SCIENTIFIC SECTION

A PHARMACOLOGICAL STUDY OF USTILAGO.*[1]

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Ustilago was defined in the United States Pharmacopœia 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 cm. 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.

A review of the literature, historical and otherwise, is presented and discussed in the original thesis.

Among the earlier investigators the work of the following merit mention in connection with the present investigation: Jussieu, Linnaeus, Leveille, Tulasne brothers, Waldheim, Wolf, Winter, Farlow, Prillieux and Spalding (2).

The exact time of the first chemical investigation of *Ustilago maydis* is obscure, but among the earlier investigators Cressler's (3) work appears to be among the first. Other investigators, such as Parsons (4), Rademaker (5), Rademaker and Fischer (6), Balch (7), Testoni (8), and Langecker (9), have also searched for active principles, which, from time to time, have been reported as alkaloidal. Recent investigations have shown that the active substance, if an alkaloid, cannot be demonstrated as such by the usual methods.

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 Pharmacopœia, 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. The clinical evidence for its admission and deletion is clearly portrayed by Leonard (10), (11), Crist (12), Cooperiden (13), Goss (14), Vailliant (15), Hale (16), and Mitchell (17).

Bogdanovitch (18) reported that fluidextract of Jugoslavian *Ustilago maydis* possessed various properties, with which the present work agrees in part. The differences occur in the results obtained by the following methods of experimentation: the action upon the isolated rabbit intestine, and the effect upon the hypertensive action of epinephrine.

Dragisic and Varicak (19) have reported that Jugoslavian *Ustilago maydis* possesses an ergotamine-like principle. In this work we have been unable to confirm their results regarding this action. However, it may be possible that there is a pronounced difference in the nature of the drug from these widely separated sources.

Since the literature reveals many conflicting reports concerning the activity of *Ustilago maydis*, a qualitative pharmacodynamic investigation of the drug was initiated. This investi-

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gation embraced studies 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.

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 50 per cent alcohol until the drug appeared to be exhausted. The percolate 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. After obtaining the control, 0.3 cc. of the percolate was injected into the femoral vein. A transitory fall of blood pressure was produced.

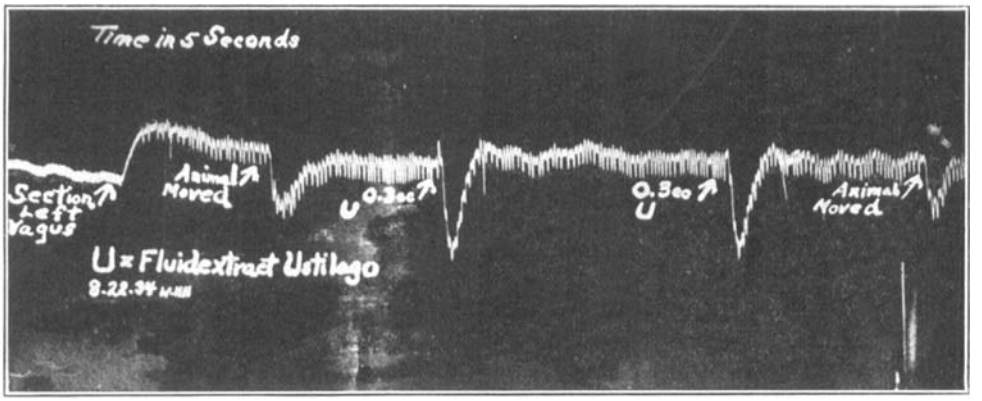

Fig. 1.

After sectioning the right vagus, a repetition of the same dose duplicated the preceding result. The left vagus was then sectioned, the results of which may be seen in Fig. 1. Following this procedure 0.3 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 correspondingly greater depressor effects.

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.

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. The employed doses of fluidextract of *Ustilago maydis* exerted no "ergot-like" influence on the pressor responses of epinephrine. In this experiment the vagi remained intact and again the depressor effect of the drug was clearly evident.

Having seen the results of the fluidextract of *Ustilago maydis* upon the blood pressure of the cat whose vagi remained intact, and in the case where the vagi were sectioned, 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 lightly anesthetized with sodium amytal and prepared in the manner of the preceding experiments. Following the intravenous administration of duplicate doses of epinephrine hydrochloride (1–50,000), 1 mg. per Kg. of atropine sulfate in saline 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, were injected intravenously. As may be seen in Fig. 2, a correspondingly greater and more prolonged depressor action was recorded. Two successive doses of epinephrine hydrochloride solution were then administered, producing the usual pressor responses. 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, 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 drugs were thoroughly mixed and percolated with petroleum ether. After this extraction the marc was exhausted with a menstruum 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 substance.

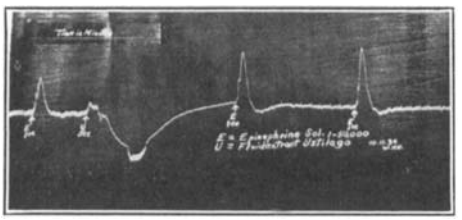

Fig. 2.

At the time of the percolation described above, 50 Gm. of unripe and ripe drug were percolated separately with 50 per cent alcoholic menstruum. The volume was finally adjusted by evaporation, without heat, such that 1 cc. represented 1 Gm. 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 substance from the petroleum-ether extraction, emulsified with saline, was administered intravenously. Since practically no effect was obtained from this dose, a larger dose, 2 cc., was administered. A definite depressor action was produced. Thereafter, repeated doses of 0.75 cc. of epinephrine hydrochloride (1:50,000), produced responses similar to those produced before the fraction was given. Since the petroleum-ether fraction neither inhibited nor augmented 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 were injected. This produced a definite depressor effect. 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 previous observations. 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. 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. 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 intravenously. The animal also received 1 mg. per Kg. of atropine

sulfate. One cubic centimeter of ripe drug extractive was administered, which produced a slight pressor action. Following this a 2-cc. dose of unripe drug fluidextract produced the characteristic depressor effect.

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.

One hundred 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 Gm. of magnesium oxide were added to alkalinize the solution. After thoroughly shaking for approximately 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.

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	White leghorn cockerel	1.17 Kg.	3.75 Gm.	0.75 cc.	Unripe 1934 Maryland, 7.25.34	25 per cent alcoholic	001	001	112	112	112	011	...	...
11.16.34	White leghorn cockerel	0.80 Kg.	3.7 Gm.	0.74 cc.	Unripe 1934 Maryland, 7.25.34	25 per cent alcoholic	001	001	112	112	123	011	...	...
2.14.35	White leghorn cockerel	1.63 Kg.	4.0 Gm.	4.00 cc.	Unripe 1934 Maryland, 7.25 34	25 per cent alcoholic	012	012	012	012	...	...	...	...
10.27.36	White leghorn cockerel	1.87 Kg.	10.0 Gm.	1.00 cc.	Unripe 1936, Maryland	95 per cent alcohol	000	011	012	...	...	...	112	001
10.28.36	White leghorn cockerel	1.25 Kg.	20.0 Gm.	2.00 cc.	Unripe 1936 Maryland	95 per cent alcohol	000	100	111	...	...	...	001	001
10.29.36	White leghorn cockerel	1.87 Kg.	20.0 Gm.	2.00 cc.	Unripe 1936 Maryland	95 per cent alcohol	001	011	112	...	...	...	223	123
11.16.36	White leghorn cockerel	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	...

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. Twenty-five cubic centimeters of a solution prepared by percolating a mixture of ripe and unripe drug with 50 per cent alcohol was evaporated to a semi-solid consistence, without heat, and then taken up in 1 cc. of 25 per cent alcohol. One cubic centimeter of this solution represented 5 Gm. of drug. Eight-tenths cubic centimeter 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 Gm. of drug. Into a third cockerel 0.74 cc. of a like solution was injected, representing 3.7 Gm. of drug. The results of these experiments may be seen in Table I. At a later date 4 cc. of fluidextract representing 4 Gm. of drug were 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. One cubic centimeter of this solution, made from unripe drug was injected. The results may be seen in Table I. Two cubic centimeters of the same solution were injected into another bird. A comparison of the results showed a definite disagreement. One cubic centimeter of the solution represented 10 Gm. of drug. To check this work 2 cc. of the same solution were 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. One cubic centimeter of this substance, representing 5 Gm. 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 dilatation rather than constriction of the blood vessels. Further, the blanching of the comb 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 Gm. 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 terminating early pregnancies than those approaching full term.

The depressor effects obtained in the earlier blood pressure experiments suggested the possibility of an acetylcholine or histamine-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 or histamine. That acetylcholine or histamine are quite devoid of significant oxytocic action has been shown in other reports from this laboratory (20).

TABLE II.

Date.	Cat Identity	Dose in Gm. 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	Alcoholic	−	.....
7.8.35	Gray-white	30.0 Gm.	10 cc. Sub. Q. thigh	Equal parts R., U. percol. with fldext., 2.15.35	Alcoholic	+	3 hairless kittens dead
7.8.35	Black-white	30.0 Gm.	10 cc. Sub. Q. thigh	Equal parts R., U. percol. with fldext., 2.15.35	Alcoholic	+	2 kittens with hair at night 1 in morn., all dead
7.25.35	Maltese	10.0 Gm.	10 cc. Sub. Q. thigh	Equal parts R. and U., 3.25.35	Alcoholic	−	Close to term
7.25.35	Maltese	10.0 Gm.	10 cc. Sub. Q. thigh	Equal parts R. and U., 3.25.35	Alcoholic	+	3 kittens, fine hair, all dead
8.13.35	Grey	10.0 Gm.	10 cc. Sub. Q. thigh	Equal parts R. and U., 3.25.35	Alcoholic	−	Close to term
5.13.36	Black	100.0 Gm.	10 cc. Sub. Q. thigh	Equal parts, 1934–1935, collect., 4.23.36	Water emulsion	−	.....
5.14.36	Same as 5.13.36	100.0 Gm.	10 cc. Sub. Q. thigh	Equal parts, 1934–1935, collect., 4.23.36	Olive oil	+	4 kittens alive but from size cat not at full term
5.22.36	Maltese	100.0 Gm.	10 cc. Sub. Q. thigh	Equal parts, 1934–1935, collect., 4.23.36	Water emulsion	−	.....
5.25.36	Same as 5.22.36	100.0 Gm.	10 cc. Sub. Q. thigh	Equal parts, 1934–1935, collect., 4.23.36	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, 1934–1935, collect., 5.27.36	Olive oil	+	Aborted during night, 7.29.36, 4 kittens about two 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.

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.	1 cc. Sub. Q. thigh	Aqueous	—
9.3.35	Same as 8.26.35	25.0 mg.	1 cc. Sub. Q. thigh	Aqueous	—
9.4.35	Same as 8.26.35	500.0 mg.	1 cc. Sub. Q. thigh	Aqueous	—

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 experiments 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. 3, had delivered two live kittens twenty-four hours before this experiment. The fluidextract used in this particular experiment

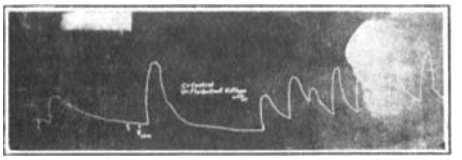

Fig. 3A.

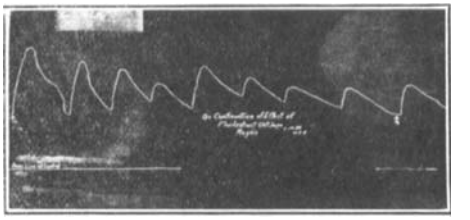

Fig. 3B.

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. 3A, B and C, the drug produced a definite action upon the uterus.

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. 3C.

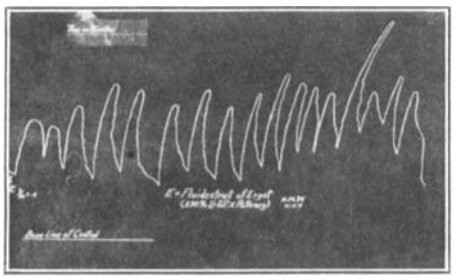

Fig. 3C.

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 Gm. 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

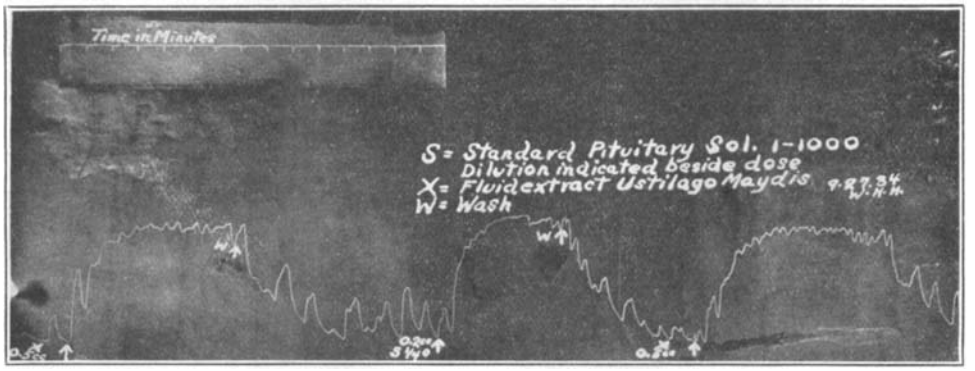

Fig. 4.

used as a means of comparison. Figure 4 clearly shows 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 Gm. 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.

The activity of the drug upon this type of tissue may be seen in Fig. 5.

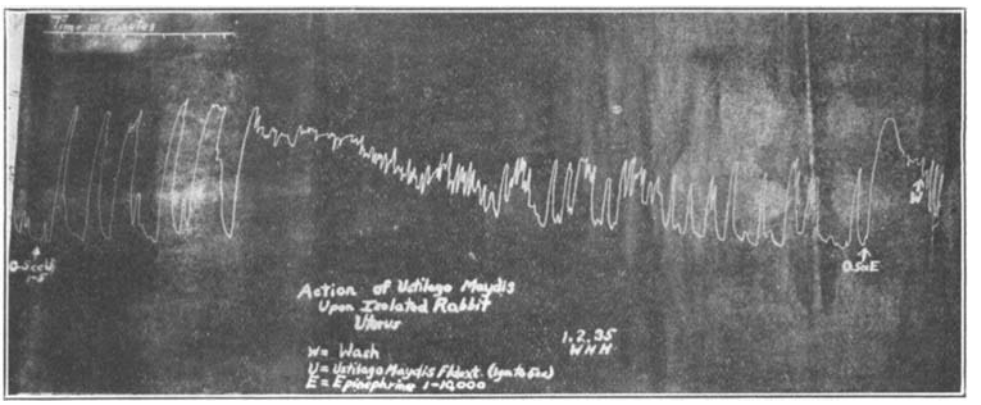

Fig. 5.

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.

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 menstruum 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.

Histamine and acetylcholine were administered to determine whether or not the results obtained in the previous isolated tissue experiments were caused by a similar or different principle.

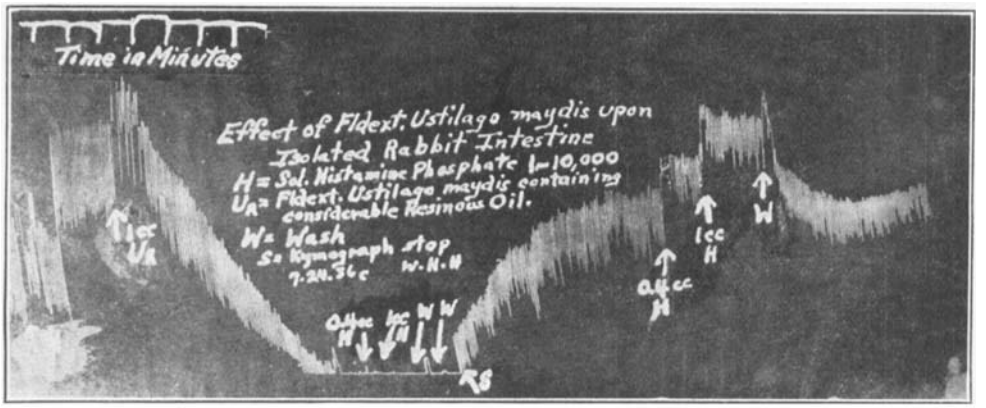

Fig. 6A.

Fig. 6B.

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 first stimulated 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.

It was 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. Further,

a dose exceeding twice the original stimulating dose of histamine failed to initiate a response. After washing the tissue with Tyrode's (the solution utilized in all of the intestinal experiments) the rhythmicity 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. Figures 6A and 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.

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. One cubic centimeter of the solution representing a dilution of 1–100 of the fluidextract was perfused through the vessels. 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 the control rate.

It was observed 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 of flow from approximately nine down 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 résumé 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.

The toxicity of *Ustilago maydis*, by oral administration, was also investigated by feeding mature white rats a mixture of fifty per cent stock rat food and fifty per cent *Ustilago maydis*.

The animals were at first limited to a definite quantity per day, but since there was a general daily decline in weight this plan was abandoned in favor of a surplus feeding. Immediately upon the increased feeding the animals showed a daily increase in weight over a period of three months and apparently maintained their normal health.

Therefore, it appears from the above experiment that *Ustilago maydis* is very low in toxicity when fed to non-pregnant rats. The influence of feeding the drug to pregnant animals is being studied further.

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 hydro-alcoholic 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 reasonably mature 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*.

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	1936 Md., ripe	P. E. ext. taken up in O	−	.....
12.7.36	B. and W. female, 1.50 Kg.	100 Gm. per Kg.	9.6 cc. I. P.	1936 Md., ripe	Al. ext. in O	+	.....
12.7.36	T. and W. female, 1.67 Kg.	120 Gm. per Kg.	13 cc. I. P.	1936 Md., ripe	Al. ext. in O	+	.....
12.8.36	B. and W. female, 1.05 Kg.	50 Gm. per Kg.	3.2 cc. I. P.	1936 Md., ripe	Al. ext. in O	−	.....

TABLE IV.—(*Continued from page 750*).

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.
12.8.36	Maltese male, 1.55 Kg.	100 Gm. per Kg.	6.5 cc. I. P.	R. and U., Md., 1936	Al. ext. in O	+	.....
12.8.36	B. and W. female, 1.45 Kg.	200 Gm. per Kg.	12 cc. I. P.	R. and U., Md., 1936	Al. ext. in O	+	.....
12.21.36	Stripe female, 1.40 Kg.	50 Gm. per Kg.	4.2 cc. I. P.	Ripe, 1936	Al. ext. in O	−	.....
12.21.36	B. and W. female, 1.76 Kg.	75 Gm. per Kg.	8 cc. I. P.	Ripe, 1936	Al. ext. in O	+	.....
12.21.36	G. and W. female, 1.63 Kg.	100 Gm. per Kg.	10 cc. I. P.	Ripe, 1936	Al. ext. in O	+	.....
12.22.36	Black female, 2.20 Kg.	100 Gm. per Kg.	14.5 cc. I. P.	Unripe, 1936	Al. ext. in O	−	.....
12.22.36	B. and W. female, 1.70 Kg.	50 Gm. per Kg.	5.6 cc. I. P.	Unripe, 1936	Al. ext. in O	−	.....
1.5.37	Black female, 1.85 Kg.	200 Gm. per Kg.	14.8 cc. I. P.	Unripe, 1936	Al. ext. in O	+	.....
1.5.37	Grey female, 1.95 Kg.	150 Gm. per Kg.	11.7 cc. I. P.	Unripe 1936	Al. ext. in O	+	.....

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.

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 the 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 Gm. of drug were required to kill, whereas intraperitoneal injections produced death in doses equivalent to approximately 75 Gm. of drug.

13. *Ustilago maydis* when fed to mature non-pregnant white female rats failed to show any significant toxicity.

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