REPORT OF

ASAP AD HOC GROUP

ON

CHEMICAL VACCINES

MEETING DATES

24-25 JULY 1973

FINAL REPORT DATE

6 AUGUST 1973

Report of Army Scientific Advisory Panel Ad Hoc Group on Chemical Vaccines, 6 August 1973:

1. Details of Meeting:

a. Persons Involved in Review: Five external scientific advisors and one Army staff assistant were involved in the review, see Incl 1.

b. Dates and Places of Meetings: The group convened on the morning of Tuesday, 24 July 1973, in Building E3100, Edgewood Arsenal, for a presentation on the subject of chemical vaccines by the staff of the Biomedical Laboratory. This presentation was followed by an executive session of the ad hoc group during the afternoon. Both during the presentation and the executive session the panel members had full opportunity to question the staff involved in the project and did so during both sessions. The group reconvened on the following morning, 25 July 1973, at the offices of the Federation of American Societies for Experimental Biology in Bethesda for further deliberations and framing the report of the group. The group was in telephone contact with the Biomedical Laboratory staff at Edgewood Arsenal during that day to resolve additional questions that had arisen. Both sessions were conducted on an "Unclassified" basis, and for that reason this report is also Unclassified. For preparation for the meetings all participants were furnished a package of material including the proposed terms of reference (see Incl 2) and summaries of the scientific data pertaining to the area reviewed (see Incls 3-7).

c. Preparation of Report: The report was prepared in draft form by the Chairman and circulated to all participants for review and corrections prior to final typing so that it represents a consensus of the ad hoc group members. The report is submitted by the Chairman of the ad hoc group to the Army Scientific Advisory Panel for appropriate action, as judged appropriate.

2. Scientific Discussion:

a. Assumptions and Facts Bearing On the Problem: As a portion of the session at Edgewood Arsenal, the Chairman requested Dr. Van Sim to brief the panel on the general position of chemical agents in the military strategy picture and to highlight the presently available methods of protection against and therapy for the organophosphate group of chemical agents. That briefing, conducted in executive session, was most valuable to the group in getting a perspective of the area reviewed. In the light of that briefing, the group operated with the following assumptions:

(1) The United States may be called upon to conduct military operations under conditions of use of organophosphate and related chemical agents by a hostile force. For that reason, it is highly desirable to maintain an effective program on research, development, test and evaluation of protective measures against chemical agents.

(2) Present protective measures are limited to mechanical barriers (gas mask and protective clothing) for prevention of injury and medical therapy (atropine, oximes and supportive measures) for those injured. The development of a means of increasing the resistance of man to these agents would be a highly important achievement with major strategic implications for the United States.

(3) It is not necessary that any means of "chemical vaccination" achieve complete protection for the soldier in order for it to be major importance. If such a vaccination program could do so, it would be a major success. Lesser degrees of success could also be very important in terms of decreasing morbidity and mortality pending the application of more conventional therapeutic measures. In this respect, such a vaccine could be likened to an emergency measure of stopping massive bleeding from a wound prior to the injured person's receiving surgical treatment. Therefore, group was urged to consider the broadest possible interpretation of benefits resulting from any vaccine program designed to provide some degree of protection against chemical agents.

(4) In its considerations of the total insult on the human organism, the group operated with the unclassified model of GD, for which the best estimate of the respiratory LD_{50} dose (the amount which would kill one half of the persons exposed) is approximately 15 micrograms (ug) per kilogram (kg) of body weight. For a 70 kg man, this corresponds to a total LD_{50} dose of 1,050 ug of agent. Assuming a molecular weight of 300, this amount, in turn, corresponds to 3.5 x 10^{-6} moles of agent. This figure is very important because it provides an estimate of the amount of the agent which must be neutralized by the antibodies which are evoked by any protective vaccine.

(5) Certain additional facts concerning antibodies are also important in the consideration of this problem. Antibodies are customarily bivalent, i.e., each molecule of antibody combines with two moleculars of antigen-agent which stimulated the production of antibody. Unfortunately, not all antibodies combine completely with antigen, a characteristic which is technically known as the "affinity" or "avidity" of the antibody. For purpose of this analysis, in order to provide the most conservative evaluation possible, it is assumed that the protective antibody against a chemical vaccine (the antibody against the chemical agent) is characterized by a high affinity, i.e., each molecule of antibody fully binds two molecules of antigen-agent, and that the affinity of the antibody produced by a derivative of the chemical agent is as high toward the chemical agent itself as it is toward the detoxified derivation of the agent used to induce immunity. Utilizing these facts, it follows that one LD_{50} dose of GD for man would be neutralized by 1.75 x 10^{-6} moles of antibody under the most favorable conditions. Should the antibody be characterized by low affinity, a larger quantity of antibody would be required to neutralize the same amount of agent.

(6) The maximum antibody production capability of the human body is not known because immunization schedules for man have been developed to achieve a desired immunization goal against infectious diseases. As long as that goal is reached, the immunization program has been considered successful in that it neutralizes an infectious agent present in relatively small amounts in the body. Hyperimmunization of man is believed to be a possible cause of a number of disease conditions, and for that reason, the minimal degree of immunity sufficient to achieve the disease prevention goal has been accepted as safe.

With diphtheria immunization, which has been studied in considerable detail, an estimate of human antibody production capabilities can be made. Recently immunized persons commonly have levels of antibody of 1 International Unit (IU) per milliliter (ml) or greater. [One-hundredth of an IU is sufficient to protect against the disease, diphtheria.] One IU of diphtheria antibody or antitoxin is equivalent to 15 ug of antibody protein per ml of serum. On the basis of an estimated 3.5 liters plasma volume and 10.5 liters interstitial fluid volume, and an antibody concentration in the interstitial fluid one-third that in plasma, the total antibody production of an adult man in moles, based upon an approximate molecular weight of 150,000, can be calculated as follows:

$$\text{Antibody Production} = \frac{[15 \times 10^{-6} \times 10^{3} \times 3.5] + [5 \times 10^{-6} \times 10^{3} \times 10.5]}{15 \times 10^{4}} =$$

$$= \frac{[52.5 \times 10^{-3}] + [52.5 \times 10^{-3}]}{15 \times 10^{4}} = 7.0 \times 10^{-7} \text{ moles.}$$

In rabbits, which can be hyperimmunized against diphtheria toxin, antibody levels as high as 310 ug per ml have been obtained. If man could be immunized to the same degree, the total antibody production in an adult, calculate in the same fashion, would be 14.5×10^{-6}, or approximately 20-fold greater. It is not at all certain that man could be immunized to this degree without experiencing severe adverse effects.

The maximum amount of antibody produced in man following immunization against a hypothetical vaccine would neutralize only 0.4 LD_{50} of agent GD if a hypothetical GD vaccine were as effective in producing immunity as diphtheria toxoid. Should man be capable of responding like the rabbit, the maximum amount of antibody produced should be capable of neutralizing 8.2 LD_{50} of agent GD, assuming a high affinity antibody. It follows that the maximum theoretical antibody production in man lies somewhere between these two limits, assuming that it is possible to produce an effective vaccine against GD and related agents.

b. Considerations Regarding Efficacy:

(1) The basic principle that antibodies may be prepared to small molecular weight compounds such as nerve gases is well established. The Edgewood staff is recommended for validation of this principle with paraoxon.

(2) Despite the fundamental soundness of the basic idea, there are inherent difficulties in the chemical vaccine approach that must not be underestimated:

(a) In comparison with immunity induced against infectious diseases, the amounts of antibody required to neutralize nerve gases are very large. As noted in the previous section, it would take 1.75×10^{-6} moles of antibody to neutralize one LD_{50} of agent GD. On the basis of an estimated molecular weight of 150,000, this corresponds to an antibody level in the serum of 37.5 ug per ml, a level which is considerably higher than that maintained for any significant period following diphtheria immunization in man.

(b) The problem is compounded by possible inherent difficulties in obtaining antibodies of the necessary avidity to firmly bind the chemical agent. In biological fluid the effectiveness of an antibody is as much a function of its avidity as of its concentration. The limited size and the absence of an aromatic ring structure of GD may prove to be a major obstacle in obtaining antibodies of the necessary affinity. In fact, there is no direct evidence that a molecule such as GD would ever produce really high affinity antibody and be useful for protection. This may explain why efforts with paraoxon, which has an aromatic ring, have been successful whereas attempts with GD have been unsuccessful to date. This is not to say that immunization with GD is not feasible, but rather that one cannot be certain that it will be feasible. This problem may be less severe for the V agents which are structually more complex.

(3) Even assuming effective antibodies for GD could be obtained, they would never be protective against more than 10 LD_{50}'s unless man were hyperimmunized to an even greater degree that the rabbit, which is unlikely. To maintain even low levels of resistance an aggressive hyperimmunization program would be necessary, accompanied by more risks in the form of adverse reactions than would be seen with the more conventional forms of immunization.

(4) One can justify an ongoing research program in this area because of possible benefits from developments in terms of better assay procedures (radioimmunoassay), as well as ultimate vaccine development (for other agents as well as GD). This is true even though the success of developing a vaccine against GD remains problematical. It must be reemphasized that the success with paraoxon in hyperimmunized animals does not prove the feasibility of obtaining practical levels of protection with agents such as GD.

c. Considerations Regarding Safety:

(1) The first consideration regarding safety of a vaccine protective against the nerve gases was that of the nature of the larger, or carrier, molecule which has been hemocyanin in the paraoxon work, and in part of the GD work. Hemocyanin has long been recognized to be immunogenic in man. A considerable body of literature exists regarding immunologic responses to keyhole lipet hemocyanin, an agent to which man has only rare contact. Hemocyanin from the keyhole limpet would appear to be good choice of carrier for the agents under study. Other carriers might also be explored, but carriers which are a part of the human diet should be avoided in order to minimize developing hypersensitivity to foods consumed. The literature is rather clear in that immunization to a small molecular weight compound coupled to a large carrier molecule is rarely successful unless the carrier itself is capable of stimulating immunity.

(2) The considered avoidance of use of an adjuvant in immunizing mixtures for animals is not sound, even though the use of adjuvants in man pose questions of safety at some point in the future. It would appear more desirable to obtain maximum immunologic response in animals at this point, even to the point of using complete Freund's adjuvant, than to dismiss adjuvant because of possible future human safety problems.

(3) The most important safety problem in possible future human use of materials developed as a result of this project lies in the need to hyperimmunize man to achieve significant protection against the oranophosphate and other chemical agents. These problems can only be weighed for man after a more successful program for the immunization of animals against these agents is achieved.

(4) There are theoretical safety hazards involved in such immunization techniques against nerve agents. The first of these is the possibility of induction of anaphylactic (allergic) shock upon the exposure of man to the free agent. The second is the possibility of breakage of the azo linkage between the chemical agent and the carrier, thus releasing toxic agent. On the basis of animal data to date neither potential hazard appears to be a significant one. Lastly, there are risks associated with the development of allergic disease as a result of hyperimmunization, such as anaphylaxis, serum sickness and vasculitis in man and amyloid disease in animals. The importance of these risks cannot be estimated at this time.

(5) It is recognized that safety considerations in the use of any vaccine coming from this project are a function of other risks to which the soldier may be exposed. It might prove to be acceptable to use a vaccine which produced significant adverse reactions in limited numbers of personnel whose ability to perform in an environment contaminated by chemical agents was critical to a military mission. Such risks might be not at all acceptable in a civilian population.

d. Considerations Regarding Laboratory Work:

(1) In general, the ad hoc study group strongly supported the objective of the project to develop protection of man to nerve gas agents. The group also endorsed the enthusiasm and capability of the scientists involved in the project who have been working near the limits of the state-of-the-art in a most persistent manner. However, the ad hoc study group does have constructive suggestions for change which are stimulated by their interest in the future goal of the project--the protection of man against chemical agents. These suggestions will be stated fully in the recommendations of this report, but the reasons for these suggestions will be presented in the following paragraphs.

(2) As noted in paragraph 2.b.(1) above, the principle that antibodies may be prepared against small molecular weight compounds such as nerve gases is well established. The achievement of this goal for the nerve gas agents may or may not be possible because of the absence of an aromatic ring in their structure as noted in paragraph 2.b.(2)(b) above. If this goal is achievable at all it will be achieved by the application of presently existing techniques. Of the five items covered in Dr. Sternberger's presentation to the group at Edgewood Arsenal, three are not essential to the achievement of the goal, e.g., predictive immunization, immunoendocrinology, and the "superantigen." All three of these areas are interesting areas for basic research, but none of them are critical to the basic objective. Specific comments will be made to each below.

(3) The electron microscopic studies of antigen-antibody reactions on cell surfaces, designed to lead to methods of predictive immunization, are most interesting. However, their prosecution is not required for the immediate attainment of the goals of the program. The success of the approach is not certain. Moreover, it represents an approach which will require much effort and considerable time to discover whether, in fact, success is attainable. In addition, at the present stage of development of the program, where feasibility studies are most urgent, it would require a significant diversion of personnel and effort from the immediate goal of protection against agents by immunization.

The proposed electron microscopic procedure would not replace the need for a conventional biological system for testing of antibody blockage of acetylcholine esterase inhibition by the nerve gas agents. This type of test is urgently needed on the project. Given its presence and existing technology for determination of antibody affinity it is possible to arrive at sound estimates of both antibody levels produced and the affinity of the antibody for both the hapten carrier moiety and the toxic agent itself. Both determinations are desirable because of the need to develop a schedule of immunization that both produces large amounts of antibody and antibody which has a high affinity for the toxic agent.

(4) The concept of utilizing polypeptide or other hormonal carriers for the nerve gas agents as a superantigen is interesting and novel. However in the experience of one of the panel members, the chemical competence required for work with the polypeptides aimed at their use as drugs is of an exceedingly high order. Several large pharmaceutical firms are active in this area and their progress has been slow. This approach does not appear to be necessary to reach the goal of the project. Further, all group members expressed extreme concern regarding the safety of utilizing such biologically active materials as carriers for the nerve gas agents. Should immunity be achieved to the releasing factor or hormonal carrier, instead of or in addition to the nerve gas hapten, the physiologic damage done to man would be major and of far greater concern than with a carrier such as keyhole limpet hemocyanin or even bovine serum albumin or lobster hemocyanin, either one of which could stimulate dietary allergic hypersensitivity. For this reason, the ad hoc group cannot recommend continuation of work in the immunoendocrinology field. Similar reservations regarding safety also concern the group members with the immunoendocrinology program in which ACTH or similar natural hormones were proposed as carriers of the hapten. Similar objections can be raised, in terms of time and resource requirements, against the superantigen and immunoendocrinology programs as were urged against the predictive immunization program in the paragraph above.

(6) In recommending decreased emphasis on the three areas mentioned above, the ad hoc group urges increased emphasis in several areas. The first of these, the use of in vitro tests for antibody activity utilizing biological indicators of activity has already been mentioned. Affinity determinations by use of serum dilutions should also be considered. There is also a possibility that the molecular modeling approach by organic chemists could contribute to the project. Further the group supports increased emphasis, as planned, in the use of a second species of animal in addition to the rabbit. Several schedules of immunization, including complete Freund's adjuvant, and at least two carrier systems should be explored. Further, for reasons already mentioned, another agent in addition to GD, possibly one in the V series, should be included in the testing. With these shifts in emphasis, an additional year's work should provide a good measure of the potential for success of this project.

(7) The ad hoc group further suggests that considerable benefit might result from periodic contact with consultants in immmology from nearby medical centers such as Johns Hopkins or Maryland University Schools of Medicine. In addition, once the possibility of clinical testing in man becomes apparent, coordination with the staff of the Office of the Surgeon General, Department of the Army is indicated. In such contact with outside consultants and Department of the Army, the preparation of more formalized reports in "publishable form" is indicated to summarize research results.

3. Recommendations: The Army Scientific Advisory Panel Ad Hoc Group on Chemical Vaccines submits the following recommendations:

a. The group recommends that the project be continued with the changes of emphasis outlined in the following recommendations. The project objective--the protection of man against chemical agents through immunization techniques--is a sound objective although it is not possible at this time to arrive at a firm estimate of the feasibility of reaching the objective.

b. The group recommends increased emphasis on the following elements more directly related to achievement of the project goals:

(1) Systematic studies of several schedules of immunization, several carrier proteins, several species of animals, and several agents with the target of measurement of both the quantity and affinity of antibody produced as a function of the pertinent variables;

(2) The development of an *in vitro* test utilizing a biological indicator of antibody activity in blocking acetylcholine esterase inhibition by the chemical agents tested, and use of serum dilution or radioimmunoassay techniques for measurement

(3) The early test of passive immunization as a protective technique in animals to confirm conclusively that the protection afforded is mediated by antibody;

(4) The exploration of molecular modeling of the combining sites of antibody and chemical agent;

(5) The greater use, on a periodic basis, of consultants in immunology from nearby medical centers; and

(6) Better summarization of research results in "publishable form" for subsequent reviews of the project.

c. The group recommends decreased emphasis on the following elements less directly related to the achievement of the project goals:

(1) The use of electron micrography as a predictive immunology technique;

(2) The use of ACTH, other hormones or releasing factors as hapten carriers in the immunoendocrine or superantigen projects.

(3) Although these concepts may be attractive in terms of basic science research, they do not, as presented at this point in time, appear to have major applicability to goals.

d. The group recommends, provided certain elements of the program are emphasized and others deemphasized, that the program funding remain approximately the same for the next year. If the changes are made as recommended, the present funding should be quite adequate for the program.

e. The group recommends that the Army Planning Staff, in its consideration of this program, recognize that the maximum theoretical antibody production capability of the human body is estimated to be in the order of neutralization of 10 LD_{50}'s of agent GD or other agents having comparable molecular weights. Realistically, such levels of antibody may never be achieved with safety in man, however, lower levels of protection may be exceedingly useful.

(It must be realized that the US Army standard operating procedures recommend that the enemy be exposed to large lethal doses of agent. For example, in a chemical attack on enemy troops with gas masks available, 15-20 rounds of 155mm shells each containing 6 lbs of GB are required to cover a hectare (100 x 100 meters). Assuming the persistance of the agent to be 10 minutes, a normal person breathing at the rate of 15 liters/minute would be exposed to 380 LD_{50}. If it took him 30 seconds to detect the presence of the agent and to mask, he would still be exposed to 20 LD_{50}. Hence, complete protection against an agent may never be attained.)

f. The group recommends that a similar review of this program be undertaken in 9 to 12 months to evaluate the progress made toward the program goals, including the feasibility of development of antibody directed toward haptens lacking an aromatic ring. At that time a more realistic estimate can be made of the time and cost required to reach the program goal, based upon the success achieved during the intervening period.

FOR THE AD HOC STUDY GROUP:

7 Incl
As Stated

Herbert L. Ley, Jr., M.D.
Chairman

JULY 1973

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ASAP STUDY

1. PROPOSED NAME: ASAP Ad Hoc Group on Chemical Vaccines

2. STATEMENT OF THE PROBLEM: To evaluate the present status of the Army program to develop vaccines against chemical agents and to estimate the future results to be expected from the program.

3. CONSIDERATIONS:

a. There is presently no acceptable form of medical prophylaxis against chemical warfare agents. Over the past few years, Army scientists at the Biomedical Laboratory, Edgewood Arsenal, have been conducting research on the possibility of using vaccines to protect individuals against chemical agents. The work has proceeded slowly since it was necessary to adapt and perfect new techniques in serology and immunochemistry in working with extremely small quantities of material.

b. Recent results indicate that some degree of protection can be demonstrated in animals when they are injected with vaccines against agent-related chemical compounds and then challenged with the toxic chemical agent. This is the first known demonstration of vaccine protection against a chemical agent. The ability to enhance the degree of protection and adapt it to man in a relatively safe procedure is the ultimate aim of the program.

c. Since the research has been entirely an in-house effort, it is advisable to have an independent group of experts review the results and advise on the likelihood of successful achievement of the objective. It is necessary that the experts be aware of the latest developments in the fields of serology and immunochemistry since success of the effort is dependent upon advancing the knowledge in these fields.

d. The ADDR&E (E&LS) has been informed that this evaluation will take place and has requested results of the study by 15 July 1973.

4. PROPOSED TERMS OF REFERENCE:

a. To review the information amassed as a result of research on vaccines against chemical agents.

b. To assess the state-of-the-art in serology and immunochemistry as it relates to vaccine development against chemical agents.

c. To recommend future direction and magnitude of effort to achieve a vaccine against chemical agents at the earliest possible time.