TABLE I.
Effect on Body Weight, Food Intake, and Liver Fat of Adding Methionine and Choline to a Diet Free of Sulfur-Containing Amino Acids.

Group	Special supplement (daily)	No. of rats	Weight of animals		Food intake,	Liver	
			Initial, g	Change, %	g/day	Wt, g	Total lipid, %
I	None	12	156 (150-164)	—32 ± 1.4	4.2 ± 0.18	4.7 ± 0.12	20.4 ± 1.6
II	50 mg Methionine	12	155 (150-165)	—26 ± 1.2	4.1 ± 0.13	4.3 ± 0.15	13.3 ± 1.3
III	100 " "	12	155 (150-160)	—27 ± 1.9	4.1 ± 0.17	4.2 ± 0.15	9.7 ± 0.75
IV	25 " Choline	12	154 (150-162)	—29 ± 1.1	4.0 ± 0.11	3.8 ± 0.12	6.8 ± 0.36

significant reduction of liver fat at the 50 mg level (average fat, 13.3%), with still lower values when 100 mg were given (average fat, 9.7%). This result, with the lower food intake and greater weight loss in this experiment, suggests that the 50 mg level of methionine was at the borderline of effective dosage, and in the present case, the higher ratio of methionine in the diet (1.2%) was sufficient to produce a lipotropic effect.

When choline was given it showed its usual efficient lipotropic action. The average liver fat content of the choline-fed group was 6.8%, an almost normal value. Methionine in the 100 mg dosage gave a 40% higher fat content of the liver. The total methyl in this amount of methionine is 25% greater than in the choline.

It may be noted that the weight of the livers paralleled the fat content consistently.

Summary. Rats fed an alipotropic diet in which a mixture of amino acids replaced protein developed fatty livers in spite of low food intake and emaciation. Methionine in adequate dosage exerted a lipotropic effect although there was still 10% of fat in the liver when the methionine constituted 2.4% of the diet. When 25 mg of choline chloride per day were fed the livers were essentially normal.

16252

Therapeutic Effectiveness of Single and Divided Doses of Penicillin in a Streptococcal Infection in Mice.

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In a preliminary study on penicillin dosage and its therapeutic effectiveness it was found that a single oral dose containing 10 mg of penicillin G per kg of body weight protected a large proportion of mice in an otherwise fatal experimental streptococcal infection.[1] The results of further studies on the effect of single doses of drug, administered orally or subcutaneously, are given in the present communication.

In addition, a comparison between the effect of a given amount of penicillin when administered as a single dose and as a series of equally spaced smaller doses has been included in this report. Such a comparison is of interest, since it is commonly assumed that the therapeutic effectiveness of penicillin in aqueous solution depends upon repeated

[1] White, H. J., Lee, M. E., and Alverson, C., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 35.

TABLE I.
Single Oral and Subcutaneous Doses of Penicillin G.
Treatment: Penicillin G sample containing 650 units per mg; administered as a single dose immediately after infection; in 0.5 cc vol. of aqueous sol. by stomach tube, or in 0.2 cc vol. subcutaneously.

Single dose* mg/kg	Oral Alive/Total†	Subcutaneous Alive/Total†
25.6	30/30	
12.8	28/30	
6.4	24/30	
3.2	23/30	30/30
1.6	5/30	23/30
0.8	0/30	18/30
0.4		7/30
0.2		1/30
0.1		0/30

	Oral mg/kg	Subcutaneous mg/kg	Oral:Subcutaneous dosage ratio
Median survival dose (19/20 limits of error)‡	2.9 (3.8-2.2)	0.78 (0.99-0.62)	3.7 (5.4-2.5)

* Dose expressed as pure penicillin G containing 1667 units per mg.
† Survival on 21st day after infection, expressed as number of mice alive out of total number tested.
‡ Interpreted as meaning that the chances are 19 to 1 that the true values fall within the indicated limits.

injections at 2- or 3-hour intervals.[2]

Methods. Our experimental infection was produced by intraperitoneal inoculation of 0.5 cc of a 10^{-5} broth dilution of a 6-hour blood broth culture of *Hemolytic streptococcus,* Group A, strain C 203, in Vanderwerken white mice of 18-22 g weight. The number of organisms in the infecting dose averaged 4000 ± 1000; virulence titrations indicated that a single organism (or chain), as determined by colony count, constituted a lethal dose. In the present studies, 80 of 80 untreated control animals died within 48 hours after infection. Treated animals alive on the 21st day after infection are considered to be cured.[3]

Tests with penicillin G were carried out with the 650 unit per mg sample previously used;[1] crystalline penicillin X was kindly supplied by E. F. Williams of the Physics Division of these Laboratories. In each case, the penicillin was dissolved in sterile distilled water and the solutions were used without filtration.

Results. The results of single dose treatment with penicillin G are given in Table I. By use of a simplified method for the quantitative evaluation of dosage-response data, devised by Wilcoxon and Litchfield[4] and illustrated in Fig. 1, the Median Survival Dose (SD_{50}) for subcutaneous administration is estimated to be 0.78 mg per kg, with 19/20 limits of error of 0.99-0.62. The corresponding values for oral administration are 2.9 and 3.8-2.2 mg per kg. The ratio of oral to subcutaneous dosage for penicillin G is thus 3.7, with 19/20 limits of error of 5.4-2.5.

In Table II, results with single doses of penicillin X are given. In this case, it is evident that subcutaneous is about 8 times as effective, therapeutically, as oral administration.

The question of single, as against divided, dosage was investigated by comparing the therapeutic effectiveness of an amount of penicillin given according to each of 4 dosage schedules, as follows: (1) total amount as single dose immediately after infection (0 hr);

[2] Dowling, H. F., Rotman-Kavka, G., Hussey, H., and Hirsh, H. L., *Am. J. Med. Sci.,* 1947, **213**, 413.

[3] Litchfield, J. T., Jr., White, H. J., and Marshall, E. K., Jr., *J. Pharm. and Exp. Therap.,* 1939, **67**, 437.

[4] Wilcoxon, F., and Litchfield, J. T., Jr., to be published.

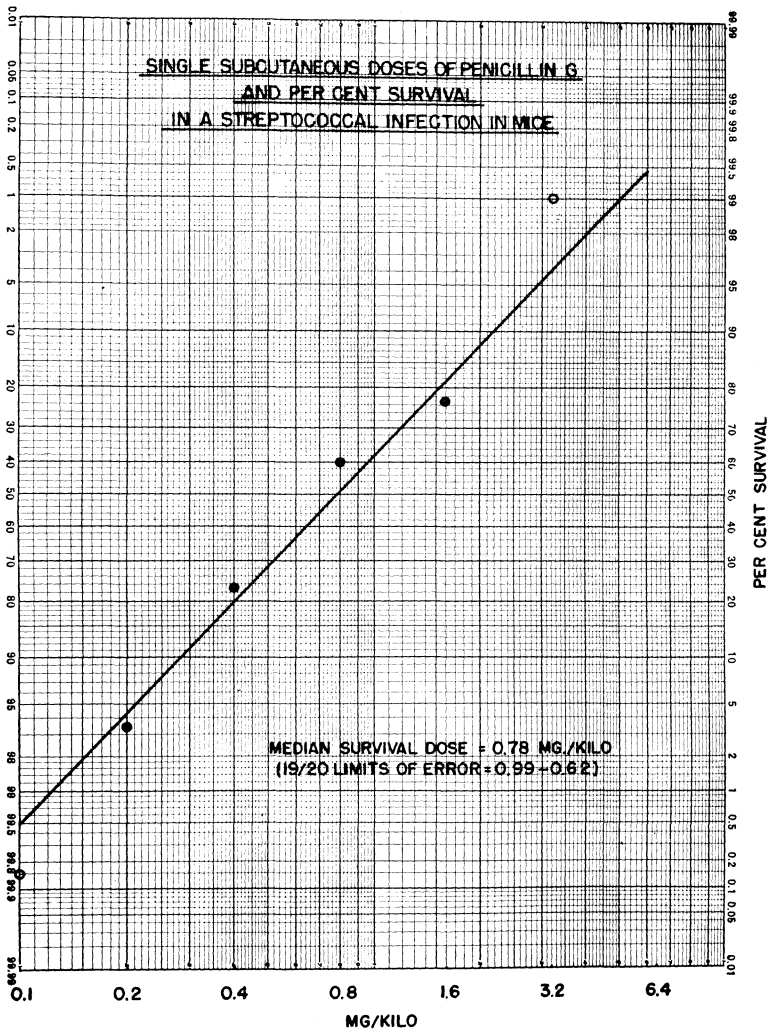

FIG. 1.

Single subcutaneous doses of penicillin G plotted against % survival on logarithmic probability paper.

TABLE II.

Single Oral and Subcutaneous Doses of Penicillin X.

Treatment: Crystalline penicillin X; administered as single dose immediately after infection; in 0.5 cc vol. of aqueous sol. by stomach tube, or in 0.2 cc vol. subcut.

Single dose mg/kg	Orally Alive/Total*	Subcutaneously Alive/Total*
16.0	10/10	10/10
8.0	9/10	10/10
4.0	8/10	10/10
2.0	4/10	10/10
1.0	1/10	9/10
0.5	0/10	8/10

* Survival on 21st day after infection, expressed as number of mice alive out of total number tested.

(2) ½ of total dose at 0 hour, the other ½, 12 hours later; (3) ¼ of total dose at 0 hour and ¼ each at 6, 12, and 18 hours later; (4) ⅛ of total dose at 0 hour and ⅛ each at 3, 6, 9, 12, 15, 18, and 21 hours later. These 4 schedules were used for each of 5 different total amounts of penicillin X, in one experiment, with 20 mice on each schedule for each amount, making a total of 20 dosage groups, each of 20 mice. Two separate experiments of this kind confirmed each other and the pooled results are given in Table III.

It is evident that no advantage was gained by splitting any amount of penicillin X into

TABLE III.
Single and Divided Doses of Penicillin X.
Treatment: Crystalline penicillin X; 0.2 cc vol. of aqueous sol. administered subcut. on dosage schedules indicated below; each dosage schedule began immediately after infection and was terminated within 24 hours.

Amt of each dose mg/kg	Number of doses: One (At time of infection)	Two (At 12-hour intervals)	Four (At 6-hour intervals)	Eight (At 3-hour intervals)
1.0	95*	....	....	....
1/2	98	98	....	....
1/4	58	95	98	....
1/8	48	73	98	100
1/16	25	48	78	100
1/32	....	13	18	35
1/64	....	....	3	8
1/128	....	....	....	8

* Percentage survival on 21st day after infection; 40 mice were used on each of the 20 dosage schedules.

TABLE IV.
Median Survival Doses for Penicillin X Administered by 4 Different Schedules.

Daily dosage schedule (Start: Immediately after infection)		Median survival dosage* mg/kg		
Frequency	No. of doses	Each dose	Total dose	19/20 Limits of error†
Once	1	.13	.13	.43-.04
q. 12 hr	2	.07	.14	.18-.11
q. 6 hr	4	.04	.16	.22-.15
q. 3 hr	8	.03	.24	.32-.21

* These values were obtained from dosage-response lines the slopes of which did not differ from each other significantly.
† The chances are 19 to 1 that the true median survival doses lie within these limits.

several small doses. For example, 8 doses of 1/8 mg per kg each were no more effective than a single dose of 1 mg per kg; and 8 doses of 1/64 mg per kg were not as effective as a single dose of 1/8 mg per kg.

As one would expect, 8 doses of 1/8 mg per kg each were more effective than 1 dose of 1/8 mg per kg; and, similarly, 8 doses of 1/16 each were more effective than a single dose of 1/16 mg per kg. However, multiple doses of 1/2 mg per kg could not improve on a single dose of 1/2 mg per kg since the maximum effect was obtained with the latter dose.

A quantitative comparison of the different dosage schedules may be based upon the Median Survival Dose for each schedule, as calculated from the dosage-response data in Table III. These values, together with their limits of error, are given in Table IV.

Discussion. The greater efficiency of subcutaneous over oral administration of penicillin, as measured by the relative amount of drug needed to produce the same degree of therapeutic effect (Tables I and II) confirms the well-known fact that about 5 times as much oral as subcutaneous dosage is needed to maintain a given concentration of the drug in the blood.[5,6] The choice between oral and parenteral dosage for clinical use would thus appear to depend upon such factors as relative cost and convenience to the patient.

The results obtained on single versus divided dosage (Tables III and IV) indicate that the total amount of penicillin X for a single day of treatment could be given in 1 or 2 doses, instead of 4 or 8 doses, without significant loss of protection. This is in agree-

[5] McDermott, W., Bunn, P. A., Benoit, M., DuBois, R., and Reynolds, M. E., *J. Clin. Invest.*, 1946, **25**, 190.

[6] Hoffman, W. S., and Volini, I. F., *Am. J. Med. Sci.*, 1947, **213**, 513.

ment with the early work of MacLeod and Stone[7] who showed that mice infected with strains of pneumococcal types I, II, or III could be effectively treated with penicillin when the total daily dose (for each of 4 days) was restricted to only 3 injections within an 8-hour period. It is of interest to note, also, that Tillett, McCormack, and Cambier[8] have concluded that in the treatment of pneumococcal pneumonia with penicillin, repetition of injections continuously throughout each 24-hour period was in many cases not necessary or advantageous. Further experimental evidence indicating that there is no advantage in splitting daily penicillin dosage into a series of small frequently repeated doses has recently been reported by Zubrod[9] who investigated penicillins G and K in mice under conditions similar to those of the present report.

In the present investigation, the comparison of single and divided dosage schedules has been confined to a maximum treatment period of only 24 hours. That different results may be obtained under other experimental conditions, is indicated by the data of MacLeod and Stone.[7] For example, these workers found that treatment with penicillin for a 4-day period could be much more effective than giving an equivalent amount of drug for a single day. In mice infected with a type I pneumococcal strain, recovery of only 74% could be obtained with a total of 600 Oxford units when treatment was restricted to a single day, whereas, recovery of 100% resulted from treatment with a total of only 60 units divided into 4 daily amounts of 15 units each. With type II pneumococcus, about the same degree of recovery (60 to 70%) was produced by a total of 300 units, regardless of whether it was administered in a single day or divided into 4 daily amounts of 75 units each.

Conclusion. From 4 to 8 times as much penicillin was needed for oral, as for subcutaneous administration, to obtain the same degree of therapeutic effect in a streptococcal infection in mice.

In this infection, the administration of daily penicillin dosage in a series of small equally spaced doses was not more effective than giving an equivalent amount of drug in 1 or 2 doses per day.

7 MacLeod, C. M., and Stone, E. R., *Bull. N. Y. Acad. Med.*, 1945, **21**, 375.

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

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

16253

Effect of Passive Sensitization and Anaphylactic Shock on Rabbit Bone Marrow.*

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Concepts relating the plasma cell to hyperglobulinemia, globulin formation, immune mechanisms, hypersensitivity, and anaphylactic shock have a firm basis in both clinical and experimental literature.[1-10] Beginning with

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1 Maciesca-Jelenska, S., *Muench. Med. Wchnschr.*, 1907, **54**, 1790.

2 Downey, H., *Folia haematol.*, 1911, **11**, 275.

3 Huebschmann, P., *Verhandl. d. deutsch. path. Gesellsch.*, 1913, **16**, 110.

4 Klein, *Z. f. d. ges. Neurol. u. Psychiat.*, 1914, **21**, 242.

5 Oeller, H., *Deutsche. Med. Wchnschr.*, 1923, **49**, 1287.

6 Siegmund, H., *Verhandl. d. deutsch. path. Gesellsch.*, 1923, **19**, 114.

7 Epstein, E., *Virch. Arch. f. path. Anat.*, 1929, **273**, 89.

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