1931 it raged in river valleys northwest of Natal, but the period between 1932 and 1937 is referred to as the "silent era" because, although it was extending its range, the mosquito caused no noticeable increase in malaria. The epidemic of 1938 in the Jaguaribe valley, in the state of Ceará and along the coast and rivers of the state of Rio Grande do Norte was catastrophic. It was estimated that in June and July there were 100,000 cases of malaria with over 14,000 deaths. In 1939 more than 185,000 people in the two states were given treatment for the disease.

The Brazilian Ministry of Education and Health and the Rockefeller Foundation collaborated in organizing the Malaria Service of the Northeast, with headquarters at Fortaleza. The basic unit of the control organization was the zone—an area in which one man could apply larvicides to all breeding places in one week; or an area in which all houses could be sprayed by a disinsectization squad in one week. The zone inspector was responsible to the chief inspector, whose district generally included five zones. Several districts were combined into a post, which was in charge of a doctor, and the posts were grouped into a total of seven divisions, each of which was in charge of a more experienced doctor.

The severity of the epidemic necessitated the distribution of quinine and atebrine, but the real offensive against the insect involved the painstaking search for larvae in all possible breeding places and the application of larvicides, especially Paris green, by the zone inspectors. The attack on the larva was supplemented by systematic spraying of adult mosquitoes resting in houses; although this measure can not be relied upon for eradication of the mosquito, it did increase the effectiveness of the larvicidal program and prevented many cases of malaria by killing infected adults. But the objective of the counter-attack against *A. gambiae* was not merely to control malaria; it was the complete extermination of the species in Brazil. After surveys had established the distribution of the mosquito, a cordon was thrown about the periphery of its range, and further infiltration into uninfested areas was prevented by the use of larvicides and pyrethrum spray in a belt eight to twenty-five miles beyond the known limits of the infested area, while transportation of the adult through the barrier was prevented by disinsectization of all planes, boats, trains and other vehicles. Control measures were intensified at the border, and, working from this frontier zone inward, one area after another was cleared of the invader, until in November, 1940, the last individuals of *A. gambiae* were destroyed.

This, in brief, is the account of the *gambiae* invasion of Brazil. But the authors do more than simply repeat this story. In addition to emphasizing the need for constant vigilance against such insect invaders, they challenge the old concept of malaria control that aims only at reducing the vector population below a certain level by drainage and other methods that require many years for their success, and which never completely eradicate the disease. Can *A. gambiae* be exterminated from regions within its natural home in Africa? Can *A. pseudopunctipennis* be eradicated from river valleys in Peru? It is true that *gambiae's* habit of breeding in small water collections free of vegetation, and its attraction to human habitations, not only cause it to be a more dangerous malaria carrier but also make it more susceptible to control by larvicides and spraying. Other anophelines which have a wider selection of breeding places and which rest in the jungle may be much more difficult to attack, but would it be feasible to attempt an all-out "blitzkrieg" instead of simply keeping down their numbers by control measures which must be continued forever? Extermination of such mosquitoes may not be possible, but no one thought that *A. gambiae* could be eradicated from Brazil in less than two years.

The book is well worth thoughtful perusal by all those interested in control of insects of economic and medical importance, whether they be doctors, scientists or legislators.

L. E. ROZEBOOM

SPECIAL ARTICLES

EXTRACTION OF A HIGHLY POTENT PENICILLIN INACTIVATOR FROM PENICILLIN RESISTANT STAPHYLOCOCCI[1]

BY grinding a suspension of *E. coli* in a crushing mill, Abraham and Chain[2] in 1940 produced an enzyme-like substance capable of completely inhibiting penicillin. This substance, called penicillinase, was presumably intracellular, for penicillin was not destroyed by the actively growing organisms, and no penicillin inactivator was present in culture filtrates. No penicillinase could be extracted from penicillin sensitive staphylococci, or, in later experiments,[3] from a strain of *Staph. aureus* made insensitive by repeated

[1] The penicillin was provided by the Office of Scientific Research and Development from supplies assigned by the Committee on Medical Research for experimental investigators recommended by the Committee on Chemotherapeutics and Other Agents of the National Research Council.

[2] E. P. Abraham and E. Chain, *Nature*, 146: 837, 1940.

[3] E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley and M. A. Jennings, *Lancet*, 2: 177, 1941.

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.*

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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.