THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Herman Hans Jensen for the degree of Master of Science. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science.

Arthur D Hirschfelder
Chairman

F. B. Kingsbury

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Dec. 2 1921

REPORT

of

COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given ~~Herman Hans Jensen~~ final oral examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota

Dec. 9 1921

Arthur D. Hirschfelder
Chairman

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REPORT

of Thesis

COMMITTEE ON ~~WRITTEN EXAMINATION~~.

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Herman Hans Jensen final written examination for the degree of Master of Science. We recommend that the degree of Master of Science be conferred upon the candidate.

Minneapolis, Minnesota.

December 9, 1921.

A. D. Kirschfelder

Chairman

E. R. Brown

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THE RELATION BETWEEN CHEMICAL STRUCTURE AND PHYSIOLOGICAL ACTION OF SOME DERIVATIVES OF ORTHO-OXY-BENZYL ALCOHOL (SALIGENIN)

HERMAN H. JENSEN.

4-21 6M

OUTLINE.

INTRODUCTION:

1.- Introductory Remarks.

2.- Review of Literature upon the Subject.

3.- Purpose and Scope of this Investigation.

I. COMPOUNDS INVESTIGATED.

1.- Chemical Structure.

2.- Methods of Synthesis.

3.- Comparison of Physical Properties of Compounds.

II. SUMMARY OF PHYSIOLOGICAL EFFECTS OF

1.- Benzyl Alcohol.

2.- Saligenin.

III. EXPERIMENTS.

1.- Toxicity and General Effects.

a) Methods and Technique
b) Data and Protocols
c) Conclusions and Comments

} Sequence in treatment of all experiments.

2.- Local Anesthesia - Frogs.

3.- Local Anesthesia - Man.

4.- Smooth Muscles - Intestinal Segments - Rabbits.

5.- Smooth Muscles - Intestines in situ - Rabbits.

6.- Exposed Heart of Frogs.

7.- Perfusion of Frog's Circulatory System.

8.- Blood Pressure and Respiration.

CONCLUSIONS:

1.- General Discussions and Comments.

2.- Summary of Deductions from Experiments.

INTRODUCTION.

With the advent of the tendency on the part of physicians to administer small doses, and the discovery that the complex molecules of alkaloids are probably superfluous, another interesting and significant chapter has undoubtedly been added to the annals of pharmacology. If the important active group or groups of the complex alkaloid molecules can be determined, then it is not at all improbable that better compounds can be developed, both in so far as efficiency is concerned as well as a minimal undesirable effect. The active group or the relation between chemical constitution and physiological action has been investigated and determined for a few of the important alkaloids and other compounds. It was with these facts and possibilities in mind that this investigation was undertaken. It is, of course, certain that a great number of compounds will have to be synthesized and studied pharmacologically to develop a limited number of useful remedial compounds. Therefore, the immediate scope of this paper is not to study these compounds with the thought that they will be used clinically, but it is rather to help extend our present knowledge of chemical pharmacology so that the possibilities of developing useful remedies will be increased and ultimately realized.

With Macht's (1) discovery of the importance of the benzyl nucleus as an effective antispasmodic, producing both a lowering of the tone and a more or less complete inhibition of the rhythmic contraction of smooth muscular tissues, a great impetus was given to chemical pharmacology. Macht demonstrated without a doubt that the complex alkaloid molecule was not necessary and probably often deletorious to its effectiveness and to its desired reactions.

4-21 6M

Furthermore, he has shown that benzyl alcohol possesses marked local anesthetic properties. Moreover, he has shown, in collaboration with C. V. Nelson (3) that benzyl alcohol possesses antiseptic properties; and in conjunction with Fischer (4) showed that the benzyl esters possess a degree of specificity for certain protozoa.

With Hirschfelder's (5) introduction of saligenin into medicine as a local anesthetic of recognized value, both in so far as effectiveness is concerned as well as its low toxicity for the tissues as compared to other accepted compounds used for local anesthesia, it has become the nucleus for a number of important derivatives. On account of the low toxicity of saligenin and its similarity to the phenol groups, which suggested its probability as an antiseptic, Hirschfelder and his collaborators (6) demonstrated that the mercury derivative had an antiseptic action which compared very favorably with that of mercuric chloride. From the study of the chemical structure of saligenin it is found to belong to the salicyl group which comprises a number of useful sedatives, antipyretics and antiseptics. This would augment the increased attention which might be given to saligenin as a nucleus for the development of useful remedial compounds.

It is noteworthy that benzoic acid has been, in general, the nucleus for the development by esterification of a large series of local anesthetics. It is of no less significance that the benzyl group has also been demonstrated to have local anesthetic properties. It was with this fact in mind that Hjort and Eagan (7) investigated further the anesthetic properties of benzyl carbinol (C_6H_5-CH_2OH) and compared its action with that of B-phenmethylol

(benzyl alcohol). Hjort in conjunction with Kaufmann (8) extended his observations further to related compounds, including the benzyl carbinol which was found to be less irritant to the tissues than its homologues, which it has been compared with.

Since Macht's study and significant discovery of the action of the benzyl group on smooth muscle, pharmacologists have been continually absorbed with the development of various esters of the benzyl group. Macht (9), with his collaborators, has reported papers from time to time concerning the pharmacological and clinical importance of these esters. With the questioning of the efficacy of benzyl benzoate and the resultant editorial comment (10) about its clinical use as an antispasmodic, a new impetus to the study of this group of compounds has arisen. Nielsen and Higgins (11) investigated the pharmacological action of a series of benzyl esters and concluded that they produced a definite inhibitory action upon the intestinal contractions and also lowered their tone. It was concluded from their observations that benzyl cinnamate was even more effective than the benzoate ester; and in agreement with Macht, they also found the benzyl esters to be comparatively non-toxic.

It will not be amiss at this point to briefly consider the work and progress made in pharmacology in relation to the chemical constitution and substitution in compounds to their physiological action, especially in so far as it borders on this study. Cushing (12) showed that, while tropine itself failed to elicit any typical atropine action, the introduction of a benzene nucleus gave the atropine action in degree. Furthermore, he demonstrated that this action was greatly augmented when an hydroxyl group and an asym-

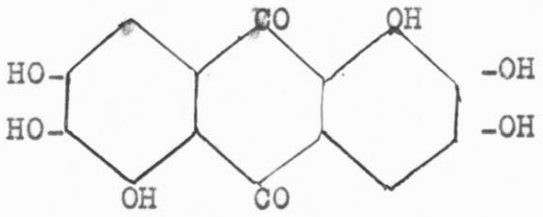
4-21 6M

metric carbon were present in the side chain. This action was further enhanced, in fact, to its maximum, only when atropine was joined to an acid of the benzene series in the side chain in which there was present an hydroxy group and an asymmetric carbon,- the whole molecule being laevorotatory. To present more direct data the work of Quigley and Hirschfelder (13) may be cited. These investigators studied the effect of substitution of one or both of the inactive hydrogens of the carbinol group of phenyl carbinols ($C_6H_5CH_2OH$). They concluded that substitution of one of the inactive hydrogens lowered the local anesthetic properties of the compound, while the substitution of both inactive hydrogens markedly diminished or completely obliterated the anesthetic effects. An examination of the compounds will point out that in the monosubstitution an asymmetric carbon is effected in the molecule and that in the disubstitution the asymmetry is again removed. Hence, this agrees in substance with the work of Cushing.

It will probably be advantageous to briefly consider the more general effects of substitution in the side chain of aromatic compounds. It is said that increasing the number of hydroxyl groups in the benzene ring decreases the convulsant action so preponderant in the phenols, but it increases the lethargic and tremor action of the compounds. However, Chassevant and Garnier (14) state that the dioxy benzols are more toxic than the phenols, but less toxic than the trioxy benzols. To be sure, the dioxy and trioxy phenols will vary in activity among the individual members of the respective groups. Binet (15) and Binet and Prevost (16) demonstrated in their investigations on the comparative toxicity of some phenol derivatives that the introduction of an aldehyde

(COH) or an alcohol (CH_2OH) group into the benzene nucleus lowered their toxicity and also their activity as convulsants. The results are relative as the introduction of the alcohol group (CH_2OH) into the phenol lowers the toxicity considerably more than the introduction of an aldehyde group,- in the former addition saligenin ($C_6H_4OH.CH_2OH$), and in the latter, salicyl aldehyde ($C_6H_4OH.COH$) would result.

The work of Ebstein (17) is imperative and fundamentally interesting as a background to the study of these compounds. He found that by substituting alkyl radicals for the hydrogen of the hydroxy group of purgatine and still better demonstrated by substituting likewise in exodine,-



Exodine.

forming ethers (B-O-R). By substituting in from one to four of the hydroxy groups their activity was greatly increased. In fact,- some of these compounds were so potent that their action had to be moderated to permit practical application. Furthermore, he found that if all the hydrogens of the hydroxy groups were replaced to form ethers, the resulting compounds were inactive. This is also evidenced by the dimethyl ether of resorcine which is far more toxic than resorcine itself. In general, however, the effect of alkylation or acylation of the hydroxy group is to render the compounds less active, chemically and pharmacologically. The above unusual phenomena are probably explained by assuming that alkylation

favors selective affinity for the substance by the sensitive membranes.

Additional interesting observations were reported by Frederico and Terroine (18) who investigated recently the cardiac action of substances of the quinolin group and found those compounds to have qualitatively an action similar to that of quinin and other important cinchona alkaloids. Their observations are especially interesting as they found that the introduction of a methyl group increased the toxicity, regardless of its position in the molecule. The introduction of methoxy groups elicited a similar augmenting effect, as did also hydrogenation of the molecule.

When we consider the substitution of the hydrogen of the hydroxy group by an acid (carboxyl) group, the change effected physiologically is comparatively surprising. It must certainly be remembered that with the compounds formed from such substitution saponification in the organism is an important factor in their reaction. Saponification, as a rule, takes place very gradually under ordinary circumstances. Hence, it might be expected that the intensity of action will decrease and duration will increase.

PURPOSE AND SCOPE OF THIS STUDY.

With the brief survey of the literature upon this subject and upon reflection of the local anesthetics, specifics, antispasmodics, and so forth, it occurred that an investigation of the relation between the substitution of the hydrogen in the phenolic hydroxyl group and in some instances also in the carbinol group (CH_7OH) of saligenin (ortho-oxy-benzyl alcohol) ($C_6H_4.OH.CH_2OH$) would prove interesting. Furthermore, it did not seem to be en-

tirely without the possibility of developing compounds of therapeutic value. Although there are available a number of effective and valuable local anesthetics and antispasmodics and other types of remedial agents, not one of these is perfect nor do they represent all that is desirable in their respective fields. Since saligenin has been demonstrated to have valuable anesthetic properties, and that the benzyl group exerts a definite effect upon the smooth musculature, saligenin was therefore taken as the nucleus for these ethers. The preference for saligenin was further strengthened as it belongs to the salicyl group. Its consequent fate in the body gives rise to a group which comprises or forms the nucleus for a large number of useful sedatives, antipyretics and antiseptics.

It is evident that a compound may be studied from so many angles. Hence, only those experiments, such as blood pressure, respiration, the effect on the smooth musculature, local anesthesia, toxicity and so forth, were investigated. It was thought that these experiments would reveal to what extent the various ethers would vary in reactions and also would help to extend our knowledge of the importance of such groups,- namely, the introduction of an ether group.

NOMENCLATURE USED.

The nomenclature is that which is in general use and derived from a consideration of the structure of ordinary sulphuric ether. Sulphuric ether is designated chemically as an organic oxide, di-ethyl oxide (C_2H_5-O-H_5C_2), that is, two alkyl groups are joined by an oxygen group. In the compounds studied we have exactly the same condition only one of the alkyl groups in ordinary ether is

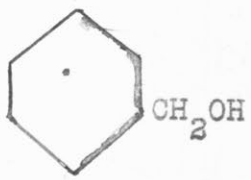
replaced by the benzyl alcohol ($C_6H_4CH_2OH$-) group. The other oxygen bond is satisfied either by an alkyl or an aromatic group. Since the compounds are made from saligenin ($C_6H_4.OH.CH_2OH$) in which the hydrogen of the phenolic hydroxyl group is replaced by an alkyl or an aromatic group, the compounds are designated as ethers of saligenin. If the hydrogen of the carbinol group (CH_2OH) of saligenin is also substituted the compound is designated as a double (di) ether of saligenin. To illustrate; if we substitute an ethyl group for the hydrogen of the hydroxy group in saligenin, we would have the ethyl ether of saligenin ($C_6H_4.CH_2OH - O-CH_2CH_3$).

COMPOUNDS INVESTIGATED.

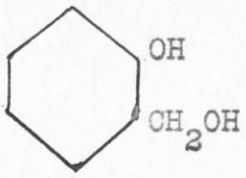
The following compounds were studied:

1) the ethyl-ether of saligenin ($C_6H_4.CH_2OH-O-CH_2CH_3$);
2) the normal (N)-Butyl-ether of saligenin ($C_6H_4.CH_2OH-O-CH_2CH_2CH_2CH_3$);
3) the Iso-Amyl-ether of saligenin ($C_6H_4.CH_2OH-O-CH_2.CH_2.CH.(CH_3)2$);
4) Benzyl-ether of saligenin ($C_6H_4-CH_2OH-O-CH_2.C_6H_5$);
5) Acetyl-ether of saligenin ($C_6H_4.CH_2OH-O-CO.CH_3$);
6) Benzoyl-ether of saligenin ($C_6H_4-CH_2OH-O-CO.C_6H_5$);
7) Di-benzoyl-ether of saligenin ($C_6H_4.CH_2-O-CO,C_6H_5.O-CO.C_6H_5$).

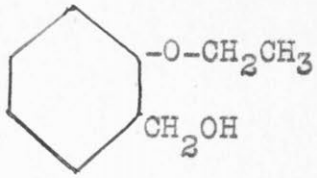
CHEMICAL STRUCTURE OF COMPOUNDS.



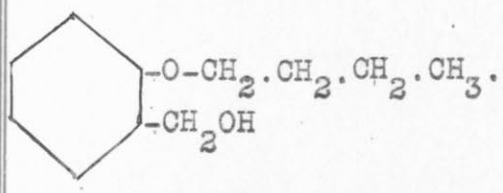
Benzyl Alcohol



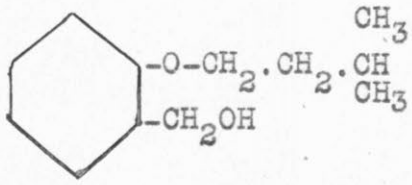
o-Oxy-Benzyl Alcohol
(Saligenin)



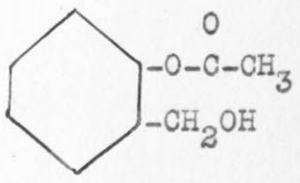
1) Ethyl-Ether of
Saligenin.



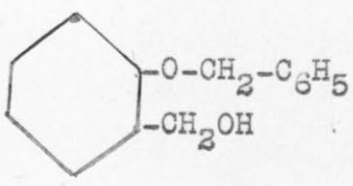
2) N-Butyl-Ether of
Saligenin



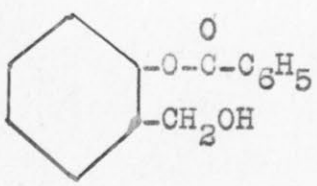
3) Iso-Amyl-Ether of
Saligenin



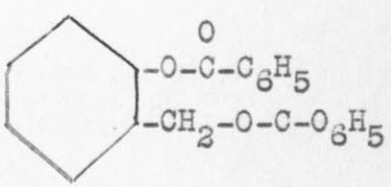
4) Acetyl Ether
of Saligenin



5) Benzyl Ether of
Saligenin



6) Benzoyl Ether of
Saligenin



7) Di-Benzoyl
Ether of
Saligenin.

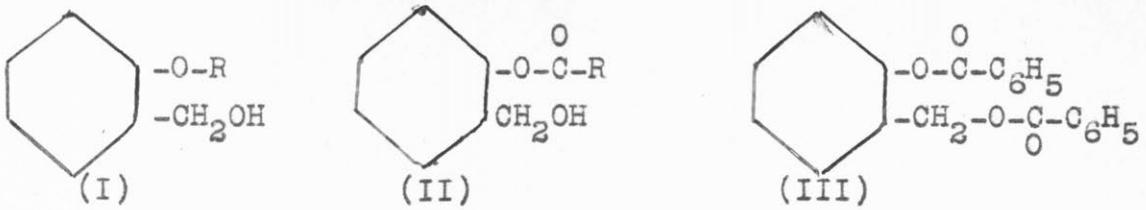
SYNTHESIS OF COMPOUNDS.

It will be noted, as pointed out by the authors of the experimental synthesis of these compounds (20) that the saligenin molecule has been varied in such a manner that the resulting compounds may be grouped conveniently into three different classes or types of compounds.

By substituting in the phenolic hydroxyl group of the saligenin molecule simple ethers were synthesized in which the hydrogen of the hydroxyl group was replaced by aliphatic and aromatic alcohol groups. This class of ethers may be graphically indicated as below in Formula (I) and have been referred to in this paper, for convenience of discussion, as a carbinol substitution of the aliphatic and aromatic compounds. The two remaining types of substituted compounds are really esters but have been, and will be, referred to in this paper as ethers inasmuch as the terminology will thus become standard throughout the discussions in this paper,- e.g. all the compounds will be regarded as ethers as already explained.

By the esterification of the saligenin molecule upon the phenolic hydroxyl group the second type (II) of compounds were effected. In this group, as in group (I). the ether group or R may be either an aliphatic (acetyl ether) or an aromatic (Benzoyl ether) group. This type of the compounds is graphically represented below in Formula (II).

The third type is also an ester but illustrates the saligenin molecule where both the phenolic hydroxyl and carbinol groups are masked. This gives rise to the di-benzoyl ether (dibenzoate) and is represented below in formula (III):



The bibliography of the literature treating the synthesis of these compounds has been omitted, but this may be obtained by referring to the published article by M. C. Hart and A. D. Hirschfelder (20) who synthesized the compounds. A brief resume of the general methods employed for the synthesis of these compounds is given together with a short abstract of the substances and their proportions used for the synthesis of the individual ether.

Potassium saligenate, prepared by treating saligenin ($C_6H_5.OH.\ CH_2OH$) in acetone solution with alcoholic potash (practically resulted with theoretical yield), was used as a source of the saligenin. The simple ethers of formula I were prepared by refluxing potassium saligenin with alkyl or aryl halides, and distilling under reduced pressure to 25 mm. of mercury. The ethers of the formula II type were prepared by refluxing potassium saligenin and the anhydrides or acid chlorides of the respective ethers desired. The ether of the formula III type was prepared by benzolation of saligenin in a pyridine solution in the presence of calcium carbonate, using a slight excess of the benzoyl chloride.

Preparation of the individual ethers:

80 Gms. of K.-Saligenate in 150 mils of ethyl iodide - yielded 47 Gms. of the ethyl ether (62.8% yield).

95 Gms. of K.-Saligenate in 100 mils n-butyl bromide- yielded 57 Gms. of the n-butyl ether (51.2% yield).

40 Gms. of K.-Saligenate in 100 gms. of iso-amyl bromide-

TABLE #1.

Comparison of the Physical Properties of the Ether Compounds.

COMPOUNDS			SOLUBILITIES					
Name	B.P.at pressure indicated mm. Hg.	Appearance of Substance	Alcohol Ether Chloroform Benzene Acetone	Water	Glycerine	Olive Oil	Petroleum Ether	$FeCl_3$ test for OH group
Ethyl Ether	265°C. 28 min.	A pleasant, ethereal smelling oil, colorless	Very sol.	Insoluble	Insoluble	Insoluble	Very sol.	Negative
N-Butyl Ether	160-162° C.25 mm.	Clear, colorless oil	Very sol.	Insoluble	Insoluble	Miscible	Very sol.	Negative
Iso-Amyl Ether	176°C. 25 mm.	Clear, colorless oil	Very sol.	Insoluble	Insoluble	Miscible	Very sol.	Negative
Benzyl Ether	221-222° C.25 mm.	Clear, colorless oil hardening to waxy crystalline solid m.p.37°C	Very sol.	Insoluble	Insoluble	Very sol.	Very sol.	Negative
Acetyl Ether	168-169° C.30 mm.	Clear, colorless oil	Very sol.	Insoluble	Insoluble	Very sol.	Very sol.	Negative immediately after preparation
Benzoyl Ether		Clear, colorless oil.	Very sol.	Insoluble	Sparingly sol.	Very sol.	Sparingly sol.	Negative

Di-Benzoyl Ether		Clear, colorless oil, becoming a waxy crystalline sub.m.p.51°C.	Very sol.	Insoluble.	Insoluble	Very sol.	Sparingly sol.	Negative

yielded 22.0 Gms. of iso-amyl ether (45.9% yield).

50 Gms. of K.-Saligenate in 100 mils of benzyl chloride.- yielded 29 Gms. of the benzyl ether (43.0% yield).

50 Gms. of K.-Saligenate was refluxed with an excess of acetic anhydride (80 mils) - yielded 33. Gms. of acetyl ether (65.4% yield).

40 Gms. of K.-Saligenate, suspended in 250 mils of alcohol, to which 27 mils of benzoyl chloride was added slowly-, extracted with ether and then with 10% sodium of carbonate solution, washed and dried over $CaCl_2$(anhydrous) - yielded 52.0 Gms.

10 Gms. of saligenin, dissolved in 35 mils of pyridine to this 10 Gms. of calcium carbonate was added and 27 mils of benzoyl chloride added slowly, extracted and treated as the benzoyl ether above,- yield 18 Gms. of di-benzoyl ether (67.2% yield).(12A)

PHYSIOLOGICAL ACTION OF BENZYL ALCOHOL AND SALIGENIN.

Since the compounds to be studied may be conveniently considered either as derivatives of benzyl alcohol or of saligenin, it would seem valuable to summarize the important physiological effects of these two compounds. A summary of the chief effects of benzyl alcohol is obtained by abstracting the results and conclusions of Macht's work (2) and these are given below.

Benzyl alcohol elicits "powerful local anesthetic properties" when applied locally to mucous membranes (tongue, lips, etc.) and also when injected subcutaneously in a one to four per cent solution. It produces considerable irritation when first applied, but this disappears as the anesthesia becomes more complete. Complete anesthesia was found to continue for thirty minutes to two

hours. When the benzyl alcohol is applied directly to the sciatic nerve of a single pithed frog, paralysis of the sensory nerve fibres was observed and with a concentration of 4% motor block resulted.

The effects from intravenous injections may be stated briefly as follows: With a 1% solution there was a fall of blood pressure due to peripheral vaso dilation. No effect upon the vaso-dilator center was observed. Continued injections produced no further effect upon the vaso-motor center and heart until toxic doses of the alcohol were administered.

The effects upon the respiration were inappreciable when fifteen mils of a 1% solution per Kg. body weight were given to a dog. Small doses reacted with a primary stimulation of the respiration which was followed by a gradual paralysis of the respiratory center as the toxic dose was approached. Death resulted from failure of the respiration, the heart continuing to beat after cessation of breathing.

Intravenous injections of 10 mils of a 1% solution per Kg. body weight in dogs produced a sedative action,- the animal appearing to be somewhat narcotized. Larger doses evidenced a deleterious action upon the central nervous system; and with toxic doses convulsions, which were undoubtedly of central origin, developed and paralysis of the respiration followed.

Rabbits usually recovered from two mils per Kg. of the alcohol; cats at times from one mil per Kg., when given subcutaneously. The doses as given intravenously to dogs were never fatal when the same dose was administered intraperitoneally, intramuscularly and subcutaneously.

Furthermore, in common with the benzyl esters, benzyl alcohol also elicits some of the characteristic group properties on smooth muscle, namely producing a lowering of tonus and an inhibitory action on the rhythmic contractions of the smooth muscular organs.

The chief and important properties of saligenin may be obtained by considering the work reported by Hirschfelder (5) who was the first to investigate this substance pharmacologically.

Saligenin is less irritating, a more effective local anesthetic and slightly less toxic than benzyl alcohol. It has on this basis been accorded a place among the local anesthetics which are used clinically.

In common with benzyl alcohol it lowers the blood pressure when given intravenously to a dog. If the 4% solution is injected slowly the fall is very gradual, and is accompanied by a slight slowing of the respiration. If a dose of 0.7 grams per Kg. is followed by a further injection, a sudden fall of the blood pressure results and the respiration stops, ending in the death of the animal. The heart continued to beat after cessation of the respiration as in the benzyl alcohol. A rapid injection of saligenin resulted in an initial slowing or paralysis of the respiration which continued for a few seconds, recovering spontaneously. These phenomena could be observed with each repitition of the same dose.

TOXICITY AND GENERAL EFFECTS.

1.- Methods and Technique:

The principles of this experiment to determine the toxicity of these ether compounds are those of Houghton's "Twelve-Hour" frog method (21) for the standardization of digitalis, with the ex-

ception that the results were recorded at the end of twenty-four hours instead of twelve hours. The twenty-four hour method was given preference on account of the physical properties of the compounds and with the expectation that more uniform results would be obtained. The method is briefly as follows.

Animals: Frogs belonging to the species of Rana esculentus were used, selecting only those that were healthy and apparently very active. They were kept under constant conditions (temperature, environment etc.) as much as facilities would permit. Five tin and wire cages, properly numbered for identification, were used to house the frogs during the experiments. The pails were kept immersed in water to a depth of from two to four centimeters and kept at uniform temperature. The water in the tank was renewed twice daily, at twelve hour intervals. The frogs were weighed and marked with various colored strings tied loosely about one hind limb and placed in the respective cages. A different colored string was used for the frogs intended for a particular drug, e.g. those frogs to be used for the ethyl ether of saligenin were all marked with a blue string; those for the N-Butyl ether with a red, etc. The frogs were all numbered on the data cards, corresponding to the number on the cage in which they were kept. To illustrate, five frogs were used in a series for each drug and were numbered on the data cards from I - V respectively. The frog designated as No. I was placed in the cage marked No. I and likewise all the frogs numbered No. I for the different compounds were placed in cage No. I, being identified by the colored string. By this arrangement it was thought that the results would be more comparative as they would all be done under exactly the same environment and under similar conditions.

Preparations of Compounds: As the various ethers were insoluble in water and saline solution, the compounds were prepared for injection as follows. A primary emulsion was made by the continental method. One part of powdered acacia (5 gms.) was triturated to a uniform mixture in a mortar with four parts of olive oil (20 mils); to this mixture two parts (10 cc.) of distilled water were added at one time and the mixture again triturated until the mass was white and the oil droplets were about the size of the red blood corpuscles of a mammal, when observed under the microscope. This primary emulsion may then be diluted indefinitely by adding distilled water or saline solution in divided portions until the desired volume or dilution is obtained. The primary emulsion, made in the proportions stated above was diluted with distilled water to a volume of about 90 cc. The desired quantity of the drugs was measured in a graduate and dissolved in 2 cc. of 95% alcohol. This alcoholic solution of the drug was incorporated into the emulsion and the latter made up to a volume of 100 cc. The emulsions were made 5% by volume. Those compounds that were rather toxic were diluted to the desired strength with the control emulsion which had been prepared exactly as the drug emulsion, only omitting the compound. It will be noted that by making the primary emulsion first, all the emulsion solutions were certain to have droplets of the same size and the same uniformity. It is also interesting to note that these emulsions were permanent from two to four weeks and even after they had separated somewhat, a little shaking would cause the emulsion to resuspend and to remain permanent for several days again. The alcohol was introduced primarily to prevent rancidity, but it was also thought to facilitate a more minute suspension

of the drug in the emulsion. The first presumption was correct as no rancidity has occurred in emulsions that have been made for eight months. The reason for the twenty-four hour method is now apparent, e.g., to permit more time for the absorption of the compounds from the emulsions and more accuracy in deducing the minimum lethal dose.

Technique and Experiments: When the frogs had been weighed and marked, the dose per gram body weight was calculated. The dose was stated in milligrams per gram body weight. It must be called to attention that the weight of the drug was determined by assuming that 1 mil equalled 1 gram or that it had a sp. gr. of 1.0 compared to water,- a fact which may or may not be correct. It was for that reason that the total dose was stated both in milligrams and mils of the emulsion used. An all glass tuberculin syringe, graduated to 1/100 of a cc., was used to make the injection whenever a fraction of a cc. was required. The strength of the solution was so regulated that the total dose injected was not less than 0.25 cc. nor more than 2.5 cc. The injections were all made into the anterior lymph sac and the animals returned to their respective cages immediately after the injection.

In the first series a large range of doses for the different frogs was used to arrive at an approximate lethal dose. The doses in the following series were graduated until the minimal lethal dose per gram body weight for twenty-four hours was determined. In each series for the individual drug one frog was always used as a control by being injected with the control emulsion and kept under the same conditions. Generally three to five series sufficed to determine the lethal dose although more were done if deemed necessary. At the end of twenty-four hours the records were taken and the condition of

the heart of the dead frogs was noted. In order to conserve animals and material the general effects (effect on pupil, reflex, etc.) were noted at this time by observing the frogs frequently during the first few hours following the injections.

2.- Data and Protocols:

The general method used for recording the data and the results for these experiments is best illustrated by inserting the records kept for one of the compounds (ethyl-ether of saligenin).

SERIES I.

Toxicity-Ethyl Ether of Saligenin - 2% Emulsion - 3/2/21.

FROGS		DOSAGE			Time of Injection	Results in 24 Hrs.	Remarks.
No.	Body wt.in Grams	Mgms. per Gm. body wt.	Total dose in mgms.	Total dose in cc. of Emul.			
I	11	1.0	11.0	0.55	11:04	Dead. Heart dilated.	Deep narcosis in 20 min. Reflexes absent.
II	10	0.8	8.0	0.40	11:06	Dead. Heart dilated	Deep narcosis in 20 min. Reflexes absent.
III	14	0.6	8.4	0.42	11:08	Dead. Heart dilated	Deep narcosis in 20 min. Reflexes absent.
IV	12	0.5	6.0	0.30	11:10	Alive. Reflexes slight. Deep stupor.	Marked narcosis in 20 min. Reflexes diminished.
V	11	0.4	4.4	0.22	11:13	Alive. Apparently normal	Marked narcosis in 20 min. Reflexes diminished.
VI	13	0.1	Control Emul.	1.3	11:15	Alive. Normal.	Normal.

4-21 6M

SERIES II.

Toxicity - Ethyl Ether of Saligenin - 2% Emulsion - 3/2/21.

FROGS			DOSAGE		Time of Injection	Results in 24 Hours.	Remarks.
No.	Body wt. in Gms.	Mgms. per Gm. Body Wt.	Total Dose in Mgms.	Total Dose in cc. of Emul.			
I	13	0.6	7.8	0.35	2:00	Alive. Very deep narcosis (apparently dead) Reflexes absent.	Deep stupor in ½ hour. Reflexes absent.
II	12	0.58	6.96	0.39	2:01	Dead. Heart dilated.	Deep narcosis in ½ hr. Reflexes absent
III	12	0.55	6.6	0.33	2:03	Alive. Normal.	Deep stupor in ½ hr. Reflexes absent
IV	12	0.50	6.0	0.3	2:06	Dead. Heart dilated.	Deep stupor in ½ hr. Reflexes absent
V	11	0.1cc.	Control Emul.	1.1cc.	2:09	Alive. Normal.	Normal at all times.
				SERIES III. 3/9/21.			
I	13	0.5	6.5	0.33	3:29	Dead. Heart dilated.	Deep stupor in ½hr. Reflexes absent.
II	13	0.45	5.4	0.27	3:30	Alive. Deep stupor. Reflexes absent.	Deep stupor in ½ hr. Reflexes absent.
III	11	0.5	6.05	0.3	3:33	Dead. Heart dilated.	Deep stupor in ½ hr. Reflexes absent.
IV	9	0.6	3.6	0.18	3:36	Alive. Normal.	Deep stupor in ½ hr. Reflexes absent.
V	15	0.2cc.	Control Emul.	3.0	3:40	Alive. Normal.	Normal at all times.

4-21 6M

The variation in the degree of toxicity of the variously substituted derivatives of saligenin is shown in table #2. The outstanding general effects are noted under remarks.

TABLE #2.

Summarized Effects of the Ether Compounds upon the Toxicity in Frogs.

Compounds	M.L.O. per Gm. Body Wt. in 24 Hrs.		Remarks.
	In milligrams.	In mils of the emulsion used.	
Ethyl Ether of Saligenin	0.5	0.025 cc. of 2% Emulsion	Deep stupor in 20 min. to 1 hr. Reflexes diminished. Pupils apparently normal. Heart diastole & dilated.
Iso-Amyl Ether of Saligenin	0.25	0.025 cc. of 1% Emulsion	Deep stupor in ½-1 hr. Decreased irritability. Pupils at times very much constricted at death and slightly in ½-1 hr. Heart-diastole and dilated.
Iso-Amyl Ether of Saligenin	0.1 - 0.12	0.015 cc. of 1% Emulsion	Deep stupor in ½ hr. Reflexes slight or absent. Pupils? Heart-general dilated. In 2 frogs Vent. in Systole.
Benzyl Ether of Saligenin	0.36-0.4	0.02 cc. of 2% Emulsion	Deep stupor in ½ hr. Reflexes generally diminished. In 2 frogs slight spasms. Marked prostration and incoordination at times. Pupils? Heart-dilated.
Acetyl Ether of Saligenin	0.9	0.045 cc. of 2% Emulsion	Deep stupor in ½-1 hr. Decreased irritability (In 3 instances increased irritibility) Pupil unaffected.
Benzoyl Ether of Saligenin	1.0	0.05 cc. of 2% Emulsion	Stupor in 20 min. to 1 hr. Reflexes diminished or absent. Pupils? Incoordination. Heart-dilated.
Di-Benzoyl Ether of Saligenin	2.0-3.0	0.1 - 0.15 cc. of 2% Emulsion.	Marked prostration. Increased irritability. Pupils apparently normal. Heart-dilated-congested.

3.- Conclusions and Comments:

As far as toxicity is concerned the compounds elicit rather interesting results and seem to group themselves into three general classes. In fact, the substitution in the phenolic hydroxyl group appears to be no small factor in determining the toxicity of a compound.

a) Alkyl substitution in the hydroxy group increases the toxicity over that of saligenin; it also seems to give rise to more toxic compounds than do the corresponding aromatic substitutions. The ethyl, N-butyl, and the iso-amyl ethers are all more toxic than saligenin; while the benzyl ether is also more toxic than saligenin itself it is considerably less toxic than the above aliphatic ethers. The toxicity of the alkyl (aliphatic) ethers seems to increase with the length of the carbon chain, as the ethyl, N-butyl , and iso-amyl ether become progressively more toxic in the order named. It is, in fact, striking that the toxicity appears to increase in a definite ratio (2:1) compared to that of the compound just below it in carbon content,- e.g. the N-butyl ether is twice as toxic as the ethyl ether and the iso-amyl ether, in turn, twice as toxic as the N-butyl ether.

b) On the other hand substitution of the hydrogen bond of the hydroxy group with a carboxylic group (-O[H -- HO]--C(=O)-) renders the compounds less toxic than saligenin. Here again the aromatic substitutions (benzoyl) appear to be less toxic than the corresponding aliphatic sub-

stitutions (acetyl). Furthermore, if the hydrogen of the carbinol group of saligenin is also masked with a carboxyllic group ($-CH_2-O[H--HO]--\overset{O}{\overset{\|}{C}}-$) (dibenzoyl), the toxicity is still further reduced as noticed by referring to Table #2.

c) All the ethers appear to produce stupor or narcosis in a degree and generally reduce the irritability (diminish reflex response). In a few instances with the benzyl, benzoyl and dibenzoyl ethers convulsions (strychnine-like) were a feature of the reactions with these aromatic substituted compounds. This is readily accounted for by the hydrolysis of the aromatic compounds, liberating the benzene nucleus which is responsible for the convulsions. There was no apparent effect upon the pupils of the eyes except the N-butyl ether produced in a number of instances a constriction of the pupil. It is interesting to note that the aromatic compounds (the benzyl, benzoyl, and the di-benzoyl ethers) produced in several instances marked incoordination and prostration. An explanation for this may probably be had in the cerebral action of the liberated benzene nucleus from the ethers. Further discussion of the significance of the pharmacological action to chemical constitution will be given in the general discussion.

LOCAL ANESTHESIA.

FROGS.

1.- Methods and Techniques:

The local anesthetic action of these compounds was first attempted in the usual manner. The sciatic nerve of a single pithed frog was exposed and the drug solution applied directly to the nerve by impregnating a pledget of cotton with the drug emulsion and inserting this underneath the nerve. The sensory response was determined from time to time (every 30 seconds) by dipping the toes equally deep into a 1% sulphuric acid solution and holding them in the acid for fifteen seconds. The acid was washed off with water after each immersion. If no response resulted in the fifteen seconds, anesthesia of the sciatic nerve was considered to be complete. The duration of the sensory anesthesia was determined in a similar manner except the toes were only immersed in the 1% sulphuric acid every five minutes. If the leg was withdrawn the anesthesia was considered incomplete (partial return) and the time intervening between the effective anesthesia and the return of sensations was considered as the duration of anesthesia. The motor anesthesia was determined by stimulating the nerve well above and below the cotton pledget with an induction coil every half minute until anesthesia was complete. When anesthesia was effected, the motor return was determined by testing similarly the response to the electrical current when this was applied above the anesthetized area. It must be noted that a control was done simultaneously on the other sciatic nerve using a cotton pledget impregnated with the control emulsion.

It was soon observed that, due to the irritant action of the ethers, the nerve was apparently killed as no return was noticed for three hours and in a couple of instances, for five hours. This was particularly true of the motor sensations; the sensory responses

were very variable and uncertain.

The above method was, therefore, abandoned and the sensory anesthesia was carried out as follows: A frog was single pithed and permitted to recover from the shock. It's normal sensory response was determined by dipping successively the toes of both limbs into a 1.0% sulphuric acid solution. The acid was washed off at once. The toes of one foot were then immersed into the drug solution (usually about 3 cm.) and the other into the control emulsion. The legs were removed from the drug and control emulsions, washed, and dipped into the 1.0% sulphuric acid solution and held there fifteen seconds or until it was removed reflexly. This was repeated every thirty seconds for the first two minutes and thereafter every minute up to a total exposure of five minutes, and after that at five minute intervals. Anesthesia was considered complete if there was no response within the fifteen seconds that it was kept in the 1.0% sulphur acid solution. The duration was determined by testing the sensory response as above every five minutes after complete anesthesia had resulted.

The drug solutions were those used for toxicity and the desired strength effected by diluting with the control emulsion. A convenient container for the emulsions in this work was made by breaking a test tube in the center. It might be well to state that the concentration or strength of the solutions was kept as low as was thought consistent with the expected results on account of the irritant action of the ethers.

2.- Data and Protocols:

When the solutions were applied the time was recorded and from this the incidence (time for the drug to take effect) and the

duration of the anesthesia was computed. Five to ten frogs were used for each drug. The problem was rather to determine if the compounds had or did not have anesthetic properties, than to determine the absolute efficiency and the optimium concentration for the particular compounds. Appended below is the data obtained with the iso-amyl ether (2% Emulsion) in five frogs:

TABLE #3.

Local Anesthesia - Frog - Iso-Amyl Ether of Saligenin - 7/28/21.

Frog No.	Time Sol. Applied	Strength of Sol. used (Emul.)	Sensory Anesthesia.	
			Incidence Minutes	Duration Minutes
I	10:10	2%	2½-3	5-10
II	10:18	2%	2½-3	25-30
III	4:23	2%	2 -2½	15-20
IV	4:28	2%	1½-2	15-20
V	4:45	2%	2 -2½	20-25

In Table #4 the results, obtained with the various ethers, are tabulated and the compounds may readily be compared as to their relative efficiency.

TABLE #4.

A Summary of the Local Anesthetic Effects of the Various Ethers of Saligenin.

COMPOUNDS USED		ANESTHESIA		REMARKS
Name	Strength of Emulsion	Incidence Minutes	Duration	
Ethyl Ether of Saligenin	2% Emul.	½-3 min.	10-25 min.	
N-Butyl Ether of Saligenin	2% Emul.	1-5 min.	1-2½ Hours.	Very irritant and when applied directly to nerve giving appearance to the tissue as phenol does
Iso-Amyl Ether of Saligenin	2% Emul.	2-3 min.	10-30 min.	
Benzyl Ether of Saligenin	2% Emul.	2-4 min.	10-25 min.	
Acetyl Ether of Saligenin	2% Emul.	1-2 min.	10-20 min.	A little free acetic acid was present due to hydrolysis.
Benzoyl Ether	2% Emul.	3-8 min.	5-20 min.	
Di-Benzoyl Ether	2% Emul. 5% Emul.			No anesthesia in 30 min. No anesthesia in 30 min.
Saligenin (2)	2% Sol. in $NaCl_2$ 0.75		60 min.	Inserted for compounds applied directly to exposed sciatic nerve.

10-20 5M

3.- Conclusions and Comments:

The local anesthetic effects of these ethers upon the sensory mechanism of the frog's hind limbs present some very interesting and significant facts. The chief generalities that can be ascertained from the results may be grouped under four salient points:

a) The substitution of the alkyl group for the hydrogen of the hydroxy group of saligenin produced a very marked diminution in the anesthetic action. It must, however, be noted that the N-butyl ether of saligenin is an exception to the above, as it produced a distinct increase in the anesthetic efficiency of saligenin. This is all the more significant as the normal butyl ether of saligenin is the most irritant of the entire series. Hirschfelder and Collaborators (5) recorded similar diminutive effects for the methyl and ethyl ethers of saligenin. This would indicate that the hydroxyl group intact enhances the local anesthetic action of the carbinol group (CH_2OH).

b) If the hydrogen bond of the hydroxy group of saligenin is neutralized by the hydroxy bond of a carboxyl group, the anesthetic action is apparently still further reduced. It would indicate that it is of little importance whether the carboxyl group be obtained from an aliphatic or an aromatic acid as the acetyl and benzoyl ethers are about of equal efficiency.

c) If in addition to the neutralization of the hydroxy group of the saligenin, the hydrogen bond of the carbinol group (CH_2O-H) is also offset with a carboxyl

group, the anesthetic action of saligenin disappears entirely. This, of course, we might expect as the esters exert their action chiefly upon the smooth musculature and the benzene group upon the brain and not upon the cord and periphery.

d) The introduction of the aromatic carbinol group (benzyl $-C_6H_5-CH_2-$) into the hydroxy group seems to produce a similar effect to that of the alkyl substitution, e.g., a diminution in the anesthetic properties and in about the same degree. Therefore, it would seem that groups belonging to the same class,-as carbinol, carboxyl and so forth, exert a similar action upon the anesthetic efficiency of saligenin. This might suggest that the destruction or blocking of the hydroxy group, regardless by what compound or what group, produces an unmistakable effect upon the local anesthetic action of saligenin,- qualitatively if not quantitatively.

LOCAL ANESTHESIA

MAN.

1.- Methods and Technique:

The anesthetic action was determined upon the mucous membrane of the tongue. A piece of cotton, impregnated with a 2% emulsion of the drug, was placed on one side of the tongue; and on the other side another piece of cotton, saturated with the control emulsion, was placed simultaneously. The results were deduced by noting the general numbness of the tongue and sensitiveness to the prick of a needle. The above tests with the needle were made every minute to

determine both incidence and duration of anesthesia up to five minutes after anesthesia was effected, then at intervals of three to five minutes. Each experiment was repeated two to four times on different days.

2.- Data and Protocols:

The data obtained with one of these compounds is given to illustrate the information that was sought and how it was recorded.

N-Butyl Ether of Saligenin - 2% Emulsion - 7/25/21.

Very irritant.

Numbness in ½ - 1 minute - which continued from 8 - 15 min.

Slightly less sensitive to prick of a needle.

A brief summary of the results with these compounds is given in the following table:

TABLE #5.

The Local Anesthetic Properties of the Ethers upon the Mucous Membrane of the Tongue.

COMPOUNDS		NUMBNESS			
Name	Strength of Emul.	Incidence Min.	Duration Min.	Sensitiveness to pin prick	REMARKS
Ethyl Ether of Saligenin	2%	3-5	5-8	No apparent change	Irritating and stings
N-Butyl Ether of Saligenin	2%	½-1	8-15	Slightly less sensitive	Very irritating, stings.
Iso-Amyl Ether of Saligenin	2%	2-4	4-5	No apparent change	Irritating and stings

Benzyl Ether of Saligenin	2%	2-3	3-5	No apparent	Very irritating and stings
Acetyl Ether of Saligenin.	2%				Acrid and biting - no apparent effects in 10 minutes
Benzoyl Ether of Saligenin	2%				Irritant and biting - no effects in 10 minutes
Dibenzoyl Ether of Saligenin	2% 5%				Non-irritant - no effects upon mucous membrane. 5% slightly irritating

3.- Conclusions and Comments:

The conclusions deduceable from the above results can not be very sharp. On account of the irritant action of these ethers satisfactory conclusions are probably warranted by the data:

a) The alkyl ethers and the benzyl ether are very irritating and produce numbness which continues only for a short time. In one or two instances an apparent diminution in the sensitiveness to the prick of a needle was evident. It is a question if the apparent anesthetic effects are not produced by the astringent action and further augmented by approaching fatigue to the irritating action of the compounds.

b) The carboxyl substitution seems to produce no discernable effects upon sensitiveness nor to produce any numbness. These compounds are either devoid of irritation or only slightly irritating. They are esters and are less active and it might be due to the absence of

irritation.

SMOOTH MUSCLES.

INTESTINAL SEGMENTS-RABBITS.

1. Methods and Technique:-

The arrangement of the apparatus is in principle the same as the method described and illustrated by Jackson (22). An elongated pan about four to six inches deep and large enough to accomodate three rather large beakers (600 cc. capacity) was filled with water. The water was kept at a constant temperature of 38-39° C. by means of a small electric thermostat which was placed centrally in the vessel. The water was agitated frequently to insure uniformity of temperature. A simple muscle lever, made from aluminium wire, was used for recording the movements or contractions of the muscle segments. A sharp point, made of medium stiff paper, was used instead of a point on the aluminium wire. It was found that the wire point was not sufficiently flexible and produced too much friction on the paper. The paper point, bent in toward the kymographion, would yield to variations in the uniformity of the smoked paper surface and produced a minimal amount of friction. Consequently, it was possible to obtain a continuous record which was more uniform and regular and which indicated more completely the changes in the segment during the experiment. A glass rod, bent at right angles and drawn out to a fine pointed hook, was attached to the lever support just back of the fulcrum to serve as the point of attachment for one end of the intestinal segment. The compressed air jet was joined with a rubber tube to the upright piece of a "T" shaped glass tube. To each of the ends of the cross piece of the

"T" shaped glass tube a suitably bent glass tube was joined so that the air would evolve through the solution near the muscle strip during its activity; and the other tube aerated the entire intestine kept in another beaker. A third beaker with Ringer-Laugendorff's solution was kept in the bath continually so that the solution used as a bath for the segment could be changed very quickly.

For the solution medium (bath) Ringer-Laugendorff's solution was used and prepared as follows:

Sodium Chloride ---------- 8.5 Gms.

Potassium Chloride ------- 0.3 Gms.

Calcium Chloride --------- 0.25 Gms.

Distilled water ------- 1000.00 cc.

The solution was warmed to 38-39°C. and kept at that temperature throughout the experiment. Of the above Ringer-Laugendorff solution 400 cc. (measured at 38°C.) were placed in the beaker to bathe the intestinal segment. The compounds to be studied were added in the form of emulsions (see Toxicity) of 2% strength. If a weaker solution of the drug seemed advisable the desired strength or per cent was obtained by diluting the 5% emulsion with the control emulsions. In most instances small quantities were added to observe better the effect upon the tonus and the rhythmic contractions of the smooth muscle. It was also thought that the isotonic equilibrium of the Ringer-Laugendorff solution would not be so apt to be disturbed and a possible effect from this source was, thus, somewhat mitigated.

The intestines from rabbits were used for the experiments. The animals were starved (given water ad libre) for twenty-four hours immediately preceding the experiment, as the activity of the

intestines was apparently increased very much by starvation. The animal was killed by a sharp blow on the head with the handle of a hammer. The abdomen was quickly opened and the intestines were removed by cutting the duodenum close to the pylorus and tearing the mesentery loose well away from the intestinal wall. The entire intestine was removed and then placed in the beaker containing the aerated Ringer-Laugendorff solution and kept in the bath at uniform temperature. The loops used were generally taken from the duodenum.

When all adjustments had been made, a segment 2-4 centimeters in length was adjusted in position for recording rhythmical contractions. This was accomplished by securing one end to the hook of the glass rod and the other end of the loop was fastened to the lever with a thread joining two pin hooks. The air was permitted to bubble through the solution at the rate of fifteen to twenty bubbles per minute, being emitted just below and a little to one side of the muscle strip. The writing point of the lever was adjusted to the smoked kymographion and a normal tracing taken. When the normal tracing had become uniform and regular the drug emulsion was added. The emulsion dispersed uniformly almost instantaneously. The effects of the compound were observed and more of the compound added when the full effects of the previous dose had been recorded. In a few instances a second dose followed the first at a short interval to obtain more distinct effects. Either before or after (generally before) the drug emulsion was added, a dose of the control emulsion was added to make sure that the changes were produced by the compounds studied and that they were not the results of secondary factors.

2.- Data and Protocols:

Typical tracings (protocols) of the effects of the individual ethers are inserted. From these the general effects might easily be deduced. Directly following the tracings a table (Table #6) is given which summarizes the results in an abstract, compact form:

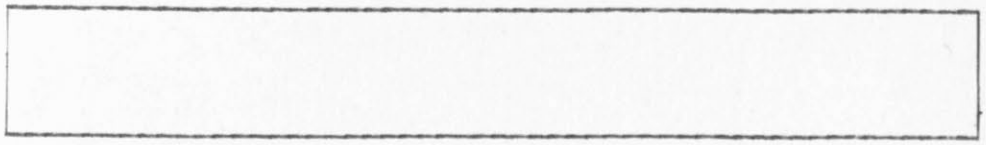


Fig. 1. Intestinal Segment from Rabbit.
Ethyl Ether of Saligenin.

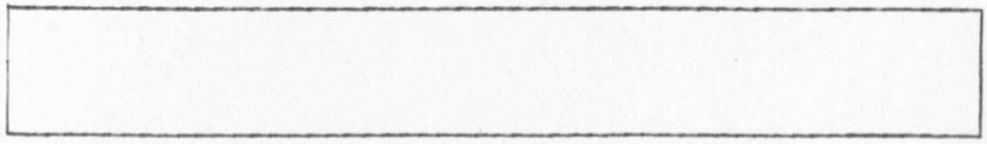


Fig. 2. Intestinal Segment from Rabbit.
N-Butyl Ether of Saligenin.

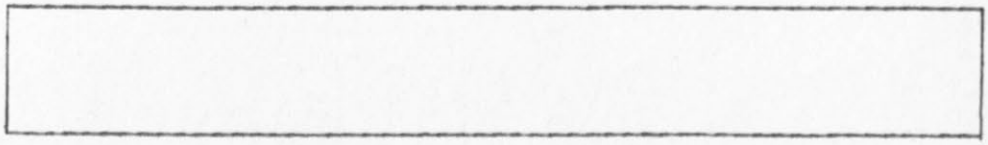


Fig. 3. Intestinal Segment from Rabbit.
Iso-Amyl Ether of Saligenin.

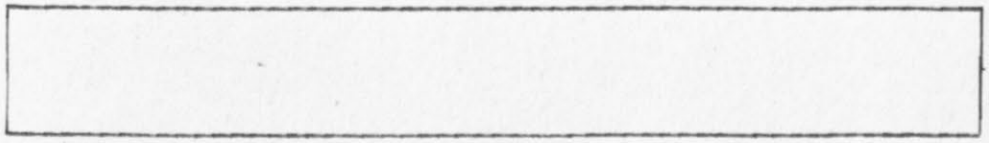


Fig. 4. Intestinal Segment from Rabbit.
Benzyl Ether of Saligenin.

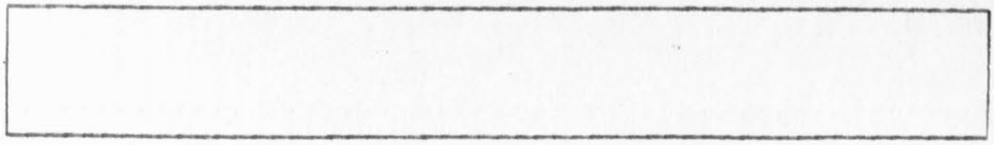


Fig. 5. Intestinal Segment from Rabbit.

Acetyl Ether of Saligenin.

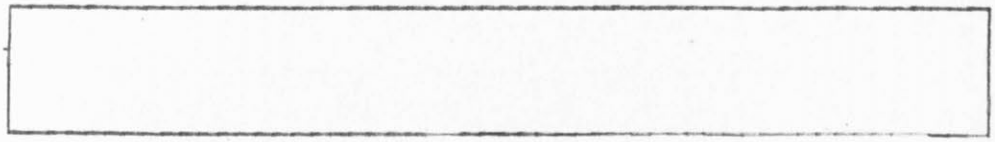


Fig. 6. Intestinal Segment from Rabbit.

Benzoyl Ether of Saligenin.

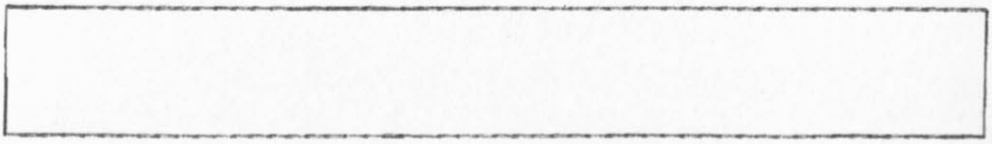


Fig. 7. Intestinal Segment from Rabbit.

Di-Benzoyl Ether of Saligenin.

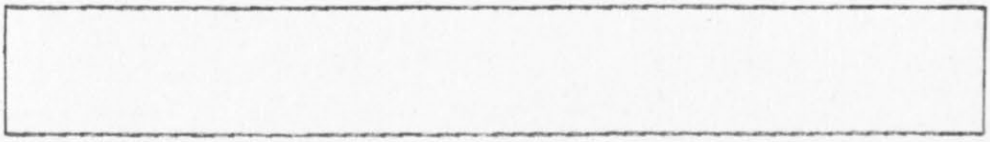


Fig. 8. Intestinal Segment from Rabbit.

Benzyl Benzoate.

TABLE #6.

Effects of the Various Ethers

upon the

Intestinal Segments-Rabbits.

COMPOUNDS		Dose(cc. of Emul.) added to 400 cc. R-L.Sol.	EFFECTS ON		REMARKS
Name	% Emul. used.		Tone of Muscle	Rhythmic Contraction.	
Ethyl Ether	2%	0.1-1.0 -2.0 cc.	No apparent effect	Decreased amplitude & slowing of rate	
N-Butyl Ether	2%	0.5-1cc.	Slight decrease	Marked decrease in amplitude. Slight slowing of rate.	
Iso-Amyl Ether	2%	0.5cc.	Marked lowering at once.	Practically complete inhibition	
Benzyl Ether	2%	0.4-0.4	Very marked decrease	Complete inhibition	Very small doses do not produce any effect on rheocord
Acetyl Ether	2%	0.5-0.5 -1.0-1.0	None or slight increase	Increased amplitude(small doses) Slowing of rate & increased amplitude then inhibition(larger doses)	
Benzoyl Ether	2%	0.3-0.4 cc.	Very slight decrease	Slowing & decreased amplitude to complete cessation	
Di-Benzoyl Ether	2%	10 cc. 10 cc. 6.5 cc.	None None None	Complete inhibition Complete inhibition Decreased amplitude.	
Benzyl Benzoate	2%	0.5 cc.	Very slight lowering	Marked decrease in amplitude	

3.- Conclusions and Comments:

It will be reacIled and it is also indicated in the protocols

that all the ethers produce an inhibitory effect upon the peristaltic rhythm of the intestinal loops. It is apparent that the aromatic ethers are the most effective and that the benzyl is the most powerful of all these compounds. In general the same identical scale of effectiveness is developed as in the preceding experiments.

a) The substitutions of the carbinol type increase progressively in activity for the alkyl ethers as the chain was lengthened. In this case it appears that the N-butyl ether does not diverge from the progressive increase in potency for the alkyl ethers. The iso-amyl ether was the only one of these alkyl ethers that produced any lowering of toxicity. They all produced a slight decrease in the amplitude and sooner or later a slowing of the rate, leading to complete cessation of movement.

b) The benzyl ether (aromatic carbinol) is the most effective and it was the only ether that had a very predominant effect upon the tone. This would indicate that the carbinol group in the benzene nucleus is the most potent in its inhibitory effects and gives greater promise for the development of valuable clinical antispasmodics.

c) The aromatic carboxyl substitutions (benzoul and dibenzoyl) also produce unmistakable effects upon the tone and the rhythmic contractions of smooth muscles. Their action is very much delayed and is not as marked as in the case of the benzyl ether. It appears that these compounds compare very favorably with benzyl benzoate as to their action on the tone and rhythmic contractions of the smooth intestinal segments.

SMOOTH MUSCLES.

INTESTINE IN SITU - RABBIT.

1.- Method and Technique:

As in the experiments on the intestinal segments the rabbit was used, so also for the observations carried on to determine the effects upon the intestines when in situ. In these experiments the rabbits were likewise starved (water ad libre) for twenty-four hours preceding the experiment. The ears were shaved to expose more distincly the ear veins into which the injections were made. The animal was weighed and then anesthetized with ether. When anesthesia was complete, the hair was removed from the abdomen from the pubis to the xyphoid process of the sternum, then shaved. An observational window was secured in the abdominal wall by making a longitudinal incision in the mid-line of the abdomen, inserting the window and fastening it in position with hemostats. The mirror was constructed of a diamond-shaped piece of glass pane fastened to a brass frame with sealing wax, a rod was soldered to the brass frame for support. When the window had been inserted it was drawn up and adjusted into a position that would afford a good view of the intestinal peristalsis. It was held in position by clamping the rod to a ring stand. If the mirror is moistened both on the inside and outside with hot water (40-50°C.) before it is secured in the abdominal wall, a cloudy mirror occurs very infrequently and a good view is insured.

Either a two or a five per cent emulsion was used and the experiments carefully controlled by injecting doses of the control emulsion before and between doses of the emulsions of the compounds.

The peristaltic waves and contractions of the intestines

were closely observed for some time (5-10 min.) before any injections were made to note any irregularities in the movements. The intestines were, as a rule, very active and the peristaltic waves rather regular. The time was noted and an injection either of the drug or control emulsion was made directly into the circulation. The effects upon the intestinal movements were closely observed. The dose was repeated at various intervals (5-30 minutes) up to an effective dose had been given or until the death of the animal resulted. It might be added at this time that the depth of the anesthesia was maintained as uniformly as possible and at no time increased immediately before an injection. Since the emulsions were injected directly into the blood stream, an effect, if any were to result, would probably have been demonstrated in a few minutes (5-10 minutes). After the completion of the observations or immediately after death of the animal, the drug and control emulsions were applied locally to determine their effects. These post-mortem observations are, of course, only very relative as death produces certain changes.

2.- Data and Protocols:

An added clarity will probably be given to the methods and general procedure as well as illustrate the data taken, if the complete data for one or two experiments is given.

EXPERIMENT #1.

Smooth Musculature-In Situ- 8/17/21.

N-Butyl-Ether of Saligenin. Rabbit-Male-Wt.1.85 Kg.-Ether Anesthesia.

9:40 A.M. - Peristalsis (normal) very rapid and forceful.

9:55 A.M. - Injected 0.8 cc. of 2% Emulsion.

Respiration stopped immediately.

Art. Respiration - revived to normal in 2-5 minutes,- no apparent effects upon peristalsis.

10:05 A.M.- Injected 1.2 cc. of 2% Emulsion.
In 2 minutes peristalsis had practically ceased.

10:20 A.M.- Injected 1.5 cc. control Emulsion.
No effects produced on peristalsis in 8-10 minutes. Peristalsis rapid.

10:30 A.M.- Injected 2 cc. of 2% Emulsion.
Peristalsis ceased almost instantly.
In 5 minutes peristalsis began to reappear very sluggishly.

10:40 A.M.- Injected 1.2 cc. of 5% Lead acetate solution in 0.9% NaCl.
Very rapid and violent peristalsis incited.

10:45 A.M.- Injected 2.0 cc. of 2% Emulsion.
Peristalsis ceased almost immediately and a dilation was apparent. Peristalsis began to return in 7 minutes - quite rapid in 10 minutes.

11:00 A.M.- Injected 3 cc. of 2% Emulsion.
Peristalsis ceased immediately. Animal died in 2 minutes. Respiration failing before heart.
In 5-10 minutes peristalsis returned in places.
The N-butyl emulsion was applied to some of these loops and the control emulsion to others.
N-butyl ether applied locally to loops dilated the intestine and inhibited the peristaltic waves. When the loop was pinched mechanically

no response resulted.

On the other hand, if the loops to which the control emulsion had been applied and other contracted parts of the intestines, even immediately beyond the loop swabbed with the N-butyl ether, gave prompt responses to mechanical stimulation.

Autopsy - Heart dilated and congested, stopped in diastole.

EXPERIMENT #2.

Smooth Musculature - In Situ -8/12/21.

Acetyl Ether of Saligenin-Rabbit-Female-Wt. 2.1 Kg.Ether Anesthesia.

3:10 - Injected 2.cc. of 2% Emulsion.
No apparent effect upon intestines. (Possibly an increased rapidity) in 10 minutes.

3:20 - Injected 5 cc. of 2% Emulsion.
No apparent effects on peristalsis in 10 - 15 min.

3:30 - Injected 3 cc. of Control Emulsion - no effects on intestine.

3:45 - Injected 5 cc. of 2% Emulsion.
In about 2-3 minutes peristalsis increased.

4:00 - Applied 2 cc. of control emulsion locally to intestine through body wall - no effect in 10 min.

4:10 - Applied locally to intestine 2 cc. of 2% Emulsion.
Immediately violent spastic contraction of intestines (very little peristalsis).

4:25 - Applied locally to intestines 2 cc. additional -
Violent continued spastic contractions of intestine resulted- peristalsis seemed to be somewhat in-

hibited (at least at first). In 5 minutes peristalsis became very rapid and powerful. The tonus of the intestine was also apparently augmented as lumen or size of intestine remained somewhat smaller after the peristaltic wave.

4:45 - Injected 2.5 cc. of 5% Emulsion.

Animal died in 2 minutes. A slight regidity of short duration followed immediately after the injection. Applied the 5% Emulsion locally over intestine through the body wall, very powerful spastic contractions which continued for several minutes,- then intestines began to dilate. (Slight peristalsis.)

Autopsy:- Heart, stopped in diastole, dilated and very congested (especially the venous system).

TABLE #7.

A Summary of the Effects of the Ether Compounds upon Smooth Musculature (Intestines-in Situ)

Rabbit-Sex Weight	COMPOUNDS			Time from first injection to death of animal.	Results And Remarks.
	Name	% of Emul. used	Doses cc.of Emul. used		
Female 1.8 Kgs.	Ethyl Ether	2%	2-2.5-3 cc.	25 min.	No appreciable effect on intestine when injected-applied locally seemed to inhibit peristalsis. Death-Resp.failing first. Autopsy-Heart dilated & congested,stopped in diastole.
Male 1.9 Kgs.	Iso-Amyl Ether	2%	1-2	15	No effects upon injection.Locally to intestines seemed to inhibit cont.& peristalsis. Autopsy-Heart dilated and con-

TABLE #7 (continued)

					gested. Stopped in diastole.
Male 1.85 Kgs.	N-Butyl Ether	2%	0.8-1.2 -2-2-3 cc.	1 hr. & 20 min.	A definite slowing or inhibition upon peristalsis after each dose. Marked inhibition(peristaltic) (block) when applied locally. Death-Resp.failing first. Autopsy-heart dilated &congested stopped in diastole.
Female 1.3 Kgs.	Benzyl Ether	2%	0.5-0.5 -0.7- 1.0- 1.0- 1.0cc.	55 min.	An apparent dilation,a degree of peristaltic inhibition when injected.Locally produced inhibition. Death-Resp.failing first. Autopsy-heart dilated & congested-Diastole.
Female 2.1 Kgs.	Acetyl Ether	2% 5%	2-5-5 2.5cc.	1 hr. 35 min.	A slight augmentation of peristalsis when injected. Locally produced spastic contractions & rapid peristalsis. Autopsy-heart dilated & congested - Diastole.
Female 1.8 Kgs.	Benzoyl Ether	2%	0.5-1- 2-3-6- 5-5	1 hr. 15 min.	Injection-Inhibits peristalsis in degree and dilates intestines Locally-entirely inhibited. Rapid peristalsis (no return in 25 min.) Autopsy-heart dilated & congested. Stopped in diastole
Female 1.9 Kgs.	Di- Benzoyl	2%	1-2- 3-2- 2.5- 2.5	1 hr. 20 min.	Injection- Inhibits or slows peristalsis. Locally - dilates and slows peristalsis. Autopsy- Heart dilated and somewhat congested. Stopped in diastole.

3.- Conclusions and Comments:

Although the number of experiments performed to determine the effects of these ether compounds upon the intestines in situ is too limited to permit comprehensive far-reaching conclusions to be deduced, enough data was obtained to indicate the possible effect of the substitution in the phenolic hydroxy group of saligenin with various alkyl and aromatic groups. The scope of these effects may be designated under the following groups:

a) The ethers composed of an alkyl substitution for the hydrogen in the hydroxy group of saligenin appear to produce little,

if any, inhibitory nor augmentatory effect upon the peristalsis or contractions of the intestines. The N-butyl ether is apparently exceptional in this respect as it produced a distinct diminution in the rate and force of the peristalsis. When these compounds are applied locally, partial inhibition of the peristalsis is demonstrated; but, here again, the N-butyl ether is the most effective. This local action might be caused by the local anesthetic properties of the compounds and, therefore, no inhibition results when they are injected directly into the blood stream. They can not be concentrated in the digestive area and some hydrolysis undoubtedly takes place.

b) The benzyl ether - an aromatic group corresponding to the alkyl group in the substituted hydroxy group - produces an apparent dilation of the intestines and a slowing of the peristaltic rate but not so marked as with the aromatic carboxyl substitutions (Benzoyl and Di-benzoyl). This is probably due to a more gradual hydrolysis of the ether.

c) With the carboxyllic substitution (aromatic and alkyl) in the hydroxy group of saligenin the reactions are even more significant and interesting. The aromatic carboxyl substitutions (benzoyl and di-benzoyl) appear to act more gradually and continue for a longer period which would, of course be expected from their chemical constitution. They are slowly hydrolized and as such liberated gradually. It seems to be more feasible to assume that hydrolysis takes place and the effects are produced by the liberated nucleated benzene compound and not due to the ether molecule, which

is probably partly responsible. The acetyl ether augmented the contractions and peristalsis due to the free acetic acid. It will be remembered that the acetyl ether hydrolized slightly almost immediately after preparation. It is not improbable, therefore, that hydrolysis is much more rapidly effected in the body and the acetic acid liberated quite rapidly and, hence, the increased peristalsis results.

EXPOSED HEART OF FROGS.

1.- Methods and Technique:

The frogs were single pithed and fastened ventral side up to a frog board. The abdominal wall was opened by a median line incision and another incision was made at right angles just back of the girdle process. The sternum was lifted up and the thoracic cavity opened by making a median line incision through the sternum and cutting the girdle of bones directly over the heart. This was done carefully so that the vago-sympathetic nerves were kept intact and the heart uninjured. The walls were pulled aside and pinned to the board when the heart is seen lying within the pericardial membrane. The pericardial membrane was picked up and the heart exposed by carefully removing the membranous sac. The rate of the normal heart beat was determined by counting the number of beats per minute. The counting was continued until the number of beats per minute became constant for three to five consecutive minutes.

When the normal rate became regular and constant, the drug solutions were applied directly to the heart in quantities of two drops at five minute intervals. The effects upon the heart, such as changes in rhythm, heart-block, slowing of the rate and other

irregularities were observed. The effect upon the rate and other observations were recorded at 1-3-5-8-10 and thereafter at five minute intervals until the heart ceased to beat. The experiments were repeated three to five times for each of the compounds, using solutions of varying strength. Simultaneously with the application of the drug solution the control emulsion was applied to the heart of other frogs, prepared exactly as those used for the drug emulsions.

The compounds were prepared in the form of emulsion (See "Toxicity") and were generally employed in the concentrations of 2% and 0.5% (volume:volume).

2.- Data and Protocols:

The experiments are self-explanatory and so simple that it seems hardly necessary to give the complete data for the individual experiment. It will, however, be valuable to give some of the experimental data and results in detail so that the methods and technique may be better understood, and also to indicate the data that was taken in each experiment. The complete data and the results obtained with the N-butyl ether of saligenin are, therefore, given below in their entirety as they were recorded during the experiments,- see table #8.

TABLE #8.

The Effects of the N-Butyl Ether of Saligenin upon the Frog's Heart (Exposed).

Minutes after first application of Emul.	Beats of Heart - per Minute. N-Butyl Ether of Saligenin						
	2% Emulsion			0.5%Emul.	Control Emul.		Remarks
	Frog #1	Frog #2	Frog #3	Frog #4	Frog #5	Frog #6	
Normal Rate	74 72 72 72	84 84 84	78 77 77 78	97 97 96 97	86 86 86	76 77 76 76	
1 Min.	66	66	64	70	86	76	
3 "	66	63	47	62	86	76	
5 "	65	52	33	58	85	76	
8 "	52	44	21	--	--	75	
10 "	42	40	16	55	85	73	
12 "	36	37	10	--	85	73	
15 "	35	35	5	47	85	73	
20 "	31	34	Heart	42	84	72	
25 "	26	32	stopped	35	84	71	
30 "	22	28	Dia-	32	82	71	
35 "	19	24	stole	27	82	70	
40 "	19	Heart	widely	27	82	71	
45 "	18	stopped	dilated	25	81	69	Frog#4 Aur: Vent- 3:2 3:1 at 70 min. the Vent. beat seemed to follow the aurl:l just before it stopped.Comp due to m.inj.
50 "	Heart	Dia-	& con-	24	80	67	
55 "	stopped	stole	gested	14	79	67	
60 "	Dia-	widely	No ef-	Aur-24	79	66	
65 "	stole	dilated	fect up-	Vent 7	78	66	
70 "	widely	& con-	on rhy-	Aur-19	65	No di-	
75 "	dilated	gested	thm &	Vent 2	No di-	lation	
	& con-	No ef-	comp.	Aur-22	lation	nor	
	gestion	fect up-	at any	Vent 15	nor	con-	
	No ef-	on rhy-	time.	Heart	con-	gestion	
	fect up-	thm at		stopped	gestion	No ef-	
	on rhy-	any		dia-	No ef-	fect	
	thm at	time.		stole	fect	upon	
	any			dilated	upon	rhythm	
	time.			& con-	rhythm	at any	
				gested.	at any	time.	
					time.		

In the preceding table (Table #8) the complete data recorded during an experiment was given. The abstracted results from all the experiments with each compound are tabulated in the following table (Table #9) so that the comparative efficiency and effectiveness of the various ether compounds may be studied.

TABLE #9.

A Summary of the Effects of the Various Ether Compounds upon the Exposed Heart of Frogs.

Compounds		Effects upon the Heart			Remarks.
Name	% Emul.	Rate of Beat	Rhythm	Compensation	(Condition of heart at Standstill)
Ethyl Ether	2%	Marked slowing from 81-36 in 1 min.	No Effect	No Effect	Heart stopped in 12-20 min. diastole-dilated widely & congested. Heart stopped in 45-60 min.-diastole-widely dilated-congested.
	0.5 %	Marked slowing			
Iso-Amyl Ether	2%	Marked slowing from 97-63 in 1 min.	No Effect	No Effect	Heart stopped-30-45 min. diastole-dilated widely-congested.
	0.5 %	Gradual slowing.			Heart stopped in 50-60 min. diastole-widely dilated-congested.
N-Butyl Ether	2%	Gradual slowing 72-66 in 3 min.	No Effect	No Effect	Heart stopped 20-45 min.-diastole-dilated widely-congested.
	0.5 %	Marked slowing 97-70 in 1min.			Heart stopped in about an hr.-diastole-dilated & congested. At one stage a decompensation-irregularity (normal at cessation of beat). Probably due to mech. injury.
Benzyl Ether	2%	Marked slowing 78-36 in 3min.	No Effect	No Effect	Heart stopped-30-50 min. diastole-dilated-widely congested.

TABLE #9 (continued).

	0.5%	Marked slowing 89-65 in 1min.			Heart stopped in an hour to 1½ hr.-diastole-dilated widely and congested.
Acetyl Ether	2%	Marked slowing 66-48 in 3min.	No Effect	No Effect	Heart stopped in 15-30 min. diastole-dilated widely-congested.
	0.5%	Gradual slowing			Heart stopped in an hour to 1½ hr.-dilated widely-congested-diastole.
	0.5%	64-50 in 3min.			
Benzoyl Ether	2.0%	Marked slowing 86-74 in 1min.	No Effect	No Effect	Heart stopped in about 1 hr. diastole-dilated widely-congested.
	0.5%	Gradual slowing 77-74 in 1min.			Heart stopped about an hour-diastole-dilated widely-congested. Note: In both instances heart stopped in diastole as drug was applied.- mech. stim. revived beat which became normal in about ½ min.
Di-Benzoyl Ether	2.0%	Very gradual slowing 84-78 in 3min.	No Effect	No Effect	Heart stopped ½-2 hrs.-diastole-dilated widely-congested.
	0.5%	Very gradual slowing 74-70 in 1min.			Heart stopped in 1½-2 hour-diastole-dilated widely-congested.
Control Emulsion.		Practically none. 86-75-76-69 in 1hour	No Effect	No Effect	No dilation and beat continues powerful.

3.- Conclusions and Comments:

In no other group of experiments do we see so clearly the results, interfering or natural sequence, from the irritating action of these compounds. They are all very irritant as was seen when the local anesthetic action on the frog's sciatic nerve was attempt-

ed. The nervous tissue was evidently destroyed and the surrounding tissue appeared to harden and blanche, resembling in appearance the tissues after the corrosive action of phenol. It is probable that the irritant action somewhat masks the true or real effect of the individual compounds upon the heart. In spite of the above possibilities the experiments reveal, at least, one or two salient points in regard to their physiological action as a relation to or an effect from the chemical consitution:

a) It appears that the substitution for the hydrogen in the hydroxy group of saligenin with an alkyl group and the combined group of the aromatic compounds (benzyl) are rendered considerably more toxic to the heart. It will also be noted that these are more irritant than the benzoyl and di-benzoyl ethers (carboxyl substitutions). The variation in toxicity is strictly one of degree and not a quantitative difference.

b) The carboxyl substitution for the hydrogen in the hydroxyl group ($-OH + HO-\overset{O}{\overset{\|}{C}}-$) does, without question, reduce the effect upon the heart. It is apparent from the table that the benzoyl ether is less active than the alkyl and aromatic carbinol substitutions (benzyl) and the di-benzoyl is still less active than the benzoyl. The di-benzoyl ether does not effect the rate of the beat so very much above that produced by the control emulsion. There is, however, a difference in that the di-benzoyl ether, like the other ethers, produces a dilation or congestion of the heart, while this aspect is lacking with the control emulsion.

c) All the ethers produce a marked slowing of the rate and cause the heart to come to a standstill in diastole, widely dilated and rather congested,- especially the venous part. This general backing up of the blood would indicate that there is a definite weakening of the cardiac musculature. They seem to exert no effect upon rhythm and compensation and the effects do not appear to be of a nervous origin.

PERFUSION OF FROG'S CIRCULATORY SYSTEM.

1. Methods and Technique:

Animal:- A frog was double pithed, the heart exposed as in studying the action on the "exposed heart". In addition the pericardial sac and other membranous tissues were carefully removed from the bi-furated aorta. A ligature was passed around the heart so that it lay in the auricular-ventricular groove. The right auricle was picked up and an incision made into it. The heart was permitted to continue to beat for a few seconds to expel as much blood as possible through the slit in the right auricle. The apex of the ventricle was picked up and the apex cut into two parts. Through this opening in the ventricle a suitable cannula was inserted into the aorta and secured in place by the ligature lying in the auricular-ventricular groove and surrounding the heart. By tying the ligature at this point the opening leading from the right auricle to the ventricle was occluded and likewise the aortic orifice leading from the ventricle is cut off. This arrangement is very simple in technique and it permits the perfusion solution to flow through both branches of the aorta.

Apparatus:- The apparatus was so arranged that a record was obtained of the rate of flow by the drop method. Two funnels of equal size (about 3-4 inches diameter) were joined together by a "T" shaped piece of glass tubing with rubber tubing of suitable size and length. A screw-clamp was used on each branch of the funnels as a stop-cock. The two funnels were supported with a ring-stand and above each of these a pressure bottle for the solution was adjusted at a proper height. A funnel (203 inches diameter) was placed in a ring support at a convenient distance below the two perfusion funnels Over this small funnel a wire mesh was fitted and the stem of the funnel was inserted into a uniform dropper. The dropper was made from a piece of glass tubing with an inside diameter that would fit the funnel stem loosely and drawn out at the other end to an internal diameter that would permit the perfusion solutions to emerge in uniform drops. The frog was placed upon the wire mesh and funnel, ventral side up, and the cannula connected to the upright piece of the "T" shaped glass tube with rubber tubing.

The recording apparatus may be very easily constructed. A light lever of aluminium wire of any desired length (preferably not too long as it loses in sensitiveness with increased length) was attached to a fulcrum at one end. The other end was hammered flat and bent downward at an angle of 50 degrees and to this flat surface a microscope slide (square) or a small piece of quartz glass was cemented. Near the distal end (end holding cover glass slide) a fine piece of wire is fixed, pointing downward. At the other end about two centimeters from the fulcrum a slit (about 2-3 mm.) is made with two pieces of stiff wire to prevent the lever from passing through too great an angle (guard). At the fulcrum a bar extends up

above the lever on which a small spring (made of very fine wire) may be moved up or down. The other end of the spring is attached to the lever four to five cm. from the fulcrum. By moving the end of the spring up or down on the supporting bar a tension will be obtained which will hold the lever against the upper wire of the guard. The tension should be great enough to overcome any inertia of the lever as the drops fall upon the glass or quartz plate. The fulcrum support is best set in hard rubber and an eye may then be made near the fulcrum base to fasten a copper wire, leading to one of the electrodes on an inverted signal magnet. A small cup, made from insulating material, is filled with metallic mercury and placed directly underneath the fine wire at distal end. The fine wire dips into the mercury when the weight of a drop of the perfusion solution forces the lever down. Into this cup of mercury another copper wire dips and leads to the other electrode of the inverted signal magnet, having a dry cell intervening in the circuit. The signal magnet was inverted so that the marks caused by each drop would point upward on the smoked kymographion in contrast to the time markings which pointed downward and imposed on the tracings just below the record of drops.

It was soon discovered that emulsions of the compounds and also the control emulsion as made and used for the other experiments were unsuitable and unsatisfactory for perfusion experiments. The emulsions seemed to lodge in the blood vessels and, hence, only a clear aqueous fluid emerged at a very irregular and slow rate. Inasmuch as solutiont intended for perfusions of the frog's vessels need to be of a very low concentration, it was thought that the compounds (the ethers) might be sufficiently soluble in Ringer's

solution for frogs without any suspending reagent or probably with a little acacia solution. The latter method was found to be quite satisfactory. All the solutions are made up and designated as percentage solutions volume to volume as heretofore. Of the various oil ethers 0.5 cc. was emulsified by shaking with 5 mils of a 10% (10% W/V) powdered acacia solution. When the emulsion appeared to be uniform and the ether compound well subdivided, this emulsion was suspended in sufficient Ringer's solution for frogs to make a total volume of 250 cc. This represented a 0.2% solution of the ether in Ringer's solution. The above solutions were rather permanent (no separation during the time required for the experiments) and were only slightly opaque. The control solution was made in exactly the same manner omitting only the 0.5 cc. of the ether compound. The solutions were all perfused at the same temperature of 25°C. When a greater dilution than 0.2% was wanted the diluent was the control solution (made as above) added to the 0.2% solution in sufficient quantity to effect the desired concentration. It is also important that the compounds were used not only in a straight percentage of 0.2% but they were also used in a one/300 molar solution (M/300) and the latter solutions reduced to percentage. The latter solution (M/300) was prepared by diluting the 0.2% solution with the control solution until the M/300 strength had been produced.

2.- Data and Protocols:

The results may be very conveniently determined by counting the number of drops per minute. In many instances a mere glance will show qualitatively the effects of the ether solutions. Typical protocols of the effects of each of the various ether compounds are inserted to allow a more careful study of their comparative efficien-

cy.

Fig. 9. Perfusion of Frog's Circulatory System.
Ethyl Ether of Saligenin(0.2%)

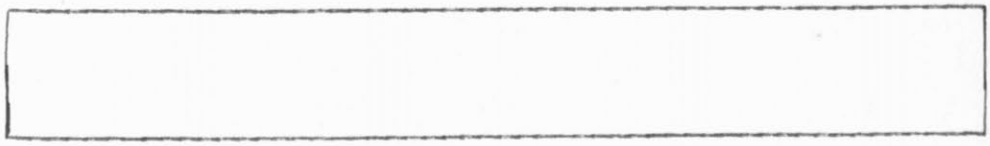


Fig.10. Perfusion of Frog's Circulatory System.
Ethyl Ether of Saligenin (M/300 or 0.05%)

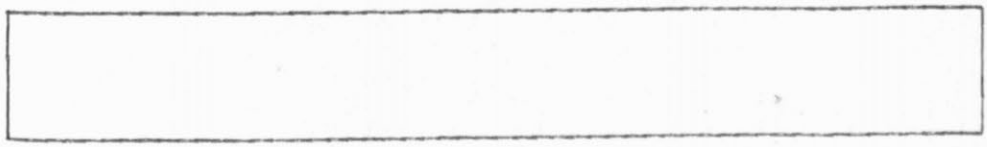


Fig.11. Perfusion of Frog's Circulatory System.
N-Butyl Ether of Saligenin(0.2%)

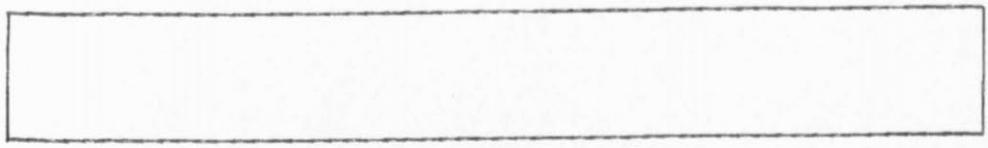


Fig. 12.Perfusion of Frog's Circulatory System.
N-Butyl Ether of Saligenin(M/300 or 0.05%)

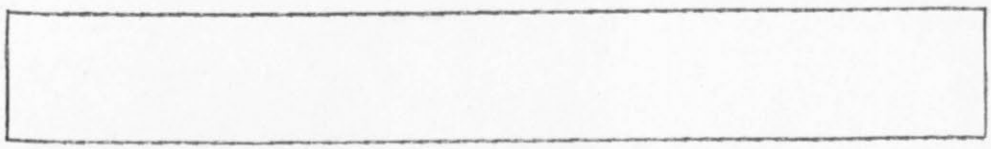


Fig.13. Perfusion of Frog's Circulatory System.
Iso-Amyl Ether of Saligenin (0.2%)

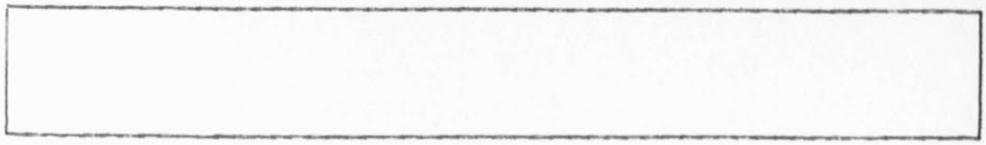


Fig. 14. Perfusion of Frog's Circulatory System.
Iso-Amyl Ether of Saligenin (M/300 or 0.064%)

Fig. 15. Perfusion of Frog's Circulatory System.
Benzyl Ether of Saligenin (0.2%)

Fig. 16. Perfusion of Frog's Circulatory System.
Benzyl Ether of Saligenin(M/300 or 0.07%)

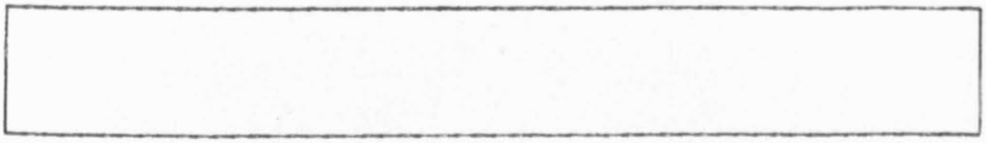


Fig. 17. Perfusion of Frog's Circulatory System.
Acetyl Ether of Saligenin(0.02%)

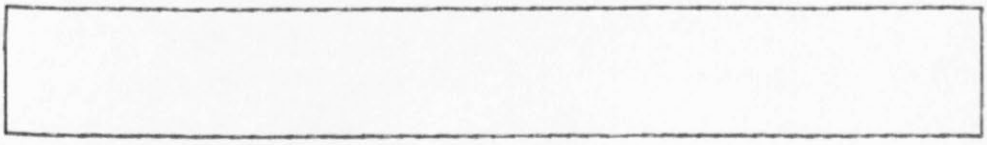


Fig. 18. Perfusion of Frog's Circulatory System.
Acetyl Ether of Saligenin(M/300 or 0.055%)

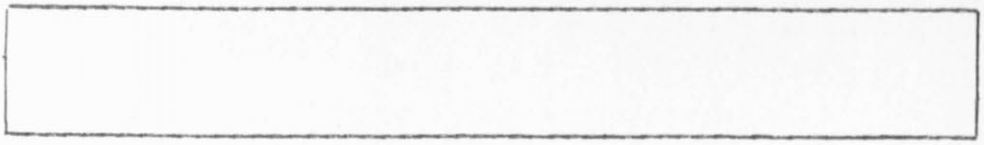


Fig.19. Perfusion of Frog's Circulatory System.
Benzyl Ether of Saligenin(0.2%)

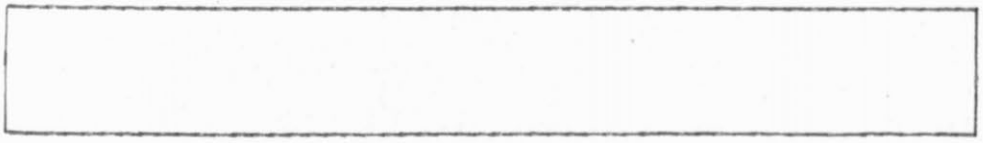


Fig.20. Perfusion of Frog's Circulatory System.
Benzoyl Ether of Saligenin (M/300 or 0.076%)

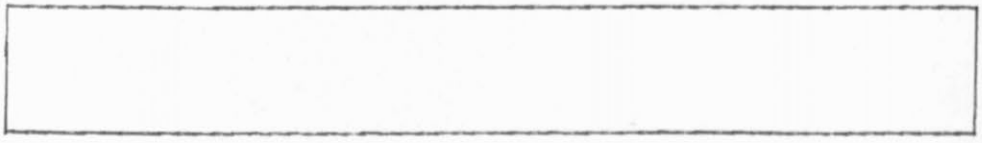


Fig.21. Perfusion of Frog's Circulatory System.
Di-benzoyl Ether of Saligenin (0.2%)

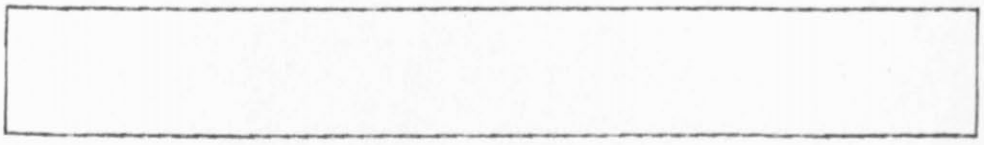


Fig.22. Perfusion of Frog's Circulatory System.
Di-benzoyl Ether of Saligenin (M/300 or 0.101%)

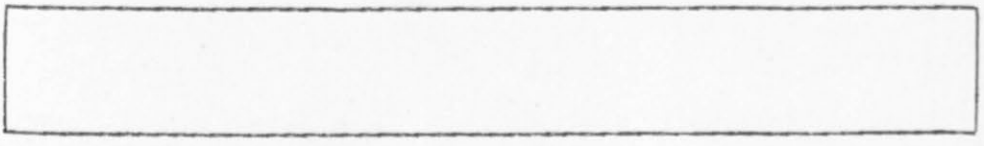


Fig.23. Perfusion of Frog's Circulatory System.
Saligenin (0.5%)

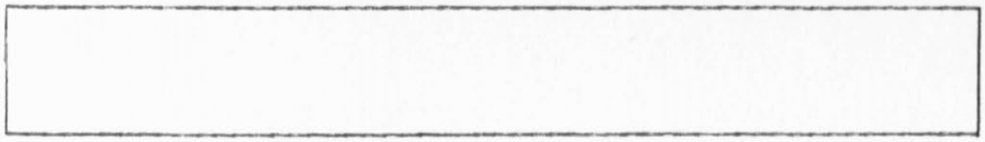


Fig.24. Perfusion of Frog's Circulatory System.
Benzyl Alcohol (0.2%)

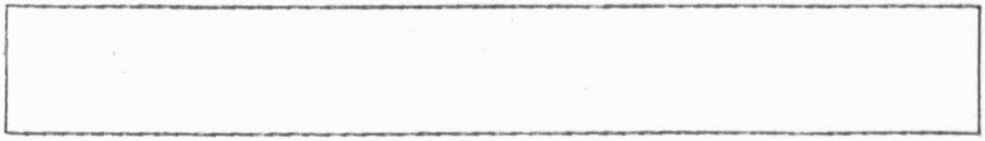


Fig.25. Perfusion of Frog's Circulatory System.
Benzyl Alcohol (M/300 or 0.03%)

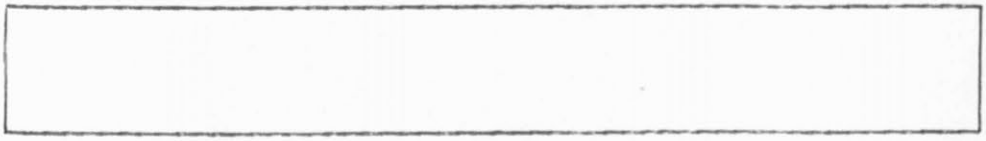


Fig.26. Perfusion of Frog's Circulatory System.
Benzyl Benzoate (0.2%)

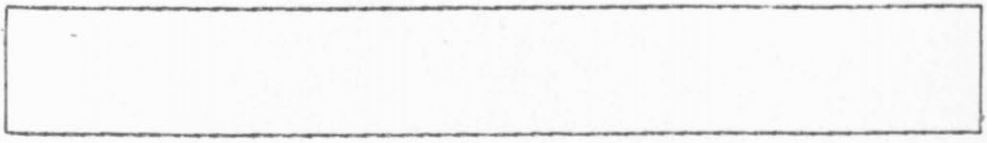


Fig.27. Perfusion of Frog's Circulatory System.
Benzyl Benzoate-(M/300 or 0.07%)

Even though the tracings indicate the results, it is rather difficult and time consuming to study these to arrive at any conclusions. For that reason a table was prepared which gives in substance the effects of the compounds. This table was further elaborated so that the relationship between molecular weight, and the percentage solution (as deduced from M/300 solution) might be more fully

comprehended and appreciated.

TABLE #10.

A Summary of the Results Deduced from the Figures.

COMPOUNDS			RESULTS AND REMARKS
Name of	Molecular Wt.	Concentration	
Ethyl Ether	152	0.2%	A vasoconstriction, slowing the flow from 39 to 29 drops in 40 seconds. Either production of no effect or a slight slowing 60-59 gtts. per 40 sec.
	152	M/300 or 0.05%	
N-Butyl Ether	180	0.2%	Marked vasoconstriction action-slowing the flow from 49-20 gtts. per 40 sec. 55-38 " " " " 47-20 " " " "
	180	M/300 or 0.06%	Marked vasoconstriction-slowing the flow from 98-34 gtts. per 40 sec. 38-24 " " " "
	180	M/3000 or 0.006%	Marked vasoconstriction-slowing the flow from 105-26 gtts. per 40 sec.
Iso-Amyl Ether	194	0.2%	A marked vasoconstriction, slowing the flow from 70-20 gtts. per 40 sec. 35- 8 " " " "
	194	M/300 or 0.064%	Marked vasoconstriction-slowing the flow from 27-13 gtts. per 40 sec. 75-33 " " " "
Benzyl Ether	209	0.2%	A vasoconstriction-slowing the flow from 98-56 gtts. per 40 sec. 15-11 " " " "
	209	M/300 or 0.07%	A vasoconstriction-slowing of the flow from 79-63 gtts. per 40 sec. 49-38 " " " " 112-70 " " " "
Acetyl Ether	168	0.2%	A vasodilation-increasing the flow from or no effect 70-70 gtts. per 40 sec. 42-56 " " " " 13-17 " " " "
	166	M/300 or 0.055%	A vasodilation-increase of the flow from 21-34 gtts. per 40 sec. (63-32 " " " ")
Benzoyl Ether	228	0.2%	A vasoconstriction-slowing of the flow from 105-77 gtts. per 40 sec. 15-13 " " " " (42-53 " " " "
	228	M/300 or 0.076%	A vasoconstriction-slowing of the flow from 33-25 gtts. per 40 sec.

TABLE #10 (continued)

Saligen-	125	0.5%	A vasodilation-increasing the flow from 49-77 gtts. per 40 sec. 78-98 " " " "
Benzyl Alcohol	108	0.2%	A vasodilation-increase in the flow from 24-215 gtts. per 40 sec. 84-105 " " " "
		M/300 or 0.03%	A vasodilation-increase in the flow from 56-63 gtts. per 40 sec.
Benzyl Benzo-	207	0.2%	Vasodilation-increase in the flow from 11-42 gtts. per 40 sec.
		M/300 or 0.07%	28-56 " " " " 112-118 " " " "

3.- Conclusions and Comments:

The conclusions that may be deduced from these experiments are interesting when compared or studied in relation to the other experiments on the circulatory and the respiratory mechanisms. It is probably well to point out that the accuracy and, consequently, the results of the perfusion experiments on frogs must be questioned and carefully scrutinized as to deductions. The fact that the experimental technique and methods were tested for efficiency and accuracy with adrenaline, nitroglycerine and benzyl benzoate,- compounds for which the reactions and effects are already known and rather predominant,- it would seem that a few general deductions might be drawn from these experiments.

a) All the ethers, except the acetyl ether, apparently produce a vasoconstriction, irrespective of the substituted ether group. The variation or exception of the acetyl group is readily accounted for by the presence of free acetic acid.

b) The alkyl ethers (-O-R)- ethyl, iso-amyl and the N-butyl ethers seem to be the most potent. Their relative activity increases progressively as in the previous experiments. It

is seen that the ethyl ether is the least active and the activity increases with increased carbon content. The N-Butyl ether probably appears to be a trifle more active than the iso-amyl ether. It is remarkable that the activity coincides with the irritant action of the compounds, e.g., the ethyl ether is least irritating, the N-Butyl most irritating and the iso-amyl ether assuming an intermediate position as to irritation and activity.

c) Of the aromatic ethers, both the carbinol ($-O-CH_2-B$) and the carboxyllic ($-O-\overset{O}{\overset{\|}{C}}-B$) types, the benzyl is probably the most effective, and the di-benzoyl the least. This demarkation line can not be drawn sharply and certainly the variation is not very great, if any at all exists.

d) The benzyl alcohol, saligenin, and benzyl benzoate, produce a vasodilation. This is, of course, in conformity with the demonstrated effects of the benzyl group upon smooth muscles

BLOOD PRESSURE AND RESPIRATION.

1.- Methods and Technique:

The blood pressure was recorded in the usual manner by joining a mercury manometer to a cannula inserted in the carotid artery of the animal.

The respiration was recorded by fastening about the animal's thorax an ordinary blood pressure bag used for the arm and joining this to a tambour. The bag was inflated with air to such an extent that the optimum magnification was obtained. This is a very simple arrangement and is probably as satisfactory as more complicated methods.

For the experiments, rabbits were used exclusively. The animals were weighed, sex noted, the ears clean shaven on the upper surface, and the animal etherized. The fur was removed from the neck and the carotid artery and the vagus nerve were separated from the surrounding tissues. A loose ligature was placed about the vagus nerve and a cannula inserted into the right carotid. The bag for respiration was inflated and adjusted.

The solutions were emulsions in olive oil and gum acacia, prepared as stated under the toxicity experiments. A control emulsion was alsoused.

As soon as the proper adjustments had been made, a normal tracing was taken. The tracings were continuous from the beginning of the blood pressure experiment until the death of the animal resulted. All injections were made into the ear veins. When a normal tracing had been recorded, a quantity of the control emulsion was injected, the effects noted and the vagus nerve was then stimulated with an induced current to determine its normal functional capacity. The injection of the ether emulsion followed and its effects recorded. The vagus nerve was stimulated when the maximal effect of the ether was noticed (immediately after injection). The injections of both emulsions and stimulation of the vagus nerve followed in a sequence which best suited the animal and the results that were recorded with previous injections. All the injections were made as much under the same conditions, such as duration of injection, depth of anesthesia, etc., as was possible. The injection of the ether emulsion was in every instance continued until death resulted. An autopsy of the animal was made to note especially the condition of the heart.

2.- Data and Protocols:

In addition to the records of the blood pressure and respiration the animal was closely observed for other symptoms, as convulsions, immediate cause of death etc. The data collected and recorded in the tracing for each of the ether compounds is given to indicate its scope.

EXPERIMENT #1.

Ethyl Ether of Saligenin - 2% Emulsion - 8/15/21.

Rabbit - Female - Wt. 2.2 Kg. Ether Anesthesia.

1.5 cc. of Control Emulsion:

No effect upon Blood Pressure and Respiration.

Vagus N. Stimulation - Marked effective response.

2.0 cc. of 2% Emulsion of Ethyl Ether:

Marked fall in blood pressure, which rises gradually to normal.

Vagus N. Stimulation - Very effective.

Marked slowing and decreased amplitude of Respiration.

2.0 cc. of Control Emulsion:

No effect upon Blood Pressure and Respiration.

Vagus N. Stimulation - Very effective.

2.5 cc. of 2% Emulsion of Ethyl Ether:

Very marked and sudden fall of Blood Pressure.

Vagus N. Stimulation - effective (slightly as animal almost dead)

Immediate cessation of Respiration with a few gasps following.

Death: Respiration fails first (Respiration and circulation practically failed simultaneously.)

Autopsy: Heart stopped in diastole - dilated and congested.

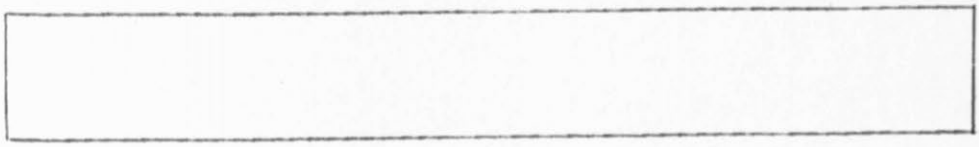


Fig. 28. Effects upon Blood Pressure and Respiration - Rabbit.

Ethyl Ether of Saligenin.

EXPERIMENT #3.

N-Butyl Ether of Saligenin - 2% Emulsion - 4/6/21.

Rabbit - Male - Wt. 2.3 Kg. Ether Anesthesia.

1 cc. Control Emulsion:

No effect.

1 cc. 2% N-Butyl Ether:

Blood pressure marked transitory fall.

Vagus N. Stimulation effective.

Respiration - Slight decrease in amplitude.

2 cc. 2% N-Butyl Ether.

Blood Pressure - marked fall.

Vagus N. Stimulation - effective.

Respiration - pronounced slowing and decreased amplitude.

Death: Respiration failed 3-5 minutes before heart ceased.

Autopsy:Heart stopped in diastole, dilated and congested.

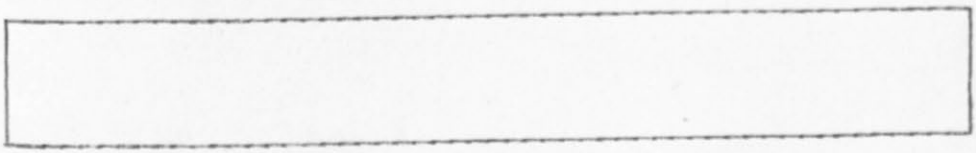


Fig. 29. Effects upon Blood Pressure and Respiration - Rabbit.

EXPERIMENT #4.

Iso-Amyl Ether of Saligenin - 2% Emulsion 3/10/21.

Rabbit - Male - Wt. 2.5 Kg. Ether Anesthesia.

1 cc. Control Emulsion:

Blood pressure - no effect.

Vagus N. Stimulation - effective.

Respiration - no effect.

1 cc. of 2% Emulsion of Iso-Amyl Ether:

Blood Pressure - fall.

Respiration - almost immediate cessation.

Artificial Respiration used.

It appeared difficult for the animal to overcome the respiratory depression.

1 cc. Control Emulsion:

Blood Pressure - no effects.

Respiration - no effect.

0.5 cc. of 2% Emulsion of Iso-Amyl Ether:

Blood Pressure - some fall- gradually occurring and recovering to normal.

Vagus N. Stimulation - very effective.

Respiration - slight decrease in amplitude.

1.2 cc. Control Emulsion:

Blood Pressure - some fall (due to approaching clot and not wholly due to the emulsion) - did not return to normal height.

Respiration - slight decrease in amplitude.

0.5 cc. of 2% Emulsion of Iso-Amyl Ether:

Blood Pressure - slight fall, followed by slight rise (vasomotor stimulation due to asphyxia) - gradually lowering of blood pressure.

Respiration: Marked decrease in amplitude and a slowing of the rate.

Death : Respiration failure.

Autopsy : Heart-diastole-dilated and somewhat congested.

Fig. #30. Effects on the Blood Pressure and Respiration - Rabbit.
Iso-Amyl Ether of Saligenin.

EXPERIMENT #5.

Benzyl Ether of Saligenin - 2% Emulsion - 3/15/21.

Rabbit - Male - Wt. 2.25 Kg. Ether Anesthesia.

1 cc. Control Emulsion:

Blood Pressure - no effect.

Respiration - no effect.

0.75 cc. of 2% Emulsion of Benzyl Ether:

Blood Pressure - sudden marked fall.

Vagus N. Stimulation - very effective.

Respiration - a slight decrease in amplitude.

1 cc. Control Emulsion:

Blood Pressure - no change.

Vagus N. Stimulation - very effective.

Respiration - no change.

1 cc. of 2% Emulsion of Benzyl Ether.

Blood Pressure - sudden marked lowering which increased gradually but never quite reached the normal pressure.

Vagus N. Stimulation - effective.

Respiration - marked slowing and a very marked decrease in

amplitude.

1 cc. of 2% Emulsion of Benzyl Ether (2 min. Blood Pressure)

Blood Pressure - an additional fall.

Vagus N. Stimulation - effective.

Respiration - almost ceased - a few gasps followed (a tonic convulsion of about 15-20 sec. followed) - a few regular breaths and then a slowing to cessation.

Death : Respiration apparently failed first.

Autopsy : Heart - diastole - dilated and somewhat congested.

Fig. 31. Effects on the Blood Pressure and Respiration - Rabbit. Benzyl Ether of Saligenin.

EXPERIMENT #6.

Acetyl Ether of Saligenin - 2% Emulsion - 8/12/21.

Rabbit - Male - Wt. 2.0 Kg. Ether Anesthesia.

1.0 cc. of 2% Emulsion of Acetyl Ether:

Blood Pressure - sudden fall, recovering to normal rapidly.

Respiration - no change apparent.

1.5 cc. Control Emulsion:

Blood Pressure - no change.

Respiration - no change.

2.0 cc. of 2% Emulsion of Acetyl Ether:

Blood Pressure - marked sudden drop, recovering to normal rapidly. A few slight irregularities occur in blood pressure and also on the heart probably.

Vagus N. Stimulation - effective.

Respiration - becomes irregular in depth.

2.0 cc. of 2% Emulsion of Acetyl Ether:

Blood Pressure - sudden drop and rise.

Vagus Stimulation - effective.

Respiration - no apparent effect.

5.0 cc. of 2% Emulsion of Acetyl Ether:

Blood Pressure - marked sudden fall, which slightly rises at once and a slight secondary fall follows.

Vagus N. Stimulation - effective.

Respiration - a slight decrease in depth.

5.0 cc. of 5% Emulsion of Acetyl Ether:

Blood Pressure - sudden marked fall, which did not rise to normal.

Respiration - no apparent effect immediately after injection. Sudden cessation followed by few gasps. (Here it seems the respiration failed on account of the failure of circulation.)

Death : Failure of circulation, followed by respiration failure.

Autopsy : Heart diastole-dilated and slight congestion.

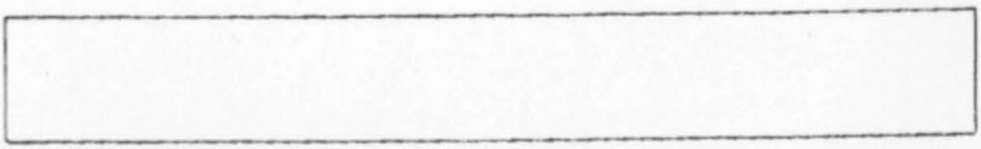


Fig. 32. Effects on the Blood Pressure and Respiration - Rabbit.
Acetyl Ether of Saligenin.

EXPERIMENT #7.

Benzoyl Ether of Saligenin - 2% Emulsion - 8/12/21.

Rabbit - Male - Wt. 2.4 Kg. Ether Anesthesia.

1.5 cc. Control Emulsion:

Blood Pressure - no change.

Respiration - no change.

2.5 cc. of 2% Emulsion of Benzoyl Ether:

Blood Pressure - a slight gradually occurring fall.

Vagus N. Stimulation - very effective.

Respiration - noticeable change.

6.0 cc. of 2% Emulsion of Benzoyl Ether:

Blood Pressure - slight immediate fall, gradually continued to fall.

Respiration - probably a slight decrease in depth.

3.0 cc. of 5% Emulsion of Benzoyl Ether:

Blood Pressure - slight fall.

Vagus N. Stimulation - effective.

Respiration - immediate decrease in depth and a slowing followed by complete failure.

Death : Failure of Respiration (Respiration failed before the heart stopped).

Autopsy : Heart stopped - diastole - dilated and congested.

Fig. 33. Effects on the Blood Pressure and Respiration - Rabbit. Benzoyl Ether of Saligenin.

EXPERIMENT #8.

Di-Benzoyl Ether of Saligenin - 2% Emulsion - 8/9/21.

Rabbit - Female - Wt. 2.4 Kg. Ether Anesthesia.

4-21 6M

1 cc. Control Emulsion:

Blood Pressure - no change.

Respiration - no change.

1.2 cc. of 2% Emulsion of Di-Benzoyl Ether:

Blood Pressure - slight fall.

Respiration - no change.

Vagus N. Stimulation - effective.

2.5 cc. of 2% Emulsion of Di-Benzoyl Ether:

Blood Pressure - gradually lowering.

Respiration - no change.

5.0 cc. of 2% Emulsion of Di-Benzoyl Ether:

Blood Pressure - fall in pressure.

Vagus N. Stimulation - effective.

Respiration - no apparent change.

2.5 cc. of Control Emulsion:

Blood Pressure - no change.

Respiration - no change.

5.0 cc. of 2% Emulsion of Di-Benzoyl Ether.

Blood Pressure - slight fall - followed shortly by an increased pressure (Increase probably due to effect upon Respiration).

Respiration - slowing and decreased depth.

5.0 cc. of 2% Emulsion of Di-Benzoyl Ether:

Blood Pressure - slight fall, followed by slight increase and then rather abrupt fall until death resulted.

Respiration - Slowing and decreased depth.

Death : Respiration and heart stopped about simultaneously (probably respiration first.)

4-21 6M

Autopsy: Heart - diastole - dilated and somewhat congested.

Fig. 34. Effects on the Blood Pressure and Respiration - Rabbit.

Di-Benzoyl Ether of Saligenin.

The results obtained in the preceding experiments are summarized in the following table. The less important results are omitted and only the salient effects of each compound are indicated. In this table the effects of saligenin and benzyl alcohol, resulting from intravenous injection, are also included to promote a better comparison with the various ethers.

TABLE #11.

A Tabulated Summary of the Effects of the Ether Compounds upon the Blood Pressure and Respiration

- Rabbits -

Name of Compound	Effects of Results			Immediate Cause of Death	Autopsy and Remarks.
	Blood Pressure	Vagus N. Stimulation.	Respiration		
Ethyl Ether	Marked lowering	Effective	Marked slowing & decreased amplitude	Respiration failure(?)	Heart stopped-diastole-dilated congested.
N-Butyl Ether	Marked transitory lowering.	Effective	Pronounced slowing of rate & decreased amplitude.	Failure Respiration	Heart stopped-diastole-dilated-congested.
Iso-Amyl Ether	Gradual lowering	Effective	Slowing of rate and decreased amplitude.	Respiration failure	Heart stopped-diastole-dilated-congested.
Benzyl Ether	Sudden marked	Effective	Pronounced slowing of	Respiratory failure (?)	Heart stopped-diastole-dilated

TABLE #11 (continued).

	lowering.		rate & marked decrease in depth.		& congested(slight) convulsion.
Acetyl Ether	Transitory lowering.	Effective	Irregularity & decreased depth	Failure of circulation	Heart stopped-diastole-dilated-slight congestion.
Benzoyl Ether	Gradual lowering	Effective	Slowing & decreased depth	Respiration failure	Heart stopped-diastole-dilated and congested.
Di-Benzoyl Ether	Gradual lowering.	Effective	Slowing & decreased depth	Resp.& heart stopped simultaneously (Resp. failure first.	Heart stopped-diastole-dilated-congested.
Control Emulsion	No Effect	Effective	No Effect		
Saligenin (5)	Gradual fall	Effective	Slight slowing	Respiration Failure	
Benzyl Alcohol (2)	Gradual lowering	Effective	Small doses a preliminary stimulation(no apparent change-slight slowing.	Respiration Failure	

3.- Conclusions and Comments:

Phenol is known to produce a somewhat deeper and quicker rate of respiration in small doses, and in larger doses to produce complete paralysis of the respiratory and circulatory systems. The blood pressure falls, but death is usually due to paralysis of the respiration.

Benzyl alcohol, in which we have a carbinol group (CH_2OH) attached to the Benzene nucleus instead of the hydroxy group in phenol, produces a gradual lowering of the blood pressure. A slight

slowing of the respiration with moderate doses is noticed when it is injected intravenously. Small doses appear to produce a slight primary stimulation. Death from benzyl alcohol appears to be caused by respiratory paralysis.

Saligenin, in which we have both the hydroxy group (OH) and the carbinol group (CH_2OH) attached to the benzene nucleus, appears to be less active and less toxic to the circulation and respiration of mammals. In comparatively large doses, when given intravenously very slowly and continuously, it produces a very gradual lowering of the blood pressure and a slight slowing of the respiration. In larger doses and with more rapid injections there is a sudden fall of blood pressure and a marked slowing of the rate and some decrease in the depth of respiration. The lowering of the blood pressure and the respiratory paralysis progress until death results from respiratory failure.

The action of the various ethers on the circulation and respiration are, therefore, interesting. The effects of substitution in the side groups of the benzene nucleus, especially with hydroxyl and the carbinol groups as found in saligenin, leads to the inference of these conclusions.

a) Whether the substitution of the hydrogen in the hydroxy group of saligenin results in the introduction of an alkyl radical ($O\text{-}C_2H_5$) or a carbinol group of the aromatic series ($-O\ H+\ HO\text{-}CH_2\text{-}$ ), or a carboxyllic group of the aliphatic and aromatic series ($-OH\ HO\text{-}\overset{O}{\overset{\|}{C}}\text{-}$) the same general effects are produced qualitatively in degree upon the circulation and respiration. They all, in common with their basic compounds (saligenin and benzyl

alcohol), produce a lowering of blood pressure, no effect upon the response of the vagus mechanism to electrical stimulation, a slight retardation of the respiratory rate and also a decrease in the depth of respiration. Death apparently results from respiratory failure, at least respiratory failure is a material factor in the toxic reactions leading to death.

b) The autopsy of the animals revealed that the heart was practically in the same condition immediately after death from the various ethers. The heart was found to have stopped in diastole, and was dilated and more or less congested. This might indicate that the ethers act directly upon the heart muscle and that the fall of blood pressure is a consequence of the weakened condition of the heart.

c) In general it appears that the substitution resulting in alkyl and benzyl ethers are more potent than those which yield the carboxyllic (-O-C-) ethers. It also seems that the action of the aromatic carboxyllic ethers (-O-C ) react for a longer period and the lowering of the blood pressure is more gradual. Furthermore, it appears that the di-carboxyllic substitution (di-benzoyl) involving both the hydroxyl and carbinol group of saligenin, prolongs this gradual action even more than the mono-carboxyllic substitution (benzoyl).

d) It occurs that the acetyl ether does not wedge in very well with either group of the ethers. This is presumably due to the presence of free acetic acid which was

formed by hydrolysis. The acid probably causes the sharp transitory fall in blood pressure, but the rapid return to normal might be due to neutralization of this free acetic acid by the blood buffers and then the effects are gradual. As a whole the acetyl ether assumes here an intermediate position as it has done in most of the other experiments.

e) It would appear that the general lowering of blood pressure was caused by the weakening of the heart and not due to vasodilation. A more comprehensive discussion of these phenomena will be given in the general discussion and conclusions that have eminated from the investigation of these compounds.

GENERAL DISCUSSION AND COMMENTS.

It will probably be advantageous at this point to call attention again to the physical properties of saligenin, benzyl alcohol and the ether compounds. Since the appearance of chemical pharmacology, it has become evident that the physiological actions may in a measure be forseen or predicted by a careful survey of the physical properties of the compounds. Even though the physical properties can not foretell entirely the physiological actions of a compound, important phases of its reaction may be suggested.

Benzyl alcohol is an oily-like liquid with a Sp.gr. of 1.0628 at 0°C. It is slightly soluble in water (4% at 17°C.) but readily soluble and miscible with the organic fat solvents,-alcohol, ether, chloroform, acetone etc.

Saligenin (24) is a shiny, flaky, crystalline compound at

ordinary temperature with a faint aromatic odor, slightly burning taste followed by a feeling of numbness. It is soluble in water, chloroform, alcohol, ether and also in the fixed and volatile oils. It melts at 85-86°C, gives the saliretin test with concentrated H_2SO_4 and yields a greenish to bluish-violet color with $FeCl_3$ solution.

The ether compounds considered in this study are either oily liquids or waxy crystalline substances which melt at a temperature slightly above the normal temperature. They are all very soluble in the organic fat solvents (alcohol, ether, chloroform, acetone etc.). They are soluble in olive oil, except the ethyl ether of saligenin, and are all insoluble in glycerine and water.

In the study on the toxicity of these ether compounds we see a striking effect of substitution in the hydroxyl group. It was stated under toxicity that alkyl substitution (ethyl ether) increased the toxicity over that of the nucleus (saligenin) and that the increased toxicity varied according to the length of the carbon chain of the alkyl radical. This would probably indicate that the alkyl ethers hydrolize in the body, forming the respective alcohols of the alkyl group and reforming saligenin. The increased toxicity, as well as the progressive increase of toxicity with the lengthened carbon chain, could then readily be explained. The toxicity would be a summation of the toxic action of the liberated saligenin and the alkyl alcohol. The progressive increase in toxicity would be due to the higher alkyl alcohols formed by hydrolysis of the respective ethers. It might be objected that the increased molecular weight would mean less of each of the hydrolytic compounds, but the difference in molecular weight is hardly great enough that this could exert much of an effect upon the toxicity. In fact, the in-

creased molecular weight would rather tend to minimize the increased toxicity.

The aromatic substitution in the hydroxy group corresponding to the alkyl group, e.g., benzyl ether, presents further data imperative to the question of toxicity. The benzyl ether, while more toxic than saligenin, is less toxic than either of the alkyl ethers. Here again the effects are probably due to the summation of the toxicity of the hydrolytic compounds. It is quite likely that the benzyl ether hydrolizes to form saligenin and benzyl alcohol. The benzyl alcohol itself has a very low toxicity which is probably due to its oxidation into benzoic acid (2). Hence, the lessened toxicity of the benzyl ether compared to that of the alkyl ethers, is undoubtedly due to the relative toxicity and fate in the body of the ether group compounds which result when the ether is hydrolized.

The same trend of information is shown by the carboxyllic substitutions, (-O-C-) whether the group be of the aliphatic or aromatic series. Here, likewise, we see that the aromatic ethers are less toxic than the similar substitutions with the aliphatic acids (acetyl). The compounds are hydrolized in the body and yield respectively the corresponding aromatic and fatty acids. Thus, the acetyl ether is saponified to form acetic acid and saligenin and the benzoyl and di-benzoyl ethers to form benzoic acid and saligenin. The acetic acid is more toxic than the benzoic acid, and hence, the acetyl ether has the greater toxicity. It must be noted, however, that the rate of hydrolysis might play an important role in the degree of toxicity. The fatty acid substitutions in general hydrolize rather rapidly and easily; while the aromatic acid substitutions hydrolize more slowly. It is, therefore, apparent

that the acetic ether and saligenin will probably be present in the same time in a greater concentration. The more gradual liberations of the aromatic acids or groups would probably keep pace with the rate of elimination from the body of these acids. It is certain that the rate of hydrolysis, the rapidity of absorption and of elimination must all modify the toxicity but this would not be contrary to the process of hydrolysis. The factors would merely have to be considered in the light of the hydrolytic products.

It is, of course, possible that the ether might exert its qualitative effects while combined with the saligenin. The former seems the more probable as it has been demonstrated that substituted compounds of this type hydrolize quite readily as a rule. If the latter view is taken, the physiological action of saligenin is modified and demonstrates itself in conformity to and in proportion to the substituted ether group. If the former view is held, the activity corresponds to the general sequence and relation existing between homologues in the aliphatic compounds and also to the comparative activity between carbinol and carboxyllic compounds.

The theory of hydrolysis seems to be further elucidated when we consider the reactions following subcutaneous injections in frogs. All of the compounds produced a degree of stupor or narcosis and reduced the reflex irritability. It was noticed, however, that all the substitutions with a benzene compound, would at times give rise to convulsions. The convulsions were usually of very short duration and strychnine-like (tonic) in character. It is known that saligenin does not induce convulsions and the assumption that the convulsions were induced by the saponified aromatic compounds is permissible. There are any number of examples (such as the benzoyl

group in cocaine) that would tend to show that the benzene nucleus does not produce convulsions when combined to other groups of the cyclic type.

The degree or depth of the narcosis does not appear to progress with any regularity or dependence upon the substituted ether groups. It is not without reason that here the rate of hydrolysis and absorption plays a greater role than in toxicity. The effects from the subcutaneous injections were largely observed for a few hours (1-4 hours) while the toxicity results were recorded at the end of twenty-four hours. It must also be borne in mind that, inasmuch as these observations were taken simultaneously with the toxicity experiments, the dose was regulated and graduated primarily in view of the toxicity rather than the general systemic effects.

According to the Meyer-Overton theory of narcosis these compounds should elicit marked effects upon the nervous system. They are all very soluble in the organic fat solvents and in olive oil (probably also in other oils). The tendency to produce a degree of stupor is probably an expression of this action on the nervous tissue. It might be added that in a few cases there seemed to be some narcotic action upon the rabbits used for the intestines in situ. The amount of ether (sulphuric ether) could at times be removed almost entirely but this was not a constant phenomenon.

In the benzyl alcohol we have somewhat similar physical properties to those of the ethers. The benzyl alcohol is an oily liquid and is slightly irritating. Its local anesthetic properties can not be disputed but the deleterious side-and after-effects (as necrosis and sloughing off etc.) are not desirable. With the introduction of the hydroxyl group to form saligenin (oxy-benzyl alcohol)

these undesirable side-effects are avoided and its anesthetic properties increased and it becomes much more water soluble. When substitution in the hydroxy group of saligenin is effected there appears to be a partial reversal back to the benzyl alcohol properties.

There does not appear to be any relation between substitution of homologues in the hydroxy group and the anesthetic properties. The ethyl, iso-amyl and the benzyl ethers seem to produce about the same degree of effectiveness; while the N-butyl ether is almost three times as effective as saligenin. Substitution with a carboxyl group further diminishes the local anesthetic action. If in addition to the above substitutions the carbinol group is also masked the anesthetic properties are entirely destroyed, as shown by the dibenzoyl ether. The substitution in the hydroxy group demonstrates that the hydroxyl group enhances the local anesthetic action of the carbinol group. All the alkyl substitutions, except N-butyl ether, as well as the a romatic substitutions (carbinol and carboxyllic alike) reduce the anesthetic action of saligenin. This same phenomenon was also demonstrated by Hirschfelder and his collaborators (5) who found that substitution with a methyl or an ethyl group reduced the anesthetic action of saligenin. It also shows that the carboxyllic substitutions reduce the effectiveness even to a lower level than do the carbinol substitutions (alkyl and aromatic). On the other hand it must not be overlooked that the possibility of increasing the local anesthetic action by substitution in the hydroxy group is not entirely without hope or reach of accomplishment. It may become the avenue of approach for the development of an effective and desirable local anesthetic compound.

From the local anesthetic action of the N-butyl ether we have a much more potent anesthetic than saligenin itself (Ration 3:1) even though the comparison is made under varying conditions. The effectiveness of the N-butyl ether was determined, as will be recalled by immersing the hind limbs of the frog into a 2% emulsion, while the effectiveness of saligenin was determined by the direct application to the sciatic nerve of a 2% solution in a physiological salt solution. It is certain that the emulsification of a compound retards its rate of diffusion and penetration, so it is not unlikely that the anesthetic properties of the two would diverge by a greater margin when determined under similar conditions. The deleterious side-effect of the N-butyl ether precludes it from practical application.

The effects of the benzyl group upon the smooth musculature, especially on the intestinal tract, is a very imperative question at this time. Macht (1-9-10-11) and other workers concluded that the benzyl esters produced a definite inhibition upon the intestinal contractions and that they also lowered the tone of the musculature. The latter of these effects of the benzylgroup is certainly of gigantic importance and significance to the profession of medicine. Not only is it clinically significant but it is also extremely interesting and striking from the physiological viewpoint. If it is borne in mind that one of the most characteristic properties of smooth muscles is the power of tonicity, and that the stretch and tone of muscle are influencing factors in determining its capacity for work, the general reduction or inhibition of this tone by drugs presents itself with a profound influence on the physiological and clinical reactions of the intestinal tract.

It might be stated that although a sufficient number of experiments were hardly done that would warrant a conclusion as to the absolute effect of the substituted group, the general effectiveness of the alkyl and aromatic substitutions is apparent. The effects seem largely to be a question of degree rather than an absolute difference. They all produce a decrease in the amplitude of the contraction and generally a slowing of the rate of the rhythmic contractions of the intestinal segments. The degree of inhibition seems to progress for the alkyl substitutions (O-R) as the carbon content is increased. This same progression also holds for the lowering of tonicity.

Saligenin itself is not very active, while benzyl alcohol has a rather marked effect. This might indicate that it is the carbinol group which is vital and essential to the benzene nucleus to produce this inhibitory effect. In the intact animal the alkyl ethers produced no apparent effects. This might suggest that the local action is due to their anesthetic properties and probably also to their marked irritating action. When the alkyl ethers are applied locally to intestinal loops in the intact animal, there is a degree of peristaltic inhibition which appears to progress with the increased length of the carbon chain. The effects with the normal-butyl ether are unmistakeable.

The acetyl ether (carboxyllic substitution with an aliphatic acid ($-O-\overset{O}{\overset{\|}{C}}-CH_3$)) does not accord with the general sequence or schema. There seems to be a general increase, although slight, both in the tone and the height of the contractions. This is unquestionably due to the presence of free acetic acid.

When we consider the aromatic ethers, carbinol ($-O-CH_2B$) and

carboxyllic types (-O-C-B), the action seems to be greater and more definite than with the alkyl ethers. The benzyl ether is the most effective as we should expect from its chemical constitution. The carbinol group is usually more active than the carboxyl group. The benzyl ether produces the greatest decrease in tone and the most marked inhibitory effect upon the contraction. This same effect of the benzene compounds is also shown by the carboxyllic substitutions even though the effects are not so predominant immediately. It was noticed that the benzoyl ether produced a lowering of the tone and a decreased amplitude as the benzyl ether, but it was not so pronounced. Nielsen and Higgins showed (11) that it was essential to allow time for the benzyl esters to act. We would naturally expect then that the effect of the benzoyl ether should be more gradual. The effect was less potent but continued and eventually produced complete inhibition. The compounds are less readily hydrolized and are available in a lower concentration. The di-benzoyl ether acts like the benzoyl ether varying in degree of intensity. In this ether both the hydroxy and the carbinol groups of saligenin are covered up and the ether is less irritating and effects its results more slowly.

The greater efficiency of the aromatic ethers is clearly seen in the intact animal. The alkyl ethers gave negative results when injected into the circulation. The aromatic ethers all produce a degree of inhibition and dilation of the intestine (decreased tone) intravenously and locally. It will be recalled that the benzoyl and the di-benzoyl ethers were devoid of any anesthetic action. The effects on the smooth musculature must, therefore, be looked for from other angles. It is probable that the effects are

produced by the ether molecule but largely from the hydrolytic products in the circulation.

The recent work of Frederick and Terroine (18) is especially significant and instructive at this time when we come to consider and discuss the actions of these ether compound upon the circulatory and respiratory systems in frogs and mammals. These investigators found that the cardiac action of substances of the quinoline group was qualitatively similar to those of quinine and other cinchona alkaloids. An introduction of a methyl group (CH_2) regardless of position in the molecule, gave rise to increased toxicity; the same phenomena were true for a methoxy group (CH_3-O-) as well as for hydrogenation of the molecule.

The ether compounds formed by substituting various carbinol and carboxyllic groups of the aliphatic and aromatic compounds present a similar augmentatory effect over that of the nucleus saligenin. They produce the same general effect as saligenin itself and also benzyl alcohol, but they vary somewhat in intensity of reaction. It appears that the alkyl ethers,- ethyl, iso-amyl and N-butyl ethers,- are more active than the aromatic ethers. It will also be noted that again the N-butyl ether seems to be the more toxic and produces the greatest effects. Benzyl alcohol and saligenin produce a gradual lowering of the blood pressure up to the point when the toxic dose is consumated, when the blood pressure drops more abruptly. In the alkyl ethers,- not so marked with the iso-amyl ether,- the lowering of the blood pressure is more marked and sudden even when small doses are used. In the aromatic ethers the lowering occurs more gradually and also returns to normal very slowly. In fact, the aromatic ethers approach more

nearly the reactions we have with the saligenin and benzyl alcohol. The aromatic ethers are, to be sure, neither so irritating nor so toxic as the alkyl ethers and it is also probable that they are less easily hydrolized and made available in the blood stream. This is especially apt to be the conditions with the benzoyl and di-benzoyl ethers as these were found to be more difficult to emulsify.

Certainly, the same general progressive increase in the effects occur as in the other experiments. The alkyl ethers have an effect which increases with the carbon content; while of the aromatic ethers the benzyl ($O-CH_2-B$) ether is more toxic than the carboxyllic ethers ($O-\overset{O}{\overset{\|}{C}}-B$). This same progression of the effects upon the blood pressure occurs when the ethers are applied directly to the frog's heart, and also in regard to the effects on the respiration.

It was observed that all the ethers apparently produced death by their paralytic effects upon the respiratory mechanism. The heart after death was found to have stopped in diastole, was more or less dilated, and in general considerably congested. This same condition of the heart resulted when the ethers were applied directly to the frog's heart. In only one instance or experiment (N-butyl ether) was any irregularity in the heart action noticed. This same irregularity was most likely caused by some mechanical injury as far as could be observed. There does not appear to be any vasodilation so the cause of the falling blood pressure must be looked for elsewhere. It will be remembered that when these ethers were perfused through the frog's circulation, a vaso-constriction resulted, e.g., at least if the drop-graphs may be taken as proof. With the exception of the acetyl ether (due to free acetic acid) the flow was decreased when the ethers were perfused. At no time during

the blood pressure experiments were there any indications of any effects upon the vagus mechanism. These conditions of the circulation indicating a gradual weakening of the heart and an apparent "backing up" of the blood would suggest that the fall in the blood pressure would be due to a cardiac weakening and not to vasodilation. It must be borne in mind, and the premises are not at all improbable, that the powerful irritation produced by the ethers was sufficient to stimulate the vaso-constrictor mechanism in the perfusion experiments and that this overshadowed the probable vasodilation. This might be an equally correct assumption when it is considered that saligenin, benzyl alcohol and benzyl benzoate all produce vaso-dilation and consequently a fall of blood pressure. These are not, or only very slightly, irritating to the tissues compared to the ether compounds of saligenin.

It might be problematic if the respiratory failure, leading to the death of the animal, was not occasioned by the depleted seration of the respiratory center. The conditions of the heart at autopsy would indicate that there was a gradual weakening of the heart action and this might account for the fall of blood pressure and would eventually lead to respiratory failure. Certainly it appears that the alkyl ethers are more toxic to the circultation and respiration and they react more quickly; while the aromatic ether compounds are less toxic to the circulation and respiration and react more gradually and for a longer period.

Since it has been found that these ethers eleicit difference in reaction depending upon the presence of an alkyl or aromatic group in the ether molecule, it might be profitable to see if the causal factors for these variations can be expounded.

In the first place it has been an outstanding feature in all the experiments that the effects of the alkyl ethers increase progressively with increased carbon content. It would suggest that this is caused by the direct transfer to the ether molecule of the principles of Richardson's law (25) for the activity of the straight chain alcohols. Whether or not these compounds are hydrolized in the body before they produce their effects is apparently of very little significance as the same relations are elicited. It will also be recalled that various observers have reported increased activity for compounds by alkylation. It is known that the antiseptic value of phenol is increased by alkylation with a methyl group. Hirschfelder (5) found that the methyl and ethyl ethers of saligenin were more toxic than saligenin itself. Likewise, the recent work of Frederico and Terroine (18) already referred to in this paper demonstrated increased activity by the introduction of a methyl, methoxy and hydrogenation of the quinoline group.

It has also been shown that alkylation of the hydroxy groups of resorcinal increasse its toxicity. It must not be unchallenged that in spite of the above changes produced by alkylation the augmentation may and actually does not materialize for all the properties of the compound. In general, alkylation actually diminishes activity as the process is usually accompanied by a decreased chemical activity, as guaiacol is less poisonous than phtnol but still has an effect similar to that of phenol. Likewise, it will be noticed that the anesthetic property of saligenin is materially decreased with the formation of alkyl ethers. Certainly, the reactions of these alkyl ethers accord with the findings of Frederico and Terroine, but they equally well accord with the other general observation that alkylation

diminishes activity.

With the introduction of a carboxyl group (attached to nucleus) the observations are usually those of a retardation or inhibition of the primary effects of a compound. With the esterification of these compounds their solubilities are generally materially modified as well as their appearance and physical properties. An excellent illustration of this is benzyl benzoage. It probably is no more efficacious than the benzyl alcohol would be if they were given in proportion to molecular weight. The former is, on the one hand, slowly saponified and as such its toxicity is materially lowered and the gradual liberation results in a prolongation of the effects. These aromatic ethers (benzoyl and di-benzoyl ethers) have also a weaker effect than the alkyl ethers, but are still more active than saligenin itself. It would seem more probable that these compounds are hydrolized in the organism, and consequently, the combined effects of the saligenin and of the ether group are consummated in the animal. This would then account for their increased activity over saligenin and the lower activity compared with the alkyl ethers. The presumption that the compounds act rather quickly to permit their effects to be explained on the supposition of the combined effects of the hydrolytic compounds does not lack entirely a basis and foundation that would command consideration.

SUMMARY

From this limited number of experiments and observations some light has been reflected upon the relation between the chemical structure (substitution) and the pharmacological action of the

saligenin derivatives. The more salient facts that occur to be tenable to deduce from this investigation are summarized and tabulated in the following conclusions:

1) Substitution in the phenolic hydroxyl group of saligenin resulting in the formation of ether compounds, increase the activity of saligenin in a degree depending upon the ether group added.

2) The activity of these substituted compounds accords with the general observation that it results in a retardation or inhibiton of the primary action of the compound, (lowers anesthetic properties of saligenin)

3) Substitutions resulting in alkyl ethers greatly augment the pharmacological action of saligenin,- the intensity of reaction depending upon the carbon content of the ether group.

4) The substitutions resulting in aromatic ethers are less active than the alkyl ethers, but still more than saligenin.

5) The substitutions resulting in aromatic ethers of a carbinol type ($-O-CH_2-B$) are more active than those aromatic ethers of a carboxyllic type ($-O-\overset{O}{\overset{\|}{C}}-B$).

6) The results of the various substitutions seem to depend upon the summation of the reactions of the saligenin and the ether compounds liberated by the hydrolysis of the compounds.

7) All of the substituted compounds exhibit the saligenin effects qualitatively, the modifications depending upon the ether group introduced into the saligenin molecule.

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Protocol.

Herman H. Jensen.

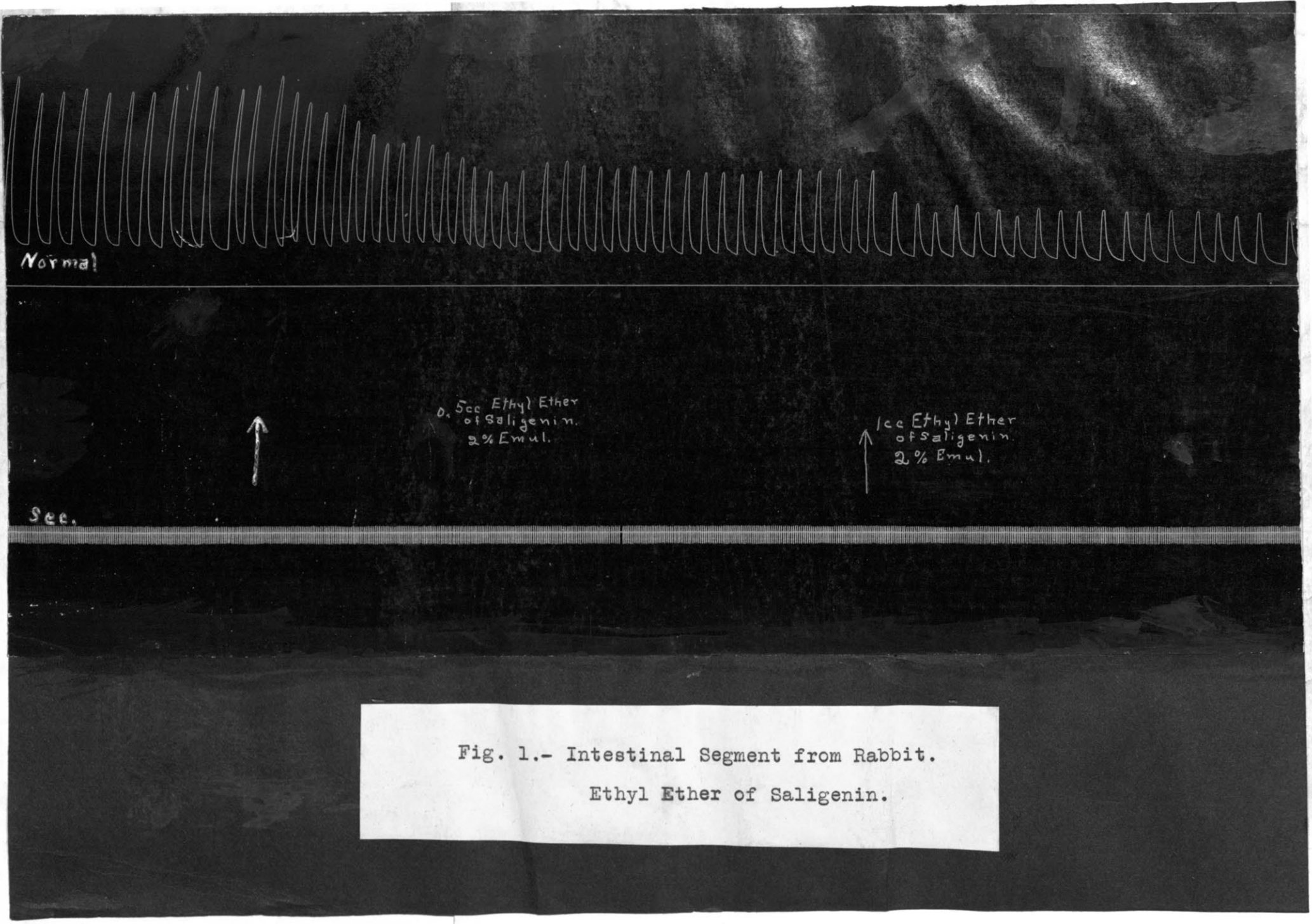

Fig. 1.- Intestinal Segment from Rabbit.

Ethyl Ether of Saligenin.

Fig. 2.- Intestinal Segment from Rabbit.

N-Butyl Ether of Saligenin.

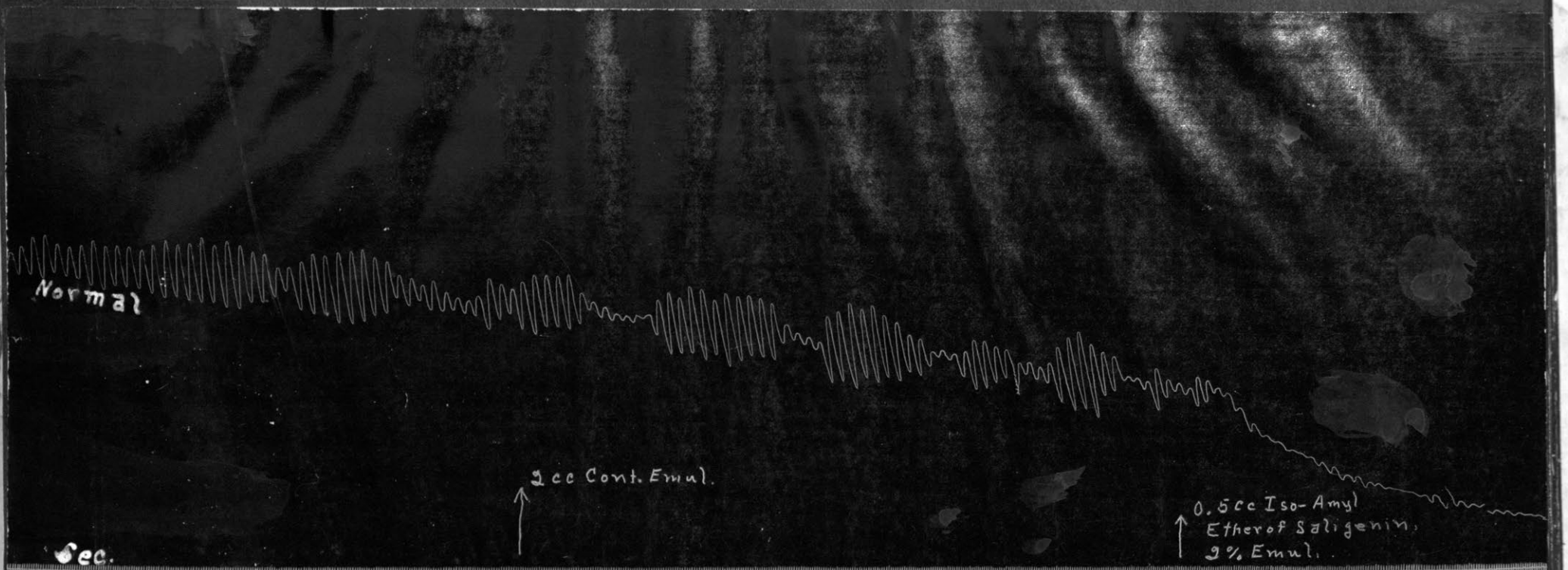

Fig. 3. - Intestinal Segment from Rabbit.

Iso-Amyl Ether of Saligenin.

Fig. 4. - Intestinal Segment from Rabbit.

Benzyl Ether of Saligenin.

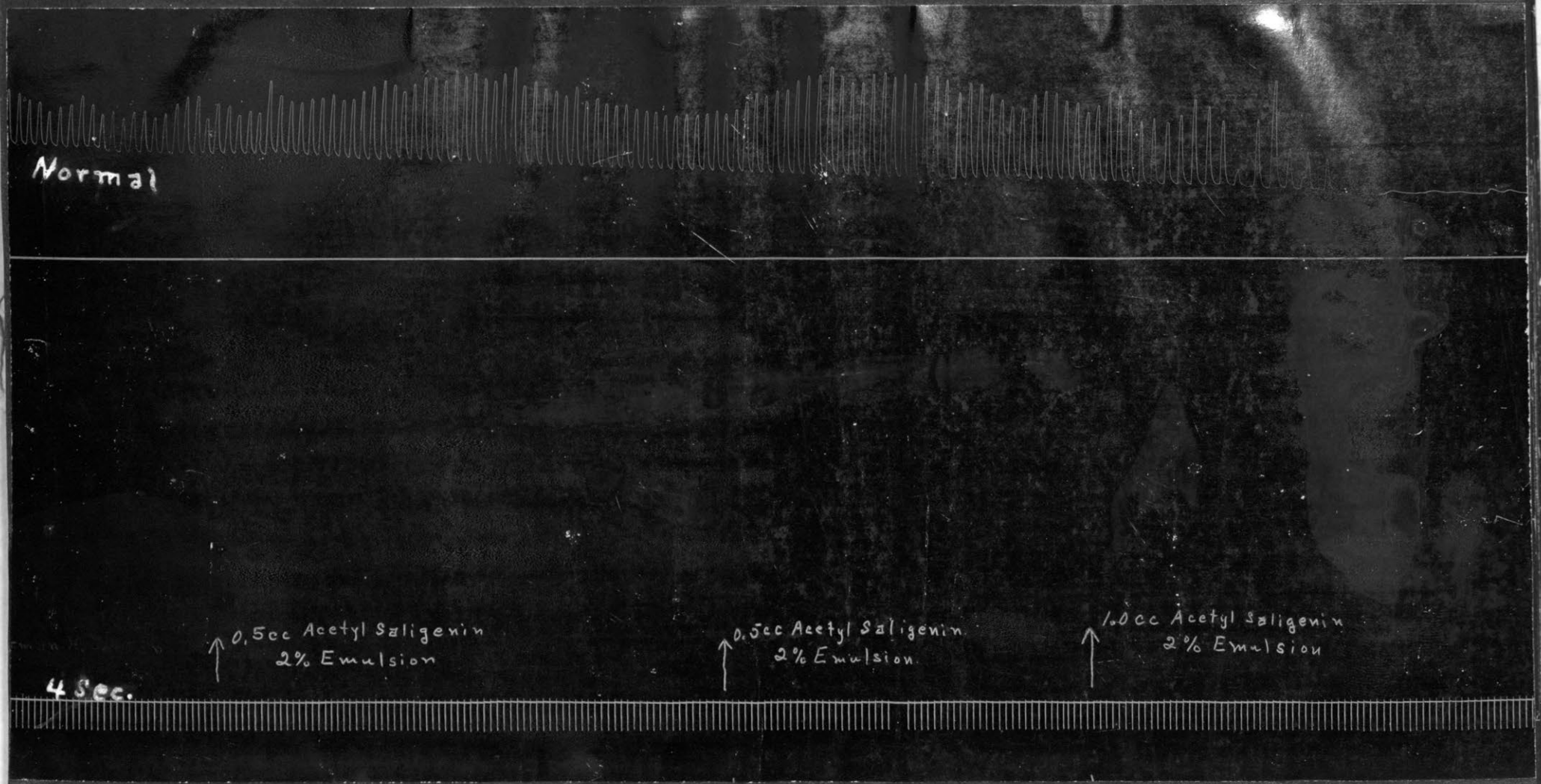

Fig. 5.- Intestinal Segment from Rabbit.
Acetyl Ether of Saligenin.

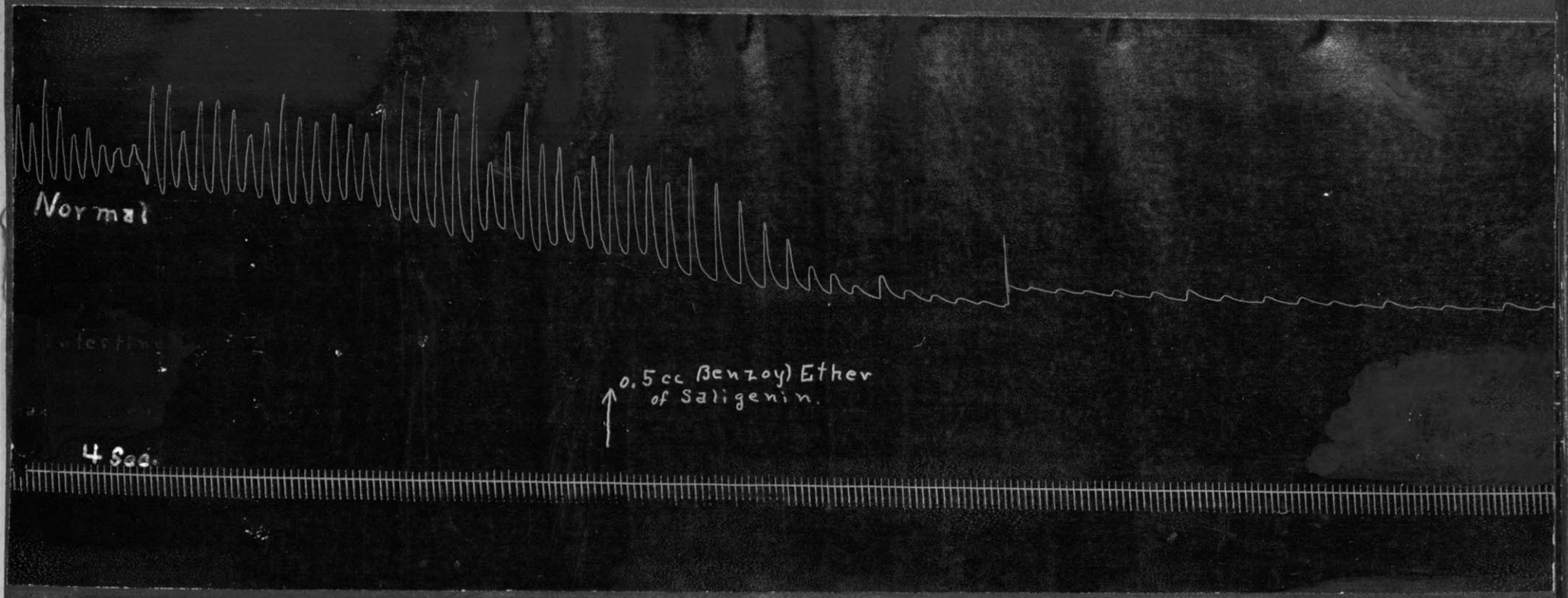

Fig. 6.- Intestinal Segment from Rabbit.
Benzoyl Ether of Saligenin.

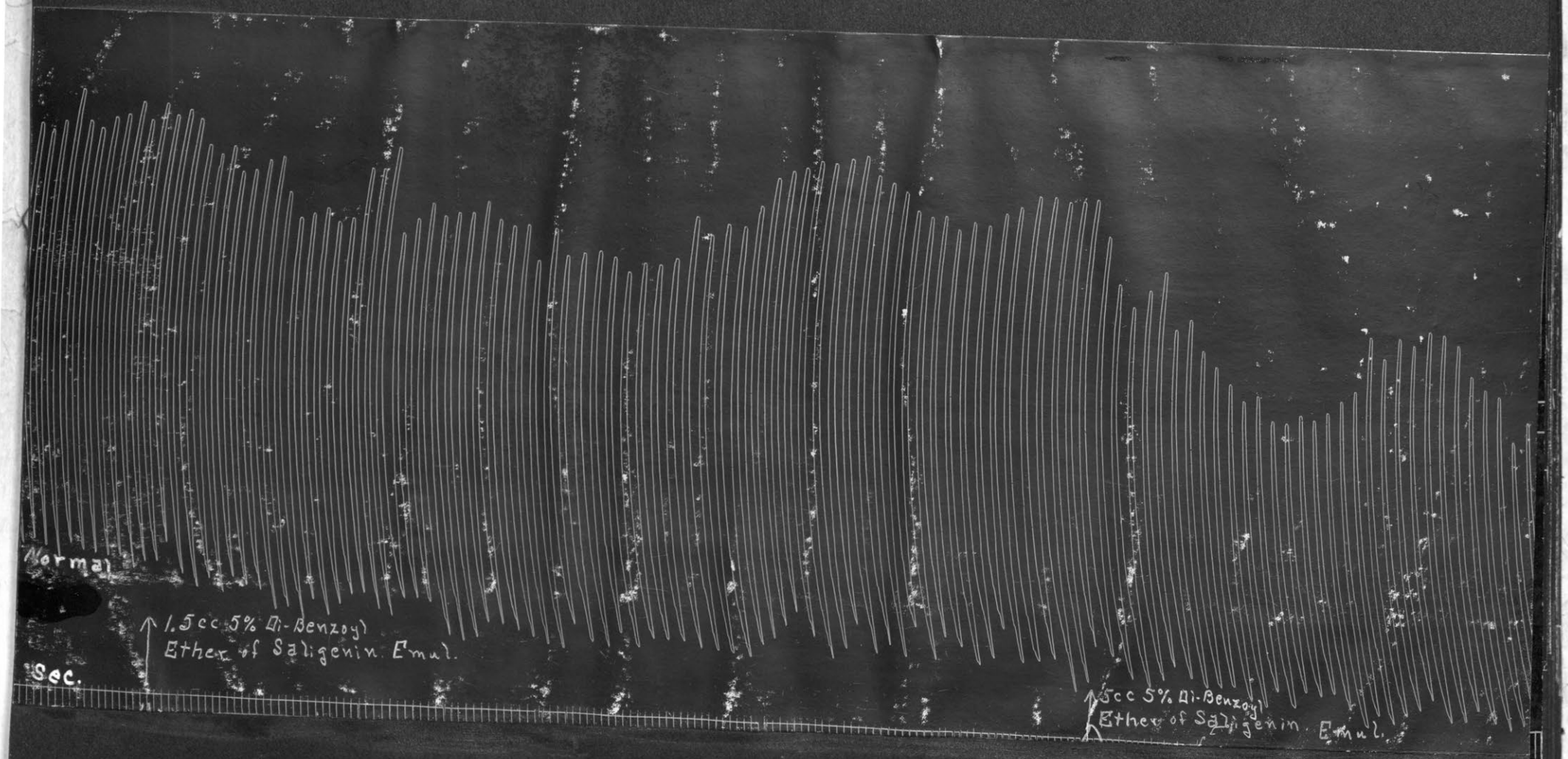

Fig. 7. - Intestinal Segment from Rabbit.
Di-Benzoyl Ether of Saligenin.

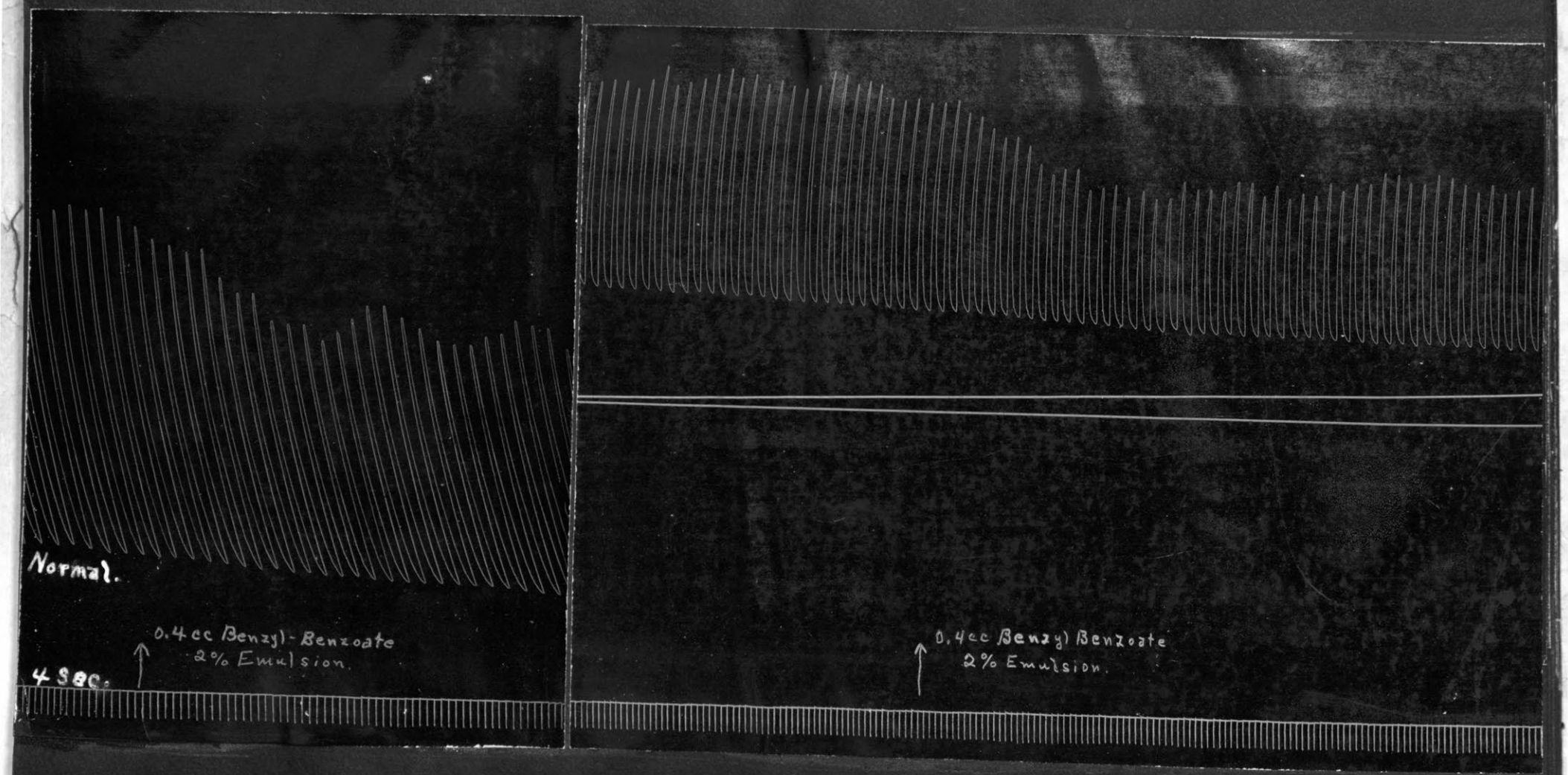

Fig. 8.- Intestinal Segment from Rabbit.

Benzyl Benzoate.

Fig. 9. - Perfusion of Frog's Circulatory System.
Ethyl Ether of Saligenin (0.2%).

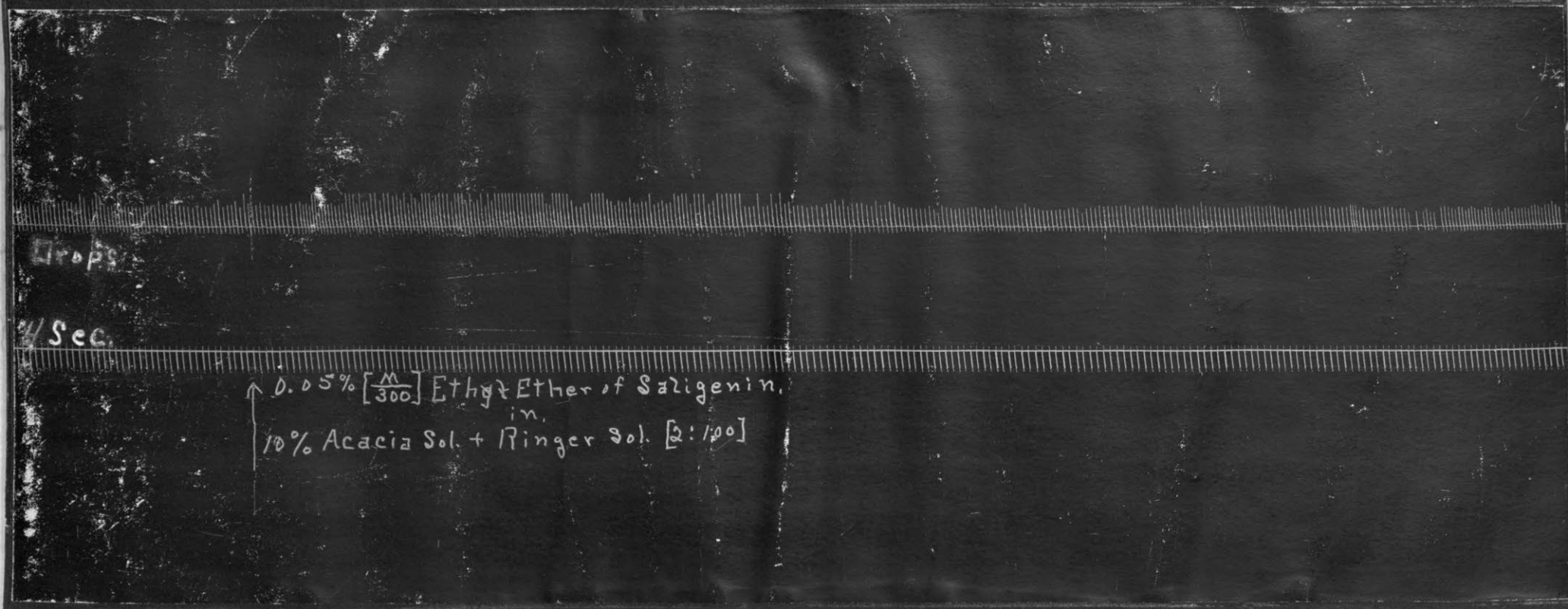

Fig. 10. - Perfusion of Frog's Circulatory System.
Ethyl Ether of Saligenin (M/300 or 0.05%).

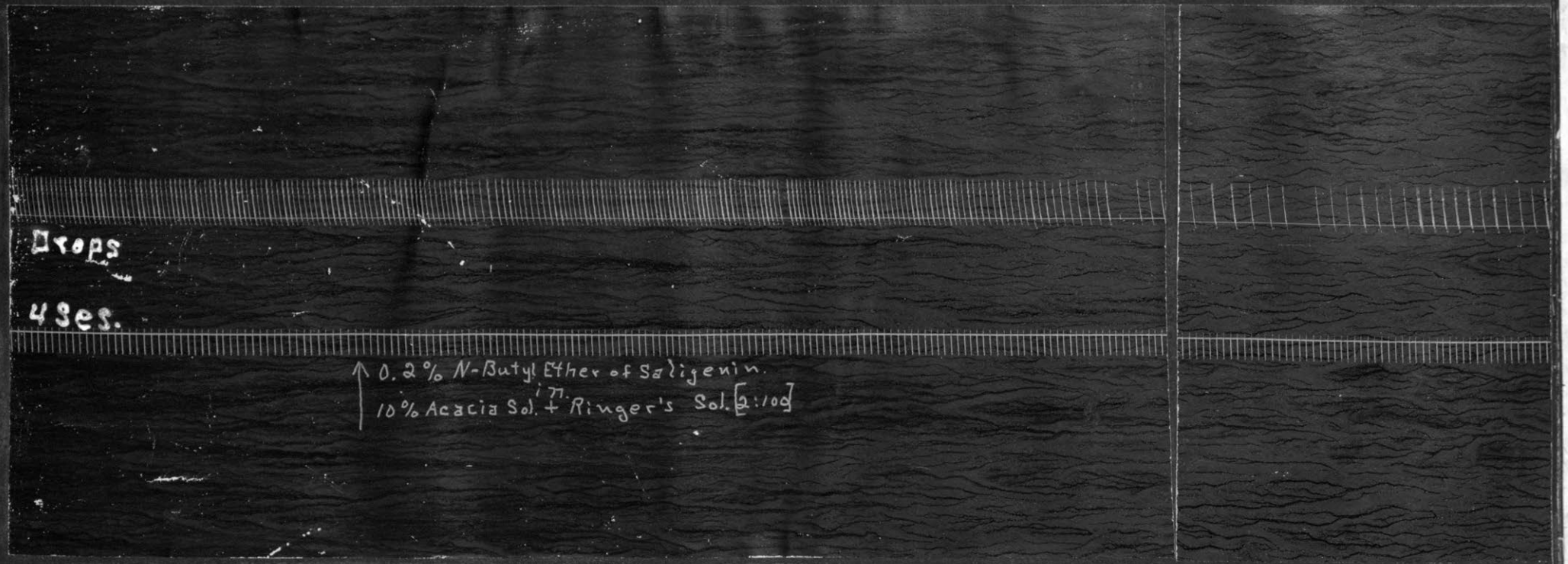

Fig. 11. - Perfusion of Frog's Circulatory System.
N-Butyl Ether of Saligenin (0.2%).

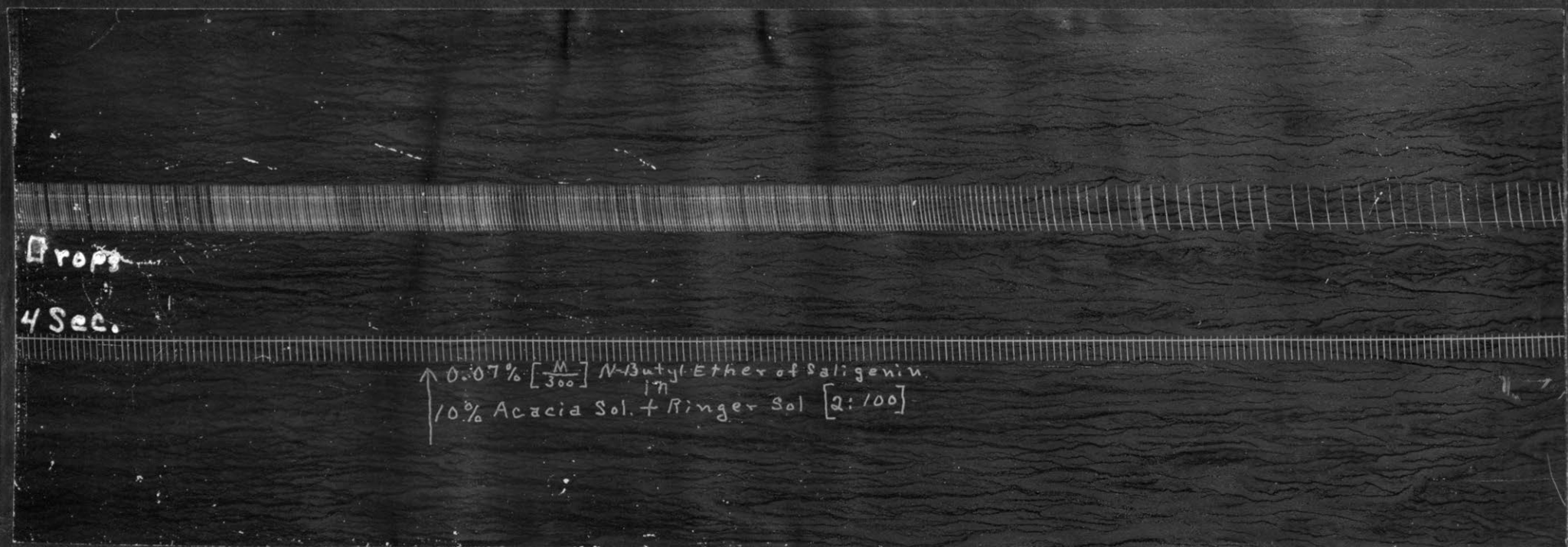

Fig. 12. - Perfusion of Frog's Circulatory System.
N-Butyl Ether of Saligenim (M/300 or 0.060%).

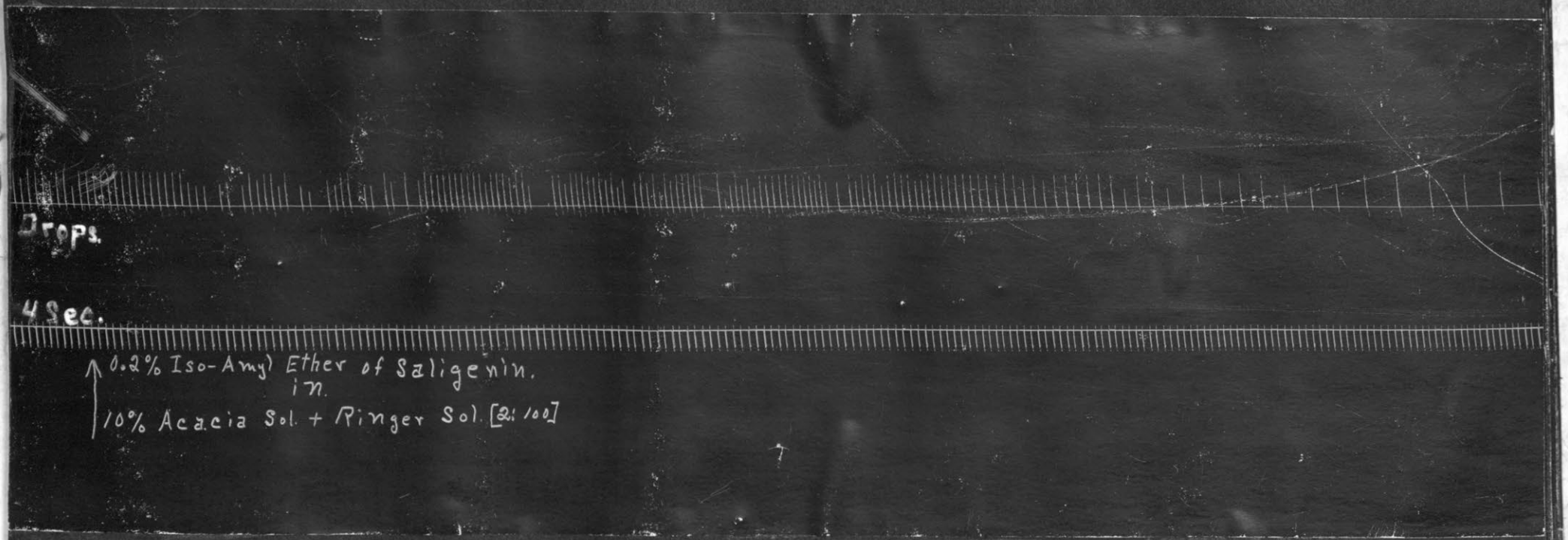

Fig. 13. - Perfusion of Frog's Circulatory System.
Iso-Amyl Ether of Saligenin (0.2%).

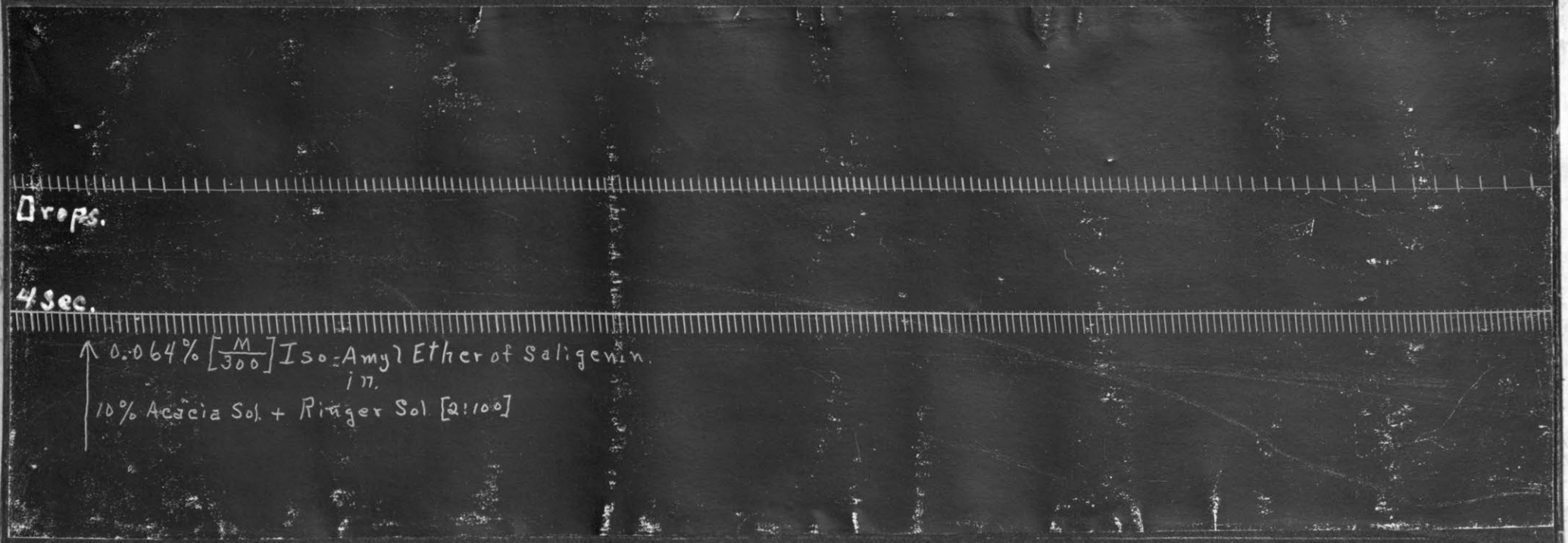

Fig. 14. - Perfusion of Frog's Circulatory System.
Iso-Amyl Ether of Saligenin (M/300 or 0.064%).

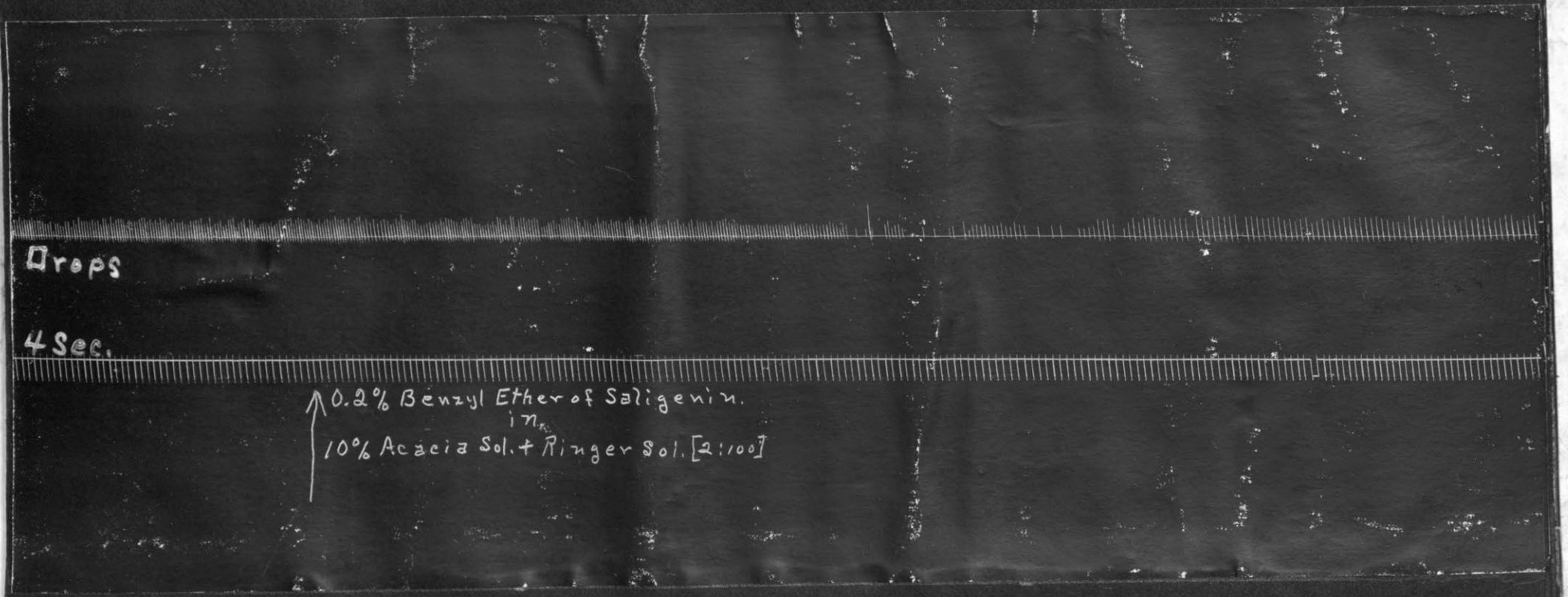

Fig. 15. - Perfusion of Frog's Circulatory System.
Benzyl Ether of Saligemin (0.2%).

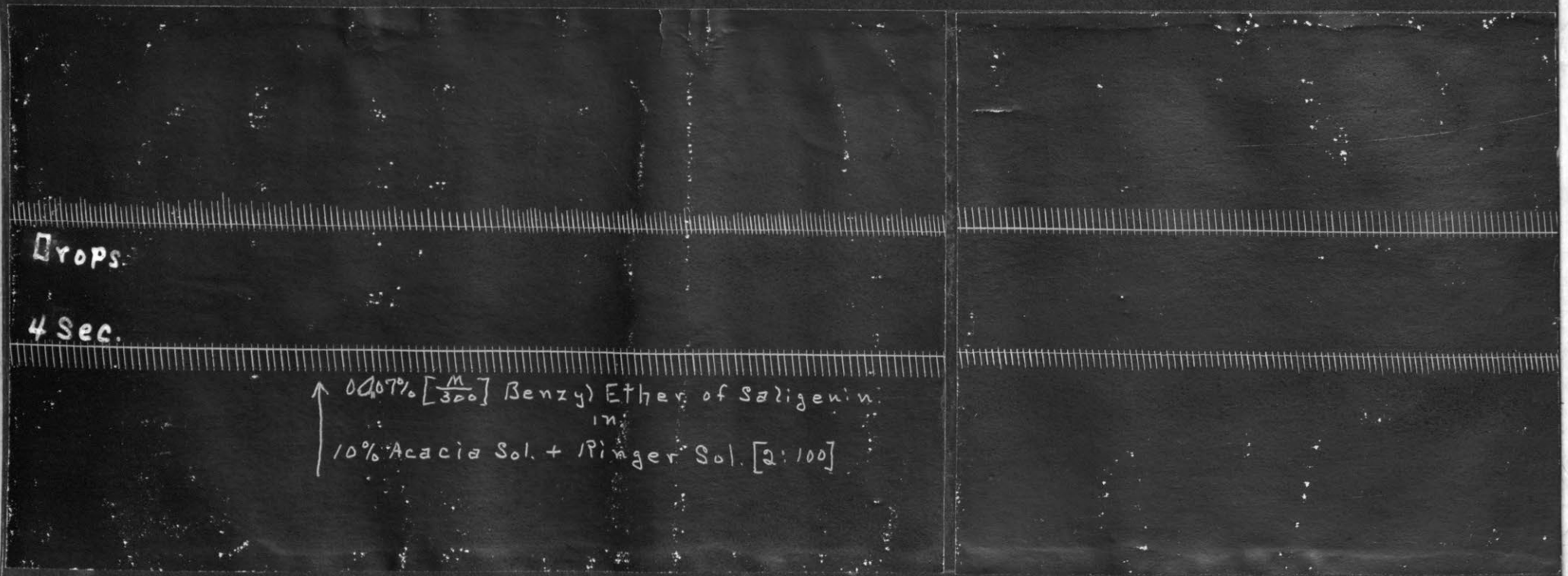

Fig. 16. - Perfusion of Frog's Circulatory System.
Benzyl Ether of Saligenin (M/300 or 0.070%).

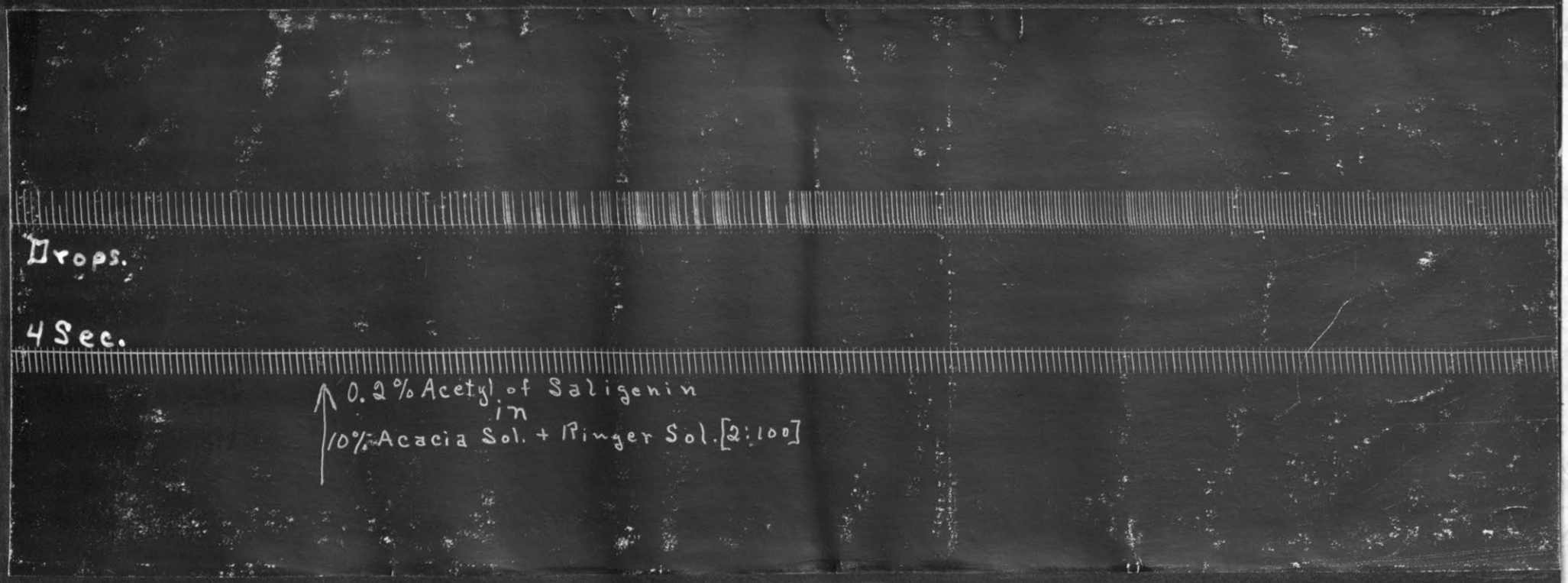

Fig. 17. - Perfusion of Frog's Circulatory System.
Acetyl Ether of Saligenin (0.2%).

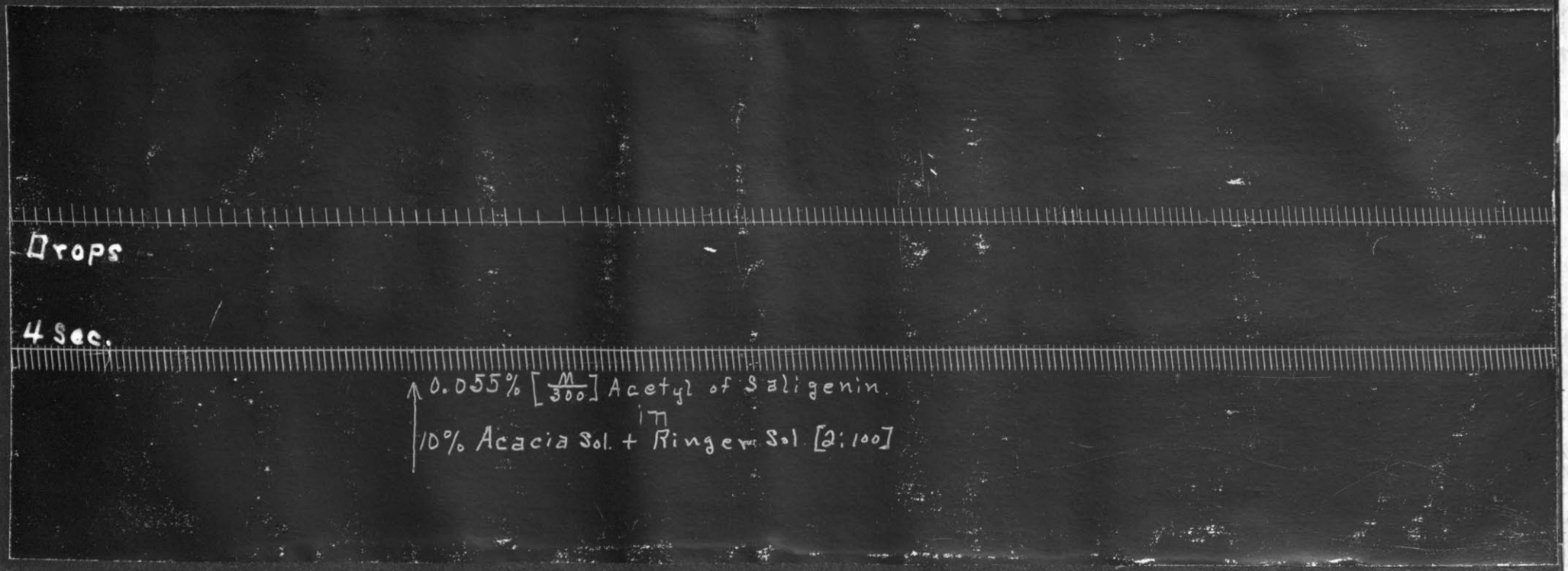

Fig. 18. - Perfusion of Frog's Circulatory System.
Acetyl Ether of Saligenim (M/300 or 0.055%).

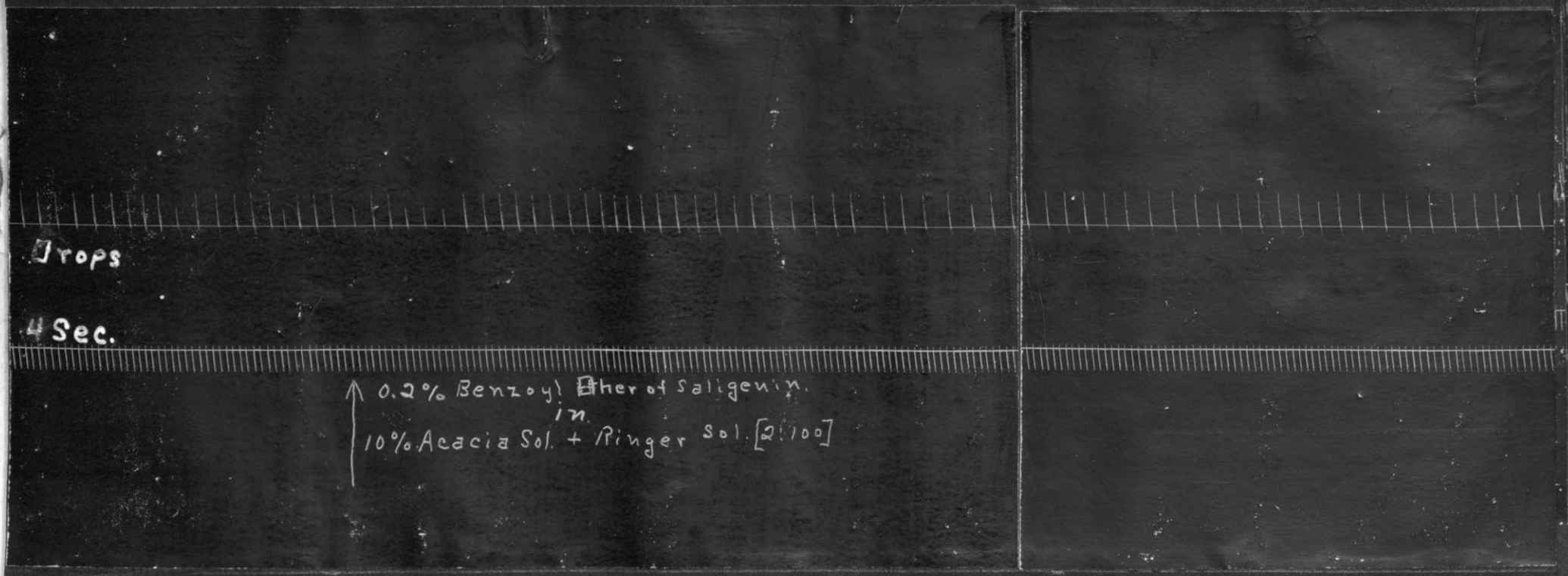

Fig. 19. - Perfusion of Frog's Circulatory System.
Benzoyl Ether of Saligenin (0.2%).

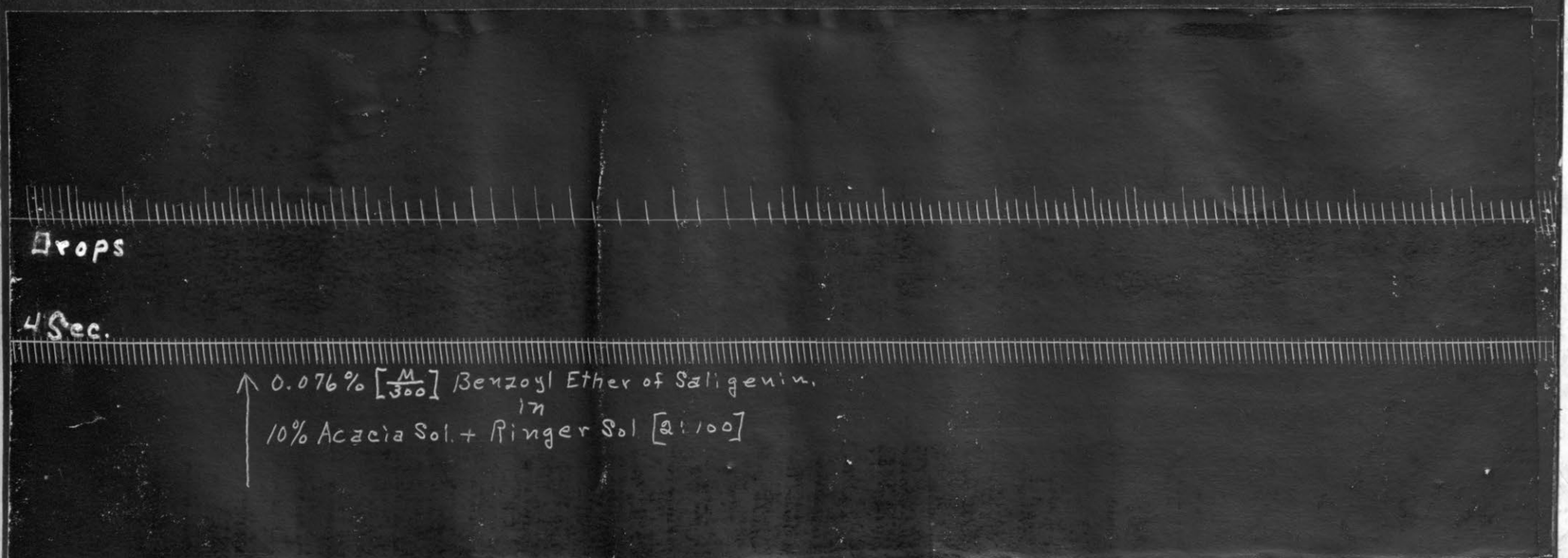

Fig. 20. - Perfusion of Frog's Circulatory System.

Benzoyl Ether of Saligenin (M/300 or 0.076%).

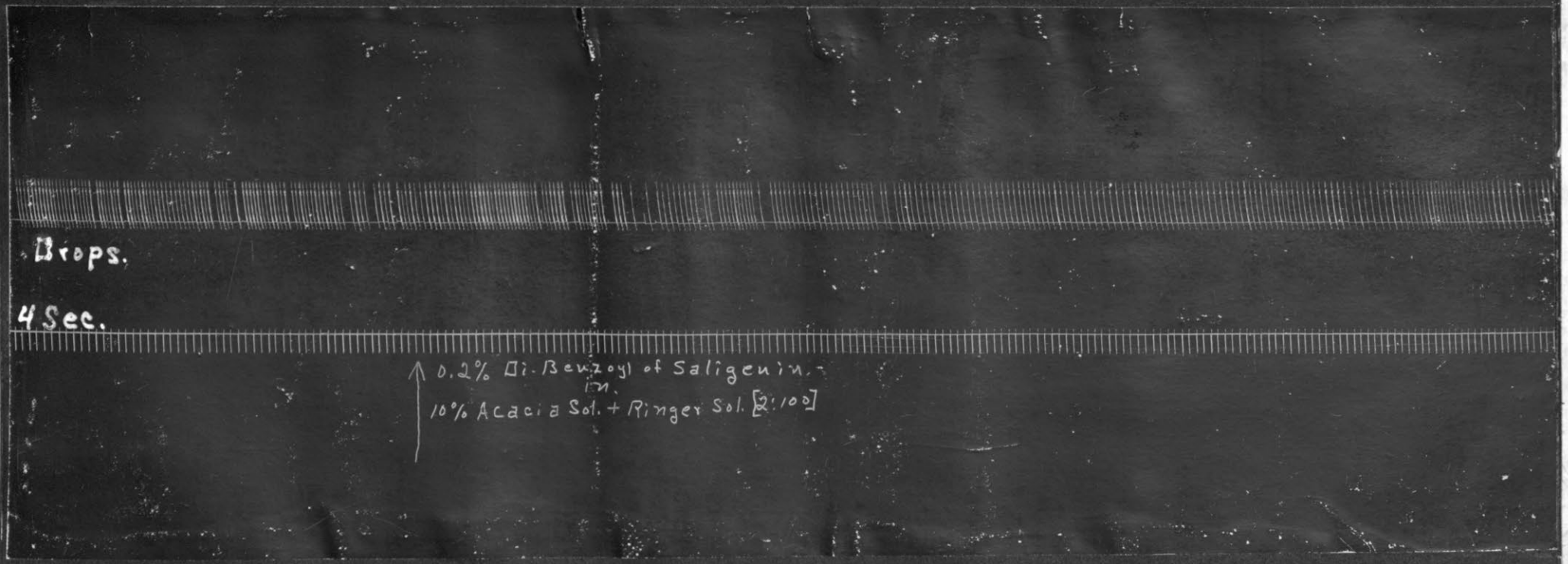

Fig. 21. - Perfusion of Frog's Circulatory System.
Di-Benzoyl Ether of Saligenin (0.2%).

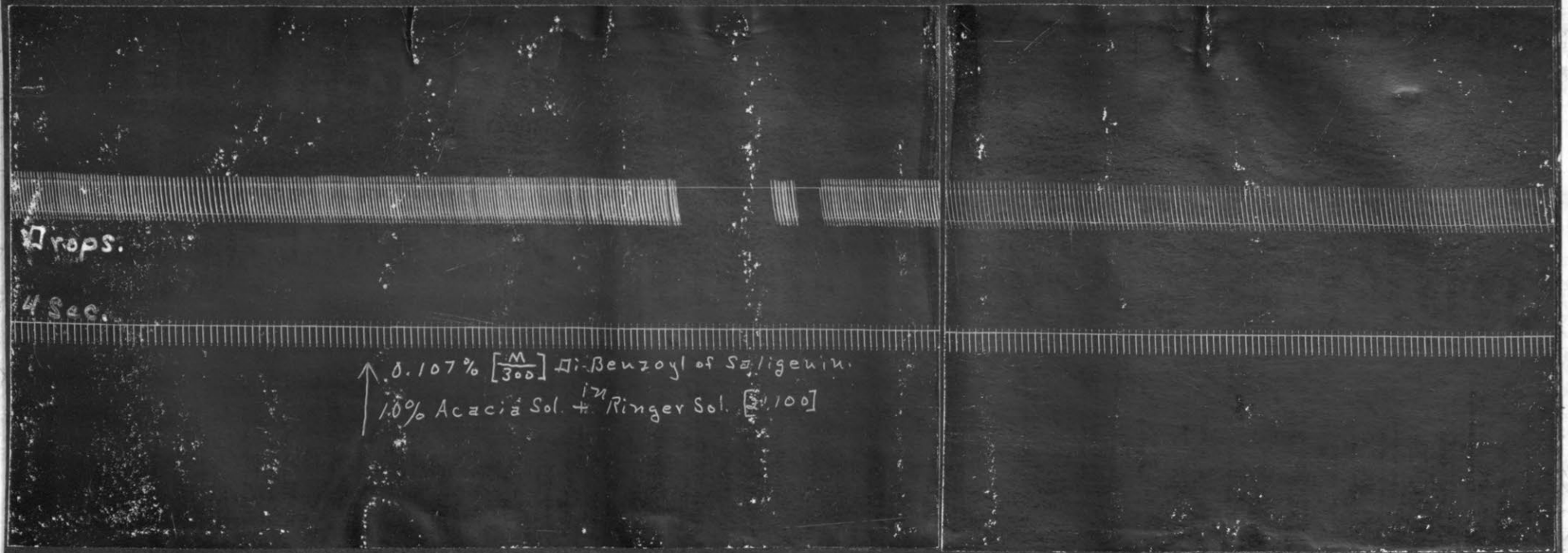

Fig. 22. - Perfusion of Frog's Circulatory System.
Di-Benzoyl Ether of Saligenin (M/300 or 0.101%).

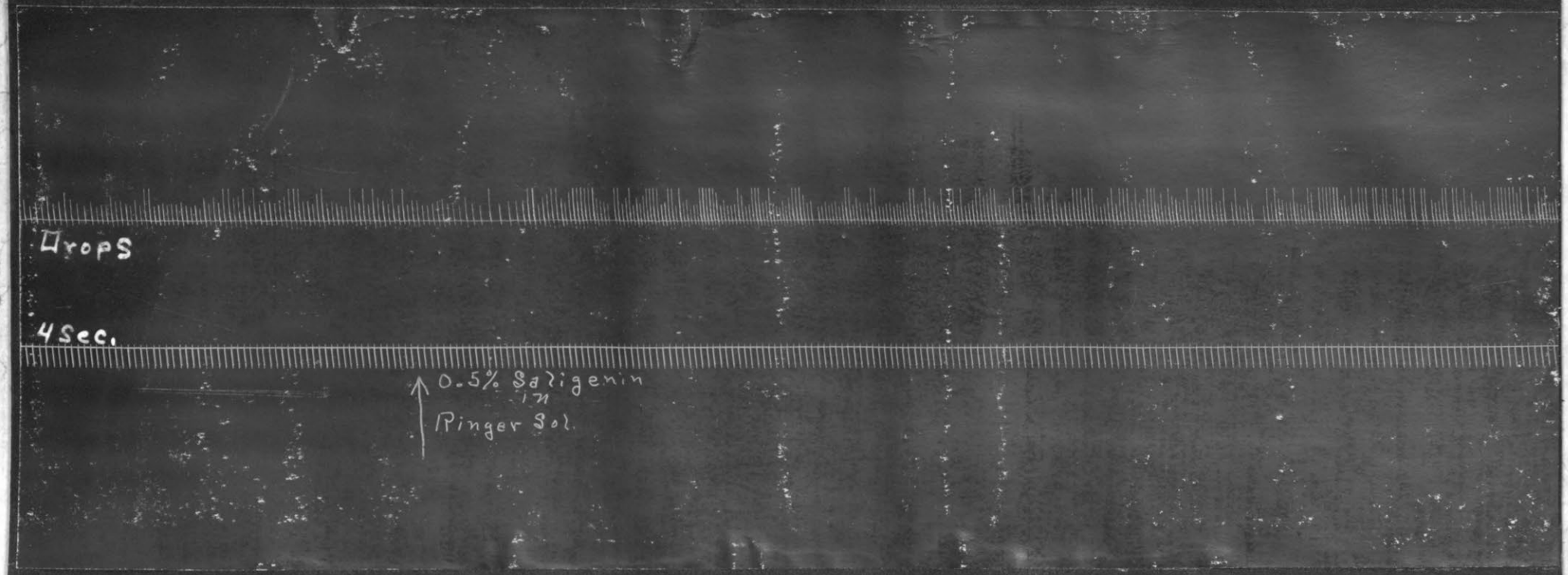

Fig. 23. - Perfusion of Frog's Circulatory System.
Saligenin (0.5%).

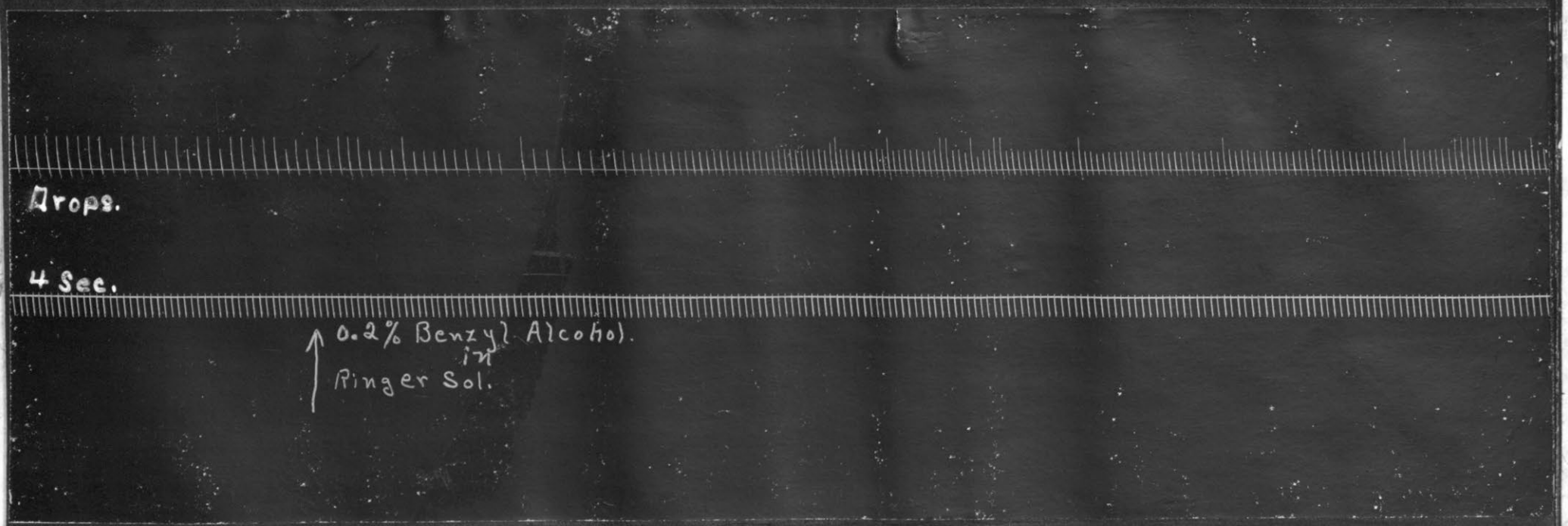

Fig. 24. - Perfusion of Frog's Circulatory System.
Benzyl Alcohol (0.2%).

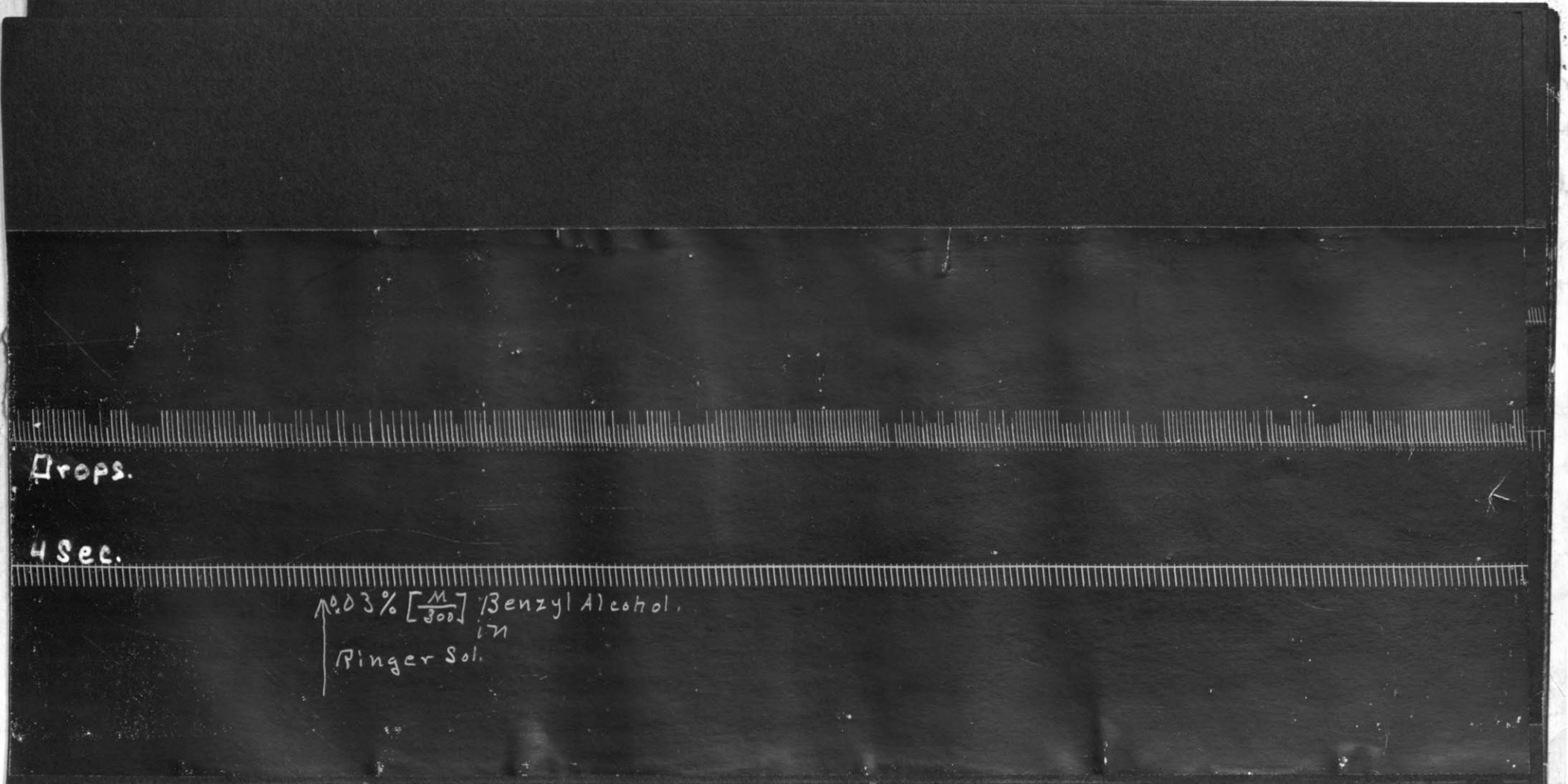

Fig. 25. - Perfusion of Frog's Circulatory System.
Benzyl Alcohol (M/300 or 0.030%).

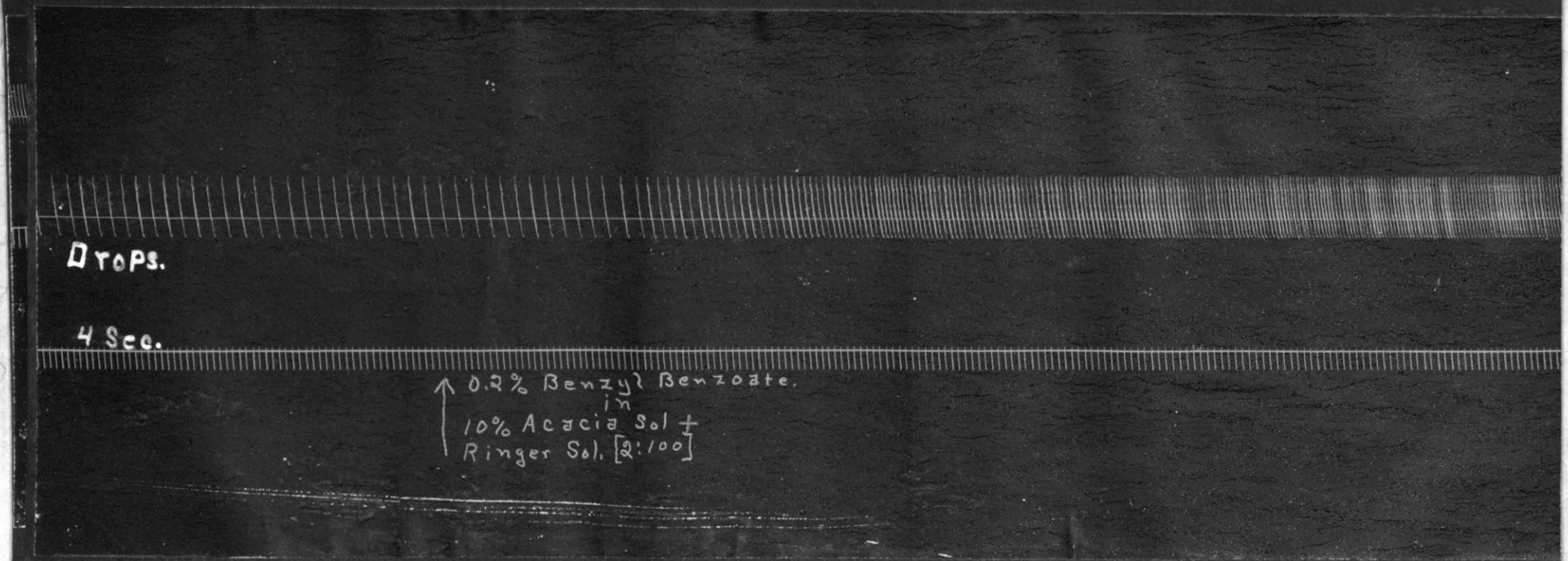

Fig. 26. - Perfusion of Frog's Circulatory System.
Benzyl Benzoate (0.2%).

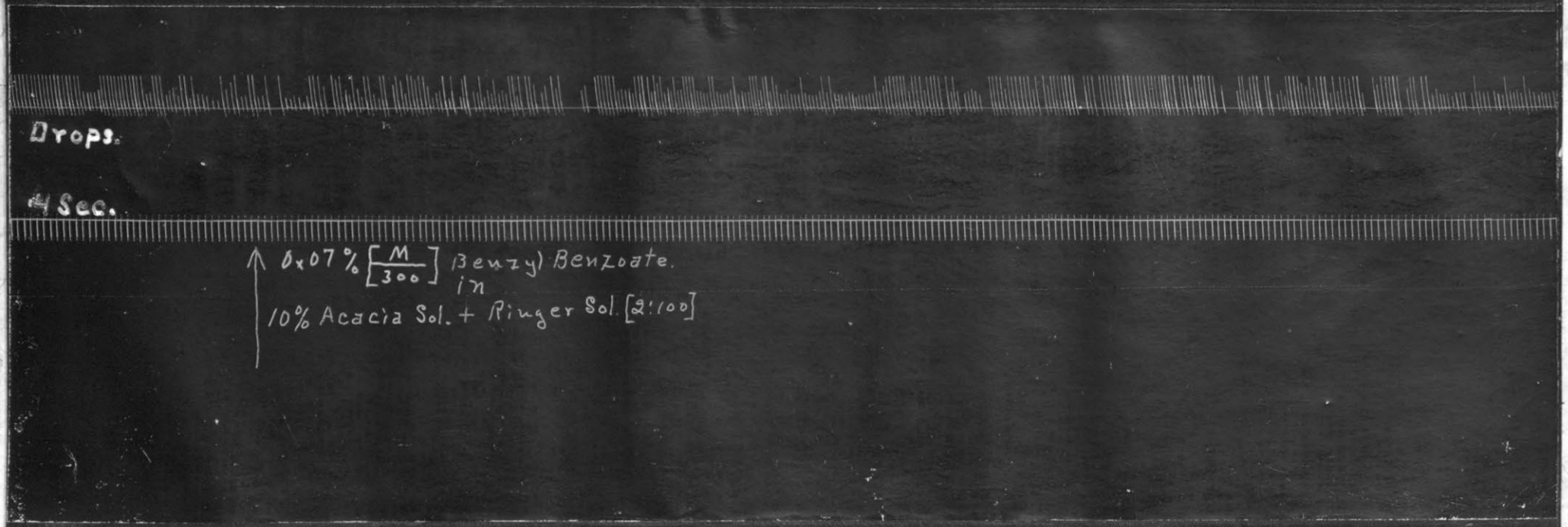

Fig. 27. - Perfusion of Frog's Circulatory System.

Benzyl Benzoate (M/300 or 0.070%).

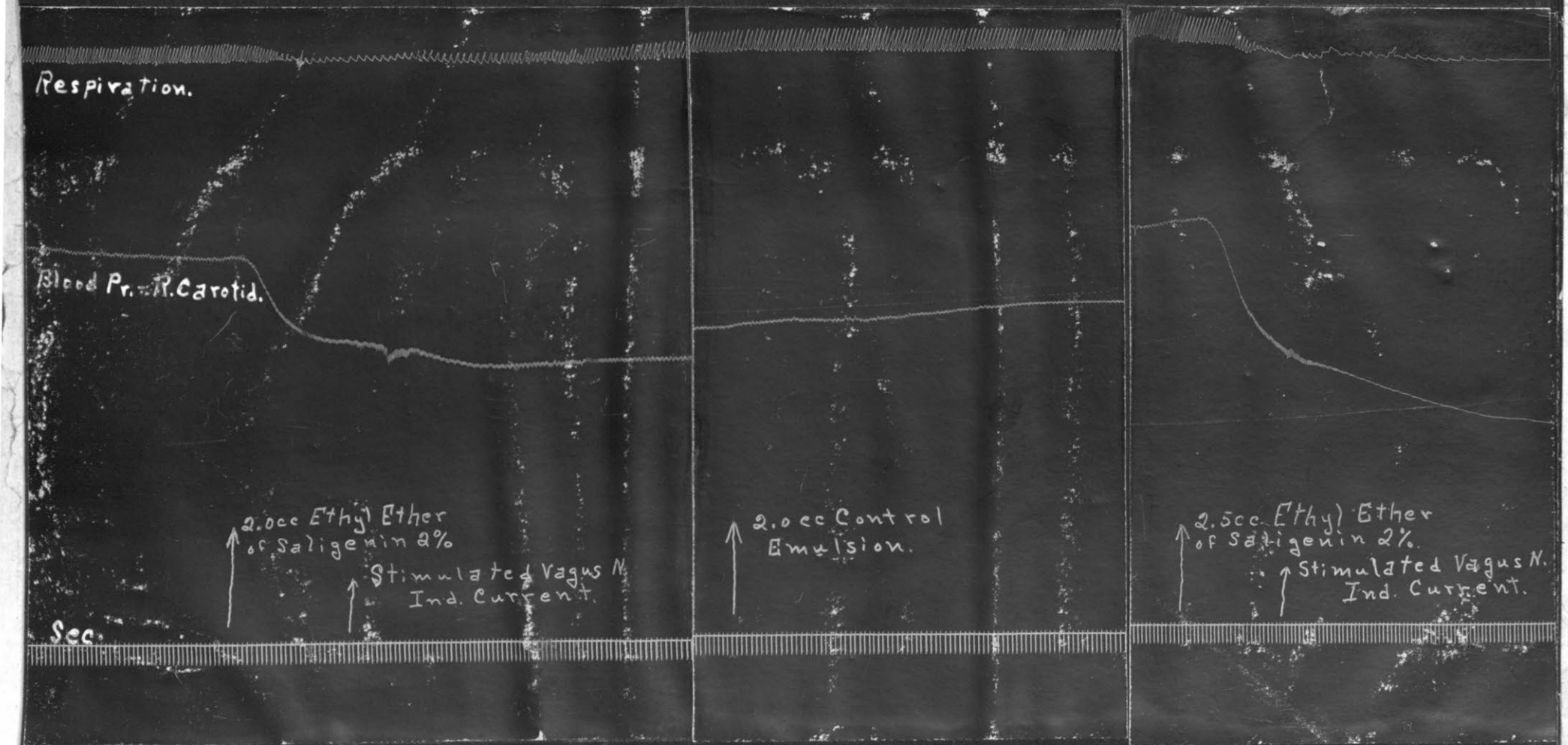

Fig. 28. - Effects upon Blood Pressure and Respiration - Rabbit.
Ethyl Ether of Saligenin.

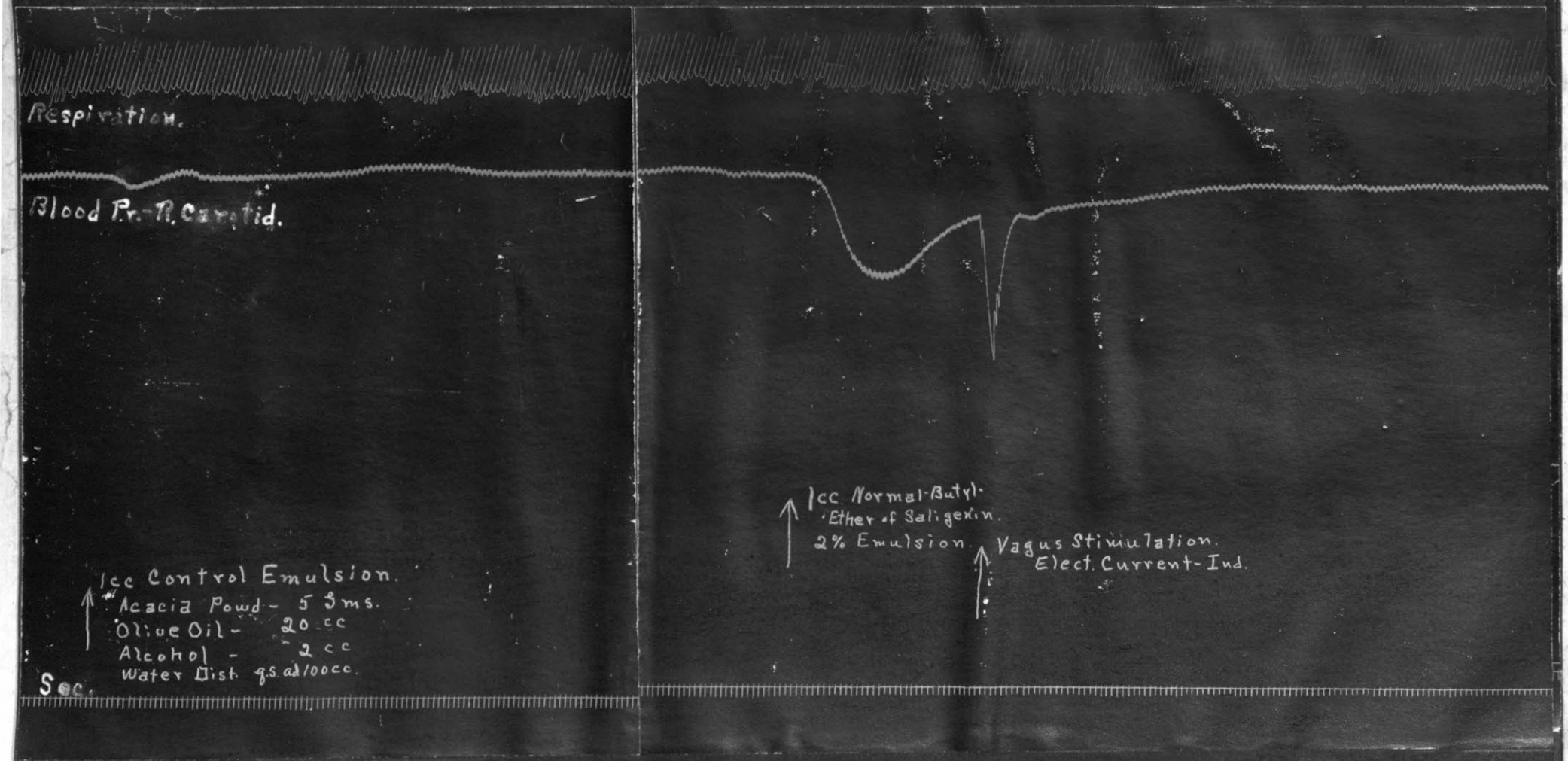

Fig. 29. - Effects upon Blood Pressure and Respiration - Rabbit.

N-Butyl Ether of Saligenin.

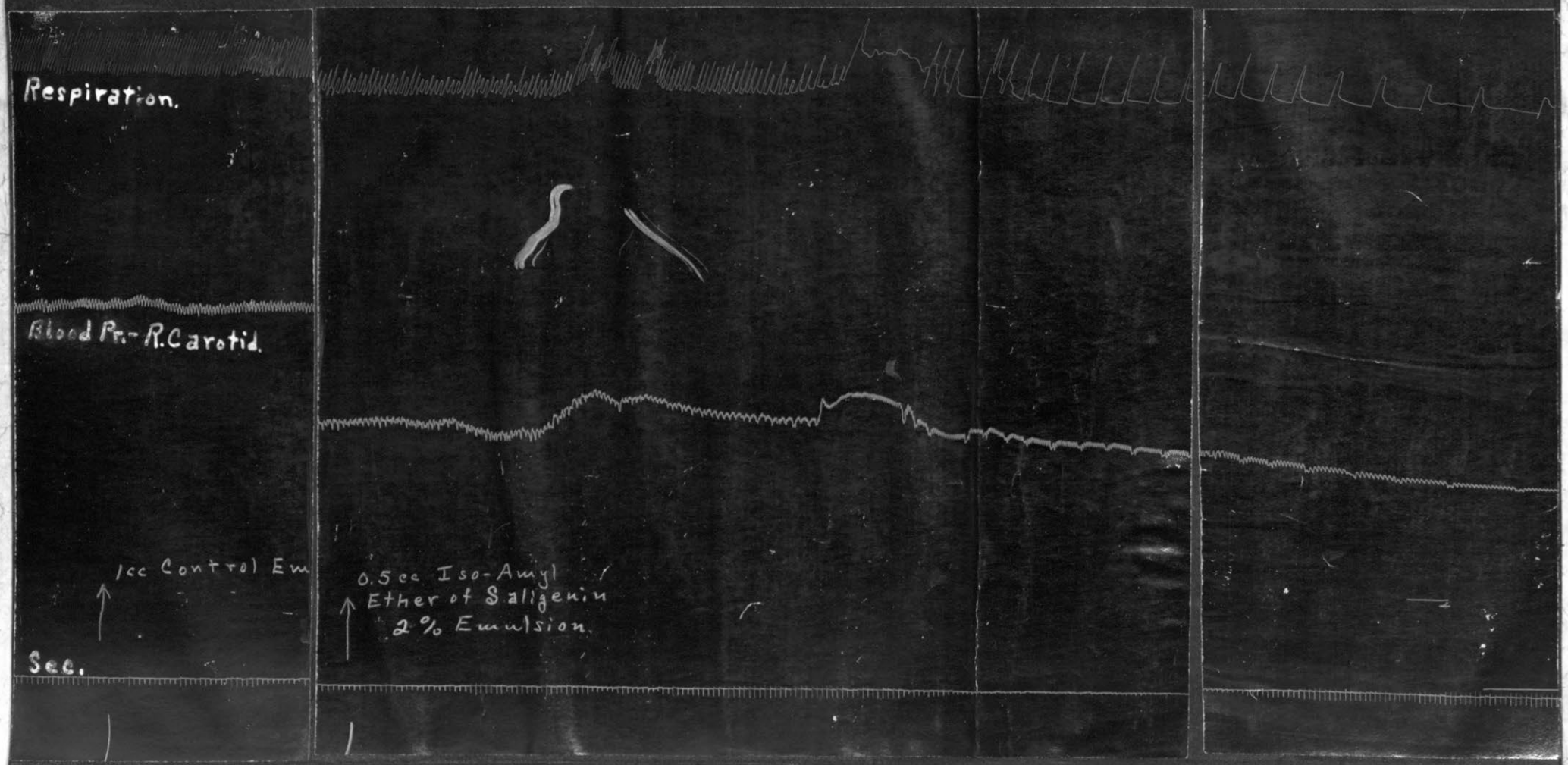

Fig. 30. - Effects upon Blood Pressure and Respiration - Rabbit.

Iso-Amyl Ether of Saligenin.

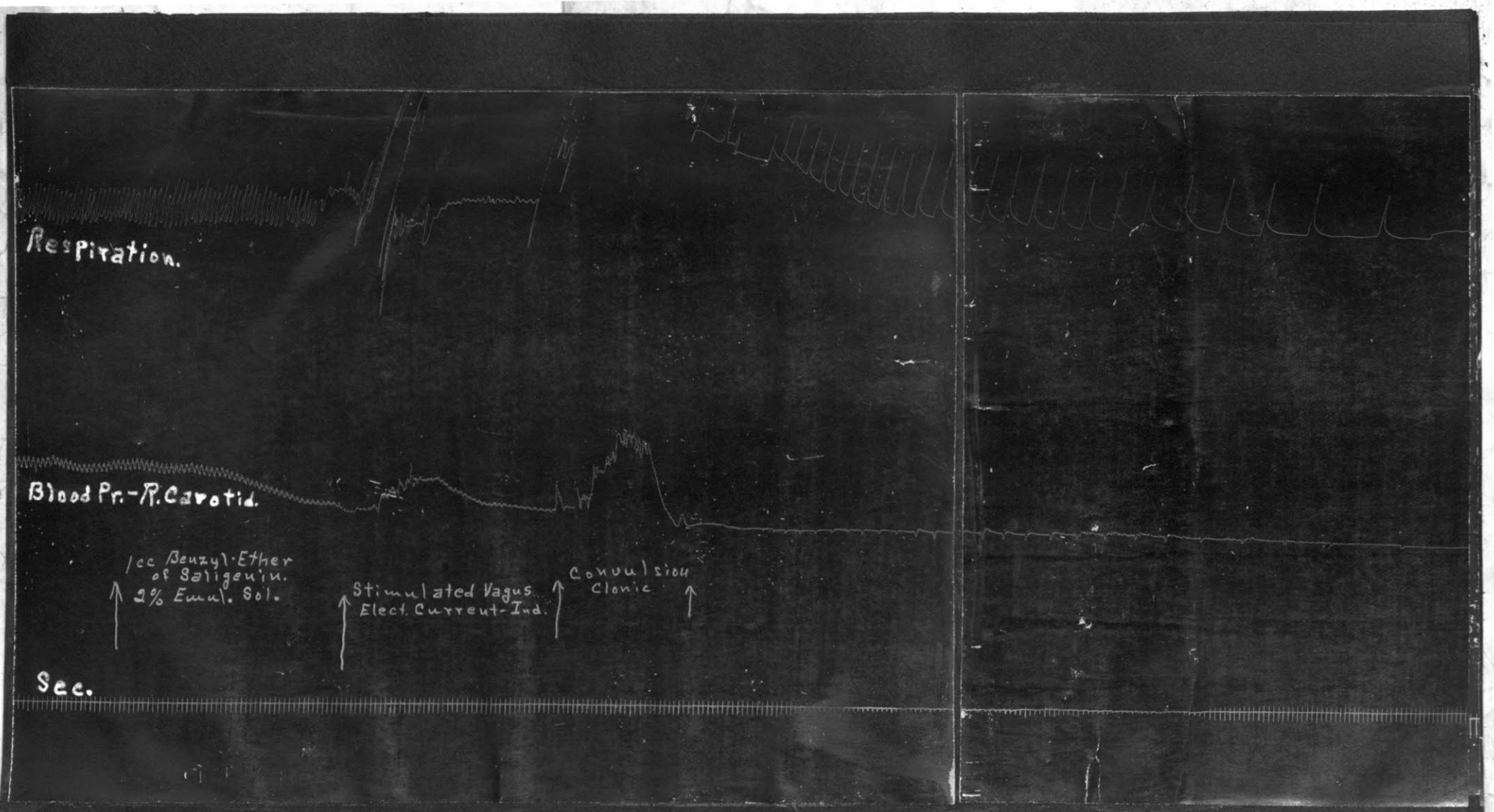

Fig. 31. - Effects upon Blood Pressure and Respiration - Rabbit.
Benzyl Ether of Saligenin.

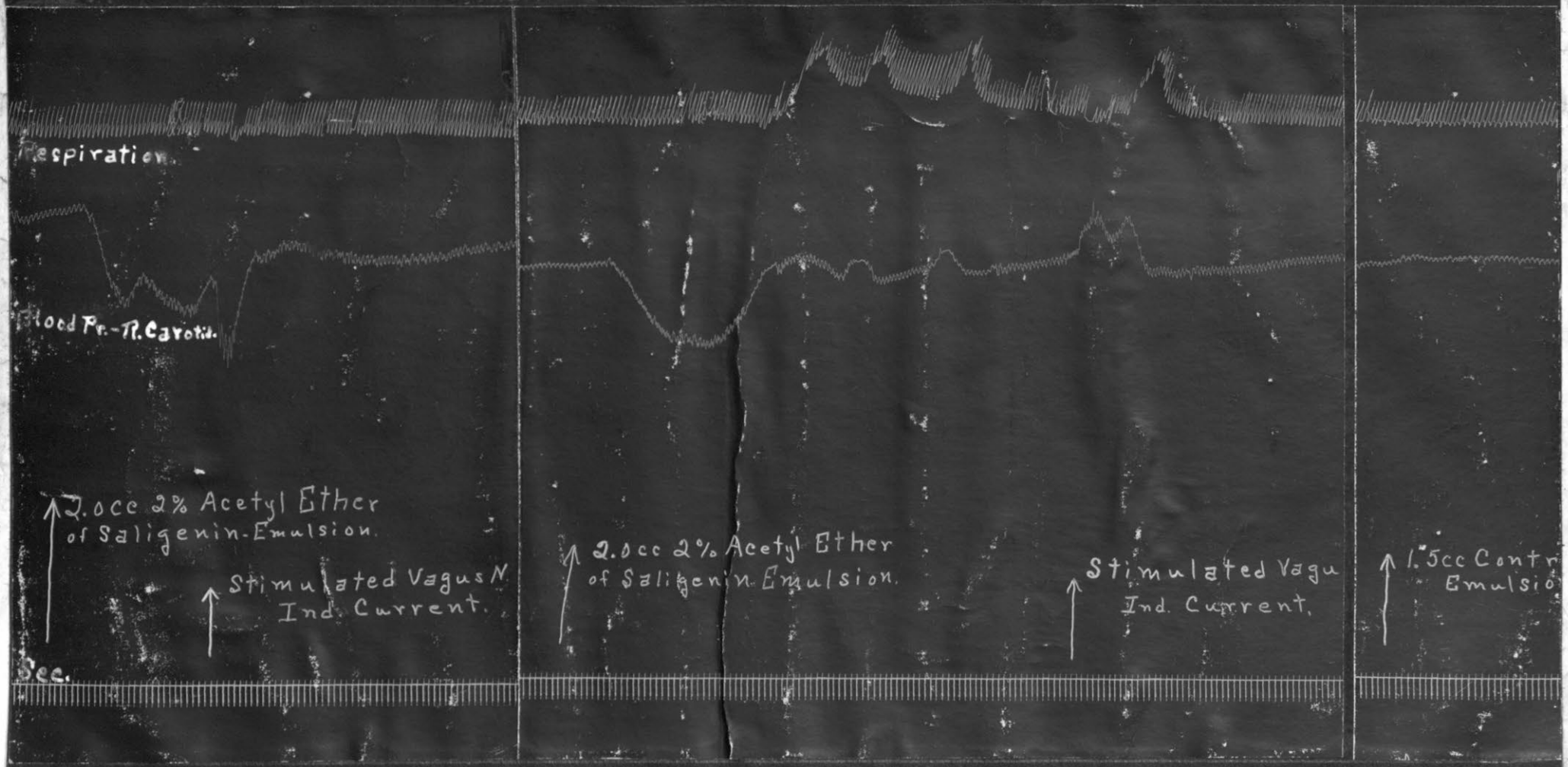

Fig. 32. - Effects upon Blood Pressure and Respiration - Rabbit.

Acetyl Ether of Saligenin.

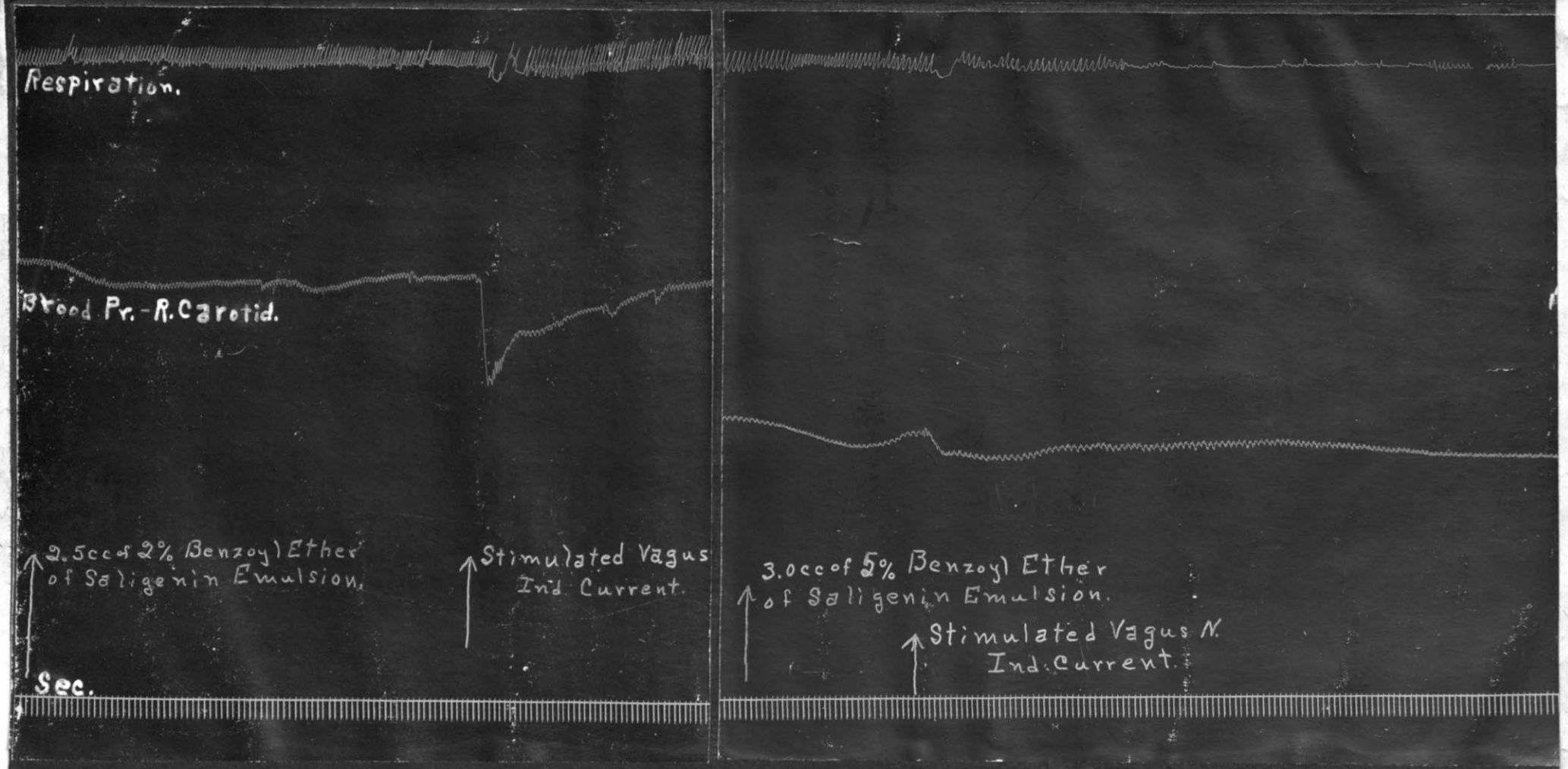

Fig. 33. - Effects upon Blood Pressure and Respiration - Rabbit..
Benzoyl Ether of Saligenin.

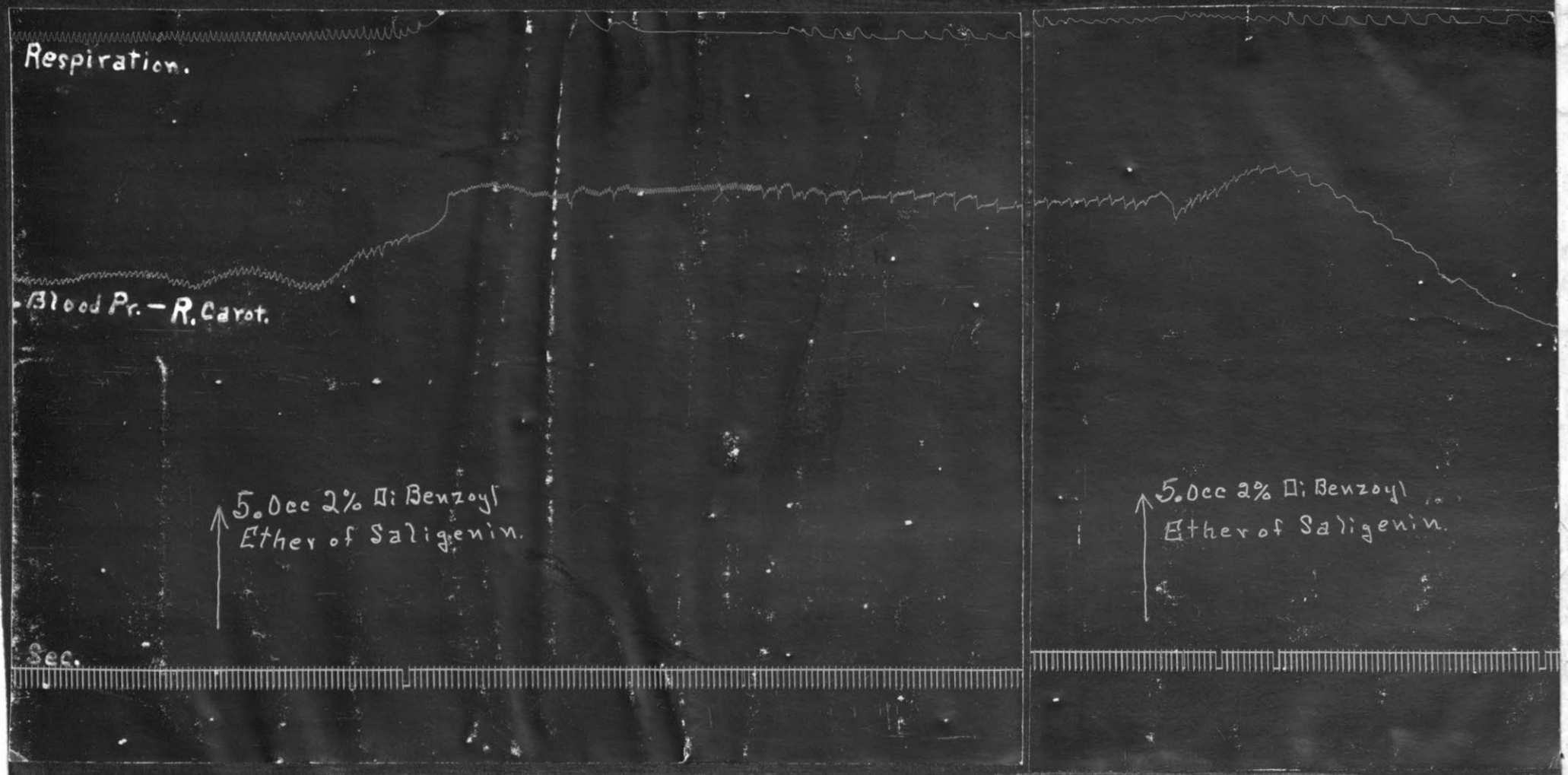

Fig. 34. - Effects upon Blood Pressure and Respiration - Rabbit.
Di-Benzoyl Ether of Saligenin.