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PREPARATION OF ANTITOXIC AND ANTIMICROBIAL VACCINES BY USE OF G--ETC(U)
JUL 77 E H RELYVELD, J TREFOVEL

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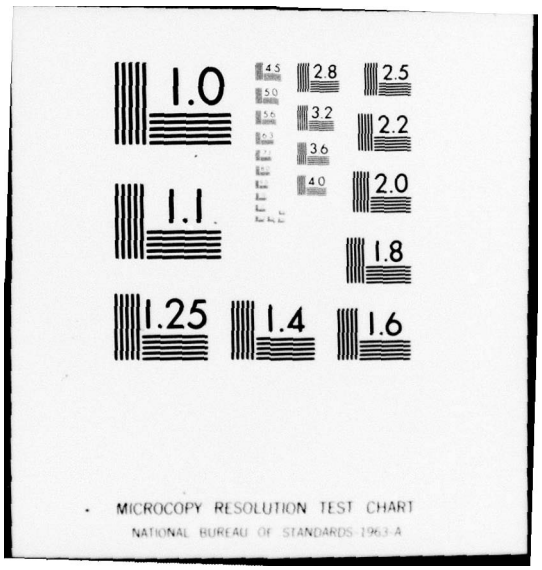
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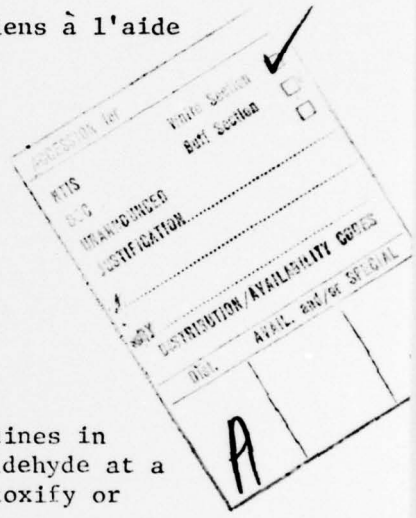
(Preparation of Antitoxic and Antimicrobial Vaccines by use of glutaraldehyde)

E. H. Relyveld[1] and J. Trefovel

Préparation de vaccins antitoxiques et antimicrobiens à l'aide du glutaraldehyde

C. R. Acad. Sci. (Paris) 277: 613-616, 1973

(Translated by Phebe W. Summers)



This article is a

Description of a procedure to obtain characterized vaccines in which one places toxins or microorganisms with glutaraldehyde at a concentration and for a period sufficiently long to detoxify or inactivate.

Transformation of diphtheria toxin to antitoxin using formol and heat simultaneously was presented by Ramon in 1923 (2). This, procedure, although applicable to preparation some excellent quality vaccines, particularly against diphtheria and tetanus, has not given complete satisfaction for all known toxins, especially against venins. Bacteria and viruses which are used in vaccine preparations are generally inactivated by heat, phenol, formol, β-propiolactone, UV light, etc., or a combination of these. These methods of inactivation have also given good results for pertussis, polio, rabies, etc., but one cannot generalize for all infectious disease agents for man and animals. The efficacy of some preparations is doubtful or of short duration. The results presented here give principles of a procedure using glutaraldehyde, applicable equally to other toxins and microorganisms. The examples concerned are: diphtheria, tetanus, and staphylococcal α and β toxins and staphylococcal and pertussis organisms. Some application to the venins and viruses under the same conditions and with comparable results will be the subject of other papers (3).

Techniques. Toxin preparations were incubated at 37°C or at ambient temperature with glutaraldehyde at various concentrations.* Samples were removed after progressive times of incubation for determination of residual toxicity and antigenicity. The reaction was stopped by elimination of glutaraldehyde excess chemically by adding to the reaction milieu agents capable of reacting with aldehydes such as amino acids (e.g., lysine or glycine), or sodium bisulfite and also by dialysis. The bacterial cultures of varying opacities treated by glutaraldehyde (GLUT), were centrifuged and washed to eliminate excess reactant. Bacterial concentration was expressed by comparison to the reference standard for opacity provided by NIH, Bethesda, Md. Measurements were carried out following directions for "Minimum Requirements: Pertussis Vaccine," treatment with glutaraldehyde was carried out under controlled conditions and according to a fundamental prodecures, i.e., one stops the reaction to remove the initial toxicity of the product, but without denaturization, and retaining the antigenicity. Study of residual toxicity of the preparations was carried out in rabbits, guinea pigs and mice, or with the help of sensitized RBC (for staph toxins). A toxicity to GLUT has been shown, but is eliminated by chemical action or dialysis. Inactivation of bacterial preparations has been verified after seeding in appropriate media. A limited time of contact for a GLUT concentration as weak as possible was chosen for vaccine preparation. The immunizing power of the preparation was studied in fluid form or after absorption on $Ca_3(PO_4)_2$. Molar concentrations of glutaraldehyde are as follows: 0.00263M = 1.07 ml/L (25% solution); 0.00526M; 0.0263M; 0.526M. Some different concentrations, going to 0.00131M, were chosen, for some preparations studied.

RESULTS - Pure diphtheria toxin -

Action of glutaraldehyde at a concentration of 0.0263M, which is equal to the quantity of formol usually employed to detoxify this toxin at 500 UF/ml for a period of 2 weeks at 37°C, caused loss of flocculation after 1 hr contact at the the same temperature. At a concentration of 0.00263M GLUT. One obtains a less pronounced lowering of flocculation titer. Study in guinea pigs and rabbits for residual toxicity showed that after 1 hr of contact the detoxification is almost complete. After 3 hr contact the preparation titering 420 UF/ml is completely detoxified (Table Ia). The immunizing power of the anatoxin obtained after 3 hr was studied after stopping the reaction with lysine and dialysis, or by dialysis alone. Table Ib shows the circulating antibody titer in International Antitoxic Units/ml (UAI/ml). The adsorbed vaccines have immungenic power greater than liquid vaccines; adsorbed anatoxin obtained by stopping the reaction with lysine gives a better immune response.

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*Toxin titers have been expressed in international flocculations units (UF), combined hemolytic doses (DCH) or minimal hemolytic doses (DMH)/ml.

Table Ia. Detoxification of pure diphtheria toxin at 500 UF/ml in 1% sodium bicarbonate solution by action of glutaraldehyde at a final concentration of 0.00263 M.

Contact Time	1 mn	1 h	3 h	6 h	24 h	48 h	1 wk	2 wk
UF/ml	500	450	420	400	350	330	325	225
Kf(*)	1 mn	6 mn	9 mn	16 mn	1 h 20	2 h 05	>3 h	>7 h
Toxicity	Toxic	Detoxified (verified in rabbit and guinea pig)						

(*)Flocculation time is 45 ° C.

Table Ib. Circulating Antidiphtheria Antibody Titers in Guinea Pigs in UAI/ml.

Incubation time at 37°C	Fluid Vaccines	Absorbed Vaccines
3 h	0.75	2
3 h + lysine 15 mn	0.75	2.5

(*) After 2 injections 15 days apart of 1 ml of vaccine at 30 UF/ml. Titers were determined on the mixture of equal volumes of blood taken 15 days after the 2nd injection.

Crude diphtheria toxin ultrafiltrate and partially purified type P_2 (4). One can obtain complete detoxification without denaturation of the toxin after 1 - 3 hr contact at a condition with respect to GLUT concentrations. compared to the degree of purification of the preparation used. Examination of the antigenicity of the anatoxins was equally effective after various times of contact without taking account of the loss of flocculant titer and depending on its starting titer. It was found that the immunogenic power decreased gradually as the time of contact increased and the titer decreased.

Purified tetanus toxin. Table II shows results of experiments carried out with a final concentration of GLUT of 0.00263 M. Complete detoxification was attained after 1 min contact. Immunizing power of the anatoxins was studied after 5 min and 1 hr of contact using as control the same anatoxin detoxified with 2% formol in the usual conditions (2 wk at 37°C). Table IIb shows the circulating antibody titers in UAI/ml. Vaccines obtained by glutaraldehyde action and in particular those obtained after 5 min contact have immunizing power higher than those obtained with formalin. Fluid or adsorbed anatoxins prepared with GLUT give practically the same immune response, whereas adsorbed anatoxin prepared with formalin give a better response than the same fluid preparation.

Table IIa. Detoxification of Tetanus Toxin Purified at 500 UF/ml in Na_2HPO_4 Solution.

Contact Time	1 mn	1 h	3 h	6 h	24 h	48 h	1 wk	2 wk
UF/ml	430	330	330	330	300	300	300	300
Kf	20 mn	40 mn	60 mn	60 mn	1 h 10	1 h 15	1 h 15	1 h 15
Toxicity	Detoxified after 1 minute (injection with 1 ml of 1/10)							

Table IIb. Circulating Antitetanus Antibodies in the Guinea Pig in UAI/ml*.

Anatoxins obtained	Fluid Vaccines	Adsorbed Vaccines
Glutaraldehyde after 5 mn contact	25	27.5
Glutaraldehyde after 1 h contact	20	20
Formol at 2%	2	8

(*) See Table Ib.

αStaphylococcal toxin. Table III shows that a concentration of 0.0263 M GLUT is sufficient to detoxify crude toxin in 5 min at ambient temperature. The effect of different concentrations of GLUT added to purified α-staphylococcal toxin at 137 DCH/ml has shown that the concentration of 0.00526 M is the best. Detoxification is complete after 1 h contact with a yield of 100%. Study of immunogenicity of crude toxin detoxified with GLUT or formol and then purified has shown that the rate of development of the antihemolysin in the rabbit is the same. Immunogenicity is decreased if detoxification is carried out in an excess of GLUT.

Table III. Detoxification of crude staphylococcal toxin at ambient temperature by action of GLUT

Concentration	Time of contact	DMH/ml	DCH/ml
Toxin without GLUT	--	2048	10
0.0131 M	5 min	64	10
	1 hr	32	10
0.0263 M	5 min	0	10
	1 hr	0	8

βStaphylococcal toxin. The detoxification of crude toxin at 10 DCH/ml using a concentration of 0.0789 M is complete in approximately 5 min with a yield of 100%, by contrast a concentration of 0.0526 M does not detoxify toxin before 24 h of contact. Results of the experiments

have presented evidence of a close relation between toxin titer, degree of purification, concentration of glutaraldehyde and contact time to obtain complete detoxification with elevated yield.

Staphylococci. A concentration of GLUT equal to 0.0131 M and 5 min contact suffices to inactivate a culture of α strain (Wood-46) to 5 units of opacity in physiological solution. The rate of agglutinins of rabbits vaccinated with organisms, inactivated, washed and readjusted to 15 opacity units increases regularly. This vaccination induced the same increased antibody titer that is effected with organisms killed by heat. Inactivation of a culture of strain β by GLUT has been studied as a function of: concentration, time of contact and opacity of the suspension. A concentration of 0.0131 M GLUT for 5 min or 0.00263 M for 1/2 h at 37°C suffices to inactivate a culture at 30 units of opacity. Study of the inactivation of a culture of 10 units of opacity at ambient temperature in relation to contact time and concentration has given these results: 0.0131 M, < 5 min., 0.0065 M, 30 min, 0.001 M, 2 h, 0.0065 M, 24 h. The action of a concentration of 0.0131 M on suspensions of different opacities has shown that 15 min suffices to inactivate at 300 units. Vaccination of rabbits with organisms inactivated with glutaraldehyde gives a rapid increase in antibody level.

Bacillus pertussis. Glutaraldehyde action has been studied on a culture of strain 134 at 200 opacity units. One obtains complete inactivation after 1 min of contact at a concentration of 0.0131 M GLUT. The titer in units of protection determined in mice was 4 international units for a vaccine at 16 units. A series of experiments was conducted to study glutaraldehyde action at less elevated concentrations. A culture of strain 509 at 84 units was equally inactivated after 1 min at the same final concentration of 0.0131 M; by contrast, 0.000131 M did not inactivate. Trials of immunizing potency in comparison to cultures inactivated by heat under the usual conditions are in progress.

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4. Relyveld, E. H. and M. Raynaud. Ann. Inst. Pasteur 107: 618, 1964.