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

TABLE I.
Effect of Ferric Citrate on the Toxicity of Lead.

	Added to 100 g diet				
	Lead* g	Ferric-citrate g	No. of rats	Mean wt† g	Mean hemoglobin‡ g/100 cc
Exp. 1	—	—	12	159 (117-196)	15.1
	0.03*	—	8	106 (85-144)	9.9
	0.03*	1.13	8	148 (111-170)	16.0
Exp. 2	—	—	10	149 (123-187)	16.6
	0.03*	—	10	126 (85-156)	12.1
	0.03*	1.13	10	141 (115-180)	14.8
	0.03*	1.47	10	142 (112-172)	15.5

* After 31 days on test, the lead content of the diets was increased to 0.09%.
† After 27 days in Experiment 1 and 56 days in Experiment 2.
‡ Determinations done after 66 days in Experiment 1 and after 75 days in Experiment 2.

TABLE II.
Effect of Ferric Citrate and Ferrous Sulfate on the Toxicity of Lead.

Added to 100 g basal diet	Mean wt after 51 days g	Mean hemoglobin after 48 days g/100 cc
(1)	213.4	15.8
(2) 0.09 g Pb (as Lead Acetate)	81.5	10.1
(3) 0.09 g Pb + 1.13 g Fe Citrate	168.0	15.3
(4) 0.09 g Pb + 0.25 g Fe Citrate (0.84 mmols)	130.4	12.9
(5) 0.09 g Pb + 0.2 g $FeSO_4$ (1.32 mmols)	133.3	15.5

In Exp. 1, the differences between the mean weights for the rats on basal diet with lead and the other two groups were examined by Fischer's *t*-technic[4] and found to be significant with a P value below 0.01. With the pair-feeding technic (Exp. 2) differences in weight gains were small enough to be disregarded. This has been observed before among lead poisoned rats.[5] But a statistically significant anemia, preventable by generous additions of ferric citrate, was obtained.

In Exp. 3, (Table II), 50 male weanling rats were divided into 5 groups equal with respect to average weight and litter distribution. It was found that an addition of 0.25 g of ferric citrate was less effective than 1.13 g in opposing the toxicity of lead. Ferrous sulfate also had some protective action. The differences in average weight gains and hemoglobin concentrations between the group given basal diet containing lead and all of the other groups were analyzed statistically and found to be significant.

Ineffectiveness of sodium citrate. Experiments were carried out to determine whether

α-tocopherol in 0.03 cc ethyl laurate. The salt mixture (in g) was composed of: NaCl, 138; $CaCO_3$, 200; $MgCO_3$, 50; KH_2PO_4, 424; $MgSO_4 \cdot 7H_2O$, 65.6; KCl, 224; $MnSO_4 \cdot H_2O$, 0.784; NaF, 2.0; KI, 0.16; $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$, 0.622; $CuSO_4 \cdot 5H_2O$, 11.95; Ferric Citrate, 16.55. This salt mixture was designed to make a low calcium-high phosphorus ratio in the completed diet. According to the work of Grant *et al.*[3] such a diet favors the deposition of lead in bone, liver and kidney. Samples of the ferric citrate used were analyzed and found to contain 18.1% of iron.

[3] Grant, R. L., Calvery, H. O., Laug, E. P., and Morris, H. J., *J. Pharm. and Exp. Therap.*, 1938, **64**, 446.

[4] Fischer, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1936.

[5] Baernstein, H. D., and Grand, J. A., *J. Pharm. and Exp. Therap.*, 1942, **74**, 18.

TABLE III. Effect of Ferric Citrate and Sodium Citrate on Depression of Growth Rate, Anemia and Red Cell Polychromasia of Lead Poisoning.*

Group	Added to 100 g, basal diet: Lead g	Ferric citrate g	Sodium citrate g	Avg wt g, Days on diet: 0	25	51	65	Hemoglobin g/100 cc, Days on diet: 26	61	Hematocrit %, Days on diet: 26	61	Polychromatic red cells per 1000r.b.c. after 62 days, avg no. and range
A	—	—	—	43.2	137.3	230.3	239.4	14.2	16.1	45	49	3.8 (2-6)
B	0.09	—	—	40.9	89.7	128.2	143.2	13.1	10.5	44	39	49.0 (12-151)
C	0.09	1.13	—	39.0	127.1	192.3	203.1	14.7	16.4	45	51	8.2 (0.1-23)
D	0.09	—	1.15	41.3	92.2	128.6	137.1	12.5	10.9	39	40	62.6 (16-180)

Significance of Difference Between Means†

	Groups compared (first term greater than second term): A-B	A-C	C-A	A-D	C-B	B-D	D-B	C-D
Weight gain, 51 days	P<0.001	P<0.02	———	P<0.001	P<0.001	P>0.3	———	P<0.001
Hemoglobin, 61 days	P<0.001	———	P>0.5	P<0.001	P<0.001	———	P>0.6	P<0.001

* Each group contained 10 rats. One rat in group A died after 24 days on experiment and one rat in group B died after 57 days on experiment.

† These P values indicate that there was no statistical significance between the weight gains or hemoglobin values of the rats receiving lead alone and those of rats getting sodium citrate added to the lead salt. The iron treated rats had hemoglobin levels not significantly different from those on basal diet. All other differences between groups were significant. Weight gains for a period of 51 days were analyzed because this covered the phase of most rapid growth for the control rats.

the citrate radicle in ferric citrate had any protective effects. It was first ascertained that sodium citrate could be fed to weanling rats at a level of 1.15 g added to 100 g of diet without depressing the rate of growth. This was equivalent to 1.13 g of ferric citrate.

In a preliminary experiment it was found that sodium citrate did not protect against the growth depressant effect of lead. Young rats were fed the basal diet, the leaded basal diet or the leaded basal diet with 1.15 g of sodium citrate added to every 100 g of diet. In one test 5 rats were placed in each group and in a second test there were 6 rats in each group. In both tests it was found that after 30 days the mean weight of the sodium citrate treated rats was approximately 10 g less than that of the rats receiving only lead.

In the next experiment 40 male weanling rats of the Wistar strain were divided into 4 groups equal with respect to litter distribution. The diets and experimental results are shown in Table III.

Discussion. In these experiments iron salts interfered in some way with the toxicity of lead. This was deduced from the fact that the depression of growth rate, anemia and red cell polychromasia, were reduced or absent in rats given supplements of ferric citrate or ferrous sulfate.

The anemia of the leaded rats, although prevented by supplements of ferric citrate, was not related to an inadequate iron content of the basal diet. The rats on the unleaded basal diet did not become anemic even when pair fed against those receiving lead.

It may be of interest to compare the content of ferric citrate in our protective diets with that obtained by one of the better known salt mixtures as ordinarily used. The McCollum-Davis salt mixture 185,[6] when fed at a 5% level, contributes 0.16% of ferric citrate to the diet. In the present study a supplement of approximately 0.25% of ferric citrate, did not bring about normal hemoglobin concentrations in leaded animals, although it had some beneficial effect. A level of 1.13% prevented the anemia entirely.

It is of interest that Miyasaki[7] found that a colloidal solution of 2% dialyzed ferric oxide reduced the absorption of lead in mice although a suspension of ferric hydroxide was ineffective. The mechanism of action of iron salts in counteracting the toxicity of orally administered lead in these studies has not been established. However, experiments in progress indicate a much lower lead content of tissues of rats whose diet contained supplements of iron citrate. A possible explanation for these findings would be an interference by iron citrate with the absorption of lead. Further studies including the use of radioactive lead (radium D) for tracing the absorption of lead in the presence of iron compounds are planned. The possibility of an interference with the absorption of iron by lead also merits investigation.

Summary. Ferric citrate as 1.12% of the diet protected young rats against the weight loss, anemia and red cell polychromasia of lead poisoning. One-fourth of this amount was less effective. About 0.2% of ferrous sulfate had a protective action. Sodium citrate was ineffective.

[6] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1918, **33**, 55.

[7] Miyasaki, S., *Arch. Exp. Path. u. Pharmakol.*, 1930, **150** (1/2), 39.

Miss Virginia T. Porterfield, Mrs. Evelyn G. Peake and Miss D. Louise Odor assisted with the experiments and helped to perform the statistical analyses.