tomycin employed in these studies. These reactions varied in frequency and severity with different lots of the material and were encountered more frequently in the earlier less refined lots than in the more recent preparations. Such undesirable effects can, we believe, be attributed to impurities rather than to the antibiotic itself.

The reactions were of two main varieties. The first was characterized by facial flushing, headache, and fall of blood pressure, and resembled the classical response to histamine. It appeared promptly following intravenous or intramuscular administration and lasted from 10 to 60 minutes. This histamine-like reaction was noted in three of the several lots which were tested, and when these same lots of streptomycin were given to experimental animals, Molitor (*2*) was able to demonstrate a similar effect.

The second type of reaction consisted of fever, invariably accompanied by myalgias and arthralgias particularly in the temporomandibular joints and suboccipital region. These symptoms appeared only after streptomycin had been given for a period of time, varying from 24 hours to several days. In this series of observations no clinical evidence of eighth-nerve toxicity was encountered. Hinshaw and Feldman (*1*) recently reported such toxic reactions in 3 of 34 patients who received streptomycin for considerable periods of time.

Skin eruptions appeared in two subjects on the third day of the experimental period. The rash of subject I.B. resembled erythema nodosum and was undoubtedly due to streptomycin. The clinical significance of the maculopapular exanthem of subject J.H. could not be satisfactorily evaluated because he had received pentobarbital concomitantly.

Local reactions, consisting of pain and tenderness at the site of intramuscular or subcutaneous injections, were related to the amount of impurities retained in the preparations used, less difficulty being encountered with the more refined lots of the antibiotic. These local effects were never severe enough to interfere with continued drug administration and could be alleviated by the local application of heat.

Further experience will be required to define the possible toxic effects of prolonged therapy with streptomycin. It is planned to extend these studies when sufficient supplies of this antibiotic are available for clinical investigation.

Summary

In this limited experience, tests of renal and hepatic function together with blood studies before and after the parenteral administration of streptomycin revealed no evidence of serious toxicity. Reactions, consisting of fever, arthralgias, and skin rashes as well as histamine-like effects, are believed to be due to impurities retained in the preparations of streptomycin employed in these studies.

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Complement-fixing and Neutralizing Antibodies Against Japanese B Virus in the Sera of Okinawan Horses[1]

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Early in July 1945, an outbreak of encephalitis appeared among natives of Okinawa. The disease, which eventually affected a number of American service men on the island as well as natives, continued to occur during August and early September. We have recently reported (*2*) that Japanese B encephalitis virus was the cause of the outbreak. This conclusion was reached by the demonstration of the development of a significant titer of complement-fixing antibodies against this active agent in the sera of convalescent patients and by the isolation, from the brain of a patient dying of encephalitis, of an active agent which was identified by specific complement-fixation and neutralization reactions as Japanese B virus.

It is known that horses are susceptible to several kinds of encephalitis. In view of this fact, evidence was sought regarding the role that horses played in the outbreak of encephalitis in Okinawa. Accordingly, sera, collected in mid-August from nine Okinawan horses in areas where encephalitis was present among human beings, were tested for complement-fixing antibodies (*1*) against antigens prepared from the viruses of Japanese B, Western equine, and Eastern equine encephalitis. Sera of five Okinawan goats from the same areas and serum obtained on Guam from a normal horse were also tested. All sera obtained from the Okinawan horses fixed complement in the presence of Japanese B virus in a final dilution of 1:16 or greater (a 2+ reaction in a dilution of 1:8 or higher is significant). The serum of one horse yielded a titer of 1:128; two, a titer of 1:64; three,

[1] The Bureau of Medicine and Surgery of the U. S. Navy does not necessarily undertake to endorse the views expressed in this paper.

a titer of 1:32; and three, a titer of 1:16. All these sera failed to fix complement to a significant degree when tested against Western equine encephalitis virus; three of the sera also gave negative results with Eastern equine encephalitis virus. Sera of five Okinawan goats failed to fix complement in the presence of Japanese B or Western equine encephalitis virus; sera of two of these animals also gave negative results with Eastern equine encephalitis virus. Serum of the normal Guamanian horse contained no complement-fixing antibodies against any of the three virus antigens employed. The results of the complement-fixation tests indicate that all nine Okinawan horses tested had had some previous contact with Japanese B virus.

TABLE 1

NEUTRALIZATION TESTS WITH SERA OF OKINAWAN HORSES AND JAPANESE B VIRUS (NAKAYAMA STRAIN)

Serum	Titer of complement-fixing antibody	Sera used in neutralization test	Fate of mice following intracerebral inoculation of serum plus virus mixture: Dilution of virus in mixture 10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	Titer of virus in presence of serum*	Neutralization index†
Okinawan Horse No. 1	More than 1:128	Horse No. 1 and Horse No. 3 (Pool I)	4/4	4/4	0/4	0/4	0/4	0/4			$10^{3.5}$	30,000
Okinawan Horse No. 3	1:32											
Okinawan Horse No. 2	1:32	Horse No. 2 and Horse No. 19 (Pool II)	4/4	2/4	0/4	0/4	0/4	0/3			$10^{3.0}$	100,000
Okinawan Horse No. 19	1:32											
Immune rabbit vaccinated against Jap B virus	Not tested	Immune rabbit	...	1/3	0/4	0/4	0/4	0/3			$10^{2.8}$	150,000
Normal Gaum Horse No. 4	0	Horse No. 4	...	4/4	4/4	4/4	4/4	4/4	2/4	0/4	$10^{8.0}$	1

* Titer of virus is expressed as the highest dilution of virus giving a 50-per cent mortality.
† Neutralization index is the ratio between the titer of the virus in the presence of the serum under test and its titer in the presence of serum of the normal horse.
4/4—Four of four mice injected died.

Evidence that the Okinawan horses under study had been in contact with Japanese B virus was obtained also by neutralization tests. In this test, serum of Okinawan horse No. 1, which had a complement-fixing titer of 1:128 against Japanese B virus, was combined with serum of Okinawan horse No. 3, which exhibited a titer of 1:32 (horse serum Pool I, Table 1). Similarly, sera of Okinawan horses No. 2 and No. 19, each having a complement-fixing titer of 1:32, were combined to form horse serum Pool II. Portions of each pool of Okinawan horse sera were mixed with equal volume of decimal dilutions of Nakayama strain of Japanese B virus, and the mixtures were incubated for 2 hours at room temperature. Serum of a normal Guamanian horse, and that of a rabbit which had received a number of inoculations of Japanese B virus were tested in the same manner. After incubation, the serum-virus mixtures were inoculated intracerebrally into mice, four animals being employed for each serum-virus dilution mixture. Mice surviving 18 days were regarded as protected. The protection afforded by each serum was estimated by the method of Reed and Muench (*3*).

An examination of Table 1 reveals that the titers of the virus in the presence of the sera comprising Pool I and Pool II were, respectively, $10^{3.5}$ and $10^{3.0}$. On the other hand, the titer of the virus mixed with serum of the normal Guamanian horse was $10^{8.0}$. The neutralization index, which is the ratio between the titer of the virus in the presence of the serum under test and its titer with normal serum, was, therefore, 30,000 for the sera of Pool I and 100,000 for Pool II. This means that these pools of sera possessed, respectively, 30,000 and 100,000 times more neutralizing power than that shown by normal horse serum. In fact, their protective action approached that of the serum of the hyperimmunized rabbit, which exhibited a protective index of 150,000. Experience has shown that a neutralization index greater than 1,000 is significant. The results recorded in Table 1 indicate that at least one of the two Okinawan horses furnishing serum for each pool had developed a high titer of neutralizing antibodies against Japanese B encephalitis virus.

The evidence thus furnished by both complement-fixation and neutralization tests supports the conclusion that the horses studied had had contact with Japanese B encephalitis virus. The nature of this contact is not known, but the results described support the idea that horses may have been of epidemio-

logic importance in the outbreak of Japanese B encephalitis which began on Okinawa during July 1945.

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The Absorption of Orally Administered Penicillin[1]

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In a previous communication (*7*), it was reported that when penicillin was administered orally to fasting subjects the concentrations attained in the blood and the range of urinary excretion were of the same order of magnitude whether the penicillin was presumably protected against destruction in the stomach by the use of oil, oil-beeswax, or an antacid, or whether it was ingested as the aqueous solution. Regardless of vehicle, it was necessary to administer approximately five times as much penicillin by the oral as by the intramuscular route to obtain comparable concentrations of penicillin in the blood. In no instance was more than 32 per cent of the ingested penicillin excreted in the urine during the 12 hours immediately following ingestion.

This low urinary excretion after oral administration is in striking contrast to the 70 to 100 per cent urinary excretion which Martin and Kirby (*9*) have demonstrated after single parenteral doses. In experiments which will be published elsewhere (*8*), there was no evidence that penicillin is destroyed in the portal circulation. Moreover, penicillin is not destroyed by whole blood *in vitro* when incubated at 37° C. for a four-hour period. It appears, therefore, that the quantitative difference between the urinary excretion of penicillin after parenteral injection and that observed after oral administration represents the penicillin which is not absorbed. Presumably the material which is not absorbed after oral administration is either destroyed or excreted in the alimentary tract.

As it appeared that the destruction of penicillin by the acid of the stomach was not an entirely satisfactory explanation of the fate of the larger part of the ingested material, an investigation of the absorption, excretion, and destruction of penicillin following oral administration has been conducted, and a preliminary report on certain of the observations is presented at this time.

A study of the urinary excretion of penicillin after both oral and intramuscular administration was made in six subjects with complete achlorhydria. Five of the subjects had pernicious anemia. On successive days, each subject received identical doses of penicillin by the oral and by the intramuscular route. Nine such experiments were performed, seven after 300,000-unit doses and two after 25,000-unit doses. The penicillin determinations were made by the Rammelkamp method of bio-assay (*10*). All subjects were in a fasting state when the penicillin was ingested and during the succeeding four hours.

The results are presented in Table 1. As may be seen, the amount of penicillin excreted in the urine (per cent of the total dose) ranged from 36 to 100 per cent following intramuscular administration, and usually was more than 60 per cent.[2] Following oral administration, the range of urinary excretion varied between 8 and 32 per cent. In the comparative studies in each individual, the differences are striking. The amounts of penicillin appearing in the urine of these achlorhydric subjects after oral administration were within exactly the same range as had been observed previously in normal subjects (*7*) and were definitely less than appeared after intramuscular administration.

TABLE 1

URINARY EXCRETION OF PENICILLIN FOLLOWING ORAL AND INTRAMUSCULAR ADMINISTRATION IN SUBJECTS WITH COMPLETE ACHLORHYDRIA

Subject	Penicillin dosage	Urinary excretion in per cent of total dose		Period of observation
	Units	Oral	Intramuscular	Hours
1	300,000	15		3
	300,000		46	5
	300,000	32	64	8
	25,000	19	100	8
2	300,000	28		8
	300,000		97	10
	25,000		36	8
3	300,000	21	68	4
	25,000	14	64	8
4	300,000	26	55	8
	25,000		52	8
5	300,000	10	96	8
6	300,000	27	73	24
7	300,000	8		3

Data on the urinary excretion of ingested penicillin obtained from the published reports and from our own observations are presented in Table 2. As may

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Cornell University Medical College.

[2] The difference between the values for the urinary excretion of penicillin in these experiments and the 70 to 100 per cent excretion noted by Martin and Kirby is presumably because fewer dilutions of a given specimen of urine were assayed in the present experiments.