The higher concentration (0.025 per cent.) may be excessive.

As was to be expected, the delayed absorption of penicillin was accompanied by a reduction in the peak concentration obtained in the blood. It is uncertain whether it will be advantageous to treat all infections by a relatively constant blood level, though the work of many investigators has indicated that measurable levels should be maintained.

SUMMARY

Blood concentrations of penicillin were maintained at measurable levels for as long as seven or eight hours following single intramuscular injections of penicillin in the dog and in patients by means of vehicles containing 6 per cent. to 20 per cent. ossein gelatin and a long-acting vasoconstrictor drug.

Intramuscular administration of penicillin can thus be carried on with three instead of eight injections per day with less variation in the extremes of penicillin blood levels.

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ACID FORMATION FROM PENICILLIN DURING ENZYMATIC INACTIVATION[1]

A CELL-FREE enzyme solution, presumably penicillinase, prepared from an aerobic spore-forming bacterium, apparently *Bacillus subtilis,* which was isolated from a contaminated *Penicillium notatum* culture, inactivates penicillin with a concomitant evolution of CO_2 from bicarbonate solutions of the mixture. The same results were obtained according to unitage with two different penicillin preparations with a sixfold difference in purity. One was the pure crystalline sodium salt of penicillin. The reaction proceeds in the absence of oxygen. In Table 1 is summarized the data of a typical experiment with pure penicillin using the Warburg respirometers. The enzyme solution, which was the cell-free bacterial culture fluid, had 0.03 per cent. $NaHCO_3$ added and was equilibrated with a gas phase consisting of 5 per cent. CO_2 in N_2. Temperature = 30° C. Gas evolution ceased after one hour at which time a sample removed for assay from manometer number 2 showed no penicillin remaining.

Since the gas phase increase in CO_2 is accounted for almost quantitatively by a corresponding decrease in bound CO_2 the only interpretation apparent is an increase in the acidity of the solution as a result of inactivation of penicillin. The boiled enzyme controls had no action on the penicillin, and CO_2 evolution by fresh enzyme occurred only in the presence of penicillin.

In a similar enzyme experiment, Abraham and Chain[2] were unable to detect appearance of acidic groups by pH measurements with the hydrogen electrode. Their enzyme was obtained from *E. coli* but in other properties is similar to the one used here. It is believed their failure to observe the effect may be due to the low degree of purity of penicillin employed and possibly because the number of units inactivated may have been too small to give a measurable change.

TABLE 1

Manometer No.	1	2	3	4	5
Main chamber	3.0 cc enzyme soln.	3.0 cc enzyme soln.	3.0 cc enzyme soln.	3.0 cc enzyme soln.	3.0 cc boiled enzyme soln.
Left side cup	0.2 cc H_2O	0.2 cc H_2O	2,700 units penicillin in 0.2 cc H_2O	2,700 units penicillin in 0.2 cc H_2O	2,700 units penicillin in 0.2 cc H_2O
Right side cup	0.2 cc 5 per cent. H_2SO_4	0.2 cc 5 per cent. H_2SO_4	0.2 cc H_2O	0.2 cc 5 per cent. H_2SO_4	0.2 cc 5 per cent. H_2SO_4
CO_2 change in gas phase during 1 hr., cmm	....	−15	+27	+27	0
Bound CO_2, final, cmm	....	463	....	399	....
Bound CO_2, initial, cmm	428	448	....	....	....
	438 average				

Total gas phase change: $+27 + 15 = +42$ cmm CO_2

Total bound CO_2 change: $438 - 399 = -39$ cmm

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[1] Received for publication December 6, 1943, by the Committee on Medical Research, Office of Scientific Research and Development.

[2] E. P. Abraham and E. Chain, *Nature,* 146: 837, 1940.