

EXPERIMENTAL STUDIES ON THE ADMINISTRATION
OF SALVARSAN BY MOUTH TO ANIMALS
AND MAN.*

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PLATE 70.

The experimental studies here described were undertaken with the view of determining whether salvarsan could be administered by mouth without producing poisonous effects, and whether, when so given, it exerted a therapeutic influence on the manifestations of syphilis. Certain studies incidental to the oral administration of salvarsan have also been made which may prove not to be devoid of interest.

ANIMAL EXPERIMENTATION.

Before any attempt was made to administer salvarsan to patients by mouth, the drug was given to experimental animals to determine whether or not it would produce deleterious effects. It was also necessary to prove that the medicament thus given was absorbed.

In administering the drug by mouth to animals, we sought to determine four points: (1) the primary and secondary toxic effects, if any, of a single large dose and of several small doses of the medicament; (2) the presence or absence of arsenic in the bile and urine and in the stomach and intestinal contents; (3) the influence of salvarsan upon the bacterial flora of the intestinal tract, especially upon that of the colon; (4) the effect of the drug upon the parenchymatous organs, especially those concerned in elimination.

TOXIC EFFECTS.

To determine the primary effects of salvarsan given by mouth, the drug was administered in various forms to a series of cats,

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rabbits, and dogs. It was given in pills, in capsules, and dissolved in milk and water.

Experiment 1.—A cat, weighing 2,650 gm., was given 0.01 gm. of salvarsan dissolved in 10 c.c. of milk and water. No other food was given for twenty-four hours. There was neither vomiting nor diarrhea. The cat was killed at the end of twenty-four hours for further study.

Experiment 2.—A cat, weighing 3,250 gm., was given 0.01 gm. of salvarsan dissolved in 10 c.c. of warm milk and water. No other food was given. The animal showed no toxic effects and was killed and autopsied forty-eight hours later.

Experiment 3.—A cat, weighing 2,550 gm., received 0.05 gm. of salvarsan dissolved in 10 c.c. of milk and water. The animal showed no toxic effects.

Experiment 4.—A cat, weighing 850 gm., received 0.05 gm. of salvarsan in a capsule. The animal was autopsied twenty-four hours later, but showed no evidence of toxic action.

Experiment 5.—A cat, weighing 2,700 gm., received 0.05 gm. of salvarsan in a capsule. The animal showed no evidence of toxic effects and was autopsied twenty-four hours later. The findings were negative.

Experiment 6.—A rabbit, weighing 2,080 gm., received 0.05 gm. of salvarsan dissolved in 20 c.c. of milk and water. The animal showed no evidence of toxic effects and was killed eighteen hours later and autopsied.

Experiment 7.—A rabbit, weighing 2,000 gm., received 0.05 gm. of salvarsan dissolved in 12 c.c. of milk and water. The animal showed no evidence of toxic effects and was autopsied at the end of forty-eight hours.

Experiment 8.—A rabbit, weighing 1,380 gm., received 0.05 gm. of salvarsan dissolved in 12 c.c. of milk and water. The animal showed no toxic effects, and was autopsied seventy-two hours later.

Experiment 9.—A rabbit, weighing 1,450 gm., received 0.05 gm. of salvarsan in a capsule. The animal showed no toxic effects, and was autopsied twenty-four hours later with negative findings.

Experiment 10.—A rabbit, weighing 1,400 gm., received 0.05 gm. of salvarsan in a capsule. The animal showed no toxic effects and was autopsied forty-eight hours later. The findings were negative.

Experiment 11.—A rabbit, weighing 1,350 gm., received 0.008 gm. of salvarsan in a capsule. The animal was autopsied ninety-six hours later. It had shown no toxic effects, and the autopsy findings were also negative.

Experiment 12.—A rabbit, weighing 1,400 gm., received 0.008 gm. of salvarsan in a capsule. The animal was autopsied ninety-six hours later. It had shown no toxic effects, and the autopsy findings were also negative.

Experiment 13.—A cat, weighing 2,550 gm., received 0.05 gm. of salvarsan dissolved in 10 c.c. of milk. The animal showed no primary toxic effects and at the present time is apparently in a normal condition.

Experiment 14.—A cat, weighing 3,550 gm., received 0.02 gm. of salvarsan dissolved in 20 c.c. of warm milk. The animal did not show any primary toxic effects and at the present time apparently is in good health.

Experiment 15.—A cat, weighing 2,850 gm., received 0.04 gm. of salvarsan dissolved in 40 c.c. of warm milk. The animal showed no toxic effects and was autopsied forty-eight hours later.

Experiment 16.—A dog, weighing 5,700 gm., received 0.025 gm. of salvarsan in the form of a pill. No toxic effects were seen, and the dose was repeated in twenty-four hours without producing any deleterious effects.

Experiment 17.—A dog, weighing 7,000 gm., received a pill containing 0.025 gm. of salvarsan and this was followed by 10 c.c. of milk. The dose was repeated at the end of twenty-four and forty-eight hours without producing any appreciable toxic effects.

Experiment 18.—A dog, weighing 7,200 gm., received in the form of a pill 0.01 gm. of salvarsan every day for twenty-two days, followed by 0.05 gm. in a capsule each day for the next seven days. The animal did not lose weight, its appetite and spirits remained perfectly normal, and at no time were toxic effects seen.

These experiments show that salvarsan is without immediate toxic effects when administered to animals by mouth in a relatively large single dose or in multiple smaller doses. In some of these experiments six weeks have elapsed since the drug was given, and the animals have shown no late or secondary poisonous effects attributable to arsenic. The solutions were made and administered while fresh, without the addition of an alkali.

Many of the pills had been made up as long as fifteen days before administration and had become very dark in color and were quite hard. These were crushed in a mortar before administration but in no instance could harmful effects be noticed.

The capsules, which contained diatomaceous earth with the salvarsan, did not undergo any change of color although exposed to light for several weeks.

ABSORPTION AND ELIMINATION.

The intravenous and intramuscular administration of salvarsan are followed by the elimination of arsenic in the urine and feces. So far there are no reliable tests for salvarsan *per se* in any of the excretions. The question of absorption of salvarsan from the intestinal tract is worthy of study. Whether or not the drug undergoes marked changes in the intestinal canal is as yet undetermined. We have examined the secretions of a number of experimental animals in order to determine whether the drug was being eliminated as arsenic. If arsenic were found, this would indicate absorption of the drug under these conditions.¹

¹We are indebted to Professor Alonzo Taylor and Dr. Rinker, of the Department of Physiological Chemistry of the University of Pennsylvania, for assistance and suggestions in conducting these tests.

Technique.—All tests were qualitative. Suitable controls were used. In brief, the material (stomach contents, feces, bile, and urine) were oxidized with concentrated nitric and sulphuric acids, after which sodium and potassium nitrate were added and the whole mass was fused with more of the same salts. The resulting product was dissolved in concentrated sulphuric acid over a water bath until all nitric acid had disappeared. The March test was then employed.

The results are given in the following table.

TABLE I.
Tests for Arsenic.

No. of experiment.	Animal.	Dose in gm.	Time of removal after administration.	Product.	Arsenic present.
3	Cat	0.05	24 hours	Stomach	Well defined trace
				Feces	Well defined trace
				Bile	Trace
				Urine	Trace
6	Rabbit	0.05	24 hours	Stomach	Well defined trace
				Feces	Well defined trace
				Bile	Trace
				Urine	Trace
7	Rabbit	0.05	48 hours	Stomach	No trace
				Feces	Trace
				Urine	Very slight trace
8	Rabbit	0.05	72 hours	Stomach	No trace
				Feces	No trace
				Bile	No trace
				Urine	No trace
18	Dog	0.01	After 3 doses	Feces	Well defined trace

These results show that within twenty-four hours after the oral administration of salvarsan, arsenic may be found in the bile and urine. Its presence in the stomach contents and feces may be indicative of failure of absorption. At the end of forty-eight hours, the quantities of arsenic found were very minute, and after seventy-two hours none was found in the stomach, feces, bile, or urine. It is probable that only a portion of the salvarsan was absorbed from the intestinal canal and that this was quickly eliminated largely in the form of arsenic.

INFLUENCE ON INTESTINAL BACTERIA.

As a matter of additional interest, a quantitative examination of the bacteria of the gastro-intestinal canal was made in a few of the

experimental animals, since arsenic and possibly some of the unchanged drug had been found in the feces twenty-four hours after the oral administration of salvarsan.

Technique.—For quantitative work with intestinal bacteria, the question of a satisfactory technique was most important. The animals were usually kept from all food for twenty-four hours before and after the administration of salvarsan. As a result, the stomach was usually empty at autopsy. Cultures were made of the stomach, ileum (close to the ileocecal valve), and the upper portion of the rectum. After asepticing the gut with a hot iron, the wall was cut through and cultures were secured by rubbing the wall or contents with a sterilized platinum loop which measured four millimeters in diameter. The loop was then thoroughly shaken in 10 cubic centimeters of sterilized salt solution. This solution was thoroughly emulsified and 0.5 of a cubic centimeter, one cubic centimeter, and two cubic centimeters were plated with 10 cubic centimeters of plain neutral agar. The plates were incubated at 37° C. for twenty-four hours and then kept at room temperature until the end of the forty-eighth hour, when counts were made. The control animals were living under exactly the same conditions and receiving the same diet. From them likewise food was kept for forty-eight hours preceding the autopsy and the taking of the cultures.

While the number of cats from which cultures were made was comparatively small, the results were fairly constant. The most

TABLE II.

The Results of Quantitative Bacteriological Examination of the Intestinal Tract of Animals Receiving Salvarsan by Mouth.

No. of experiment.	Animal.	Dose in gm.	No. of hours separating the administration of the drug and the taking of cultures.	Organ.	No. of colonies of bacteria per c.c. of emulsion.
1	Cat	0.01	24	Stomach	10
				Ileum	450
				Colon	14,000
3	Cat	0.05	24	Stomach	None
				Ileum	270
				Colon	10,500
15	Cat	0.04	48	Stomach	18
				Ileum	1,200
				Colon	17,000

TABLE III.

Table of Control Bacteriological Examinations.

No. of control.	Animal.	Organ.	No. of colonies of bacteria per c.c. of emulsion.
1	Cat	Stomach	24
		Ileum	11,000
		Colon	19,000
2	Cat	Stomach	19
		Ileum	12,200
		Colon	20,000
3	Cat	Stomach	50
		Ileum	17,000
		Colon	19,500

marked influence was seen in the lower end of the ileum, where, within twenty-four hours after the administration of salvarsan, the total number of bacteria was found much decreased. In the colon, the decrease in the number of bacteria was about 30 per cent. At the end of forty-eight hours, however, the differences were less marked.

EFFECTS OF SALVARSAN ON PARENCHYMATOUS ORGANS, ESPECIALLY THOSE OF ELIMINATION.

Urinary Examinations.—The urine of the six animals that received salvarsan by mouth, Nos. 4, 5, 9, 10, 11, and 12, and of four others, Nos. 19, 20, 21, and 22, that received the drug intravenously, was examined, but in no instance was the presence of albumin or casts demonstrated, the findings being, in fact, entirely similar to those in the normal controls. In all the animals mentioned, the special test for salvarsan in the urine advocated by Abelin was tried. In these tests, we secured a clear dark brownish discoloration in all specimens of urine from the experimental and control animals, but in no instance did we find the red color described as a positive reaction in this test.

Autopsy Protocols.—Twelve of the eighteen experimental animals were autopsied at intervals varying from eighteen to ninety-six hours after the administration of salvarsan. In none of these animals were there any gross evidences of deleterious effects. The heart, lungs, liver, spleen, kidneys, and the mucosa of the stomach and of the entire intestinal tract were found to be normal. The liver and kidneys of many of the animals were examined in the

fresh condition for albuminous degeneration of the cells, but the findings did not differ from those of the control animals. The liver of one cat (experiment 4) had the gross appearance of fatty changes, but a control animal showed an exactly similar condition.

Histological Examination.—Sections of the heart, lungs, liver, spleen, kidneys, stomach, and ileum from experimental animals 1, 2, 4, 5, 9, 10, 11, and 12 were fixed, stained, and examined histologically for evidence of vascular disturbances and degenerative changes. After examining the sections from animals 1, 2, 4, and 5, which were cats, we at first thought that, in addition to the fatty infiltration found normally in a large proportion of cats, important albuminous and fatty degenerative changes had been produced. But after studying the organs of three control cats living under the same conditions, it was found that no degenerative or vascular changes could be ascribed to the influence of the salvarsan.

In order to study this question more thoroughly, salvarsan was administered intravenously in alkaline solution to the following four rabbits:

Experiment 19.—A rabbit, weighing 1,500 gm., received intravenously 0.006 gm. of salvarsan dissolved in 16.8 c.c. of water. The animal was autopsied forty-eight hours later.

Experiment 20.—A rabbit, weighing 2,500 gm., received intravenously 0.01 gm. of salvarsan dissolved in 28 c.c. of water. The animal was autopsied ninety-six hours later.

Experiment 21.—A rabbit, weighing 1,400 gm., received intravenously 0.0056 gm. of salvarsan dissolved in 15.6 c.c. of water. It was autopsied eighteen hours later.

Experiment 22.—A rabbit, weighing 1,000 gm., received 0.004 gm. of salvarsan dissolved in 11.2 c.c. of water, and was autopsied twenty-four hours later.

According to body weight, the doses administered were about equal to those administered intravenously to man. None of these animals showed any toxic effects whatsoever, the urine examinations were negative, and the gross autopsy findings were entirely negative. Sections of the heart, lungs, liver, kidneys, spleen, stomach, and ileum of these four rabbits and of rabbits 9, 10, 11, and 12, were examined histologically and contrasted with preparations of the organs from our normal rabbits. While the sections from the hearts showed in some instances a loss of muscle striations and fragmentation of the muscle cells with slight fatty

changes, entirely analogous changes were found in the controls. The lungs showed no changes. The stomach and ileum appeared entirely normal, there being no evidences of vascular changes or degeneration of cells. Sections of the liver showed in many instances marked granularity of the cells with perivascular interstitial connective tissue proliferation, but in the normal controls entirely analogous conditions were found. The kidneys showed some cloudy swelling of cells in a few of the sections, but no vascular changes. The controls were similar. The spleen showed in a few instances a large number of eosinophil cells and occasionally apparent enlargement of the Malpighian bodies. There was no congestion or endothelial cell proliferation. The normal controls were similar in all particulars.

As a result of these studies, we must conclude that salvarsan in the doses in which we administered it does not produce appreciable degenerative changes in the principal organs within ninety-six hours following its administration.

ORAL ADMINISTRATION OF SALVARSAN TO MAN.

As it had been demonstrated that salvarsan could be given by mouth to cats, dogs, and rabbits in relatively large doses without producing toxic effects, or, indeed, untoward symptoms of any kind, we proceeded to administer it to human subjects.

A decigram of salvarsan dissolved in hot milk was first given to three men suffering from secondary and tertiary syphilis. In two of these patients the drug was well borne, no symptoms of gastrointestinal irritation and no other disturbing symptoms developing.

Case 1.—In a man thirty years old, vomiting, followed by diarrhea, occurred two hours after the ingestion of the salvarsan, but the patient had no pain and was not otherwise disturbed.

On the following day, a second dose of the same amount was administered to this patient without producing vomiting or diarrhea. Four days later, 0.2 gm. of salvarsan dissolved in hot water to which a little peppermint water was added, was given. This was followed by some vomiting and diarrhea, but the patient had no pain and did not feel ill in any way. On the two succeeding days, he received 0.2 gm. and 0.15 gm., respectively, without vomiting or diarrhea.

This patient had an extensive small papular syphilide (follicular syphilide), for which he had a short time before received nine mercurial inunctions, with but slight influence on the eruption. A mucous patch on the lip healed promptly after the salvarsan was given and the eruption was perceptibly improved.

Case 2.—A man, twenty years old. He had a chancre and a macular syphilide with beginning papular lesions.

On January 21, 1912, the patient was given 0.1 gm. of salvarsan in 90 c.c. of milk. On January 20, the dose was repeated. On January 24, 0.2 gm. was given in water with peppermint. On January 25, 0.2 gram was again administered.

All of these doses were well borne, the patient experiencing no unpleasant symptoms whatsoever. The treatment was discontinued owing to a misunderstanding of orders.

During the administration of the drug, the macular eruption disappeared, but a large flat papular syphilide continued to make its appearance.

Case 3.—The patient was a man, thirty-eight years old. He has been a hard drinker. Twelve years ago he had a chancre and secondaries, and then had received mercurial inunctions, but in the past twelve years he has received no treatment. The patient has a large ulcerating gumma below the knee which is deeply excavated and discharges profusely (figure 1). The gumma has existed since September, 1911.

Treatment.—On January 19, 1912, and again on January 20, 0.1 gm. of salvarsan was administered in milk. Healthy granulations were observed on January 20. On January 25, 0.2 gm. of salvarsan was administered in water, and on this date the first photograph (figure 1) was taken. On January 26, 0.3 gm. of salvarsan was given in water. On January 27, the gumma was healing quickly, and granulations were appearing rapidly. On the 27th, the 28th, and again on the 29th of January, a capsule containing 0.1 gm. of salvarsan and 0.2 gm. of diatomaceous earth was given.

On February 2, the ulcer was about half its former size. The granulations were on a level with the skin. The discharge was very scanty, and cicatrization from the border was advancing. The local treatment consisted merely of the employment of a wet boric acid dressing. The patient stated that he felt well and that his appetite was most keen.

On February 2, the full dose of 0.6 gm. of salvarsan in 120 c.c. of water flavored with peppermint was administered. No unpleasant symptoms followed. The ulcer was healing rapidly.

On February 10, another full dose of 0.6 gm. of salvarsan was given. No unpleasant symptoms followed.

The second photograph (figure 2), was taken on March 1, just five weeks after the first. It shows the ulceration to be practically healed although epidermization is not yet complete.

Case 4.—A young woman, with a recurrent papulosquamous syphilide of the wrists and palms, received 0.1 gm. of salvarsan in a capsule, once a day for ten days. The drug was well borne, but as the improvement was but slight, intravenous administration was resorted to.

Case 5.—A young negro, with a beginning follicular syphilide on the trunk and an annulo-papular syphilide of the penis and scrotum, received the full dose of 0.6 gm. of salvarsan in 200 c.c. of water. About three quarters of an hour later he vomited, and after this had seven loose bowel movements.

It was subsequently ascertained that prior to the ingestion of the drug, he had had no food for eighteen hours. Three days later, the follicular syphilide

had almost disappeared, but the patches on the penis and scrotum were but slightly influenced.

A week after the first administration, a second dose of 0.6 gm. of salvarsan in 200 c.c. of water was given. This was followed by slight nausea, but no vomiting. The patient had four loose bowel movements, but no soreness or pain.

After the second dose of salvarsan, the genital eruption improved but did not disappear. Other treatment was therefore substituted.

Case 6.—The patient was a woman, forty years old, who had on her back a palm-sized tubercular syphilide that had come on three years after infection. She received four capsules of 0.1 gm. of salvarsan on successive days, and three days later took 0.6 gm. dissolved in 200 c.c. of water. The patient had two loose bowel movements, but no vomiting. There was practically no improvement in the eruption.

Case 7.—A mulatto girl, aged nineteen years, with an extensive pustular syphilide of the varicelliform type, was given 0.6 gm. of salvarsan in 200 c.c. of water. Three hours later she vomited twice, and had some cramps, but no diarrhea. Three days later, 0.6 gm. of salvarsan was again administered. Some hours later the patient had nausea and slight vomiting.

The influence of the salvarsan on the eruption in this patient was extremely slight.

Urinary Examinations.—Twenty-four hours after the full doses of salvarsan had been administered by mouth, the urine of the patients treated by mouth was subjected to careful chemical and microscopical examination. Albumen was tested by the nitric acid, heat, and potassium ferrocyanid tests and was absent in all the examinations except one in which a trace was disclosed by the potassium ferrocyanid reaction only. In this specimen of urine, a single hyaline cast was found. With this exception, the microscopical examinations were negative.

SUMMARY.

Salvarsan in capsule and in solution in doses varying from 0.1 to 0.6 of a gram was administered by mouth to seven patients suffering from syphilis. Five patients were given full doses of 0.6 of a gram. One patient (case 3) received in the aggregate within four weeks 2.4 grams without loss of weight or any disturbing effects. An ulcerating gumma of the knee was practically healed after a period of five weeks. This was the only patient of the seven, however, in whom notable improvement in the cutaneous manifestations followed the administration of the drug. This ulceration

would probably have healed as soon, or possibly sooner, under the influence of mercury and the iodids.

From the above observations, it would appear that salvarsan administered by mouth up to the full dose of 0.6 of a gram, has a perceptible though feeble therapeutic effect on cutaneous syphilitic manifestations. We should naturally expect the influence of any drug to be more evident in the case of a gumma, in which spirochætes are only sparsely present, than in the secondary syphilides in which the spirochætes are present in enormous numbers. Cutaneous gummata are readily healed at times by very small doses of mercury or iodids.

It is interesting to note that the administration of the full dose of the drug by mouth does not appear to give rise to symptoms of systemic arsenical poisoning, unless the vomiting and diarrhea be regarded as such. After some of the full dose administrations, there was neither vomiting nor diarrhea. As salvarsan contains 34 per cent. of arsenic, these patients received the equivalent of five grains of arsenic. In case 3, in the course of four weeks the patient took by mouth the equivalent of over twelve grains of arsenic, without vomiting or having diarrhea, and with no loss of weight or disturbance of health. The urine of this patient was entirely normal. It would seem, therefore, that salvarsan is not converted in the gastro-intestinal tract into an organotropic arsenical compound, and it is possible that it may remain practically unchanged.

CONCLUSIONS.

1. Salvarsan can be administered in pills, in capsules, and in solution to lower animals in dosage of 0.02 to 0.03 of a gram per kilo of body weight, without producing toxic symptoms. A dog received 0.57 of a gram of salvarsan in pill and capsule form in the course of twenty-nine days without any disturbing effects.
2. After the oral administration of salvarsan, arsenic is found in the bile and urine at the end of twenty-four hours, but it disappears by the end of seventy-two hours.
3. Twenty-four hours after the oral administration of salvarsan to cats, the number of bacteria in the intestinal tract appears to be

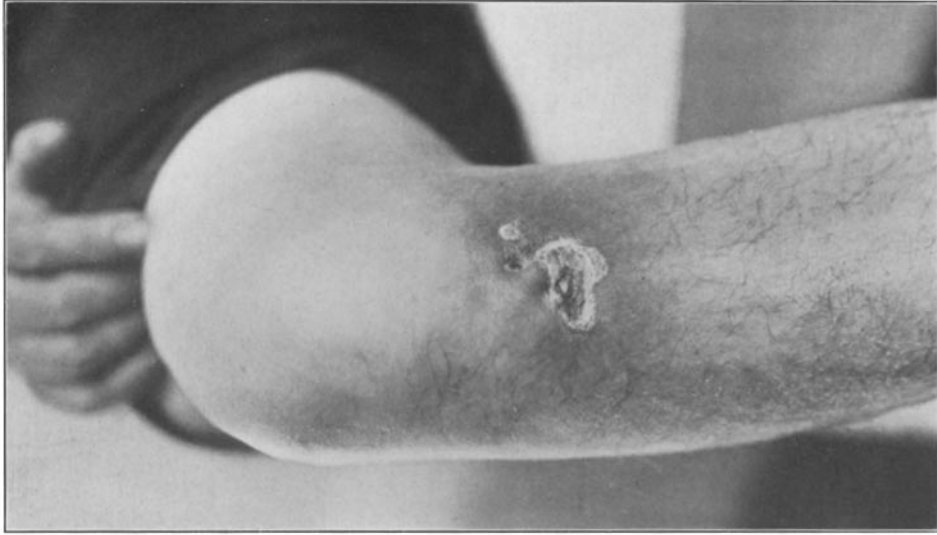


FIG. 2.



FIG. 1.

reduced, the reduction being most striking in the lower end of the ileum.

4. Salvarsan administered by mouth and likewise intravenously to rabbits in doses approximating those employed in human subjects, does not produce, at least within ninety-six hours following its administration, any appreciable microscopic changes in the important viscera.

5. With the exception of a little vomiting and diarrhea in some cases, salvarsan can be given by mouth up to 0.6 of a gram to human subjects without producing toxic symptoms. Thus administered, the drug exerts a therapeutic influence, but this influence is too feeble to warrant its use by this route.

EXPLANATION OF PLATE 70.

FIG. 1. Case 3. First photograph, taken January 25, 1912. Gummatous ulcer developing twelve years after infection. No treatment was given after the initial inunctions. This patient received 2.4 grams of salvarsan by mouth within a period of four weeks.

FIG. 2. Case 3. Second photograph, taken March 1, 1912.