UMBELLULONE: PHARMACOLOGICAL AND BACTERICIDAL PROPERTIES

by

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In Charge of Major

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Chairman of School Graduate Committee

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This investigation was made possible through the generosity of Fairchild and Foster in offering the Fairchild Scholarship. The author wishes to take this opportunity to thank the doners.

INTRODUCTION

The literature on the oil of California laurel, Umbellularia californica (Hook and Arn.) Nutt., includes very little information on the pharmacology(20) of the oil or the ketone (Umbellulone) obtained from it. Likewise, no reference could be found in the literature on volatile or essential oils regarding the germicidal or fungicidal activity of the oil of California laurel or umbellulone, although extensive research on the chemistry of the constituents present in this oil has been pursued by various investigators.

Persons who have come in close proximity with the oil or its vapors report discomforts, and in some cases death has been attributed to the contact with the vapors while traveling through regions densely covered with Umbellularia californica. It was reported that in 1925 a worker, during distillation of a large quantity of oil, became unconscious and remained so for six hours. It was assumed that the unconsciousness was produced by the vapors of the volatile oil. Other reports show that the fruits of the tree have been eaten by Indians, and that in some localities the leaves have been used as flavoring agents in soups(2) Sawyer(22) reports that Indians used Myrtle leaves in cases of nervous headaches and nasal catarrh. Myrtle oil has been used in M.O. cough drops and in Myrtle Balm, an ointment manufactured in Portland, Oregon.

With such seemingly conflicting reports, there is evidently a need for research on the oil of California laurel in an attempt to ascertain the value or danger of this oil, and the effects of the oil on the body mechanism. It is with this aim in view that this study is presented.

UMBELLULONE: PHARMACOLOGICAL AND BACTERICIDAL PROPERTIES

HISTORICAL

Umbellularia californica (Hook and Arn.) Nutt., was first collected in California by Menzies (1) in the latter part of the eighteenth century. Prior to Menzies the Spaniards of California knew the tree as Laurel Silvestre.

In 1826 Douglas (6) classified this evergreen tree as a laurel, Laurus regia (the regal laurel), probably intending to indicate the beauty and splendor of the tree.

In 1833 Hooker and Arnott (1) classified this evergreen as Tetranthera californica. Later Nutall (1) gave it the present name of Umbellularia californica. As far as has been reported this is the only representative of the genus Umbellularia.

Figures 1 and 1-a show tree and detail of leaf.

WORK OF PREVIOUS INVESTIGATORS

The first work reported on the oil of California laurel was that of Heaney (9) who, in 1875, by fractionation under reduced pressure obtained a colorless liquid possessing a pungent odor. To this product he gave the name Oreodaphenol.

In 1880 Stillman (26) obtained by fractionation at 215-216° centigrade, a colorless, mobile liquid which he named umbelloe, possessing an aromatic but pungent odor. He observed that excessive inhalation of the vapors produced a headache.

In 1904 Powers and Lee (19) found that the oil of the

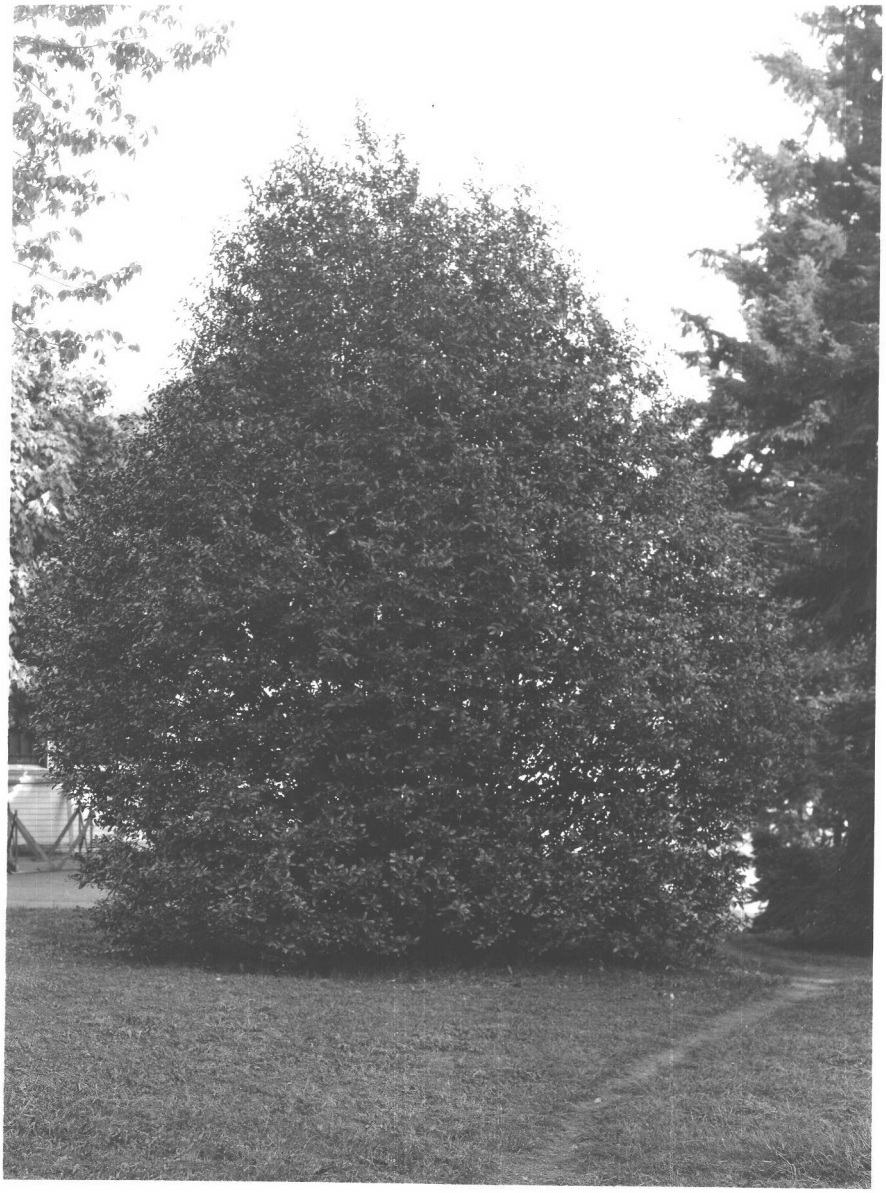
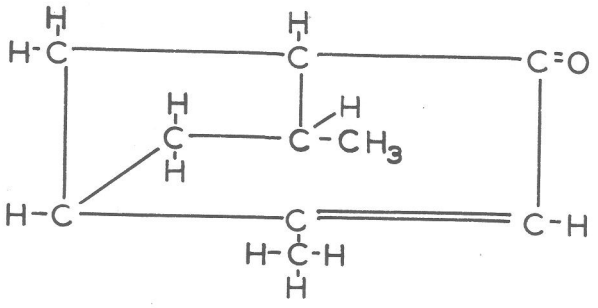


Figure 1

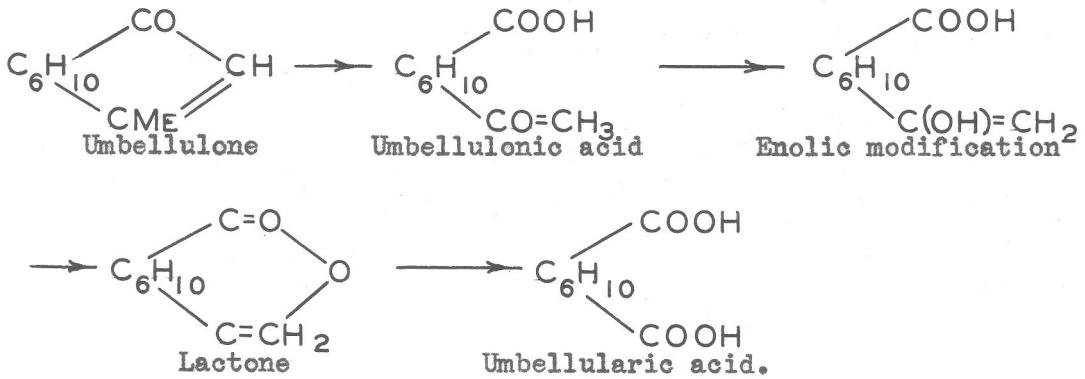
Figure 1-a

California laurel yielded on fractionation the following constituents; pinene, cineol, eugenol, methyl eugenol and a ketone, umbellulone. This ketone fraction was collected at 217-222° centigrade and was purified by regeneration from its semicarbazone. The purified ketone had a specific gravity of 0.9584 at 15/15, alpha $_D$ -37° in a 100 mm tube.

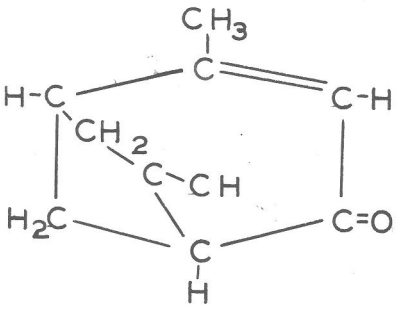
In addition to this Powers and Lee (19) observed that on treating umbellulone with bromine, a liquid dibromide of umbellulone was formed. On distillation under reduced pressure the liquid dibromide gave a solid de-bromodihydroumbellulone ($C_{10}H_{14}O\ Br_2$), an unsaturated liquid monobromoumbellulone ($C_{10}H_{13}O\ Br$), and a small amount of a hydrocarbon ($C_{10}H_{14}$). They also found that umbellulone did not give a test for aldehyde by Schiff's rosaniline test. Umbellulone does not give all the characteristic ketone reactions as do the other known ketones. Umbellulone is isomeric with carvone, obtained from oil of caraway and other oils. Umbellulone is the only known naturally occurring ketone that has such a pungent odor and nauseating effect. Powers and Lee (19) concluded that umbellulone had one ethylenic linkage, and gave it the following formula.



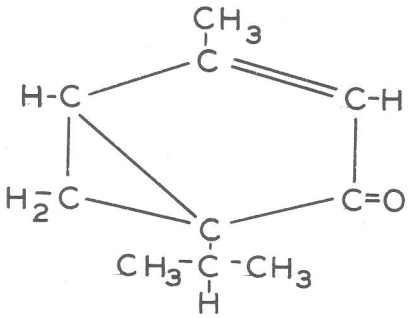
Tutin (29), in 1908, showed that when umbellulone was oxidized with potassium permanganate there was formed a keto acid, umbellulonic acid, $C_9H_{14}O_3$, which on distillation passed into an unsaturated lactone, $C_9H_{12}O_2$. The latter on oxidation yielded umbellularic acid, a polymethylenedicarboxylic acid, possessing the formula $C_8H_{12}O_4$. These changes may be represented as follows:



Tutin gave to umbellulone the following structure:

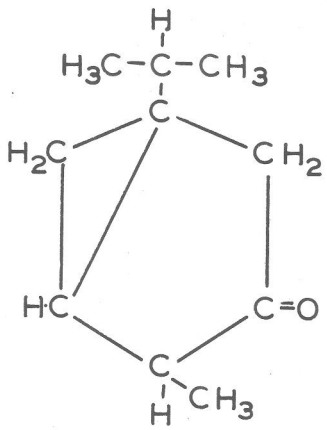


Semmler (23), in 1908, gave umbellulone the structural formula that is accepted today.

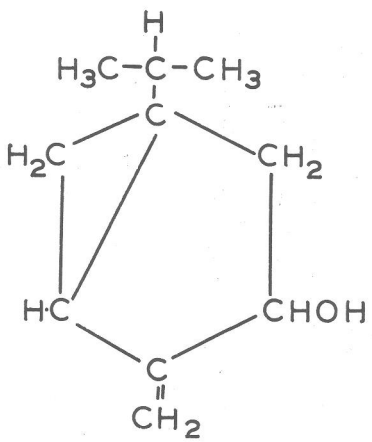


Wienhaus and Todenhofer (30), in 1929, obtained the ketone, umbellulone, from oil of myrtle by preparing a sodium sulphite addition product, which is water soluble. This was steam distilled to get the ketone which was purified by fractionation under reduced pressure. From umbellulone they were able to prepare dihydro umbellulone, a hexahydroderivative of umbellulone and other products.

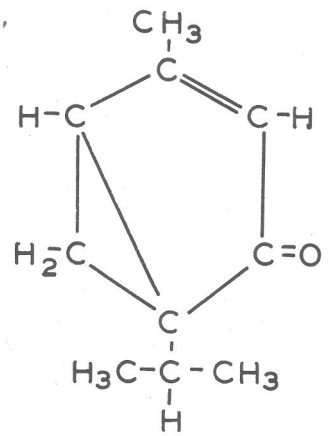
According to Spiegel (25), sabinol, thujone and umbellulone are very similar in structure.



THUJONE



SABINOL



UMBELLULONE

DISTILLATION OF THE LEAVES OF THE CALIFORNIA LAUREL TO OBTAIN THE OIL

The leaves are gathered and dried completely or to a semi-dry condition (16), and then steam distilled. A mixture of oil and water is obtained, which, on standing, separates, leaving the oil as the top layer. This is decanted and dried over anhydrous sodium sulphate. The yield of the oil according to Parry (16) varies from 2.5 to 5.5 percent.

BRIEF DESCRIPTION OF THE OIL OF CALIFORNIA LAUREL

The oil is clear, brownish-yellow in color and possesses a very pungent odor. It is extremely irritating to the mucous membrane of the nasal cavity and the throat. It produces headaches, the pain of which progresses from the upper region of the nasal bone up to the frontal region, ending in the temporal regions where the pains are of a throbbing nature. The odor is at first pleasant, followed by intense irritation, the effects of which may be likened to the odor of formaldehyde, which is of a delayed nature.

According to Parry (16) the oil has a specific gravity of 0.935 to 0.950 and optical rotation of -22°. Sawyer (22) states that the dry oil has a solubility of 1:1000 in water and a specific gravity of 0.936.

The oil used in the present research was distilled from the Coos County myrtle trees.

OBTAINING THE UMBELLULONE FROM OIL OF MYRTLE

Following the method of Wienhaus and Todenhofer(30) no appreciable ketone yield was obtained.

Method A:

A modified Wienhaus and Todenhofer method was employed. 350 grams of myrtle oil were shaken out twice with a saturated solution composed of 200 grams of sodium sulphite and 80 grams of sodium bicarbonate in 400 cubic centimeters of distilled water. The first washing of the oil was carried out when the solution, neutral to five drops of phenolphthalein, was shaken vigorously for thirty minutes. On standing the oil which separated from the water solution was decanted and added to another 400 cubic centimeters of saturated sodium sulphite and sodium bicarbonate solution and permitted to remain twenty-four hours. After the oil had been decanted from the water solution the aqueous portion of washing number one was combined with the aqueous portion of washing number two and steam distilled in a neutral state for two hours, in order to free the water solution of any excess oil. To the aqueous solution, freed of the oil, was added 40 grams of stick sodium hydroxide and the mixture was steam distilled for three hours. The distillate obtained was shaken out with an equal volume of ether, which was then dried over night, using ten or twelve grams of anhydrous calcium chloride. The next morning the ether was evaporated, leaving 53 grams of a yellow, mobile liquid which had an odor resembling that of the oil. This material was fractionated at four mm pressure. The first two cubic centimeters came over at 75° centigrade (Uncorrected) at four mm pressure. There was obtained

between 77.5 - 77.8° centigrade (Uncorrected), 48 grams of a colorless liquid which had the odor of the oil and possessed the same similar pungency and irritating effect.

Physical constants of umbellulone:

$a_{D_{21}}$ -38.50; n_D 1.48285; $d_{25°}$ 0.9465, agreeing fairly well with the reports of Wienhaus and Todenhofer (30).

Table 1 gives the constants reported by various investigators.

TABLE 1

	Sp.G.	Op.Act.	R.I	B.P. 4mm
Wienhaus and Todenhofer(30)	20°.949	-38.51	20°1.48315	850
Stillman(26)		-36.30		
Powers and Lee(19)	15.5°C .9614	-36.33	20°1.18325	
Author's	25°C .9465	-38.50	21°1.48285	77.5-77.8

Method B: Fractionation of oil of California Laurel

300 grams of the oil were fractionated under reduced pressure and fractions collected as shown in Table 2.

TABLE 2

Fraction No.	Amount obtained	Temp. in C.	Pressure in M.M
I	46.0 Gms	37-48	4 mm
II	34.75 "	48-65	4 mm
III	11.1 "	65-74	4 mm
IV	130.1 "	74-78	4 mm
V	52.1 "	78-80	4 mm
VI	11.8 "	80-82	4 mm
VII	7.5 "	82-86	4 mm
VIII	2.1 "	86-95	4 mm
IX	3.7 "	95-110	4 mm

No. IV was refractionated and fractions collected according to the following table.

Frac. No.	Amt. in Gms	Temp. in C.	Pressure
I	72.8	74-77.1	4
II	53.4	77.1-77.6	4
III	3.5 Gms left in distilling flask		

No. V was refractionated and fractions collected according to the following table.

Frac. No.	Amt. in Gms	Temp. in C.	Pressure
I	12.0	77.2-77.7	4
II	38.0	77.7-80	4
III	1.6 Gms left in distilling flask		

Fraction II obtained from No. IV was mixed with Fraction I obtained from V and the mixture refractionated under reduced pressure. A total of 61.0 grams was collected between 77.1 and 77.7° centigrade at 4 mm pressure. This 61 grams was considered as the ketone, umbellulone.

Only two physical constants were run on this fraction which are given below:

The boiling point, determined by the capillary method of Kamm (12), was found to lie between 216-217° centigrade (Corr.). The refractive index was found to be 1.4830 at 21° centigrade. The yield in neither case was what might be expected, as only 13.71 percent was obtained by the sodium sulphite method and 20.3 percent by fractionation. This is out of all agreement with the reports of other investigators, Wienhaus and Todenhofer (30) getting around 24.1 percent,

Powers and Lee (19) about 60 percent, Stillman (26) 40 percent of umbellulone from the oil of California Laurel, and Russell (20) 28 percent.

Solubility of Umbellulone

Umbellulone is freely soluble in 70 percent alcohol, in olive oil and in Petrolatum Liquidum; also in the special solvent of Miller (15), which is composed of 33 parts alcohol, 33 parts glycerol, 33 parts HOH and 6.6 parts soap and diluted to 50 percent with water.

EXPERIMENTAL

Effects of Umbellulone Upon the Blood

For the action of umbellulone upon defibrinated blood in vitro the method of Dessemontet (4) was used.

Method:

To one cubic centimeter of horse blood was added three cubic centimeters of 0.9 percent sodium chloride solution and 0.06 cubic centimeters of umbellulone. This mixture was warmed gently and then placed in a Sedgwick counter and observed through a Bausch and Lomb No. 22 spectrometer.

Experimental:

Diluted blood was heated gently and then observed with the spectrometer. There were no bands present that were within the range of methemoglobin.

To the diluted horse blood, as specified above, was added 0.06 cubic centimeters of a ten percent solution of potassium ferricyanide. This mixture was warmed gently. The blood became a mahogony brown. This was placed in a Sedgwick counting chamber and observed with the spectrometer. The following bands were noted:

First Trial		Second Trial	
First band	635-631 mu mu	First band	636-632 mu mu
Second band	588-572 " "	Second band	587-572 " "
Third band	554-532 " "	Third band	554-532 " "

To a similar dilution of horse blood was added 0.06 cubic centimeters of umbellulone. This mixture was warmed gently. The blood also became a chocolate brown color. Part of this blood was placed in a Sedgwick counting chamber and observed through a

spectrometer. The following bands were recorded:

First Trial		Second Trial	
First band	635-631 mu mu	First band	634-631 mu mu
Second band	587-571 " "	Second band	589-573 " "
Third band	555-536 " "	Third band	554-532 " "

Effects of Umbellulone on Guinea Pig Blood

The blood was obtained from a normal guinea pig by the heart stab method (24).

The guinea pig blood was diluted as above. To this diluted blood was added 0.06 cubic centimeters of umbellulone. This mixture was heated gently and then observed with a spectrometer. The following results were obtained:

	First Trial	Second Trial	Third Trial
First band	634-630 mu mu	634-631 mu mu	635-630 mu mu
Second band	586-572 " "	588-571 " "	589-572 " "
Third band	553-533 " "	553-534 " "	553-534 " "

The bands obtained on horse blood and guinea pig blood are in fair agreement with those reported by Halliburton (8).

The center of the absorption band considered as characteristic, had a wave-length of 630-634 mu mu, according to Dessemontet (4).

Effects of Umbellulone on the Blood in Vivo

A guinea pig was given two cubic centimeters of a 1-1000 dilution of umbellulone in olive oil intraperitoneally, three times a week for six weeks. At the end of six weeks the blood was obtained by the heart stab method. This blood was kept at 37.5° centigrade until used. To one cubic centimeter of blood was added three cubic

centimeters of 0.9 percent sodium chloride solution. This mixture was heated gently and placed in a Sedgwick counting chamber and observed through a spectrometer.

Results:

It was found that umbellulone produced methemoglobin in vivo. The following bands were recorded:

First band	634-630 mu mu
Second band	587-573 " "
Third band	554-532 " "

Figure 2 shows the absorption band at 630-634 mu mu. The guide lines above are the neon lines.

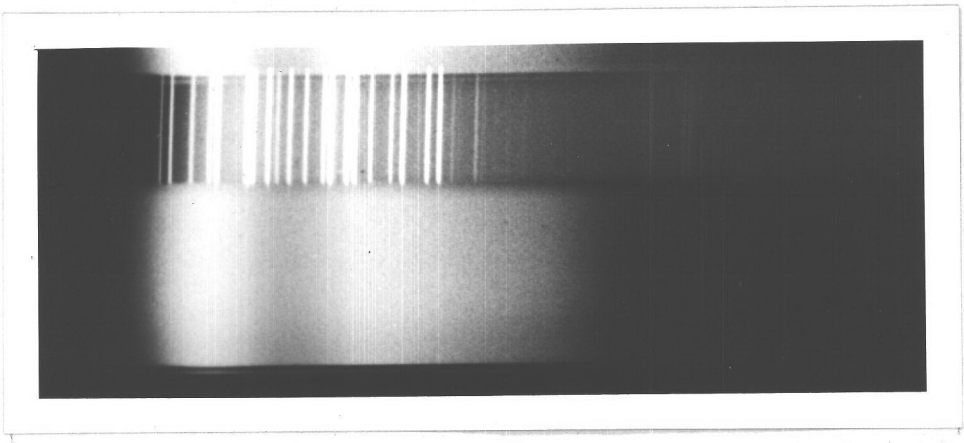


Figure 2

Showing absorption band at 630-634 mu mu as produced by umbellulone in vivo on guinea pig blood

Further work showed that umbellulone produced very decided hemolysis of human, guinea pig and horse blood.

Effects of Umbellulone on Intact Frog Heart

Method:

The frogs were pithed and placed on their backs, and by a median incision (17) of the skin the sternum was exposed. The skin on each side was removed and the sternum carefully cut away without any damage to the heart. The pericardium was slit open and the beating heart exposed. A fine hook was placed through the top of the ventricle and connected to a universal heart lever. The recordings were made on a single drum kymograph.

Results:

A normal tracing was obtained and then 0.2 cubic centimeters of a ten percent umbellulone in a one percent acacia emulsion dropped on the heart.

Protocol of frog No. 2

Weight	13.0 grams
Normal pulse	78 per minute
7:29	0.2 cubic centimeters of ten percent umbellulone added to the intact heart.
7:31	Pulse 92 per minute
7:36	Pulse 75 per minute
7:38	Pulse 30 per minute Heart slow, with complete relaxation
7:40	Pulse 10 per minute
7:43	Heart washed with 0.65 percent sodium chloride solution producing only a very slight movement of the heart.
7:45	The addition of 0.1 cubic centimeter of 1-1000 caffeine had no effect on the paralyzed heart.

7:48 The injection of 0.2 cubic centimeters of 1-1000 adrenaline hydrochloride into the right atrium produced no effect upon the paralyzed heart.

In all cases the addition of 0.2 cubic centimeters of a 10 per cent umbellulone in a one per cent acacia emulsion produced at first a quickening of the pulse, with no apparent change in tonus, (Fig. 3). This was followed by a slowing effect which was progressive in nature. As the experiment progressed there was a decided loss in tonus of the heart, and a decreased frequency with a decreased contraction of the ventricle (Fig. 4, 5 and 6). This was followed by a pronounced slowing of the heart with a beat occuring about every three seconds, during which time the heart was stopped in diastole. These beats were regular for a period of some 15 seconds, when the heart stopped in diastole (Fig. 7). Washing the heart with 0.65 per cent sodium chloride solution produced no effect, and the addition of 0.1 cubic centimeter of 1-1000 caffeine likewise produced no reviving effect. The injection of 0.2 cubic centimeters of 1-1000 adrenaline hydrochloride into the right atrium produced no reviving effect upon the paralyzed heart.

Effects of Umbellulone on Atropinized Frog Heart

Method:

The frogs were pithed and the hearts exposed. The exposed hearts were connected to a universal heart lever and recordings made on a single drum kymograph.

A normal tracing of the heart was taken and 0.1 cubic

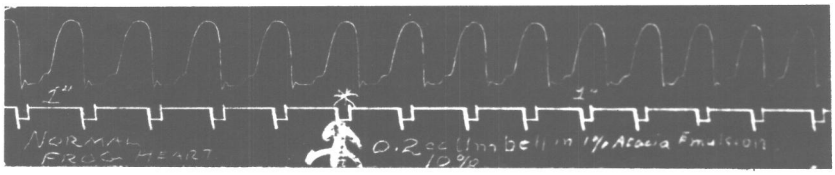


Figure 3 - Normal tracing of frog heart. * The addition of 0.2 cubic centimeters of 10 per cent umbellulone to the intact heart. Time in seconds.

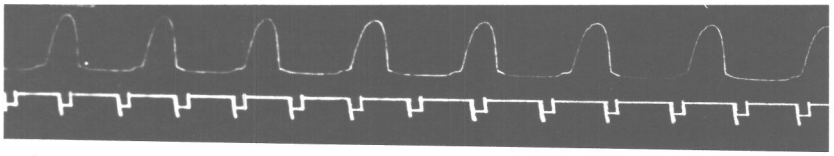


Figure 4 - Showing loss in tonus of the heart, decrease in frequency and decrease in contraction of the ventricle of frog heart. Time in seconds.

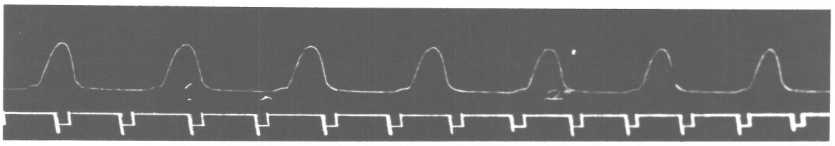


Figure 5 - Showing further loss in tonus, decrease in frequency and decrease in contraction of the ventricle of frog heart. Time in seconds.

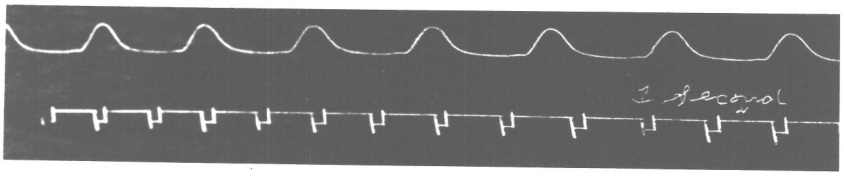


Figure 6 - Showing rather pronounced loss in tonus, decrease in frequency and decrease in contraction of the ventricle of frog heart. Time in seconds.

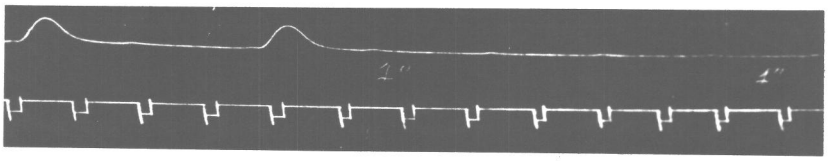


Figure 7 - Pronounced slowing of the heart, with stoppage in diastole. Time in seconds.

centimeters of 1-1000 atropine dropped upon the exposed heart (Fig. 8).

Results:

Protocol of frog No. 2

Weight	15.0 grams
Normal pulse	40 per minute
3:15	0.1 cubic centimeter of 1-1000 atropine dropped upon exposed heart
3:22	Pulse 28 per minute
3:23	0.2 cubic centimeters of 10 per cent umbellulone in a one per cent acacia emulsion dropped upon the exposed heart.
3:23:20	Pulse 15 per minute
3:26	Pulse 14 per minute Two contractions, then a pause
3:34	Pulse 30 per minute, followed in about two minutes with failure of heart.

To the atropinized frog's heart (Fig. 9) was added 0.2 cubic centimeters of a 10 per cent umbellulone solution. Twenty seconds after dropping the umbellulone solution upon the exposed heart there was a decided decrease in the frequency of the heart, a decrease in the ventricular contraction and evidence of an interference between the auricular and ventricular contractions (Fig. 10). This was followed by a decided slowing of the heart, there being two successive contractions, then a pause in diastole (Fig. 11). This was followed by a more rythmatic contraction, but a decrease in both auricular and ventricular contraction which finally resulted in stoppage of the heart in diastole (Fig. 12).

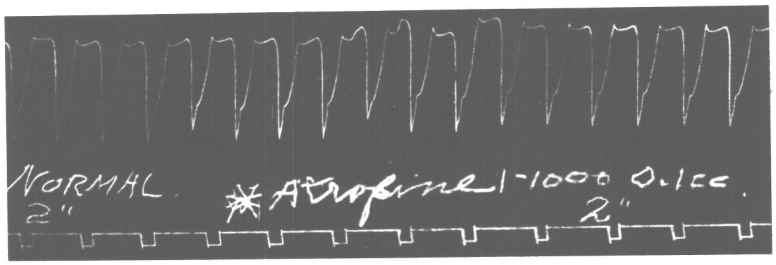


Figure 8 - Showing normal frog heart. * Indicates the addition of 0.1 cubic centimeter of a 1-1000 solution of atropine upon the exposed frog heart. Time in two seconds.

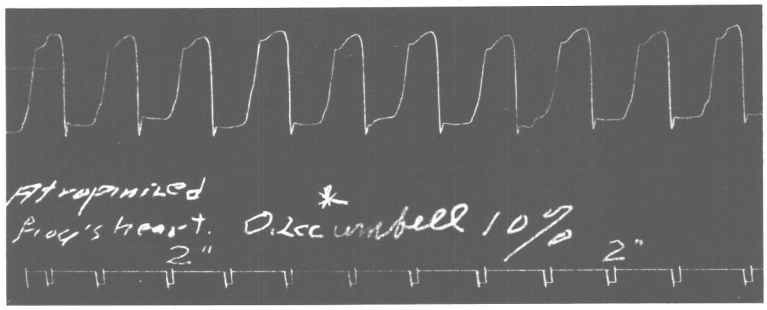


Figure 9 - Showing atropinized frog heart and the * indicates the dropping of 0.2 cubic centimeters of 10 per cent umbellulone upon the exposed frog heart. Time in two seconds.

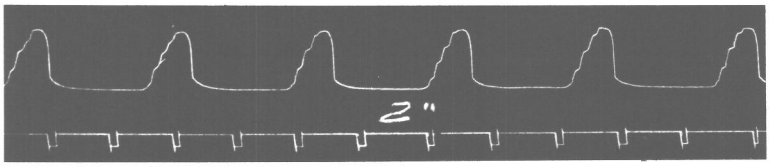


Figure 10 - Showing decrease in frequency of frog heart, with decrease in ventricular contraction and interference between auricular and ventricular contraction. Time in two seconds.

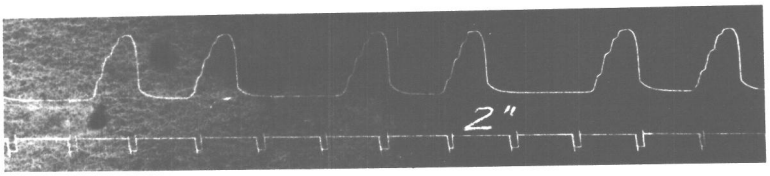


Figure 11 - Showing slow heart action with pause in diastole between each two beats. Time in two seconds.

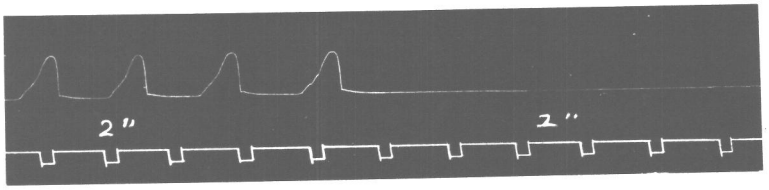


Figure 12 - Showing increased heart action, with decrease in both auricular and ventricular contraction, and finally stoppage of the frog heart in diastole. Time in two seconds.

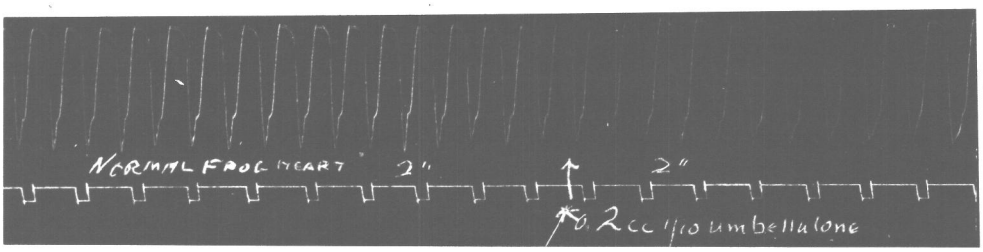


Figure 13 - Showing normal frog heart and the * indicates the addition of 0.2 cubic centimeter of 10 per cent umbellulone to the exposed frog heart. Also showing initial stimulation. Time in two seconds.

Effects of Physostigmine Upon the Umbellulonized Frog Heart

Method:

The frogs were pithed and the hearts exposed. The exposed hearts were connected to a universal heart lever and recordings made on a single drum kymograph.

A normal tracing of the frog heart was obtained and then 0.2 cubic centimeters of a 10 per cent umbellulone solution in a one per cent acacia emulsion dropped upon the exposed heart. (Fig. 13).

Results:

Protocol of frog No. 5.

Weight	38 grams
Normal pulse	54 per minute
9:24:30	0.2 cubic centimeters of ten per cent umbellulone solution dropped upon the exposed frog heart.
9:25	Pulse 60 per minute
9:25:30	Pulse 42 per minute
9:38:30	Pulse 24 per minute
9:29:30	Heart stopped, with two feeble attempts to contract.
9:32:30	Two drops of 1-1000 physostigmine dropped upon the exposed heart.
9:33	Heart contracted once, followed in nine seconds by an increase in contraction and tonus of the heart.

Thirty seconds after adding the 10 per cent umbellulone solution, the heart showed an increased rate or frequency, which was followed by an immediate decrease in frequency (Fig. 14). This decrease in frequency was progressive over a period of five minutes, when the

heart apparently stopped, followed by two feeble attempts to contract. (Fig.15).

Three minutes after the stoppage of the heart, two drops of 1-1000 physostigmine were dropped upon the paralyzed frog heart and twenty-eight seconds later the heart contracted once, followed in nine seconds by an increase in frequency and tonus of the frog heart. (Fig. 16).

Discussion:

The injection of 0.2 cubic centimeters of 1-1000 adrenaline hydrochloride into the right atrium produced no revival of the frog heart paralyzed by umbellulone. According to Dixon (5) adrenaline will not accelerate or increase the force of the heart if the sympathetic nerve-endings are paralyzed.

The addition of atrophine to the frog heart paralyzed by umbellulone produced no revival, indicating that umbellulone acted upon the vagal endings and cardiac muscles as does atropine.

The addition of umbellulone to an atropinized frog heart produced results similar to those obtained on frog hearts not treated with atropine, again indicating that umbellulone acts upon the same mechanisms as does atropine.

The addition of physostigmine to the umbellulonized frog heart produced a revival in contraction and an increase in tonus of the frog heart. The fact that physostigmine revived the frog heart paralyzed by embellulone indicated that umbellulone must act upon the vagal endings and the cardiac muscles as does atropine.

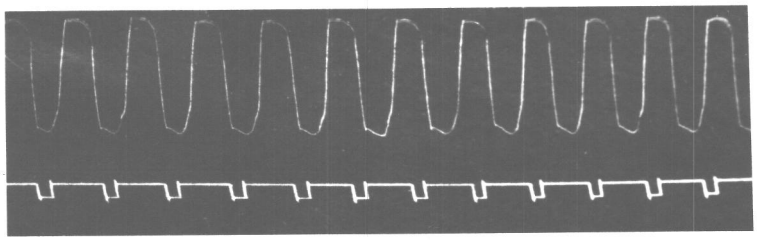


Figure 14 - Showing the decrease in frequency of the frog heart after the addition of umbellulone solution. Time in two seconds.

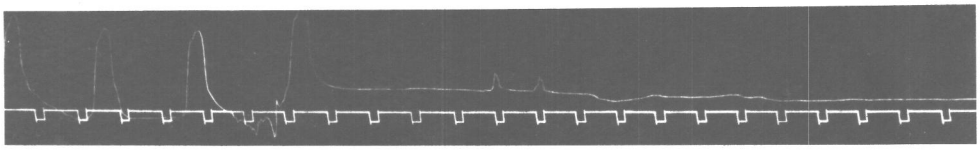


Figure 15 - Showing decreased frequency of the frog heart, followed by apparent arrest and then two feeble contractions, followed by complete paralysis of the frog heart. Time in two seconds.

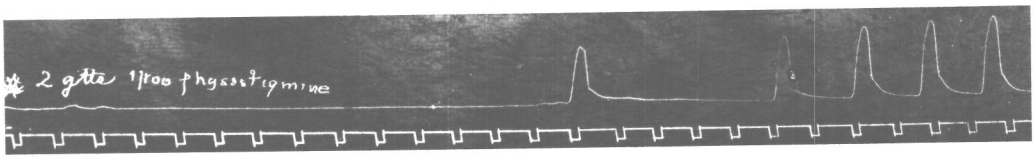


Figure 16 - Showing paralyzed heart and the * indicates the addition of two drops of 1-100 physostigmine to the paralyzed frog heart and the increased frequency and tonus following the physostigmine addition. Time in two seconds.

Effects of Umbellulone on Unanesthetized Animals

Two guinea pigs were used, each pig receiving an intraperitoneal injection of four cubic centimeters of a 10 per cent solution of umbellulone in olive oil.

Results:

Protocol of guinea pig No. 1.

3:15	Pulse 75, respiration 80.
3:16	Four cubic centimeters of 10 per cent umbellulone in olive oil injected intraperitoneally, the injection requiring two minutes for completion.
3:18	Hind legs of pig showed signs of paralysis.
3:19	Pig clawing at head, indicating signs of asphyxia.
3:20	Pulse 48 per minute.
3:21	Respiration 75, irregular, bordering on cheyne-stokes type.
3:24	Signs of asphyxia convulsions.
3:26	Cornea reflex absent.
3:27	Onset of another asphyxia convulsion, lasting about one minute.
3:30	Pulse 24 per minute.
3:31:30	Onset of asphyxia convulsions, lasting for thirty seconds.
3:32	Respiration very shallow and irregular, with a spasmodic gasp occurring about every three seconds.
3:35	Asphyxia convulsions, lasting for thirty seconds.
3:36	Onset of another asphyxia convulsion, lasting for nearly two minutes.
3:40	Pulse 128 per minute.
3:43	Respiration ceased.
3:44	Pulse stopped.
3:46	Post mortem: The odor of umbellulone was present in both the peritoneal and pericardial cavities. The heart stopped in diastole and was dilated, as were the aorta and the inferior and superior vena cava. Lungs showed decided congestion. The blood was extremely dark, indicating methemoglobin formation.

Protocol of guinea pig No. 2.

3:55	Pulse 70, respiration 85.
3:57	Injection of four cubic centimeters of 10 per cent umbellulone in olive oil intraperitoneally.
3:59	Apparent paralysis of hind legs.

4:01 Pig clawing at head, indicating asphyxia.
4:04 Pulse 50 per minute.
4:05 Respiration 79, very irregular.
4:06 Convulsions of asphyxia type.
4:09 Onset of convulsions, lasting about thirty seconds.
4:12 Pulse 27 per minute
4:15 Onset of convulsions, lasting about thirty seconds.
4:20 Pulse 125 per minute.
4:21 Respiration shallow and rapid, 90 per minute.
4:24 Arrest of respiration.
4:25 Stoppage of the heart.
4:27 Post mortem:
Odor of umbellulone pronounced in both peritoneal and pericardial cavities.
Heart stopped in diastole, aorta and superior and inferior vena cava dilated as was the heart.
Lungs showed decided congestion.
Blood very dark.

Discussion:

Two minutes after injection the guinea pigs showed a slight paralysis of the hind quarters. This was followed in both cases by a decrease in pulse rate with a slight change in respiration, which rapidly became very irregular. Both pigs showed asphyxia convulsions, lasting over a period of thirty seconds to one minute. These convulsions became more pronounced and frequent as the experiment progressed. Following the decrease in heart rate there was a marked increase in the pulse, and in the case of pig No. 1, the respiration failed in about one minute, while guinea pig No. 2 showed a rapid and shallow respiration, with failure of respiration in about three minutes. In both guinea pigs the heart stopped about a minute after respiratory failure.

Effects of Inhaled Umbellulone on Guinea Pigs

Method:

The umbellulone was heated over a water bath in an evaporating dish and then placed in a large dessicator to vaporize. The guinea pig was then placed in the dessicator and maintained under these conditions, with oxygenation of the dessicator about every fifteen or twenty minutes for four hours.

Results:

It was found that the only apparent results were irritation to the mucous membranes of the eyes and nose. The respiration was at times irregular but at no time showed signs of failure.

BACTERICIDAL ASPECTS

Fungicidal Action of the Oil of Myrtle and Umbellulone

Method:

The organisms were transferred each day from broth of pH 5.0 for a period of five days, as specified by the F.D.A. method (7), using a standard loop for transferring.

The organisms used were Monilia tropicalis (1885) and Trychophyton interdigetale (2284).

The solvent used to dissolve the umbellulone was composed of 33 parts respectively of 95 per cent alcohol, glycerol, distilled water and 6.6 parts of castile soap. (15). This mixture was sterilized by placing in a three liter flask, sealing tightly and sterilizing at ten pounds pressure for 45 minutes. The solvent was tested for fungicidal action when diluted fifty per cent and found to have none. This is in agreement with the reports of Miller (15).

Dilutions of 1-10, 1-50, 1-100, 1-1000 of umbellulone were made and used in the experiments.

The method used for determining the fungicidal powers of umbellulone was a slight modification of Kingery and Adkison (14). Two cubic centimeters of umbellulone solution were added to a one cubic centimeter of twenty-four hour broth culture, which had been transferred for five consecutive days at twenty-four hour intervals. The umbellulone was allowed to remain in contact with the broth culture for intervals of one, thirty and sixty minutes, then transferred to Sabouraud's Agar. Each dilution was streaked on a single plate. The plate was divided into quarters and marked with one,

thirty, sixty and check. The one minute interval was transferred to that portion of the plate marked one, thirty to the portion marked thirty and sixty to that portion marked sixty. The check was obtained by adding two cubic centimeters of sterile distilled water to one cubic centimeter of twenty-four hour broth culture, and at the end of the sixty minute period a portion was transferred to that part of the plate marked check. The plates were incubated for forty-eight hours at 37° centigrade and then read.

Results:

The following Table 3 is a tabulation of typical results obtained. A total of ten experiments were run.

Table 3

		Oil of Myrtle				Umbellulone			
No.	Dilution	Time in minutes				Time in minutes			
		1	30	60	Check	1	30	60	Check
1885	1/10	-	-	-	//////	-	-	-	//////
	1/50	//	-	-	//////	-	-	-	//////
	1/100	//////	//	/	///	/	-	-	//////
	1/1000	//////	///	//	//////	//////	/	/	//////
2284	1/10	-	-	-	//////	-	-	-	//////
	1/50	/	-	-	//////	-	-	-	//////
	1/100	///	//	/	//////	/	-	-	//////
	1/1000	//////	///	//	//////	//////	//	//	//////

Key to the tabulation above:

-	No growth recorded
/	1-4 colonies on the quarter observed
//	5-10 colonies on the quarter observed.
///	11-24 colonies on the quarter observed.
////	25 or more colonies on the quarter observed.

Discussion:

In the absence of protein the oil and umbellulone killed the specific organisms used in dilutions of 1/10 for intervals of one, thirty and sixty minute contacts, but in a dilution of 1/50, growth was recorded on the one minute contact for the oil of myrtle, but not for umbellulone. In the 1/100 dilution there was recorded growth in all intervals of time used for oil of myrtle, while the ketone, umbellulone, showed no growth on thirty and sixty minute contact. In 1/1000 dilution all contacts were positive for both oil and umbellulone.

The fact that the organisms were killed and not inhibited was proven by taking a loop from each dilution for the various intervals of time and transferring to a sterile tube of broth. No growth was recorded in forty-eight hours time when incubated at 37° centigrade.

Fungicidal Action of Umbellulone in the Presence of Protein

The same method as used above was used with the same technique but using mediums which contained protein or protein like substances.

Medium I:
10 Gms of flake agar.
10 per cent horse blood, added after medium had cooled to 50° centigrade.
16.5 Gms of Dextrose (Merk C.P.)
500 cubic centimeters of 0.85 per cent salt solution.
pH adjusted to 5.5 and sterilized at 15 pounds pressure for eighteen minutes.

Medium II:
10 Gms of flake agar.
5 Gms of peptone (Bacto)
16.5 Gms Dextrose (Merk C.P.)
500 cubic centimeters of tap water.
pH adjusted to 5.5 and sterilized at fifteen pounds pressure for eighteen minutes.

Medium III: 10 Gms gelatine (Bacto)
16.5 Gms Dextrose (Mark C.P.)
500 cubic centimeters of distilled water.
pH adjusted to 5.5, sterilized at fifteen pounds pressure for eighteen minutes.

Fifteen experiments were run using the above media. Table 4 is typical of the results obtained. The first five experiments showed a slight enhancing in the fungicidal action of the ketone, umbellulone; in none of the experiments was there a great decrease in the fungicidal action of umbellulone, which is contrary to what might be expected.

Table 4

Org.	Dil.	Medium I				Medium II				Medium III			
		Time in minutes				Time in minutes				Time in minutes			
		1	30	60	Check	1	30	60	Check	1	30	60	Check
1885	1/10	-	-	-	////	-	-	-	////	-	-	-	////
	1/100	//	/	-	////	/	-	-	////	/	-	-	////
	1/1000	////	///	/	////	////	///	//	////	////	///	//	////
2284	1/10	-	-	-	////	-	-	-	////	-	-	-	////
	1/100	//	/	-	////	/	-	-	////	/	-	-	////
	1/1000	////	////	//	////	////	////	//	////	////	////	//	////

Umbellulone in the presence of ten per cent blood agar, Medium I, appeared to be slightly decreased in action, as in the 1/100 dilution for contacts of one and thirty minute intervals there is about double the growth as compared to Sabouraud's Dextrose Agar. On the other two media there is a slight decrease in action, but not as great as would be expected, considering that there was present one per cent peptone in Medium II, and two per cent gelatine in Medium III.

Medium III was incubated at 21° centigrade for three days and then recorded. All others were incubated at 37° centigrade for forty-eight hours.

The dilutions as specified in all the experiments are as before adding to the twenty-four hour broth culture. In each case the original dilution is diluted one-half, thus making 1/10 dilution actually 1/15, etc.

Germicidal Action of Umbellulone

Due to the high insolubility of the ketone, (umbellulone), in water, the Wet Filter-Paper Method of the F.D.A. (7) was used instead of using a standard phenol coefficient.

No. 2 Whatman filter papers are cut into squares and sterilized in a sterile petri dish at fifteen pounds pressure for twenty minutes. The sterile squares are then impregnated with the desired organism, which has been transferred every twenty-four hours for five days in the medium specified by the F.D.A. (7). The squares impregnated with the microorganism are then placed in the antiseptic and left in contact with it for the specified interval of time. They

are then washed off in a sterile tube of broth and transferred to a second subculture or tube of broth, both tubes being then incubated for twenty-four hours at 37° centigrade. It is essential that the second subculture be made, otherwise there is liable to be enough disinfectant adhering to the squares to prevent growth in the first tube.

The organisms used in the test were Eberthella typhi (Sears) and a standard stock culture of Staphlococcus albus, both having been transferred from broth every twenty-four hours for five consecutive days before the organism was used. Both organisms were not inhibited or killed by a five minute exposure to 1-70 solution of phenol, which had a potency in agreement with the U.S.P. IX standard. Both organisms were killed by a fifteen minute exposure to a 1-90 dilution of phenol.

Table 5 shows the typical results obtained.

Table 5

Organism	Time	Dilution					
		1/10	1/20	1/100	1/500	1/1000	1/2000
E. Typhi	1	-	-	-	≠	≠	≠
	2	-	-	-	≠	≠	≠
	5	-	-	-	-	≠	≠
	7½	-	-	-	-	≠	≠
	15	-	-	-	-	-	≠
	30	-	-	-	-	-	≠
	60	-	-	-	-	-	≠
Staph.Albus	1	-	-	-	≠	≠	≠
	2	-	-	-	≠	≠	≠
	5	-	-	-	≠	≠	≠
	7½	-	-	-	≠	≠	≠
	15	-	-	-	-	≠	≠
	30	-	-	-	-	-	≠
	60	-	-	-	-	-	≠

Staphlococcus albus showed more resistance to umbellulone than did E. typhi (Sears). This was rather surprising, as both were inhibited by 1-80 dilution of a standard phenol solution for five minutes contact.

It might be worthy of note here to state that the ketone, umbellulone, was dissolved in olive oil and used as the antiseptic agent. The results were decidedly discouraging, as both E. typhi (Sears) and Staph. albus were not inhibited or killed by a 1/10 dilution in five minutes contact. The ketone was then dissolved in petrolatum liquidum and the above results obtained. This is in agreement with the results reported by Koch and McMaster (28).

The above experiment was performed using the solvent of Miller (15) for dissolving the ketone. The results obtained were almost identical.

Estimated phenol coefficient:

The estimated phenol coefficient was obtained by dividing the greatest dilution of umbellulone capable of killing in less than fifteen minutes but in more than five minutes.

E. typhi $\frac{500}{80} = 6.25$

Staph.albus $\frac{500}{80} = 6.25$

The Agar-Plate Method for Determination of Germicidal Action of Umbellulone

The agar-plate method is a test for inhibitory properties and is used for substances remaining in contact with the body in the absence of serous body fluids. Such materials as salves, dusting powders, creams, plasters, pads, etc. may be tested by this method.

The umbellulone was made into an ointment, using "Lanolin" as a base. The strengths used were ten per cent, five per cent, one per cent and 0.1 per cent.

The method as outlined by the F.D.A. (7) is as follows: Fifteen to twenty cubic centimeters of agar is melted and cooled to 42-45° centigrade. To this is added 0.1 cubic centimeter of a twenty-four hour broth culture of the test organism. The inoculated agar is then poured into a sterile petri plate and allowed to harden. As soon as the agar has hardened, the test substance is placed in intimate contact with the surface of the agar. If a salve, it is first warmed just sufficiently to soften it and thus secure a complete peripheral contact. As a control, sterile petrolatum may be placed on another portion of the plate or on another plate. The plates are incubated twenty-four to forty-eight hours at 37° centigrade and then examined for evidence of inhibition. If the preparation is antiseptic or inhibitory a zone of clear agar will be noted around the place where the substance has been in contact, and the width of the zone will indicate the diffusibility of the inhibitory (antiseptic) agent. If there is no inhibition, growth of the test organism will be observed

adjacent to and even under the test substance.

The organisms used were Staph. albus and E. typhi (Sears). Umbellulone inhibited and showed good diffusibility on both E. typhi (Sears) and Staph. albus. The ten per cent ointment inhibited and showed good diffusibility on both Staph. albus and E. typhi. The same was recorded for the five per cent, one per cent and 0.1 per cent ointments. Figure 17 shows the inhibitory and diffusibility action of umbellulone for a 0.1 per cent ointment with E. typhi (Sears). Figure 18 is the check with the "Lanolin". The dark ring around the antiseptic material shows clearly that umbellulone diffuses nicely and also inhibits the growth of E. typhi (Sears) in a 0.1 per cent ointment.

The fact that E. typhi (Sears) and Staph. albus were killed was determined by taking a portion of the material from the clear zone and transferring to sterile tubes of broth and incubating for twenty-four hours at 37° centigrade.

Figure 17

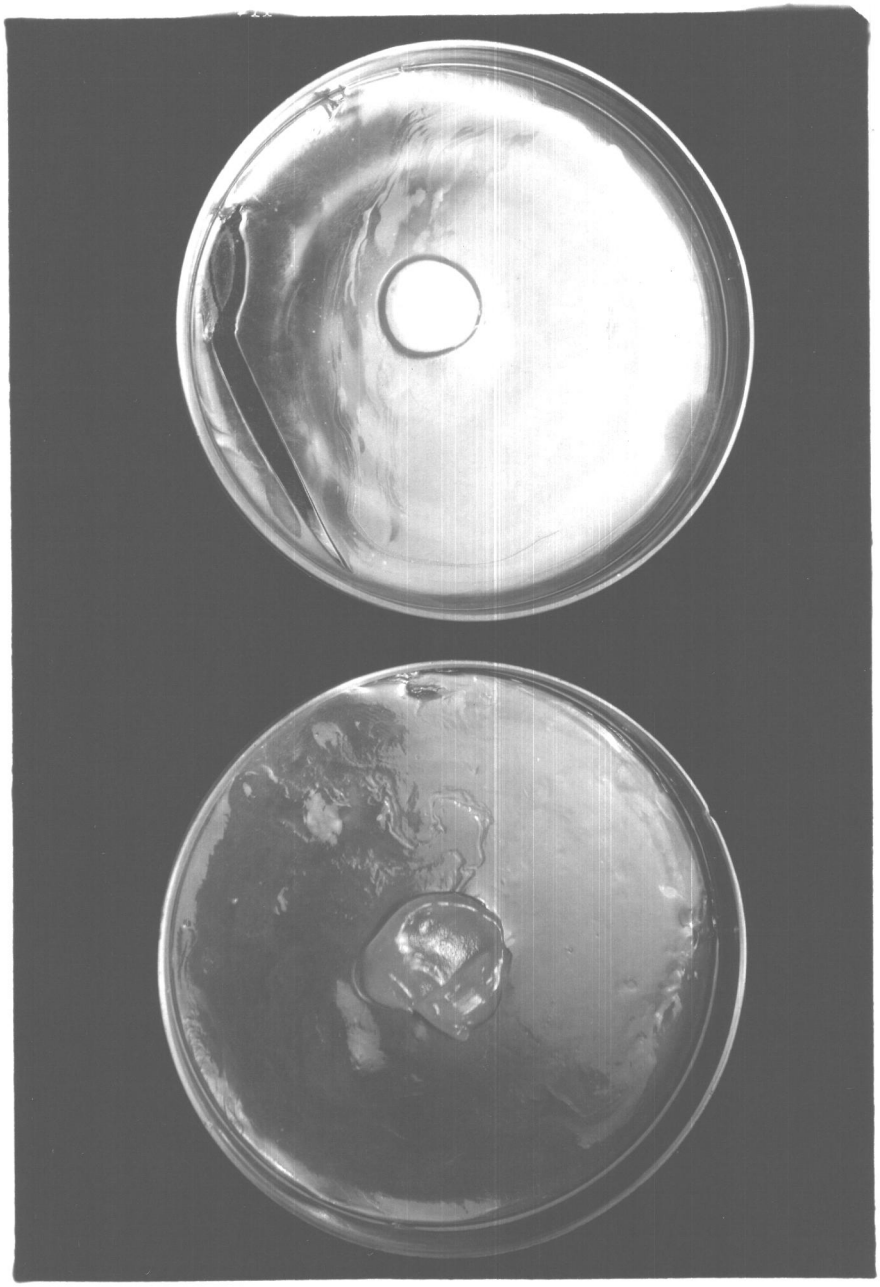


Figure 18

EFFECTS OF UMBELLULONE ON ISOLATED INTESTINAL SEGMENT

The following experiments were carried out by using a modification of the procedure of Salant and Mitchell (21).

Method:

The rabbits and cats were killed by admininstering an excessive dose of chloroform. The small intestines were immediately removed, care being taken to avoid injury to the intestines. The removed intestines were placed in fresh, oxygenated Locke's solution and kept at 37.5° centigrade until used.

The segments of the intestines, 2.5 to 4 centimeters in length were suspended in four hundred cubic centimeters of Locke's solution through which a stream of oxygen was continuously bubbled. The temperature was maintained at 37.5° centigrade by using an electrically controlled water bath. Recordings were made on a single drum kymograph, using a Becker universal lever. The umbellulone was prepared in the form of a one per cent acacia emulsion. Dilutions of 1-10, 1-100 and 1-1000 were prepared for use in the experiments.

Results obtained on rabbit gut:

It was found that umbellulone in a dilution of 1-100,000 produced a slight stimulation. There was a slight increase in contraction, with tonus and amplitude remaining nearly constant.

Umbellulone in a 1-40,000 dilution produced a very decided decrease in contraction, but no apparent change in tonus or amplitude. This partial paralysis of the intestinal segment lasted for a period of five minutes when the tissue began to revive, and at the end of seven minutes the tissue was functioning normally. (Fig. 19,(A),(B),(C).

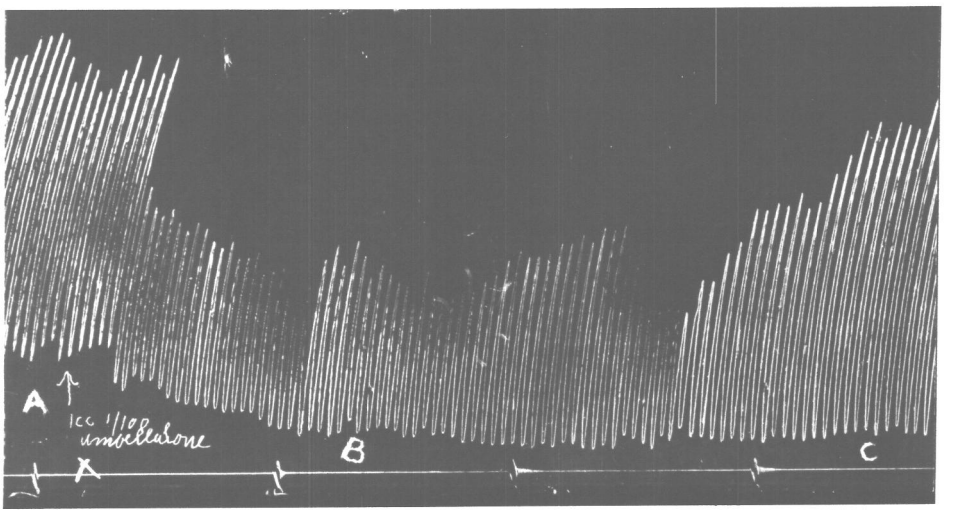


Figure 19 - Rabbit gut - Showing effect of one cubic centimeter of 1-100 dilution of umbellulone, giving a dilution of 1-40,000. A- normal tracing, B- partial paralysis, C- segment normal. Base line indicates two minute intervals.

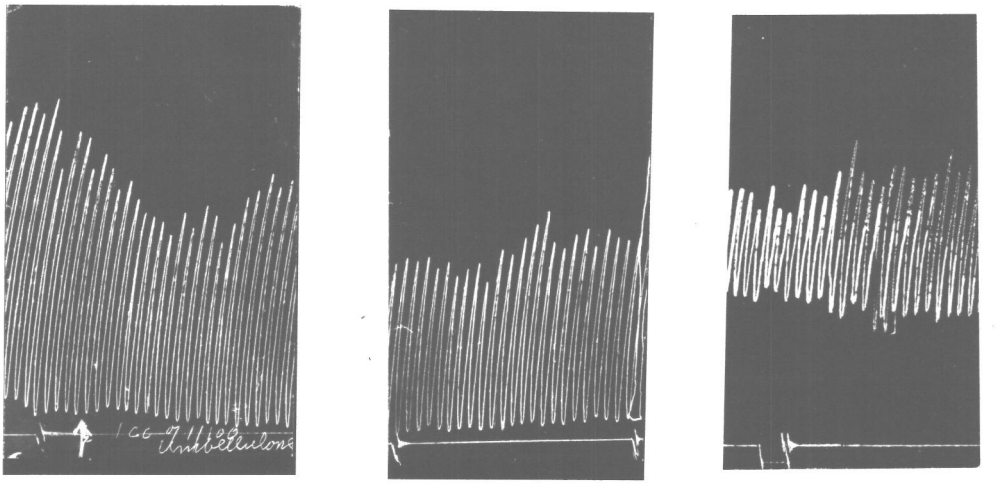


Figure 20 - Showing progressive decrease in contraction of rabbit intestinal segment when subjected to 1-20,000 solution of umbellulone. Base line indicates two minute intervals.

Umbellulone in a 1-20,000 dilution produced a decrease in contraction which was progressive over a period of eight minutes. There was perhaps a slight decrease in tonus with no apparent change in amplitude (Fig. 20).

Umbellulone in a dilution of 1-13,333 produced a marked decrease in contraction in conjunction with a loss of tonus and amplitude. This was followed by a spasmodic stimulation which lasted for two minutes, then there was apparently a complete loss of contraction, tonus and amplitude, which was followed by a very slight contraction, there being twenty-two successive contractions and relaxations over a period of two minutes. At this point the muscle lost its contractibility, tonus and amplitude. The segment was observed for a period of ten minutes with no signs of reviving. (Fig. 21). The segment was then washed and fresh Locke's solution added. The segment showed immediate signs of reviving, with progressive increase in contraction, amplitude and tonus (Fig. 22).

Results obtained on cat gut:

Three cats were used in this phase of the work. The average weight of each cat was 2100 grams (2.1 kilograms).

Method:

Same as used for rabbit intestinal segments.

Results:

Umbellulone in 1-100,000 dilution produced a slight stimulation in contraction with little change in amplitude and tonus.

Umbellulone in 1-40,000 dilution produced an almost immediate loss in tonus, amplitude and contraction; this was followed by one

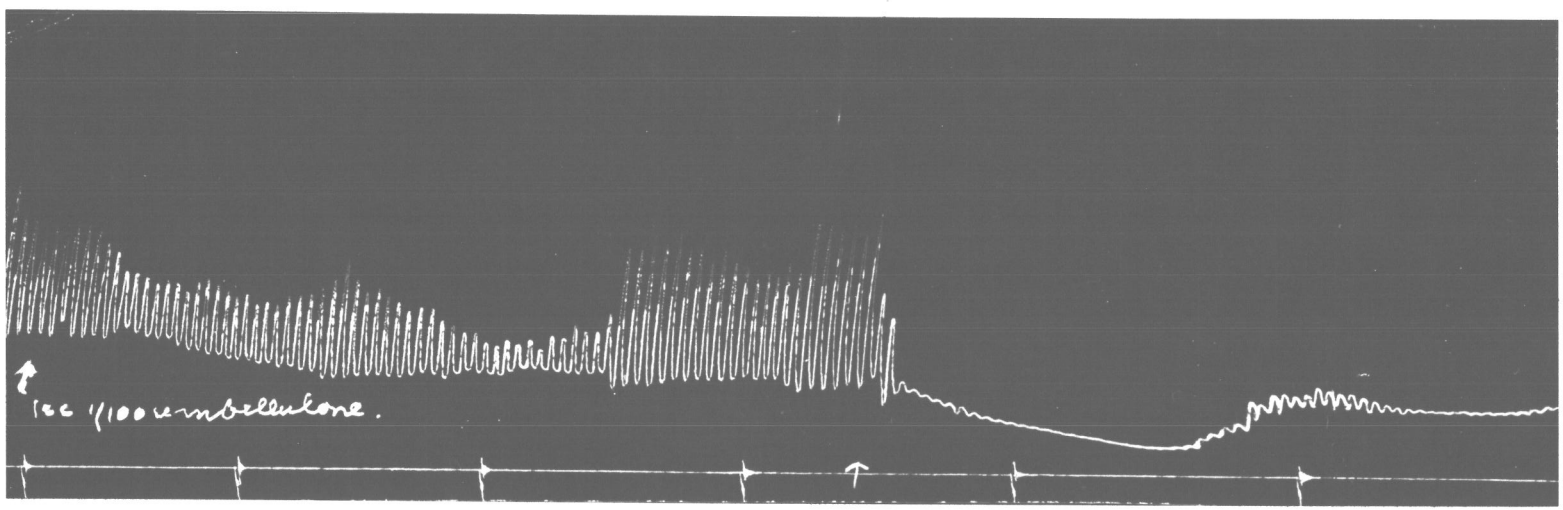


Figure 21 - Rabbit gut - Showing progressive loss of contraction (in dilution of 1-13,333 of umbellulone), with period of slight stimulation and complete paralysis of intestinal segment. Base line indicates two minute intervals.

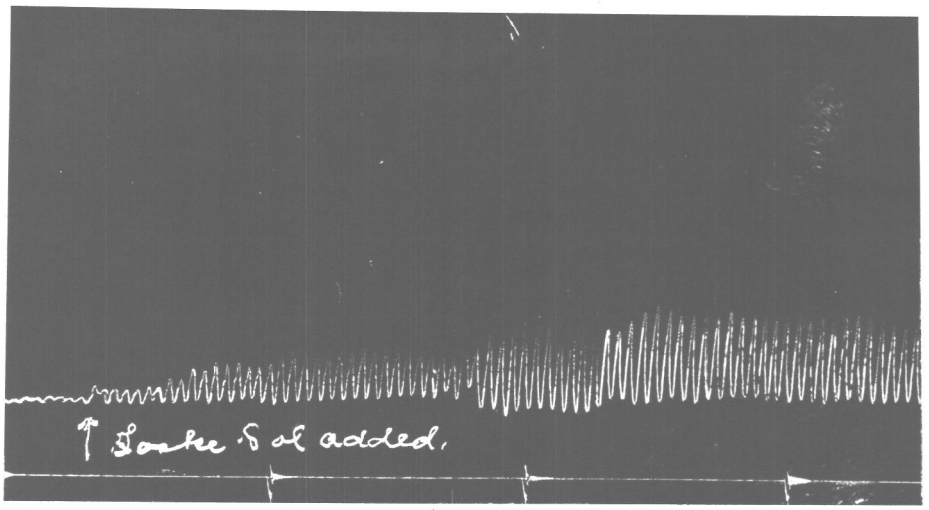


Figure 22 - Rabbit gut - Intestinal segment revived in Locke's solution. Base line is time in two minute intervals.

vigorous contraction and relaxation. The gut then appeared to be dead for a period of about one minute when the segment began contracting in a spasmodic fashion with a slight increase in contraction and tonus, but a slight decrease in amplitude (Fig. 23).

Umbellulone in 1-20,000 dilution produced an apparent loss of tonus, amplitude and contraction for a period of about two minutes. This was followed by a slow, vigorous contraction and relaxation, which gradually became very even and rhythmic with perhaps a slight increase in tonus and amplitude but a gradual or progressive decrease in contraction. This effect was evident over a period of some six minutes (Fig. 24).

Umbellulone in 1-10,000 dilution produced a loss of tonus, amplitude and contraction, which lasted for a period of five minutes when the segment began contracting spasmodically with a decrease in amplitude and tonus but no apparent change in contraction (Fig. 25).

Umbellulone in a 1-5000 dilution produced a decided decrease in tonus and amplitude with a progressive decrease in contraction, which lasted over a period of twenty-two minutes when the tracing became little more than a straight line, showing loss of tonus, amplitude and contraction (Fig. 26,27,28). At this point the segment was washed and fresh Locke's solution added, showing almost immediate revival of the segment.

All the above results were obtained from the same strip of intestinal segment; that is, they are progressive results as recorded on continued addition of umbellulone in the form of a one per cent acacia emulsion.

In order to check the above results, separate pieces of intestinal segments were used for dilutions of 1-10,000 and 1-5000. The results obtained from 1-10,000 dilution were almost identical with those recorded above.

In the dilution of 1-5000 the paralyzing effect took place over a longer period of time, with intervening actions similar to those recorded above (Fig. 29).

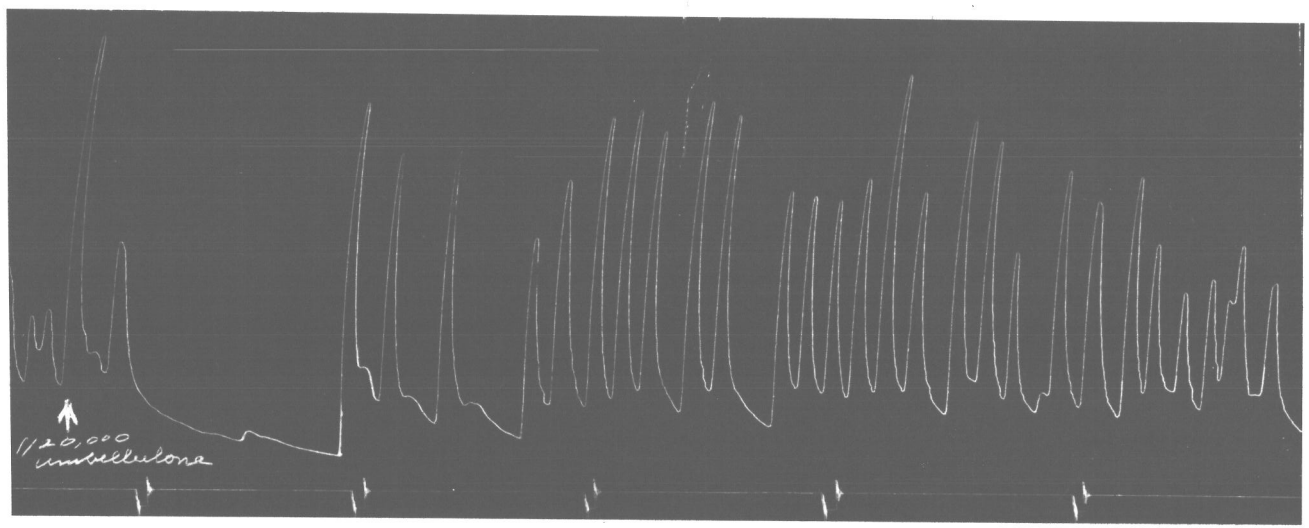


Figure 24 - Showing action of umbellulone in 1-20,000 dilution on cat gut. The above tracing is a continuance of Figure 23, with enough umbellulone added to make a total dilution of 1-20,000. Base line is time in two minute intervals.

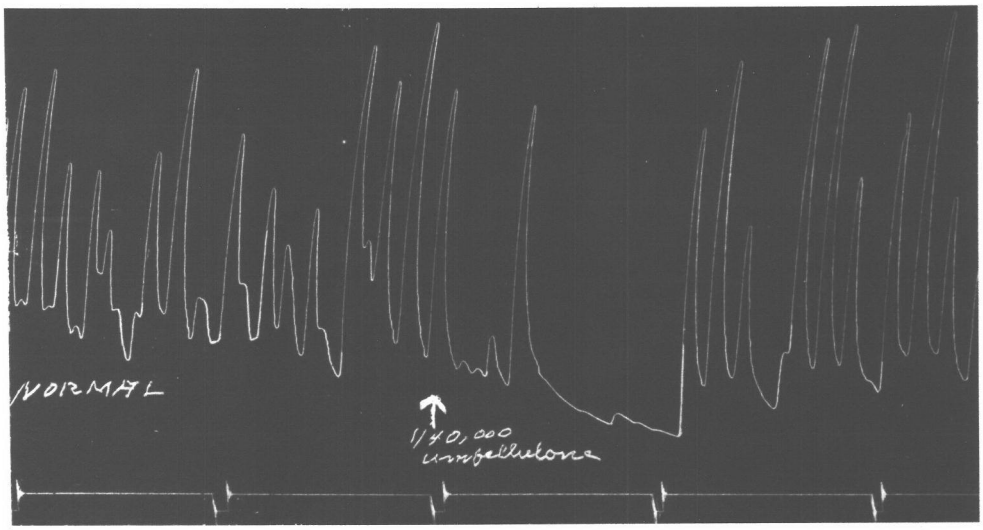


Figure 23 - Showing normal tracing and first effects of umbellulone on cat gut. Base line is time in two minute intervals.

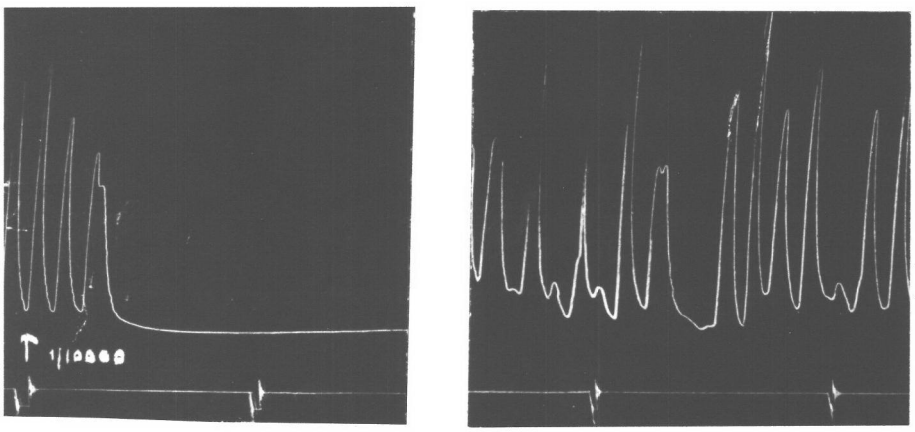


Figure 25 - 1-10,000 dilution of umbellulone on cat gut. Continuation of Figures 1 and 2. Sections to show effects. Base line is time in two minute intervals.

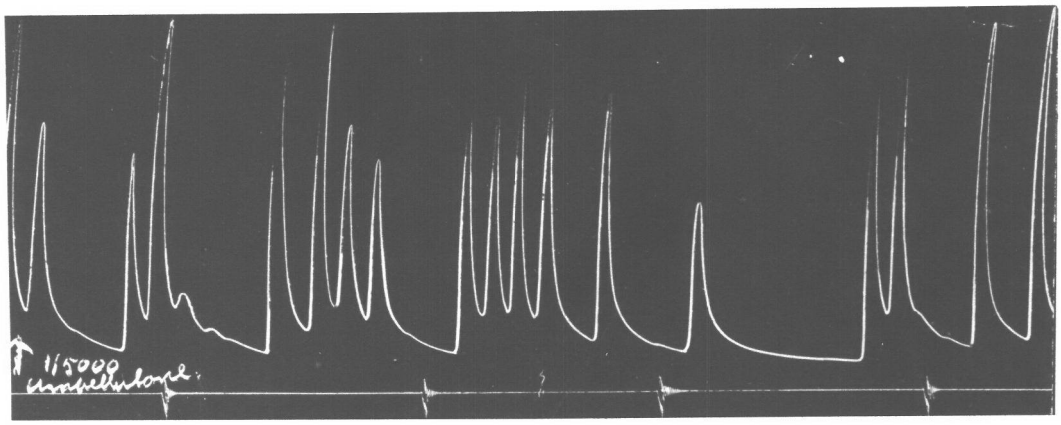


Figure 26 - 1-5000 dilution of umbellulone on cat gut. Continuance of Figures 23, 24 and 25. Base line is time in two minute intervals.

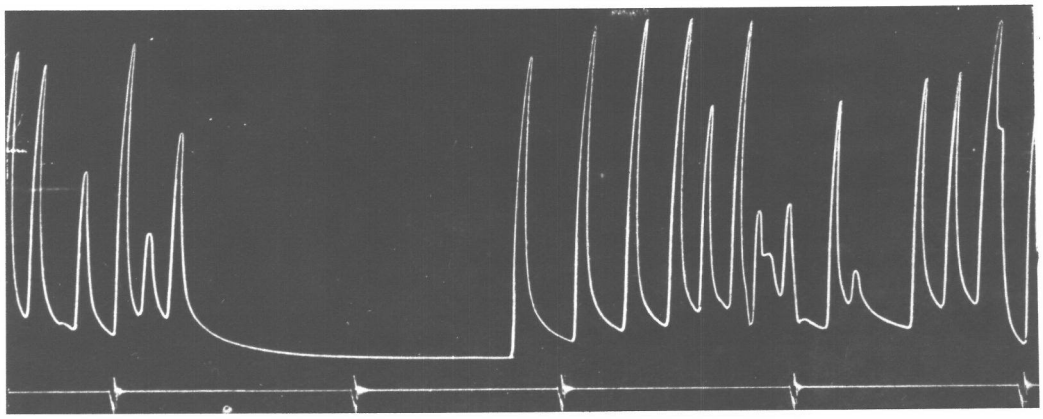


Figure 27 - Later effects of 1-5000 dilution of umbellulone on cat gut. Base line is time in two minute intervals.

Figure 28 - Final effects of 1-5000 dilution of umbellulone on isolated cat gut. Base line is time in two minute intervals.

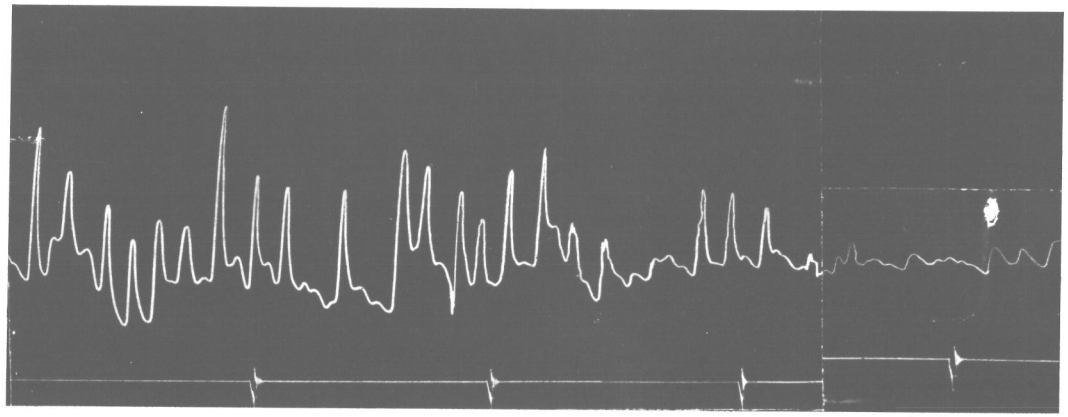
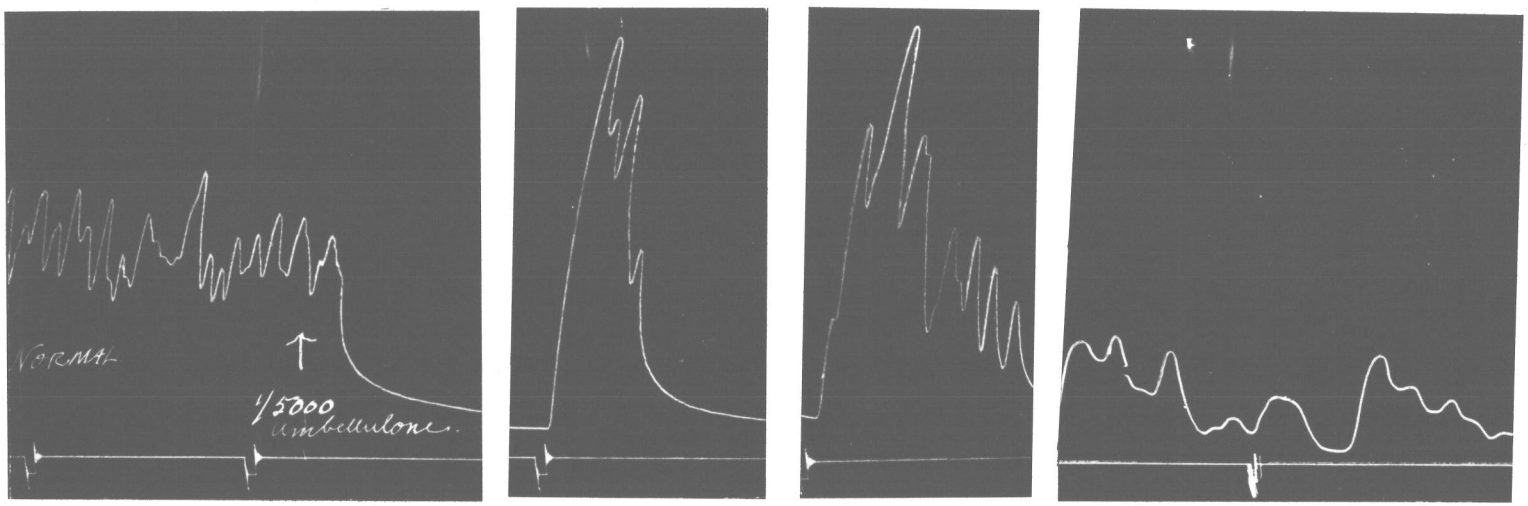


Figure 29 - Cat gut - Effect of 1-5000 dilution of umbellulone on isolated intestinal segment. Time in two minute intervals.



EFFECTS OF UMBELLULONE ON RESPIRATION AND BLOOD-PRESSURE

Dogs were the only animals used in this phase of the work. A total of seven dogs was used.

Method:

The dogs were weighed and 1.2 cubic centimeters of 40 per cent chlorotone in 40 per cent ethyl alcohol (13) per kilogram of body weight were injected intraperitoneally. Two and one-half hours later the dogs were prepared for blood-pressure recordings (10) (18) by using a Becker U tube mercury manometer for blood-pressure and Becker respiration plethysmograph for respiration. Both respiration and blood-pressure were recorded on a single drum kymograph.

Results:

It was found that umbellulone, when given intravenously, in a dost of .089 cubic centimeters per kilogram of body weight produced a rapid and decided fall in blood-pressure, followed by a progressive rise in the blood-pressure, which at no time regained the mean pressure. The injection of a second dose of similar size, as stated above, produced death in a few minutes with failure in respiration, followed shortly by arrest of the heart.

Protocol of Dog No. 3:

Sex	Female
Weight	22.5 pounds (11.2 kilograms)
Normal respiration	24 per minute
Normal pulse	90 per minute

5:37	13.5 cubic centimeters of 40 per cent chlorotone intraperitoneally.
5:55	Respiration 26 per minute.
5:56	Pulse 90 per minute.
7:12	Respiration 38 per minute.
7:13	Pulse 90 per minute.
8:07	Carotid incision and femoral incision.

8:09	Respiration 27 per minute
8:10	Blood-pressure 192 mm of Hg.
	Pulse 90 per minute.
8:11	Injection of seven cubic centimeters of 20 per cent umbellulone into right femoral vein.
8:13	Blood-pressure 140 mm of Hg.
8:17	Heart slower but apparently stronger.
	Pulse 81 per minute
8:22	Blood-pressure 135 mm of Hg.
8:32	Injection of seven cubic centimeters of 20 per cent umbellulone in olive oil into right femoral vein.
8:33	Blood-pressure 135 mm of Hg.
	Respiration 23 per minute
8:56	Respiration slow and weak.
8:58	Respiration ceased.
	Blood-pressure 127 mm of Hg.
8:59	Arrest of heart, blood-pressure 110 mm of Hg.

After the intravenous injection of seven cubic centimeters of 20 per cent umbellulone in olive oil, (an equivalent of 1.2 cubic centimeters of umbellulone) there was a rapid and decided fall in blood-pressure, from 192 mm to 135 mm of mercury, with a decrease in heart action and a slight slowing of the respiration (Fig. 30,31,32(A)).

Figure 32 B shows the injection of an additional seven cubic centimeters of 20 per cent umbellulone in olive oil and the almost immediate fall in blood-pressure from 187 mm to 135 mm of mercury, with a decided decrease in heart action. The respiration is a little faster and more shallow. This decrease in heart action was followed by a slight increase in action of the heart and a slow but deep respiration. The respiration became progressively slower and deeper until failure finally resulted. The heart also became progressively weaker, and failure of the heart occurred about one minute after respiration failure (Fig. 33).

The same results were obtained when using a 10 per cent

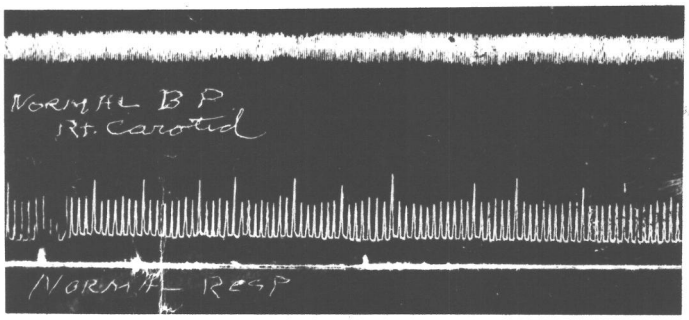


Figure 30 - Normal respiration and blood-pressure of dog. Base line is time in two minute intervals.

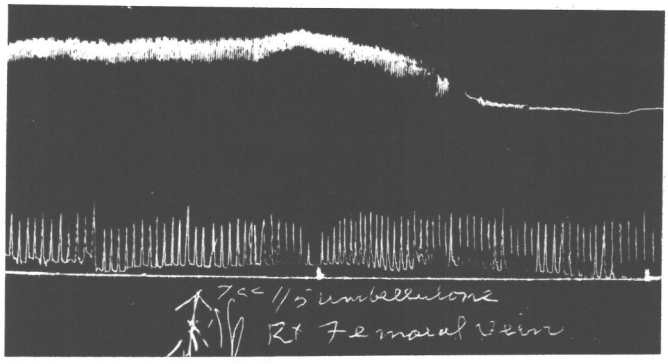


Figure 31 - Injection of seven cubic centimeters of 20 per cent umbellulone at point marked with arrow into right femoral vein of dog. Base line is time in two minute intervals.

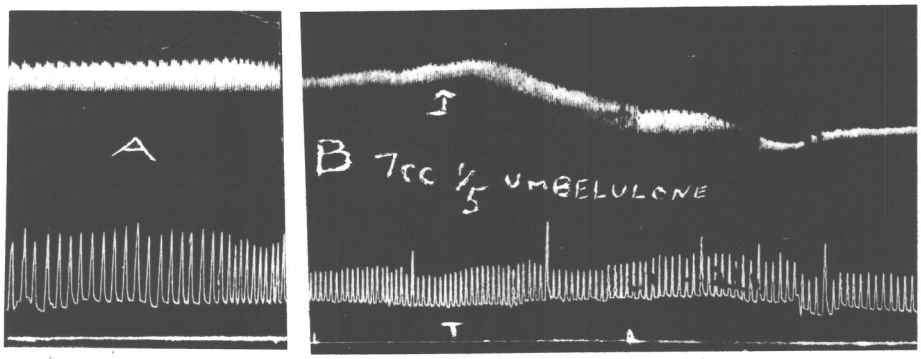


Figure 32 (A-B) - Showing increased heart action and deep respiration in (A) and the decided fall in blood-pressure in (B) on administering seven cubic centimeters of 20 per cent umbellulone into the femoral vein of a dog. Base line is time in two minute intervals.

solution of umbellulone in olive oil, and the dosage was found to be similar to that reported above.

To check the results obtained on the first four dogs, see Fig. 38, which had been given various dilutions of umbellulone in olive oil, dogs No. 1 and 2 received 10 per cent umbellulone in olive oil, dogs No. 3 and 4 received 20 per cent umbellulone in olive oil, dogs No. 5,6 and 7 received undiluted umbellulone, which was injected into the right femoral vein.

Results from dog No. 5:

Sex	Male
Weight	22.5 pounds (11.2 kilograms)
Normal respiration	28 per minute
Normal pulse	90 per minute

5:30	13.8 cubic centimeters of 40 per cent chlorotone injected intraperitoneally.
7:58	Pulse 90 per minute.
7:59	Respiration 30 per minute.
8:00	Carotid and femoral incisions.
8:01	Blood-pressure 190 mm of Hg. Respiration 28 per minute. Pulse 90 per minute
8:03	Two cubic centimeters of undiluted umbellulone injected into right femoral vein.
8:04	Blood-pressure 110 mm of Hg.
8:05	Respiration 5 per minute. Pulse 45 per minute.
8:06	Respiration failed.
8:06:30	Heart stopped. Blood-pressure 110 mm of Hg.

The injection of two cubic centimeters of undiluted umbellulone into the right femoral vein caused a very decided drop in blood-pressure from 190 mm to 105 mm of mercury. This took place within fifteen seconds after the injection was completed. There was also a decrease in heart action and a temporary arrest of the respiration, which was followed by a slow but deep respiration. Both respiration and heart action became progressively weaker, and finally stoppage of respiration

and arrest of heart resulted, with respiration failing a few seconds before heart arrest (Fig. 34-A and B).

Dog No. 6 was anaesthized with 40 per cent chlorotone and arranged for blood-pressure, respiration and intestinal contractions.

Results obtained from dog No. 6:

Sex	Male
Weight	22.5 pounds (11.2 kilograms)
Normal Respiration	58 per minute
Normal pulse	90 per minute

5:30	13 cubic centimeters of 40 per cent chlorotone injected intraperitoneally.
7:37	Respiration 75 per minute.
7:38	Pulse 99 per minute.
8:00	Carotid and femoral incision.
8:50	Blood-pressure 187 mm of Hg.
8:51	Respiration 60 per minute.
8:57	Injection of one cubic centimeter of undiluted umbellulone into right femoral vein.
9:00	Blood-pressure 140 mm of Hg. Respiration 78 per minute. Pulse 90 per minute.
9:01	Blood-pressure 130 mm of Hg. Respiration 156 per minute.
9:03	Second injection of one cubic centimeter of undiluted umbellulone. Blood-pressure 135 mm of Hg. Respiration 84 per minute.
9:05	Blood-pressure 120 mm of Hg.
9:06	Respiration 18 per minute, slow and deep. Blood-pressure 110 mm of Hg.
9:07	Respiration ceased, followed in a few seconds by arrest of the heart.

The injection of one cubic centimeter of undiluted umbellulone into the femoral vein produced a very decided drop in blood-pressure, from 187 mm to 140 mm of mercury, and produced a slight increase in intestinal contraction. The respiration became very rapid and shallow with the same partial paralysis as reported on dog No. 5 (Fig.35). The respiration continued to be rather rapid and shallow, with intestinal contractions becoming more vigorous with increase in contraction,

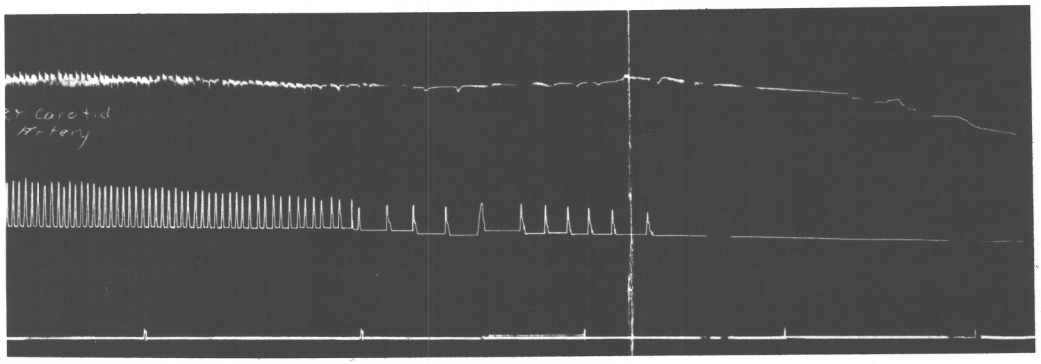


Figure 33 - Showing increase in heart action of dog following the decrease produced by the injection of umbellulone. Showing the progressive decrease in heart action and respiration, which is finally terminated by failure of respiration, followed by arrest of the heart. Base line is time in two minute intervals.

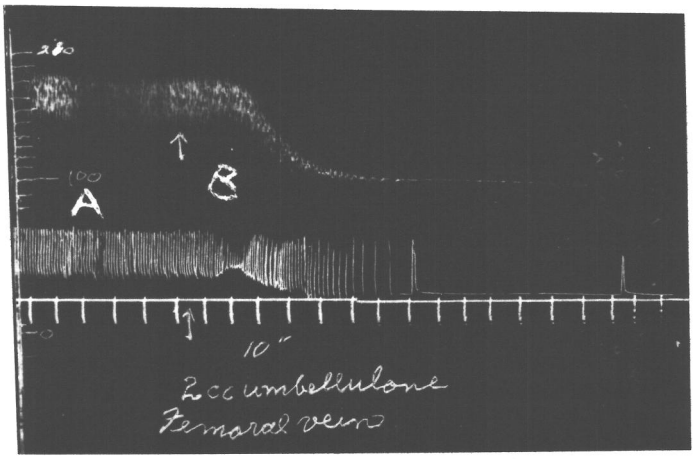


Figure 34 (A and B) - Showing normal respiration and blood-pressure of dog from right carotid artery. (B) shows injection of undiluted umbellulone and subsequent drop in pressure, decrease in heart action and finally failure of respiration, followed by stoppage of the heart. Time in ten seconds.

tonus and amplitude and then becoming progressively weaker. The blood-pressure rose slightly and the heart became stronger (Fig. 36).

The injection of an additional cubic centimeter of undiluted umbellulone produced a drop in blood-pressure from 165 mm to 115 mm of mercury. The intestinal contractions became little more than a straight line and finally ceased. The respiration became progressively slower and deeper and finally failed. This was followed in about fifty seconds by stoppage of the heart (Fig. 37).

Table 6 shows normal respiration and blood-pressure. With the lowest respiration and blood-pressure recorded following the first injection of umbellulone and the lowest respiration and blood-pressure just before death of the dogs following the second injection of umbellulone.

Table 7 shows the chlorotone anasthesia comparison on dogs.

Figure 38 shows the fall of the blood-pressure following the initial injection of umbellulone, the subsequent rise and then the sudden fall following the second injection. All injections were made into the right femoral vein of the dogs.

Post Mortem:

The autopsies of dogs No. 2, 3, 4, 5 and 7 showed congestion of the lungs with blood clots. The heart stopped in diastole. The inferior and superior vena cava and heart were dilated as were the other great vessels.

Discussion:

The presence of the blood clots may be explained by the fact that the pulmonary circulation is blocked, thus causing a rupturing of

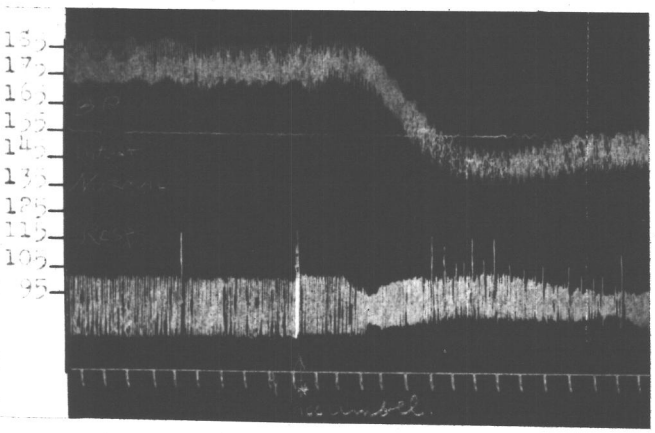


Figure 35 - Dog blood-pressure, respiration and intestinal contractions. * Marks the point of injection of one cubic centimeter of undiluted umbellulone. Scale at side indicates drop in blood-pressure in mm of mercury. Time is ten seconds.

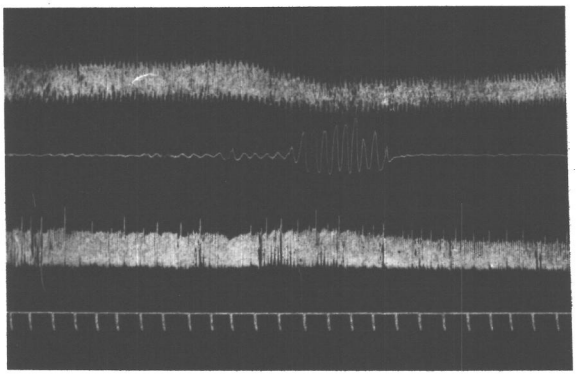


Figure 36 - Dog blood-pressure, respiration and intestinal contraction. Showing increased heart action, stimulation of intestines and rapid but shallow respiration. Time is ten seconds.

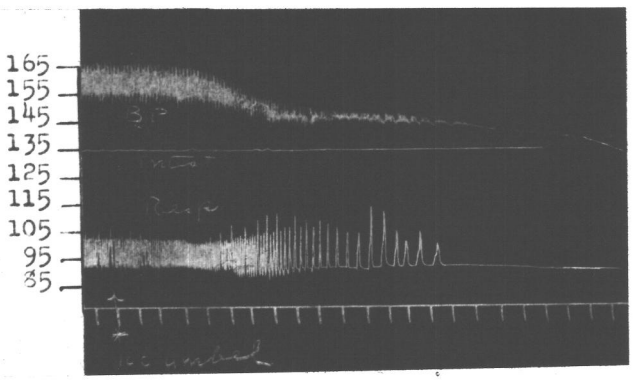


Figure 37 - Dog blood-pressure, respiration and intestinal contraction. Arrow indicates the injection of an additional cubic centimeter of undiluted umbellulone into femoral vein. Time is ten seconds.

Table 6

			10% UMBELLULONE IN OLIVE OIL				20% UMBELLULONE IN OLIVE OIL				UNDILUTED UMBELLULONE				
	NORMAL		10CC INJECTION		10CC INJECTION		7CC INJECTION		7CC INJECTION		1CC INJECTION		1CC INJECTION		DEATH
DOG	RESP.	B.P.	RESP.	B.P.	RESP.	B.P.	RESP.	B.P.	RESP.	B.P.	RESP.	B.P.	RESP.	B.P.	RESULTED
1	40	200	24	140	3	110									89 MIN.
2	37	195	22	138	4	110									90 MIN.
3	27	192					18	135	23	110					48 MIN.
4	35	195					21	138	25	110					40 MIN.
*5	28	190									5	110			3 MIN.
6	58	187									156	135	18	110	9 MIN.
7	72	180									66	138	12	112	16 MIN.

*RECEIVED 2CC UNDILUTED UMBELLULONE INTRAVENOUSLY

Effects of Umbellulone on the B. P. and resp. of Dogs.

Table 7

DOG	AGE	SEX	WEIGHT	40% CHLOROTONE	ANAESTHESIA TIME
	MONTHS		KILOS	C.C.	MINUTES
1	24	MALE	17.0	20.4	8
2	12	MALE	11.2	13.5	9
3	15	FEMALE	11.2	13.5	8
4	10	FEMALE	11.2	13.5	15
5	12	MALE	11.3	13.8	8
6	9	MALE	11.2	13.0	18
7	8	MALE	9.7	11.0	20

NOTE: TIME ELAPSING BETWEEN INJECTION OF ANAESTHETIC AND OPERATION WAS $2\frac{1}{2}$ HOURS IN EACH CASE.

Chlorotone anasthesia comparison on dogs.

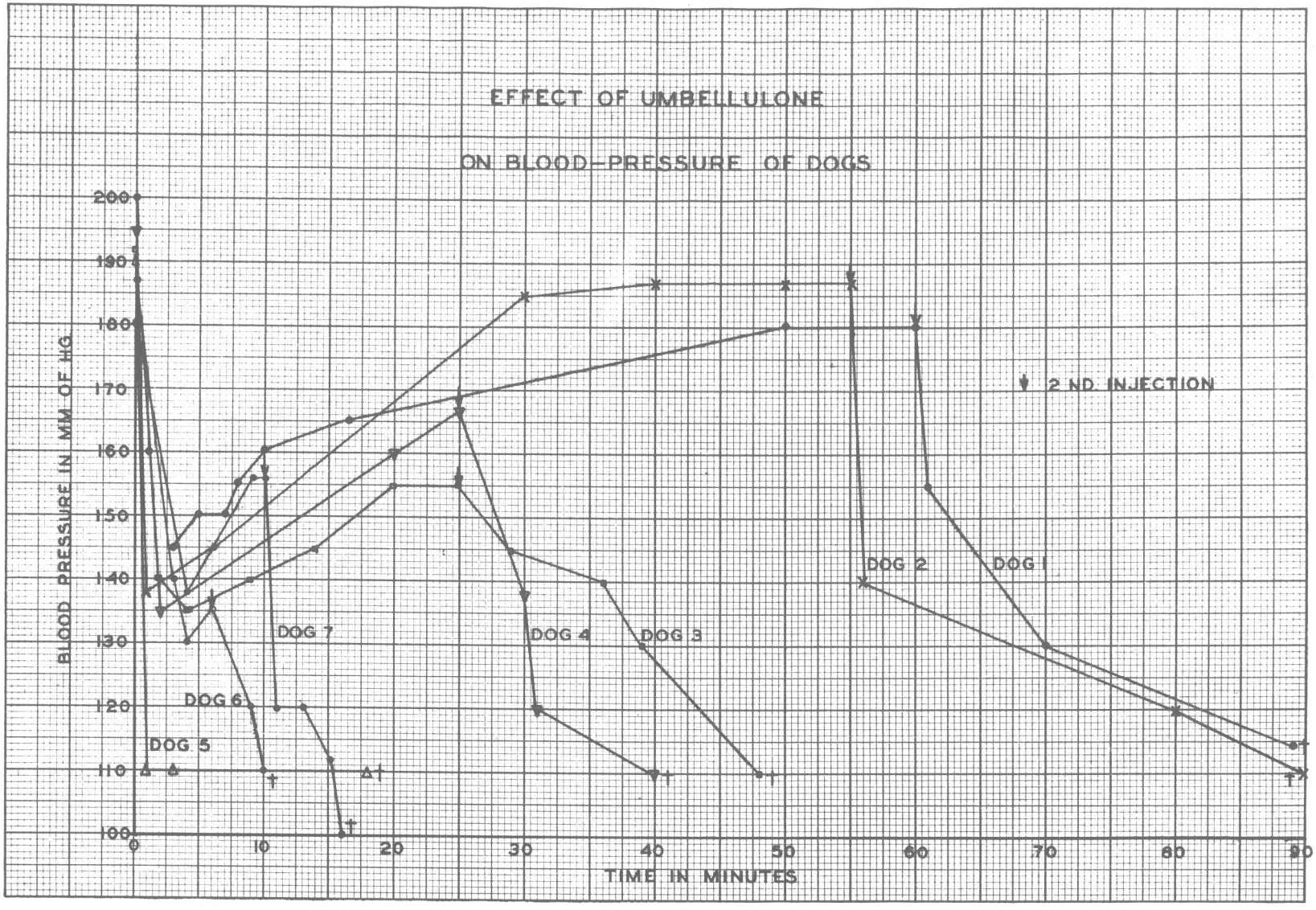

EFFECT OF UMBELLULONE
ON BLOOD-PRESSURE OF DOGS
BLOOD PRESSURE IN MM OF HG.
200
190
180
170
160
150
140
130
120
110
100
0
10
20
30
40
50
60
70
80
90
TIME IN MINUTES
2 ND INJECTION
DOG 1
DOG 2
DOG 3
DOG 4
DOG 5
DOG 6
DOG 7

Figure 38

the arterioles and small vessels of the lungs. Also, according to Sollmann (27), cardiac dilation diminishes the efficiency of the heart, producing a rise in pulmonary pressure with a fall in arterial pressure, thus favoring the production of pulmonary edema and consequent rupturing of the small vessels of the lungs.

Conclusions:

The failure of respiration caused by umbellulone apparently involves several factors. In all probability umbellulone acts as a respiratory depressant. Thus when the respiration becomes inadequate to oxygenate the blood sufficiently the heart fails and death occurs. The fact that umbellulone produces rapid methemoglobin also probably contributes to the failure of respiration. From the autopsies it appears that pulmonary circulation is blocked, thus leading to respiratory failure. It was found that 0.75 gram of caffeine injected intravenously did not stimulate the respiration, thus showing that other factors besides the depression of the respiratory center were involved.

The rapid and decided fall in blood-pressure is probably due to a vaso-dilation effect of umbellulone. The autopsies showed a very decided dilation of the great vessels and heart. The fact that the heart and the great vessels are filled with blood rules out the possibility of capillary dilation, as is produced by histamine (3), which produces capillary dilation and subsequent fall in blood-pressure. However, in the case of histamine, the heart will be found to be beating strongly, but forwarding no blood. The increased heart action following the drop in blood pressure is probably a secondary effect

caused by the fall in blood-pressure, thus removing the normal inhibitory action of the vagus.

The minimal lethal intravenous dose of umbellulone in dogs is about 0.178 cubic centimeters per kilogram of body weight, death being due to failure of the respiration, followed in a few seconds by stoppage of the heart.

CONCLUSIONS AND SUMMARY

1. Umbellulone in blood produces methemoglobin in vitro and vivo.
2. Umbellulone produces decided hemolysis of human, guinea pig and horse blood.
3. Umbellulone injected intraperitoneally into guinea pigs produces asphyxiation followed shortly by death.
4. Inhalation of umbellulone by guinea pigs irritates mucous membranes of eyes and nose and causes irregular respiration, but there is no indication of respiratory failure.
5. Umbellulone in dilutions of 1-50 kills Monilia tropicalis and Trychophyton interdigetale in one, thirty and sixty minute contacts. In dilutions of 1-100 the one minute contact is positive and the thirty and sixty minute contact negative.
6. Umbellulone in the presence of blood, peptone and gelatine shows but a slight loss in fungicidal power.
7. Umbellulone kills E. typhi (Sears) and Staph. albus in dilutions as high as 1-500 for fifteen minutes contact. The estimated phenol coefficient is 6.25.
8. Umbellulone in the form of an ointment, using "Lanolin" as a base shows inhibitory and diffusibility properties.
9. Umbellulone produces a decrease in frequency of the frog heart, loss of tonus and a decrease in the ventricle contraction. Stoppage of the heart occurs in diastole. Atropine, caffeine or adrenaline injected into the right atrium has no effect upon the paralyzed heart.
10. Results obtained on frog heart show that umbellulone probably acts

upon the same nerves and fibers as does atropine, since physostigmine revived the embellulonized frog heart.

11. Umbellulone on isolated segment of rabbit and cat gut in 1-100,000 dilution produces a slight stimulation; in dilutions of 1-40,000 or less, produces an apparent state of depression which progresses into partial paralysis. The duration of the paralysis depends on the degree of concentration of the solution; the more concentrated the solution the longer and the more complete is the paralysis. Washing with fresh Locke's solution revives the segments.
12. Intravenous injections of umbellulone causes a lowering of the blood-pressure and failure of respiration in dogs.
13. The clinical results and post mortem examination indicate that:
    a. Umbellulone probably acts as a respiratory and heart depressant.
    b. Umbellulone produces rapid methemoglobin.
    c. Umbellulone apparently causes blocking of the pulmonary circulation.
    d. Umbellulone causes vaso-dilation of the heart and great vessels.
    e. The minimal lethal dose of umbellulone in dogs is about 0.178 cubic centimeters per kilogram of body weight, death being due to failure of the respiration, followed in a few minutes by stoppage of the heart.

BIBLIOGRAPHY

1. Benson, G. T. "Trees and Shrubs of Western Oregon", 2, 67, Stanford Publication. (1930)

2. Britton, N. L. "North American Trees", 404. H. Holt and Co. (1901)

3. Clark, A. J. "Applied Pharmacology", 242. Blakiston (1932)

4. Dessemontet, E. "Methemoglobin Formation", Journ. Pharm, and Exp. Therap. 31, 377, (1927)

5. Dixon, W. E. "A Manual of Pharmacology" Ed. 5, 174. Edward Arnold and Co. (1921)

6. Douglas, D. "Douglass' Journal", 67, (1823-1827) W. Wesley and Son (1914)

7. F. D. A. U.S.D.A. Circular No. 198, 4, 11 and 12. (Dec. 1931)

8. Halliburton, W. D. "The Essentials of Chemical Physiology", Ed. 2, 249, Longmans, Green and Co.(1922)

9. Heaney, J. P. "Oil of California Laurel" Proc. of Am. Ph. Assoc. 23, 105, (1875)

10. Jackson, D. E. "Experimental Pharmacology", 37, 85. Mosby and Co. (1917)

11. Jepson, W. L. "The Silvia of California", 2, 243. University Press, Berkley (1919)

12. Kamm, O. "Qualitative Organic Analysis", Ed.2,129. John Wiley and Son (1932)

13. Kamm, O., Aldrich, T.B., Grote, I.W., Rowe, L.W., and Bugbee, E.P. "The Active Principle of the Posterior Lobe of the Pituitary Gland", J. Am. Chem. Soc. 50, 581, (Jan.-June 1928)

14. Kingery and Adkison "Certain Volatile Oils and Stearoptenes as Fungicides", Arch, Dermat. and Syph. 17, 499 (Apr. 1928)

15. Miller, Ruth "Bacterial Efficiency of Essential Oils", Am. J. of Phar. 103, 324, (1931)

16. Parry, E. J. "Chemistry of Essential Oils", 237. Scott, Greenwood and Sons (1908)

17. Pembrey, M.S. and Phillips, C.D.F. "The Physiological Action of Drugs", 5. Edward Arnold and Co. (1901)

18. Pittinger, P. S. "Biologic Assays", Ed. 2, 67, 68. P. Blakiston and Sons (1928)

19. Powers, F.B. and Le, F.H.-Constituents of the Essential Oil of California Laurel. J. Chem. Soc. 85, 629, (1904)

20. Russell, E. R. Unpublished Thesis. Oregon State College (1929)

21. Salant, W. and Mitchell, C. W. Influence of Heavy Metals on Isolated Intestines. Journ. of Physiology, 39, 355-374(Feb.1916)

22. Sawyer, W. T. "Odorographia", "A Natural History of Raw Materials and Drugs Used in the Perfume Industry", 64. Gurney and Jackson (1894)

23. Semmler, F. W. Constitution of Etherial Oils, Umbellulone Chem. Abs. 2, 837 (1908)

24. Simmons, J. E. Department of Bacteriology, O. S. C. Personal Communication

25. Spiegel, L. "Chemical Constitution and Physiological Action of Drugs", 73-76. D. Van Nostrand Co. (1915)

26. Stillman, J. M. Etherial Oil of California Bay Tree. Am. Chem. Journ. 2, 38, (1880)

27. Sollmann, T. A Manual of Pharmacology, 429, 4 ed. Saunders and Co. (1932)

28. Topley, W.C. and Wilson, G.S. "The Principles of Bacteriology and Immunity" 1, 117. William Wood and Co. (1929)

29. Tutin, F. Constitution of Umbellulone. J. of Chem. Soc. 89, 1104, (1906) J. of Chem. Soc. 93, 252, (1908)

30. Wienhaus, H. and Todenhofer, K.
Untersuchungen uber Umbellulone und Umbellularia-Ol Berichte der Schimmel and Co., 285 (Jubilaums-Ausgable 1929)