A PRELIMINARY NOTE ON THE PREPARATION OF NON-TOXIC SHIGA DYSENTERY VACCINES BY IRRADIATION WITH SOFT X-RAYS

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It is known that intravenous injections of heat-killed suspensions of *Shigella dysenteriae* (Shiga) are so toxic that rabbits are first paralyzed and later succumb with typical symptoms. Non-toxic vaccines may be prepared by treatment with formalin (Costa, Boyer and Placidi, 1925; Durand, 1925; Enlows, 1925; Ramon and Dumas, 1924; Wherry and Bowen, 1925), nitrous acid, (Foshay) or, according to Manoussakis (1932), by permitting the suspensions to remain at ice-box temperatures for five months.

It is also known that bacteria may be killed with x-rays, and that the soft or long wave-length part of the x-ray spectrum is especially lethal. It is possible that the changes accompanying the killing of bacteria with heat are not identical with those produced with x-ray killing and that a vaccine prepared by irradiation of organisms with soft x-rays might be less toxic than one prepared by killing with heat, yet retain all of its antigenic properties. It is the purpose of this paper to describe some experiments which were carried out to test this possibility.

METHODS AND APPARATUS

The bacteria were prepared for irradiation by wiping off eighteen-hour slant cultures of *S. dysenteriae* (Shiga) and bringing the resulting suspensions in salt solution to a turbidity of 500 on the Fuller's scale. In order that the suspension during irradiation would occupy a small enough space to be placed in the most intense part of the x-ray beam near the focal spot of

the tube, it was concentrated by centrifuging for ninety minutes at 4000 r.p.m., decanting the supernatent liquid and resuspending in 1 cc. of sterile saline. These concentrated suspensions were irradiated in small glass dishes placed at a distance of 5 cm. from the focal spot of a copper target gas x-ray tube (Kersten, 1934) whose radiation had the K_α(1.54 Å) and the K_β(1.38 Å) lines of copper as its most intense parts. The tube was operated at 38 peak kilovolts, with a current of 20 m.a. and the time of irradiation varied from 30 to 300 minutes. After irradiation the suspensions were made up to 10 cc. with sterile saline and tested for sterility before use. All the heat-killed suspensions were treated in the same manner, i.e., centrifuged, decanted and resuspended and then heated at a temperature between 55° and 60°C. for one hour. Suspensions of living organisms used as tests for protection were brought to a turbidity of 500 on the Fuller's scale before injection.

During the time of irradiation, the sample, being very near the focal spot of the x-ray tube, was slightly warmed by heat transmitted through the thin window, even though the target and body of the tube were cooled with running water. The approximate temperature of the sample was measured by replacing the suspension of bacteria in the small glass dish, with an equal volume of oil in which was immersed a thermocouple. The replacement of the saline suspension by the oil was necessitated by the fact that the former is an electrical conductor. The temperature was found to rise approximately 3°C. above room temperature under the same conditions of irradiation as that used for the bacterial suspensions.

As a test for the antigenic power of the x-ray vaccines, the animals were given 0.4 cc. of living organisms intravenously. Control animals were given equal doses to determine whether the toxicity of these amounts was sufficient to kill.

RESULTS

Table 1 gives a summary of the results of the inoculation of 45 rabbits with x-ray treated vaccines, and of 12 controls given heat-killed or living organisms. Of 30 animals receiving from

1 to 4 cc. (1 cc. doses) of vaccine irradiated from 30 to 100 minutes, 17 died after receiving one to three inoculations and 13

TABLE 1

Showing the immunizing effects of Shiga dysentery vaccine irradiated with soft x-rays for 30 to 300 minutes

MINUTES OF IRRADIATION	NUMBER OF RABBITS	TOTAL AMOUNT OF VACCINE	DEATH FROM VACCINE	IMMUNITY TO 0.4 CC. LIVING S. DYSENTERIAE (SHIGA) GIVEN INTRAVENOUSLY (DENSITY EQUAL TO VACCINE)
30–60	4	4 cc. (1 cc. every 7 days)	2 (after first inoculation)	2
60	7	4 cc. (1 cc. every 7 days)	0	5 survived. 2 died of pneumonia 10 days after living organisms were given
60	1	3 cc. (1 cc. every 7 days)	1 (after third inoculation)	
60	3	2 cc. (1 cc. every 7 days)	3 (after second inoculation)	
60	10	1 cc.	8 (dead in one to six days	2
70–100	5	4 cc. (1 cc. every 7 days)	3	2
120–300	13	4 cc. (1 cc. every 7 days)	0	13
120–300	1	2 cc. (1 cc. every 7 days)	1 (died after second dose. Gm. + staphylococcus in heart's blood)	
120–300	1	3 cc. (1 cc. every 7 days)	1 (died after third dose. Coccidia in liver. Gm. + coccus in heart's blood)	
Heat-killed vaccine controls	8	1 cc.	8 (died 24 to 36 hours after inoculation)	
Living *S. dysenteriae* (Shiga) suspensions controls	4	0.4 cc.	4 (died 24 to 48 hours after inoculation)	

N.B.: All survivors were watched for three weeks.

survived the test dose of living organisms. Of 15 animals receiving from 1 to 4 cc. (1 cc. doses) of vaccine irradiated for 120

to 300 minutes, 2 died as a result of intercurrent infections and the remainder (13) survived the test dose for immunity. All control animals receiving heat-killed or living organisms died within 24 to 36 hours after inoculation.

From these results it appears that vaccines receiving large doses of x-rays are rendered non-toxic and retain their antigenic properties, while vaccines receiving small doses of x-rays, even though these doses are more than sufficient to merely kill the organism, produce vaccines which are still toxic.

CONCLUSIONS

A non-toxic vaccine of *Shigella dysenteriae* Shiga has been prepared by irradiation of saline suspensions of the organisms with soft x-rays. This vaccine retains its antigenic properties as evidenced by the protection of rabbits against subsequent fatal doses of heat-killed and living suspensions of the organisms.

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