ACCUMULATION OF DIETHYLAMINO-ETHANOL ESTER OF PENICILLIN IN INFLAMED LUNG TISSUE

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Since the inception of widespread penicillin therapy, attempts have been made to dispense with frequent intermittent injections of aqueous solutions of penicillin salts by injection in an oily medium or as water-insoluble salts such as procaine penicillin. With the same end in view it was suggested that penicillin esters, by virtue of their greater stability and slower absorption and excretion, might offer some advantage (Meyer, Hobby, and Dawson, 1943). It was subsequently found, however, that the methyl, ethyl, and benzyl esters investigated at that time were unlikely to have therapeutic value in man, since no hydrolysis took place in the human body, although the esters of penicillin were effective in the treatment of experimental infection in mice, rats, and guinea-pigs (Richardson *et al.*, 1945; Ungar, 1947).

More recently, Carpenter (1948) prepared a dimethyl-aminoethyl ester hydrochloride of penicillin, which was readily hydrolysed *in vitro* in aqueous buffer at pH 7.3. Jensen *et al.* (1950) have subsequently reported that after injection of the analogous diethyl ester into guinea-pigs higher levels of penicillin can be detected in the lungs than in the blood at the corresponding time, and Heathcote and Nassau (1951) reported effective levels in sputum of patients treated with this ester. This remarkable observation particularly attracted our attention in view of past experience of the accumulation of penicillin in inflamed tissue (Ungar, 1950).

In the present communication a short account is given of our laboratory findings with the hydriodide of the same ester ("estopen").

Properties of the Ester

While the free base is readily soluble in water the hydriodide is only sparingly soluble (about 1% at 20° C.). This compound was injected as aqueous suspensions. The ester is stable in dry powder form, but is hydrolysed spontaneously in aqueous solution, hydrolysis increasing with rise in temperature and pH. The hydrolytic process brings about a fall in pH to about 4, where the rate of hydrolysis is small. At pH 7.35 the ester has a "half-life" of about 30 minutes at 37° C.

The unhydrolysed ester is not attacked by penicillinase—for example, that produced by penicillin-resistant staphylococci—but in aqueous solutions de-esterification takes place and the liberated benzyl-penicillin is inactivated by the enzyme. Also, in the animal body the ester is without effect against bacteria producing penicillinase. Groups of mice infected with a lethal dose of penicillin-resistant *Staphylococcus aureus* were treated with benzyl-penicillin or with its ester; treatment was ineffective with either.

The ester is apparently slightly more toxic than is benzyl-penicillin, particularly to guinea-pigs. In the first experiments guinea-pigs were injected with 100,000 units in 0.5 ml. of saline, which occasionally caused immediate reactions in the central nervous system and subsequent death (within

about 10 minutes of injection). Accordingly the standard dose was reduced to 50,000 units in later experiments. The toxicity after intravenous administration is entirely due to the ester linkage, for the components of the ester (benzyl-penicillin and diethylaminoethanol hydriodide) are non-toxic when injected together at equivalent concentrations.

The bacterial spectrum of the ester is identical with that of benzyl-penicillin. It has been shown, however, that the intact ester is biologically inactive until hydrolysis liberates free benzyl-penicillin.

In laboratory animals and in human volunteers parenteral injection of the ester was found to produce penicillin levels in the blood intermediate between those produced by the same number of units of benzyl-penicillin or of procaine penicillin. Rather less penicillin was recoverable from the urine after injection of the ester than after injection of the other two preparations. On injection of the ester there was some slight antidiuretic effect, especially in rats; it could also be produced by injecting an equivalent amount of diethylaminoethanol. After intramuscular injection of the ester into normal rabbits it was found that penetration into the cerebrospinal fluid occurred to the same extent as with the same dose of procaine penicillin. When administered orally to rabbits (200,000 units in 5 ml. of water by stomach tube) the ester produced levels in the blood similar to those in control animals given the same dose of penicillin by the same route. The levels in rabbits treated with benzyl-penicillin, however, appeared to persist for rather longer.

Distribution of Penicillin after Administration of the Ester Levels in Blood, Lung, and Other Tissues

The plate assay method was used with *Staph. aureus* as the test organism, and an average of five readings was taken at each test level. An account of the methods of preparing extracts of organs and the technique used in inducing local lesions is being prepared for publication elsewhere.

Bronchopneumonia was induced in mice and guinea-pigs by intranasal instillation, under ether-chloroform anaesthesia, of two drops of a saline suspension of *Haemophilus pertussis*, which resulted in marked consolidation of the lung in two to three days. Table I gives the results of levels in normal and infected mice and Table II in guinea-

TABLE I.—Mice Injected Subcutaneously with 5,000 Units of Penicillin in 0.2 ml. of Saline. Average of 10 Mice for Each Level

	Penicillin	Tissue	Penicillin Levels (units/g.) at (hours):										
			½	1	2	4	6	10	12	16	22		
Normal mice	Ester	Blood	267	108	28	7.5	0	Not tested					
		Lung	180	75	17	1.8	0						
	Procaine	Blood	160	150	9	3.2	0	,,					
		Lung	87	66	18	1.2	0						
Infected mice	Ester	Blood	182	253	87	15	12	0.11	0	0	0	0	0
		Lung	141	411	198	63	31	1.64	4.6	0	0	0	0
	Procaine	Blood	171	214	152	27	9	0.1	0	0	0	0	0
		Lung	158	123	123	21	7	0.2	0	0	0	0	0
	Sodium salt	Blood	—	114	62	4	0.4	0.2	0	0	0	0	0
		Lung	—	176	96	6	2.4	2	0	0	0	0	0

TABLE II.—Guinea-pigs; Dose 100,000 Units in 0.5 ml. of Saline Subcutaneously. Average of Three Guinea-pigs for Each Level

	Penicillin	Tissue	Penicillin Levels (units/g.) at (Hours):							
			½	1	2	4	6	10	12	
Normal guinea-pigs	Ester	Blood	26	22	44	22	5	0	0	
		Lung	10	24	40	11	5	0	0	
	Procaine	Blood	16	35	44	15	23	2.6	1.3	
		Lung	9	18	13	5	5	0.6	0.3	
Infected guinea-pigs	Ester	Blood	9	8	11	7	4	2.1	0	
		Lung	60	51	36	13	5	1.5	0	
	Procaine	Blood	12	10	15	6.5	8	1.5	0.4	
		Lung	<6	<6	<6	6	5	0.4	0.2	
	Sodium salt	Blood	6	35	6.6	1	<0.2	0	0	
		Lung	0	17	4	1.6	<0.2	0	0	

pigs, after injection of the ester and procaine penicillin. They indicate that after injection of the ester significantly higher penicillin levels are detectable in the lungs than in the blood of the infected animals, particularly during the first six hours. Animals treated with procaine penicillin show consistently lower lung levels than do those treated with the ester, though the levels in the blood and lung tend to persist longer in guinea-pigs; this can be explained by the lower solubility of the procaine salt (about 0.6% at 15° C.) and its greater ability to form depots.

It can be seen (Table I) that with penicillin sodium salt we secured a concentration effect in infected lungs of mice, but far below the levels and for a shorter period (two to six hours only) than after the injection of the ester.

Specific Concentration in the Lungs

Further experiments showed that after injection the ester was concentrated only in the lungs, and not in other tissues, of those suffering from lung infection (Table III). Mice infected intracerebrally (Kendrick *et al.*, 1947) (Table IV) or rabbits with exudate due to turpentine injections (Ungar,

degree of concentration of penicillin in the lungs was directly related to the total dose of the ester injected and to the extent of the lesions in the lungs, and also that in mice recovered from a lung infection injecting the ester two to three weeks later still induced a marked concentration of penicillin in the lungs.

Mode of Action of the Ester

Having established this basic property of the ester, we have tried to discover its mode of action and the reasons for its selective concentration. Two points had especially to be kept in mind: (a) the structure of the penicillin ester, especially in relation to its pharmacological action and those of its components; and (b) the special nature of lung tissue in contrast with other organs and its peculiarities when inflamed.

It should be mentioned that there seems to be a species specificity for penicillin accumulation in the lungs. It was noticed that in normal guinea-pigs, rabbits, and rats higher levels in the lungs were recorded than in the blood in the first 30 to 60 minutes after injection of the ester, but that no such phenomenon was seen in mice.

Though no detailed theory can yet be advanced to explain the mode of action of the ester, because of the complexity of factors involved, certain aspects of the matter have been elucidated, of which a brief account follows.

On injection of the ester some of it dissolves (about 0.9% at 15° C.) and ionizes in the blood, circulating as PD⁺ and I⁻ (P=benzyl-penicillin; D=diethylaminoethanol; I=iodine). The PD⁺ (ester) enters the lung and is there fixed by tissue constituents, particularly in inflamed tissue, from which it is gradually released, hydrolysis of the ester link liberating free P⁻. The lung constituent that first fixes the PD⁺ may be increased in amount, or its activity may be altered by pH or enzymatic changes in infected lungs. The equilibria concerned can thus be suggested as follows:

Ester injected $\longrightarrow$ carried to the lungs
Ester+lung constituents $\rightleftharpoons$ ester-tissue complex (1)
Free ester $\longrightarrow$ D+P (2)

Penicillin is removed by the kidney via the blood stream, therefore reaction proceeds thus $\longrightarrow$ in (2)

The ester-tissue complex $\longrightarrow$ ester+lung tissue components
reaction will proceed in this direction if ester is removed, the liberated ester being hydrolysed as in (2).

The lung may therefore contain, at any given time after injection, the following entities: (1) intact ester (as hydriodide or free base); (2) ester complex with the lung constituents; (3) free benzyl-penicillin; (4) benzyl-penicillin-tissue complex; (5) diethylaminoethanol-lung-tissue complex; (6) free diethylaminoethanol; (7) free I⁻.

The effect of diethylaminoethanol on benzyl-penicillin was investigated, since this compound is a common constituent of both estopen and procaine penicillin. Some of the action of the ester might be caused by the presence of the alcohol (see experiments with procaine esterases by Kisch, Koster, and Strauss, 1943), which could exert an independent pharmacological action (Hauschild, 1950).

In other experiments (of which details are also being prepared for publication elsewhere) we found that simultaneous administration of the alcohol and the benzyl-penicillin prolonged the circulation of penicillin in the blood of rabbits and guinea-pigs; but this was not due to delayed excretion of penicillin in the urine, although in rats an antidiuretic action of the alcohol was noticed. In independent experiments, using specially prepared salts of diethylaminoethanol and benzyl-penicillin, we found that the compounds behaved in the matter of lung concentration exactly as penicillin sodium salt, which indicated that the effect in the lungs is dependent on the intact ester link within the molecule. It occurred to us that the selective concentration of penicillin in the lungs might be due to the conditions of enzymatic hydrolysis of the ester in the lungs differing from those in other organs. Investigation was therefore made into the

TABLE III.—Distribution of Penicillin after Subcutaneous Injection of the Ester, 5,000 Units, in the Tissues of Normal and Lung-infected Animals. Average of 10 Mice for Each Level

		Penicillin Levels in Units/g. of Tissue at (Hours):		
		1½	3	5
Normal	Blood	37	13	0.4
	Lung	11	5	1.5
	Liver	24	4	0.8
	Spleen	5	2	<0.5
	Kidney	82	18	<6
	Muscle	<6	5	0.6
Infected	Blood	90	10	3
	Lung	766	96	131
	Liver	25	4	<2
	Spleen	14	<3	<2
	Kidney	145	14	<3
	Muscle	22	1	2

TABLE IV.—Distribution of Penicillin in Tissues of Mice Infected Intracerebrally with *H. pertussis* after Injection of the Ester and Penicillin Sodium Salt. Average of 10 Mice for Each Level.

Penicillin	Mice	Tissue	Penicillin Levels in Units/g. of Tissue at (Hours):					
			1	2	4	7	12	15
Sodium salt (5,000 units)	Normal	Blood	24	94	0.8	0.8	0	0
		Lung	13	23	0	0	0	0
		Brain	0.8	1.4	0	0	0	0
	Intracerebrally infected	Blood	500	5	1.5	8	2.2	0.6
		Lung	400	50	31	28	0	1.4
		Brain	32	36	12	34	0	1
Ester (5,000 units)	Normal	Blood	110	82	6.6	1.7	0	0
		Lung	56	35	3	0	0	0
		Brain	3	2	0	0	0	0
	Intracerebrally infected	Blood	123	216	10	1.7	1	0.8
		Lung	70	200	48	6.7	0	0
		Brain	7	9	5	3.4	0	0

1950) did not show any selective concentration of the ester at the inflamed site. Levels of penicillin in the other organs of animals injected with the ester were similar to those recorded after injection of procaine penicillin or penicillin sodium salt.

In the intracerebrally infected mice there was, after injection of the ester, no increase in the concentration of penicillin in the lungs compared with the blood, nor was a selective concentration noticed in brain tissue. It should be noted that the blood and tissue levels of penicillin were higher in infected animals than in normal mice, irrespective of the type of penicillin used. It is therefore justifiable to conclude that the selective concentration of estopen is a specific property of lung tissue, particularly of diseased lung tissue, and is not shown to the same extent by any other organ. We observed that in our experiments the

esterase activities of tissues from a number of animals (mouse, rat, guinea-pig, and rabbit) and from man. The method of Knafl-Lenz as modified by Harrer and King (1941), in which the activity of the esterase is measured by the rate of hydrolysis of ethyl butyrate, was employed. The results showed that esterases varying in activity from species to species were present in lung tissues. Inflamed lung tissue, in contrast with normal, however, showed esterase activity decreased to about 50% per unit weight of tissue. It is probable that the reduction of esterase in the diseased lung is at any rate in part responsible for the longer persistence of the ester in these lungs than in the healthy ones.

Following the hydrolysis of the ester in tissues and in human blood by an ether-extraction technique, we found that the soluble ester was hydrolysed rapidly at pH 7.35 at 37° C., but the hydrolysis curve began to flatten out at about 60% hydrolysis. It was therefore evident that part of the ester was being protected from hydrolysis owing to its being bound on constituents of the inflamed lung tissue, particularly on proteins. Using a modification of the ultra-filtration method described by Sirota and Saltzman (1950) (for measurements with aureomycin) we found that benzyl-penicillin was bound on the tissue to the extent of 23.5%, whereas the ester at the same unitage was bound to the extent of 71%. Of the blood constituents, globulin was the fraction that bound 50% more ester than it did benzyl-penicillin. In addition, we ascertained that 1% globulin bound as much ester as did 4% albumin.

Discussion and Comments

We noticed in our experiments that the ester injected into animals suffering from bronchopneumonia gave consistently higher penicillin levels in the lung tissue than did procaine penicillin or soluble penicillin salts. In none of the other inflamed organs (brain or muscle exudate) was this phenomenon noticed, the ester behaving in this and other respects similarly to procaine penicillin. Heathcote and Nassau (1951) have shown in the sputa of patients treated with the ester effective concentrations of penicillin higher than any observed in the past after injection of benzyl-penicillin or procaine penicillin.

The reasons for the selective accumulation of penicillin in the lung tissue are many. We have found that the rate of hydrolysis in the lung may be affected by the amount of esterase available, which decreases in inflamed lungs, thus presumably causing persistence of unhydrolysed ester. The antidiuretic action of the diethylaminoethanol can be demonstrated in animals, and this might have a contributory effect in prolonging the levels of penicillin in the lungs. A number of physico-chemical interactions between the ester and lung tissue probably play a significant part; one of these is the binding on tissue constituents, and we have shown that the ester was bound on the tissue proteins to a greater extent than was benzyl-penicillin. Although the binding of certain drugs on proteins (sulphonamides, acridines, metallic compounds, etc.) is reputed to result in a decrease of their biological activity, experimental evidence and clinical experience with antibiotics seem rather to indicate that with them the process is usually reversible and that the bound drug is gradually released.

We found that hydrolysis of the ester takes place more rapidly at pH 7.3 than in a more acid solution. The pH of acutely inflamed tissue is about 7.2–7.5, whereas in chronic inflammation the pH may fall to about 6.5 (Menkin, 1950). This fact may partly explain the better therapeutic effect in acute than in chronic inflammation of the lungs (Jensen *et al.*, 1950).

The lung is an organ with distinct anatomical and physiological characteristics. In lower animals and plants diffusion takes place through cell membranes, but in higher animals diffusion of certain substances occurs only across the microscopic spaces between the blood and pulmonary alveoli (Kety, 1951). It seems that the lungs have for many

substances passing through the capillaries an absorptive capacity that might differ in certain species—for example, the affinity of lung tissue for Koch's bacillus. Bacteria, viruses, and insoluble particles (Markham and Florey, 1951) are known to accumulate in inflamed areas, and this phenomenon has been observed by a number of authors, particularly in inflammation of the lung. According to Aschoff (1926), monocytes accumulate during the process of phagocytosis in the lung capillaries and disappear from the blood circulation. Siegmund (1926) also reported many monocyte-fibrin thrombi in the lung veins, a phenomenon that obviously affects blood circulation and causes venostasis in the inflamed lungs. Accumulation of monocytes, disintegration of phagocytes, and venostasis in inflamed tissue together result in changes in the globulin-fibrinogen-albumin ratio. The degree of binding of drugs on tissue constituents (proteins, cells) will therefore vary, depending on the composition and activity of the tissue and also on the physico-chemical properties of the drug; moreover, the rates of gradual release from the bound condition will also vary correspondingly. It is known that in tissue exudates the total protein is over 3% (specific gravity over 1.018), but in some chronic lesions (tuberculosis) the protein content is only 0.1–0.5 g.%, in contrast with normal tissue transudates (protein content under 3% and specific gravity 1.006–1.015), which contain mainly albumin with only traces of fibrinogen. In exudates the proportions of globulin and fibrinogen are much increased. The lung being an organ with a wide net of capillaries, any inflammation there will cause a proportionately larger increase of the inflammatory exudate than in other organs, particularly in the early stages of inflammation, owing to an increase in the capillary permeability followed by mechanical obstruction of venous circulation (venostasis, lymphostasis, small venous thrombi) and to the pressure of the exudate. Any of the antibiotics circulating in the blood will diffuse into the inflamed tissue, will be taken up by the tissue constituents to a degree depending on their physico-chemical affinity, and will be slowly released as a result of venostasis. Probably different drugs will vary in extent of accumulation, depending on their molecular size and structure. In addition, the types of exudate, either acute or chronic, differing widely in their chemical composition and cell constituents will have a pronounced effect on the accumulation of the antibiotic.

Summary

Experiments on laboratory animals have shown that: (1) diethylaminoethylbenzyl-penicillinate hydriodide (estopen) is selectively concentrated in lung tissue, particularly when inflamed, the degree of accumulation being related to the extent of the lesions. (2) Selective accumulation is more pronounced with estopen than with procaine penicillin or the sodium salt of benzyl-penicillin. (3) The factors involved in this accumulation include the different chemical properties of the drug used and the specific feature of lung structure and function.

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