TABLE I.
Diameters of Cleared Areas Produced by a Standard of Mixtures of Streptothricin and Streptomycin with Water as the Diluent.

Concentration of antibiotic agents	Zonation
1000 units streptomycin per ml only	0 mm
" " " " " plus 20 units streptothricin per ml	18.0 mm
" " " " " " 10 " " " "	16.5 "
" " " " " " 8 " " " "	15.5 "
" " " " " " 4 " " " "	13.5 "
" " " " " " 2 " " " "	12.0 "
" " " " " " 1 " " " "	8.5 "

of streptomycin per ml. However, concentrations of over 1000 units per ml produced moderate zones, for example 7.8 mm for 5000 units and 10.5 mm for 10,000 units per ml.

This culture has been maintained by daily transfer in this laboratory for a period of 8 weeks without showing any indications of changing in its response to streptomycin and streptothricin.

With this test one unit of streptothricin can be measured in the presence of 1000 units of streptomycin. Therefore it would be possible to detect as little as 0.1% of streptothricin if it were present as a contaminant in lots of streptomycin.

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Biological Conversion of n-Butyl Penicillin into a Chemotherapeutically Active Substance.

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Due to its rapid rate of absorption and excretion, it is impossible to maintain satisfactory blood levels of penicillin for prolonged periods after parenteral injection of an aqueous solution of penicillin. These characteristics necessitate either a frequent intermittent or a continuous type of injection. Attempts have been made to prolong the blood levels after parenteral injection of salts of penicillin by decreasing either their rate of absorption[1-4] or their rate of excretion.[5,6]

Since the rapid rate of elimination of penicillin following its parenteral injection is due in part to its extreme solubility in aqueous phases, it would appear advantageous to modify the penicillin molecule in such a manner as to form water insoluble derivatives. This goal has been accomplished by the production of various esters of penicillin. Meyer and his co-workers have reported the preparation of a number of such esters including the methyl, ethyl, n-butyl and benzhydryl esters.[7] All of these compounds were de-

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1 Fisk, R. T., Foord, A. C., and Alles, G., *Science*, 1945, **101**, 124.

2 Parkins, W. M., Wiley, M., Chandy, J., and Zintel, H. A., *Science*, 1945, **101**, 203.

3 Romansky, M. J., and Rittman, G. E., *Bull. U. S. Army Med. Dept.*, 1944, **81**, 43.

4 Trumper, M., and Hutter, A. M., *Science*, 1944, **100**, 432.

5 Beyer, K. R., Peters, L., Woodward, R., and Verway, W. F., *J. Pharmacol.*, 1944, **82**, 310.

6 Rammelkamp, C. H., and Bradley, S. E., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 30.

7 Meyer, K., Hobby, G. L., and Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 100.

TABLE I.
The Protective Action of Subcutaneous Injections of n-Butyl Penicillin Against Streptococcal Infections in the Mouse.

Wt of Ester injected, mg	Dilution of culture inj.	No. of mice inj.	No. of mice survived	No. of mice died
0	10^{-6}	3	1	2
	10^{-5}	3	0	3
	10^{-4}	3	0	3
0.1	10^{-6}	3	2	1
	10^{-5}	3	2	1
	10^{-4}	3	1	2
0.5	10^{-6}	3	3	0
	10^{-5}	3	3	0
	10^{-4}	3	3	0

Note. Doses of 1.0 mg and 5.0 also gave complete protection.

scribed as insoluble in neutral or slightly alkaline buffers but were quite soluble in fat solvents. They were almost devoid of any activity *in vitro* but apparently could be hydrolyzed in alkaline solution to regenerate an active principle, presumably the non-esterified penicillin.[8]

In contrast to their relative inactivity *in vitro,* the ethyl and n-butyl esters were demonstrated to be effective chemotherapeutic agents in mice infected with large doses of virulent hemolytic streptococci.[7] Thus, single doses of these esters, given either orally or subcutaneously, protected mice against intraperitoneal injections of streptococci. The protective action of methyl penicillin in mice infected with spirochetes has also been demonstrated.[9] It appeared, therefore, that these esters were hydrolyzed *in vivo* with the slow liberation of active penicillin. Such results suggested the possibility of the successful use of penicillin esters as chemotherapeutic agents in man.

More recently, following the preparation of the benzyl ester of penicillin,[10] reference was made to unpublished results[11] of the successful use of this ester in clinical infections and high promise was held forth for the effectiveness of penicillin esters against infections in man. In contrast to these results, previous work in our own laboratory had indicated that higher mammals were unable to effect the conversion of alkyl penicillin esters into chemotherapeutically active substances. Accordingly, we report a few preliminary experiments obtained with the n-butyl ester in the dog, monkey and man.

Methods and Results. Four of the butyl penicillin samples were dark brown viscid oils; one was a yellowish powder. Their *in vitro* activity was tested by dissolving a weighed amount in absolute alcohol and diluting further with broth. Their activity was then assayed against the C203 MV strain of hemolytic streptococcus and all samples showed an activity of 5 to 10 units per mg as opposed to 600 to 1100 units per mg of the original free penicillin from which they had been prepared.

In work with dogs and monkeys, weighed amounts of the ester were dissolved in absolute alcohol and diluted with 4 parts of propylene glycol. The alcohol-glycol solutions were then injected without further sterilization. Preliminary to injection into man, the ester was dissolved in the alcohol-glycol mixture as described above and sterilized by filtration through a small sterile sintered glass filter. The filter was washed with small amounts of absolute alcohol until the washings showed no traces of color. The solution was concentrated in the cold under high vacuum until alcohol was no longer detectable. The residue was then assumed to contain the total amount of ester originally

[8] Hickey, R. J., *Science,* 1945, **101**, 462.

[9] Richardson, A. P., Walker, H. A., Loeb, P., and Miller, I., *J. Pharm.*, 1945, **85**, 23.

[10] Cavallito, C. J., Kirchner, F. K., Miller, L. C., Bailey, J. H., Klimek, J. W., Warner, W. F., Sutler, C. M., and Tainter, M. T., *Science,* 1945, **102**, 150.

[11] Gamble, T. O., Miller, L. C., and Tainter, M. L., *Am. J. Obst. and Gynec.,* in press.

TABLE II.
Recovery of Free Penicillin from the Urine and Blood after Subcutaneous Injection of n-Butyl Penicillin.

Animal	Wt of Ester inj. mg	Duration of urine collection after inj. of Ester, hr	Total free penicillin recovered in urine units	Serum penicillin levels hr after inj. 1	2	3	4	8	12	24
Dog	100	48	22	0	*	0	*	0	*	0
Dog	200	48	31	*	0	*	0	*	0	*
Monkey	100	36	4	*	*	*	*	*	*	*
Monkey	200	48	17	*	*	*	*	*	*	*
Man	200	48	14	0	0	0	0	0	0	0
Man	200	48	46	0	0	0	0	0	0	0

* Indicates no determination made.

weighed out and was used, in appropriate quantities, for parenteral injection into man.

Penicillin assays on blood samples and urine specimens were performed by a serial dilution technic. Since it was believed the urine assays offer a more reliable index of the total absorption of penicillin,[12] such assays were used to indicate the degree of conversion of the ester in the body.

We were able to confirm the results of Meyer, Hobby and Dawson[7] in regard to the chemotherapeutic activity of n-butyl penicillin in mice. For this purpose, mice weighing approximately 20 g were given intraperitoneal injections of 0.5 ml of a 10^{-4}, 10^{-5} and 10^{-6} dilution of a 15 hour culture of the virulent C203 MV strain of the Group A *Streptococcus*. One-half hour later, the treated animals each received a single subcutaneous injection of the propylene glycol solution of the ester. The results given in Table I indicate that a single injection of 0.5 mg of ester protected all of the mice against 0.5 ml of a 10^{-4} dilution of the culture. Only moderate protection was afforded by 0.1 mg of ester.

No studies on the chemotherapeutic activity of the butyl penicillin were performed on the dog or the monkey, but these animals were used to investigate the pharmacology of this compound and to determine their reaction to large doses subcutaneously.

Two dogs weighing approximately 5 and 7 kg were given subcutaneous injections of 100 mg and 200 mg, respectively, of the ester. The injections apparently produced a transient moderate local irritation. Observations of both dogs failed to reveal any evidences of a systemic reaction to the injections. Blood and urine assays for free penicillin were performed and the results in Table II indicate failure to produce demonstrable blood levels or more than minute quantities of excreted penicillin.

Similarly, 2 monkeys *(M. mulatta)* weighing approximately 4 kg received 100 and 200 mg each of the butyl penicillin subcutaneously. As was the case in the dogs, the monkeys failed to evidence any reactions to the injections other than a transient mild local irritation. Blood assays were not performed. However, as in the dogs, composite urine samples did not reveal appreciable excretion of free penicillin (Table II).

Inasmuch as the dogs and monkeys appeared to tolerate the injections of penicillin ester, it was considered safe to determine their effect in man. In order to investigate chemotherapeutic activity of the ester, male patients with acute gonorrheal urethritis were chosen since this disease is so sensitive to the activity of penicillin. Two such patients were each given subcutaneous injections of 100 mg of the ester followed 6 hours later by another similar injection. None of the injections produced immediately more than an extremely mild irritation. The following day, however, the site of the injections was swollen, hot and very tender. In addition, there was a moderate febrile reaction accompanied by some malaise. Both the systemic and local reactions had disappeared 48 hours later. Due to the impurity of the preparation, it was difficult to assess the

[12] Pedrick, R. F., and Broh-Kahn, R. H., *AAF School of Aviation Medicine Res. Rep.*, 1945, 388-1.

role of the solvent or of the impurities in this moderately toxic reaction. Urine and blood assays were performed and the effect on the disease determined. The results of the assays (Table II) again revealed little or no free penicillin. Clinically, the urethral discharges showed no improvement and smears and cultures continued to reveal the presence of viable gonococci. These observations were continued for 3 days after the injections. If the greater part of the ester had been hydrolyzed, the quantity of free penicillin so liberated might have been expected to produce a demonstrable improvement in the clinical condition. At the end of this period, both patients were treated with 500,000 units of oral penicillin[13] and their disease responded satisfactorily, thus affording evidence that the infecting strains were not or had not become refractory to penicillin. In order to determine whether the method of preparation of the solution of ester for injection into man had resulted in its inactivation, the same solution was later demonstrated to protect mice against the intraperitoneal injections of 0.5 ml of a 10^{-4} dilution of a 15 hour culture of a virulent hemolytic streptococcus.

Discussion. There would appear to be little doubt that subcutaneous injections of butyl penicillin protect mice against infections by hemolytic streptococci. This protection obviously cannot be attributed to any impurity of unchanged penicillin since 1 mg of the ester contains a maximum of 10 units of activity. Inasmuch as 0.5 mg is sufficient to protect mice, the protective dose contains a maximum of 5 units of free penicillin. Approximately 200 units of free penicillin are required for protection of the mouse against lethal doses of hemolytic streptococci.[14,15]

The possibility exists either that the esters *per se* exert a chemotherapeutic action or that they, after injection, are converted in the body into active substances. It appears doubtful that the esters themselves are the actual chemotherapeutic substances since they failed to display any activity against human gonococcal infections and since they are almost devoid of activity *in vitro*. Accordingly, it appears most plausible to assume that, subsequent to absorption, the esters are converted into the active agent. The most probable conversion would undoubtedly be a regeneration of free penicillin. *In vitro* studies[16] have already demonstrated that this reaction occurs. The most reasonable explanation for the mechanism of the activity of the ester in mice would be its constant and somewhat slow conversion, in the body, to free penicillin. If this conversion occurred at an appropriate rate, adequate levels of free penicillin might be maintained in the blood for prolonged periods following a single injection of ester. Once the hydrolysis had occurred the resulting penicillin would be expected to be excreted in the urine at its normal rate.

On the other hand, following subcutaneous injections of the ester in the dog, the monkey and in man, the observations of this study indicate the inability to recover appreciable quantities of penicillin in either the blood or the urine. It would seem, therefore, that the tissues of these mammals are unable to effect the conversion of the ester into free penicillin at an appreciable rate.

In general, the velocity of hydrolysis of aliphatic esters by tissues varies inversely with the molecular weight of the esterifying alcohol.[17] It was therefore believed that efforts should be directed towards investigation of simpler esters. However, Richardson *et al.*[18] report results with both the methyl and benzyl esters of penicillin comparable to our own findings with the n-butyl ester. In view of the fact that the benzyl radical behaves as an aliphatic derivative, it would have been somewhat surprising to learn that tissues unable to hydrolyze methyl and butyl penicillin could effect the hydrolysis of the

[13] Broh-Kahn, R. H., *AAF School of Aviation Medicine Res. Rep.*, 1945, 404-1.

[14] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 285.

[15] Robinson, H. J., *J. Pharm.*, 1943, **77**, 70.

[16] Broh-Kahn, R. H., and Smith, P. K., unpublished observations.

[17] Hass, G. M., *Arch. Path.*, 1938, **26**, 1183.

[18] Richardson, A. P., Walker, H. A., Miller, I., and Hansen, R., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 272.

higher benzyl homologue.

Summary and Conclusions. 1. A subcutaneous injection of butyl penicillin conferred marked protection against hemolytic streptococcal infections in the mouse. 2. Subcutaneous injections of 200 mg exerted no appreciable effect on gonococcal infections in 2 men. 3. Following subcutaneous injections of butyl penicillin into the dog, the monkey, and man, appreciable quantities of penicillin were not recovered in the blood or urine. 4. The subcutaneous injection of from 100 to 200 mg of butyl penicillin produced no evidences of marked toxicity in the dog or the monkey. Two men reacted with evidences of moderate toxicity. 5. The tissues of the mouse can convert the chemotherapeutically inactive butyl penicillin into a chemotherapeutically active substance, presumably penicillin. The tissues of man, the monkey, and the dog cannot effect this conversion at an appreciable rate.

Dr. Karl Meyer kindly furnished us with the butyl penicillin used in this study and supplied the data concerning the *in vitro* activity against the streptococcus.

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Influence of Iron Salts on the Toxicity of Lead.

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The relationship between the toxicity of lead and the dietary level of other elements has been studied for many years. The extensive literature has been reviewed by Cantarow and Trumper.[1] Calcium and phosphate have been especially implicated in modifying the effects of oral administration of lead salts. The influence of iron compounds on the development of lead poisoning has received little study. Our attention was directed to this problem during the course of work on blood formation in various toxic states. In the present study it was found that iron salts prevented the weight depression and anemia in rats fed diets containing lead acetate.

Experimental. Albino rats of Wistar and Osborne and Mendel strains were fed the experimental diets at weaning. Feeding was *ad libitum* except in Exp. 2. The animals were weighed at intervals of 4 to 7 days. Tail blood was used for micro-determinations of the hematocrit (Van Allen) and for hemoglobin determinations by the oxyhemoglobin method of Sanford *et al.*[2] Blood smears were fixed in absolute methanol and stained for 1½ hours with dilute giemsa solution buffered to pH 7.2. The total number of basophilic red cells per 1000 red cells were counted with the aid of a Whipple disc.

Effects of ferric citrate and ferrous sulfate. In a preliminary study with semipurified diets it was found that supplements of ferric citrate and copper sulfate protected against the depression in weight gain and anemia of lead poisoning. Subsequent tests showed that ferric citrate alone was equally effective.

Table I shows the results of 2 experiments in which male weanling rats were used.*

[1] Cantarow, A., and Trumper, M., *Lead Poisoning,* The Williams and Wilkins Co., Baltimore, 1944.

[2] Sanford, A. H., Sheard, C., and Osterberg, A. E., *Am. J. Clin. Path.,* 1933, **3**, 405.

* The percentage composition of the basal diet for these and all remaining experiments was as follows: purified casein (Smaco) 30.0, hydrogenated cottonseed oil (Crisco) 10.0, U.S.P. cod liver oil 5.0, choline chloride 0.2, salt mixture 1.9, cane sugar 52.9. In addition, the vitamin supplement (in mg per kilo) was thiamine hydrochloride 10, nicotinic acid 40, pyridoxine hydrochloride 10, calcium pantothenate 40, and riboflavin 20. Each rat received a weekly oral supplement of 3 mg of