times after vaccination. The mice that received the virus in saline were resistant to about 100 MLD of virus at 4 and 8 weeks after vaccination, but after 26 weeks no immunity could be detected. The mice that received the virus plus adjuvants, on the other hand, were resistant to about 1,000,000 MLD of virus at 4 and 8 weeks after vaccination and even after 26 weeks they were resistant to at least 1,000 MLD.

The antibody response in ferrets following intranasal instillation of active PR8 virus was compared with the amount of antibody elicited by a single subcutaneous inoculation (2 cc) of formalinized PR8 virus with and without the above-mentioned adjuvants. The results are shown in Table 1. The antibody titers elicited in rabbits by allantoic fluid suspensions of influenza virus and by concentrated preparations of the virus[5] were likewise increased and maintained at high levels by these adjuvants. The experiments indicated clearly that the adjuvants provide a much more effective method of increasing antibody production to the virus than the use of concentrated preparations of virus alone.

TABLE 1

SERUM ANTIBODY TITERS IN FERRETS FOLLOWING SUBCUTANEOUS INOCULATION OF FORMALINIZED INFLUENZA VIRUS WITH AND WITHOUT ADJUVANTS AND FOLLOWING INTRANASAL INSTILLATION OF ACTIVE VIRUS

Test bleeding weeks	Mean serum antibody titer* of ferrets inoculated with		
	Formalized virus subtaneously		Active virus intranasally
	+ saline	+ adjuvants	
0	< 32	< 32	< 32
2	388	3,010	10,800
4	169	10,100	3,580
6	147	7,650	2,306
10	128	3,010	1,670
14	104	2,200	1,350
18	91	2,520	1,270

* The titers were determined by means of a standard red cell agglutination inhibition test (G. K. Hirst and E. G. Pickels, *Jour. Immunol.*, 45: 273, 1942), and are expressed as the reciprocal of the serum dilution end point. Four ferrets were used for each group.

Further experiments have shown that another acid-fast organism, *Mycobacterium butyricum,* could be substituted for the tubercle bacilli in the emulsions with the same degree of enhancement of immunity against the virus as described above. The acid-fast bacteria were essential in the vaccines, for paraffin oil and Falba alone were less effective. Aleuronat, broth and plain diphtheria toxoid had no detectable effect on the antigenicity of the virus. When influenza virus was sedimented from allantoic fluid by high-speed centrifugation and resuspended in sesame oil together with dried, heat-killed *M. butyricum,* it elicited antibody titers in rabbits which were about 4 times higher than when the sedimented virus was resuspended in saline.

The results make plain that the addition of certain adjuvants to influenza virus vaccines not only greatly increases the immunizing capacity of the virus in experimental animals but maintains the immunity at a high level over a long period. It seems unlikely that the adjuvants employed in the above experiments can be safely used in human beings. Further study of the phenomenon, however, may provide materials which can be utilized in human vaccination. A more complete report will be published at a later date.

WILLIAM F. FRIEDEWALD

[5] G. K. Hirst, *Jour. Exp. Med.*, 76: 195, 1942; T. Francis, Jr. and J. E. Salk, SCIENCE, 96: 499, 1942.

ASCORBIC ACID LOSSES IN MINCING FRESH VEGETABLES[1]

DURING a period of shortage of fresh vegetables, the importance of conserving vitamins is evident. In the preparation of many salads, raw vegetables and fruits are finely minced. In many mess halls vegetables are minced in a machine called the "Buffalo chopper." This machine is merely a bowl set under a pair of rotating blades. These function like the old-fashioned wooden bowl used with a hand chopper for mincing cabbage.

Numerous studies have indicated that maceration speeds the rate of disappearance of ascorbic acid in fresh plant products.[2, 3] Enzymes, metallic catalysts and fine division favor oxidation.

A series of studies to learn methods of conserving vitamin C have been completed in the naval hospital cafeteria of the National Naval Medical Center. Spe-

TABLE 1

ASCORBIC ACID LOSSES FROM CUTTING FRESH VEGETABLES (MG/100 GM)

Vegetable	Cutting tool	Freshly cut	30 mins. after cutting	2 hrs. after cutting
Green peppers	Plastic knife	130	128	87
	Steel knife		118	53
	"Chopper"		84	31
Radish	Plastic knife	52	49	35
	Steel knife		41	8
	"Chopper"		36	5
Cabbage	Plastic knife	27	26	19
	Steel knife		8	8
	"Chopper"		7	6
Cucumbers	Plastic knife	14	12	7
	Steel knife		10	5
	"Chopper"		3	2
Onions	Plastic knife	11	10	8
	"Chopper"		2	2
Lettuce	Plastic knife	4	2	1
	Steel knife		1	1
Tomatoes	Plastic knife	13	9	9
	Steel knife		8	8

[1] The opinions and views set forth in this article are those of the writers and are not to be considered as reflecting the policies of the Navy Department.

[2] C. G. King, "Physiology of Vitamin C. The Vitamins." Amer. Med. Assn., Chicago, p. 331, 1939.

[3] M. Pyke, *Nature,* 149: 499, 1942.

cial attention has been given to the knives used for mincing vegetables, since their composition may be important. Furthermore, in large-scale cookery salads are often prepared one or more hours before they are served. Therefore, attention was given to the rate of disappearance of ascorbic acid. All analyses were run by the method of Bessey.[4]

The procedure followed was to remove the vegetables from cold storage. A liberal sample was set aside without cutting. Another sample was thinly sliced with a plastic knife. A third was sliced with a steel knife. A fourth was put through the Buffalo grinder. The samples were then taken to the laboratory. The original time of cutting was recorded.

At the laboratory samples of the intact vegetable were prepared for immediate analysis by mincing on a wooden board with a plastic knife. The other samples were run one half hour and two hours after the initial slicing.

Typical data are shown in Table 1. These data indicate the losses that result from both the knives used in cutting and from the time of standing of the cut vegetable. Possibly some form of plastic bowl and knife can be devised for the "Buffalo chopper." Wherever possible salads should be prepared with large pieces of fruits or vegetables prepared just before serving.

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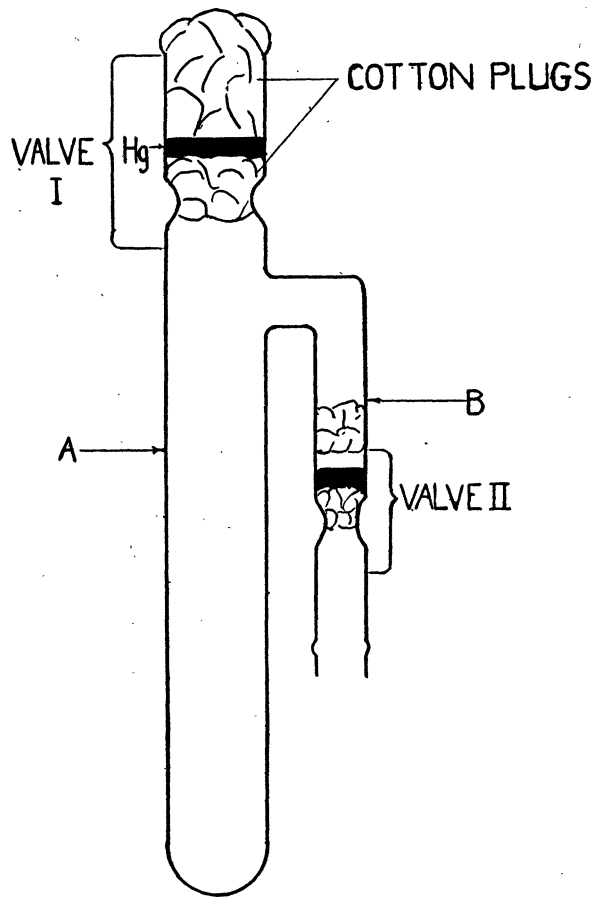
SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLIFIED LABORATORY CHECK VALVE AND ITS APPLICATION IN THE CONSTRUCTION OF ANAEROBIC CULTURE TUBES

SINTERED glass filters have been used as one-way or check-valves in a variety of ways.[1] However, besides being difficult to construct, these are relatively expensive. An inexpensive substitute may readily be realized in any laboratory simply by floating mercury over a cotton or glass wool plug contained in the constricted portion of a tube. This valve will not permit the passage of air into the tube, but will relieve the slightest pressure of gas within.

In certain applications a check valve of the type described above possesses an advantage over the sintered glass type. This is particularly true of its application in the construction of bacteriological tubes for the growth of anaerobes. Fig. 1 shows a tube constructed in this laboratory for the cultivation of *Clostridium acetobutylicum*. *A* is a 22 × 175 mm test tube to which tube *B* (approximately 10 mm diameter) is sealed as a side arm. Tube *A* contains a constriction in which a cotton or glass wool plug fits snugly. Mercury is floated over this plug to a depth of at least ¼″; and above this mercury a cotton plug is inserted to prevent splattering. A sheet of paper fastened over the open end may be used to accomplish the same purpose. Tube *B* is bent at right angles. Below the bend a check valve is assembled in the same manner as indicated above. The use of the tube is illustrated in what follows.

Medium is introduced into tube A, and the tube and its contents are sterilized with the cotton plugs of valve I, and the plugs and mercury of valve II in place. Following sterilization and cooling, the in-



[4] O. A. Bessey, *Jour. Biol. Chem.*, 126: 771, 1938.

[1] A. A. Morton, "Laboratory Technique, Organic Chemistry," McGraw-Hill. 1938.