subcultures in the presence of penicillin. Harper[4] has recently prepared acetone-ether extracts of paracolon bacilli which were more effective penicillin inhibitors than were extracts of *E. coli.*

The purpose of this report is to describe the extraction of a highly potent penicillin inactivator from 7 strains of *Staph. aureus* (coagulase positive). Details of the strains will be presented elsewhere. Briefly, they were "naturally" penicillin resistant; all were isolated from patients who had not received penicillin. The method of extraction was that used by Harper.[3] Saline suspensions of 24-hour plate cultures were precipitated with 7 volumes of acetone. After a change of acetone, and two of ether, the precipitate was dried quickly *in vacuo* and stored at room temperature. For tests of potency, broth suspensions of the powder were added to broth cultures containing a constant inoculum of hemolytic streptococci (about 1 million organisms per cc) and varying amounts of penicillin. A typical experiment is presented in Table I.

TABLE I

PROTOCOL OF A TYPICAL EXPERIMENT SHOWING RAPID, COMPLETE DESTRUCTION OF 100 UNITS OF PENICILLIN BY 1 MGM OF THE POWDERED EXTRACT OF A PENICILLIN RESISTANT STRAIN OF STAPH. AUREUS. TURBIDITIES ARE EXPRESSED IN TERMS OF OPTICAL DENSITY

Tube	Broth plus hemolytic streptococcus	Penicillin	Penicillinase 1 mgm/cc	Sterile broth
1	10 cc	0	0	0
2	9 "	1 cc (1 μ/cc)	0	0
3	8 "	1 cc (100 μ/cc)	1 cc	0
4	0 "	0	1 cc	9 cc

	RESULTS			
Tube	Initial	4 hours	8 hours	12 hours
1	.07	.29	1.0	1.0
2	.04	.06	.06	.06
3	.14	.17	.30	1.0
4	.18	.18	.19	.18

Optical densities (turbidities) were measured with a Coleman universal spectrophotometer every few hours while the solutions were incubated at 37° C. As indicated in the table, complete destruction of 100 units of penicillin by 1 mgm of the powder was so rapid that at the end of 12 hours growth in this tube was equal to that of the control. Although there were some variations, this same high degree of potency was shown by the extracts of all 7 strains.

Extracts of 7 penicillin sensitive strains of *Staph. aureus* (coagulase positive) were tested in the same manner, using 2 mgms of the powder and only 1 unit of penicillin. In no instance was there any inactivation of penicillin.

Actively growing cultures of the resistant strains caused complete destruction of penicillin in the culture fluid, but the seitz filtrate of the fluid contained no penicillin inactivator. Ability to destroy penicillin was completely lost when a broth suspension of the powder was left at 56° C for 1 hour. Further studies of the properties of this substance are in progress; it is not possible at present to say whether it is the same thing as the extracts of the colon and paracolon bacilli.

The powdered extracts are now being used routinely in this clinic for all cultures of patients who are receiving penicillin.

[4] G. J. Harper, *Lancet*, 2: 569, 1943.

SUMMARY

A highly potent penicillin inactivator has been extracted from 7 strains of *Staph. aureus* (coagulase positive), all of which were naturally penicillin resistant. No such inhibitor was present in extracts of 7 penicillin sensitive strains of *Staph. aureus.*

Acknowledgment: I am indebted to Miss Mary Beach for technical assistance.

WILLIAM M. M. KIRBY

STANFORD UNIVERSITY SCHOOL OF MEDICINE,
SAN FRANCISCO, CALIF.

ENHANCEMENT OF THE IMMUNIZING CAPACITY OF INFLUENZA VIRUS VACCINES WITH ADJUVANTS[1]

FREUND and McDermott[2] reported that an intense, prolonged sensitization to horse serum and increased production of antibody occurred when the serum was combined with a lanolin-like substance and killed tubercle bacilli suspended in paraffin oil. The present report describes the effect of various adjuvants on the antibody production and immunizing capacity of a single subcutaneous inoculation of formalinized influenza virus in animals.

Allantoic fluid suspensions of PR8 virus, which had been rendered non-infectious by the addition of 0.1 per cent. formaldehyde, were blended with paraffin oil containing dead tubercle bacilli[3] and an absorption base known as Falba.[4] Each cc of the emulsion contained 0.4 cc of the allantoic fluid, 0.4 cc of paraffin oil, 0.2 cc of Falba and 1.4 mg of dried, heat-killed tubercle bacilli. The immunizing capacity of a subcutaneous inoculation of 0.5 cc of the emulsion was tested in young adult Swiss mice. For controls a comparable group of mice received the same amount of virus suspended in saline, and a third group received only saline. Mice from each of the three groups were tested for resistance to intranasal instillation of graded amounts of PR8 virus at various

[1] From the Laboratories of the International Health Division, The Rockefeller Foundation, New York.

[2] J. Freund and K. McDermott, *Proc. Soc. Exp. Biol. and Med.*, 49: 548, 1942.

[3] The tubercle bacilli, which were the virulent human Jamaica No. 22 strain, were kindly supplied by Dr. M. W. Chase. They were heated at 100° C in the Arnold sterilizer for 30 minutes and after being dried were incorporated in sterile paraffin oil.

[4] Distributed by Pflatz and Bauer, Inc., New York.

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.