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Author(s): Georges Knaysi
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THE USE OF IODINE AND OF CERTAIN IODINE COMPOUNDS IN EXPERIMENTAL TUBERCULOSIS*

GEORGES KNAYSI
ITHACA, N. Y.

Iodine has been used in a variety of forms in the treatments for a number of diseases. In the present investigation I have attempted to study principally the possibility of injecting elemental iodine into the animal body and, if practical, to use it in the treatment for tuberculosis. Of course, it is known that elemental iodine does not remain as such in the animal body, but is changed for the most part into sodium iodide, while some unites with the proteins and lipoids of the blood. However, the speed with which this transformation takes place is not known, and if enough iodine can be injected, it might be expected that some will reach the tubercles in the free condition and there exert its disinfecting power, which I [1] have shown to be great for the tubercle bacillus.

TOLERANCE OF RABBITS TOWARD ELEMENTAL IODINE

A 1 per cent iodine solution was prepared by dissolving 1 Gm. of iodine in 100 cc. of a 3 per cent aqueous potassium iodide. Of this solution three rabbits received intravenously amounts that were equivalent to 10, 20 and 30 mg. of free iodine per kilogram of body weight. All three rabbits survived the injection. The rabbit that received the 30 mg. showed more apparent uneasiness during the twenty-four hours following the administration of the iodine.

ATTEMPT AT IODINE THERAPY IN TUBERCULOSIS

EXPERIMENT 1.—Forty-three rabbits were weighed and divided into eight groups. On Nov. 10, 1930, each animal received 500,000 tubercle bacillus cells per kilogram of body weight from a 4 week old culture of the bovine strain BR. This was derived from the Saranac B_1 bovine strain of tubercle bacillus obtained from Dr. S. A. Petroff of the Trudeau Sanatorium Laboratory. It was found to have lost

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* From the Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y., and the Department of Dairy Industry, Cornell University, Ithaca, N. Y.

1. J. Infect. Dis., this issue, p. 255.

much of its virulence. The cells were injected into the marginal veins of the ear from a suspension prepared as follows:

Some of the growth was scraped off a glycerin egg slant with a sterile platinum loop and transferred to the bottom of a sterile, graduated centrifuge tube, where it was worked into a thick paste with a sterile glass rod, the end of which had been carefully rounded. Small droplets of sterile water were then added, one at a time, and the working was continued. Finally, the paste was diluted with from 7 to 8 cc. of sterile, distilled water and mixed well. The suspension was then filtered through sterile Whatman filter paper, and the number of cells in the clear suspension counted by mixing 0.1 cc. of the suspension with 0.9 cc. of sterile skim milk, which before sterilization had been diluted with four times its volume of distilled water. Of this mixture, 0.01 cc. was spread over 1 sq. cm., allowed to dry, fixed over the flame and stained by the Ziehl-Neelsen method. The acid-fast cells were then counted under the oil-immersion objective. A definite volume of the filtered suspension was then diluted with the necessary volume of 1 per cent sterile solution of sodium chloride to bring down the number of cells per cubic centimeter to the desired figure, and the diluted suspension counted as a check.

In an emulsion prepared in this manner, the bacterial mass was broken mostly into single cells, and none of the occasional groups encountered numbered more than 3 cells.

During the week preceding infection, group 1 was given daily intravenous injections of iodine in increasing doses. Each animal received 0.5, 1, 1.5 and 2 mg. per kilogram of body weight during the first four days, respectively. The latter dose of 2 mg. was then maintained for the rest of the week; it was repeated one hour after infection and twice a week thereafter. Group 2 was first given an injection of iodine one hour after infection, daily during the following week and twice a week thereafter, according to the scheme just described. Group 3 received its first injection of iodine one week after infection, daily during the following week and twice a week thereafter, as outlined. Group 4 received the same iodine treatment as group 2, but each animal was inoculated with 1,700,000 iodized tubercle bacillus cells four times during the week preceding infection. This was done in the hope that the antibodies formed against the iodized proteins of the tubercle bacillus cells might be effective against the infecting organisms following the treatment with iodine.

The iodine was injected in aqueous solution containing 1 Gm. of iodine and 3 Gm. of potassium iodide per liter. This was found to be the highest concentration that did not unduly injure the blood vessels into which it was injected. A fresh solution was prepared every week.

The iodized cell suspension was prepared as follows: Some of the growth was scraped off a glycerin egg medium slant into a sterile, graduated centrifuge tube, worked into a thick paste in a droplet of sterile water, covered to the 10 cc. mark with a 1 per cent iodine solution in 3 per cent aqueous potassium iodide, and allowed to stand twenty-four hours, with occasional stirring. The tube was then centrifugated at a high speed, the supernatant iodine decanted and the precipitate suspended in 1:10,000 solution of iodine in potassium iodide and filtered through sterile filter paper. The cells in a cubic centimeter of the suspension were counted by mixing a 1 cc. portion with an equal volume of a sterile 1 per cent egg

albumin, spreading 0.01 cc. of the mixture over 1 sq. cm., fixing with the flame and counting with the oil-immersion objective, with and without staining with a dilute crystal violet solution.

Groups 5 and 6 were treated intravenously with an iodine compound, amiodoxyl benzoate, instead of free iodine. Amiodoxyl benzoate is ammonium of *O*-iodoxy benzoate (for description, see "New and Nonofficial Remedies," p. 219). It was injected in 0.5 per cent aqueous solution, group 5 receiving its first treatment one hour, and group 6 one week, after infection. The injections were repeated daily during the week following the first treatment and twice a week thereafter, at the rate of 10 mg. per kilogram of body weight. Amiodoxyl benzoate is painful on injection.

Group 7 was forced to thirty inhalations of ethyl iodide every day. The treatment began one hour after infection and was continued till December 24.

All animals were tested with tuberculin before the experiment and did not react, and twice during the investigation. The test was carried out by injecting intradermally 0.1 cc. of a 5 per cent solution of human old tuberculin, prepared by the Mulford Company, in sterile physiologic solution of sodium chloride.

Comment on the Technic of Intravenous Injection of Iodine.—Iodine injected intravenously in aqueous solution is not too painful to the animal. However, it slowly reacts with the vein, and if the solution is too concentrated, profound injury may result. This effect is relatively weak when the concentration of iodine is below 1 : 1,000. Injected into the muscle, small amounts of iodine result in severe inflammations or even necrosis, and care must be taken that no laceration of the vein takes place during the injection, and that no iodine thus escapes into the surrounding tissue. Therefore, it is necessary that the animal should remain motionless during the injection. In the present work the animal was placed in an injection box, and the head and ear were held by an assistant. A 26 G one-half inch hypodermic needle was found to be most suitable. When an injection is well made with this needle, no immediate mark can be seen at the site of the injection.

Amiodoxyl benzoate is more painful than iodine on injection, the pain developing from one-half to one minute after the injection is begun, as judged by the restlessness of the animal. However, iodine produces a more severe local reaction.

Results.—Table 1 shows that the majority of the animals reacted positively to tuberculin. However, none of the animals that died or were killed during the investigation or afterward showed any lesions of tuberculosis. The organism, which seems to have lost its pathogenic power, must have been successfully walled off in the animal body. A negative reaction to tuberculin may mean that the organism has been completely destroyed or eliminated by the animal, or that the animal is in too poor a physical condition to react positively. As a matter of fact, the negatively reacting animals were generally weak or suffered from nontuberculous infections. A very striking example is afforded in the next experiment.

A significant fact brought out in table 1 is that, aside from a few hypersensitive animals, the majority were able to stand frequent injections of the doses of iodine used throughout the experiment without apparent harm. The animals survived

TABLE 1.—*Rabbits Infected with the Bovine Strain BR* *

Group and Manner of Treatment	Animal	Weight in Kg. Nov. 11, 1930	Weight in Kg. Dec. 3, 1930	Weight in Kg. Jan. 22, 1931	Tuberculin Test Nov. 26, 1930	Tuberculin Test Jan. 19, 1931	Comment
1							
Received iodine daily during week previous to infection and then twice a week at the rate of 2 mg. per kilogram of body weight, beginning one hour after infection; iodine treatment discontinued Dec. 24	24	2.6	2.8	2.6	—	—	
	25	3.2	3.5	4.0	+	+	
	26	3.0	3.1	3.4	±†	+	
	27	2.4	2.5	2.7	+	+	
	29	2.8	3.0	3.6	±	+	
2							
First iodine treatment given one hour after infection, daily during the following week and biweekly thereafter till Dec. 24, at the rate of 2 mg. per kilogram of body weight	30	2.7	2.4	2.6	—	—	
	31	2.6	2.9	3.2	+	+	
	32	2.4	2.7	3.1	+	±	
	33	2.9	3.0	3.1	±	+	
	34	2.4	2.4	2.7	—	—	Died Feb. 22; areas of pneumonia in lungs; cause of death not obvious from autopsy; no tuberculosis
3							
First iodine treatment given one week after infection, daily during the following week and biweekly thereafter till Dec. 24, at the rate of 2 mg. per kilogram of body weight	35	2.8	3.0	3.4	+	+	
	36	2.9	2.9	3.3	+	+	
	37	3.0	3.3	3.7	+	+	
	38	2.8	2.7	3.5	+	+	
	39	2.5	2.5	2.2	—	—	Killed on Jan. 27 to relieve it from encephalitis; no tuberculous lesions
4							
Each rabbit received 1,700,000 iodized cells four times during week previous to infection; first dose of iodine given one hour after infection, daily during the next week and biweekly till Dec. 24, at the rate of 2 mg. per kilogram of body weight	18	2.5	2.6	2.8	—	—	Hypersensitive to iodine; iodine treatment discontinued at end of second week
	19	3.0	3.3	3.6	±	+	
	20	3.4	...	...	..	..	Died Nov. 19 of polyserositis; a beta hemolytic streptococcus was isolated from fibrinous exudate and blood; no signs of tuberculosis
	21	2.5	2.7	3.1	—	—	
	22	2.3	1.9	...	—	—	Died Dec. 5; extensive abscesses of lymph glands and on both sides of chest; pus contained minute diplococcus; no tuberculosis
	23	3.1	3.5	4.0	+	+	
5							
Received amiodoxyl benzoate one hour after infection, daily during the next week and twice weekly till Dec. 24, at the rate of 10 mg. per kilogram of body weight	40	2.8	3.1	3.8	—	+	
	41	2.8	3.1	3.8	+	+	
	42	3.0	3.0	3.4	—	+	
	43	2.5	2.7	3.1	+	+	
	44	2.9	3.3	4.0	±	—	

* Each animal received 500,000 cells per kilogram of body weight on Nov. 10, 1930.
† ± means doubtful.

TABLE 1.—*Rabbits Infected with the Bovine Strain BR*—Continued*

Group and Manner of Treatment	Animal	Weight in Kg. Nov. 11, 1930	Dec. 3, 1930	Jan. 22, 1931	Tuberculin Test Nov. 26, 1930	Jan. 19, 1931	Comment
6							
Received amiodoxyl benzoate one week after infection, daily during the next week and biweekly till Dec. 24, at the rate of 10 mg. per kilogram of body weight	45	2.5	2.8	3.3	—	±	
	46	3.0	3.3	3.9	+	+	
	47	2.2	2.8	3.2	—	—	Died Feb. 24, 1931; large, confluent, caseous lesions in lower lobes of both lungs; fibrous pleurisy; no acid-fast organisms
	48	2.6	2.5	3.2	—	+	
	49	3.1	3.2	3.7	—	±	
7							
Received daily 30 inhalations of ethyl iodide, beginning one hour after infection	1	2.2	2.5	3.0	+	±	
	2	2.7	2.8	3.0	+	—	Died Feb. 1, 1931; animal long sick with encephalitis; no tuberculosis
	3	2.7	3.0	...	..	..	Died Jan. 15, 1931; extensive subcutaneous abscesses along both sides of chest and neck; beta hemolytic streptococcus isolated from pus laden with it; no tuberculosis
	4	2.6	2.5	2.8	+	+	
	5	2.9	3.0	3.2	+	±	
	6	3.0	3.2	3.6	+	+	
	7	2.4	2.7	3.0	±	±	
8							
Controls; no treatment	50	2.8	3.2	3.0	±	—	
	51	3.0	3.3	3.9	+	+	
	52	2.4	2.6	3.0	±	±	
	53	2.5	2.7	3.0	+	—	
	54	2.2	...	...	..	..	Died 10 days after infection, probably from streptococcus infection; not examined post mortem

* Each animal received 500,000 cells per kilogram of body weight on Nov. 10, 1930.
† ± means doubtful.

well, consistently increased in weight and showed no apparent alterations in their thyroid glands when examined for that purpose. Rabbit 18 was so sensitive, however, that injections had to be discontinued at the end of two weeks. Rabbit 24 was sensitive, but not to the extent of making further injections undesirable.

Experiment 2.—In view of the failure to infect the animals in the previous experiment, it was thought desirable to use a strain of high pathogenic power. Accordingly, eight rabbits were weighed and inoculated with a suspension of a bovine strain of tubercle bacillus obtained from Saranac Lake, to which I shall

refer as strain BS. The suspension was prepared as described, and was given to the animals intravenously at the rate of 100,000 cells per kilogram of body weight.

Four of the animals (group 9) received each 5 mg. of iodine one week after infection, 10 mg. the second week, and 3 mg. the third week, per kilogram of body

TABLE 2.—*Rabbits Infected with the Highly Pathogenic Bovine Strain BS* *

Group and Treatment	Animal	Weight in Kg. Feb. 13	Weight in Kg. Feb. 27	Tuberculin Test, March 12	Comment
9					
Each rabbit received 5 mg. of iodine one week after infection, 10 mg. the second week, and 3 mg. the third week, per kilogram of body weight; on March 20, each was given an injection of 20 mg. of iodine per kilogram of body weight	8	3.3	3.3	+	Died March 22; considerable number of small tubercles in lungs; few superficial larger ones; spleen considerably enlarged and full of small nodules; no enlargement of thyroid gland; miliary tuberculosis; acid-fast organisms found
	9	3.8	3.7	+	Died March 24; thyroid gland not enlarged; lymph nodes slightly enlarged; spleen enlarged, but contained no tubercles; no tubercles in liver or kidney; extensive miliary tuberculosis of both lungs with acute pleurisy; acid-fast cells found
	10	3.1	3.0	—	Died, March 25; swollen lymph nodes with caseous areas in left axilla; numerous fibrinous, slightly caseous nodules in all lobes of pale, edematous left lung; liver swollen, with small scars and irregular caseous areas; spleen slightly enlarged, soft, with few small nodules; kidneys normal; tuberculosis; acid-fast organisms found
	12	3.0	2.8	+	Died March 21; peculiar swelling of head, involving jaw; subcutaneous tissue edematous; no tubercles in lungs, spleen, liver or kidneys; spleen enlarged and fibrotic; no smears made
10					
Controls; no treatment	13	3.6	3.6	+	Killed April 10; extensive miliary tuberculosis of both lungs; no tubercles in spleen or liver, both of which were normal; acid-fast organisms found
	14	3.6	3.5	+	Killed April 10; extensive miliary tuberculosis of both lungs; liver enlarged, with few white areas along margin; no tubercles; tubercles in slightly enlarged spleen; acid-fast cells found
	17	3.5	3.0	—	Died March 18; animal very thin; large pyogenic abscess on left side of head; fibrinous pleurisy of both sides; numerous tubercles in lungs and large caseous area; other organs normal; acid-fast organisms found
	55	...	1.5	—	Died March 10; very emaciated; all lymph nodes slightly enlarged; spleen, liver and kidneys normal; acute fibrinous pleurisy numerous small and medium-sized nodules; caseation in some tubercles; thyroid gland normal; acid-fast organisms found

* Each animal received 100,000 cells per kilogram of body weight, on Feb. 13, 1931.

weight. The remaining four animals (group 10) were left without treatment, as controls. When a tuberculin test was given on March 12 and all but one of group 9 gave positive reactions, it was decided to administer to these animals what I call a "sterilizing" dose of iodine; each received 20 mg. per kilogram of body weight

from a 1 per cent solution of iodine in a 3 per cent aqueous potassium iodide solution. This dose gives in the blood a concentration of approximately 1 : 5,000, if, as is assumed, no transformation of iodine takes place. This dose is strongly toxic to the tubercle bacillus and well below the limit of tolerance in a normal rabbit. The results recorded in table 2 show that all treated animals succumbed to the dose administered. The weakening effects of the severe tuberculosis which these animals had, added to the toxic effect of iodine, were more than the animals could stand.

It is interesting to note that animals 17 and 55, which had an extensive miliary tuberculosis, did not react to tuberculin. These animals were very weak and emaciated even before infection.

COMMENT

The experiments reported in this paper show that iodine, when administered under the conditions described, is not effective in the treatment for tuberculosis, in spite of its relatively low toxicity for the animal body and its strong germicidal action against the tubercle bacillus.

The low toxicity of iodine for the animal body is made evident by the tolerance of normal rabbits to injections of 30 mg. of elemental iodine and 90 mg. of potassium iodide per kilogram of body weight. If, as is assumed, the blood forms 10 per cent of the rabbit's weight, and no neutralization of elemental iodine takes place, this amounts to a concentration of approximately 1 part of free iodine in 3,300 parts of blood or about ten-thousandth molecular, and this concentration has been shown [1] to be amply sufficient to destroy millions of tubercle bacillus cells within a short period of time. In these experiments, the doses administered were too low; for a dose of 1 mg. per kilogram of body weight is equivalent to approximately 1 part of free iodine in 100,000 parts of blood. Such a small amount is probably quickly neutralized into sodium iodide by the alkali reserve of the blood, and even if there is no immediate neutralization, such a concentration may not be toxic to the cells of Mycobacterium tuberculosis. The only adequate dose given was administered to rabbits already severely sick from tuberculosis and too weakened to withstand an additional intoxication with iodine. It seems, therefore, probable that, aside from this last instance, the injections had the same effect as a treatment with sodium and potassium iodides, which have been shown to have harmful effects on tuberculous animals by causing a breaking down of tuberculous tissues (Cantacuzène [2]) and causing more extensive tuberculous processes (Sorel [3]).

2. Ann. Inst. Pasteur **19**:699, 1905.
3. Ann. Inst. Pasteur **23**:533, 1909.

The mechanism of this action can be attributed to the saturation of the fatty acids of the tubercles, allowing the tryptic ferments there present to exert their digestive action (Jobling and Petersen [4]). It is therefore felt that free iodine (administered directly or in such a way that it will be liberated under conditions present in the tubercle) has not yet been used under the proper conditions in the treatment for tuberculosis and deserves further consideration.

SUMMARY

The experiments reported in this paper were carried out to test the therapeutic value of iodine in the treatment for experimental tuberculosis in rabbits.

Iodine may be injected intravenously into the body of the rabbit over relatively long periods of time without apparent harm to the health of the animal or injury to the thyroid gland. The dose used in most of these experiments was 2 mg. per kilogram of body weight, administered twice a week over a period of about two months from an aqueous solution containing three times as much of potassium iodide.

The results show that such a small dose is not effective in the treatment for tuberculosis, probably because it is quickly neutralized by the alkali reserve of the blood.

A dose of 30 mg. of iodine per kilogram of body weight does not kill a normal animal, but one of 20 mg. per kilogram is fatal to a rabbit with a severe miliary tuberculosis.

It is thought that this subject deserves further consideration under more appropriate conditions.

4. J. Exper. Med. **19**:383, 1914.