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Henry Welch; ... et. al

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INHIBITION OF PHAGOCYTOSIS BY PENICILLIN

HENRY WELCH, RUTH P. DAVIS AND CLIFFORD W. PRICE

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In a previous publication (1) the relative toxicities of six salts of penicillin were reported. Toxicity was determined by intravenous injection of mice and it was demonstrated that the toxicities of the various salts of penicillin tested were primarily due to the respective cations used in their preparation. In continuing our studies on the toxicity of penicillin, a large number of lots of penicillin sodium as well as seven different salts of penicillin[1] have been studied for their influence on the inhibition of phagocytosis.

The method used for determining loss of phagocytic activity in the presence of penicillin has been reported previously (2). It consists primarily of mixing the penicillin with antigen (*Staphylococcus aureus*) and citrated human blood followed by rotation on a leucocyte-serum mixing machine for 30 minutes at 37 C. Smears are then prepared, stained with methylene blue, and the concentration of penicillin causing complete inhibition of phagocytosis determined by microscopic examination.

Sixty lots of penicillin sodium from ten manufacturers, varying in potency from 86 to 930 units per mg, have been studied for their effect on phagocytic activity in an attempt to determine the relation of purity to inhibition of phagocytosis. The results are given in table 1 where it will be noted that with low potency penicillin sodium (86 units per mg) complete inhibition of phagocytosis occurred with a correspondingly low concentration (1,200 units), while high potency material (930 units per mg) brought about inhibition of phagocytosis at correspondingly high concentrations (16,000 units). Between these low and high potency products there is a gradual decrease in toxicity per unit of penicillin with increase in potency. These results are in accord with those obtained previously (3) on the relationship of purity of penicillin preparations to intramuscular pain in human subjects where it was shown that there was a correlation between the potency of the product and intramuscular pain produced.

It should be noted that even though the low potency material required fewer units to inhibit phagocytosis, the weight, i.e., milligrams of commercial penicillin, responsible for inhibition of phagocytosis in most cases is practically the same regardless of the purity of the product. This is particularly true for those products of average potency between 86 units per mg and 471 units per mg.

Although it was possible with only a few of the lots of penicillin sodium reported in table 1 to obtain the number of mg of cation present at the toxic dose, the indications were, however, that the cation was not involved in this toxic

[1] These salts of penicillin were prepared by Chas. Pfizer & Co., Inc., from a single master lot of penicillin sodium. This investigation was made at the request of the Office of Scientific Research and Development.

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effect, in contrast to the previously reported (1) results where the toxic effect was measured by determining the LD-50 of the various salts of penicillin in mice.

In continuing this investigation similar studies have been made of the sodium, ammonium, lithium, strontium, calcium, potassium and magnesium salts. The salts studied were either prepared in our small pilot plant or were products of commercial manufacture. In the case of each salt the number of units completely inhibiting phagocytosis was determined by the method previously described[2] and the results are given in table 2-A and B. The data in table 2-A show that as the potency of the product increased, regardless of the cation, the toxicity decreased, e.g., penicillin calcium with a potency of 176 units per mg inhibited phagocytosis at a concentration of 2,000 units while penicillin magnesium having a potency of 1,028 units per mg inhibited phagocytosis at 12,000 units. In between these extremes of purity, with the exception of the ammonium salt (table 2-A), an increase in potency resulted in a corresponding decrease in toxicity. As with the sodium salts reported in table 1, the number of mg of penicillin responsible for complete inhibition of phagocytosis was again, within experimental error, approximately the same, an average of 11.2 mg. The commercial salts of penicillin were, with the exception of the strontium salt, approximately equal in potency (table 2-B). When these high potency salts of penicillin are tested for their toxic effect on phagocytosis all inhibit this activity of the leucocyte at approximately the same concentration of units. Similarly, the high potency penicillin magnesium made in our pilot plant (table 2-A) completely inhibited phagocytosis at approximately the same concentration of units and at approximately the same weight.

TABLE 1

Effect of purity on the toxicity of penicillin sodium (commercial samples) for human leucocytes

AVERAGE POTENCY	COMPLETE INHIBITION OF PHAGOCYTOSIS		NUMBER OF	
	No. units	No. mg.	Lots tested	Manufacturers
units/mg				
86	1,200	13.9	1	1 (E)
155	2,200	14.2	20	4 (A, G, I & J)
244	3,000	12.3	9	5 (C, E, G, H & J)
359	4,300	12.0	10	3 (B, C & E)
471	6,000	12.7	9	4 (C, E, F & H)
697	11,000	15.8	8	3 (D, H & F)
930	16,000	17.2	3	1 (D)
Average		14.0		

In order to demonstrate that the amount of cation at the "toxic dose" had little or no effect on phagocytosis both the amount and twice the amount of the cation present at this dose of penicillin were tested as the acetates of these cations to determine their effect on phagocytic activity. The acetates of NH_4, Sr, K, Na, Mg and Li caused no inhibition of phagocytosis at either of the concentra-

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tions tested while calcium acetate did cause appreciable but not complete inhibition. Since the concentrations of cations present at the toxic dose of crude penicillin and the number of units of active penicillin are not responsible for inhibition of phagocytosis it would seem that the whole penicillin molecule and the extraneous matter accompanying it are involved in this phenomenon. The average weight of the six salts of penicillin prepared in the pilot plant which completely inhibited phagocytosis was 11.2 mg. Similarly, the average weight of seven commercial salts of penicillin bringing about this effect was 13.1 mg, while 60 commercial salts of penicillin sodium completely inhibited phagocytosis

TABLE 2

Effect of purity on the toxicity of various salts of penicillin for human leucocytes

PENICILLIN	POTENCY	COMPLETE INHIBITION OF PHAGOCYTOSIS		CATION AT TOXIC DOSE	
		No. units	No. mg	Percent	Milligrams
A. Pilot plant samples					
Ca	176	2,000	11.4	8.16	0.93
NH_4	332	3,000	9.0	6.96	0.63
Sr	285	3,500	12.3	13.53	1.66
K	479	5,100	10.7	12.45	1.33
Na	656	8,000	12.2	7.72	0.94
Mg	1,028	12,000	11.7	4.23	0.49
Average			11.2		
B. Commercial samples					
Sr	835	12,800	15.3	13.74	2.11
Mg	970	12,800	13.2	3.72	0.49
Ca	973	12,800	13.2	6.27	0.83
NH_4	1,010	11,200	11.1	7.54	0.84
K	1,020	14,400	14.1	11.67	1.65
Li	1,033	12,800	12.4	2.39	0.30
Na	1,037	12,800	12.3	7.22	0.89
Average			12.1		

at an average concentration of 14.0 mg. When consideration is given to the error inherent in the assay of penicillin, it appears that the weight of crude penicillin which completely inhibits phagocytosis is for all practical purposes the same regardless of the cation used in its preparation or its potency. It is unlikely that the toxic factor responsible for inhibition of phagocytosis can be predicated on the presence of toxic extraneous material present in low potency salts as a result of the process of extraction. As shown in table 2-A, the low potency penicillin calcium (176 units per mg) caused complete inhibition of phagocytosis only when in a concentration of 11.4 mg, while the high potency penicillin magnesium (1028/mg) completely inhibited phagocytosis at a concentration of 11.7

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mg. In view of the above it is suggested that the inhibition of phagocytosis demonstrated in these studies may be the result of an osmotic pressure effect where the leucocyte is prevented from performing its normal function in the presence of a definite weight of impure or commercial penicillin.

The test used to determine the concentration of penicillin which inhibits phagocytosis measures relative toxicity only and the effective concentrations are extremely high in comparison to those concentrations normally obtained when penicillin is used therapeutically. Under the ordinary conditions of use it would be practically impossible to obtain concentrations of penicillin in man that would have a deleterious effect on phagocytosis.

SUMMARY AND CONCLUSIONS

The toxicity, as determined by inhibition of phagocytosis, of 60 lots of penicillin sodium from ten manufacturers has been studied to determine the effect of purity on the toxicity of this salt of penicillin. Similar studies have been made of six different salts of penicillin prepared on a pilot plant basis and of seven different salts of penicillin prepared commercially. There is a correlation between the toxicity of penicillin preparations as measured by inhibition of phagocytosis and potency. The toxicity for leucocytes of the penicillin preparations studied is not dependent upon the cation used in their production. The weight of the penicillin preparations studied which completely inhibits phagocytosis appears to be the same, regardless of the type or potency of the salt tested. It is suggested that the inhibition of phagocytosis demonstrated in these studies may be the result of an osmotic pressure effect. The concentrations of crude penicillin necessary to inhibit phagocytosis are not obtained in the ordinary clinical use of this material.

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