THE ENHANCEMENT OF PENICILLIN EFFECTIVENESS IN VIVO BY TRACES OF COBALT[1]

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It has been shown that the *in vitro* activity of dilute solutions of penicillin against *Staphylococcus aureus* may be increased 4- to 8-fold in the presence of trace amounts of cobalt chloride (Strait, Dufrenoy, and Pratt, 1947) and that the enhancing effect is even more pronounced when gram-negative, penicillin-resistant organisms such as *Eberthella typhosa*, *Proteus vulgaris*, or *Escherichia coli* are used as test organisms (Pratt and Dufrenoy, 1947*b*). This effect seems to be specific to cobalt among the ions studied, since salts of Ni, Mn, Pt, Ir, Fe, Zn, Sr, Cd, Li, Cu, Ag, Au, and Bi have been studied, and none has been so effective in producing a similar enhancement of penicillin activity in the range from 0.01 to 10 ppm.

A similar action of cobalt on penicillin activity has now been demonstrated in mice inoculated with lethal doses of *E. typhosa*.

Different groups of 30 adult mice (Swiss Kloke) were injected intraperitoneally with 0.5 ml aqueous solutions of crystalline sodium benzyl penicillin alone (2,000 units per animal) or with the same dose of the penicillin combined with different amounts of cobalt ranging from 4 to 256 micrograms of $CoCl_2 \cdot 6H_2O$ per animal, or with the solutions of cobalt chloride alone. Thirty minutes later the animals were inoculated intraperitoneally with 0.4 ml of a 6-hour broth culture of *Eberthella typhosa* (approximately 2×10^8 organisms). This procedure is essentially similar to that proposed by Welch, Randall, and Price (1947) for the detection of an enhancing "factor" found in certain commercial lots of amorphous penicillin.

Experimental results are shown in table 1. Five experiments with the same procedure but with different control conditions all yielded similar results.

The data for 24 hours show that 64 micrograms of cobalt chloride administered simultaneously with 2,000 units of crystalline benzyl penicillin enhanced the activity of the penicillin approximately 50 per cent, i.e., 2,000 units with cobalt chloride protected animals for 24 hours as well as did 3,000 units without cobalt. Larger amounts of $CoCl_2 \cdot 6H_2O$ administered with the penicillin caused greater enhancement. Thus with 256 micrograms of cobalt chloride administered with 2,000 units of penicillin, the enhancement of the activity as seen from the 48-hour mortality data is of the order of 100 per cent, i.e., 2,000 units of penicillin plus 256 micrograms of cobalt chloride protected animals for 48 hours as well as

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did 4,000 units without cobalt. There were no further deaths after 48 hours. The percentage of enhancement at 24 hours was much greater than at 48 hours. Administration of cobalt chloride alone conferred no protection on the infected animals. Therefore, the result that was observed cannot be interpreted merely as the additive effect of two antibiotics acting simultaneously.

It seems likely that the schedule of injection that was used failed to provide a sufficient concentration of cobalt over a long enough period of time to sustain adequately the enhancing action. Copp and Greenberg (1941) showed that in rats small doses of intraperitoneally injected cobalt were excreted largely in the

TABLE 1

The effect of cobalt with penicillin

TEST MATERIAL INJECTED 30 MINUTES BEFORE INOCULUM OF E. TYPHOSA	NUMBER OF MICE	PERCENTAGE OF MORTALITY AFTER	
		24 hours	48 hours
Control (inoculum only)*	555		90*
Control (inoculum only)	100	92	92
Penicillin (2,000 units)*	530		78*
Penicillin (2,000 units)	90	77	80
Penicillin (3,000 units)*	560		67*
Penicillin (3,000 units)	60	72	77
Penicillin (4,000 units)*	80		45*
Penicillin (2,000 units) plus $CoCl_2 \cdot 6H_2O$ (0.064 mg)	60	69	79
Penicillin (2,000 units) plus $CoCl_2 \cdot 6H_2O$ (0.256 mg)	90	27	39
$CoCl_2 \cdot 6H_2O$ (0.256 mg)	60	95	96
Cobalt control: $CoCl_2 \cdot 6H_2O$ (0.256 mg)† with no subsequent inoculation with *E. typhosa*	10	0	0

* Accumulated data from Microbiological Research Department of Cutter Laboratories, Berkeley, California.

† No deaths occurred when doses as high as 0.512 mg per animal were administered, but the animals seemed drowsy and disinclined to move voluntarily for 24 hours, although they were active if prodded slightly. Higher doses were not used. No symptoms of toxicity or altered action were seen in animals receiving 0.256 mg.

first few hours: 70 per cent via the urine in the first 10 hours and approximately only an additional 10 per cent in the next 14 hours.

It was observed *in vitro* (Strait, Dufrenoy, and Pratt, 1947) that there is an optimum concentration of cobalt for securing the maximum enhancement of penicillin activity and that to secure the best effect a period during which the cobalt is in contact with the pathogen before the application of penicillin is desirable. Thus it may be surmised that, *in vivo*, appropriate doses of cobalt chloride administered before and periodically for several hours after appropriate doses of penicillin would be effective in increasing and prolonging the effect of the penicillin. We are investigating this matter as well as the use of cobalt-penicillin mixtures in the treatment of acutely and chronically infected animals.

The enhancement of penicillin activity effected by traces of cobalt *in vitro* and *in vivo* differs from that induced by the "factor" reported by Welch, Randall, and Price (1947), which is detectable only *in vivo*.

These experiments were conceived because of the demonstrated effect (Albert, 1947) of cations on the bacteriostatic activity of agents with which they had the capacity to form complexes. Evidence has been presented (Dufrenoy and Pratt, 1947; Pratt and Dufrenoy, 1947*a*) that agrees with the hypothesis that the bacteriostatic activity of penicillin can be correlated with a shift in the oxidation-reduction potential, possibly attributable to dehydrogenation of the —SH groups from sulfhydryl- containing proteins. Thus it may be proposed that the synergistic effect of cations may be ultimately associated with the formation of complexes with —SH-containing groups or with some other essential component of an energy-providing oxidation-reduction system. The degree of inhibition effects exhibited by the various cations may be related to the degree of binding of the cations in the complex. Thus Cd and Ag, which form stable complexes, are highly toxic, whereas Co, which forms loose complexes with —SH groups, is much less toxic.

SUMMARY

The enhancement of penicillin effectiveness by the addition of small amounts of cobalt, previously observed *in vitro*, has been demonstrated *in vivo*. Administration of 256 micrograms $CoCl_2 \cdot 6H_2O$ with 2,000 units of crystalline sodium benzyl penicillin per animal exerted a protective action against *Eberthella typhosa* in adult mice equivalent to at least 4,000 units of the penicillin alone. Concentrations as low as 64 micrograms of cobalt chloride produced some enhancement of penicillin effectiveness. Cobalt chloride alone in these concentrations was not toxic and conferred no protection on the animals.

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