With regard to influenza, the first evidence that this virus can have an immunosuppressive effect was reported in 1919 by Bloomfield and Mateer with their description of loss of skin reactivity to Old Tuberculin by persons experiencing acute influenza (2). More recent studies have confirmed the association of influenza virus infection in humans with decreased cutaneous reactivity to routine skin test antigens (3,4), and also with impaired responsiveness of peripheral lymphocytes to phytohemagglutinin (4,5).

Other data have shown sensitization of humans to influenza virus itself by means of delayed cutaneous reactions (6,7), and by proliferative responses of peripheral lymphocytes cultured with virus antigen (8,9). However, despite the apparent impairment of established cell mediate immunological responsiveness during influenza, evidence concerning the significance of this immunological system in the host response to influenza infection itself is conflicting (7, 10, 11, 12).

In summary, the available information about the effect of influenza on immunologic function suggests a depression of the cell-mediated system during acute infection, and sensitization of this system to the virus in addition to the well-known induction of antibody formation following exposure. Information concerning effects of influenza infection on primary induction of delayed hypersensitivity and on primary and secondary antibody responses to heterologous antigens is meager or lacking although studies with other viruses suggest that such effects might occur.

The functional status of the immunologic system seems likely to be important both in the host response to influenza virus infection and in the development of post-influenzal complications such as secondary bacterial infections. Therefore, further information about the effect of influenza infection on immunologic function is desired.

Keyhole limpet hemocyanin (KLH) is a protein antigen which has been shown to be a suitable immunologic probe for study of both delayed hypersensitivity and antibody production in humans (13). Naturally acquired sensitization to KLH is rare, and the antigen can be used to investigate primary and secondary immunologic responses. We propose to use KLH as well as 4 routine skin test antigens to evaluate the effects of influenza infection on immunological functions. Correlation of the results with responses of the subjects to replicating influenza viruses (virus shedding, illness, antibody response) will also be investigated.

Purpose:

To re-evaluate the previous reports of depression of established delayed hypersensitivity during influenza infection, and to explore the effect of this virus infection on the primary induction of delayed hypersensitivity and on primary and secondary antibody responses.

Description of Study:

This investigation will be performed on volunteer inmates at the Ramsey Unit of the Texas Department of Corrections in conjunction with

previously approved protocols entitled "Immunogenicity and Clinical Efficacy of Influenza Type A Whole Virus and Subunit Vaccines" and "Comparison of Homotypic and Heterotypic Immunity to Type A Influenza Virus Infection" (principal investigator: Robert B. Couch, M. D.). Where challenge with influenza (Scotland strain of influenza A) is indicated for subjects in this investigation (Influenza Groups), the men will have already volunteered for participation in one of the earlier protocols, will be free of detectable antibody to the challenge virus, and, as described below, will have additionally agreed to participate in this investigation after a full explanation. These men will be confined to a wing set aside for this purpose at the Ramsey Unit for 2 days prior to intranasal challenge with Scotland influenza virus and for approximately 10 days after the challenge as per previous protocols.

Those subjects who will not be challenged with influenza (Control Groups) will be matched in their influenza antibody status as closely as possible with the Influenza Groups before being offered the opportunity to participate. Members of the Control Groups will continue in their usual quarters and daily routines during this period.

The design of the study is shown in the following table:

Groups of 15 volunteers	Days				
	0	28	32	38	49
1. Control	KLH	--	KLH	Blood only	KLH
2. Influenza	KLH	Influenza challenge	KLH	Blood only	KLH
3. Control	--	--	KLH, PPD, C, T, SK-SD	--	KLH, PPD, C, T, SK-SD
4. Influenza	--	Influenza challenge	KLH, PPD. C, T. SK-SD	--	KLH, PPD. C, T, SK-SD

Abbreviations: KLH = Keyhole limpet hemocyanin, prepared by Miss Sarah Dyre in the laboratory of Dr. Evan M. Hersh, Dept. of Developmental Therapeutics, Univ. of Texas, M. D. Anderson Hospital, Houston, Texas 77025, for continuing clinical investigations of that laboratory (13). Doses of 100 micrograms to be used.
PPD = Purified protein derivative (5 T. U.), obtained commercially.
C = Candidin (1:100), obtained commercially.
SK-SD = Streptokinase-streptodornase (4 units), obtained commercially.

All of the test antigens shown by abbreviation in the table are to be administered in 0.1 ml intradermal injections on the volar aspect of the forearm. Each volunteer is to have a 20 ml blood sample for KLH antibody assay collected prior to each set of intradermal injections; the total blood collection for this investigation in combination with the other studies in which the two Influenza Groups will be participating will be well within the 450 ml limit per 6 weeks. KLH antibody assays will be by a double antibody radioimmunoassay already in use in one of our laboratories (R.D.R.) and permitting quantitation of the antibody response according to immunoglobulin class. Cutaneous reactivity to each intradermal injection will be measured as the mean of 2 perpendicular diameters of induration at 24 and 48 hours.

Answers to the following questions will be sought by comparing serum antibody titers to KLH and cutaneous reactivities to the indicated antigens in Control and Influenza Groups, and by correlating these measurements with responses to influenza challenge (virus shedding, illness, antibody responses) in the Influenza Groups:

1. Does influenza infection alter primary induction of delayed hypersensitivity? (Compare KLH skin reaction at 49 days in groups 3 and 4.)

2. Does influenza infection alter the primary antibody response? (Compare KLH antibody titers at 49 days in groups 3 and 4.)

3. Does influenza infection alter the secondary antibody response? (Compare KLH antibody titer changes between days 32, 38, and 49 in groups 1 and 2.)

4. Does influenza infection depress established delayed hypersensitivity as previously reported (3, 4)? (Compare KLH skin reactions at day 32 in groups 1 and 2, and changes in skin reactions to other test antigens between days 32 and 49 in groups 3 and 4.)

5. How do the above immunologic responses to non-replicating antigens correlate with responses to replicating influenza virus?

6. Is the presumed depression of delayed cutaneous reactivity during active influenza infection gone by 3 weeks after challenge as data of others (4) suggests should occur? Any volunteers who still exhibit depression of delayed cutaneous reactivity at 3 weeks will be retested with the pertinent antigen(s) 6 weeks after influenza challenge

Subject Population:

Volunteers will be 18 to 40 year old adult male inmates of the Purdy Unit of the Texas Department of Corrections. Details of the selection process have been previously approved.

Those men who lack antibody to the Scotland strain of influenza A, who have already been selected as possible control subjects for intranasal challenge studies with this virus as described in earlier protocols, and who have volunteered to participate in those studies will be offered the opportunity of participating in the present investigation as a member of

one of the Influenza Groups. No men will be challenged intranasally with influenza virus for the sole purpose of the present investigation.

Men who lack antibody to Scotland influenza but who were not selected as control subjects for intranasal challenge with this virus will be offered the opportunity of participating in the present investigation as a member of one of the Control Groups. Other men in the Control Groups will be selected from among those with low titers of antibody to Scotland influenza.

Volunteers will be reimbursed at the rate of $4 per blood sample and $2 per individual skin test for each portion of the study in which they participate.

Discomfort and Potential Hazard to Subjects:

The discomforts associated with this study are those of venipuncture and intradermal skin tests. All skin tests will be administered by a physician and emergency medications will be on hand in the event they are needed. The volunteers will be seen by a physician 24 and 48 hours after their intradermal injections; volunteers also have immediate access 24 hours a day to a medical officer who will notify the responsible physician, and care will be administered as indicated.

Some soreness may develop at sites of intradermal injections. Any excessive reactions (vessiculation, symptomatic regional adenopathy, fever, etc.) will be treated with topical steroids and/or symptomatic medications as indicated, and the offending antigen will not be administered again.

Method of Obtaining Informed Consent:

A detailed description of the study to be performed will be given to potential volunteers verbally prior to the beginning of the study with explanation that they may elect not to participate while continuing with any other investigations for which they have volunteered. Volunteers will be given the opportunity to ask any questions they wish, and it will be made clear that they can remove themselves from the study at any time. They will then be asked to read and sign the appended written consent form if they wish to participate.

Procedures to Minimize Potential Risks:

See previous section on potential hazards. Privacy of the volunteers will be strictly protected in presenting the results of this study.

Benefits to the Subject:

The only potential direct benefits of this study to the individual volunteers are knowledge of how their immunological systems are able to respond to a foreign antigen, and knowledge of tuberculin reactivity in those subjects who receive the PPD skin test. The benefits to society are in the potential of increased understanding of the pathogenesis of influenza illness and post-influenzal complications.

Risk-Benefit Ratio:

The risks involved in this study are negligible and are felt to be justified by the information to be obtained.

Thomas R. Cate, M. D.
Principal Investigator

Warner Knight, M. D.
Chairman, Department of
Microbiology & Immunology

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REVISED

BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77025

[illegible] OF MICROBIOLOGY AND IMMUNOLOGY
(713) 790-4469

VOLUNTEER'S CONSENT FORM

Immunoresponsiveness during Influenza Virus Infection (KLH plus other skin tests)

I understand that I am consenting to the injection into the skin of my forearm of an investigational antigen and 4 commercially available skin test antigens on 2 to 3 occasions according to a schedule that has been explained to me. I understand that the injections may produce pain, swelling, redness, heat and/or a blister at the site of the injection and that I may feel bad or develop fever. It is possible that other symptoms may occur.

I understand that 20 ml of blood will be obtained 2 to 3 times for tests.

I understand that the results of these studies are not likely to help me directly, but that the studies are aimed at helping to understand why some people get sick with influenza virus infection and/or develop complications after the infection.

I understand that I will receive $4 for each blood sample drawn and $2 for each individual skin test performed.

[illegible] has been clearly explained to me and I understand the [illegible] have been given the opportunity to ask any questions I might have. I understand that I have the right to withdraw from the study at any time without prejudice to me.

Signature_____

Date_____

Witness_____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the normal volunteers.

Signature_____

Date_____

REVISED

BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77025

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
(713) 790-4469

VOLUNTEER'S CONSENT FORM

Immunoresponsiveness during Influenza Virus Infection (KLH only)

I understand that I am consenting to the injection into the skin of my forearm of an investigational antigen on 3 to 4 occasions according to a schedule that has been explained to me. I understand that the injection may produce pain, swelling, redness, heat and/or a blister at the site of the injection and that I may feel bad or develop fever. It is possible that other symptoms may occur.

I understand that 20 ml of blood will be obtained 4 to 5 times for tests.

I understand that the results of these studies are not likely to help me directly, but that the studies are aimed at helping to understand why some people get so ill with influenza virus infection and/or develop complications after the infection.

I understand that I will receive $4 for each blood sample drawn and $2 for each individual skin test performed.

The proposed study has been clearly explained to me and I understand the hazards involved. I have been given the opportunity to ask any questions I might desire and I understand that I have the right to withdraw from the study at any time without prejudice to me.

Signature_____

Date_____

Witness_____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the normal volunteer.

Signature_____

Date_____

BAYLOR COLLEGE OF MEDICINE
TEXAS MEDICAL CENTER
HOUSTON, TEXAS 77025

DEPT OF INTERNAL MEDICINE
DEPT OFFICE (713) 790-3215
LAB OFFICE (713) 790-4761

October 15, 1975

Thomas R. Cate, M.D.
Department of Microbiology & Immunology
Baylor College of Medicine
Houston, Texas 77025

Dear Dr. Cate:

The Baylor Institutional Review Board for Human Research is pleased to inform you that your research proposal Immunoresponsiveness During Influenza Virus Infection (NIH contract AI-42528) (RAMSEY)

was approved on October 14, 1975 according to institutional guidelines and provided it receives the unaltered approval of the institutional committee in which it is involved.

1. Continued review will be required
   - ( ) a. After each subject's exposure
   - ( ) b. Quarterly
   - ( ) c. Semi-annually
   - (x) d. Annually
   - (x) e. Change in Protocol
   - (x) f. Development of unexpected problems or unusual complications
   - ( ) g. Other

2. Method of Review
   - (x) a. Questionnaire (example enclosed)
   - ( ) b. New Protocol
   - ( ) c. Interview with principal investigator
   - ( ) d. Other

Sincerely yours,

H. Brown

Harold Brown, M.D., Chairman
Baylor Institutional Review Board
for Human Research

HB:ib

Protocol #2

Reference: Request for Approval of Clinical Investigation Involving Human Beings

Title: Effect of Corticosteroids and Related Compounds on Immune Reactions in Man.

Principal Investigator: William T. Butler, M.D. William T Butler

Co-investigators: Roger D. [illegible], M.D. [signature]

Evan M. [illegible], M.D. [signature]

Harry S. Lipscomb, M.D. [signature]

Experimental Protocol: See attached

Duration of Study: 2 years

Chairman, Microbiology Department,
Baylor College of Medicine
Vernon Knight, M.D. [signature]

Committee Approvals: Protocols are being submitted simultaneously to the Committee on Research Involving Human Beings of Baylor College of Medicine and the General Clinical Research Center Committee.

(1) Consent: See attached consent form.

(2) Procedure to be used:

Effects of corticosteroids on the immune response

It has been known for nearly 2 decades that cortisone causes prolongation of skin allografts in laboratory animals (1, 2), although it is less effective in so doing in man (3). Moreover, one particularly useful property of corticosteroids is that of being able to reverse established states of sensitization (4). Clinically, corticosteroids clearly can reverse acute renal allograft rejection (3, 5, 6).

Antibody formation is suppressed in animals given corticosteroids under appropriate conditions. Results of antibody studies in man are less convincing, [illegible]ily because most studies have been done using dosage schedules more likely [illegible] in physiologic rather than pharmacologic effects (3). Moreover, it appears [illegible]ome systems, the timing of administration of corticosteroids in relation to the [illegible]dose is crucial: once antibody synthesis is under way, corticosteroids may be less effective (3). A related finding is that established delayed hypersensitivity reactions in man are suppressed by daily, but not by alternate-day administration of corticosteroids (7).

Only little is known about the mechanisms by which corticosteroids [illegible] with the immune response. Actually, the net effect may be the result of a number of individual actions (3 4). Direct destruction of lymphoid tissues, perhaps as a result of binding to specific receptor sites in thymocytes or lymphocytes (8, 9), inhibition of phagocytosis, anti-inflammatory effects, stabilization of membranes of lysosomes to effector cells, and inhibition of cellular metabolism have all been implicated as possible mechanisms of actions of corticosteroids on immune reactions (5). Important to note however, is the fact that considerable differences of action exist in different species and after different types of antigenic stimuli. Therefore, for any particular set of circumstances, the effects of corticosteroids on the immune system can be determined only by investigation.

Purpose of study

Despite the widespread clinical use of corticosteroids, the immunologic effects of these agents in man are not clearly understood. Adequate data does not exist to answer the following questions:

1. How effective are corticosteroids in suppressing the immune response?
2. Which of the many possible corticosteroids available are the most effective agents in suppressing immune reactions?
3. What doses are required to cause substantiated immunosuppression?

4. At what intervals should individual doses be given to obtain maximum effect for the particular response in question?

5. Do progesteroids have immunosuppressive properties? Do they act synergistically with corticosteroids?

6. Is immunosuppression per se the mode of action of steroids? Or, do the steroids exert their effects by anti-inflammatory or other actions?

Summary of procedures

Patients or volunteers selected for study will be admitted to the General Clinical Research Center of the Methodist Hospital. Persons with a history of duodenal ulcers, diabetes, glaucoma and hypertension will not be accepted for study. After baseline studies, corticosteroids will be given according to protocol for 7 - 14 days; studies will be repeated during and after drug administration. Patients will be examined frequently throughout the study. If any untoward findings appear, corticosteroids will be immediately withdrawn without regard to the status of the experiment. All studies will be done with the full informed consent of the patient (see attached consent form).

Immunologic, hematologic, endocrinologic, microbiologic, metabolic and psychologic studies are planned. Not all studies will be done at one time, but rather each experiment will be designed to incorporate as many of the studies as possible without interfering with the comfort of the patient. The types of studies include:

A. Immunologic studies

1. Effect of corticosteroids on humoral immunity
   a. Immunoglobulin metabolism
      (1) Serum concentrations of IgG, IgA and IgM
      (2). Plasma disappearance rates and turnover of isotopically labeled immunoglobulins
   b. Antibody formation
      (1) Primary response to keyhole limpet hemocyanin (KLH), GLAT, etc.
      (2) Secondary response to diphtheria and tetanus toxoids

2. Effect of corticosteroids on cell-mediated immunity
   a. Delayed-type hypersensitivity
   b. Lymphocyte reactivity, in vitro
   c. Skin allograft survival - Determine survival of grafts matched and unmatched for one or more histocompatibility antigens.

3. Effect on immediate - type hypersensitivity

4. Effect on inflammatory response
   a. Cellular response determined by Rebuck skin window technique
   b. Phagocytic capacity determined quantitatively by uptake of bacteria by leukocytes.

5. Effect on complement system
   a. Quantitative measurement of individual components
   b. Turnover of isotopically labeled complement components (later, if feasible)

B. Hematologic studies

1. Complete blood counts
2. Bone marrow examination (selected cases) before and once either during or after corticosteroids.

C. Endocrinologic studies - Effect of corticosteroids on the major endocrine systems:

1. Hypothalamus and pituitary axis - radioimmunoassay of serum concentrations of ACTH, LH and FSH
2. Thyroid - Serum protein bound iodide; T3 and T4 uptake
3. Adrenal - Analysis of blood and urine for 17 hydroxy corticosteroids and 17 ketosteroids, total and fractional; urinary epinephrine and nor-epinephrine
4. Gonadal - Measurement of estriol or estradiol
5. Metabolic conversion products - pregnanediol/pregnanetriol, and testosterone/epitestosterone
6. Free-fatty acids

D. Microbiologic studies

Cultures of nasopharynx and urine for bacteria, fungi, mycobacteria and viruses

E. Metabolic studies

Serum electrolytes, blood and urine glucose, serum transaminases, serum lactic acid dehydrogenases, alkaline phosphatase, bilirubin, calcium, phosphorus and creatinine and blood urea nitrogen; bone density, weight, height and girth; chest x-ray.

F. Psychologic studies

1. EEG
2. Evaluation by psychologic testing (later, in selected cases)

Patient discomforts and hazards

The study requires frequent blood and urine collections. It is anticipated that on some days large amounts (50 cc) of blood will be required; on many others, none. At no time will more than 50 cc be drawn on any one day.

Numerous skin tests will be applied. These may on occasion cause transient redness, swelling and local discomfort.

Less than one percent of adults develop reactions to immunization with antigens such as diphtheria toxoid. These reactions may be local or systemic, the latter characterized by fever and chills of several days duration.

Selected patients who receive a Rebuck skin window (10) or skin allograft (11) have minimal local irritation at the site of application which will disappear when the test is removed.

Side effects are known to occur during corticosteroid administration. However, these most often occur after long-term administration of steroids. Patients will be monitored carefully for any evidence of the development of diabetes, hypertension, peptic ulcer, and other untoward effects. At the first sign of serious side effects, corticosteroids will be withdrawn and appropriate therapy instituted.

(3) Potential benefits

The major benefits will be

(a) Elucidation of the effects of corticosteroids on the immune response
(b) Definition of the optimal conditions for administration of corticosteroids to suppress immune reactions both in transplant patients and in patients with immunologic disorders requiring immunosuppression.

(4) Invasion of privacy

[illegible] procedures outlined above will invade privacy. If in the future psychological testing is done, it will be done primarily to determine whether or not corticosteroids alter the baseline ability to carry out simple mental functions. If further in depth studies appear warranted, a separate detailed protocol will be submitted.

[illegible] will strictly adhere to the protocol of procedures described in this application for research support and will not alter those procedures in any way concerned with human beings, without prior submission to and receipt of approval from the Faculty Committee on Research Involving Human Beings".

CONSENT FORM

Title of Study: Effect of Corticosteroids and Related Compounds on Immune Reactions in Man.

Immunizations: Patients will be immunized with a variety of antigens by one of the following routes: intramuscular, intradermal or subcutaneous. In some cases antigen will be placed directly on the mucous membranes. An example of the latter would be the application of antigen in nose drops. The antigens to be used include those which are approved for use by the Food and Drug Administration and those which are still considered experimental but which have had extensive use in man without adverse side effects.

In general, reactions to the antigens will be infrequent. Local redness, swelling, tenderness and itching may last for about 24 hours after intracutaneous injections of antigen, although no reaction occurs in most persons. Intramuscularly injected vaccines can produce local soreness and redness lasting two to three days, occasionally irritability and anorexia, rarely vomiting and occasionally febrile reactions.

Skin Tests: The patient will be skin tested for allergy to numerous substances such as ragweed, foods, dusts, molds, and bacterial viral and fungal products. The extent of the reaction will depend on the degree of sensitivity present, and may include localized swelling, redness and pain at the injection site. Systemic reaction with prostration and fever may occur in highly allergic persons, but this is rare. Scratch tests will be done prior to immediate-type skin tests to exclude test materials to which subjects are highly sensitive.

The inflammatory response is studied by making an abrasion of a 1 cm^2 area of the skin of the forearm. The abrasion elicits a brisk inflammatory response. A glass cover slip is taped over the lesion; this is replaced at timed intervals by new cover slips until the abrasion heals. Skin grafts no larger than 1 cm^2 will be applied using surgical aseptic techniques. The graft may become inflamed and reject; the wound will then heal, possibly leaving a scar.

Radioisotope Studies: Metabolism of gamma globulin will be studied by injecting radioiodine - labeled purified gamma globulin. Material to be injected will be sterile by bacterial culture and will be pyrogen free, based on the standard U.S.P. pyrogen test in rabbits. Despite these negative tests, an occasional person may develop fever and chills following the injection.

Steroid Administration: Cortisone or one of the related corticosteroids or progesteroids will be given daily in tablet or by injection. These drugs can cause toxic reactions, including diabetes, high blood pressure, peptic ulcers, psychologic disturbances, skin rashes, acne, swelling of the body tissues, weakness of muscles and a number of other unpredictable reactions. In general, these reactions occur principally in patients on

prolonged treatment, that is, patients treated for periods of many weeks or months. An occasional patient, however, may develop symptoms after only a short time, especially if he has a history of having had peptic ulcers and so forth.

All immunosuppressive drugs tend to lower the body's resistance to infection and make it more difficult for the body's own defense mechanisms to fight an established infection. If any evidence of infection develops during the study, steroids will be stopped immediately and appropriate therapy instituted.

Patient Discomfort: In addition to the above mentioned items, the study requires frequent blood and urine collections.

Institutional Authorization: All studies to be done have been approved by the Radioisotope Committee and the Human Studies Committee of Baylor College of Medicine.

Signatures: The nature and demands of the study have been clearly explained to me, and I understand and accept the hazards involved. I also understand that if some unforeseen complication occurs, it, too, is considered to be one of the hazards of the experiment for which I volunteer as endorsed by my signature below. A patient may withdraw from the study at any time of his own choosing.

Signature _____

Date _____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the patient.

Signature _____

Date _____

References

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Pre-drug treatment	Drug – treatment	Post-drug treatment	
			Corticosteroid Administration
			Skin Tests – delayed (tuberculin + 6 others)
			Skin Test DNCB
			Skin Tests– Immediate type
			Antigen injections (SQ) (ST=skin test ID)
			Skin window applied
			^{125}I-labeled human IgG (IV)
			Serum for Ig, Ig turnover, and complement level
			Serum for antibody titrations
			Blood for phagocytic studies of leukocytes
			Blood for lymphocyte culture studies
			Blood for complete blood counts
			Blood for endocrinologic assays
			Urine for endocrinologic assays
			Microbiologic cultures of nasopharynx and urine
			Blood for metabolic studies
			Weight and girth measurements
			Chest – x-ray

Addendum

Title: Effect of Corticosteroids and Related Compounds on Immune Reactions in Man.

Principal Investigator: William T. Butler, M.D. William T. Butler, M.D.

Approvals:

Chief of Medicine, Methodist Hospital,
H. D. McIntosh, M.D. H. D. McIntosh, M.D.

Chairman, Microbiology Department,
Baylor College of Medicine
Vernon Knight, M.D. Vernon Knight, M.D.

Purpose: To request permission to incorporate administration of aspirin to patients as a control in the above experimental protocol.

The attached above named protocol was submitted in its original form to the Committee on Research Involving Human Beings of Baylor College of Medicine on March 20, 1970, and was approved on March 25, 1970. The same protocol has also been approved by the General Clinical Research Center Committee and by the Committee for Clinical Investigation Involving Human Beings of Methodist Hospital.

Two experiments have been carried out so far. Preliminary analysis of results indicate that methyl prednisolone (72-96 mg, po, daily for 3 days) has profound suppressive effects on cellular and humoral immunity. In order to obtain a reasonable control for the cortisone study we would like at this point to compare those effects found with corticosteroids with those which would occur following similar treatment with aspirin. Aspirin has been chosen principally because of its anti-inflammatory effects and because it has been shown only questionably to alter immune mechanisms in animal experiments. The experimental protocol would be precisely the same as outlined in the attached protocol.

Proposed dosage of aspirin. We would aim for a blood salicylate level of about 30 mg/100 ml. Blood levels will be measured twice-daily during the period of administration of aspirin. According to data supplied by the Bayer Company Division

of Sterling Drug Inc., this level is usually reached by the third day by giving 65 mg/pound body weight per 24 hours, given in divided doses every 4 hours. We will monitor the dosage according to blood salicylate level and maintain a level of about 30 mg/100 ml for no longer than 5 days. As was the case in the corticosteroid studies, patients will be monitored closely for adverse reactions, and studies will include daily evaluation of acid-base balance.

Attachment to Addendum

Title: Effect of Corticosteroids and Related Compounds on Immune Reactions in Man.

Dosage of Aspirin

The ultimate purpose of studying the effect of aspirin on the immune response is to determine whether it will be useful in the treatment of acute allograft rejection. It would follow, therefore, that we need to study a dose of aspirin which would correspond roughly to that used in the treatment of other severe acute inflammatory lesions, such as acute rheumatic fever. It is stated in Gordman and Gilman (Third Edition, page 329), "For maximal suppression of rheumatic inflammation, doses that provide a plasma salicylate level of 25-35 mg % should be maintained....For adults, a total daily dosage of 5 to 8 g., given at intervals in 1-g. amounts, usually suffices". This statement is consistent with other studies reported in the review of the literature by M. L. Tainter and A. J. Ferris (Aspirin in Modern Therapy, A Review, Sterling Drug Inc., New York, 1969).

In order to achieve this therapeutic dose level it is our anticipated plan to give aspirin according to the following schedule: (B. Beckman, in Pharmacology, 2nd edition, W. B. Saunders, Philadelphia, 1961) "On a 24-hour dosage of 65 mg (1 gr.) per pound of body weight, administered fractionally by mouth at 4-hour intervals around the clock, this titer of 30 to 35 mg per 100 ml is usually reached by the third day. Once the level is achieved it can usually be maintained by the same dosage at 6-hour intervals instead of 4-hour intervals".

Modulation of Dosage of Aspirin

1. If persistent tinnitus and gastrointestinal irritation occur, dosage will be reduced accordingly.
2. If any sign of a serious reaction occurs, aspirin treatment will immediately cease. Included in this category are idiosyncratic reactions, dermatologic reactions, allergic reactions, gastrointestinal hemorrhage, renal reactions, respiratory reactions and disturbances in acid-base balance.
3. If the blood salicylate level exceeds 40 mg % at any time during the study, all aspirin will be stopped until the blood level falls to below 30 mg % at which time treatment will be re-started at a lower dose level.

Patient Selection

In addition to excluding patients from the study with a history of duodenal ulcers, diabetes, glaucoma and hypertension as outlined in the original proposal, we will also exclude patients from the aspirin study with a history of asthma, allergic disorders, and deafness as determined by audiometer testing.

CONSENT FORM

Title of Study: Effect of Aspirin on Immune Reactions in Man.

Immunizations: Patients will be immunized with a variety of antigens by one of the following routes: intramuscular, intravenous, intradermal or subcutaneous. The antigens to be used include those which are approved for use by the Food and Drug Administration and those which are still considered experimental but which have had extensive use in man without adverse side effects.

In general, reactions to the antigens will be infrequent. Local redness, swelling, tenderness and itching may last for about 24 hours after intracutaneous injections of antigen, although no reaction occurs in most persons. Intramuscularly injected vaccines can produce local soreness and redness lasting two to three days, occasionally irritability and anorexia, rarely vomiting and occasionally febrile reactions.

Skin Tests: The patient will be skin tested for allergy to numerous substances such as ragweed, foods, dusts, molds and bacterial viral and fungal products. The ...ent of the reaction will depend on the degree of sensitivity present, and may ...localized swelling, redness and pair at the injection site. Systemic reaction ...prostration and fever may occur in highly allergic persons, but this is rare.

Radioisotope Studies:

Metabolism of gamma globulin will be studied by injecting radioiodine - labeled purified gamma globulin. Material to be injected will be sterile by bacterial culture and will be pyrogen free, based on the standard tests of the United States Pharmacopeia. Despite the negative tests, an occasional person may develop fever and chills following the injection.

Aspirin Administration:

Aspirin will be given in tablets at 4-hour intervals for a period no longer than 5 days. Many persons will develop gastrointestinal irritation or ringing in the ears; if so, the dosage will be reduced. If any sign of serious reaction occurs, aspirin treatment will immediately stop and appropriate corrective treatment will be given. The types of reactions that have occurred, but only very rarely in comparison to the total amount of aspirin that is consumed daily in the United States (approximately 30 tons) include skin rashes, allergic reactions, bleeding in the stomach and disturbances in the acid-base balance in the blood. The overall incidence of all types of hypersensitivity to aspirin has been estimated at about 2 per 1000 population.

Patient Discomfort: In addition to the above mentioned items, the study requires frequent blood and urine collections.

Consent Form - Effect of Aspirin on Immune Reactions in Man (Continued)

Institutional Authorization:

All studies to be done have been approved by the Radioisotope Committee and the Human Studies Committee of Baylor College of Medicine.

Signatures: The nature and demands of the study have been clearly explained to me, and I understand and accept the hazards involved. I also understand that if some unforeseen complication occurs, it, too, is considered to be one of the hazards of the experiment for which I volunteer as endorsed by my signature below. A patient may withdraw from the study at any time of his own choosing.

Signature _____

Date _____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the patient.

Signature _____

Date _____

To: Committee on Research Involving Human Beings
Baylor College of Medicine

Title: Determination of the ability of known antigens to induce antibody formation in man.

Investigators: William T. Butler, M.D.
Evan M. Hersh, M.D.
Roger D. Rossen, M.D.

1. Method of obtaining informed consent: See attached sample.

2. Experimental Protocol:

To fully evaluate the effectiveness of certain immunosuppressive agents, a quantitative measure of the capability of an individual to produce specific antibody is required. This is done by injecting antigens at periodic intervals and by measuring the circulating antibody that subsequently develops. Antigen injections are given intradermally, subcutaneously or intramuscularly in doses of 0.1 to 0.5 ml using sterile techniques. In some cases, antigens will also be applied directly to mucous membranes such as the nasal mucosa. Local redness, swelling, tenderness and itching may last for about 24 hours after intracutaneous injections of antigen, although no reaction occurs in most persons. Intramuscularly injected antigens can produce local soreness and redness lasting two to three days, occasionally irritability and anorexia, rarely vomiting and occasionally febrile reactions.

The antigens to be used can be categorized into 2 groups, those which are FDA approved and commercially available, and those which are still experimental but have had extensive use in man without adverse side effects.

The first group of antigens includes dermatophytin, dermatophytin O, candida, varidase, streptococcal toxin, trichinella extract, brucellergen, histoplasmin, coccidiodin, tube culin, mumps antigen, blastomycin, diphtheria toxin and toxoid, tetanus toxoid, typhoid-paratyphoid, and so forth.

The second group includes keyhole limpet hemocyanin (KLH), other hemocyanins and synthetic amino acid co-polymers such as glutamic-lysine-alanine-tryptophan (GLAT). These compounds have been given in doses of 1 to 5000 μg, intradermally or subcutaneously, without serious side effect by the following investigators:

1. Swanson, M.A. and Schwartz, R.S. Immunosuppressive Therapy. New England J. Med. 277:163-170, 1967.

2. Maurer, P.H., Gerulat, B.F., and Pincluck, P. Antigenicity of Polypeptides. VII. Studies in Humans. J. Expt. Med. 116:524-533, 1962.

3. Curtis, J.E., Hersh, E.M., Harris, J.E., McBride, C. and Freireich, E.J. The Human Primary Immune Response to Keyhole Limpet Hemocyanin, Interrelationship of Delayed Hypersensitivity, Antibody Response and in vitro Blastogenesis. (Submitted for publication).

4. Hersh, E.M. Kinetics of the Normal Human Immune Response. Annual Report to the NIAID. U.S. Govt. Printing Office. 1969.

3. No invasion of individual privacy will occur.

"I certify that I will strictly adhere to the protocol of procedures described in this application for research support and will not alter those procedures in any way concerned with human beings, without prior submission to and receipt of approval from the Faculty Committee on Research Involving Human Beings."

4. Signatures:

Principal Investigator E.M. Hersh, MD

Department Chairman Vernon Knight

August 26, 1969

CONSENT FORM

Title of Study: Determination of the ability of known antigens to induce antibody formation in man.

General Description of Research Procedure:

Patients will be immunized with a variety of antigens by one of the following routes: intramuscular, intradermal or subcutaneous. In some cases antigen will be placed directly on the mucous membranes. An example of the latter would be the application of antigen in nose drops. The antigens to be used include those which are approved for use by the Food and Drug Administration and those which are still considered experimental but which have had extensive use in man without adverse side effects.

All procedures undertaken will be done with the approval of the Human Studies Committees of Baylor College of Medicine and of the Hospital.

Potential Hazards of the Study:

In general, reactions to the antigens will be infrequent. Local redness, swelling, tenderness and itching may last for about 24 hours after intracutaneous injection of antigen, although no reaction occurs in most persons. Intramuscularly injected vaccines can produce local soreness and redness lasting two to three days, occasionally irritability and anorexia, rarely vomiting and occasionally febrile reactions.

Authorizations

The nature and demands of the study have been clearly explained to me and I understand and accept the hazards involved. I also understand that if some unforeseen complication occurs, it, too, is considered to be one of the hazards of the experiment for which I volunteer as endorsed by my signature below.

Signature _____

Date _____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the patient.

Signature _____

Date _____

To: Dr. Robert B. Couch, Program Director
General Clinical Research Center
Baylor University College of Medicine

From: William T. Butler, M.D.

Subject: Proposed Study of Diphtheria Immunization in Volunteers

Investigators: William T. Butler, M.D.
Roger D. Rossen, M.D.
Reuben D. Wende

Purpose:

To study the systemic and local antibody responses to non-replicating protein antigens applied to the nasal membranes and tonsils.

Background

Previous studies have indicated that following experimentally-induced upper respiratory virus infection, the IgA content of nasal secretions increases at a sustained level for at least two weeks, and is associated with locally produced specific antibody. The proposed studies are planned to determine whether locally produced antibody can be stimulated by non-replicating antigen as well.

Volunteers.

1. Type. Volunteers will be 18- to 40-year-old inmates of the Texas State Department of Correction.

2. Number of volunteers and duration of study. Nine volunteers; six weeks.

3. Selection. Volunteers will be solicited from the general population of one or more institutions of the Texas State Department of Correction. The investigators will visit the institution and describe the research protocol to prospective volunteers. Medical records will be screened on those men who volunteer. Final selection will be based on willingness to participate and good general emotional and physical health. The Department of Correction will then screen suitable candidates from the custodial point of view.

4. Medical procedures. Suitable volunteers will be admitted to Methodist Hospital in Houston for final medical examination and screening. On admission, complete medical histories and physical examinations will be performed. Laboratory studies must be within normal limits and will include urinalysis, complete blood count, electrocardiogram, chest and abdominal x-rays, blood urea nitrogen, blood sugar, transaminase (SGOT), serologic test for syphilis, total serum protein concentration and albumin-globulin ratio, serum electrophoresis, and serum immunoglobulin levels (IgG, IgA, and IgM).

Specimens:

1. Blood specimens (5 ml) will be obtained daily. In no case will more than 600 ml blood be obtained from a subject during a single study. Volunteers will be advised not to donate blood for three months after completion of the study.

2. Nasal wash specimens will be obtained daily. These will be done by instilling 5 ml lactated Ringer's solution into each nostril, and having the volunteer lean forward and blow the secretions into a beaker.

Biologic Reagents:

Immunizations will be done with purified diphtheria fluid toxoid, obtained from the Texas State Department of Health. Control immunizations will contain the same broth medium used in the preparation of the fluid diphtheria toxoid.

Experimental Protocol:

Following collection of baseline blood and nasal secretion specimens, six volunteers will be inoculated with a standard immunizing dose of fluid diphtheria toxoid and three with control medium as follows:

Volunteer	Immunizing Agent	Route of Immunization
1	Diphtheria toxoid	Intramuscular
2	Diphtheria toxoid	Intramuscular
3	Control medium	Intramuscular

Volunteer	Immunizing Agent	Route of Immunization
4	Diphtheria toxoid	Intranasal
5	Diphtheria toxoid	Intranasal
6	Control medium	Intranasal
7	Diphtheria toxoid	Intratonsillar
8	Diphtheria toxoid	Intratonsillar
9	Control medium	Intratonsillar

The intranasal and intratonsillar immunizations will be done by swabbing the fluid toxoid onto cotton swabs and placing the swab in the nasal passages and into the tonsillar crypts, respectively.

Hazards to the Volunteer:

Purified fluid diphtheria toxoid prepared by the Texas State Department of Health is known to give a local or systemic reaction in less than one per cent of the adult population. It can produce a local soreness and redness lasting two to three days, occasionally irritability and anorexia, and rarely, vomiting.

In general, toxoids precipitated with alum (i.e., those commonly used in the U. S. for immunization) cause more reaction than the fluid toxoids that we will use in the present study.

Benefits to the Subject:

The booster immunization to diphtheria should provided added protection against this disease.

VOLUNTEER CONSENT FORM

Title of Study: Diphtheria Immunization

General Description of Research Procedure:

Volunteers will be immunized with purified diphtheria toxoid by one of three routes: intramuscular, intranasal, and intratonsillar. Blood specimens and nasal secretions will be collected daily for about six weeks.

All procedures undertaken will be done with the approval of the Human Volunteer Studies Committee of the Baylor University College of Medicine to ensure safety of the experiment as well as its scientific value.

Potential Hazards of the Study:

Purified fluid diphtheria toxoid prepared by the Texas State Department of Health is known to give a local or systemic reaction in less than one per cent of the adult population. It can produce a local soreness and redness lasting two to three days, occasionally irritability and anorexia, and rarely, vomiting.

In general, toxoids precipitated with alum (i.e., those commonly used in the U.S. for immunization) cause more reaction than the fluid toxoids that we will use in the present study.

Authorizations:

The nature and demands of the study have been clearly explained to me and I understand and accept the hazards involved. I also understand that if some unforeseen complication occurs, it, too, is considered to be one of the hazards of being a volunteer. Furthermore, I understand that I may withdraw from the study if I find that I am unable to continue.

Volunteer's Signature _____

Date _____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the normal volunteer.

Investigator's Signature _____

Date _____

VOLUNTEER CONSENT FORM

Title of Study: Diphtheria Immunization

General Description of Research Procedure:

Volunteers will be immunized with purified diphtheria toxoid by either the intramuscular or intranasal route. In some cases the diphtheria toxoid will be labelled with radioactive iodine. The length of the study will be about two weeks. Blood specimens will be taken frequently and nasal secretions daily.

All procedures undertaken will be done with the approval of the Human Volunteer Studies Committees of the Baylor University College of Medicine and Methodist Hospital and by the Radio Isotope approval committees of the same institutions.

Potential Hazards of the Study:

Purified diphtheria toxoid prepared by the Texas State Department of Health is known to give a local or systemic reaction in less than one per cent of the adult population. It can produce a local soreness and redness lasting two to three days, occasionally irritability and anorexia, and rarely, vomiting.

The vaccine preparation volunteers will receive is the same one which [illegible] able for use by private physicians in Texas.

The amount of radioisotope approved for administration is very small and not considered to be hazardous.

Authorizations:

The nature and demands of the study have been clearly explained [illegible] understand and accept the hazards involved. I also understand that if some unforeseen complication occurs, it, too, is considered to be one of the hazards [illegible] volunteer. Furthermore, I understand that I may withdraw from the study if I find that I am unable to continue.

Volunteer's Signature _____

Date _____

I have carefully explained the nature, demands, and foreseeable risks of the above study to the normal volunteer.

Investigator's Signature _____

Date _____

Form B

BAYLOR UNIVERSITY COLLEGE OF MEDICINE
Houston, Texas

Date October 25, 1967

TO: ----------
Granting Agency (Use Specific Title)

Title of Proposed Research Project: Proposed Study of Diphtheria Immunization in Volunteers

Grant Number: ----------

Principal Investigator William T. Butler, M.D.
Department Microbiology

The attached research grant proposal has been reviewed by the Faculty Committee on Research Involving Human Beings, and is considered to take proper cognizance of the personal rights and welfare of the individuals concerned. We are assured that the principal investigator has developed appropriate procedures for obtaining informed consent of the proposed experimental subjects. We judge the experiments proposed to have potential usefulness and have satisfied ourselves that proper precautions and safeguards have been devised to protect against [illegible] risk. Further, the matter of individual privacy of subjects has been considered and we have accepted and approved the principal investigator's [illegible] for the rights of both adults and underaged subjects.

The principal investigator, and his departmental chairman, have been advised not to alter the procedures herein without specific prior approval of the Faculty Committee.

The Faculty Committee on Research Involving Human Beings consists of senior Professors or Associate Professors drawn from the Departments of Medicine, Pediatrics, Rehabilitation, Physiology, Radiology, Surgery, Pharmacology, and Psychiatry/Neurology.

Signed H. Shannon
Chairman, Committee on Research Involving Human Beings

Signed [illegible]
Institutional Official

Form B

BAYLOR UNIVERSITY COLLEGE OF MEDICINE
Houston, Texas

Date October 25, 1957

TO: ----------
Granting Agency (Use Specific Title)

Title of Proposed Research Project: Proposed Study of Diphtheria Immunization in Volunteers

Grant Number: ----------

Principal Investigator William T. Butler, M.D.
Department Microbiology

The attached research grant proposal has been reviewed by the Faculty Committee on Research Involving Human Beings, and is considered to take proper cognizance of the personal rights and welfare of the individuals concerned. We are assured that the principal investigator has developed appropriate procedures for obtaining informed consent of the proposed experimental subjects. We judge the experiments proposed to have potential usefulness and have satisfied ourselves that proper precautions and safeguards have been devised to protect against hazards. Further, the matter of individual privacy of subjects has been considered and we have accepted and approved the principal investigator's statement for the rights of both adults and underaged subjects.

The principal investigator, and his departmental chairman, have pledged themselves not to alter the procedures herein without specific prior approval of the Faculty Committee.

The Faculty Committee on Research Involving Human Beings consists of senior Professors or Associate Professors drawn from the Departments of Medicine, Pediatrics, Rehabilitation, Physiology, Radiology, Surgery, Pharmacology, and Psychiatry/Neurology.

Signed [signature]
Chairman, Committee on Research Involving Human Beings

Signed [signature]
Institutional Official