51 healthy adult males on their regular diets was 38.3 ± 1.7 mg. The 24 hour excretion of any individual was remarkably constant over a three week period. The daily ingestion of 4 or more g of ascorbic acid increased urinary excretion of oxalic acid; whereas the daily ingestion of less than 4 g of ascorbic acid produced no significant increase in oxalate excretion.

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An Antipyretic Action of Aureomycin. Inhibition of Febrile Response to Influenza Virus in Rabbits.* (20828)

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Aureomycin has been reported to influence favorably the course of certain viral infections in man(1-3). Except for the psittacosis-lymphogranuloma group(4), however, there is no evidence that this drug either limits viral multiplication(5) or prevents production of lesions by these agents(6). It seemed possible, therefore, that the effect of aureomycin in these instances might be non-specific; that is, its action might be mediated through a mechanism other than one which influences the pathogenesis of the disease. For example, in those circumstances in which the major objective evidence for the effectiveness of aureomycin is provided by an early return of temperature to normal, the question of an antipyretic action may be raised. As an approach to this problem, an investigation was carried out to determine whether aureomycin could inhibit the pyrogenic response to a viral agent in a situation where infection was not concerned. The experimental model chosen for study was the febrile reaction produced in rabbits by intravenous inoculation of influenza virus(7).

Materials and methods. Virus. The virus employed was influenza B, Lee strain, which was propagated in the allantoic sac of chick embryos 9 to 11 days of age. Methods of cultivation were identical to those previously described(8). Infected allantoic fluids were stored at -30°C(9). Fluids were clarified by centrifugation at 3000 RPM for 10 minutes at 4°C and tested for bacteriological sterility on blood agar plates at 37°C for 24 hours. All virus infected allantoic fluids employed had hemagglutination titers of 1:512, using methods previously described(8). *Normal allantoic fluid.* Allantoic fluid was obtained from chick embryos handled in the same manner as described above except that they were not inoculated with virus. *Aureomycin.* Each injection of aureomycin consisted of 100

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mg of aureomycin hydrochloride† dissolved in 10 cc of 1.95% l-leucine diluent. Aureomycin of a single lot number was employed. Care was taken that the drug was completely dissolved before it was injected since when visible suspended particles were present, intravenous injection resulted in fever. *Animals.* Adult male or female rabbits of mixed breeds, weighing 2-3 kg, were employed. The animals' temperatures were taken at various intervals for at least two days prior to using them in an experiment in order to accustom them to the procedure. They were kept in individual cages without access to food or water during experiments and were removed from the cages when temperatures were taken or injections given. Rabbits which had been given influenza virus were not used in subsequent experiments for at least 4 weeks in order to avoid the period of resistance to influenza virus pyrogen which is present for 2 weeks after inoculation(10). *Design of experiments.* Four groups of 3-6 rabbits each were employed in each experiment. Rabbits of 2 groups were inoculated intravenously with 5 ml of allantoic fluid infected with influenza virus. Animals of the other 2 groups each received 5 ml of normal allantoic fluid intravenously. One of the groups inoculated with virus and one inoculated with normal allantoic fluid were given aureomycin either intravenously or intraperitoneally; the other 2 groups received 10 ml of l-leucine diluent by the same route. Aureomycin or diluent was administered at various times before and after inoculation of virus in different experiments. A fifth group of rabbits which received no injections was employed in most experiments. *Rectal temperatures* were taken every 30-60 minutes for several hours before and six hours after inoculation of virus or normal allantoic fluid. Temperatures were recorded as changes from the initial value, and the mean change in temperature at each time interval was calculated for each group of rabbits. Experiments were conducted at room temperature.

Experimental. Effect of intraperitoneal aureomycin before and after virus. To determine whether or not aureomycin could alter the pyrogenic reaction to intravenous inoculation of influenza virus, 100 mg of the drug was injected intraperitoneally 2 hours before and 1½ hours after intravenous inoculation of virus. The results of a typical experiment are presented graphically in Fig. 1. Two similar experiments yielded entirely comparable results. Control rabbits given diluent intraperitoneally and virus intravenously exhibited a characteristic febrile response with a maximum mean temperature increase of 2.8°F 5 hours after injection of virus. This temperature elevation was blocked completely in rabbits given aureomycin. In addition to this antipyretic effect, all rabbits injected with aureomycin intraperitoneally showed a marked fall in rectal temperature which was of the same magnitude in animals given aureomycin and virus as in those given aureomycin and normal allantoic fluid. The similarity of these temperature curves suggested that the antipyretic effect did not depend quantitatively upon the hypothermic action of the drug administered intraperitoneally. Rabbits which received no injections had little change in rectal temperature. Aside from temperature differences, rabbits injected with aureomycin reacted and appeared no differently from those given diluent; there was no indication of any discomfort or untoward effect. Peritoneal cavities of rabbits autopsied ½ to 3 hours after intraperitoneal administration of either aureomycin or diluent showed no grossly discernible differences save for the occasional presence of some residual drug in those injected with aureomycin. Thus, there was nothing to suggest that the observed effects of intraperitoneal aureomycin were produced by central vasomotor collapse or local irritation resulting in a state of shock or altered reactivity.

Effect of intraperitoneal aureomycin after virus. The effect of aureomycin when administered after the pyrogenic reaction was evident was next determined. The results of an experiment in which aureomycin was injected intraperitoneally 1½ hours after virus are presented in Fig. 2. Temperatures of all rabbits were similar 1½ hours after influenza

† The aureomycin was kindly furnished by Dr. James M. Ruegsegger of Lederle Laboratories, Pearl River, N. Y.

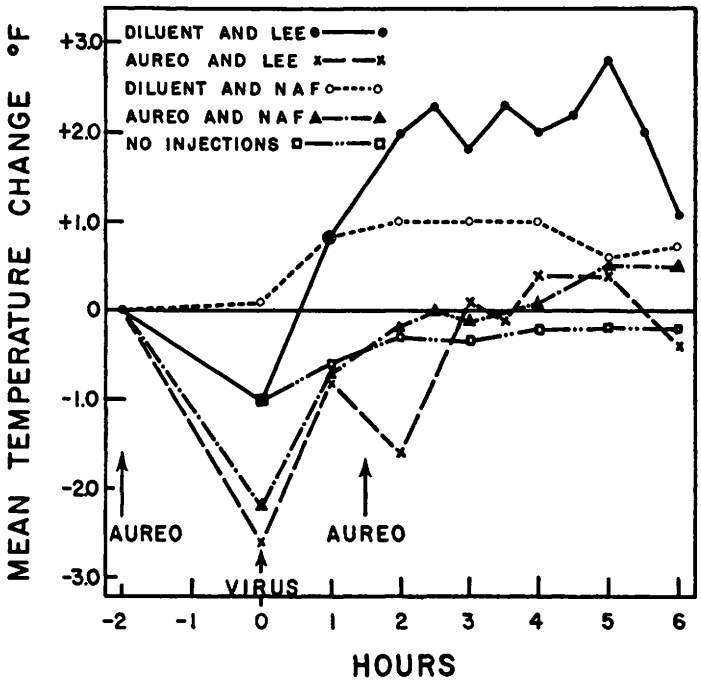

FIG. 1. Effect of intraperitoneal injection of 100 mg of aureomycin upon pyrogenic response of rabbits to intravenous inoculation of 5 ml of Lee virus. Aureomycin was injected 2 hr before and 1½ hr after virus. Controls injected with combinations of virus, diluent, normal allantoic fluid (NAF) and aureomycin are included.

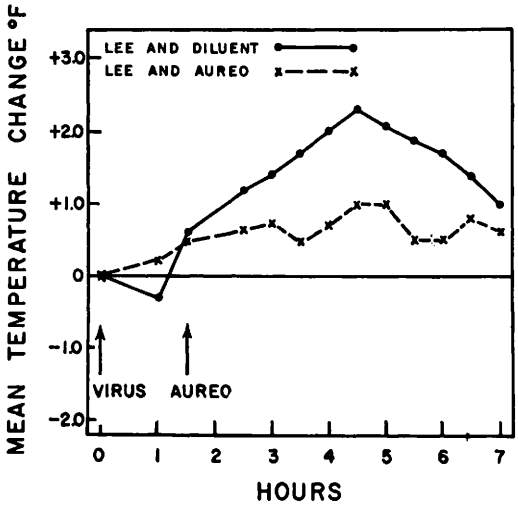

FIG. 2. Mean temperature change of rabbits injected intraperitoneally with 100 mg of aureomycin 1½ hr after intravenous inoculation of 5 ml of Lee virus.

B was inoculated, but animals given aureomycin at that time failed to develop the subsequent increase in fever shown by those which received diluent after virus. One similar experiment showed comparable results. These data indicate that aureomycin did exhibit antipyretic activity when administered after the pyrogenic response was established even though the temperature did not return to a normal value.

Effect of simultaneous intravenous inoculation of aureomycin and influenza B virus. To determine whether the hypothermic and antipyretic effects of aureomycin observed after its intraperitoneal injection depended in some specific manner upon the route of its administration rather than upon some pharmacologic property of the drug, other routes of injection were employed. Data from an experiment in which aureomycin was injected intravenously at the same time as virus are presented in Fig. 3. Two additional experiments conducted in the same manner yielded similar results. Aureomycin given intravenously prevented the febrile response of rabbits to influenza B virus. These data indicated that the antipyretic action of this drug was independent of the route of parenteral adminis-

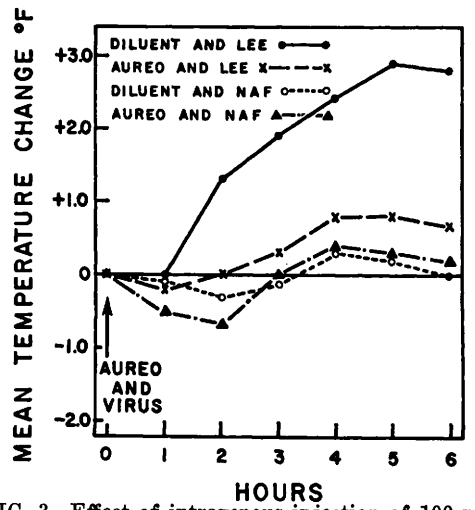

FIG. 3. Effect of intravenous injection of 100 mg of aureomycin upon febrile response of rabbits to intravenous inoculation of 5 ml of Lee virus.

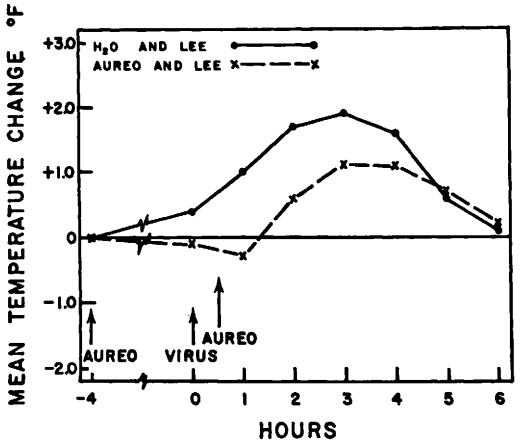

FIG. 4. Effect upon febrile response of rabbits given 1 g of aureomycin by gavage 4 hr before and ½ hr after intravenous inoculation of 5 ml of Lee virus.

tration. A hypothermic action of aureomycin was not evident by this route since there was little reduction of rectal temperatures below normal in rabbits given intravenous aureomycin and normal allantoic fluid. This observation gave additional evidence that the antipyretic activity was not a direct function of the hypothermic effect of the drug.

Effect of aureomycin by gavage before and after Lee virus. To determine the effect of oral aureomycin on the viral pyrogenic response, 1 g of aureomycin was given in 30 ml of water through a stomach tube 4 hours before and ½ hour after virus. The results of one experiment are presented in Fig. 4. The degree of fever produced in the treated rabbits was less than in controls. Four other similar experiments were done: in two the results were similar to those presented in Fig. 4, in one experiment no difference between aureomycin treated and control animals was apparent, and in one experiment the aureomycin treated animals developed more fever than controls. The marked variation in antipyretic effect achieved within the treated group in each experiment was responsible for failure to obtain reproducible results. This variability may have been the effect of variable absorption of the drug although experimental determinations to elucidate this possibility were not done. In order to reduce the mathematical effect of variation, geometric means were used to calculate temperature changes for all 5 gavage experiments combined. This procedure yielded temperature curves substantially identical to those presented in Fig. 4. However, since aureomycin was given at various times in different experiments these data could not be presented in a summarized form. Another difficulty encountered in gavage experiments was that the amount of fever produced in control animals was regularly less than that expected on the basis of previous experiments. Apparently the procedure of gavage itself reduced the pyrogenic response. In spite of these experimental difficulties, the trend was for aureomycin treated rabbits to manifest less fever than controls.

Effect of aureomycin on development of tolerance to the pyrogenic property of influenza virus. A febrile response to intravenous inoculation of influenza virus renders rabbits resistant to the pyrogenic action of the agent for a period of approximately 2 weeks(10). This tolerance is not related to immunity. To obtain evidence concerning the mechanism of the antipyretic action of aureomycin, experiments were designed to determine whether or not rabbits become resistant to the pyrogenic effect of influenza virus even if the febrile response were inhibited by the drug. Influenza B virus was inoculated intravenously into rabbits which

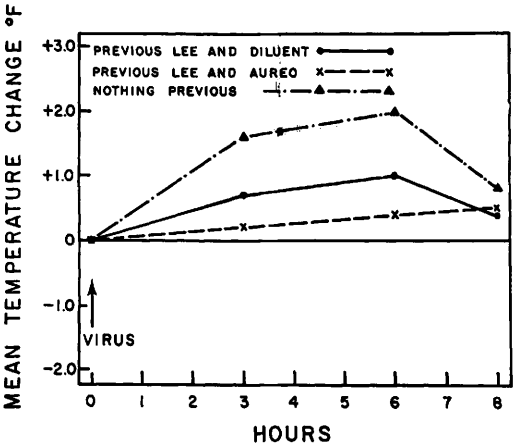

FIG. 5. Effect of antipyretic action of aureomycin upon acquisition of resistance to the pyrogenic property of influenza virus. Rabbits were inoculated intravenously with 5 ml of Lee virus 48 hr after intravenous inoculation of Lee virus and intraperitoneal injection of 100 mg of aureomycin or diluent.

received an intraperitoneal injection of aureomycin 2 hours before and a second injection 1½ hours after virus. The febrile response to virus was inhibited by aureomycin. Two days after the first viral inoculation these rabbits and controls which had been inoculated with virus and diluent received a second inoculation of virus. The results of this experiment are presented in Fig. 5. Rabbits inoculated with virus but in whom the occurrence of fever had been prevented by aureomycin behaved in the same manner toward subsequent inoculation of virus as those which had received virus and diluent and had experienced the febrile reaction. That is, both groups failed to develop fever. In this experiment temperatures were recorded only 0, 3, 6, and 8 hours after inoculation of virus so that peaks in the temperature curves may have been missed. At the times tested, however, refractoriness of rabbits to the pyrogen was apparent when compared to the response of animals which had not previously been inoculated with virus. These observations provided evidence that aureomycin like antipyrine(10) did not directly alter the host reaction to virus other than to inhibit the appearance of fever.

Effect of intraperitoneal aureomycin before or after typhoid vaccine. To determine whether aureomycin could exhibit antipyretic activity against a bacterial pyrogen, rabbits were inoculated intravenously with 1 ml of typhoid vaccine, containing 10^8 killed organisms. Aureomycin was injected intraperitoneally 2 hours before and 1 hour after vaccine in one group of rabbits and 1 hour after vaccine in another. The results of this experiment are shown in Fig. 6. Fever was markedly reduced in animals given aureomycin both before and after vaccine. When aureomycin was administered after the pyrogenic reaction was established, although an immediate reduction in temperature was observed and subsequent fever was less than that of controls, the febrile response was not entirely inhibited. The effect of aureomycin was not as marked as in experiments in which influenza virus was the pyrogenic stimulus, but this experiment does indicate that aureomycin can abolish or modify the reaction to a bacterial pyrogen.

Discussion. The original pharmacological investigation of aureomycin by Harned *et al.* (11) failed to disclose evidence of an antipyretic activity of this drug. Indeed, rabbits which were given 25 mg of aureomycin per kg intravenously 2 hours after intravenous inoculation of typhoid vaccine, experienced more fever than the controls. Since intravenous administration of some lots of aureomycin

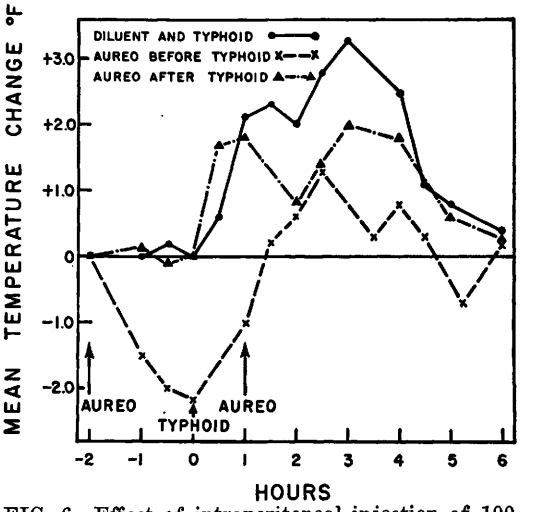

FIG. 6. Effect of intraperitoneal injection of 100 mg of aureomycin upon pyrogenic response of rabbits to intravenous injection of 1 ml of typhoid vaccine. Aureomycin was given 2 hr before and/or 1 hr after typhoid bacilli.

may produce fever, it seems possible that the preparation of aureomycin used in the experiments reported by these workers may have itself been pyrogenic, and thus obscured any antipyretic effect of the drug. Furthermore, the drug was given only after establishment of the pyrogenic reaction, a situation in which less antipyretic effect is observed.

Beeson(12) administered 2 g of aureomycin by gavage to 2 rabbits 2 hours before injection of typhoid vaccine intravenously and found no evidence of antipyretic activity of the drug. A similar lack of effect in individual rabbits given aureomycin by gavage was noted in the experiments reported in this paper although good protection was obtained in other rabbits. Consequently, this mode of administration of the drug cannot be considered the optimal one to demonstrate this property of aureomycin.

The pyrogenic effect of influenza virus is closely associated with its capacity to agglutinate erythrocytes but is unrelated to the infectivity of viral particles(9). Since viral multiplication does not occur in rabbits, the question of a chemotherapeutic action in this experimental model does not arise. The similarity of the effect of aureomycin to that of antipyrine against influenza virus pyrogen has been commented upon, and by analogy suggests a non-specific antipyretic action.

The experimental data presented do not permit conclusions concerning the mechanism of this action of the drug, but irrespective of mechanism, it is apparent that there are circumstances, however specialized these may be, in which aureomycin can exhibit an antipyretic effect. Certainly the evidence does not indicate whether or not this pharmacological property of aureomycin is capable of expression in any clinical situation. However, in the absence of direct evidence of a specific effect of aureomycin in viral diseases, the data do suggest that the possibility of an antipyretic action of this drug should receive consideration in the evaluation of its action.

Summary. Intraperitoneal or intravenous injection of aureomycin prevented the febrile reaction of rabbits to intravenous inoculation of influenza B virus, but administration of aureomycin by gavage did not consistently prevent fever. Although intraperitoneal injection of aureomycin reduced rectal temperature below normal, the data suggest that this hypothermic action of the drug was not responsible for the antipyretic effect observed. Aureomycin did not alter the development of tolerance to the pyrogen. Intraperitoneal aureomycin also modified the febrile response of rabbits to a bacterial pyrogen, typhoid bacilli.

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