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Fate and Distribution of Penicillin in the Body. II. Duration of Blood Concentration and Chemotherapeutic Effectiveness.*

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The original concept that the continuous presence of penicillin in the blood is essential for therapeutic effectiveness has been substantially weakened since the clinical reports of Tillett, McCormack, and Cambier.[1,2] These authors obtained excellent results in the treatment of lobar pneumonia on a regimen which gave detectable blood levels only intermittently. These findings were confirmed by the experimental studies of Zubrod,[3] White, Baker and Jackson[4] and Gibson.[5] The implications of such observations were discussed by Marshall[6] and Eagle.[7]

Our studies[8] on the distribution of penicillin in the organism required an analysis of the relationship between duration of therapeutic effectiveness and "blood level" as determined by *in vitro* methods.

Material and Methods. Albino rats of 60 to 220 g body weight were injected intramuscularly with a repository preparation consisting of a suspension of crystalline potassium penicillin G in peanut oil containing 1 mg/cc epinephrine.[9] At various intervals after injection the animals were etherized and bled from the subaxillary artery for the determination of the penicillin blood concentration by the method in current use.[10] At corresponding intervals other groups were infected. One cc of an 18 hour serum-broth culture of Streptococcus strain 4 (Group A, Type 3) and Pneumococcus Type 1 strain 6301 was inoculated intraperitoneally into the rats previously treated with the penicillin preparation. The inocula varied from 10^{-5} to 10^{-7} for the pneumococcus and from 10^{-4} to 10^{-6} for the streptococcus, corresponding to 100 - 1000 LD. Groups of 10 to 20 rats were used for control and for each alteration in the interval, or dosage, in any single experiment. Most of the untreated control animals died within 2 to 3 days. The rats which survived a three-week period were adjudged cured; occasionally the effect of treatment was verified by heart cultures of the animals sacrificed at the end of the observation period.

Results. (*a*) *Blood levels.* In our initial attempts to establish the duration of the penicillin levels, we encountered a number of

TABLE I.

Duration of Detectable Penicillin Levels in Rats Treated with Penicillin in Oil + 1 mg/cc Epinephrine.

Injection: 0.1 cc/100 g, intramuscular.

Rats of 80-150 g weight.

Dose/100 g	Hr duration in 50% rats	Hr duration in 90% rats
5 × 1000	13	14
10 × 1000	14	16
20 × 1000	16	18

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2 Tillett, W. S., McCormack, J. E., and Cambier, M. J., *J. Clin. Invest.*, 1945, **24**, 589.

3 Zubrod, C. G., *Bull. Johns Hopkins Hosp.*, 1947, **81**, 400.

4 White, H. J., Baker, M. J., and Jackson, E. R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 199.

5 Gibson, C. D., Jr., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 278.

6 Marshall, E. K., Jr., *Bull. Johns Hopkins Hosp.*, 1948, **82**, 403.

7 Eagle, H., *Ann. Int. Med.*, 1948, **28**, 260.

8 Schachter, R. J., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 29.

9 Ercoli, N., Hueper, W. C., Landis, L., Schwartz, B. S., and Queally, F. J., *J.A.M.A.*, 1948, **138**, 115.

10 Rammelkamp, C. H., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 95.

TABLE II.
Protection of Rats Against Pneumococcus Type I Infection at Various Intervals After Repository Penicillin Treatment.

Dose u/100 g‡	Hr after treatment	Blood negative for hr	Survivors after 6 days: Survivors infected	Survivors after 6 days: % survivors	Survivors after 21 days: Survivors infected	Survivors after 21 days: % survivors
Controls	—	—	26/134	19.4	14/134	10.4
10,000	14	*	8/ 10	80	8/ 10	80
	17	1	11/ 20	55	6/ 20	30
	20	4	8/ 20	40	4/ 20	20
	22	6	7/ 10	70	6/ 10	60
	24	8	14/ 25	56	7/ 25	28
	28	12	2/ 10	20	2/ 10	20
20,000	16	*	7/ 10	70	6/ 10	60
	18	†	20/ 30	66.6	16/ 30	53
	20	2	31/ 50	62	21/ 50	42
	25	7	6/ 13	46	2/ 13	15.4
	35	17	4/ 13	31	1/ 13	7.7

* 50% of rats negative.
† 90% " " "
‡ in 0.1 cc/100 g.

discrepancies. From experiments conducted on a large number of rats, it became apparent that the absolute weight of the animal —using the same dose/weight—influences the duration of blood levels. The larger animals (over 150 g) gave longer durations due to the greater absolute dose injected. In order to minimize variables, only experiments conducted on rats of 80-150 g weight are considered in this paper.

The duration of blood level is expressed in the most significant manner by indicating the end period of detectable penicillin in 50% and 90% of the animals. (Table I). The establishment of this effect in 100% of the animals is exposed to greater error, as is the case in other dose-response relationships.

(b) *Pneumococcus Type I Infection.* From the experiments presented in Table II, *it is evident that the therapeutic activity outlasts the duration of the detectable blood level.* For instance, rats infected 20 hours after treatment had no penicillin in their blood for the last 2 hours before infection, yet in 62% death was significantly delayed and 42% were cured. In general, a definite protective effect was given by the repository treatment 6-8 hours after the end of the penicillin blood level.

(c) *Hemolytic Streptococcus Infection.* Four experiments were carried out on a total of 250 rats. The same bacterial strain was used for *in vitro* as for *in vivo* experiments. The results are not as clear-cut as in the pneumococcal infection, due probably to the heavier inocula used in order to compensate for the less *regular* susceptibility of rats to streptococcal infection. However, in this infection also, chemotherapeutic effectiveness appears to be present for relatively long periods (4-8 hours) following non-detectable blood levels. In one experiment, for instance, out of 30 rats infected 2 to 6 hours after the disappearance of penicillin from the blood, 10 rats survived an observation period of 21 days. Seventy-five percent of a group of 20 controls died within 2 days; the entire group within 8 days.

Conclusion. It may be concluded that our present bacteriological methods cannot give the value of the *minimal* penicillin concentrations required for therapeutic activity. It is certain that this concentration in the heavy bacterial infection used is below 0.015–0.03 u/cc serum, which is the sensitivity of the bacteriological method.

Our present finding explains only in part the well-documented opinion of other authors that it is not necessary to maintain continuous penicillin blood levels for the whole period of treatment. A more important factor responsible for this is probably the *additive chemo-*

therapeutic effect of repeated doses. This effect is obvious from the experiments reported in the literature indicating that *fractions* of the curative dose, repeated at such intervals as to leave the organism without penicillin for the greatest part of the treatment period, result in cure. This is the case in spirochetal[11] as well as in bacterial[3] infections. The *additive effect* might depend on biological changes induced by sub-curative doses on the micro-organism, on reduction of the bacterial population, and on the immunological response of the host. The experiments of Kelly and Schnitzer[12] would suggest furthermore that the immunological response might influence the susceptibility of the micro-organism to chemotherapy.

The immunological response itself can be influenced by the treatment schedule, according to the recent observations of Kilbourne and Loge.[13] In hemolytic streptococcal pharyngitis, the antistreptolysin titer is higher with *intermittent* penicillin injections than it is with continuously sustained blood levels.

Another phenomenon which accounts for the independence of therapeutic effect duration from blood level is revealed in the experiments of Grunberg, Schnitzer, and Unger.[14] Penicillinase injected *after* the total disappearance of penicillin from the host tissue still blocks the therapeutic effect in the local streptococcal infection of mice. Thus, the presence of the penicillin in the micro-organism is more prolonged than in the host.

In our own experiments, part of the therapeutic effect existing beyond the blood level is due to penicillin accumulated in the tissues. How much of this prolonged therapeutic effect is dependent upon the undetectable amounts present in the blood, in comparison with the penicillin accumulated in the organs and tissue fluids, remains an open question.

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4-Amino Pteroylglutamic Acid (Aminopterin), Pteroylglutamic (Folic) Acid, and Response of Frogs, *Rana clamitans*, to Estrogens.*

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It has been shown that chicks and monkeys (*Macaccus rhesus*) maintained on a diet deficient in pteroylglutamic (folic) acid exhibit a decreased response to estrogenic substances.[1-3] It has been further demonstrated by Franklin, Stokstad, and Jukes[4] that folic acid deficiency in mice may be accelerated by a chemical antagonist of folic acid ("crude antagonist", Lederle). Hertz,[5] using the same antagonist, has shown that it interferes with the estrogen response of the chick genital

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