A COMPARATIVE STUDY OF THE PHARMACOLOGICAL PROPERTIES OF ISOARTEMISIN, SANTONINAMINE, AND SANTONIN

BY

William Ellsworth Evans, Jr.

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
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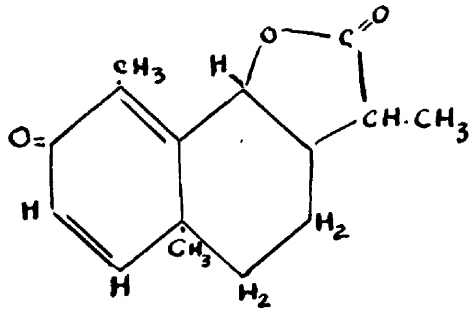
I. Introduction:- A study of the pharmacological properties of δ-oxysantonin and the sulfate of santoninamine has been undertaken, not only to extend our knowledge of these compounds, but to compare their properties with those of the parent substance, santonin.

I-A. Review of Literature:-

1. Chemical.

Santonin[1,2] is obtained from the unexpanded flowerheads of Artemisia pauciflora, Artemisia Cina, and Artemisia maritima. It crystallizes in colorless platelets which have a melting point of 170°. It is soluble in alcohol, benzene, and solutions of alkalies, but almost insoluble in water. The yellow photosantonin is formed on exposure of the crystals to light.

Various graphical formulae have been assigned to santonin, the most important of which, until recently, was that of Cannizzaro[3]. Clemo, Haworth, and Walton[4] have now definitely proved the following structure for santonin:

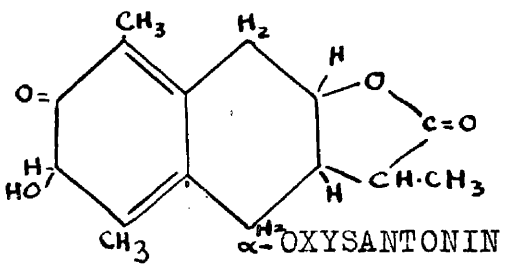


SANTONIN

Clemo[5] has also isolated a β-santonin, a stereo-isomeride of santonin. It occurs as colorless prisms which melt between 216° and 218° and have an $[\alpha]_D$ of -137.2° at 19°.

Five oxides of santonin which have the constitutional formula of $C_{15}H_{18}O_4$ have been described in the literature.

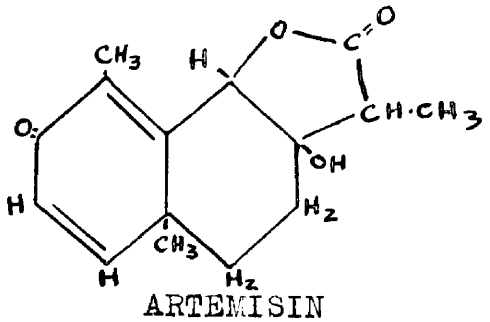
α-oxysantonin was isolated by Jaffe[6] from the urine of large meat-fed dogs which had received capsules containing one to two grams of santonin daily. The pure compound was difficulty soluble in boiling alcohol, difficulty soluble in chloroform and almost insoluble in ether. It was partly soluble in water on long heating but separated out on cooling. The aqueous solution was neutral in reaction. The product was easily soluble in hot glacial acetic acid but very difficulty soluble in the cold acid. Solutions of the salts which were formed on long heating with dilute alkalies or alkaline earths remained clear at first on addition of acids but the anhydride gradually separated out. The colorless needle-like crystals of α-oxysantonin melted with decomposition between 280° and 286° and had an $[\alpha]_D$ of -115° in alcohol. These results were confirmed by D. Lo Monoca[7] who suggested the following graphical formula:



α-OXYSANTONIN

β-oxysantonin was obtained by Jaffe[6] from the ether extract of the urine of rabbits which had been fed santonin. This substance was easily soluble in cold alcohol, ether, chloroform, and in hot water, but insoluble in petroleum ether and difficultly soluble in cold water. It crystallized in yellow platelets or leaves, was laevorotary, and melted to an oily droplet between 128° and 130°.

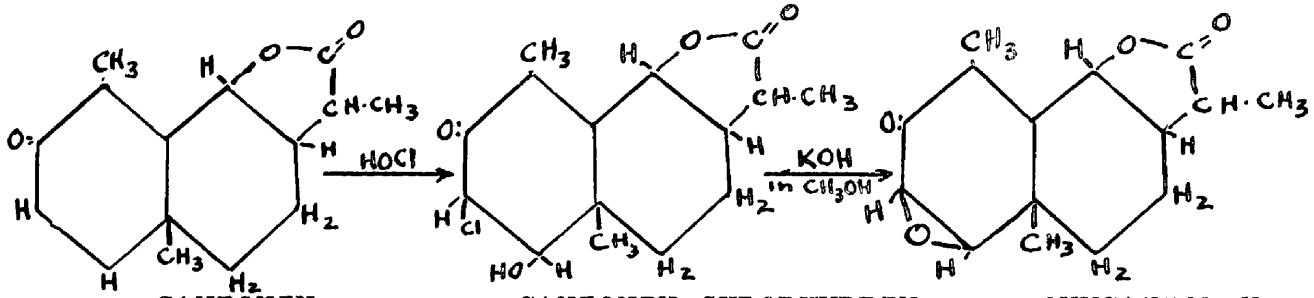
γ-oxysantonin or artemisin was isolated by Merck from the seeds of Artemesia maritima. It was soluble in hot water and alkali solutions. The compound had $[\alpha]_D$ of -84.3° and crystallized in massive needles or prisms which melted at 200°. The structure of artemisin has been studied by Freund and Mai[9]. They obtained an oily product on distillation of artemisin in a stream of hydrogen which yielded β-dimethylnaphthalene[10] on fractional distillation over sodium. This derivitive had a boiling point of 264° and formed a picrate which melted at 119°. The 1,4-dimethylnaphthalene obtained by Cannizzaro and Carnelutti[11] on reduction of santonin formed a picrate which melted at 139°. The more recent and more exact chemical research of Wedekind[12] has proved the following graphical formula for artemisin to be correct:



ARTEMISIN

Two oxysantonins have been prepared synthetically by Wedekind and his co-workers. These were termed δ-oxysantonin or isoartemisin[13] (later called α-oxysantonin[14]) and β-oxysantonin[15].

Wedekind and Tettweiler obtained a chlorhydrin by the action of chlorine water on finely powdered santonin. The product was boiled thoroughly with benzene to remove unattacked santonin. The chlorhydrin which separated out on cooling was recrystallized from dilute alcohol. It was then converted into δ-oxysantonin by the action of potassium hydroxide in methyl alcohol between 50° and 60°. The long colorless needle-like crystals which were obtained on repeated recrystallization from boiling absolute alcohol had a melting point of 214° and an $[\alpha]_D$ of -108.6°. It was difficultly soluble in water and alkali solutions but easily soluble in chloroform.

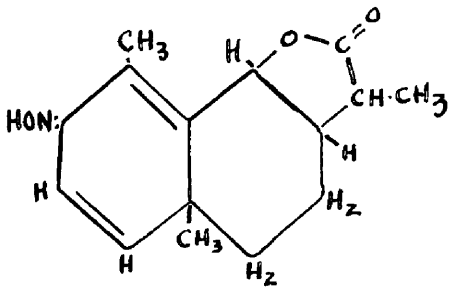


SANTONIN SANTONIN CHLORHYDRIN OXYSANTONIN

A mixture of α- and β-oxysantonin was obtained by the action of perbenzoic acid on santonin in boiling chloroform.

β-oxysantonin, a spatial isomer of α-oxysantonin, was separated by difference of solubility in alcohol. It crystallized in colorless leaves and melted at 157°.

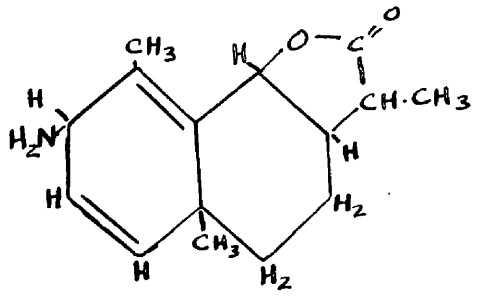
Santoninoxime was first prepared by Gucci[16] by the use of 5 parts of santonin (1 mol.), 4 parts of hydroxylamine hydrochloride (2 mols.), and 3-4 parts of precipitated calcium carbonate suspended in 50 parts of 90% alcohol. The mixture was heated for 3-4 days on a water bath, the temperature of which was maintained between 75°-80°. The suspended matter was then filtered out and the filtrate was added to 4-5 volumes of boiling hot water. The lacy, silky white product which was obtained was purified by recrystallization from cold alcohol. It melted between 216°-217°. The following graphical formula was assigned to the oxime:



SANTONINOXIME

The sulfate of santoninamine was prepared from the oxime by Gucci and Grassi-Crislaldi[17]. About 400 c.c. of 90% alcohol were added to 10 gm. of the oxime. Then 20 c.c. of concentrated sulfuric acid and 60 gm. of powdered zinc were added a little at a time. The mixture was shaken frequently and the temperature was not allowed to exceed 30°-40°. Several drops of 10% platinum chloride were added to facilitate reduction.

After the reaction was complete, the remaining zinc was filtered from the alcoholic solution. Finally the alcohol was removed by distilling under reduced pressure. A light yellow mass which was suspended in a very acid liquid remained. The amine was extracted from this mass by repeatedly dissolving in absolute alcohol and precipitating with 3-4 volumes of anhydrous ether. Isolation of the amine is rendered difficult due to its extreme solubility in water. The purified product melted between 145° and 146°, had a specific rotation ($[\alpha]_D$) of -103.67° and the constitutional formula of $C_{15}H_{21}NO_2.H_2SO_4.H_2O$. The following graphical formula was assigned to the amine.



SANTONINAMINE

I-A. 2. Pharmacological:

Santonin has been used as a remedy against round worms since ancient times[18], for which purpose it is still considered a very valuable drug. It is reputed to have been used by the Russian peasants as a folk-remedy for diabetes. It also acts as a peripheral vasodilator[19], a diuretic[20,21,22], and increases uric acid elimination[23,24]. In toxic dosages it produces violent gastro-intestinal irritation, hematuria, xanthopsia, muscular weakness, convulsions, and fall of body temperature[25,26,27]. A more detailed review of the literature on santonin will be given under the various sections of the experimental part.

Trendelenburg[28] has reported that both δ-oxysantonin and γ-oxysantonin had a strong action on the earthworm muscle preparation, as might be expected from their lactone character. The δ-oxysantonin was found to be only a little weaker than santonin when isomolecular solutions were used. The convulsant action of isoartemisin on the mouse was shown to be very much weaker than that of santonin. The fatal dose is above 22 mgm. The effect of both oxysantonins on the heart was somewhat weaker than that of santonin when used in equivalent concentra-

tion (1:5000). A 1:500 solution of artemisin, however, was very toxic, causing diastolic arrest in a few seconds.

Coppola[29] recommended that santoninoxime be used in treatment of ascaridiasis rather than satonin because of its lower toxicity. Schmidt[30] injected 0.05 to 0.2 gm. of the oxime and of the amine intravenously in rabbits and observed no toxic effects except with one rabbit. This animal died 6 hours after injection of 0.2 gm. of the aminocompound. Weak convulsions were observed in this case. Lo Monoco[31] observed that the sulfate of santoninamine and soda were as effective as santonin and soda. The effect of administration of the sulfate of santoninamine on blood-sugar level has not been previously reported.

I-B. Scope of Problem.

The object of this research is to determine certain of the pharmacological properties of (a) δ-oxysantonin, namely:

1. Anthelmintic Activity.
2. Influence on Cardiac Rate, Amplitude of Contraction, and Output.
3. Effect on Peripheral Circulation.
4. Ability to Act as a Diuretic.
5. Ability to Lower the Blood-Sugar Level.
    a. Of Fasted Animals.
    b. Of Animals which had Received Dextrose.

6. Toxicity and Pathological Effects Produced on Liver and Kidney.

7. Excretion and Fate.

B. The Sulfate of Santoninamine:

1. Ability to lower the Blood-Sugar Level of Fasted Animals.

2. Pathological Effects Produced on Liver and Kidney.

II Experimental:-

1. Anthelmintic Activity.

The anthelmintic activity of δ-oxysantonin was determined on earthworms, according to the method employed by Sollman[32]. Five earthworms, each about 10 cm. long, were placed in 100 cc. of the solution to be tested. The control solution contained 1% of sodium bicarbonate and 0.04% of bile salts in order to approximate the alkalinity of the pancreatic and intestinal juices and the solvent activity of the bile. The drug stock solution was a suspension of 0.1% of finely divided δ-oxysantonin in the control solution. The isoartemisin was only completely soluble in the lower dilutions.

Observations of the activity and appearance of the earthworms were made at the end of 3, 5, and 10 hours in the case of the control solutions, and

within a minute and after 2, 5, and 10 hours when the drug was employed. In order to make the final observations, each worm was lifted out by means of a hook from the beaker containing it and placed on a towel. The movements recorded in tables I and II were either spontaneous or the response to mechanical (φ_M), galvanic (φ_G), or weak faradic stimulation (φ_F). There was an almost immediate appearance of a web-like precipitated secretion around the worms which were placed in the higher concentrations of the isoartemisin. This was followed in several hours by liquifaction of the external musculature which was especially marked in the 0.1% δ-oxysantonin suspension, leading to rupture of many of the worms. Little secretion and no liquifaction were observed with the control worms.

The fatal concentration of δ-oxysantonin was found to be between 0.0333% and 0.0666%, which placed the drug in Group III of Sollmann's classification. Hence, it may be regarded as a rather weak vermicide as compared with santonin[32], the fatal concentration of which is 0.001% to 0.009%. The violent movements of the worms placed in solutions of oxysantonin indicated that it would

be an excellent vermicide. This was confirmed by the following experiment.

An aspirator bottle was connected to a piece of glass tubing about 75 cm. long and so arranged that the open end of the tubing was higher than the outlet tube of the aspirator bottle. Forty cc. of water was poured into the bottle, partially filling the glass tubing. A large earthworm was then placed in the bottle and, after ascertaining that the worm would not attempt to pass through the outlet tube, 10 cc. of the 0.1% solution of oxy-santonin was added. After some agitation, the head end of the worm (in 3 out of 4 cases) sought and passed through the outlet tube into the glass tubing connected to it. The head end of the fourth worm appeared to seek the outlet tube but finally the tail end passed through into the glass tubing.

Table I

Stock BB solution: 1% $NaHCO_3$, 0.04% Bile Salts. Concentration in Percent of Stock Solution.

Elapsed Time (hrs.)	100	66.6	33.3	10	Water
3	depressed but move actively on φ_M	active	appear depressed but move on φ_M	appear depressed but move on φ_M	active
5	all active no liquifaction	all active	all active	all active	sluggish
10	11 dead 4 responded to φ_F	2 move actively 3 respond feebly to φ_M. Move spontaneously several minutes after φ_F. 3 do not respond to φ but move spontaneously on return to solution. 2 dead.	6 show normal movements. 3 respond to φ_F. 1 dead	6 show normal movements 4 respond to φ_F.	4 move normally 1 shows sluggish response to φ_F.

Table II

S-oxysantonin Stock Solution: 0.1% Oxys., 1% $NaH \cdot Co_3$, 0.04% Bile Salts.

Concentration in Percent of Oxysantonin Stock Solution

Elapsed Time	100	66.6	33.3	10	6.66
Within 1 min.	violent agitation and appearance of web-like, precipitated secretion.	violent agitation and appearance of web-like precipitated secretion	violent agitation. precipitated secretion not observed.	active	active
2 hours	depressed. respond to φ_F after long latent period (1 min.) Slimy due to partial liquifaction causing rupture of many.	depressed but respond to φ_M	depressed. respond on active agitation of container	active	active
5 hours	3 move slowly, respond to φ_M sluggishly 7 dead	6 move feebly, show little response to φ_M 4 dead	all very depressed but move on agitation of container	still moving, but slowly.	active
10 hours	10 dead	10 dead	8 responded to φ_F 7 dead	12 responded to φ_F 4 dead	14 respond normally 1 reacts sluggishly to φ_F

II 2. Influence on Cardiac Rate, Amplitude of Contraction, and Output:-

The influence on cardiac rate, force, and output was determined on the frog heart. The method employed was essentially the same as that described by Sollmann and Barlow[33]. A Y-cannula which was provided with a wash-out side arm and with a tube for measuring the perfusion pressure was connected to two special Mariotte bottles containing iso-artemisin in Howell-Ringer solution (80 mgm. per l.), and Howell-Ringer solution, respectively. The Mariotte bottles were provided with glass tubes for the maintenance of constant pressure and for aëration of the fluid.

After removing the ventral abdominal wall and pectoral girdle of a pithed frog, the pericardium was cut away from the heart and the plica v. bulbi post. severed from the base of the ventricle. The Y-cannula was inserted and tied into the inferior vena cava at the point of junction of the two hepatic veins with it. The height of the Mariotte bottles was adjusted until the perfusion pressure was 15 mm., which was sufficient to properly fill the heart. The right aorta was ligated as close to the heart as possible and an outflow cannula was tied into the left aorta, also close to the bifurcation of the aorta in order

to avoid loss of fluid through any of the branches. Both cannulae were fixed in position by means of pins. The perfusate was collected in a 50 cc. graduated cylinder. The heart was connected to a recording lever by means of a thread tied to the apex of the ventricle. Howell-Ringer solution was dropped on the surface of the heart to prevent drying.

After making a normal tracing of the heart and observing the output per 5 minutes while perfusing Howell-Ringer solution, the saturated solution of isoartemisin was perfused and observations at appropriate intervals were recorded, as shown by the tracings (No. 1 to 10) and tables III, IV, V, and VI. Control experiments were performed in which only Howell-Ringer solution was perfused.

Although there was occasionally an initial decrease in amplitude and an irregularity of contraction when the drug was perfused, the heart beat soon returned to normal. There was a continuous decrease in output in both the control and the isoartemisin experiments. In conclusion, it may be stated that δ-oxysantonin does not significantly affect the heart when perfused in a concentration of 0.008%.

Table III

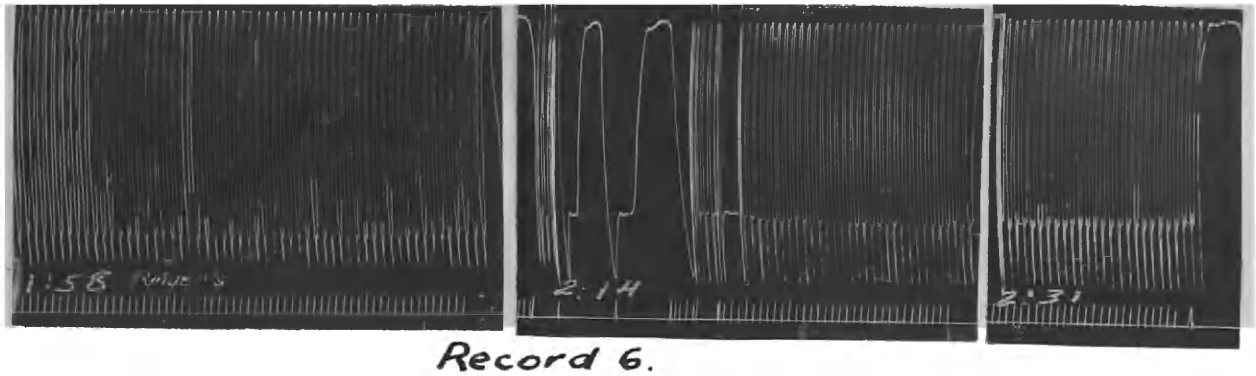
Howell-Ringer Perfusion

	Control		15 minutes		30 minutes		Change		Per Cent Change	
	rate	amp.	rate	amp.	rate	amp.	rate	amp.	rate	amp.
(6)	52	59	57	26	54	26	2	-3	3.8	-10.
(7)	52	25	52	25	52	27	0	2	0	8.0
(8)	57	15	62	12	60	12	3	-3	5.3	20
(9)	52	17	51	16	62	12	10	-5	19	29
(10)	56	22	60	21	60	21	5	-1	8.9	-4.5

Table IV

Drug Perfusion

	Control		15 minutes		30 minutes		Change		Per Cent Change	
	rate	amp.	rate	amp.	rate	amp.	rate	amp.	rate	amp.
(1)	38	31	34	30	32	28	-6	-3	-16	-9.7
(2)	52	21	42	17	42	17	-10	-3	-19	-14
(3)	50	22	48	22	51	20	1	-2	2.0	-9.1
(4)	46	17	48	15	48	15	2	-2	4.4	-12
(5)	46	16	50	14	51	14	5	-2	11	-12



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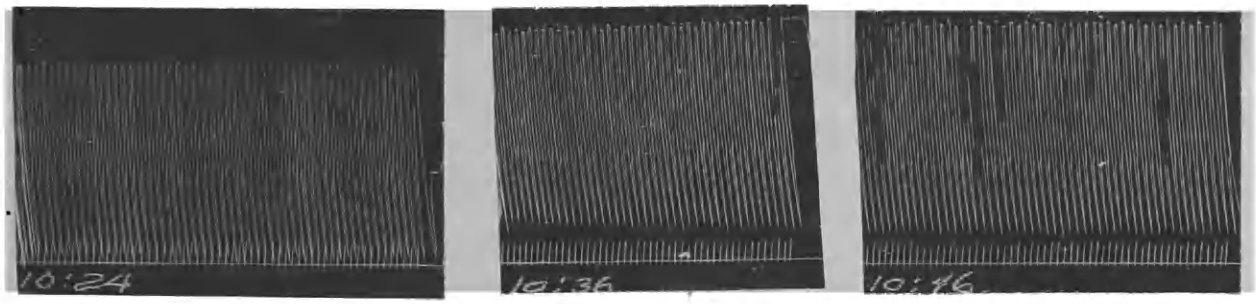

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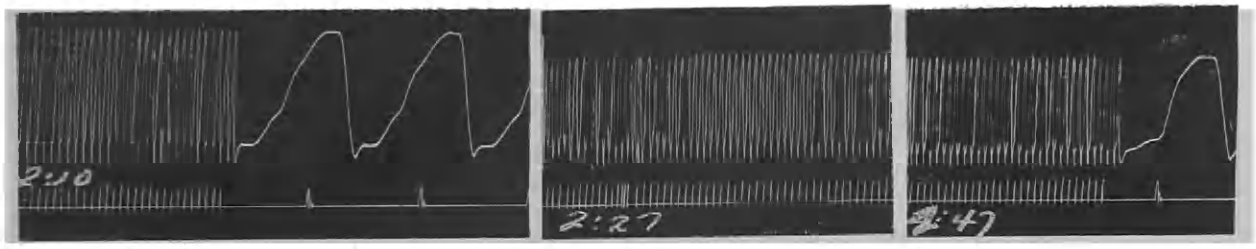

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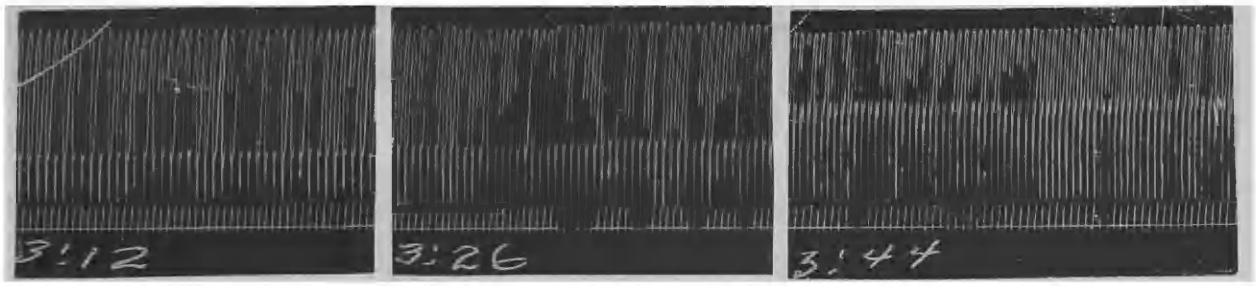

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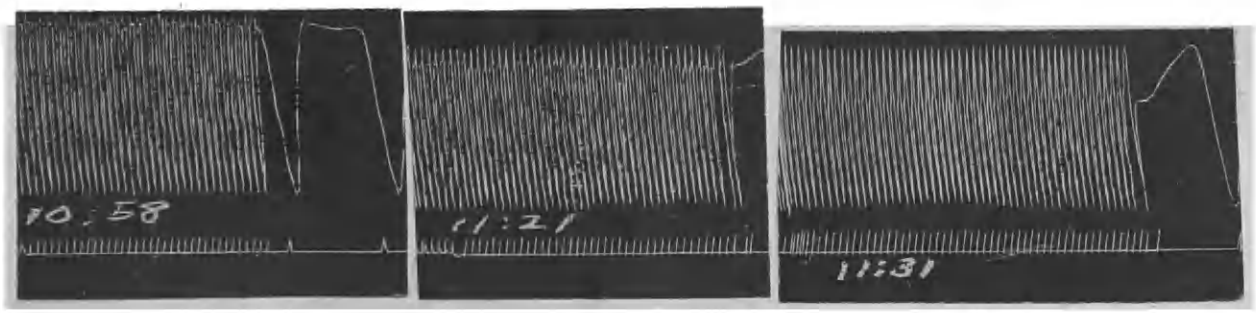
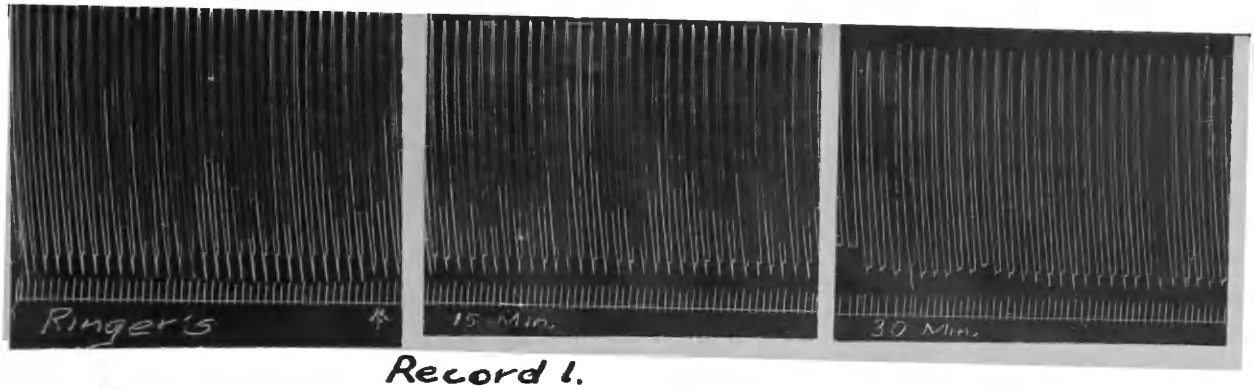


Record 10.

Perfusion of Frog Heart with Howell-Ringer Solution



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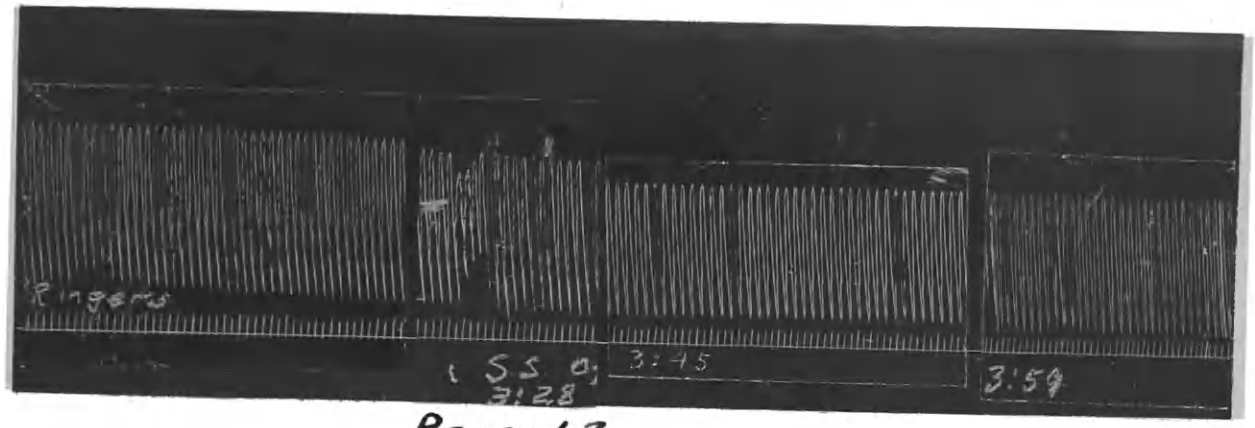

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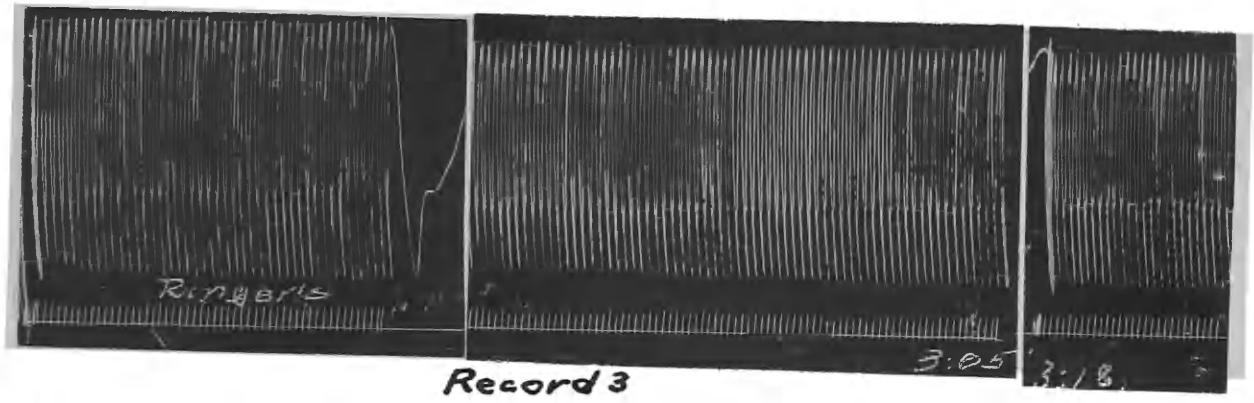

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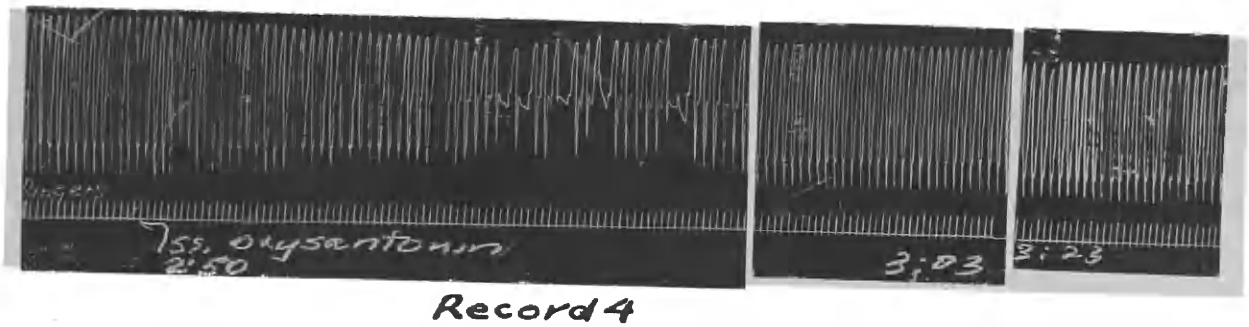

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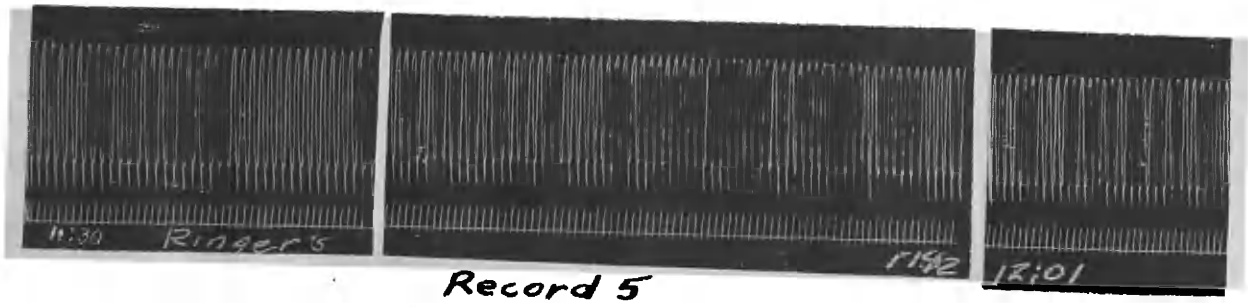


Record 5

Perfusion of Frog Heart
with Isoartemisin

Table V

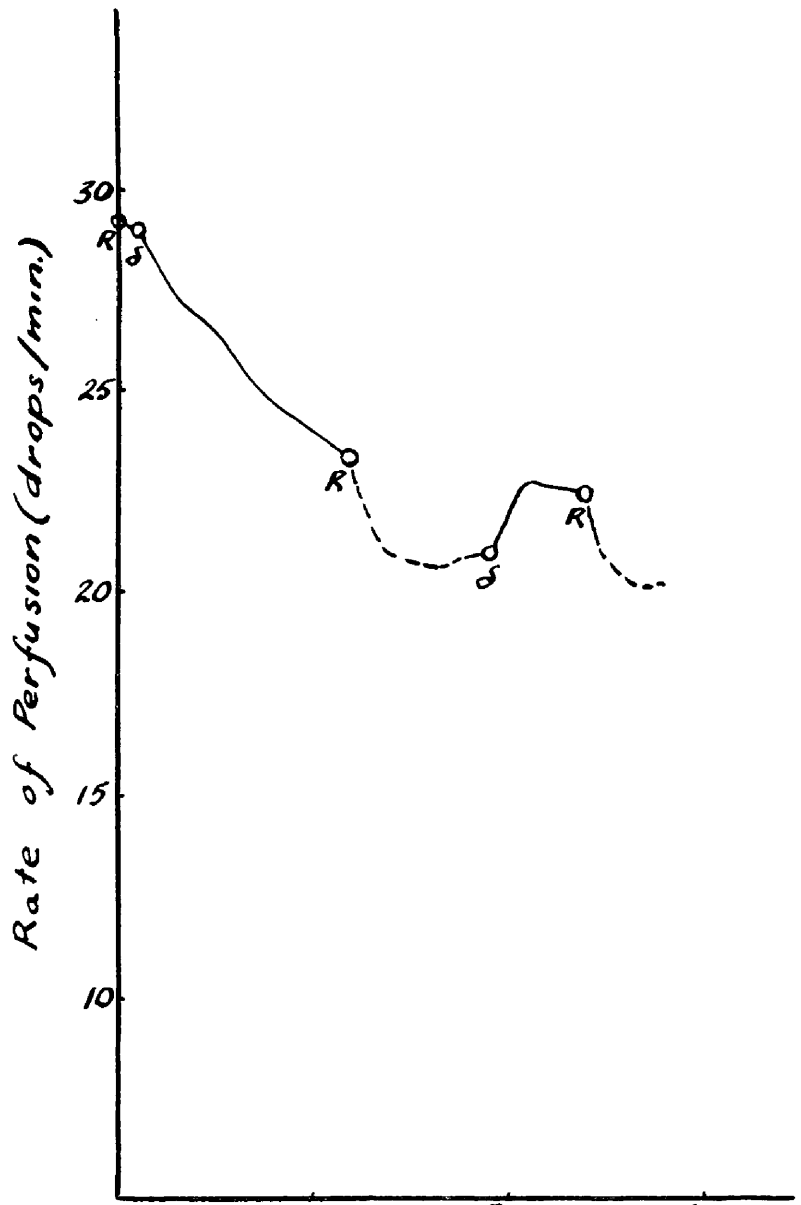
Howell-Ringer Perfusion in cc. per 5 minutes.

	Control rate	15 min. (app.) rate	30 min. (app.) rate	change	Per Cent change	45 min. rate
(6)	11.7	12.5	11.8	plus 0.1	0.854	10.0
(7)	25.8	21.5	18.0	- 7.8	30.2	16.8
(8)	18.8	20.6	16.1	- 2.7	14.4	10.3
(9)	16.7	16.9	16.4	- 0.3	1.11	16.2
(10)	27.0	23.1	18.3	- 8.7	32.2	15.9

Table VI

Drug Perfusion in cc. per 5 minutes.

	Control rate	15 min. (app.) rate		30 min. (app.) rate	change	Per Cent change	45 min. rate
(1)	13.6	8.3	-5.3	5.2	- 8.4	61.8	--
(2)	20.4	4.5	-15.9	6.1	-14.3	70.0	--
(3)	27.0	24.0	-3.0	23.2	- 3.8	14.1	18.2
(4)	16.8	15.2	-1.6	10.8	- 6.0	35.7	8.7
(5)	14.0	16.0	plus 2.0	16.5	plus 2.5	17.8	15.3



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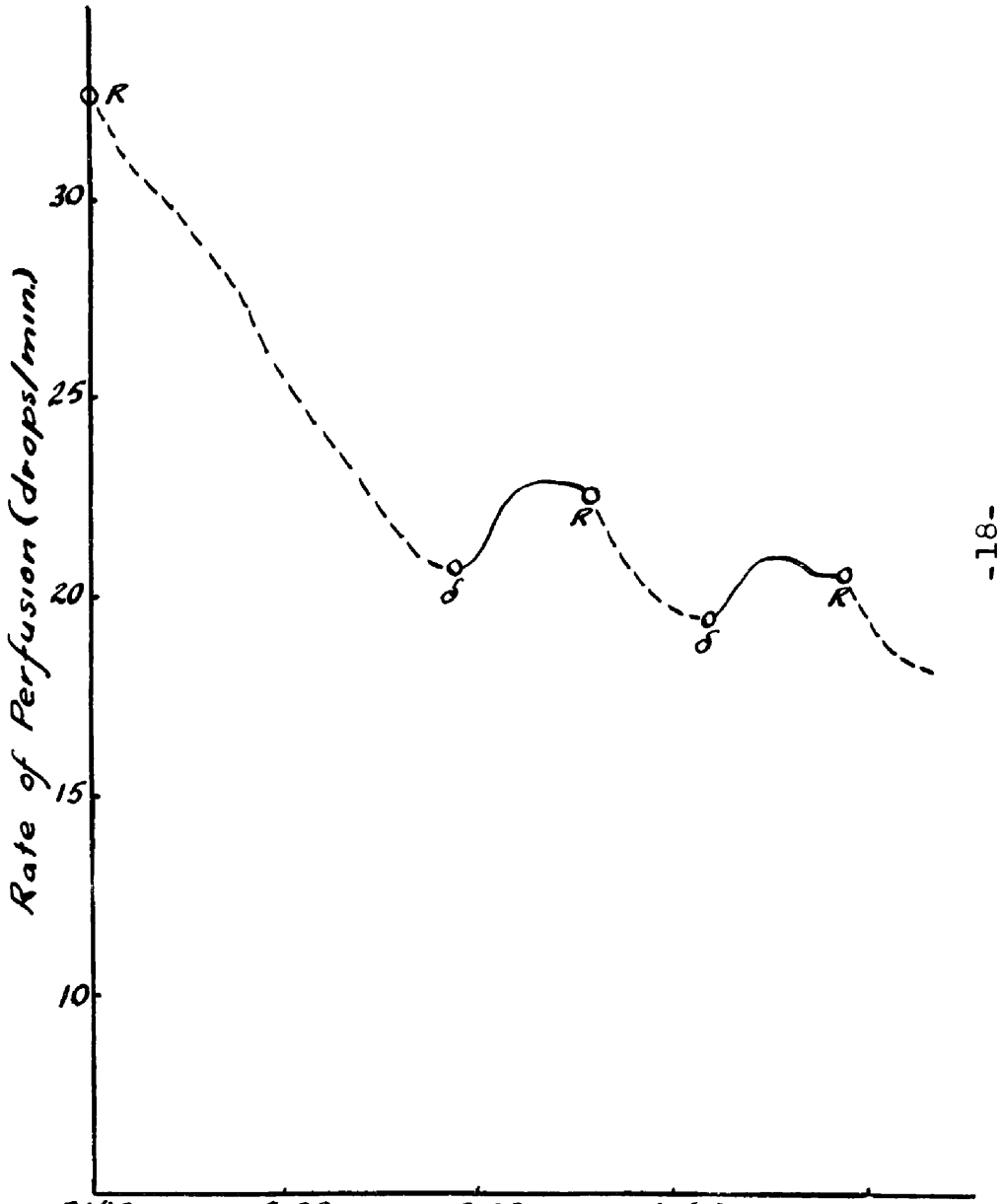

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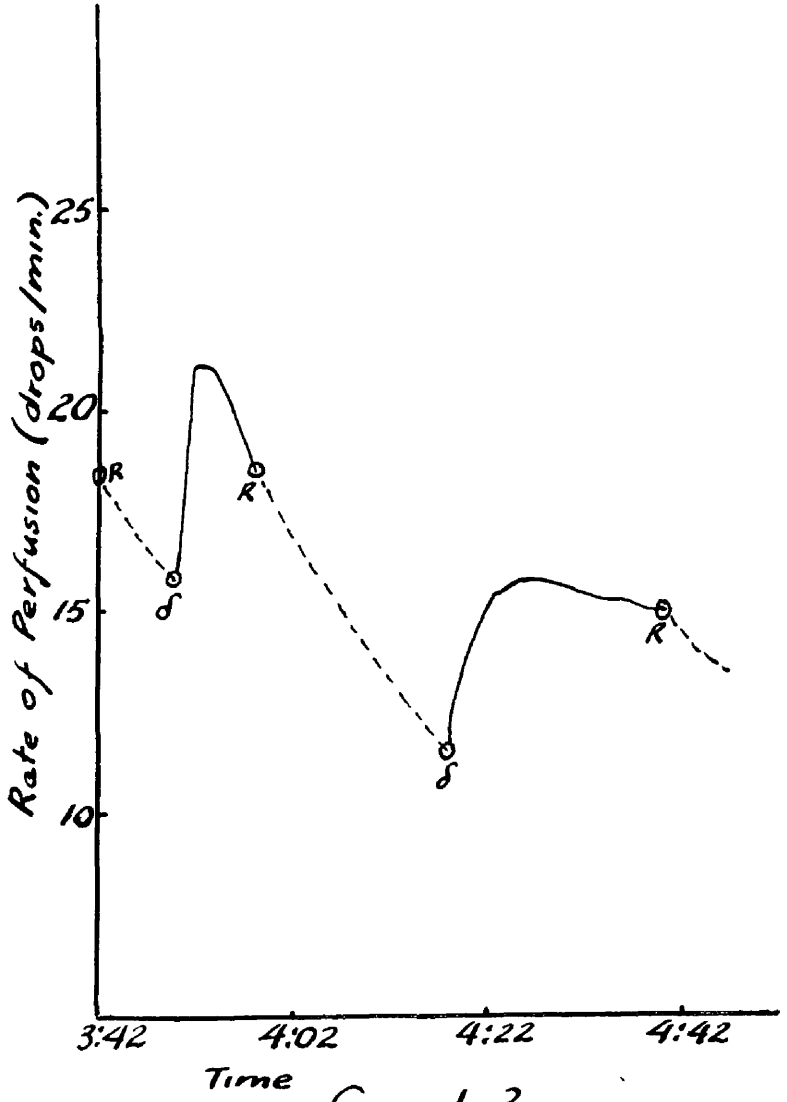

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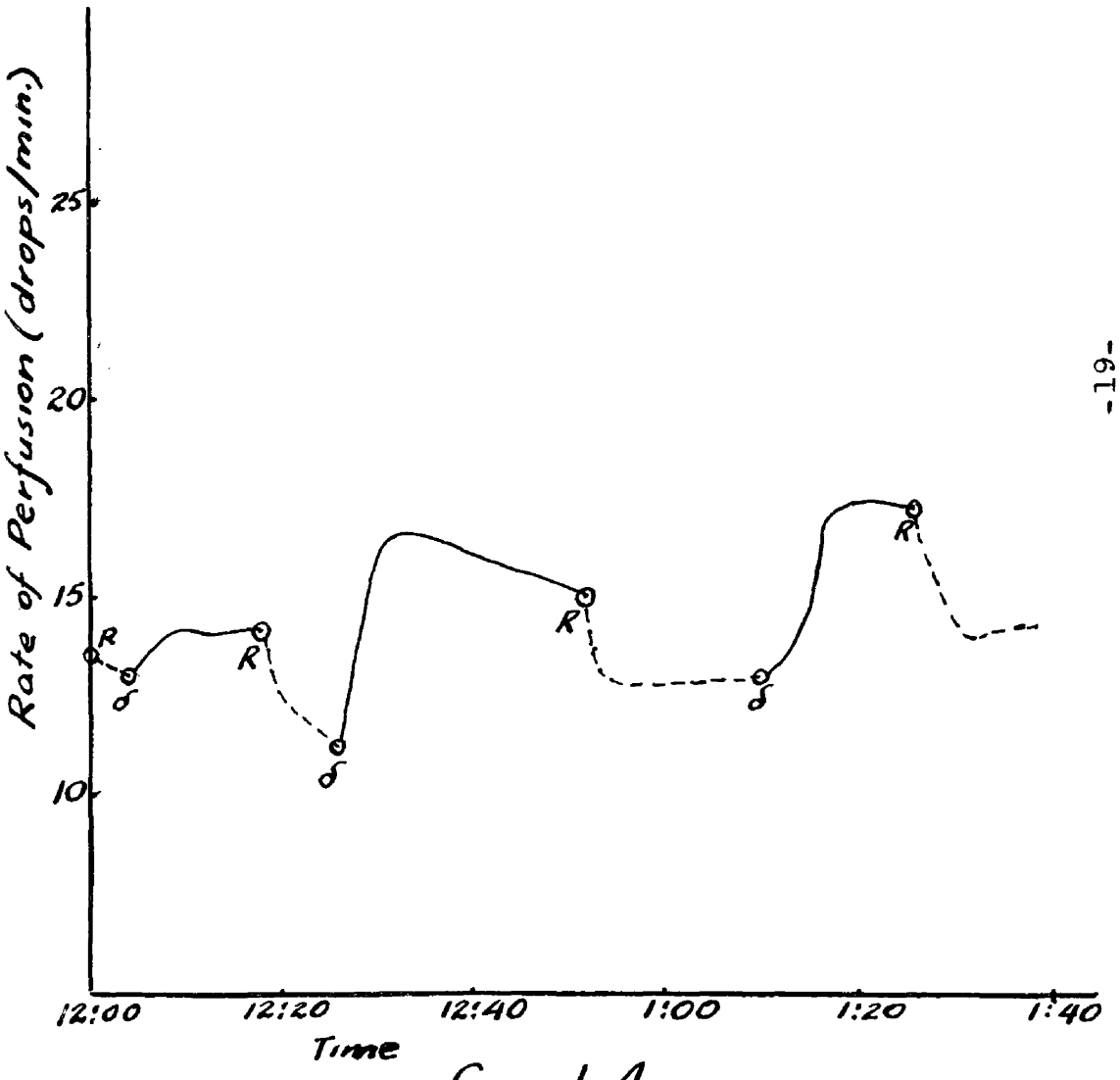

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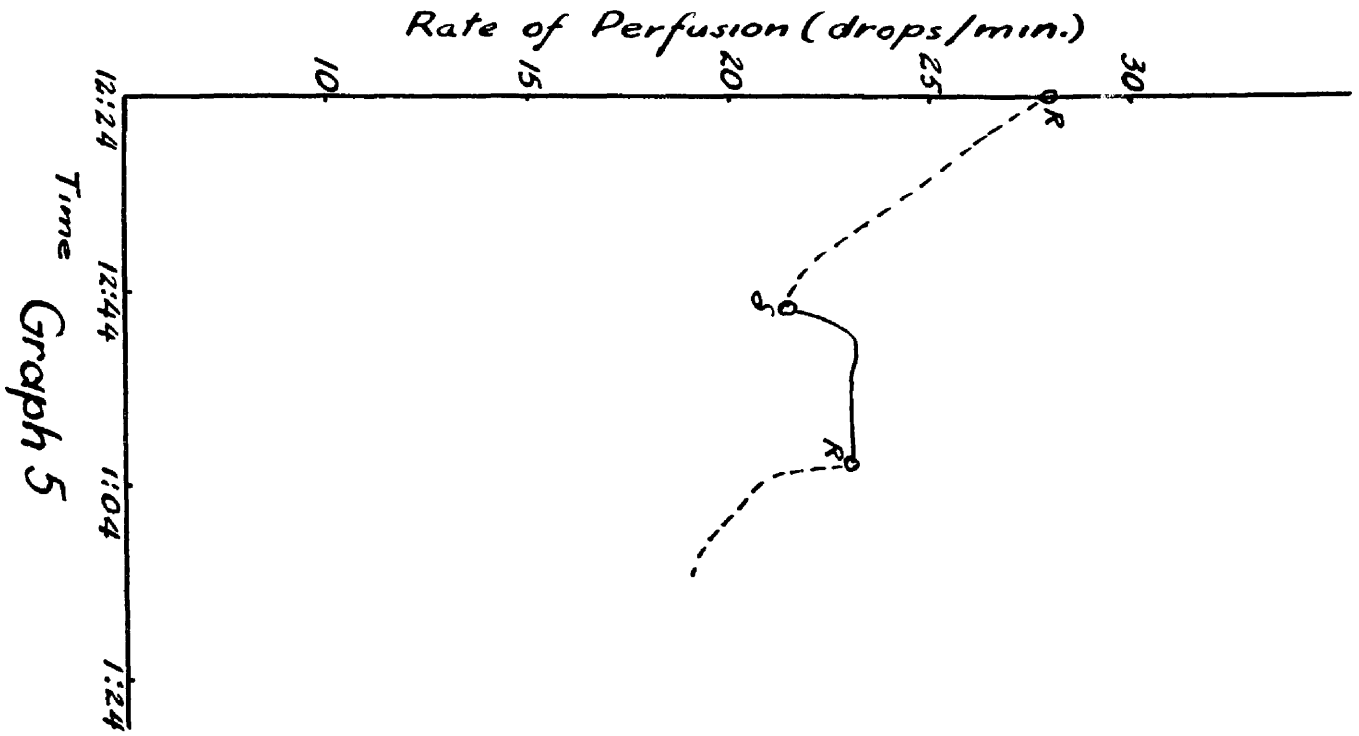

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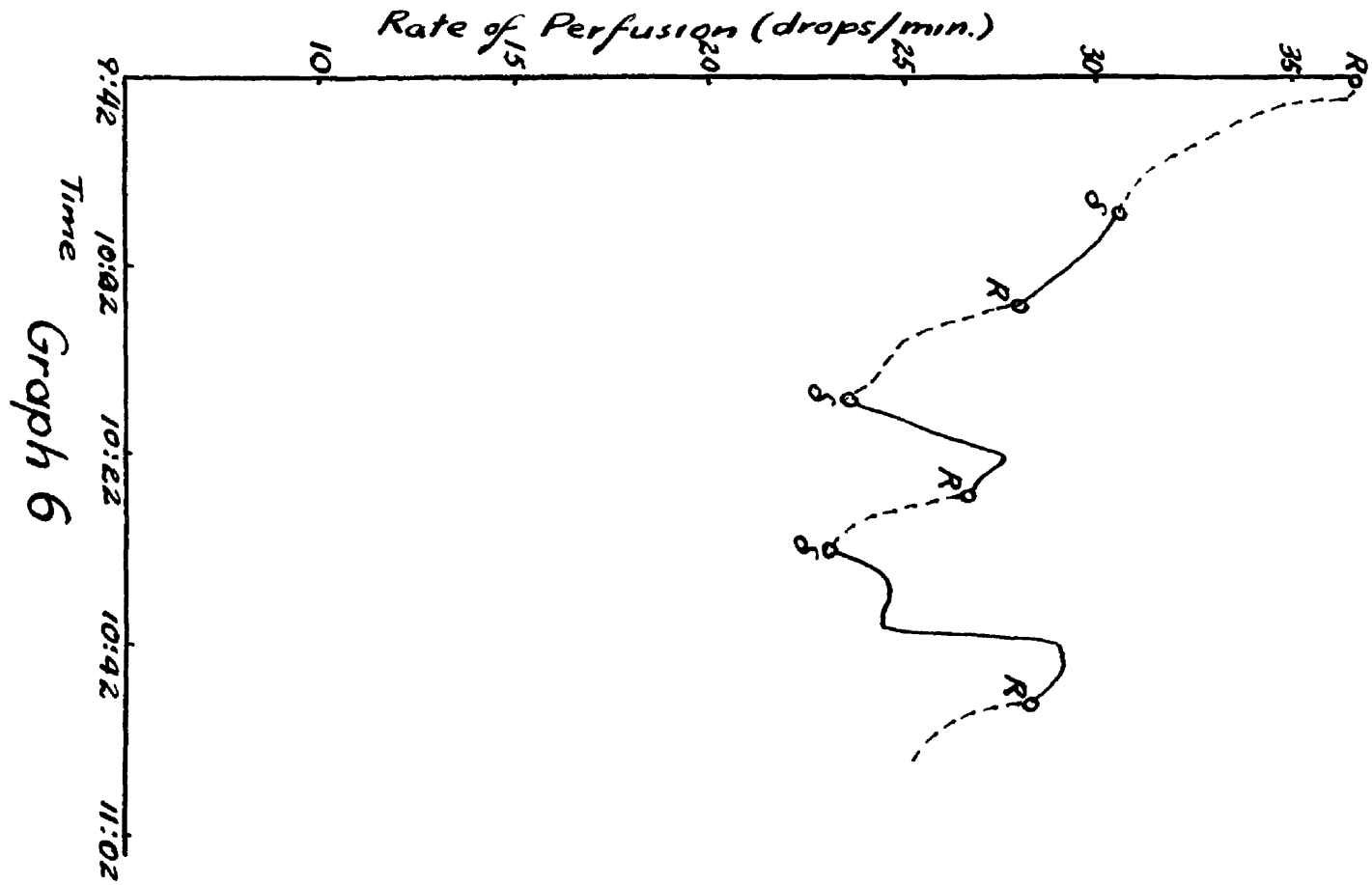

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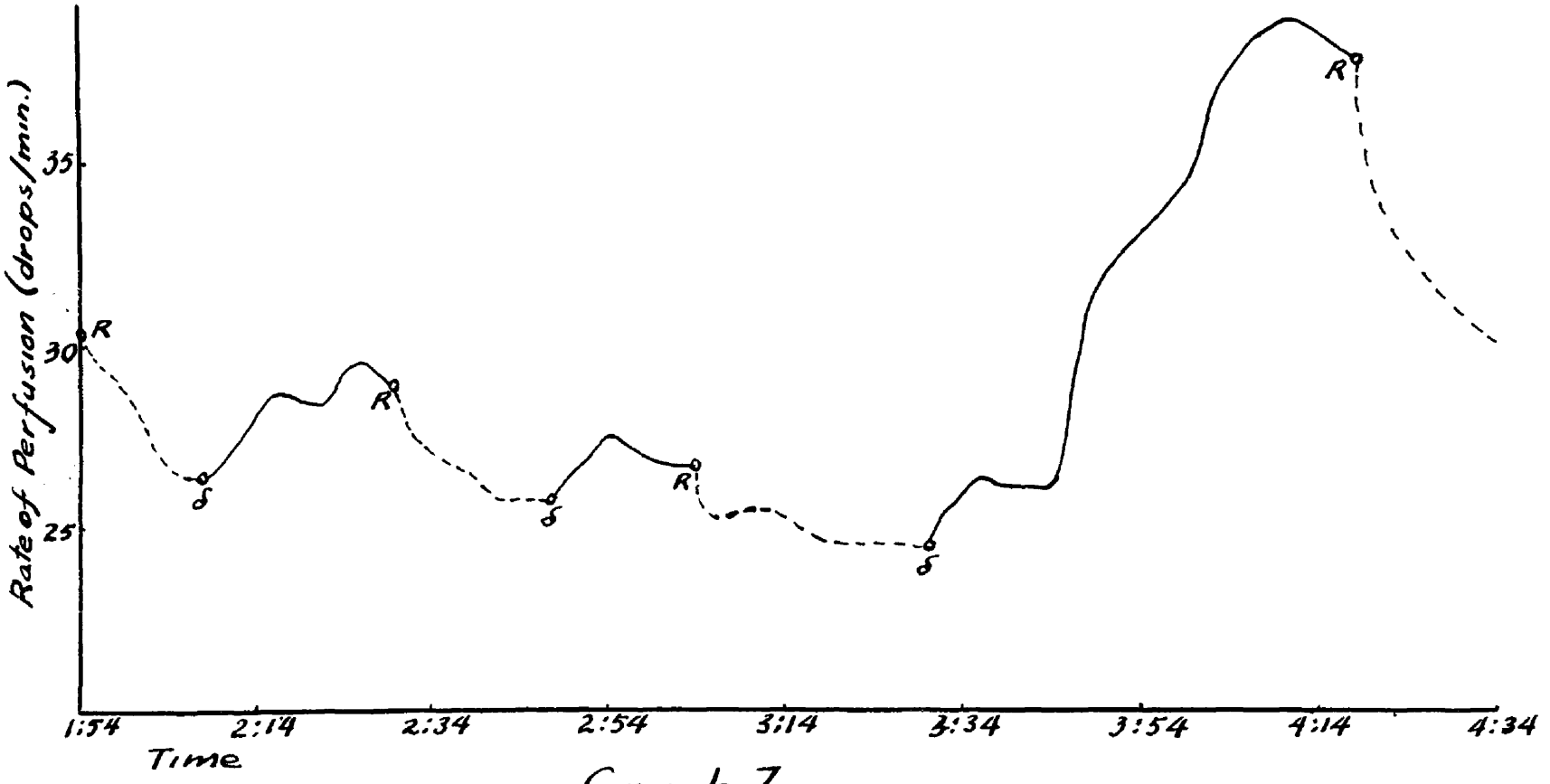
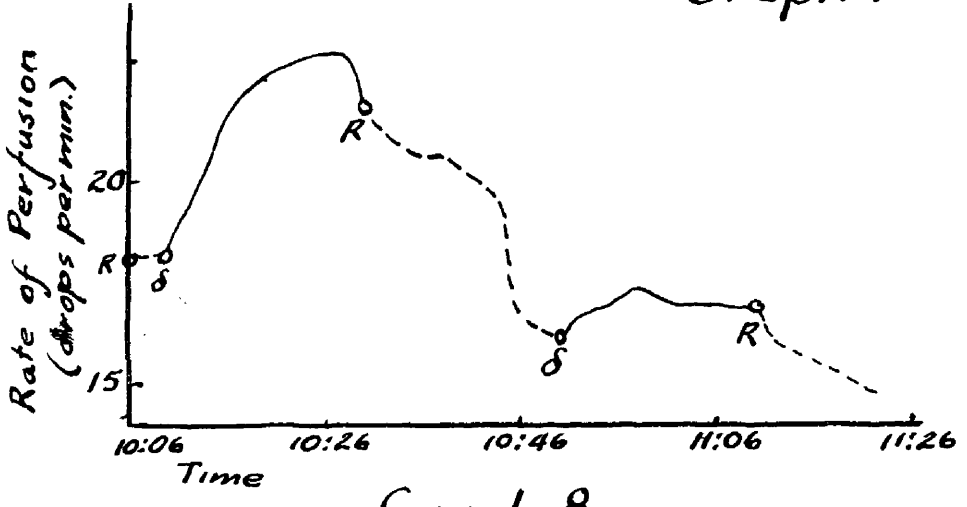
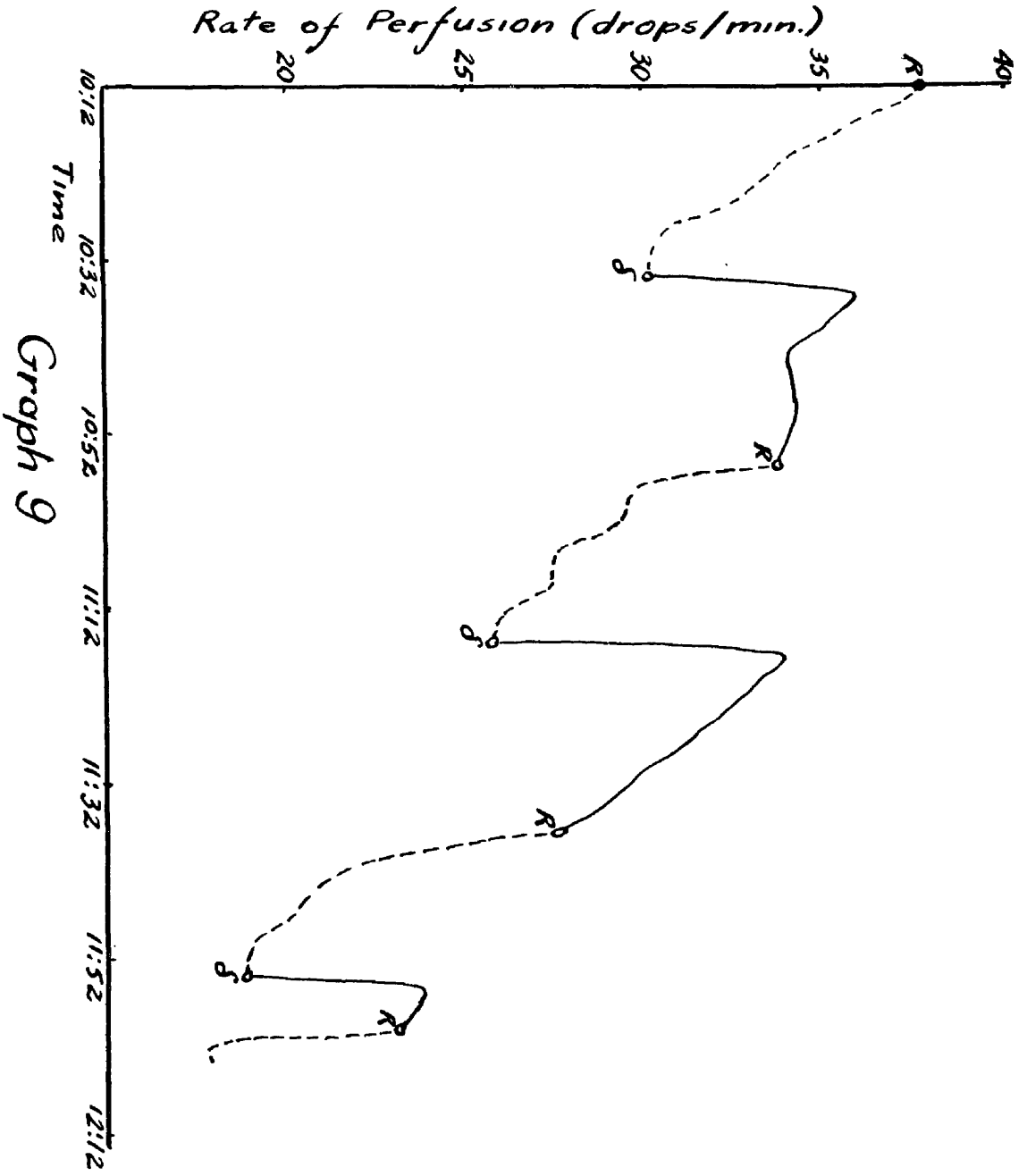


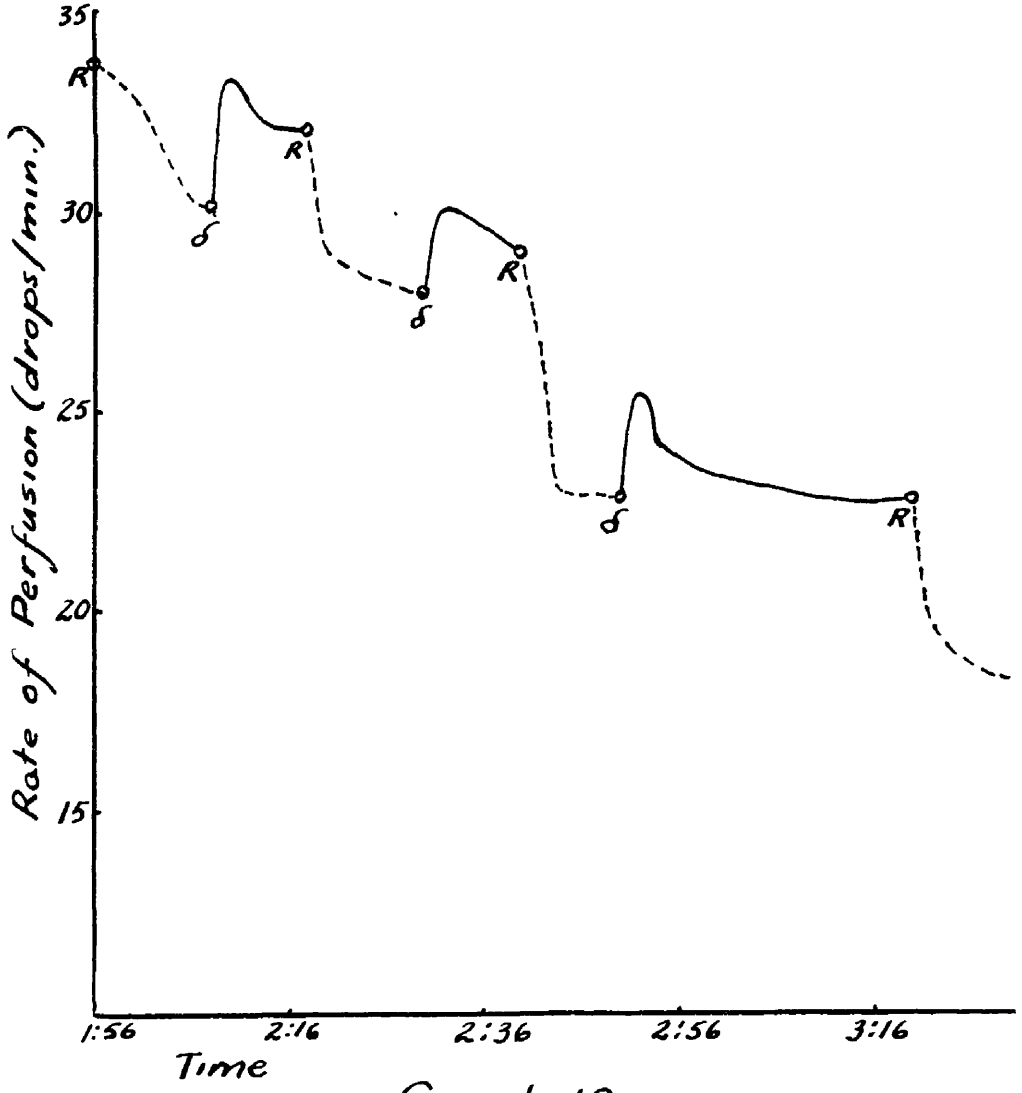
Graph 7



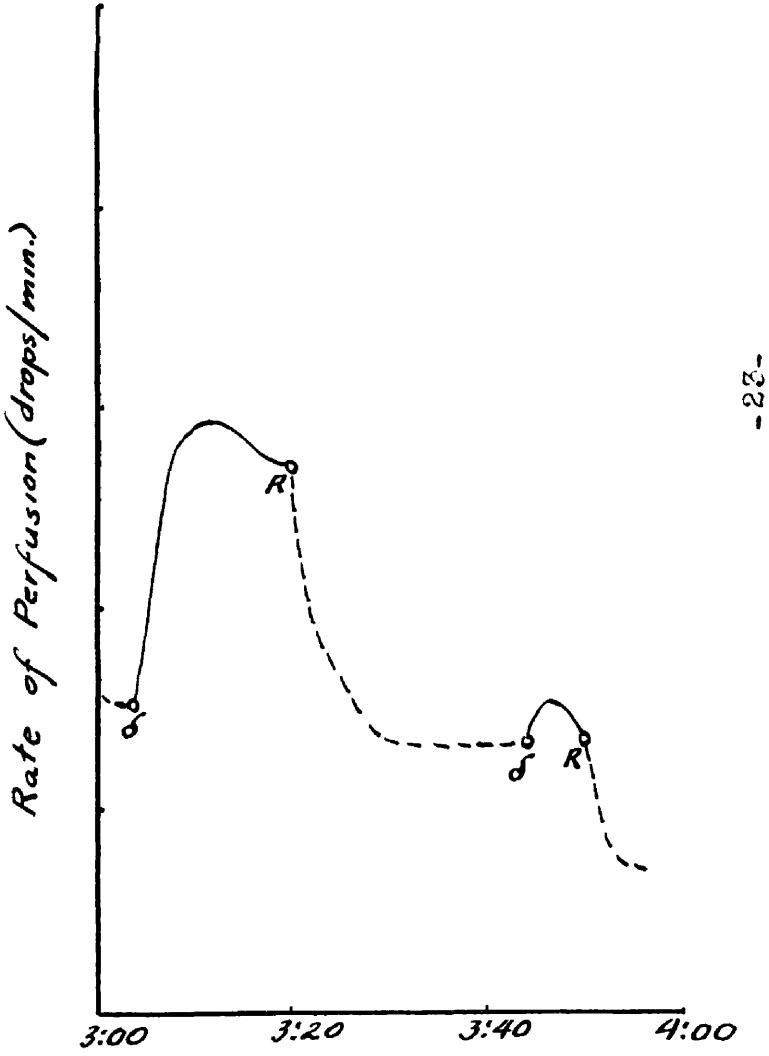
Graph 8


Rate of Perfusion (drops/min.)
20
25
30
35
40
R
S
R
S
R
S
R
10:12
10:32
10:52
11:12
11:32
11:52
12:12
Time

Graph 9



Graph 10



Graph 11

II 3. Effect on Peripheral Circulation:-

The technique employed for perfusion of frog legs was that devised by A. Laewen[34] and improved by P. Trendelenburg[35]. Instead of injecting the drug solution into the perfusion cannula, however, it was thought preferable to use a Y-cannula and perfuse the saturated solution of isoartemisin (80 mgm. per 1.) and the Howell-Ringer solution separately from Mariotte bottles. The perfusion pressure of 150 mm. was kept constant by adjusting the glass tubes in the Mariotte bottles to the same level. The rate of perfusion was determined indirectly every two minutes by finding the time required for twenty drops to be delivered from the cannula tied in the median abdominal vein. The perfusate was collected in a 50 cc. graduated cylinder and the amount recorded.

As will be seen on the graphs (1 to 11) and table (No.VII) perfusion of isoartemisin caused a variable increase in rate in all but two cases. It is believed that in these two cases not enough time was allowed for the rate of perfusion of the Howell-Ringer solution to become approximately constant. This is indicated by the sudden decrease in rate on returning from the drug solution to Howell-Ringer solution. No entirely satisfactory explanation

can be offered for the variable increase in rate of perfusion obtained with the 0.008% isoartemisin solution. It is possibly due to the variable extent of the edema present. The swelling is certainly an objectionable feature of the experiment and tends to obscure the results.

The vasodilator action of isoartemisin is a property of santonin which has not been modified by oxidation. S. Bertino[36] has shown that 1:5000 to 1:10000 solutions of santonin have a marked dilator action on the blood vessels of the frog when tested by the Laewen-Trendelenburg method. He also reports a further dilation on reperfusing Ringer's solution after santonin. This was not observed after perfusing with isoartemisin.

Table VII, Exp. 1, 2 and 3.

	Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
R	4	22	41.0	29.3
		24	41.2	29.2
D		26	42.4	28.3
		28	44.2	27.2
		30	44.6	27.0
		32	45.0	26.7
		34	45.8	26.2
		36	47.6	25.2
		38	48.2	24.9
		40	49.2	24.4
		42	50.4	23.8
		44	50.6	23.8
		46	51.0	23.5
R		50	57.4	20.9
		52	57.2	21.0
		54	58.0	20.7
		56	58.0	20.7
		58	57.0	21.0
	5	00	57.0	21.0
		2	54.0	22.2
D		4	52.6	22.8
		6	53.0	22.6
		8	53.4	22.5
		10	53.0	22.6
		12	57.4	20.9
R		14	58.6	20.5
		16	59.6	20.2
		18	59.2	20.3

	Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
	3	42	36.4	33.0
		46	38.8	30.9
R		48	39.8	30.2
		50	39.8	30.2
		52	40.7	29.5
		54	41.8	28.7
		56	42.5	28.2
		58	43.5	27.6
	4	00	45.4	26.4
		4	48.5	24.8
		6	50.4	23.8
		10	51.8	23.2
		12	53.4	22.4
		14	55.0	21.8
		16	56.8	21.1
		18	57.6	20.9
		20	57.8	20.8
		22	57.2	21.0
D		24	54.4	22.0
		26	52.8	22.7
		28	52.6	22.8
		30	52.4	22.9
		32	52.6	22.8
		34	53.0	22.6
		36	55.4	21.6
R		38	58.2	20.6
		40	59.3	20.2
		42	60.1	20.0
		44	61.2	19.6
		46	62.0	19.4
		48	59.8	20.1
D		50	57.6	20.8
		52	57.0	21.0
		54	57.3	20.9
		56	58.0	20.7
		58	58.2	20.6
	5	00	58.4	20.6
		2	61.2	19.6
R		4	64.0	18.8
		6	65.2	18.4
		8	66.0	18.2
		10	66.8	18.0

	Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
	3	42	65.0	18.5
		44	65.4	18.3
R		46	71.0	16.9
		48	73.0	16.4
		50	76.0	15.8
		52	56.6	21.2
D		54	56.7	21.2
		56	59.3	20.2
		58	65.5	18.3
			R	
	4	16	98.2	12.2
		18	103.2	11.6
		22	78.6	15.3
D		24	76.8	15.6
		26	76.0	15.8
		28	76.2	15.8
		30	76.5	15.7
		34	78.4	15.3
		36	78.2	15.4
		38	79.4	15.1
		40	79.6	15.1
		42	83.7	14.4
R		44	86.8	14.0
		46	88.0	13.6
		48	88.4	13.6

Table IV, Exp. 4, 5 and 6.

Time	Hr.	Min.	Sec./ 20 drops	Drops/ min.
12		00	88.2	13.6
R		2	90.2	13.3
		4	91.6	13.1
		6	88.0	13.6
D		8	85.2	14.1
		10	84.6	14.2
		12	85.0	14.1
		14	85.2	14.1
		16	84.8	14.2
		18	84.8	14.2
		20	96.6	12.4
R		22	101.0	11.9
		24	103.4	11.6
		26	107.6	11.2
		28	87.2	13.8
D		30	75.0	16.0
		32	72.6	16.6
		34	73.2	16.4
		36	72.4	16.6
		38	73.8	16.3
		40	75.6	15.9
		42	75.4	15.9
		44	76.7	15.7
		46	76.8	15.6
		48	78.2	15.4
		50	79.2	15.2
		52	80.0	15.0
		54	93.4	12.9
R		56	94.0	12.8
		58	93.8	12.8
1		00	95.4	12.6
		2	94.2	12.7
		4	92.6	13.0
		6	92.6	13.0
		10	92.6	13.0
		14	85.2	14.1
D		16	71.2	16.9
		18	68.8	17.4
		20	69.8	17.2
		22	70.0	17.2
		24	69.4	17.3
		26	70.0	17.2
		28	78.4	15.3
R		30	85.1	14.1
		32	88.8	13.5
		34	85.2	14.1
		36	84.8	14.2
		38	84.8	14.2
12		24	40.8	29.4
R		26	44.0	27.3
		28	45.6	26.4
		30	46.6	25.8
		32	47.5	25.3
		34	49.0	24.5
		36	50.4	23.8
		38	53.0	22.6
		40	53.7	22.4
		42	56.4	21.3
		44	55.0	21.8
		46	56.0	21.4
D		~~45~~	~~52.4~~	~~22.9~~
		50	52.6	22.8
		52	51.6	23.3
		54	53.0	22.6
		56	52.2	23.0
		58	53.0	22.6
1		00	52.3	23.0
		2	52.3	23.0
		4	57.9	20.7
R		6	59.2	20.3
		8	60.1	20.0
		10	63.6	18.9
		12	62.0	19.4
		14	63.2	19.0
		42	32.8	36.6
R		44	32.8	36.6
		46	35.5	33.8
		48	36.4	33.0
		52	38.6	31.1
		54	39.0	30.8
		56	39.2	30.6
		58	39.8	30.2
	10	00	40.0	30.0
D		2	41.6	28.9
		4	42.2	28.5
		6	43.2	27.8
		8	46.2	26.0
R		10	48.2	24.9
		12	49.1	24.4
		14	49.6	24.2
		16	51.6	23.3
		20	46.2	26.0
D		22	43.4	27.6
		24	44.4	27.0
		26	45.2	26.6
		28	49.4	24.3
R		30	51.2	23.4
		32	51.9	23.1
		34	49.4	24.3
D		36	48.8	24.6
		38	49.2	24.4
		40	49.6	24.2
		42	41.4	29.0
		44	41.2	29.1
		46	41.8	28.7
		48	42.6	28.2
		50	46.0	26.1
R		52	47.3	25.4
		54	47.8	25.1

Table VII, Exp. 7 and 8.

	Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
	1	54	39.2	30.6
R		56	40.7	29.5
		58	41.2	29.2
	2	02	43.2	27.8
		4	45.4	26.4
		6	45.2	26.6
		8	45.6	26.4
		10	43.6	26.8
D		12	43.8	27.4
		14	43.6	27.6
		16	41.8	28.7
		18	42.0	28.6
		20	42.2	28.5
		22	42.4	28.3
		24	41.0	29.3
		26	40.6	29.6
		28	41.8	28.7
		30	41.6	28.9
		32	43.4	27.6
R		34	44.7	26.9
		36	45.1	26.6
		38	44.8	26.8
		40	46.0	26.1
		42	46.6	25.8
		44	47.2	25.5
		46	46.4	25.9
		48	46.2	26.0
		50	45.1	26.6
D		52	44.4	27.0
		54	43.4	27.6
	3	00	45.2	26.6
		2	44.8	26.8
		4	44.8	26.8
		6	47.5	25.3
R		8	47.6	25.3
		10	46.8	25.6
		12	46.8	25.6
		14	48.0	25.0
		16	47.6	25.3
		24	48.8	24.6
		26	48.8	24.6
		28	48.2	24.9
		30	49.0	24.5
		32	46.9	25.5
D		34	46.3	25.9
		36	45.4	26.4
		38	45.9	26.1
		40	46.0	26.1
		44	46.0	26.1
		48	38.6	31.1
		50	37.6	31.9
		52	36.0	32.3
		54	35.8	33.5
		56	35.8	33.5
		58	35.8	33.5
	4	00	34.4	34.9
		2	32.6	36.8
		4	31.5	38.1
		6	31.6	38.0
		8	31.2	38.5
		10	30.8	38.9
		12	31.0	38.7
		14	31.6	38.0
		16	31.4	38.2
		18	31.8	37.7
		20	35.2	34.1
R		22	36.4	33.0
		24	37.2	32.3
		26	37.6	31.9
		28	38.6	31.1
		30	38.8	30.9
		32	39.8	30.2
		34	39.9	30.1
		36	40.4	29.7
		38	40.6	29.6
		40	42.0	28.6
		42	42.2	28.4
	10	06	66.3	18.1
R		8	65.8	18.2
		10	66.2	18.2
		12	62.0	19.4
D		14	59.0	20.4
		16	55.2	21.7
		18	53.9	22.2
		20	52.7	22.8
		22	53.2	22.6
		24	54.9	21.9
		26	53.0	22.7
		28	52.9	22.7
		30	55.3	21.7
		32	56.0	21.4
R		34	57.9	20.7
		36	58.2	20.6
		38	58.0	20.7
		40	58.9	20.4
		42	60.2	19.9
		44	61.5	19.5
		46	72.5	16.6
		48	73.0	16.4
		50	74.2	16.2
		52	72.0	16.7
D		54	71.2	16.8
		56	71.2	16.8
		58	68.8	17.4
	11	00	70.6	17.0
		2	71.0	16.9
		4	70.8	17.0
		6	71.2	16.8
		8	71.6	16.8
		10	71.2	16.9
		12	75.0	16.0
R		14	75.6	15.9
		16	77.2	15.6
		18	77.8	15.4
		20	79.8	15.0
		22	81.0	14.8

Table VII, Exp. 9.

Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
10	12	31.8	37.7
R	14	32.2	37.3
	16	33.3	36.0
	18	34.0	35.3
	20	35.2	34.1
	22	36.4	33.0
	24	36.2	33.2
	26	37.0	32.4
	28	39.0	30.8
	30	39.4	30.4
	32	39.8	30.2
	34	39.8	30.2
	36	33.4	35.9
D	38	33.8	35.5
	40	34.2	35.1
	42	35.2	34.1
	44	35.4	33.9
	46	35.1	34.2
	48	35.1	34.2
	50	35.0	34.3
	52	35.4	33.9
	54	35.4	33.9
	56	35.6	33.7
	58	40.2	29.9
R 11	00	40.6	29.6
	2	40.6	29.6
	4	41.6	28.9
	6	44.0	27.3
	8	43.9	27.4
	10	45.4	26.4
	12	46.0	26.1
	16	46.8	25.7
	18	35.4	33.9
D	20	36.2	33.2
	22	36.6	32.8
	24	37.3	32.2
	26	38.0	31.6
	28	38.6	31.1
	30	40.0	30.0
	32	42.3	28.4
	34	41.2	29.2
	36	42.0	28.6
	38	44.0	27.3
11	40	50.8	23.6
R	42	55.8	21.5
	44	57.8	20.8
	46	58.8	20.4
	48	60.1	20.0
	50	63.0	19.0
	52	63.4	18.9
	54	64.0	18.8
	56	50.4	23.8
D	58	50.8	23.6
12	00	51.8	23.2
	2	67.4	17.8
R	4	67.2	17.9

Table VII, Exp. 10 and 11.

	Time Hr.	Min.	Sec. / 20 drops	Drops/ min.
	1	56	35.6	33.7
R		58	36.0	33.4
	2	00	36.4	33.0
		2	37.2	32.3
		4	38.6	31.1
		6	39.6	30.3
		8	39.8	30.2
		10	36.1	33.3
D		12	36.7	32.7
		14	37.4	32.1
		16	37.2	32.3
		18	37.4	32.1
		20	41.6	28.9
R		22	42.1	28.6
		24	42.8	28.0
		28	42.3	28.4
		30	42.8	28.0
		32	39.9	30.1
D		34	40.2	29.9
		36	40.6	29.6
		38	41.0	29.3
		40	41.6	28.9
		42	44.9	26.7
R		44	52.6	22.8
		46	52.4	22.9
		48	52.3	22.9
		50	52.6	22.8
		52	47.0	25.5
D		54	49.8	24.1
		56	49.8	24.1
		58	51.2	23.5
	3	04	51.8	23.2
		16	52.8	22.7
		20	53.6	22.8
		22	62.0	19.7
R		24	63.6	18.9
		28	65.4	18.4
		30	65.6	18.3

	Time Hr.	Min.	Sec./ 20 drops	Drops/ min.
	2	40	52.4	22.9
R		42	56.3	21.3
		44	55.2	21.8
		52	58.6	21.5
		58	64.9	18.5
	3	00	67.4	17.8
		2	66.2	17.6
		4	67.8	17.7
		8	50.0	24.0
D		10	49.4	24.3
		12	48.7	24.7
		14	49.1	24.5
		16	49.8	24.1
		18	50.6	23.7
		20	51.2	23.5
		22	60.6	19.8
R		24	64.2	18.7
		26	67.2	17.9
		28	70.2	17.1
		30	71.8	16.7
		44	72.0	16.7
		46	67.8	17.7
D		48	69.0	17.4
		50	72.0	16.7
		52	85.2	14.1
R		54	88.2	13.6
		56	89.0	13.5

Table VIII.

Rate of Perfusion (drops/min.)

Experiment No.		Ringer's	S.S. of -Oxysantanin in Ringer's	Change in rate (drops/min.)	Per Cent change in rate
1	a	29.3	29.2	-0.1	--
	b	21.0	22.8	1.8	8.6
2	a	20.8	22.9	2.1	10.1
	b	19.4	21.0	1.6	8.2
3	a	15.8	21.2	5.4	34.2
	b	11.6	15.8	4.2	36.2
4	a	13.1	14.2	1.1	8.4
	b	11.2	16.6	5.4	48.2
	c	13.0	17.4	4.4	33.8
5	a	21.4	23.3	1.9	8.9
6	a	30.6	30.2	-0.4	--
	b	23.3	27.0	3.7	15.9
	c	23.1	29.1	6.0	26.0
7	a	26.4	29.6	3.2	12.1
	b	26.0	27.6	1.6	6.2
	c	24.5	38.9	14.4	58.8
8	a	18.2	22.8	4.6	25.3
	b	16.2	17.4	1.2	7.4
9	a	30.2	35.9	5.7	18.9
	b	25.7	33.9	8.2	31.9
	c	18.8	23.8	5.0	26.6
10	a	30.2	33.3	3.1	10.3
	b	28.0	30.1	2.1	7.5
	c	22.8	25.5	2.7	11.8
11	a	17.7	24.7	7.0	39.6
	b	16.7	17.7	1.0	6.0

II 4. Ability to Act as a Diuretic:-

Diuresis has frequently been observed after the oral administration of santonin[20,21,22]. Knipping and Seel [22] reported a 10% - 20% increase in the volume of urine excreted after the drug was ingested by humans and rabbits. The diuresis began in ½ hour and lasted for 24 hours.

It had been observed in the first group of experiments that the urine volume was greater after administration of δ-oxysantonin than in the untreated animals. Consequently the amount of urine excreted was measured in the cases of animals (11) and (12).

The increase was found to be especially marked during the second day of fasting. The increased urine output may be caused by injury of the kidney tubules.

Protocol #1 - Oxysantomin Diuresis:-

Rabbit #11.

Date	Urine Volume	Remarks
3/30 - 3/31	26	
3/31 - 4/1	29	First day of fasting.
4/1 - 4/2	235	Second day of fasting.
4/2 - 4/3	26	Animal fed.
4/3 - 4/6	245	Animal fasted 4/5 and 6
4/6 - 4/7	27	Oxys. adm. B.S. determined.
4/7 - 4/8	22	Animal fed. Urine pH-6 Sugar neg. to Fehling's.
4/8 -4/9	23	
4/9 - 4/11	425	Fasted 9-11.
4/11 - 4/14	74	Fed. Urine evap. in vac.
4/14 - 4/15	170	Fasted. B.S. determined. Oxys. adm.
4/15 - 4/16	57	Fed. Urine orange-red in color.
4/16 - 4/17	17	
4/17 - 4/18	43	Fasted 24 hours. B.S.O.
4/18 - 4/20	79	Fed. Food withdrawn from cage at 9 A.M. on 4/20.
4/20 -4/21	4	Cage contained no feces.
4/21 - 4/22	385	Fed 4/23 after B.S.
4/22 - 4/25	240	Food removed at 12 noon 4/25.
4/25 - 4/27	275	
4/27 - 4/28	185	Fed after B.S. at 10 A.M. 4/28.
4/28 - 5/2	175	Fed. Food withdrawn at noon 5/2.
5/2 - 5/4	145	

Protocol #2 - Oxysantonin Diuresis:-

Rabbit #12.

Date	Urine Volume	Remarks
4/9 to 4/11	210	Fasted. B.S. determined. Oxys. adm.
4/11 to 4/14	115	Fed. Urine evap.
4/14 to 4/15	350	Fasted. B.S. determined. Oxys. adm.
4/15 to 4/16	20	Fed.
4/16 to 4/17	--	Urine lost.
4/17 to 4/18	100	Some lost. Fasted 24 hours B.S.O.
4/18 to 4/20	41	Fed. Food withdrawn from cage at 9 A.M. on 4/20
4/20 to 4/21	110	Urine had extremely large amount of ppt. in it.
4/21 to 4/22	390	
4/22 to 4/25	210	Fed 4/23 after B.S.
4/25 to 4/27	205	Food removed 12 noon 4/25.
4/27 to 4/28	115	Fed after B.S. at 10 A.M. 4/28.
4/28 to 5/2	240	Fed. Food withdrawn at noon 5/2.
5/2 to 5/4	210	

II 5. Ability to Lower the Blood-Sugar Level:-

A search of the literature for a basis for a pharmacological classification of drugs (other than insulin) which lower blood sugar indicates that most of these substances cause increased glycogen deposition in the liver by direct or reflex stimulation of the parasympathetic nervous system. Many of the reflex stimulants also produce degenerative changes in the liver. The more important of these drugs have been listed in table IX. On the whole, the results obtained by use of these substances have been rather disappointing, both experimentally and clinically. Such effects which have been obtained depend on their ability to stimulate the remaining healthy islet tissue[50].

The role played by the vagus in blood-sugar regulation has been the subject of much experimental work. de Corral[51] obtained a small decrease in blood-sugar level within an hour in five out of eight experiments in which the right vagus of anesthetized dogs was stimulated after section of the hepatic plexus. McCormick and co-workers[52] stimulated the right

vagus electrically at the oesophageal end of the stomach of anesthetized dogs and of decerebrated cats. A prompt fall was noted in one experiment and a less decided fall in four other experiments. Britton[53] anesthetized cats with barbituric acid and cut the branches of the right vagus to the stomach and upper intestine, sectioned the hepatic plexus as a rule, and removed the left adrenal or cut its nerve supply. Several hours after the operation was performed, he stimulated the right vagus at the oesophageal end of the stomach for a period of one to two hours. He obtained a fall of blood sugar of 18 mgm. per hour in eleven experiments in which the hepatic nerves were cut and a fall of 27 mgm. per hour in six experiments in which the hepatic nerves were intact. No effect was produced on the blood-sugar level if the blood vessels and nerves of the pancreas were tied off before stimulation. Britton concluded that impulses to the islet tissue through the vagus might come into play only when hormonic regulation was inadequate to maintain the normal blood-sugar level or as an emergency measure.

La Barre[54] and Zunz and La Barre[55] proved by means of transfusion experiments the importance of the vagus mechanism in blood-sugar regulation.

Table IX.

A. Central Parasympathetic Stimulants.

1. Codeine[37]
2. Opium[38]
3. Salicylic Acid[39]

B. Reflex Parasympathetic Stimulants.

1. Gastric Lavage[40]
2. Bitter Blaar[41]
3. Synthalin[42]
4. Sulfonated Bitumen[43]
5. Solanum[44]
6. Anticoman[45]
7. Iodoform[46]

C. Peripheral Parasympathetic Stimulants.

1. Vagotonine[47]
2. Pilocarpine[48]
3. Choline[49]

G. A. Clark[56] showed in the rabbit that drugs which stimulate parasympathetic nerve endings caused lowering of blood-sugar level but usually failed to do so after section of the right vagus. After section of the right vagus below the cardiac branches, the blood sugar fell almost immediately. The tolerance for glucose on injection of 1 Gm. per kilo. was not decreased but the blood sugar returned to the normal level more rapidly than before vagotomy. After two months, however, this condition was reversed: hyperglycemia lasted considerably longer than in the normal. Clark stated that the regulatory fibers of the vagus appear to be of two kinds: tonic inhibitory and secretory.

Quigley et al.[57] reported only that the ability of double vagotomized dogs to remove glucose from the circulation was greater than that of the controls. The tests were performed two weeks after the operation.

Santonoise and co-workers[47] extracted and purified a solution of a substance, vagotonine, from the pancreas which he believed to be the hormone which caused glycogen storage in the liver. He found, clinically, that the dosage of insulin could be greatly reduced in favorable cases if it were supplemented by injections of vagotonine.

The hypoglycemic action of santonin has been studied both experimentally and clinically. Extremely variable results have been reported. Stasiak[58] adminstered

daily doses of 0.2 Gm. to 0.4 Gm. of the sodium salt in aqueous or oily suspension by stomach tube to rabbits over a period of about three weeks. The animals were fasted for 10 to 15 hours before each injection. No change in blood-sugar level occurred at first but at the end of this time hypotonicity, paresis, fits of convulsions of the extremities, lowering of body temperature, and hypoglycemia (in all but one case) resulted. The symptoms were relieved temporarily by the injection of dextrose in all except the case in which there was no hypoglycemia. Death resulted when the administration of dextrose was discontinued. Degeneration of the liver and absence of liver glycogen were found in every case. The kidneys showed small hemorrhagic spots and slight parenchymatous degeneration. The pancreases were normal. No sugar was found in the urine. He assumed that the disturbance of hepatic function was responsible for the symptoms observed. This conclusion is supported by the work of Mann and Magoth[59] on dogs which were totally hepatectomized by a special method. The same symptom complex was observed within an hour after the final operation, which involved removal of the liver. In addition to a progressive fall of blood-sugar level, they noted an increase of uric acid in the blood, the appearance of bilirubin in the plasma and urine, and a fall of muscle glycogen to 50% of the normal. The symptoms disappeared temporarily and the muscle-glycogen content rose to normal

on the injection of glucose.

Walterhöfer[60] treated 8 diabetics with santonin in doses of 0.025 Gm. The time of treatment was 15 days when one dose was given daily or 5 days when three doses were given daily. The drug was used in the form of Dr. Sejournet's pills[61] , pure santonin, and the sodium salt. The urine sugar was sometimes reduced for a few days but then returned to the former level or higher. There was no reduction in amount of acetoacetic acid in the urine nor was there any improvement in carbohydrate tolerance. Blood sugar was not determined.

Leulier and Roche[62] found that the injection of approximately 0.25 Gm. of santonin per kilo. arrested or decreased the glycosuria of phlorizinized rabbits without affecting the hyperglycemia. They formulated the hypothesis that the antiglycosuric action was due to a raising of the renal threshold for glucose, accompanied by oliguria, and suggested that santonin might be a useful adjuvant in the treatment of certain diabetics.

Sei'ichi Ishikawa[63] reported that 0.05 to 0.1 Gm. of santonin per kilo. perorally caused no change in the blood-sugar level of rabbits, while 0.9 Gm. per kilo. caused a temporary hyperglycemia. Santonin had no effect on the hyperglycemia of adrenalin or glucose. Continued administration in man caused no change in blood-sugar level.

Ijuro Fujii[64] and Bomer[65] proved experimentally that large doses of santonin cause an increase of blood sugar and lactic acid independent of the convulsant effect. Fujii demonstrated, in addition, that the hyperglycemia was independent of the effect on the respiratory center and on body temperature and that the alkali reserve was lowered.

In view of the above experimental and clinical results, the administration of santonin in diabetes would seem to be wholly undesirable.

The study of the action of δ-oxysantonin on the blood-sugar level of the rabbit was therefore undertaken to determine the effect of oxidation on the santonin molecule.

II 5-a. Ability to Lower the Blood-Sugar Level of Fasted Animals:-

Rabbits were fasted for at least 48 hours and blood-sugar estimations were made, using the recently developed Folin ferricyanide method[66].

A 0.1 cc. sample of blood is rendered protein-free by means of tungstic acid. Potassium ferricyanide is reduced in a sodium cyanide-sodium carbonate medium by the reducing substances of the blood. Colloidal Prussian blue is formed by adding ferric iron in 3% acacia and is compared colorimetrically with an appropriate standard which has been treated in the same manner.

After the control determination, δ-oxysantonin was administered by stomach tube. Samples were then taken after ½ hour, 1 hour, 2 hours, 3 hours, 4 hours, and in some cases after 5 hours.

Neither small nor large doses of isoartemisin had any effect on the blood-sugar level within four to five hours. Animals (1), (2), (3), and (7) died during the night so that the data are necessarily incomplete in these cases. Blood-sugar determinations were made on rabbit (6) when the level was critically low. Test of the urine with Fehling's solution showed no reducing substance. Three hours after the control level was determined, clonic convulsions set in with intervening periods of extreme muscular weakness. These convulsions were relieved by the intravenous administration of dextrose. The animal was fed after the blood sample for the next hour was taken. On the following morning a value of 112 mgm. per cent. was obtained. The animals treated with santonin by Stasiak, as well as those on which Mann and Magoth performed a total hepatectomy, reacted similarly.

Table IX. Blood-Sugar Level after Administration of Isoartemisin

No. and Sex	Date 1935	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube.
	5/27	1.9	136	128	121	120	112	--	103	0.1 Gm./ kilo.after c
1 ♀	5/29	1.9	112	133	141	117	100	--	112	0.4 Gm./ kilo.after c Boil emulsion
	6/24	1.67	119	--	--	--	--	--	--	
	7/9	1.71	106	124	119	124	119	--	--	0.18 Gm. after c
	7/10-11		--	--	--	--	--	--	--	0.18 Gm. daily
	7/12		106	104	103	--	--	111	--	0.18 Gm. after c
	7/13		--	--	--	--	--	--	--	0.18 Gm.
	7/15		100	114	110	114	112	--	--	0.18 Gm. after c
	7/16-17									0.18 Gm.daily
	7/18	1.72	116	110	111	110	117	115	--	0.18 Gm. after c
	7/19									0.18 Gm.
	7/20									0.36 Gm.
	7/22	1.70	106	108	110	107	112	109	--	0.18 Gm. after c
	7/23-24									0.18 Gm.daily
	7/25		99	101	113	123	121	110	--	0.18 Gm. after c
	7/29		103	115	113	101	--	--	--	0.18 Gm.daily 7/26,(27,28), 29 after c
	8/1		107	98.5	100	103	99.0	90.9	--	0.18 Gm.after c
	8/5		94.8	98.6						No drug
	8/15									Dead. Organs removed and preserved in H.CHO

Table IX. Blood-Sugar Level After Administration of Isoartemisin

No. and Sex	Date 1935	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube
2 ♀	5/27	1.82	136	125	116	132	132	--	116	0.1 Gm./ kilo after c
	5/29	1.82	124	125	122	124	124	--	117	0.4 Gm./ kilo (oil emul.) after c
	6/24	1.47	100	--	--	--	--	--	--	
	7/5									Dead. Organs removed and preserved in H·CHO
3 ♀	5/30	2.0	118	141	132	122	127	--	102	1.2 Gm./ kilo (oil emuls) after c
	6/20	2.37	104	132	115	123	142	123	--	0.18 Gm. after c
	7/8	2.4	101	110	114	112	110	107	--	1.2 Gm./ kilo after c
	7/12		103	--	--	--	--	--	--	Died same night. Organs removed and preserved in H·CHO
4 ♀	5/28	1.83	117	121	122	123	123	--	123	0.2 Gm./ kilo after c
5 ♀	5/28	1.87	130	128	123	119	--	--	119	0.2 Gm./ kilo after c

Table IX. Blood-Sugar Level after Administration of Isoartemisin

No. and Sex	Date 1935	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube
6 ♂	5/30	1.8	103	129	127	125	129	--	107	1.2 Gm./kilo (oil emuls.) after c
	6/20	1.53	plus 46	50	45.9	32	*35.4	*196	--	0.18 Gm. after c Plus ½ Std. for comparison *clonic convulsion ∝: after intravenous glucose
	6/21		112							
	6/25		--	--	--	--	--	--	--	Dead. Organs removed and preserved in H CHO
7 ♂	6/26	1.83	110	--	128	123	124	--	--	0.18 Gm. after c
	6/27-28									0.18 Gm. daily
	6/29									0.36 Gm.
	7/1		121	149	146	149	150	146	--	0.18 Gm. after c
	7/2									0.18 Gm.
	7/3									0.36 Gm.
	7/5		--	--	--	--	--	--	--	Dead. Organs removed and preserved in H CHO

Table IX. Blood-Sugar Level After Administration of Isoartemisin

No. and Sex	Date 1935	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube
	6/26	2.22	114	--	132	119	115	--	--	0.18 Gm. after c
♀	6/27-28									0.18 Gm. daily
♂	6/29									0.36 Gm.
	7/1		120	127	126	116	114	115	--	0.18 Gm. after c
	7/2									0.18 Gm.
	7/3									0.36 Gm.
	7/5		--	--	--	--	--	--	--	0.36 Gm.
	7/6-7		--	--	--	--	--	--	--	0.18 Gm. daily
	7/8	2.33	102	110	111	117	114	108	--	0.18 Gm. after c
	7/9-10-11									0.18 Gm. daily
	7/12		101	109	116	--	115	114	--	0.18 Gm. after c
	7/13									0.18 Gm.
	7/15		112	123	118	117	112	--	--	0.18 Gm. after c
	7/16-17									0.18 Gm. daily
	7/18	2.11	108	120	116	120	123	129	--	0.18 Gm. after c
	7/19									0.18 Gm.
	7/20									0.36 Gm.
	7/22	2.28	100	106	107	112	--	113	--	0.18 Gm. after c
	7/23-24									0.18 Gm. daily
	7/25		101	102	108	108	112	114	--	0.18 Gm. after c
	7/26									0.18 Gm.
	7/27									0.36 Gm.
	7/29		86.3	98.1	113	102	109	--	--	0.18 Gm. after c
	8/1		103	109	100	107	100	96.7	--	0.18 Gm. after c
	8/5		96.3	98.6						No drug
	8/21		106							No drug
	9/16									Sacrificed. Liver saved for path. exam.

Table IX. Blood-Sugar Level after Administration of Isoartemisin

No. and Sex	Date 1936	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube
11 ♂	4/6	1.6	98.6	110	118	--	--	--	--	1 Gm. O/ kilo after c B.S. 110 after 17 hrs.
	4/11	1.6	109	137	137	126	--	--	--	1 Gm. O/ kilo after c
	4/15		97	115	118	114	--	--	106	1 Gm.O/ kilo after c
	4/18	1.9	101	114	118	113	--	--	--	Fasted 24 hrs. 1 Gm.O/ kilo after c
	4/21		101	--	--	--	--	--	103	Fasted 24 hrs.
	4/22		106	--	--	--	--	--	--	Fasted 53 hrs.
	4/23		106	--	--	--	--	--	--	Fasted 71 hrs.
	4/28		108	--	--	--	--	--	--	Fasted 72 hrs.
	5/5		118	--	--	--	--	--	--	Fasted 72 hrs.
	5/11		96	--	--	--	--	--	89	Fasted 48 hrs. Urine reduced Fehling's sol'n.
	5/12	1.62	95	128	128	--	114	--	110	1 Gm. O/ kilo after c

Table IX. Blood-Sugar Level after Administration of Isoartemisin

No. and Sex	Date 1936	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2hrs.	3hrs.	4 hrs.	5 hrs.	Remarks Oxys. adm. by stomach tube
12 ♂	4/11	1.52	98	121	120	114	--	--	--	1 Gm.O/ kilo after c
	4/15		97	116	110	108	--	--	101	1 Gm.O/ kilo after c
	4/18	1.7	96	107	118	112	--	--	--	1 Gm. O/ kilo after c
	4/21		96	--	--	--	--	--	96	Fasted 24 hrs.
	4/22		118	--	--	--	--	--	--	Fasted 53 hrs.
	4/23		112	--	--	--	--	--	--	Fasted 71 hrs.
	4/28		106	--	--	--	--	--	--	Fasted 72 hrs.
	5/5		107	--	--	--	--	--	--	Fasted 72 hrs.
	5/11		100	--	--	--	--	--	102	Fasted 48 hrs. Urine reduced Fehling's solution
	5/12		113	159	159	--	129	--	118	1 Gm. O/ kilo after c

II 5b. Ability to Lower Blood-Sugar Level of Animals which had Received Dextrose:-

Since no lowering of blood-sugar level was obtained on administration of δ-oxysantonin by stomach tube to fasting rabbits, it was thought advisable to find whether isoartemisin would affect the alimentary hyperglycemia caused by the administration of a 20% solution of dextrose in water.

Control experiments were performed in which 4 Gm./kilo. of dextrose was given by stomach tube. Then 4 Gm./kilo. of dextrose and varying doses of δ-oxysantonin were administered simultaneously.

The isoartemisin did not affect the blood-sugar level in alimentary hyperglycemia significantly. Bertino[67] found that 0.15 Gm./kilo. of santonin when dissolved in ethylene glycol (3 cc./kilo.) and administered to rabbits intramuscularly produced an exaltation after 1 hour of the hyperglycemia caused by the oral administration of 6 Gm./kilo. of dextrose. He did not observe a significant increase after 0.01 Gm./kilo. of santonin and 3 Gm./kilo. of dextrose.

Table X. Blood-Sugar Level after Isoartemisin and Glucose

No. and Sex	Date 1935	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	Remarks. Oxys. adm. by stomach tube.
9 ♀	7/19	1.4	108	110	116	110	115	116	Control exp. 10 cc. water given by s.t. after c
	7/23	1.48	138	189	194	164	185	144	4 Gm. dextrose/ kilo after c
	7/26		107	203	222	205	164	113	(4 Gm.dextrose/ (after c (0.1 Gm.Oxys/ kilo
	8/2		115	170	180	170	115	110	(4 Gm.dextrose/ kilo after c (0.2 Gm. Oxys/ kilo
	8/21	1.70	119	230	248	210	135	110	(4 Gm.dextrose/ kilo after c (0.4 Gm. Oxys/ kilo.
10 ♂	7/19	1.96	100	106	109	109	113	112	Control exp. 10 cc. water given in s.t. after c
	7/23	2.08	128	187	198	162	154	130	4 Gm. dextrose/ kilo after c
	7/26		102	212	217	190	175	94.3	(4 Gm.dextrose/ kilo 0.1 Gm.Oxys./ kilo
	8/2		100	175	196	179	142	122	4 Gm. dextrose/ kilo after c 0.2 Gm. Oxys/ kilo
	8/21	2.13	114	197	231	208	192	107	4 Gm. dextrose/ kilo after c 0.4 Gm.Oxys/ kilo

II 6a. Toxicity and Pathological Effects Produced on Liver and Kidney of Rabbits:-

Orally administered doses of 0.4 Gm. per kilo. of δ-oxysantonin were given to rabbits twice, 1 Gm./ kilo. nine times, and 1.2 Gm./kilo. given three times without appearance of convulsions or significant change of blood-sugar level within five hours. Santonin, on the other hand, is a convulsant poison when 0.5 Gm./kilo, is given by stomach to the rabbit[31]. The insolubility of the compound may, however, have prevented the appearance of acute toxic effects. Convulsions with hypoglycemia were noted in the case of rabbit (6) 21 days after the administration of 1.2 Gms. of the drug per kilo. of body weight.

The livers and kidneys of rabbits (1), (2), (3), (6), (7), and (8) were examined histologically by Dr. C. G. Warner. Advanced degenerative changes were found in the livers of (1), (2), and (8) and in the tubules of kidneys (1), (3), (6), and (7). One plus degeneration was observed in the remaining organs. The following report was submitted:

> RABBIT #1: Liver 92 Gms.
> Two sections from liver, both show advanced degenerative changes in the liver cells. This is generalized and evenly distributed throughout the liver, without regard to lobular zone or periphery or central part of liver. Not only is the cytoplasm granular and vacuolated but there is picnosis and

nuclear destruction. At least 10% of the cells are disintegrated. In a comparative way this liver represents 4 plus degeneration.

KIDNEY: A section from the kidney shows about two plus degeneration of the convoluted tubules only.

RABBIT #II: Liver 61 Gms.
Degeneration in this organ is three plus with some pigmentation and nuclear destruction, 5 to 10%. The degeneration in this section is more pronounced about the central zone.

KIDNEY: Shows one plus tubular degeneration.

RABBIT #III: Liver 42 Gms.
Section of liver shows marked congestion but less than one plus degeneration. There are practically no degenerative nuclear changes. The kidney shows three plus tubular degeneration.

RABBIT #VI: Liver 40 Gms.
Liver shows one plus degeneration, which is more marked about the central vein zone. Tubular epithelium in the kidney is degenerated to the extent of two plus.

RABBIT #VII: Liver 56 Gms.
One plus degeneration liver cells which is slightly more evident in the central zone, with pigmentation and slight nuclear degeneration.

KIDNEY: Shows three plus tubular changes.

RABBIT #VIII: This liver fixed in formalin immediately after death to serve as a control for autolysis, shows extensive degeneration which must have been ante-mortem. Four plus cytoplasmic changes are present with 25 to 50% nuclear degeneration. Incidentally there are two other apparently unrelated lesions, Tuberculosis and Adenoma.

IIA. 6b. Toxicity to Salamanders:-

Ten animals each were placed in 400 cc. of water in one liter beakers marked (O) and (S), respectively. Two salamanders in beaker (C) served as controls. Those in (O) were treated from time to time with 6-oxysantonin while the animals in (S) were treated with equal amounts of santonin per animal. Observations were made on their appearance, activity, and heart rate, as noted in protocol 3. Only slight differences were observed when small doses of the respective drugs were administered. With larger doses, the animals in (S) became hyperirritable and excreted large amounts of fecal matter. Those in (O) did not appear to be more active than the controls and excreted less matter than those in (S). After prolonged treatment, muscular weakness and loss of equilibrium were much more pronounced in the animals which were treated with isoartemisin. No significant changes in heart rate were observed. Santonin appeared to be more toxic than the oxide.

Protocol 3.

11/14/35 10 mgm. of oxysantonin added to (O)
10 mgm. of santonin added to (S)

11/16/35 (O): 3 animals appear weak and float near surface
(S): No observable difference from (C)

11/18/35 All inactive

11/19/35 Water changed and 10 mgm. of drug added to respective beakers. Show some activity after 5 minutes.

11/20/35 All appear normal

11/21/35 (S): 1 salamander found dead. Heart rates determined before and 1¾ hours after changing water. All animals fed with Tubafex worms.

Heart Rates at 22.2°	Heart Rates at 16.3°
(O) 36-39/min.	(O) 32-35
(S) 30-35/min.	(S) 30-32
(C) 28-32/min.	(C) 27-30

11/23/35 10 mgm. drug added to fresh water in respective beakers

11/25/35 (S): All but one animal floating near surface. When disturbed appear very weak. Water very cloudy, possibly from dead Tubafex.
(O): Two animals floating near surface; one very weak. Dead Tubafex in ball on bottom of beaker.
(C): Both animals on bottom of beaker. Swim actively when disturbed.
Water changed immediately. Slimy deposit on walls of (S) removed. All recover after changing water.

11/26/35 When first observed, all animals were on the bottoms of the beakers. Later in the day, 1 salamander in (S) was found dead. Water changed in all beakers every 2 or 3 days until 12/17/35.

12/17/35 Water changed. 10 mgm. drug added to respective beakers. No immediate symptoms.

12/19/35 Water changed in all beakers every 3 or 4 days until 1/8/36

1/9/36 Heart Rates (averages) at 19.4°
(O) 37/min.
(S) 42/min.
(C) 42/min.
20 mgm. of drug added to respective beaker.

1/10/36 (S): Animals hyperirritable. Large amounts of fecal matter in beaker.
(O): Animals appear normal. Smaller amounts of fecal matter present.
(C): No change.

1/11/36 (S): Animals still somewhat hyperirritable.
(O): No change.
(C): No change.
Heart Rates (averages) at 19.5°
(O) 40/min.
(S) 43/min.
(C) 40/min.

1/13/36 Water changed in all beakers.
32 mgm. of santonin added to (S)
40 mgm. of oxysantonin added to (O)
(S): Animals appear greatly agitated. Swim about rapidly,and go to surface of water, apparently for air.
(O): Less marked but similar reaction took place later.

1/14/36 (O): Two animals found dead. Three others apparently suffering from anoxemia and very weak. Gills of all animals fluffed. Animals recover on oxygenating solution. Did not show hyperirritability. Beaker very dirty.
(S): Animals hyperirritable. Rise occasionally to surface and appear to swallow air.
(C): No change.
Heart Rates before Oxygenating Solutions (21°)
(O) 45/min.
(S) 38/min.
(C) 35/min.
Tubafex put in beakers. Only controls eat.
Water changed and Tubafex added to each beaker.

1/15/36 (O): Two animals die during night.
(S): Animals no longer hyperirritable.
On feeding with Tubafex, animals in (C) and (S) eat readily. Those in (O) show little desire to eat.
Water changed in all beakers. One of the control animals lost.

1/18/36 Heart Rates at 19.6°
(O) 45/min.
(S) 40/min.
(C) 43/min.
Water changed.

1/20/36 Heart Rates at 17.4°
(O) 40/min.
(S) 32/min.
(C) 36/min.

1/21/36 Water changed
24 mgm. of oxysantonin added to (O)
32 mgm. of santonin added to (S)

1/22/36 (O): One animal very weak. No hyperirritability observed.
(S): Animals "start" on tapping beaker.

1/23/36 Water changed in all beakers
24 mgm. of oxysantonin added to (O)
32 mgm. of santonin added to (S)

1/25/36 (O): Three animals very weak. Have difficulty in maintaining normal position.
(S): No apparent change.
Water changed. Tubafex fed. Only control shows desire to eat.

1/27/36 Heart Rates at 17.5°
(O) 38/min.
(S) 36/min.
(C) 31/min.
30 mgm. of oxysantonin added to (O)
40 mgm. of santonin added to (S)

1/29/36 (S): 6 animals dead. 1 very weak.
(O): 3 animals very weak. Have difficulty in maintaining normal position.
Water changed in all beakers.

1/30/36 (O): 2 animals dead.
Water changed in all beakers.

2/1/36 (O): 1 animal dead. Others weak.
Heart Rates of Remaining Animals (16.7°)
(O) 35/min.
35/min.
39/min.
(S) 37/min.
43/min.
(C) 37/min.
All animals sacrificed for histological examination.

IIA. 7. Excretion and Fate:-

The urine of rabbits which had been fed δ-oxysantonin was collected and reduced to a light brown mass by distillation in vacuo. This residue was extracted with boiling alcohol and the process was repeated until a small amount of brown oily residue remained. This oil was soluble in cold alcohol, ether, chloroform, and carbon tetrachloride and insoluble in water, acetone, and petroleum ether. A small amount of gelatinous yellow precipitate was obtained by fractional precipitation of a solution of the oil in chloroform by the addition of petroleum ether. The precipitate was soluble in cold alcohol.

The product was too impure and the amount of it was too small to attempt an analysis.

IIB. 1. Ability of Santoninamine to Lower the Blood-Sugar Level of Fasted Animals:-

Doses of 0.5 Gm./kilo. of the sulfate of santoninamine were given to four animals which had been fasted for 18 hours. In three out of four cases a pronounced lowering of blood-sugar level was observed. In the fourth case, the last determination made showed an unusually high blood-sugar level. Since no immediate effects were noted after the administration, it was concluded that the lowering was due to a toxic action on the liver, such as is obtained with hydrazine[68,69],

guanidine[70,71], synthalin[72,73,74], and neosynthalin[75].

The urine of rabbits which had received injections of santoninamine reduced Fehling's solution. No reduction was observed when the urine was tested before the injection of this drug.

Table XI. Effect of Santoninamine on Blood-Sugar Level

No. and Sex	Date 1936	Wt. in kilos	Control (c)	½ hr.	1 hr.	2 hrs.	5 hrs.	Remarks Sulfate of samine adm. by subcut. inj.
13 ♂	5/5	1.42	92.3	98.6	100	94.4	95.3	0.5 Gm. Samine/ kilo. after c Fasted 18 hrs.
	5/6		74.2	--	--	--	--	Fasted 42 hrs.
			44.2	--	--	--	--	Fasted 52 hrs.
	5/7		--	--	--	--	--	Found dead. No gas distension.
	5/6	1.55	110	113	110	106	81.7	0.5 Gm. Samine/ kilo. after c Fasted 18 hrs.
14 ♂	5/7		91.3	--	--	--	115	Fasted 44 hrs.
	5/8		191	--	--	--	184	Fasted 67 hrs. Animal very irritable. 0.5 Gm. Samine/ kilo. injected after B.S. det'n. Fed.
	5/9		--	--	--	--	--	Found dead. No gas distension.

Table XI. Effect of Santoninamine on Blood-Sugar Level

No. and Sex	Date 1936	Wt. in kilos.	Control (c)	½ hr.	1 hr.	2 hrs.	5 hrs.	Remarks Sulfate of Samine adm. by subcut. inj.
15 ♂	5/7	1.63	103	105	104	93.5	--	0.5 Gm. Samine/ kilo. after c. Fasted 18 hrs.
	5/8		76.1	--	--	--	81.9	Fasted 38 hrs.
	5/9		42.9	--	--	--	--	Fasted 62 hrs. Animal sacrificed.
16 ♂	5/7	1.54	93.4	104	106	97.2	--	0.5 Gm. Samine/ kilo. after c. Fasted 18 hrs.
	5/8		100	--	--	--	109	0.5 Gm. Samine/ kilo. after last B.S. det'n. Fasted 38 hrs.
	5/9		124	--	--	--	124	Animal fed after B.S. det'ns.
	5/11		113	--	--	--	109	Animal had not been fasted.
	5/12		76.3	--	--	--	65.3	Fasted total of 30 hrs. Animal sacrificed.

IIb. 2. Pathological Effects Produced on Liver and Kidney:-

Histological studies of the livers and kidneys of rabbits (13), (14), (15), and (16) revealed little evidence of hepatic injury. There was marked congestion and coagulation of the tissue surrounding the gall bladder, however. The kidneys showed slight to marked tubular degeneration.

The following report was submitted by Dr. Warner:

(13) Liver: There is rather marked congestion and enlargement of the sinusoid throughout the section. The liver cells themselves appear slightly swollen but are well preserved. There is a coagulation of the liver tissue surrounding the gall bladder.

(13) Kidney: Section shows an even, thin capsule. The convoluted tubular epithelium appears pale and pink, granular cytoplasm.

(14) Liver: Sinusoidal congestion. Pallor and swelling of liver cells, most marked about the periphery of each lobule.
(14) Kidney: Marked tubular degeneration.

(15) Liver: No abnormal congestion. No apparent degeneration. Several focal areas of necrosis and inflammatory reaction (apparently parasitic disease).
(15) Kidney: Slight tubular degeneration.

(16) Liver: No congestion. No apparent degeneration.
(16) Kidney: Moderate tubular degeneration. Irregularity in structure suggests old rabbit.

III Conclusions:-

A. Isoartemisin has the following pharmacological actions:

1. The vermicidal action is weaker than that of santonin. However, it is an excellent vermifuge.
2. Cardiac rate, amplitude of contraction, and output are not significantly affected on perfusing an 0.008% solution.
3. A slight but significant dilating action on the vessels of the frog leg was observed.
4. A marked diuresis was observed on the second day of fasting after administration of the drug.

5a. No effect was found on the blood-sugar level of fasting animals. In one animal a terminal hypoglycemia was recorded.

5b. The blood-sugar level in alimentary hyperglycemia was not significantly affected by the simultaneous administration of isoartemisin and dextrose.

6. No acute toxic effects were produced in either rabbits or salamanders. However, after prolonged administration the liver and kidneys showed marked degeneration in many cases.

7. No δ-oxysantonin was recovered from the urine, indicating that it was either excreted in very small amounts or had been altered chemically.

B-1. The subcutaneous administration of a 20% solution of the sulfate of santoninamine produced no immediate effect on the blood-sugar level of rabbits. A pronounced hypoglycemia occurred later in three out of four cases. In the fourth case the last blood-sugar estimation showed a marked rise.

2. Santoninamine produced slight to marked tubular degeneration of the kidney. No hepatotoxic action was observed on study of histological sections.

IV Summary:-

Isoartemisin is an excellent vermifuge and is devoid of the convulsant action of santonin. Toxic effects are not observed unless it is administered over long periods and in relatively enormous doses. It has no significant effect on blood-sugar level.

The sulfate of santoninamine, like other amino derivatives, which have been used in treatment of diabetes, lowers blood-sugar level, possibly through a hepatotoxic action. Histological studies have not confirmed this hypothesis.

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