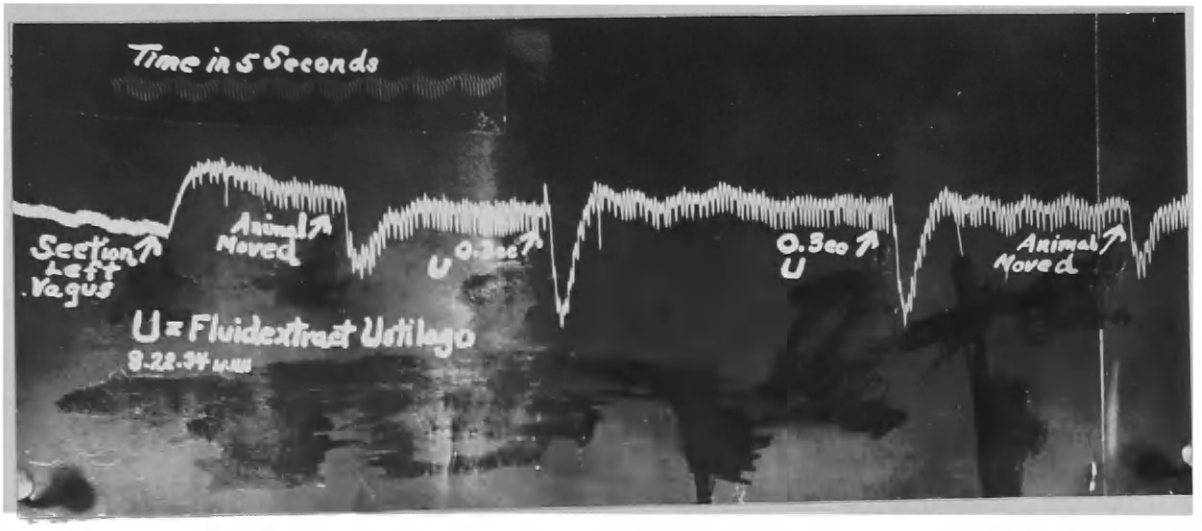

Fig. 1 B

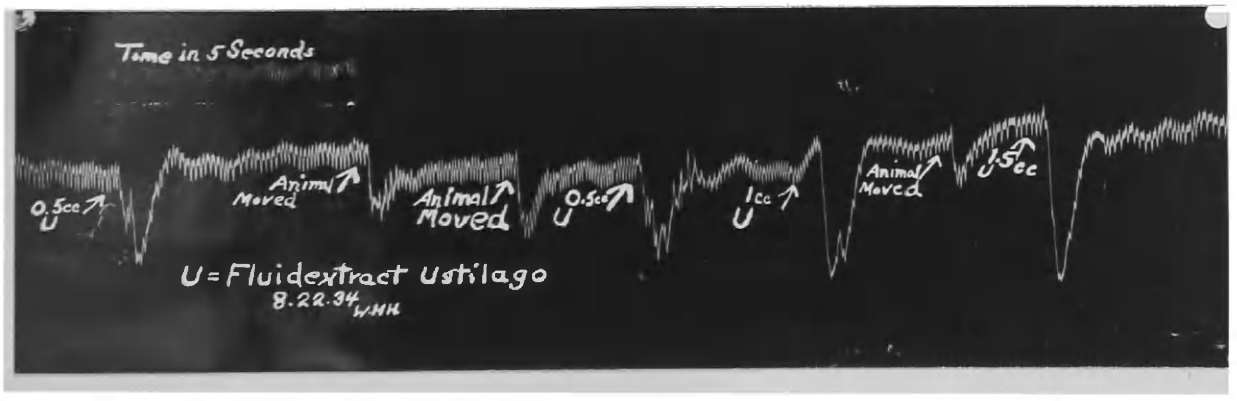

Fig. 1 C

The above experiment having shown that the drug possessed a principle which produced a transitory depressor effect, it was decided to determine the action of the fluidextract upon the pressor effect produced by epinephrine hydrochloride.

A non-pregnant female cat, weighing 2.65 Kg. was anesthetized with ether and prepared in the same manner as the preceding experiment. Repeated doses of epinephrine hydrochloride solution were administered intravenously until consistent results were obtained. As may be seen in Figs. 2 A and 2 B, the employed doses of fluidextract of Ustilago maydis exerted no "ergot-like" influence on the pressor response of epinephrine. In this experiment the vagi remained intact and again the depressor effect of the drug is clearly evident.

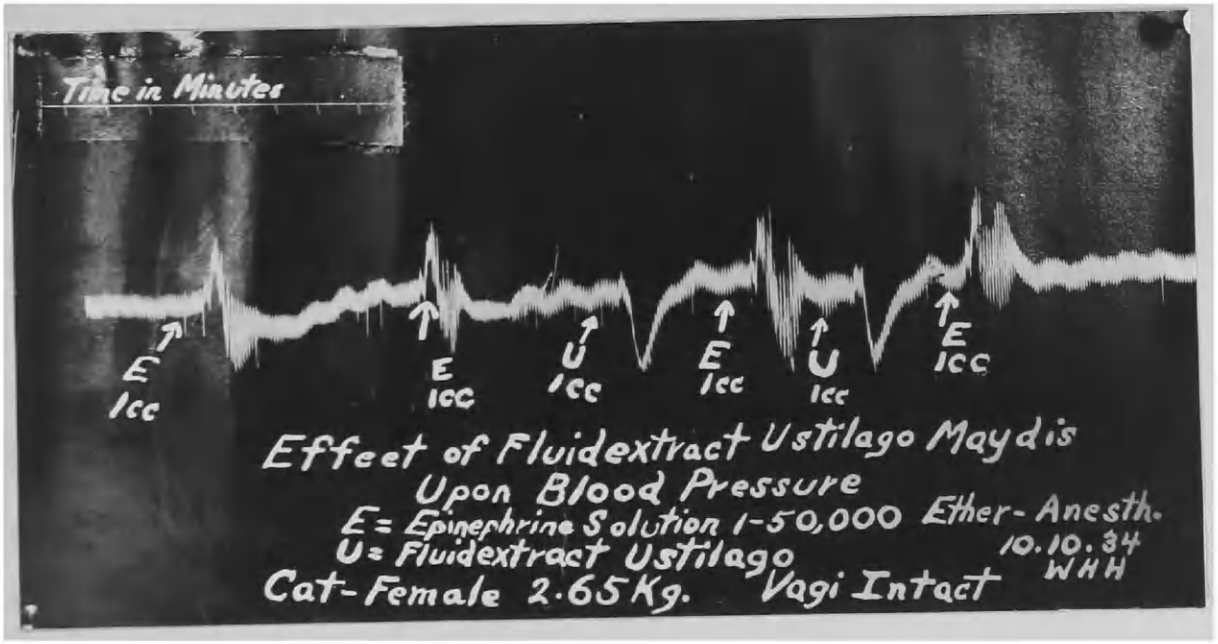

Fig. 2 A

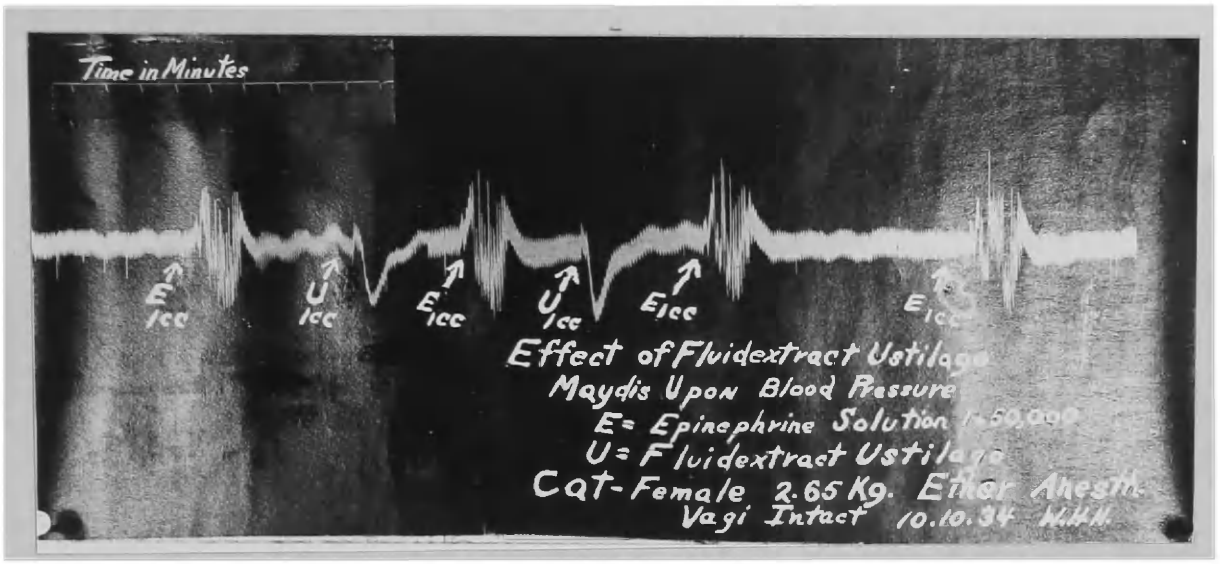

Fig. 2 B

Having seen the results of the fluidextract of Ustilago maydis upon the blood pressure of the cat whose vagi were sectioned, and in the case where the vagi remained intact, it was decided to determine the effect of the fluidextract following the administration of atropine sulfate.

A non-pregnant female cat, weighing 2.62 Kg. was anesthetized with sodium amytal and prepared in the manner of the preceding experiments. As may be seen in Fig. 3 B, following the intravenous administration of duplicate doses of epinephrine hydrochloride (1-50,000), 1 mg. per Kg. of atropine sulfate in distilled water was administered intravenously. After the blood pressure returned to a constant level, 1 cc. of epinephrine hydrochloride solution was administered intravenously until consistent results were obtained. At this time 5 cc. of the fluidextract, used in the previous experiments, was injected intravenously. As may be seen in Fig. 3 C a correspondingly greater and more prolonged depressor action was recorded. Two successive doses of epinephrine hydrochloride

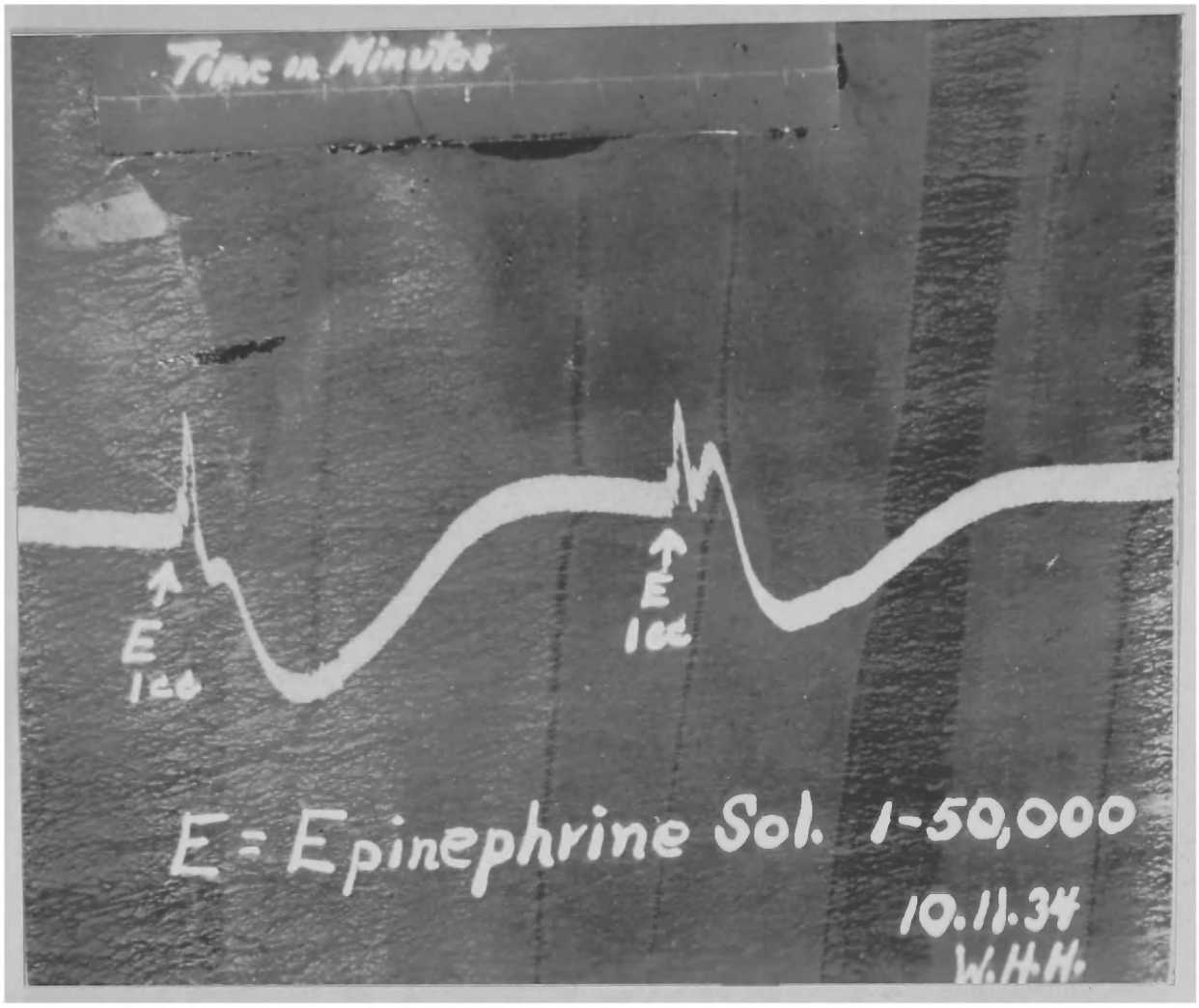
Time in Minutes
E
1cc
E
1cc
E = Epinephrine Sol. 1-50,000
10.11.34
W.H.H.

Fig. 3 A

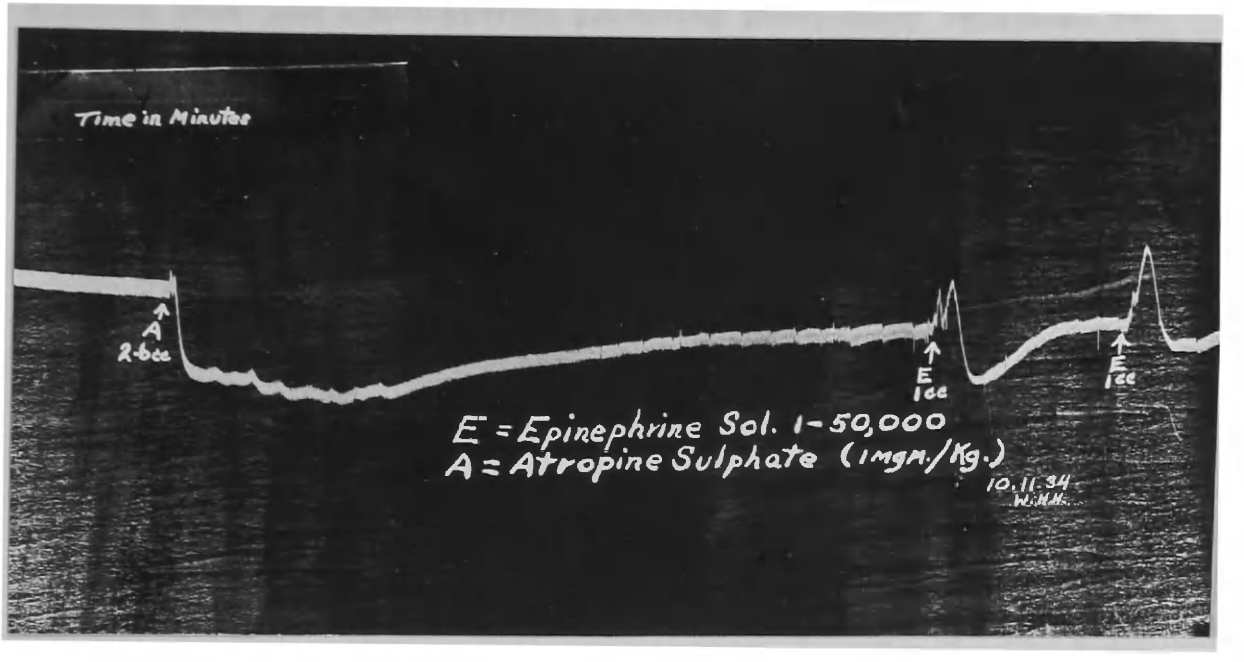
Time in Minutes
A
2-bcc
E
1cc
E
1cc
E = Epinephrine Sol. 1-50,000
A = Atropine Sulphate (1mgm./Kg.)
10.11.34
W.H.H.

Fig. 3 B

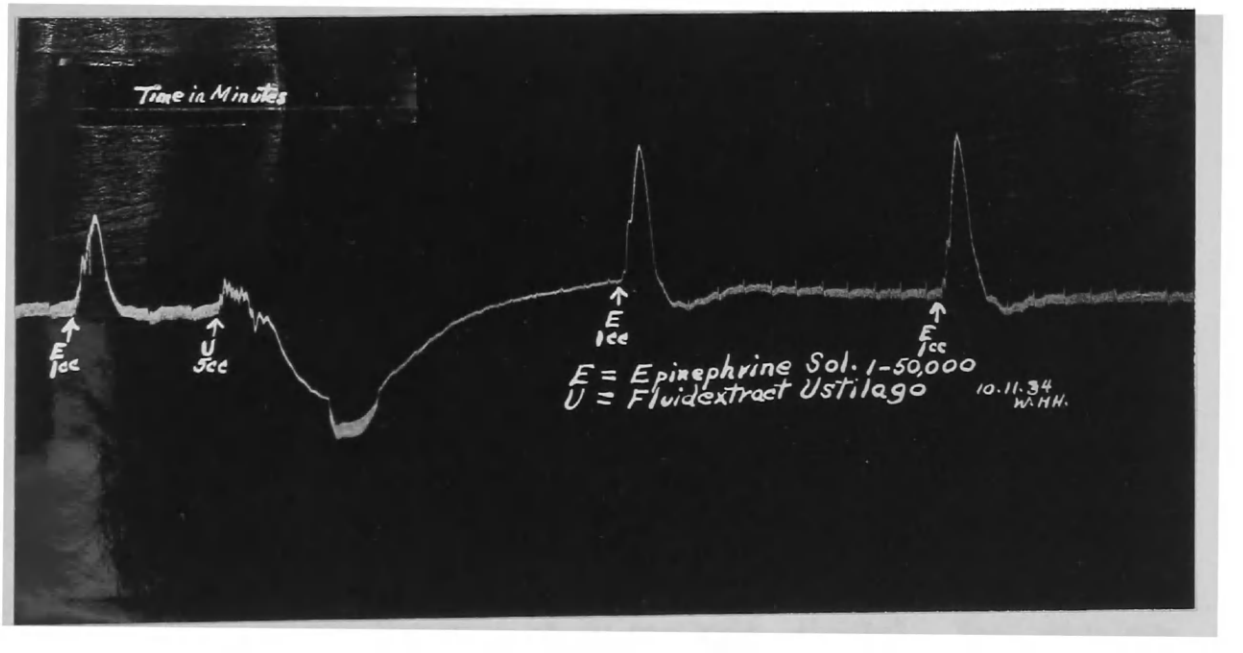
Time in Minutes
E
1cc
U
5cc
E
1cc
E
1cc
E = Epinephrine Sol. 1-50,000
U = Fluidextract Ustilago
10.11.34
W.H.H.

Fig. 3 C

solution were then administered, producing practically identical results. This confirmed the previous observation that the fluidextract produced no "ergot-like" influence on the action of epinephrine hydrochloride.

Since section of the vagi and the effect of atropine sulfate both inhibit vagal effects, as well as general parasympathetic effects, it appears that the depressor effect produced by the fluidextract is the result of an action upon a site other than the parasympathetic innervation of the heart and arterial system. Since the parasympathetic nervous system, which when stimulated causes a dilatation of blood vessels, is depressed by the action of atropine sulfate, and since the prior administration of the fluidextract neither inhibits nor augments the subsequent action of epinephrine hydrochloride (showing no action upon the sympathetic innervation of the blood vessels) it may be concluded that the fluidextract probably causes its depressor effect by acting directly upon the blood vessels themselves. In support of this view, a carotid blood pressure experiment upon a 3.1 Kg. cat showed that ergotoxine, in dosage sufficient to produce a "reversal" of the effect of epinephrine, failed to influence the depressor action of the fluidextract.

In an effort to determine the nature of the principle responsible for the depressor activity, ripe and unripe drug were thoroughly mixed and percolated with petroleum ether. After this extraction the marc was exhausted with a menstrum of 50 per cent. alcohol. The petroleum ether solution was allowed to evaporate until all traces of its odor were removed, after which the residue was taken up in 95 per cent. alcohol. This in turn, when evaporated, left a clear yellow oily-like

substance.

At the time of the percolation described above, 50 grams of unripe and ripe drug were percolated separately with 50 per cent. alcoholic menstrum. The volume was finally adjusted by evaporation, without heat, such that 1 cc. represented 1 gram of the drug.

A male cat weighing 2.8 Kg. was anesthetized with sodium amytal, administered intraperitoneally and prepared in the manner of the previous experiments. After obtaining a control, 1 mg. per Kg. of atropine sulfate was administered intravenously. Allowing a sufficient length of time for the completion of this reaction, 1 cc. of the clear yellow oily-like substance from the petroleum ether extraction, emulsified with saline, was administered intravenously. The result of this injection may be seen in Fig. 4 A. Since practically no effect was obtained from this dose, a larger dose, 2 cc. was administered. As shown in Fig. 4 B, a definite depressor action was produced. Thereafter, repeated doses of 0.75 cc.

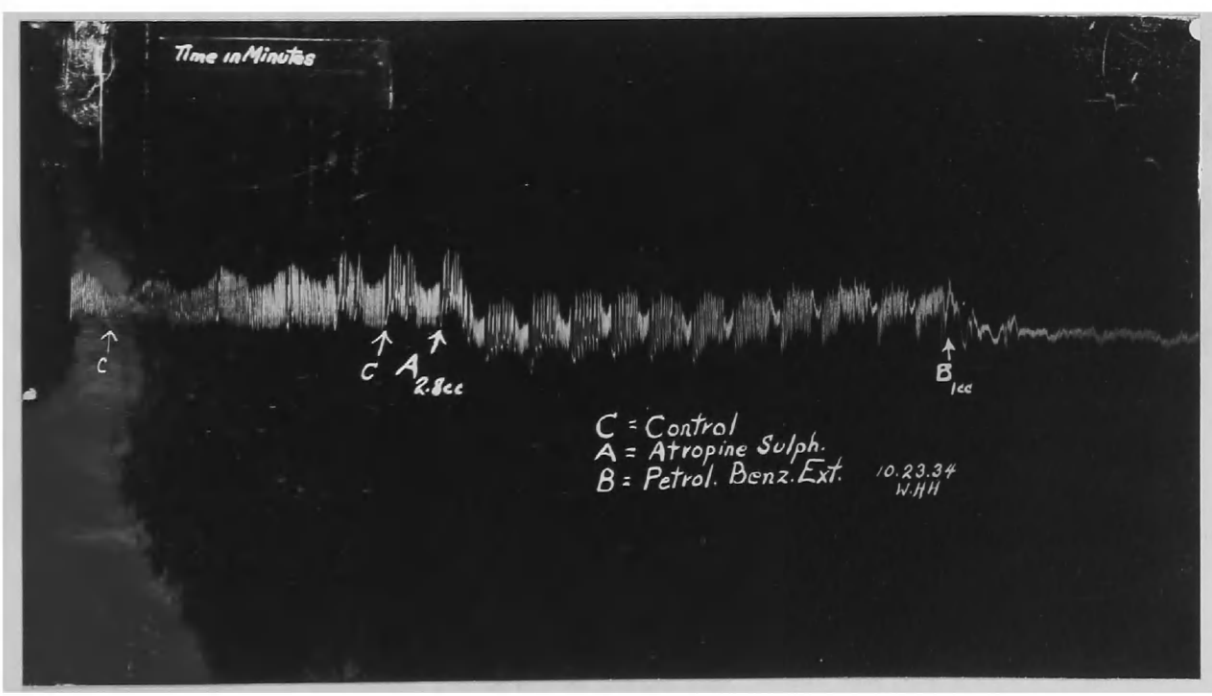

Fig. 4 A

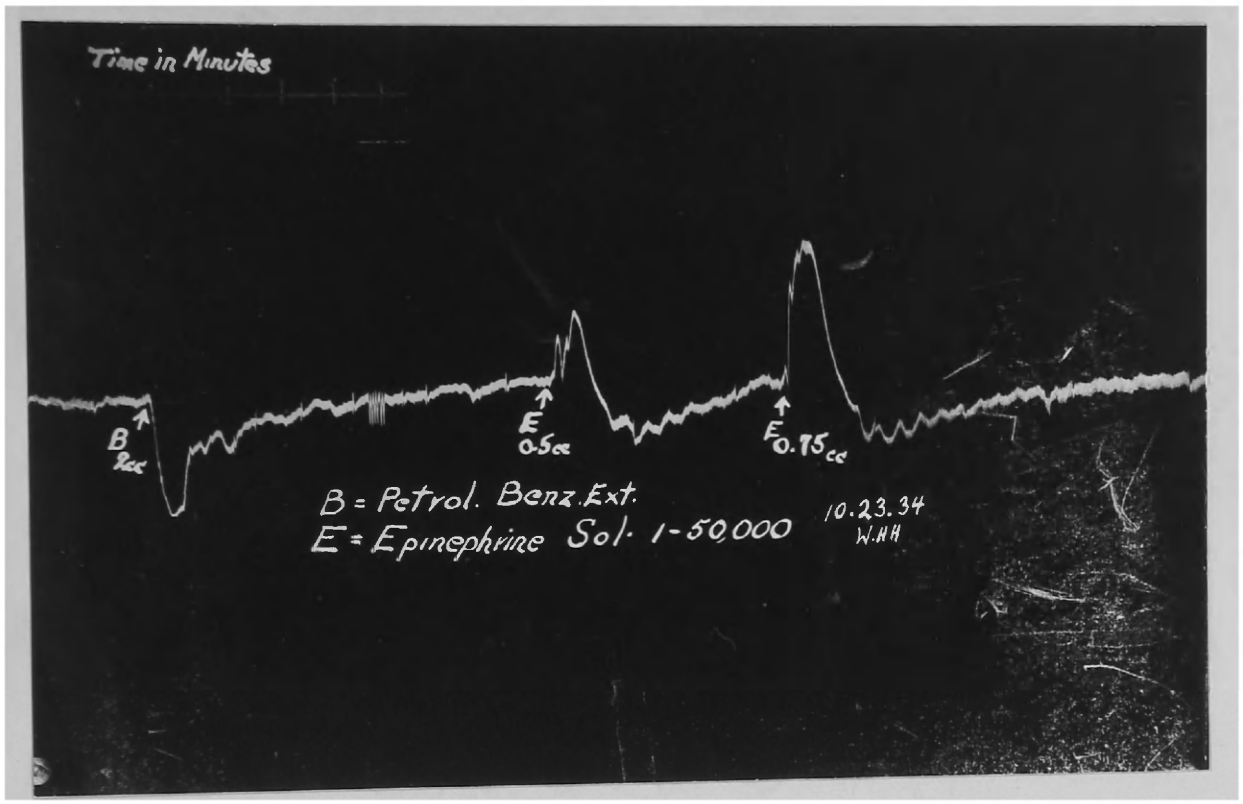

Fig. 4 B

of epinephrine hydrochloride (1:50,000), as shown in Fig. 4 C, produced responses similar to those produced before the fraction was given. Since the petroleum ether fraction neither inhibited nor accelerated the activity of epinephrine hydrochloride, it appeared that this extractive must likewise contain the same depressor substance as was present in the 50 per cent. alcoholic extraction. At this time 2 cc. of the 50 per cent. alcoholic extraction of the unripe drug was injected. This produced a definite depressor effect, as may be seen in Fig. 4 C. The effect of 0.75 cc. of the epinephrine hydrochloride solution was again apparently not modified. Repeated doses of both the extractive and epinephrine hydrochloride solution were given and these confirmed pre-

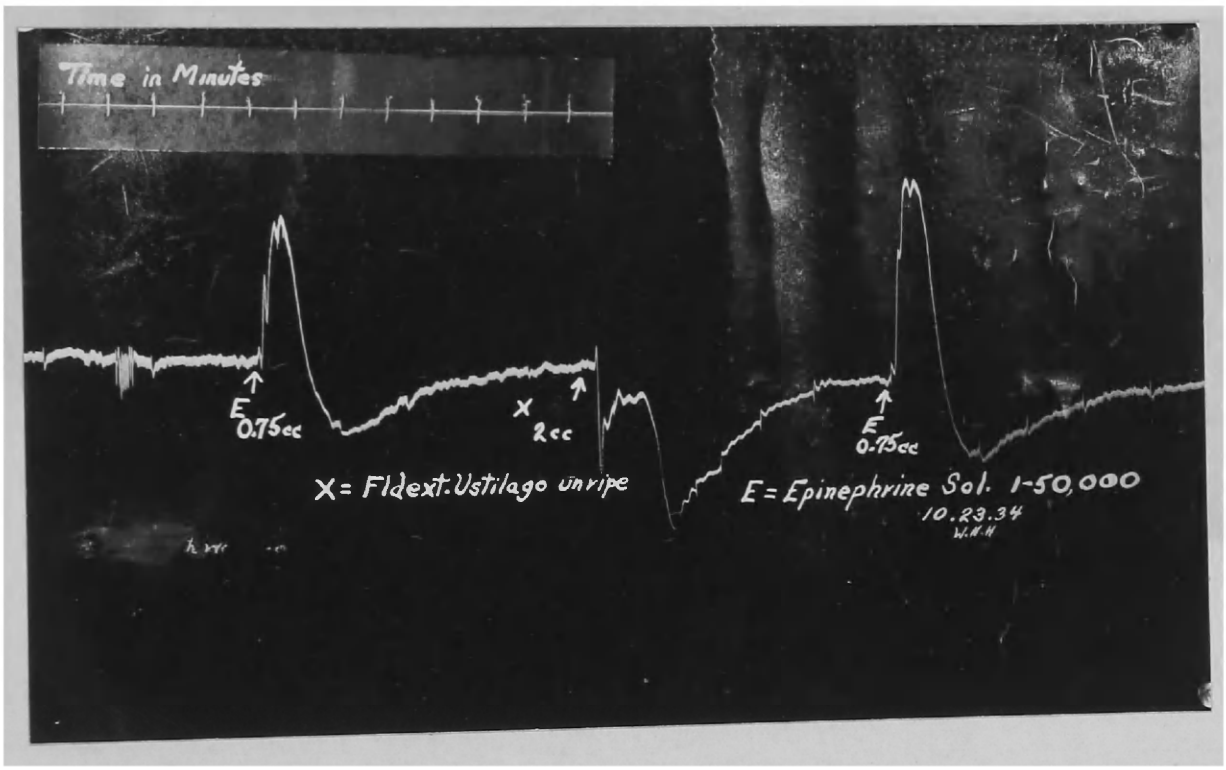

Fig. 4 C

vious observations, as may be seen in Fig. 4 D. A 2 cc. dose of the ripe drug extractive was then administered intravenously and the results obtained were almost identical with those obtained from the unripe drug. These results may be seen in Fig. 4 D. A repetition of the dose duplicated the original response. Two more 2 cc. doses of the unripe drug extractive were injected alternately with doses of 0.75 cc. of epinephrine hydrochloride solution (Fig. 4 E). The results confirmed the previous observations on the activity of this preparation.

Using the same preparations of Ustilago maydis as were used in the above experiments upon cats, confirmatory evidence was obtained upon a dog. The animal was anesthetized with chloretone, administered intraperitoneally, and supplemented with phanodorn intravenously. The animal

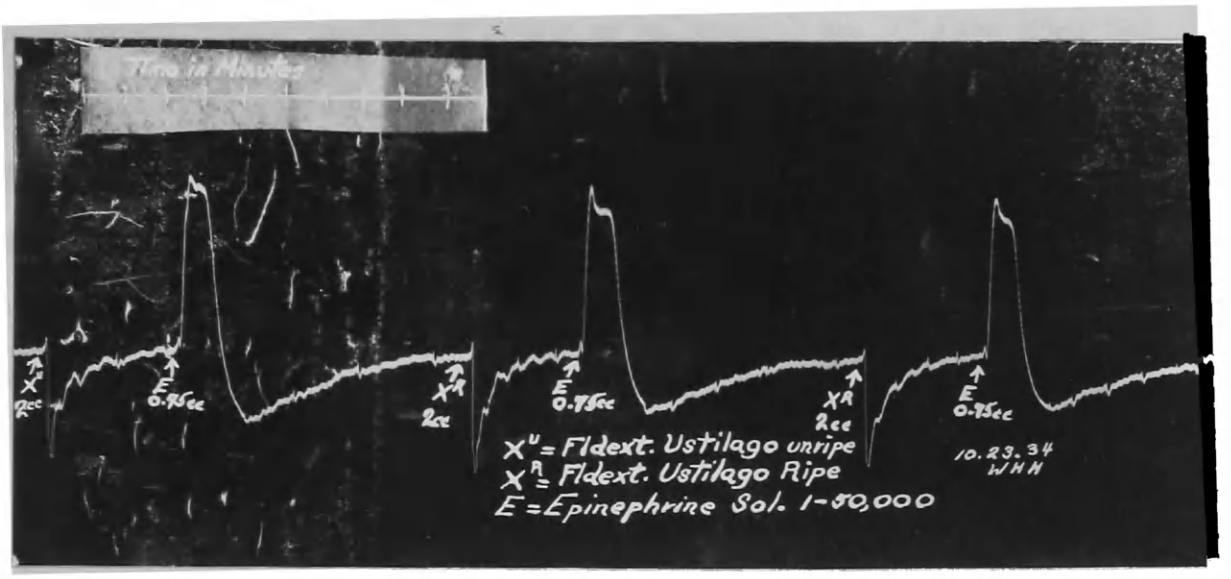
Time in Minutes
E
0.75cc
X^R
2cc
E
0.75cc
X^R
2cc
E
0.75cc
X^U = Fldext. Ustilago unripe
X^R = Fldext. Ustilago Ripe
E = Epinephrine Sol. 1-50,000
10.23.34
WHH

Fig. 4 D

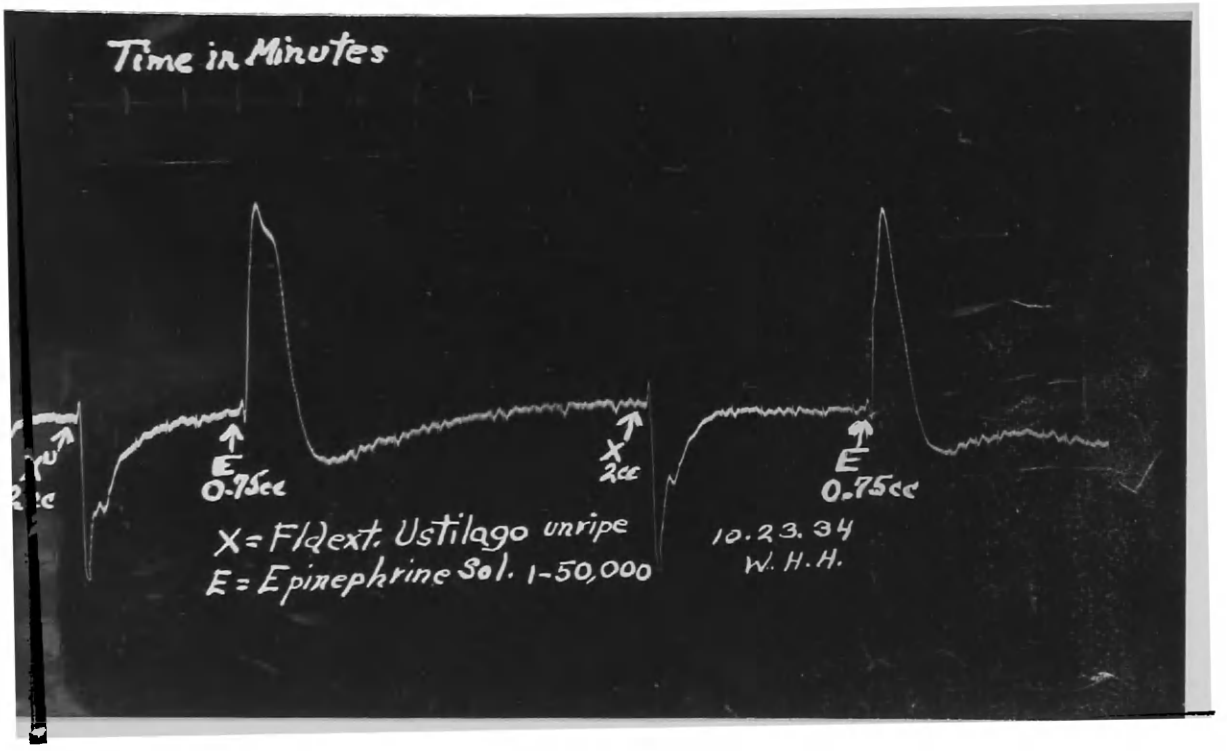
Time in Minutes
E
0.75cc
X
2cc
E
0.75cc
X = Fldext. Ustilago unripe
E = Epinephrine Sol. 1-50,000
10.23.34
W. H. H.

Fig. 4 E

also received 1 mg. per Kg. of atropine sulfate. 1 cc. of ripe drug extractive was administered, which, as may be seen in Fig. 5, produced a slight pressor action. It may be noted that upon the injection of extracts of Ustilago maydis a slight pressor response preceded the depressor effect. Following this a 2 cc. dose of unripe drug fluidextract produced the characteristic depressor effect.

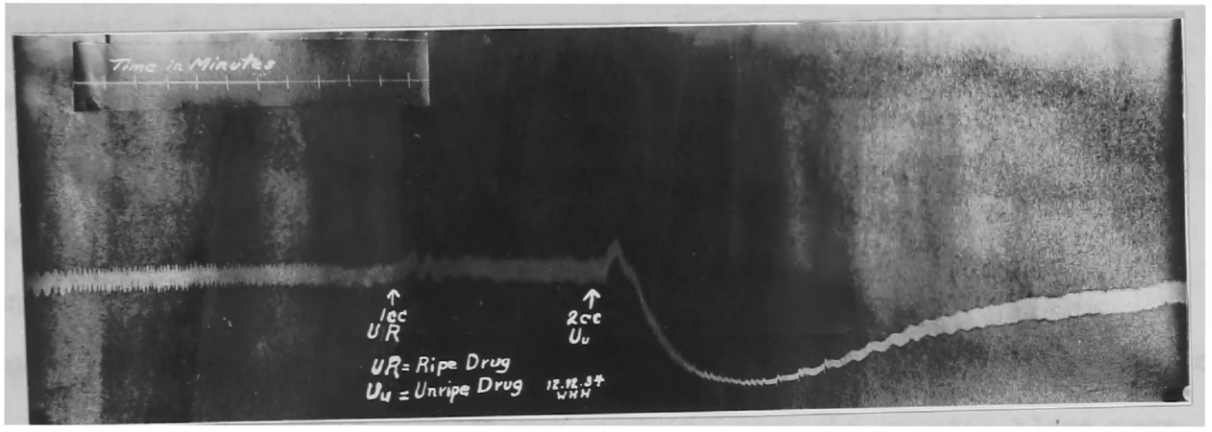

Fig. 5

The above observations indicated that the principle responsible for the depressor activity of Ustilago maydis is soluble in both 50 per cent. alcohol and petroleum ether.

The presence of one or more alkaloids in Ustilago has been suggested from time to time by various workers. Since the above experiments indicated that the activity resided in a non- alkaloidal fraction (solubility in petroleum ether), this problem was pursued further. 100 grams each of ripe and unripe Ustilago maydis were thoroughly mixed and macerated with one liter of ethyl ether, which was added in 75 cc. portions over a period of two days. To this mixture, 30 grams of magnesium oxide were added to alkalinize the solution. After thoroughly shaking for ap-

proximately thirty minutes the solution was allowed to stand for separation of the layers. The ethereal solution was then drawn off and shaken with 1 per cent. tartaric acid solution. The acidified aqueous portion was then separated and tested for the presence of alkaloids by the following alkaloidal methods: to a portion, on a spot plate, a drop of N/10 iodine test solution was added, yielding a very slight fine precipitate. Mayer's Reagent was then tried in the same manner and this produced an extremely fine precipitate which was barely perceptible. Finally, tannic acid was used as a test solution and a negative result was obtained.

The above acid aqueous solution, which should have contained any alkaloids present in the drug, failed to produce any significant effect upon the isolated rabbit uterus in doses representing concentrations of the drug as high as 1:50 in the tissue chambers. Similarly this solution proved to be devoid of significant activity upon the carotid blood pressure of anesthetized cats.

The above tests, it is believed, rather conclusively show that the activity of the drug is not derived from constituents of an alkaloidal nature.

ACTION ON COCKSCOMB

The cockerels used in the following experiments were those used routinely in this laboratory for assaying ergot by the U. S. P. method. 25 cc. of a solution prepared by percolating a mixture of ripe and unripe drug with 50 per cent. alcohol was evaporated to semi-solid consistence, without heat, and then taken up in 1 cc. of 25 per cent. alcohol. 1 cc. of this solution represented 5 grams of drug. 0.8 cc. of this solution was injected into the breast muscles of the cockerel. Into the

second cockerel 0.75 cc. of a like solution was injected, representing 3.75 grams of drug. Into a third cockerel 0.74 cc. of a like solution was injected, representing 3.7 grams of drug. The results of these experiments may be seen in Table I. At a later date 4 cc. of fluidextract representing 4 grams of drug was injected into the breast muscles of a white Leghorn cockerel. The results of this experiment may be found in Table I. At the time of an evaporation of a 95 per cent. alcohol percolate, some of this concentrate was injected into the breast muscles of a cockerel. 1 cc. of this solution represented 10 grams of drug. 1 cc. of this solution, made from unripe drug was injected. The results may be seen in Table I. 2 cc. of the same solution was injected into another bird. A comparison of these results showed a definite disagreement. To check this work 2 cc. of the same solution was injected into the same bird which had received the 1 cc. dose and a positive response was noted. This showed that the threshold of the bird which first received the 2 cc. dose was much greater than the one receiving the 1 cc. dose. After evaporating the alcohol from the fluidextract the heavy viscid oily fraction remaining was used for the next test. 1 cc. of this substance, representing 5 grams of the drug, was injected into the cockerel which had originally received the 1 cc. of the fluidextract. The results may be seen in Table I.

From the above results it appears that some substance present in the drug caused a bluing of the combs of these cockerels. It was observed that this bluing was different from that of ergot in that there appeared to be a stasis of blood in the comb due to excessive dilation rather than constriction of the blood vessels. Further, the blanching of the comb

TABLE I

Date	Animal	Weight	Dose in Gm. of Drug	cc. of fluid injected	Drug from which extraction was made and when extracted	Type of Solution	Orig. Reading	Reading 1 hr.	Reading 2 hr.	Reading 3 hr.	Reading 4 hr.	Reading 6 hr.	Next Morn.	2nd Day
11/16/34	White Leghorn Cockerel	1.95 Kg.	4.0 Gm.	0.8 cc.	Unripe 1934 Maryland 7/25/34	25 per cent. alcoholic	001	001	112	113	113	012	---	---
11/16/34	"	1.17 Kg.	3.75 Gm.	0.75 cc.	"	"	001	001	112	112	112	011	---	---
11/16/34	"	0.80 Kg.	3.7 Gm.	0.74 cc.	"	"	001	001	112	112	123	011	---	---
2/14/35	"	1.63 Kg.	4.0 Gm.	4.00 cc.	"	"	012	012	012	012	---	---	---	---
10/27/36	"	1.87 Kg.	10.0 Gm.	1.00 cc.	Unripe 1936 Maryland 10/7/36	95 per cent. alcohol	000	011	012	---	---	---	112	001
10/28/36	"	1.25 Kg.	20.0 Gm.	2.00 cc.	"	"	000	100	111	---	---	---	001	001
10/29/36	"	1.87 Kg.	20.0 Gm.	2.00 cc.	"	"	001	011	112	---	---	---	223	123
11/16/36	"	1.15 Kg.	5.0 Gm.	1.00 cc.	Ripe 1936 Maryland 10/30/36	Oil fract. after evap of alcohol	111	123	123	---	---	---	111	---

characteristic of ergot action was not observed, nor was there fluffing of the feathers (which is pronounced in ergot action). It appears therefore, that although Ustilago can cause a cyanosis of the cockscomb, its action is relatively weak and possibly qualitatively dissimilar to ergot in this effect.

EFFECT OF USTILAGO ON PREGNANCY IN CATS

A fluidextract was made from 500 grams of an equal mixture of ripe and unripe drug by percolation with 95 per cent. alcohol until the drug was exhausted. The percolation of this preparation was preceded by forty-eight hours of maceration with the solvent. Following the percolation the solution was adjusted to fluidextract strength by evaporation with the aid of a current of air and without heat.

Table II embraces the results obtained in these experiments.

As may be seen in Table II, small doses of the drug failed to produce abortion and, furthermore, failed to produce any other symptoms of activity in the animals. By increasing the dosage sufficiently, an abortive action was clearly demonstrable. Of striking significance is the influence of the different solvents on the activity observed. The alcoholic extracts and the olive oil suspensions were possessed of definite abortive activity, whereas the aqueous solutions, prepared by removal of alcohol from the extracts without heat and diluting with water, and using only the clear supernatant aqueous liquid, were apparently quite devoid of any type of activity.

Although it was difficult to ascertain the stage of pregnancy in the animals, it was apparent that the drug was more effective in ter-

TABLE II

Date	Cat Identity	Dose in grams of crude drug	cc. of fluid injected and area	Drug from which ext. was made and date of extraction	Type of Solution	Result	Remarks
2/25/35	Black	0.5 gm	2 cc. Sub. Q. Abdomen	R., U., Equal parts 2/15/35	Alcoholic	—	
2/25/35	Black	2.5 gm	5 cc. Sub. Q. Abdomen	E.U.; L.U. L.R. in like parts 2/15/35	"	—	
7/8/35	Gray-White	30.0 gm	10 cc. Sub. Q. Thigh	Equal parts R., U. Percol. with Fldext. 2/15/35	"	+	3 Hairless Kittens. Dead
7/8/35	Black-White	"	"	"	"	+	2 Kittens with hair at night 1 in morn All Dead
7/25/35	Maltese	10.0 gm	"	Equal parts R. and U. 3/25/35	"	—	Close to term
"	"	"	"	"	"	+	3 Kittens fine hair All Dead
8/13/35	Grey	"	"	"	"	—	Close to term
5/13/36	Black	100.0 gm	"	Equal parts 1934-35 Collect. 4/23/36	Water Emulsion	—	
5/14/36	Same Cat As 5/13/36	"	"	"	Olive Oil	+	4 Kittens alive but from size cat not at full term
5/22/36	Maltese	"	"	"	Water Emulsion	—	
5/25/36	Same as 5/22/36	"	"	"	Olive Oil	—	Close to term
7/28/36	Maltese	20.0 gm	20.0 cc. 10.0 cc. Sub.Q. in each thigh	Equal parts 1934-35 Collect. 5/27/36	Olive Oil	+	Aborted during night 7/29/36. 4 Kittens about 2 weeks of pregnancy
11/18/36	Tiger Stripe	33.0 gm	6.6 cc. Sub. Q. Thigh	Ripe 1936	Oil Fraction after evap. of alcohol	—	Close to term. Animal died 11/21/36, without giving birth to kittens. Blood in nose and mouth.

R = Ripe; U = Unripe; E = Early; L = Late; Collect. = Collection

minating early pregnancies than those approaching full term.

The depressor effects obtained in the blood pressure experiments suggested the possibility of an acetylcholine like principle as being responsible for the abortive action. However, the results in Table III show acetylcholine, even in enormous doses, to be ineffective in terminating pregnancy in the cat. The insolubility of the active principle in water also proves lack of similarity to cholines. That acetylcholine is quite devoid of significant oxytocic action has been shown in other reports from this laboratory (20).

EFFECT OF USTILAGO ON CAT UTERUS IN SITU

In attempting to record the drug action upon the non-pregnant uterus it was found that the tissue was non-responsive to varied doses of the drug when injected subcutaneously or intravenously. After six ex-

TABLE III

Date	Cat Identity	Dose in mg. of drug	cc. of fluid injected and area	Type of Solution	Result
8/26/35	Grey	0.5 mg.	1 cc. Sub. Q. Thigh	Aqueous	—
8/27/35	Same as 8/26/35	4.5 mg.	"	"	—
9/3/35	Same as 8/26/35	25.0 mg.	"	"	—
9/4/35	Same as 8/26/35	500.0 mg.	"	"	—

periments upon six different cats this type of approach was abandoned.

Experimental work upon the pregnant cat uterus in situ showed that there was a slight drug action, which, however, was not sufficiently consistent to accept as conclusive evidence of definite uterine activity. Inasmuch as a slight activity was noted it should be mentioned that the animals used were in the late stage of pregnancy. Animals in the earlier stages of pregnancy were not available for these experiments.

However, the experimental work upon the puerperal uterus in situ clearly showed oxytocic activity. The animal used in the experiment illustrated in Fig. 6, had delivered two live kittens twenty-four hours before this experiment. The fluidextract used in this particular experiment was made from an equal mixture of ripe and unripe drug, percolated with 50 per cent alcohol. The animal was anesthetized with phanodorn (40 mg. per Kg.) which was administered subcutaneously. The drug was administered intravenously. As may be seen in Figs. 6 A, B, C, D the drug produced a definite action upon the uterus.

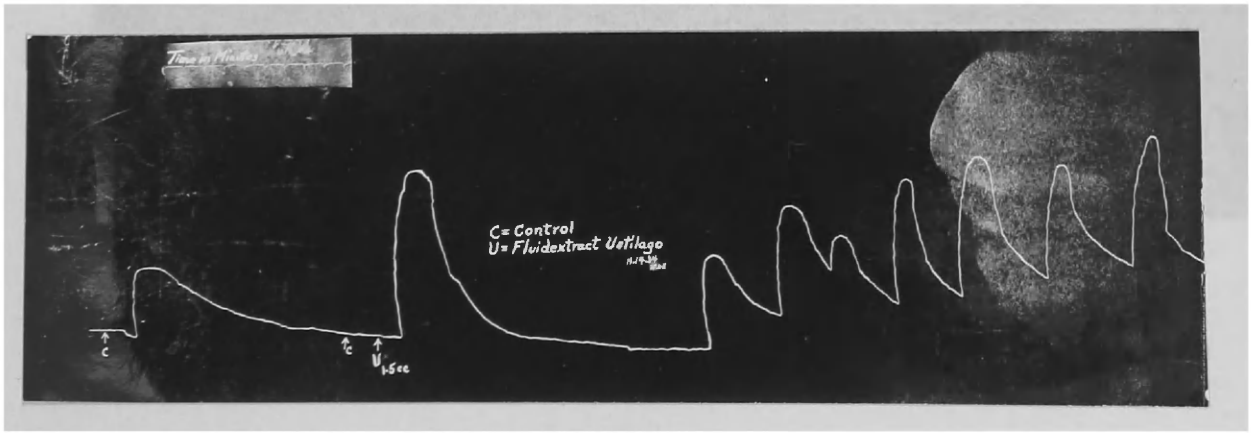

Fig. 6 A

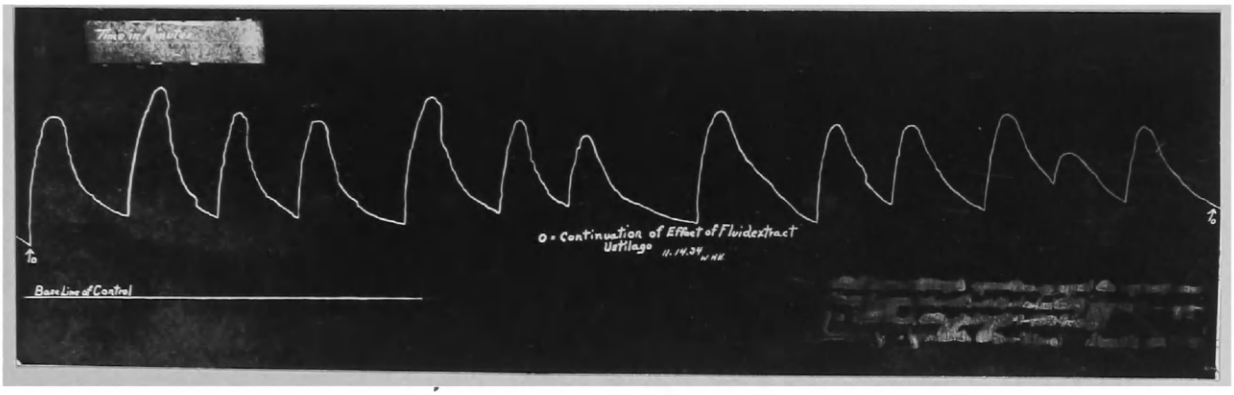
O = Continuation of Effect of Fluidextract Ustilago
Base Line of Control

Fig. 6 B

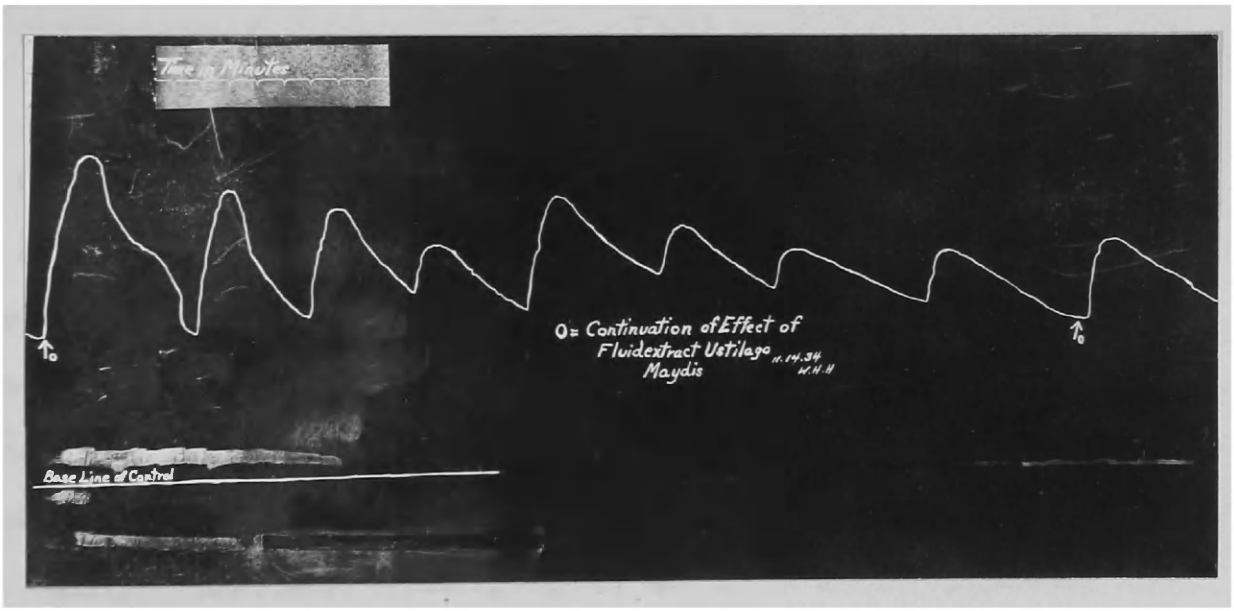
Time in Minutes
O= Continuation of Effect of Fluidextract Ustilago Maydis
Base Line of Control

Fig. 6 C

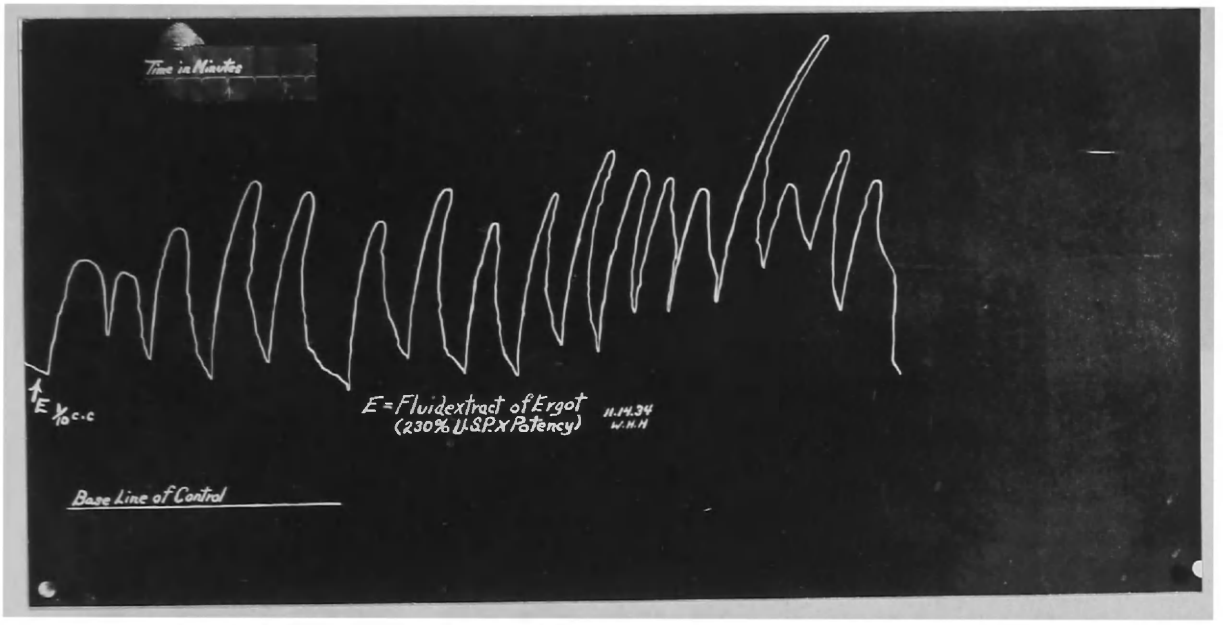

Fig. 6 D

It not only increased the rhythmicity, but also the tonus of the muscle. It may further be seen that this preparation maintained this increased rhythm and tonicity over a considerable period. After recording this result a dose of fluidextract of ergot was given for comparative purposes. This drug further increased the rhythmicity and tonus of the muscle as shown in Fig. 6 D.

Another experiment illustrating the effect of a fluidextract of Ustilago maydis may be seen in Figs. 7 A, B, C, D, E, F. The cat was used five days post-partum.

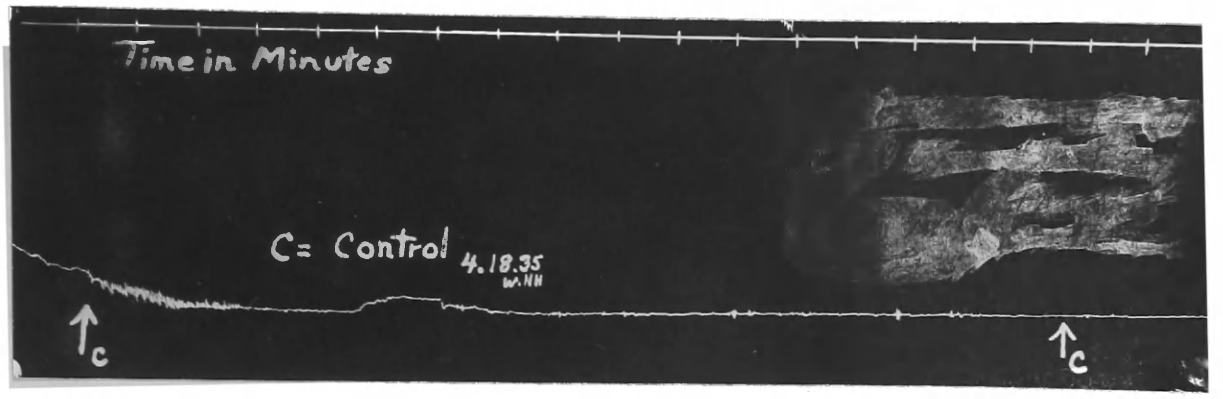

Fig. 7 A

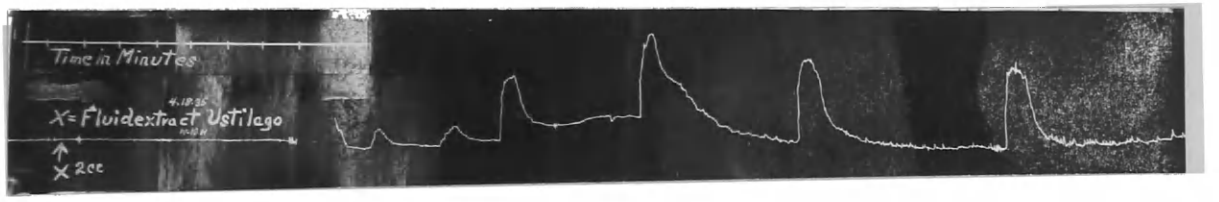

Fig. 7 B

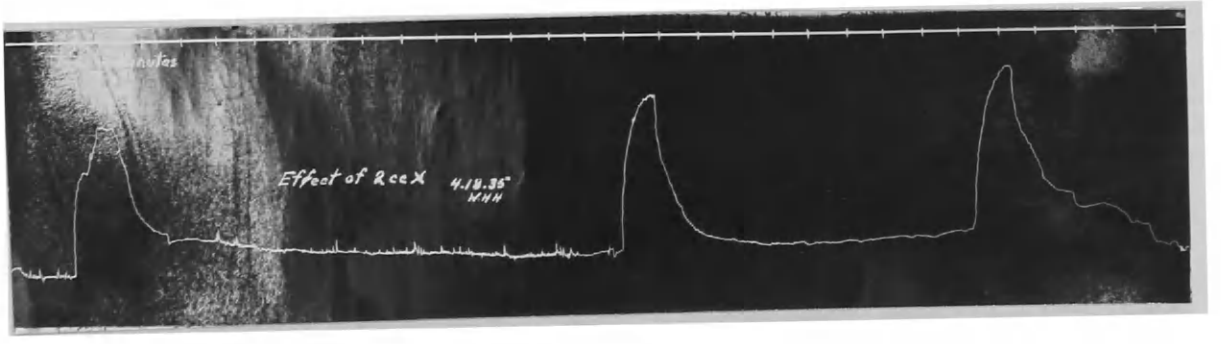

Fig. 7 C

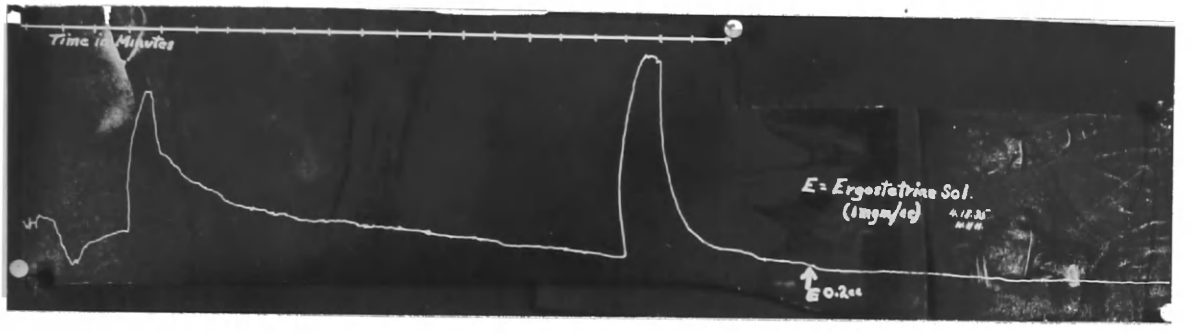

Fig. 7 D

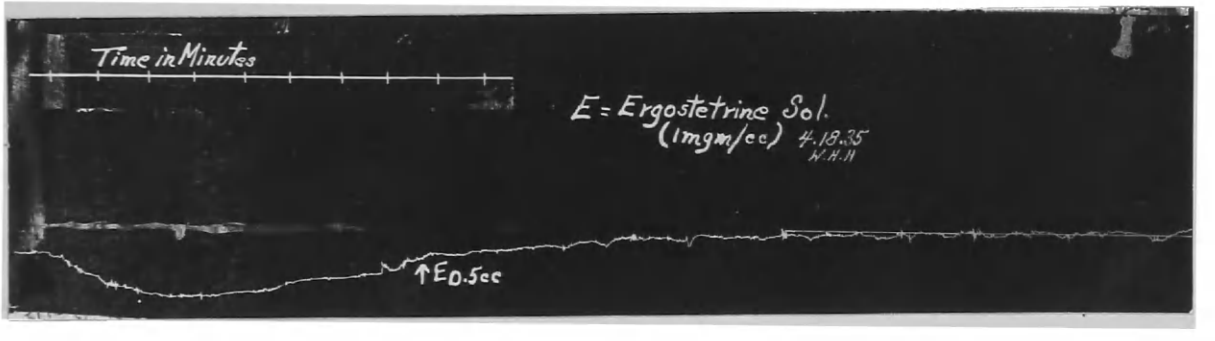

Fig. 7 E

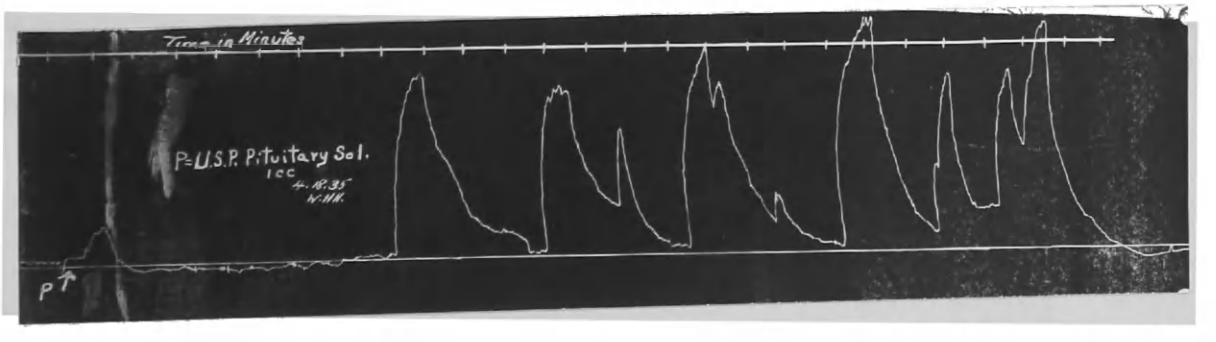

Fig. 7 F

EFFECT OF USTILAGO ON THE ISOLATED GUINEA PIG UTERUS

The tests upon isolated guinea pig uteri involved the use of virgin guinea pig uteri as in the U. S. P. assay for Solution of Posterior Pituitary. These strips of uteri were mounted in an isolated tissue bath, containing 50 cc. chambers for the 37.5° C. Locke-Ringer solution, in the conventional manner for this type of experiment.

The preparation to be tested consisted of mixed equal quantities of ripe and unripe drug collected in the fall of the year, approximately one month prior to the test. The drug was extracted with 95 per cent alcohol and then adjusted so that 1 cc. represented 1 gram of drug. Before using this highly alcoholic solution, the alcohol was driven off and its volume replaced by distilled water, the dilution forming an emulsion. A U.S.P. Solution of Posterior Pituitary was used as a means of comparison. Figs. 8 A, B, C clearly show the results of the action of this preparation upon the guinea pig uterus.

It may be seen that this fluidextract, although much weaker than the pituitary solution, definitely possessed a stimulating influence upon the isolated guinea pig uterus, qualitatively comparable to that of pituitary. Based on this experiment, 1 cc. of U.S.P. Solution of Posterior Pituitary was equivalent in activity to approximately 80 grams of Ustilago.

EFFECT OF USTILAGO ON THE ISOLATED RABBIT UTERUS

The preparation of drug used was the same as that used in the preceding experiment, except that 1 cc. represented 200 mg. of drug. A solution of epinephrine hydrochloride (1 - 10,000) was used as a comparative drug for these experiments.

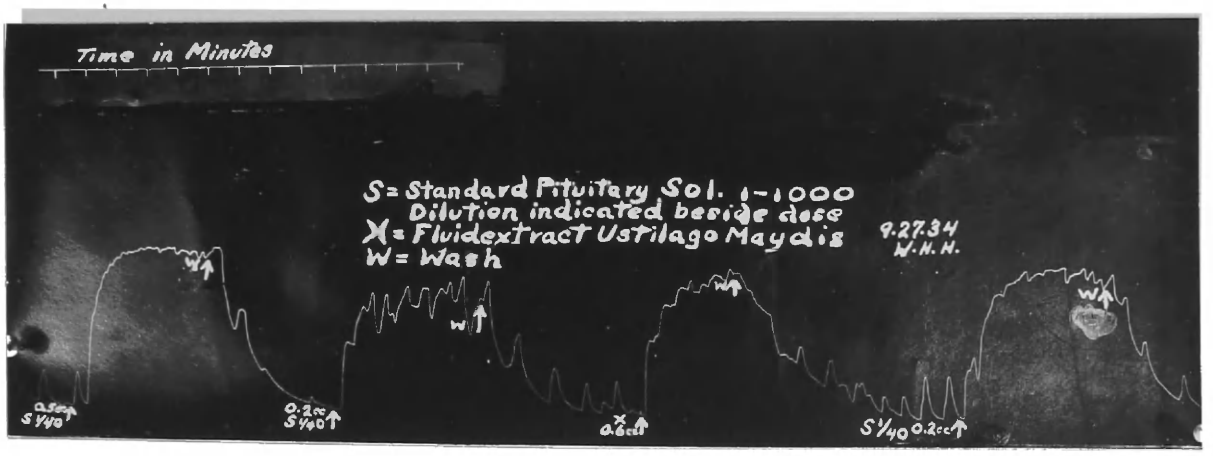

Fig. 8 A

Fig. 8 B

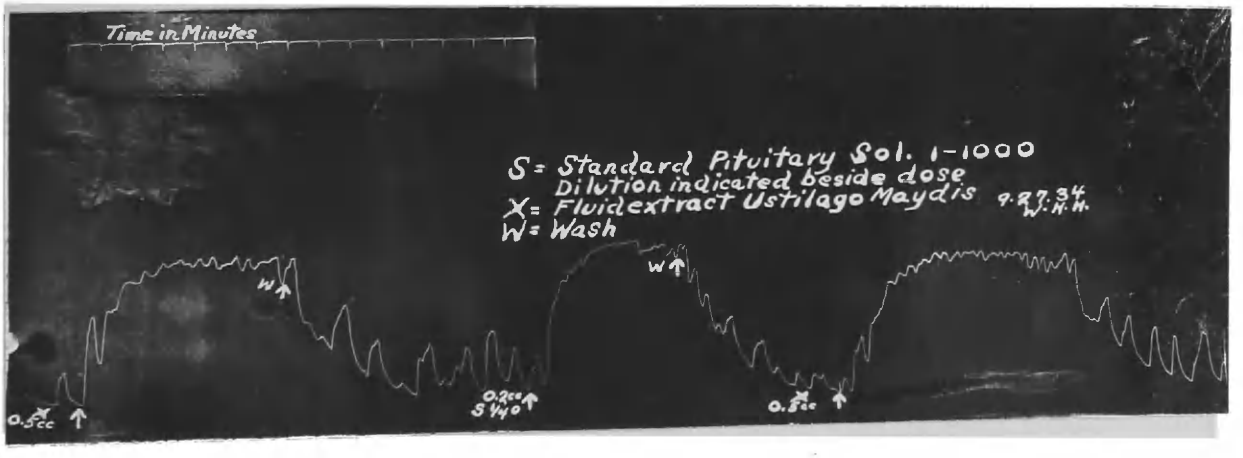

Fig. 8 C

The activity of the drug upon this type of tissue may be seen in Figs. 9 A, B.

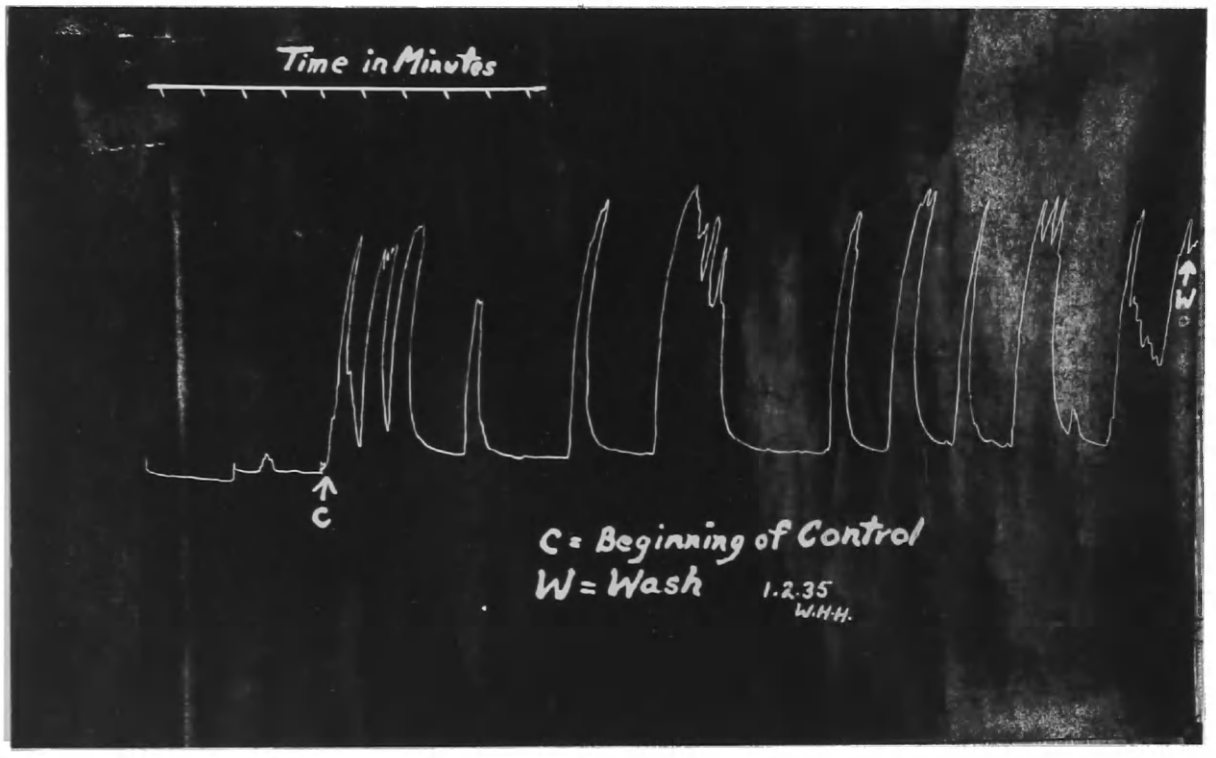

Fig. 9 A

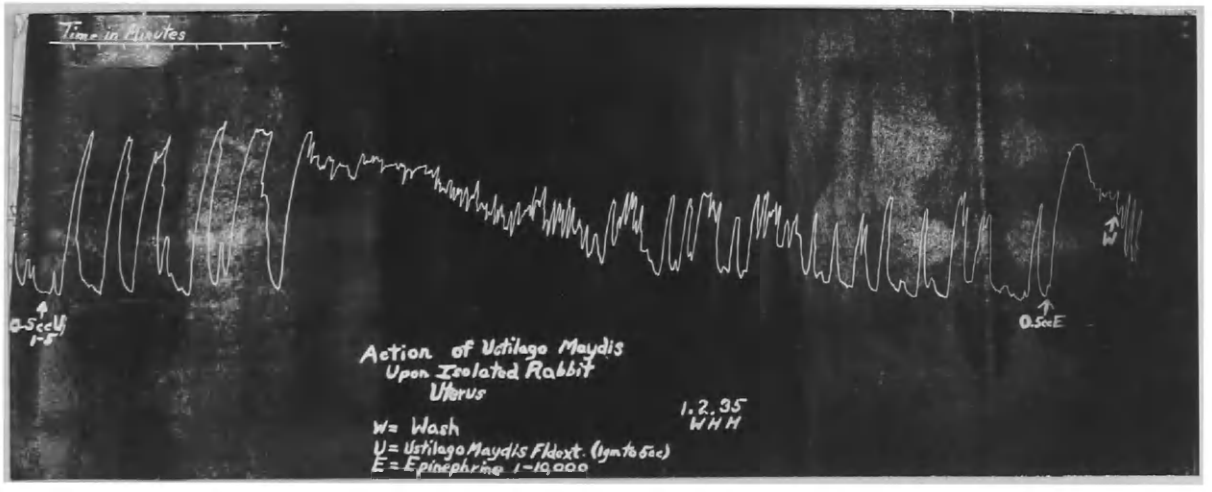

Fig. 9 B

Since the action of epinephrine hydrochloride solution was not diminished, it is apparent that the principle which is responsible for the oxytocic activity of Ustilago does not resemble ergotamine or ergotoxine, both of which inhibit the epinephrine activity.

In Fig. 10, the uterus employed was approximately twenty-four to thirty-six hours old and had been stored in the refrigerator in normal saline solution. Consequently, its irritability had been reduced. Although there appears to be an inhibition of the epinephrine action, it is believed that the length of time of storage of the tissue in the refrigerator had much to do with magnifying the ergotoxine-like activity.

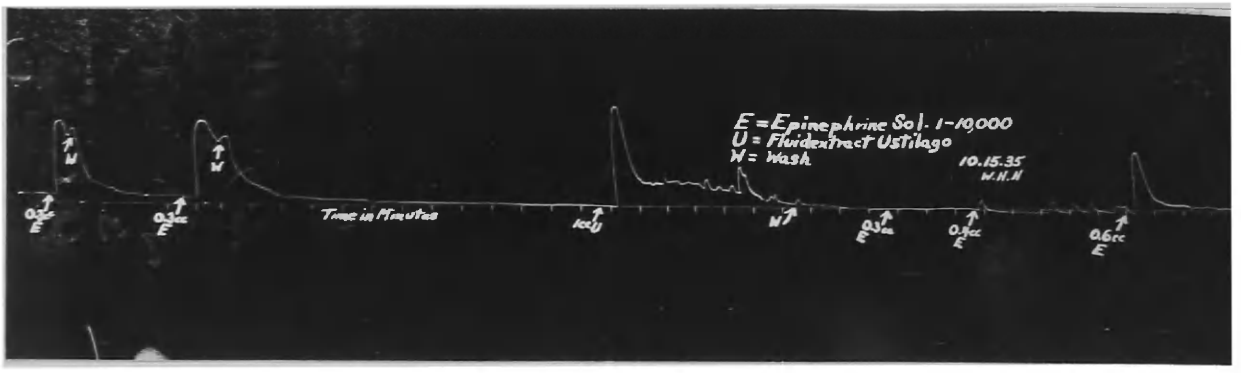

Fig. 10

Fig. 11

The reason for this belief may be seen by referring to Figs. 9 and 11. The strength of the drug utilized in this experiment was that of a fluid-extract, 1 cc. representing 1 gram of drug. This fluidextract was prepared approximately one month prior to this test.

THE EFFECT OF USTILAGO ON ISOLATED RABBIT INTESTINE

The fluidextract of Ustilago maydis employed in all of the experiments upon isolated rabbit intestine was prepared from equal mixtures of one and two year old ripe and unripe drug. The menstrum used was 95 per cent alcohol. After percolation to exhaustion of the drug the alcohol was driven off by a current of air, without heat and the volume restored with distilled water to make the preparation of fluidextract strength. Upon adding water to this extract a milky emulsion formed, which, upon standing, broke down yielding a pale yellowish to light orange colored aqueous layer and a gummy resinous layer. This same condition was noted in the uterine experiments. Consequently, the solution was constantly agitated to maintain the emulsion form which was utilized in the experiments. These layers were also studied separately in order to determine which of the layers carried the active substance.

Figs. 12 A, B show the action of both histamine and acetylcholine upon the isolated rabbit intestine. These drugs were administered to determine whether or not the results obtained in the previous isolated tissue experiments were caused by a similar or different principle. Having obtained the action of both histamine and acetylcholine, a portion of the aqueous layer, containing a slight amount of the oily substance, was administered. The drug, it will be noted, first stimulated

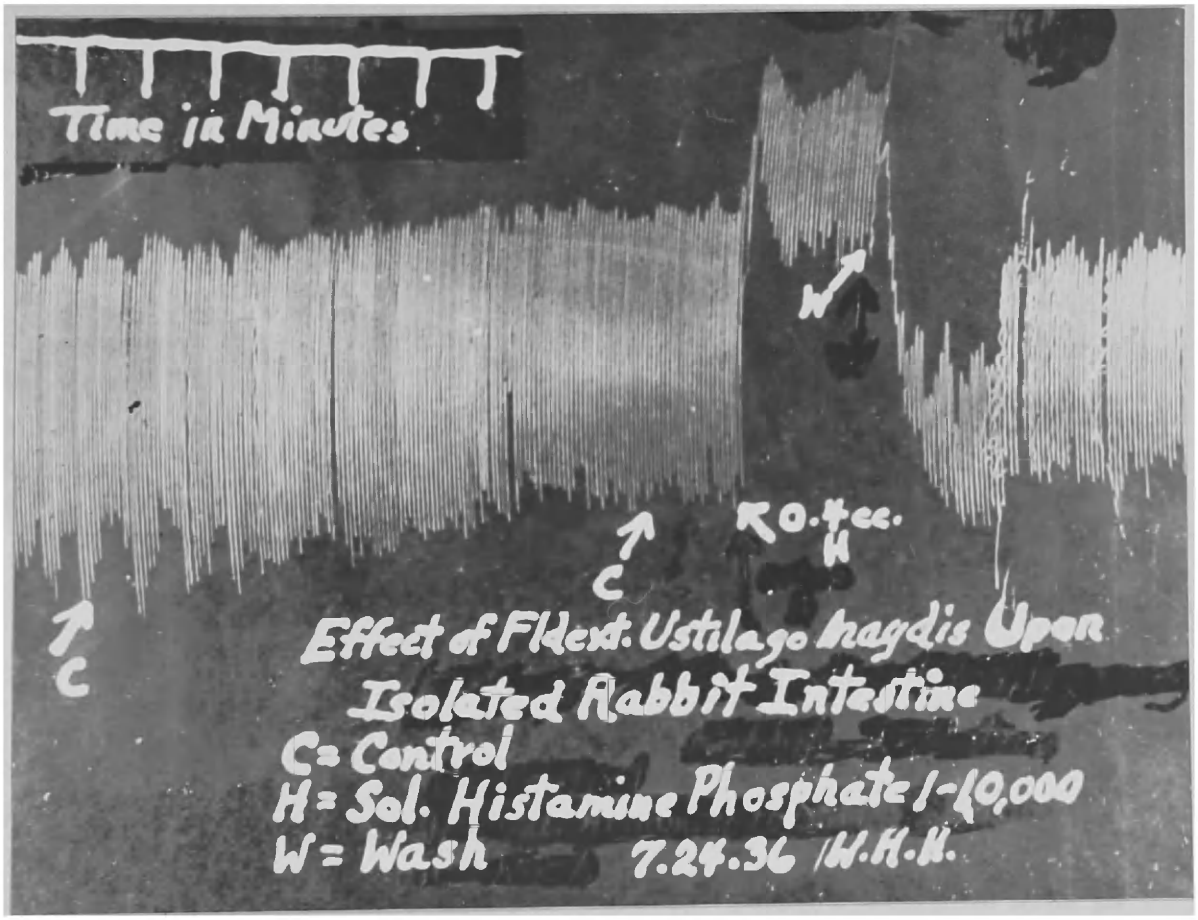
Time in Minutes
W
0.4cc.
H
C
C
Effect of Fl.ext. Ustilago Maydis Upon
Isolated Rabbit Intestine
C= Control
H= Sol. Histamine Phosphate 1-10,000
W= Wash
7.24.36 W.H.H.

Fig. 12 A

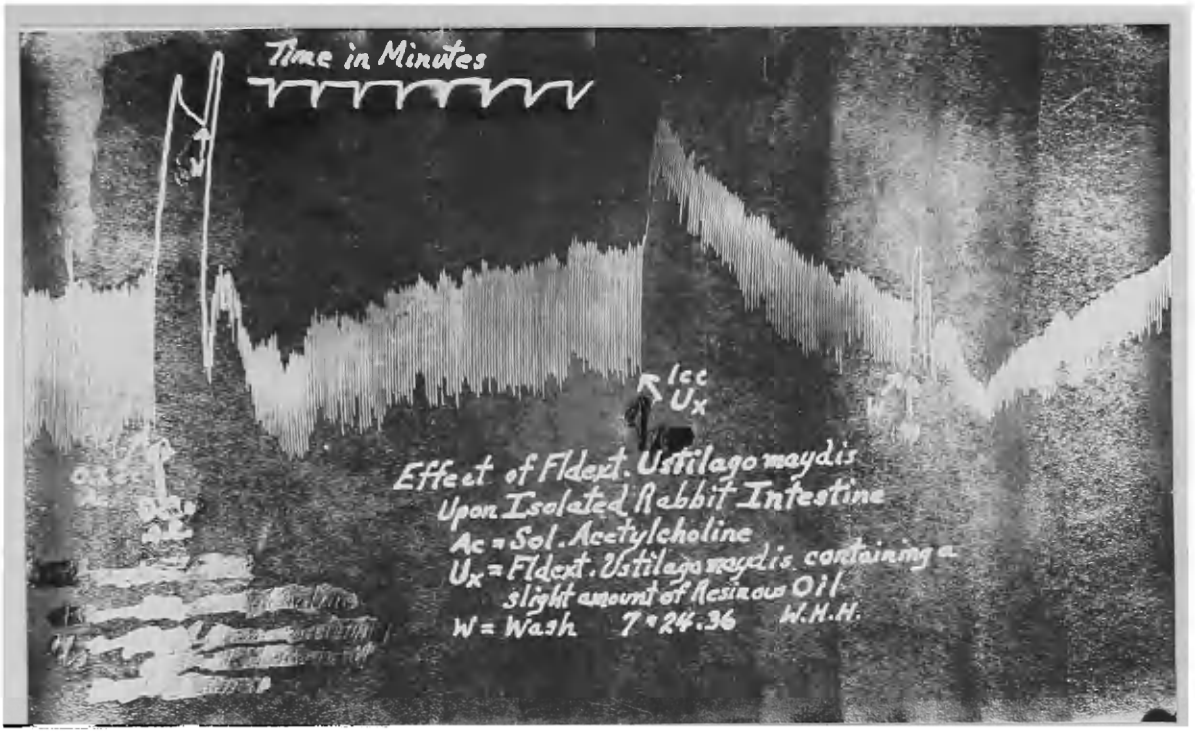

Fig. 12 B

and then gradually inhibited the intestinal movements.

Since this aqueous layer possessed little or no convincing action upon the isolated rabbit intestine it was decided to administer some of the resinous oily portion in the form of an aqueous emulsion. The same technique was used except that the administration of acetylcholine was omitted. The results of this experiment may be seen in Figs. 13 A, B, C.

It may be observed that this resinous oily substance contained a principle which abolished the activity of the intestine and also the stimulant effect of histamine phosphate solution.

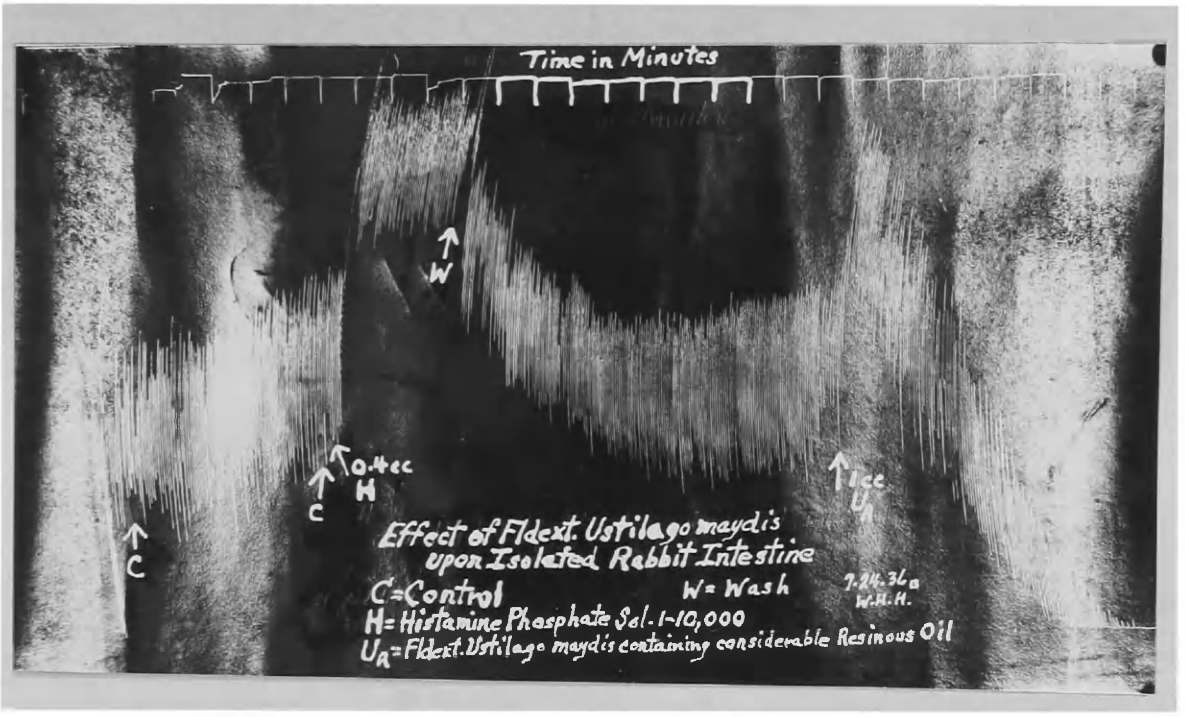
Time in Minutes
W
0.4cc
H
C
C
1cc
U_A
Effect of Fldext. Ustilago maydis
upon Isolated Rabbit Intestine
C=Control
W= Wash
7.24.36a
W.H.H.
H= Histamine Phosphate Sol. 1-10,000
U_A= Fldext. Ustilago maydis containing considerable Resinous Oil

Fig. 13 A

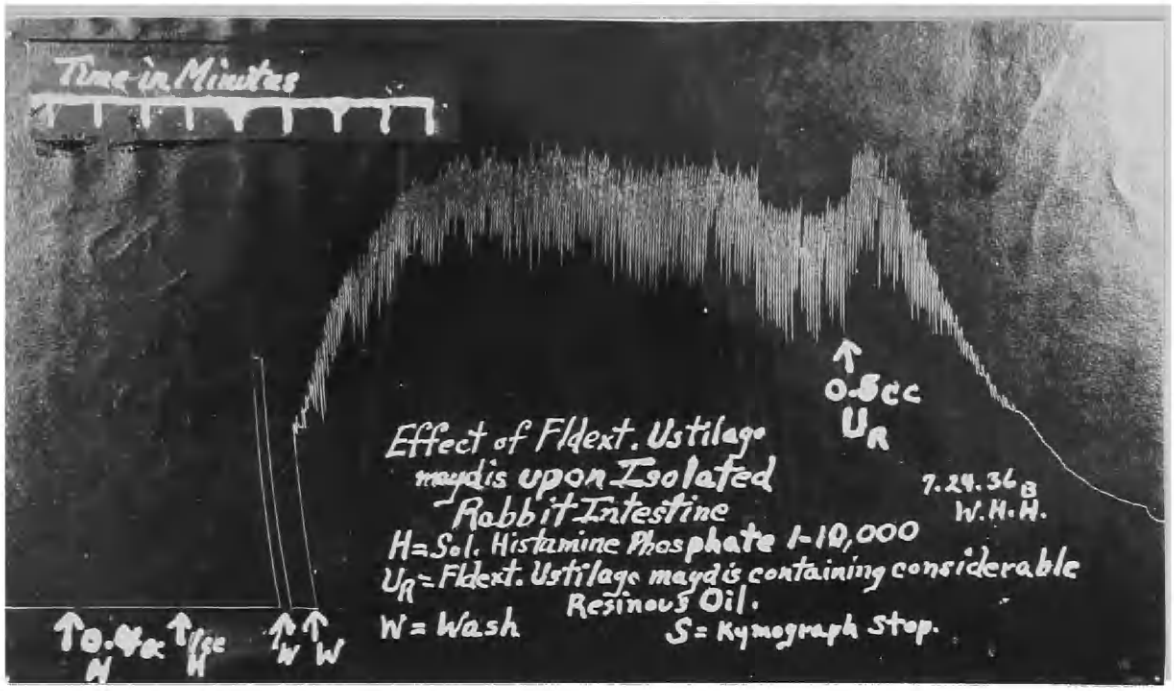

Fig. 13 B

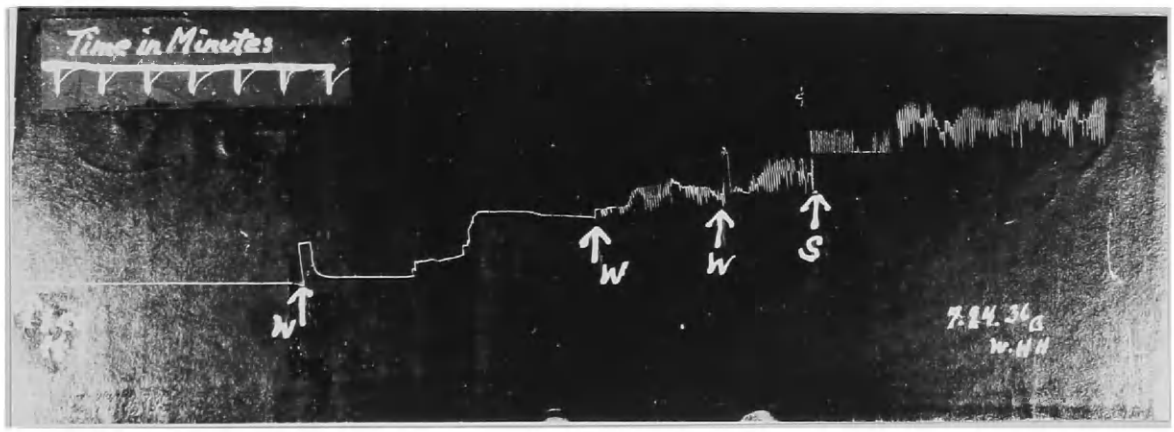

Fig. 13 C

Further, a dose exceeding twice the original stimulating dose of histamine failed to initiate a response. After washing the tissue with Tyrode's solution (the solution utilized in all of the intestinal experiments) the rythmicity and tonus of the tissue returned. Another experiment was performed to check the above result, using one-half the quantity of the emulsion. The expected response was obtained. Finally, to check these two results a fresh piece of tissue was mounted in the bath and, after the administration of the histamine, first the aqueous portion containing a slight amount of the resinous oil was administered, then after the tissue returned to the control level the resinous oil was administered in the emulsion form. Figs. 14 A, B illustrate the results. From these experiments it may be concluded that the activity of this preparation was contained for the most part in the resinous oily portion.

Fig. 14 A

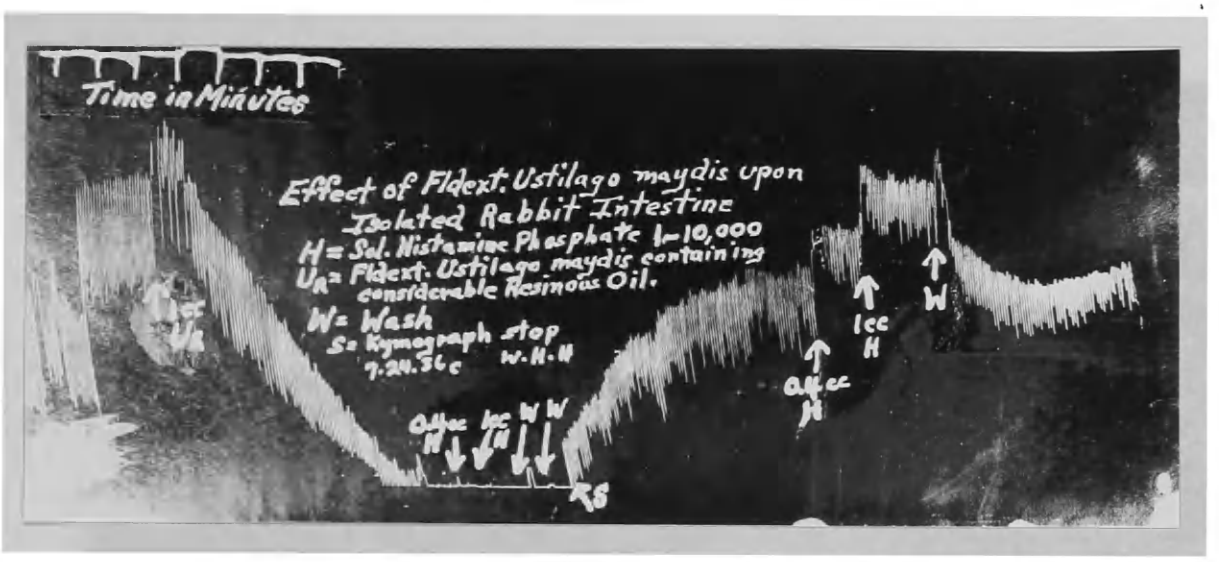

Fig. 14 B

EFFECT OF USTILAGO ON THE PERFUSED LEG VESSELS OF THE FROG

The method employed for the perfusion experiments was that of Lewen-Trendelenberg (21). The preparation employed in this work was the same alcohol-free fluidextract as was employed in the intestinal experiments. Upon examining Figs. 15 A, B and Figs. 16 A, B it may be seen that the drug caused a constriction of the leg vessels. Fig. 15 A shows a control tracing taken for a period of approximately fourteen minutes. As may be noted in Fig. 15 B, a definite slowing of the flow occurred after the administration of 1 cc. of the fluidextract. This action was interrupted by the appearance of a blood clot in the vein near the point of cannulation. Upon removal of the clot, 1 cc. of the solution representing a dilution of 1-100 of the fluidextract was then perfused through the vessels. As may be seen in Fig. 16 A the rate of flow remained practically unchanged. After injecting the second 1 cc. dose a definite slowing of the flow was observed, which after a few minutes increased slightly above

Fig. 15 A

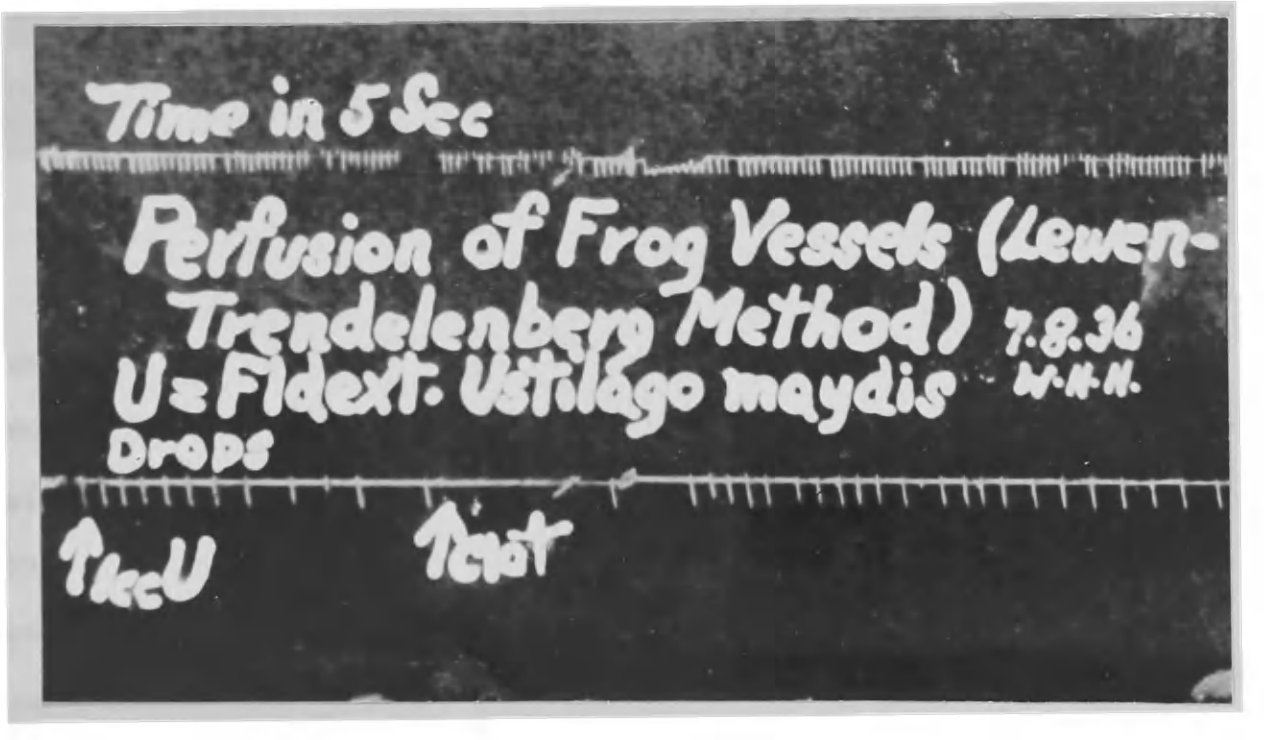

Fig. 15 B

Fig. 16 A

Fig. 16 B

the control rate. Referring to Figs. 16 A and 16 B, it may be noted that this increased rate was maintained for approximately ten minutes, at which time 1 cc. of the undiluted fluidextract was administered. This immediately slowed the rate from approximately nine to five drops per minute. Thus it may be concluded that the fluidextract, by this particular perfusion method, has a weak action in constricting the leg vessels of the frog.

TOXICITY OF USTILAGO

Since the cat was used as the test animal for uterine and abortion studies, this animal was the logical choice for the investigation of the toxicity of Ustilago maydis. Table IV embraces a concise yet comprehensive resume of the toxicity experiments. It may be noted that three of the recorded deaths involved pregnant animals. These animals are included here as well as in Table II, inasmuch as they died while under the influence of Ustilago maydis in an attempt to induce abortion.

TABLE IV

Date	Type of animal and Wt.	Dose in gm. of drug	cc. of fluid injected and area	Drug from which ext. was made Date of ext.	Type of Solution	Result	Remarks
4/20/36	Maltese Pregnant 2.55 Kg.	500 gm.	20 cc. Sub. Q. Thigh	1935 Md 1934 Md 1935 Io E.P.	A. 10 cc. 0.10 cc.	+	10 cc. A. R.T. 10 cc. O L.T.
5/7/36	Maltese Female 2.35 Kg.	400 gm.	30 cc. Sub. Q. Thigh	1934 Md 1935 Md E.P.	A. 10 cc. O 10 cc.	+	10 cc. of three percola.
7/30/36	Maltese Pregnant 2.40 Kg.	400 gm.	10 cc. Sub. Q. Thigh	1934 Md 1935 Md E.P.	Olive Oil	+	
7/30/36	Stripe Female 1.95 Kg.	400 gm.	10 cc. Sub. Q. Thigh	1934 Md 1935 Md E.P.	Olive Oil	+	
11/18/36	Stripe Pregnant 3.00 Kg.	33 gm.	6.6 cc. Sub. Q. Thigh	1936 Md Ripe	Oil Frac. after evap.	+	Animal died 11/21/36
12/2/36	B. and W. Female 1.50 Kg.	100 gm. per Kg.	4.5 cc. I. M. Thigh	1936 Md Ripe	P.E. ext. taken up in O	—	
12/2/36	T. and W. Female 1.77 Kg.	50 gm. per Kg.	2.66 cc. I. M. Thigh	"	"	—	
12/7/36	B. and W. Female 1.50 Kg.	100 gm. per Kg.	9.6 cc. I.P.	"	Al. ext. in O	+	
12/7/36	T. and W. Female 1.67 Kg.	120 gm. per Kg.	13 cc. I.P.	"	"	+	
12/8/36	B. and W. Female 1.05 Kg.	50 gm. per Kg.	3.2 cc. I.P.	"	"	—	
12/8/36	Maltese Male 1.55 Kg.	100 gm. per Kg.	6.5 cc. I.P.	R. and U. Md 1936	"	+	
12/8/36	B. and W. Female 1.45 Kg.	200 gm. per Kg.	12 cc. I.P.	"	"	+	
12/21/36	Stripe Female 1.40 Kg.	50 gm. per Kg.	4.2 cc. I.P.	Ripe 1936	"	—	
12/21/36	B. and W. Female 1.76 Kg.	75 gm. per Kg.	8 cc. I.P.	"	"	+	
12/21/36	G. and W. Female 1.63 Kg.	100 gm. per Kg.	10 cc. I.P.	"	"	+	
12/22/36	Black Female 2.20 Kg.	100 gm. per Kg.	14.5 cc. I.P.	Unripe 1936	"	—	
12/22/36	B. and W. Female 1.70 Kg.	50 gm. per Kg.	5.6 cc. I.P.	"	"	—	
1/5/37	Black Female 1.85 Kg.	200 gm. per Kg.	14.8 cc. I.P.	"	"	+	
1/5/37	Grey Female 1.95 Kg.	150 gm. per Kg.	11.7 cc. I.P.	"	"	+	

Sub. Q. = Subcutaneous; I.M. = Intramuscular; I.P. = Intraperitoneal

B. = Black; W. = White; T. = Tan; G. = Grey; A. = Aqueous;

Al = Alcohol; E.P. = Equal Parts; Io = Iowa; O = Olive Oil; R = Ripe;

U = Unripe; R.T. = Right Thigh; L.T. = Left Thigh; ext. = extraction

SUMMARY

Ustilago maydis, ripe and unripe, has been studied by a variety of pharmacological procedures for the purpose of determining the presence or absence of activity in this drug, and the qualitative nature of this activity.

CONCLUSIONS

1 - Ustilago maydis, when administered intravenously in an aqueous suspension of a hydroalcoholic fluidextract produces a depressor effect upon the carotid blood pressure of the cat and dog without significantly inhibiting the pressor activity of epinephrine.

2 - Ripe and unripe samples of Ustilago maydis appeared to be of equal activity.

3 - It was impossible to demonstrate, by the methods employed, the presence of any active alkaloid in Ustilago maydis.

4 - Tested by the U. S. P. Cockscomb method for Ergot, Ustilago produces a cyanotic reaction, but relatively large doses were required. The cyanosis, as well as the symptoms induced in the cockerels, differed considerably from the effects produced by Ergot.

5 - Ustilago causes abortion in cats especially in the early stages of pregnancy. Quantitatively this drug is many times weaker than Ergot in this respect.

6 - The active substance of Ustilago maydis is not of an acetylcholine or histamine-like nature.

7 - It can be demonstrated that Ustilago maydis causes contractions of the pregnant and puerperal cat uterus in situ.

8 - Ustilago maydis stimulates contractions of the isolated guinea pig and rabbit uterus.

9 - Ustilago maydis inhibits the movements of isolated rabbit intestine as well as the stimulating effect induced by solutions of histamine phosphate.

10 - It has been found after fractional separation of the alcoholic fluidextract of Ustilago maydis, that the greater portion of the activity resides in the resinous oily-like fraction rather than in the aqueous fraction. Petroleum ether extracts have been found to possess the same activity as that of alcoholic extracts.

11 - Ustilago maydis preparations have been found by perfusion experiments to produce a constriction of the leg vessels of frogs.

12 - Ustilago maydis exhibits toxicity when administered intramuscularly, intraperitoneally or subcutaneously to cats. By the subcutaneous or intramuscular route, doses equivalent to 400 grams of drug were required to kill, whereas intraperitoneal injections produced death in doses equivalent to approximately 75 grams of drug.

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