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TRANSLATION NO. 2677

DATE: 2/9/70

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2677

USE OF IONIZING RADIATIONS FOR THE PREPARATION OF RADIO VACCINES AND RADIO-ANTIGENS

Proceedings of a Symposium,
International Atomic Energy Agency,
Vienna, 1967, pp 231-241.

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Abstract

The possibility of employing ionising radiations in certain doses to kill microorganisms was used to produce vaccines against intestinal infections and also to obtain from these bacteria antigens capable of being used as chemical vaccines. Typhoid fever and dysentery radiovaccines and radio-antigens were prepared and the effect of various gamma-ray doses on their toxicity and their antigenic and immunogenic properties was tested. The doses used did not change the properties of these products as compared with those of vaccines and antigens produced by conventional means. The paper also discusses the possibility of using radiation to sterilise the already prepared vaccines and antigens including radiovaccines and radio-antigens, anitoxins, antitoxic serums and nutrient media for the culture of microorganisms. Data on the irradiation apparatus used for these investigations are reported.

The property of ionising radiations to produce bacteriostatic or bactericidal effect with certain irradiation doses may be utilised in the production of bacterial preparations. Two ways of utilising the radiation are contemplated: firstly, for sterilising vaccines, antigens, serums and nutrient media in the process of their preparation or in finished form and, secondly, for obtaining vaccines and antigens from microorganisms killed by irradiation.

At the present time, the expediency of radiation sterilisation in various branches of medical industry, including the production of bacterial preparations, does not need confirmation. However, the use of radiation for killing microorganisms with the aim of preparing new types of corpuscular and chemical vaccines, the so-called "radiovaccines" and "radioantigens" needs serious proof.

Conventional methods of killing microorganisms for the manufacture of preparations widely used in the prevention of infectious diseases of man and animals are for the most part unsatisfactory. The most widely used methods are the action of various chemical substances on a bacterial cell and effect of heating. In doing so, many cellular antigens responsible for the creation of complete immunity in the organism are destroyed. Therefore, the search for new chemical substances or new physical factors which would have bactericidal effect while retaining in doing so the high antigenic and immunogenic properties of a live microorganism, continues. In spite of the relatively high radio resistance of microorganisms to ionising radiation, irradiation of microorganisms has drawn the attention of a considerable number of researchers.

We used ionising radiations for the preparation of vaccines (antityphoid-fever and antidysentery vaccines). Controulis et al. and Troitskiy et al. used radiation for the preparation of diphtheria anatoxin; Traub et al. obtain a vaccine against rabies; Polley furnished experimental proof for vaccine against influenza and parotitis.

Data furnished by Schwartz, Tisler, Golds et al. and by others on the use of radiation for the preparation of vaccines from helminths are of considerable interest.

The work on the preparation of a vaccine against hookworm (Dow et al.) with the help of ionising radiations is extremely important.

However, radiovaccines and radioantigens must meet the main requirement — not to be more toxic in comparison with the preparations made by conventional methods and not to impair their immunogenic properties.

In view of the fact that vaccines are widely used for the prevention of intestinal infections we set for ourselves the task of investigating the possibility of using radiation for the preparation of antityphoid-fever and antidysentery radiovaccines.

Doses of from $4 \cdot 10^5$ to $6 \cdot 10^5$ rad killed all of the strains of the typhoid-fever and dysentery bacteria that we tested. However, for the preparation of radiovaccine irradiation was done in the doses of 1.5-2.0 Mrad.

Irradiation was done on an experimentation apparatus with radioactive cobalt. The apparatus consists of two adjacent pools filled with water. The small pool is designed for auxiliary operations: reception, assembly and storage of the sources. In the wall adjacent to the operating pool is arranged a sluice gate designed for the transmission by the source from the auxiliary pool into the operating pool.

Two chambers for irradiation of the objects are built-in hermetically in the front wall of the large operating pool. Two pipes which serve as the guides for the carriages carrying the irradiation sources are installed on the edges of the pool. Radiation sources are assembled into devices resembling

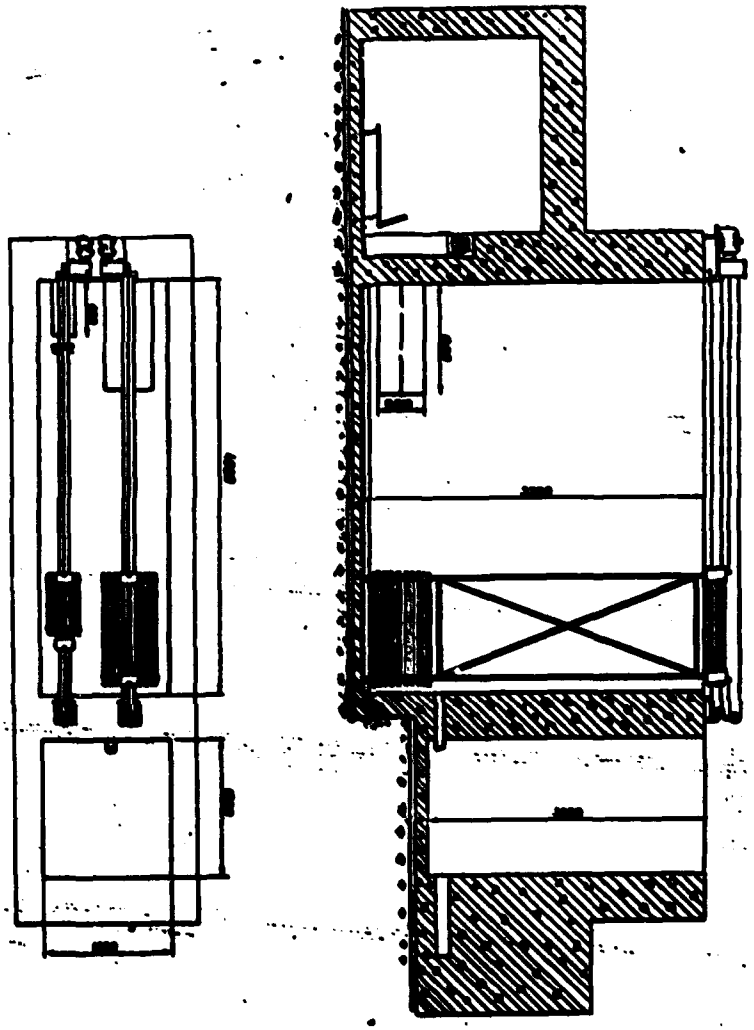

Рис.1. Экспериментальная установка.

Figure 1. Experimentation apparatus.

a "squirrel wheel". They are moved up to the chambers for irradiation. Moving of the carriages with the sources is accomplished by electromechanical drives by means of chain traction. Controlling of the motion of radiation sources is done from a benchboard. A set of cobalt-60 preparations has a total activity of 30,000 curie and provides a power of a dose of up to 10,000 rad/min.

The loading of the objects into the chambers for irradiation is done from the side of the front wall of the operating pool. At this time the pro-

tective door covering the hatches of the chambers is open and the radiation sources are at the pool end which is away from the chambers. Protection of personnel doing the loading is done by a layer of water with a thickness of 2,500 mm. After loading the objects the personnel moves into the benchboard room and from there controls the apparatus: closes the protective door and moves the radiation source into the operating position.

TABLE 1. TOXICITY OF VACCINES

Vaccines	Radiation dose (Mrad)	Mice		
		Total (No.)	Deaths (No.)	Survivors (%)
P. Vaccine Plomer 4437	0	30	2	93,3
Radiovaccine Plomer 4437	1.0	30	3	90,0
P. Vaccine Ty 2	0	55	33	40,0
W. Vaccine Ty 2	0	40	17	56,0
Radiovaccine Ty 2	1.5	80	23	71,0
P. Vaccine Ty 2 (Irradiated)	1,7	40	8	80,0

The apparatus is provided with blocking and signaling devices. Ionizing pickups marking the excess of gamma-ray background over the permissible level with a light or audible signal are installed in all rooms. The audible signal is duplicated in the control room. Signaling of the position of the radiation sources and protective doors is grouped on the control board. The apparatus is provided with two time relays which automatically turn it off upon the expiration of the exposure time.

The wash-off of the daily agar culture of typhoid-fever or dysentery bacteria (30-70 billion microbe bodies in 1 milliliter of physiological solution) was irradiated in glass flasks (100-200 milliliters) or retorts (1 liter). Irradiated bacteria were used for vaccine preparation or else various antigens or antigenic complexes were extracted from them.

To judge the quality of the preparations obtained their antigenic and immunogenic properties were tested and also their capacity to retain V antigen and toxicity.

Toxicity of the preparations was determined by the deaths of the animals with intraperitoneal and subcutaneous administration of various doses of the preparations being investigated, in a volume of 0.5 milliliters for mice and 1 milliliter for rats, and also by cutaneous reaction to intracutaneous administration of the preparations to rabbits. A comparison was made of the

TABLE 2. TOXICITY OF ANTIGENS

Antigen from bacteria treated as below	Radiation dose (Mrad)	Dose (mg)	Mice		
			Total (No.)	Deaths (No.)	Survivors (%)
F. bacteria Flexner 170	0	1.0	10	0	100
		2.0	10	6	40
		4.0	10	10	0
Irradiated bacteria Flexner 170	1.5	1.0	10	0	100
		2.0	10	2	80
		4.0	10	8	20
F.a. irradiated bacteria Flexner 170	1.5	1.0	10	4	60
		2.0	10	5	50
		4.0	10	5	50
Bacteria Ty 2	0	1.0	20	3	85
		2.0	20	4	80
		4.0	20	12	40
Irradiated bacteria Ty 2	2.0	1.0	20	0	100
		2.0	20	1	95
		4.0	20	3	85

usual formalin-treated and heated vaccines with the vaccines prepared from microbes killed by irradiation ("radiovaccines") and with the usual formalin-treated vaccines subjected to irradiation with the aim of sterilization (Table 1).

It was found that radiovaccines or vaccines subjected to irradiation with the aim of sterilization did not differ in toxicity from the usual formalin-treated vaccines.

The toxicity of typhoid-fever vaccines was also investigated by the cutaneous reaction on rabbits. The vaccines were administered intracutaneously. With respect to the degree of cutaneous reaction the vaccines may be arranged in the following order: formaldehyde-killed vaccine, radiovaccine prepared of microbes killed by 1.5 Mrad, heated vaccine and radiovaccine made of microbes killed by 1 Mrad. In comparison with the reaction to the administration of conventional vaccines reaction to the administration of radiovaccines was characterized by a later appearance of infiltrate, by its smaller dimensions and by an absence of necrosis.

The liquid portion of radiovaccines did not produce the formation of infiltrate whereas the liquid portion of vaccines prepared by conventional method produced formation of a dense infiltrate of considerable dimensions,

TABLE 3. TOXICITY OF ANTIGENS

Antigen from bacteria treated as below	Radiation dose (Mrad)	Dose (mg)	Rats		
			Total (No.)	Deaths (No.)	Survivors (%)
F. bacteria Flexner 2a	0	0.5	10	10	0
		1.0	10	10	0
		2.0	10	10	0
Irradiated bacteria Flexner 2a	1.5	0.5	10	6	40
		1.0	10	8	20
		2.0	10	6	40
F.a. irradiated bacteria Flexner 2a	1.5	0.5	10	6	40
		1.0	10	10	0
		2.0	10	10	0
F. bacteria Ty 2	0	0.5	10	0	100
		1.0	10	2	20
		2.0	10	2	20
		4.0	10	10	0
		6.0	10	10	0
Irradiated bacteria Ty 2	1.5	0.5	10	0	100
		1.0	10	0	100
		2.0	10	0	100
		4.0	10	8	20
		6.0	10	8	20
F.a. irradiated bacteria Ty 2	1.5	0.5	10	0	100
		1.0	10	0	100
		2.0	10	2	20
		4.0	10	10	0
		6.0	10	10	0

which would not disappear for a week. This makes it possible to consider radiovaccines to be less reactogenic in comparison with heated and formalin-treated vaccines.

Anaphylactogenic properties of the vaccines prepared were tested in a series of experiments. Formaldehyde-killed vaccine and vaccine made of microbes killed with 1.5 Mrad produced a reaction with necrosis in Snarelli-Schwartzman phenomenon when investigation was conducted in the course of one month after the preparation of vaccines. However, if the vaccines were examined in 2 or more months, then neither the radiovaccines nor the conventionally prepared vaccines produced Sanarelli-Schwartzman phenomenon.

In testing the toxic properties of antigens prepared from microbes killed by irradiation it was found that radioantigens are less toxic in com-

parison with antigens prepared from formalin-treated microbes or from microbes not subjected to killing before the preparation of the antigen (Table 2). However, if irradiation was used on an already prepared antigen obtained from formalin-treated microbes, then with respect to toxic properties it either did not differ from an unirradiated antigen or was more toxic than the latter. The same results were obtained in the experiments on rats (Table 3).

Investigation of antigenic properties of radio vaccines was carried out on rabbits. Groups of rabbits (of 5 animals in each group) were immunized three times with 7-day intervals by means of subcutaneous injections of the vaccine (Table 4).

TABLE 4. AGGLUTININS (RABBITS

Immunization	Radiation dose (Mrad)	Average titres						
		7 days after injection			14	21	36	50
		I	II	III	days after 3 injections			
W. vaccine Ty 2	0	1:5120	1:5120	1:10000	1:8000	1:2560	1:2560	1:1120
F. vaccine Ty 2	0	1:5120	1:5120	1:10000	1:16000	1:5120	1:5120	1:640
Radiovaccine Ty 2	1.5	1:2560	1:5120	1:8000	1:5120	1:2560	1:2560	1:640
Radiovaccine Ty 2	1.0	1:2560	1:5120	1:8000	1:5120	1:2560	1:2560	1:640

TABLE 5. IMMUNOGENICITY OF RADIOVACCINES

Vaccines	Radiation dose (Mrad)	No. of mice	LD_m (10^6 bacteria)	R
Radiovaccine Flexner 4497	1.0	50	1000	2.9
F. vaccine Flexner 4497	0	50	1000	2.9
Irradiated F. vaccine Ty 2	1.7	50	212	4.2
F. vaccine Ty 2	0	50	274	5.4

We determined agglutinins in the serum of immunized rabbits in 7 days after the first injection, in 7 days after the second injection and in 7, 14, 21, 36 and 50 days after the third injection of the vaccine.

Radiovaccines, both the dysentery and typhoid-fever vaccines, produced formation of antibodies (agglutinins). The average titers of serums in rabbits immunized with typhoid-fever radiovaccine hardly differed from those found in rabbits immunized with the usual typhoid-fever formaldehyde-killed vaccine. In regard to dysentery radiovaccine observations showed even a certain increase in the average titers of the serums in comparison with those of the serums of rabbits immunized with the usual dysentery formaldehyde-killed vaccine. Thus, microbes killed by irradiation did not lose their antigenic properties and radiovaccines produced formation of antibodies like the usual vaccines.

TABLE 6. IMMUNOGENICITY OF RADIOVACCINES

Vaccines	Radiation dose (Mrad)	No. of mice	LD_{50} (10^6 bacteria)	IR
W. vaccine Ty 2	0	50	25	2
F. vaccine Ty 2	0	50	15.5	1.2
Radiovaccine Ty 2	1.0	50	22.5	1.8
Radiovaccine Ty 2	1.5	50	22.5	1.8
Control	-	50	12.5	-

TABLE 7. IMMUNOGENICITY OF RADIOANTIGENS

Antigens from bacteria treated as below	Radiation dose (Mrad)	No. of animals	LD_{50} (10^6 bacteria)	IR
F. bacteria Ty 2	0	40 rats	2200	16
Irradiated bacteria Ty 2	1.5	30	2600	18.8
Irradiated antigen from F. bacteria Ty 2	1.5	30	2400	18
Bacteria Ty 2	0	30 mice	250	1000
Irradiated bacteria Ty 2	2.0	30	275	1131

TABLE 8. PREVENTIVE PROPERTIES OF SERA

Sera of rabbits (immunized by)	Radiation dose (Mrad)	LD_{50} (10^8 bacteria)	IR
W. vaccine Ty 2	0	60	42
Radiovaccine Ty 2	1.0	70	50
Radiovaccine Ty 2	2.0	8.6	6
Control	-	1.12	-
Antigen from bacteria Ty 2	0	32	42.6
Antigen from irradiated bacteria	2.0	32	42.6
Control	-	0.75	-

Immunogenic properties were studied on mice. Immunization with dysentery vaccines was done subcutaneously, twice with an interval of 5 days.

Inoculation with different doses of dysentery bacteria was done intraperitoneally 7 days after the completion of immunization. Immunization with typhoid-fever vaccines was done once under the skin. Immunized mice were infected intraperitoneally 10 days after the injection of the vaccine.

Dysentery radiovaccine did not differ in its immunogenic properties from the usual formalin-treated vaccine (Table 8). For example, the resistance index of dysentery radiovaccine was equal to 2.9 and that of formaldehyde-killed vaccine — to 3. However, if an already prepared formaldehyde-killed vaccine was subjected to irradiation its immunogenic properties declined in comparison with the usual formaldehyde-killed vaccine which had not been subjected to irradiation (resistance index was 4.2 and 5.4 respectively).

Tests were made of the typhoid-fever vaccine made from the microbes of the strain Ty_2 using different methods of preparation: heated, formalin-treated, radiovaccine made from microbes killed with 1 Mrad and a radiovaccine made from microbes killed with 1.5 Mrad (Table 6). Immunization of mice and rats was done once, subcutaneously.

Inoculation was done 10 days after immunization with different doses of a live culture of typhoid-fever bacteria in a semiliquid agar. In testing the immunogenic properties 50 animals were used for each vaccine.

TABLE 9. V ANTIGEN IN IRRADIATED MICROORGANISMS

Antigen	Radiation dose (Mrad)	Vi-ccm						
		1:2 to 1:16	1:32	1:64	1:128	1:256	1:512	1:1024
Salmonella Ty 2	1.5							
micro-organisms		+	+	+	+	-	-	-
liquid part		++++	++	++	++	++	++	-
F. vaccine Ty 2	0							
micro-organisms		-	-	-	-	-	-	-
liquid part		++++	++++	++	++	+	+	-
Suspension of bacteria Ty 2	0							
micro-organisms		+	+	+	-	-	-	-
liquid part		++++	++++	++++	++++	++	++	-

The data cited indicate that irradiation-killed microbes retained longer than the usual vaccines the capacity to produce in organism upon immunization a state of immunity to an infection with live microbes.

The same conclusion may be drawn both for antigens prepared from irradiation-killed microbes and for irradiated antigens prepared from formalin-treated microbes (Table 7).

Rats were immunized with typhoid-fever antigens twice subcutaneously with a 7-day interval at a rate of 0.25 milligrams of antigen. Infection was done intraperitoneally 10 days after the completion of immunization with different doses of a live culture of typhoid-fever microbes.

Mice were immunized with typhoid-fever antigens twice subcutaneously with an interval of 7 days at a rate of 0.1 milligram. Infection was done intra

immunity was lower with respect to the level of immunity in animals immunized with antigen obtained from formalin-treated typhoid-treated microbes (a resistance index of more than 16). In the experiment on mice the radioantigen proved to be more effective than the usual antigen. The resistance index was 1131 and 1000.

To determine the capacity of radiovaccines to produce in the organism being inoculated the formation of preventive antibodies, rabbits were immunized thrice subcutaneously with the vaccine in the doses of 0.5 and 1 billion microbe bodies with an interval of 7 days. One week after the completion of immunization the preventive properties of rabbit serums were tested: a mixture of serums was administered to mice subcutaneously in the amount of 0.25 milliliters; 4 hours later mice were infected intraperitoneally with different doses of a live typhoid-fever culture in agar. A determination was made of the dose of microbes producing a 50-percent loss of the animals to which serum had been administered before the infection, and that of the control animals to which serum had not been administered (Table 8).

With the aim of determining the capacity of radioantigens to produce in the organism the formation of preventive antibodies, rabbits were immunized in the following manner: first injection — 0.1 milligram subcutaneously, the following 6 injections were made intravenously with an interval of 5 days in increasing doses — 0.2, 0.4, 0.6, 0.8, 1 and 1 milligram. A test of the preventive properties of the serums was made 2 weeks after the completion of immunization.

With respect to preventive properties the serums of rabbits obtained by immunization with radiovaccine from microbes killed with 1 Mrad did not differ from the serums obtained by immunization of the rabbits with heated vaccine. An increase of irradiation dose to 2 Mrad brought about a lowering of the capacity of the radiovaccine to produce the formation of preventive antibodies in the organism. However, antigens obtained from microbes irrad-

iated with 2 Mrad did not differ from the usual antigens — in both cases the resistance index was equal to 42.6.

For a characterization of the properties of radiovaccines the question of retention in them of V antigen is of great interest. The effect of irradiation on V antigen in microbe bodies was determined with the aid of hemagglutination reaction (Table 9).

The wash-off from agar of a 24-hour culture of typhoid-fever microbes (Ty_2) was divided into 3 portions out of which one was subjected to irradiation with 1.5 Mrad, the second was treated with formalin and the third was left in the form of a suspension of live microbes.

With an irradiated culture of microbes the hemagglutination reaction was both quantitatively and qualitatively nearly the same as with a culture of microbes not subjected to any influences.

The results of the experiments carried out give us grounds to consider that gamma radiation may be used both for the preparation of intestinal vaccines and antigens and for the sterilization of respective finished bacterial preparations since even large doses of gamma rays did not change or hardly changed the antigenic, immunogenic and toxic properties of bacteria in the intestinal group.