the latter stages of gestation augmented significantly the vitamin A concentration in the blood and the livers of their newborn calves, but pasture grazing, providing an abundance of carotene in the prepartum diet of the dams, failed to effect an increase over that observed in calves from dams restricted to a standard winter ration. The explanation for these divergent results is obscure, but it is suggested that the placental membrane may be more permeable to the ester form of vitamin A than to the alcohol form. This high initial vitamin A reserve in the newborn calf should have practical value in the maintenance of its postnatal health.

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The Low Therapeutic Activity of Penicillin K Relative to That of Penicillins F, G, and X, and Its Pharmacological Basis

HARRY EAGLE and ARLYNE MUSSELMAN

The Johns Hopkins University and *U. S. Public Health Service Venereal Disease Research Laboratory and Postgraduate Training Center, Baltimore, Maryland*

Commercial penicillin contains at least four molecular species, identified as F, G, K, and X and differing from each other in the nature of the side group attached to a common nuclear structure (*8*). These are known to vary significantly in their bactericidal activity *in vitro*. Thus, referred to penicillin G as 100, the relative activities per milligram of penicillins F, G, K, and X against *Staphylococcus aureus* are reported to be 90, 100, 140, and 55, respectively (1,550, 1,667, 2,300, and 900 units/mg.) (*7*). In this laboratory, crystalline samples of F, G, K, and X were found to have relative gravimetric activities *in vitro* of 82, 100, 120, and 140 against the C-203 strain of hemolytic streptococcus, and 53, 100, 75, and 50, respectively, against a cultured strain of *Spirochaeta pallida* (Reiter) (*3*).

Subsequently, however, results obtained in the treatment of experimental syphilis indicated differences in the relative activity of the several penicillins *in vivo* far exceeding those hitherto observed *in vitro*. The curative dose (CD_{50}) of commercial penicillins, which probably consisted largely of penicillin G, had been found by Eagle, Magnuson, and Fleischman (*2*) to be 1,650 units/kg. when given every four hours for 20 injections. Almost identical results were obtained by Fleming (*4*), using both commercial penicillin and crystalline G. However, with penicillin K similarly administered preliminary data provided by Chesney (*1*) and confirmed by Mahoney and Arnold (*5*) indicated that even 16,000 units/kg. were not curative.

The obvious explanation for this marked discrepancy between the activities of penicillins G and K seemed to be that penicillin K, despite its definite activity against cultured, nonpathogenic spirochetes, was relatively inactive against pathogenic *S. pallida*. An alternative, if less likely, explanation was that penicillin K might be excreted or destroyed in the body more rapidly than the other penicillins and would therefore be relatively ineffective not only in the treatment of syphilis but in other infections as well. The data here presented indicate that this is in fact the case. Penicillin K disappears from the blood, and presumably the tissue fluids as well, far more rapidly than do the other penicillins; and the relatively small amount excreted in the urine suggests that it is inactivated *in vivo* to a greater degree than penicillins F, G, or X. Finally, corresponding to its pharmacologic behavior, and in complete agreement with the results in experimental syphilis (*5, 9*), it has proved approximately 9 to 15 per cent as active as penicillins F, G, or X in the treatment of experimental pneumococcus or streptococcus infections in white mice.

Preliminary data indicate that the rapid disappearance of penicillin K from rabbit blood and its relatively low recovery in the urine are due to its inactivation by the circulating blood. In rabbits the inactivating agent appears to be a relatively thermolabile, nondialyzable constituent of plasma. It is not present in any of the highly purified protein fractions of human plasma so far tested.

Blood levels and urinary excretion in rabbits. When rabbits were injected intramuscularly with crystalline penicillins F, G, K, and X[1] at equivalent dos-

[1] The following firms have made available samples of crystalline penicillin used in this study: Upjohn (F), Squibb (G), Lederle (X), and Abbott (K). Their cooperation is gratefully acknowledged.

age (0.6 mg./kg.), essentially similar blood levels were obtained with F, G, and X.

With penicillin K, on the other hand, the blood level fell far more precipitously than with the other three. Thus, one hour after the injection, the blood level in 7 animals injected with K ranged between a minimum of < 0.02 and a maximum of 0.031 μg./cc., averaging approximately 0.02; while in 10 animals receiving the other three penicillins, the blood levels at one hour varied between 0.07 and 0.45 μg./cc., averaging 0.23, or 11 times the average level of K. The length of time for which penicillin remained at a measurable level, e.g. 0.04 μg./cc., averaged 45 minutes with penicillin K, and two hours with the other three penicillins.

The data on the urinary excretion of penicillin in rabbits showed equally pronounced differences between penicillin K, on the one hand, and the other three penicillins on the other. The total cumulative excretion over a period of six hours in six rabbits receiving K varied between 18 and 42 per cent of the total injected and averaged 33 per cent; while in nine rabbits receiving penicillins F, G, and X the corresponding cumulative excretion varied between 39 and 100 per cent and averaged 74 per cent.

Blood levels and urinary excretion in man. Results qualitatively similar to those in rabbits were obtained in six men injected with identical doses of F, G, K, and X (0.6 mg./kg.). With penicillin K, the blood penicillin level one hour after injection varied between < 0.036 and 0.14 μg./cc., averaging 0.08; the corresponding values in the same four patients similarly injected with G were 0.14 to 0.57, with an average of 0.31 μg./cc. In man, the length of time for which the blood contained measurable amounts of penicillin, e.g. 0.04 μg./cc., averaged approximately 70 minutes in the case of penicillin K and was more than two hours with penicillin G.

The differences in the urinary excretion of G and K in man were consistent and marked. In four patients injected with 0.6 mg./kg. of penicillin G, the cumulative percentages excreted in the urine in six hours were 95, 99, 73, and 99, the average being 91 per cent. When the same four patients were injected with an equal amount of K, the corresponding percentages recovered in the urine in six hours were 39, 31, 31, and 22, the average being 31 per cent.

Therapeutic activity of penicillins F, G, K, and X in experimental infections in white mice. One would anticipate from the foregoing data that penicillin K would be relatively inactive *in vivo* in any infection, and regardless of its bactericidal activity *in vitro*. To determine this point, mice heavily infected with pneumococcus Type I and with *Streptococcus pyogenes* were treated with varying doses of penicillins F, G, K, and X. The curative doses (CD_{50}) of penicillins F, G, K, and X in pneumococcus Type I infection were, by the particular method of treatment used, totals of 4, 3.4, 20, and 2.45 mg./kg., respectively. Expressed relative to G as 100, these are gravimetric activities of 85, 100, 17, and 140. Penicillin K was thus one-sixth as active per milligram as penicillin G and one-eighth as active as penicillin K. The sample of penicillin K used in this experiment contained up to 10 per cent of some contaminating penicillin other than K. It follows that the therapeutic activity of pure K would have been even less than that observed.

An even more striking difference between the activity of K and the other three penicillins was observed in the treatment of streptococcal infections in mice. Against that same strain of streptococcus the relative activities of crytsalline F, G, K, and X *in vitro* had been found to be 82, 100, 120, and 140, respectively. Their curative doses (CD_{50}) in infected mice were 2.6, 1.3, 14.9, and 0.5 mg./kg., or relative activities of 50, 100, 9, and 260. Penicillin K was therefore only one-eleventh as active as penicillin G and one-thirtieth as active as penicillin X *in vivo,* despite its high activity against the same organism *in vitro*.

Practical implications. The strain of penicillium most widely used at the present time in the commercial production of penicillin is the Q-176 strain of *Penicillium chrysogenum.* It is stated that up to 50 per cent of the penicillins produced by this strain in the absence of specific precursor substances may be penicillin K. The present data indicate that, with "penicillin" containing such large amounts of K, the actual therapeutic potency *in vivo* may be far less than its activity *in vitro,* measured in staphylococcidal units. With such penicillins, previously effective doses may be therapeutically inadequate, and larger amounts must be administered to achieve the same results.

It is clearly desirable to modify the method of producing penicillin in order to minimize the proportion of K in the final product. If a single molecular species of penicillin cannot be provided, whether F, G, or X, it would be desirable also to use methods of standardization which bear a more direct relationship to therapeutic activity than does the determination of bactericidal activity *in vitro*. The determination in experimental animals of either therapeutic activity or residual blood penicillin, e.g. one hour after the injection of a standard test dose, might suffice for this purpose.

Summary

One hour after the injection into rabbits or man of penicillins F, G, K, and X at 0.6 mg./kg., blood

levels of K were one-fourth to one-eleventh of those observed with the other penicillins, and K persisted at demonstrable levels for relatively short periods.

In both rabbits and man the recovery of K in the urine averaged 30–35 per cent. This compares with an average recovery for F, G, and X of 74 per cent in rabbits and 91 per cent in man.

In the treatment of experimental pneumococcal infections in white mice, an impure preparation of K was one-sixth as active as G and one-eighth as active as X. In the treatment of experimental streptococcal infections in white mice, a pure preparation of K was one-eleventh as active as G, and one-thirtieth as active as X.

The above data suggest that penicillin K is inactivated in the body to a greater extent and more rapidly than either F, G, or X, resulting in a far lower therapeutic activity than would be anticipated from its bactericidal action *in vitro*. It seems clear that the amount of K in commercial penicillin should be minimized; and it would seem desirable to standardize impure mixtures of penicillins for therapeutic use by some method other than their bactericidal activity *in vitro*.

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Direct Culture of Rheumatic Virus[1]

WARD J. MAC NEAL, KATHERINE WARDELL, ANNE BLEVINS, and MARCELLO R. PACIS

Department of Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University

In March 1945 there was presented before the New York Pathological Society (*4*) a demonstration of specimens and a discussion concerning the production of nonbacterial endocarditis of a verrucous type in experimental animals following the injection of pericardial fluid, whole blood, and blood plasma from patients with rheumatic fever, and the subsequent propagation of the supposed pathogenic agent or agents in embryonated eggs and transfer back to the small mammals to reproduce the disease. It was recognized that virus diseases naturally present in the experimental animals may give rise to difficulty in interpretation of the morphological results, as had been noted by De Vecchi (*3*) and Andrei and Ravenna (*1*); hence the need of supporting evidence.

J. L., girl aged 16, with severe rheumatic endocarditis and pericarditis and temperature of 105.2°, was admitted to hospital on 12 January 1946. The plasma of her blood, drawn that evening, was injected into five embryonated eggs on 13 January. Of these eggs, two died on the first day, one on the fourth day, and two were killed on the sixth day, so that the result was briefly recorded as $D_1 - D_1 - D_4 - K_6 - K_6$. Another specimen of plasma, taken on the morning of 13 January and injected into five eggs on this day, gave the result $D_1 - K_6 - K_6 - K_6 - K_6$; and the third specimen, taken on the afternoon of 13 January and injected without delay into five eggs, gave the result $D_2 - D_2 - K_6 - K_6 - K_6$. On 14 January a fourth specimen, taken in the morning, supplied plasma for five eggs with the result $D_1 - D_3 - K_6 - K_6 - K_6$. A fifth specimen, taken that afternoon, supplied plasma for five more eggs and resulted in $D_2 - D_3 - D_3 - K_6 - K_6$.

None of these eggs showed any recognized pathological changes. The extraembryonic fluids were harvested promptly and found bacteria free by aerobic culture.

On 21 January the pooled fluids from these 25 eggs were used in part for the inoculation of rabbits and guinea pigs and later for inoculation of more embryonated eggs (see below).

Meanwhile, on 15 January some of the citrated plasma saved from the blood drawn on 12 January was used for inoculation of the chorioallantoic membranes of five embryonated eggs, aged 9 days. All five of these eggs survived to be harvested on 18 January ($K_3 - K_3 - K_3 - K_3 - K_3$), and all exhibited remarkable localized thickening of the chorioallantoic membrane and intense reddish-pink discoloration of the embryo proper. Aerobic bacterial cultures remained free from growth. One membrane of this lot was ground in a mortar and suspended in saline solution for inoculation on the chorioallantois of five additional embryonated eggs on 29 January. All these survived to be harvested on 2 February ($K_4 - K_4 - K_4 - K_4 - K_4$), and each exhibited a thickened chorioallantoic membrane with local nodules and general reddening of the live embryo itself.

The pooled fluids of the first 25 eggs mentioned above (21 January) were used in part for injection into five eggs on 26 January, with the result $D_2 - D_2 - D_4 - K_6 - K_6$. No pathological changes were recognized in these eggs. On 26 January, also, this

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