Methionine was also studied because it is important as a methyl donator, and because it contains sulphur. Unfortunately, the solubility of methionine is limited to about .22 mM per cc at 25° C, and in order to inject a dose of 7.5 mM/kg, each rat (average weight of 350 g) would have to have been injected with about 10 cc of fluid intravenously. Consequently, the dose of methionine used in the present experiment was not greater than 2.9 mM/kg. At this dose, no effect was observed; whereas, at corresponding doses of cysteine and glutathione,[2] 100% protection was noted. Thiourea was also studied because of its sulphur content, inasmuch as its sulphur is not in the SH form. Although thiourea did not protect against alloxan damage, some animals given thiourea and alloxan died within the first 1-2 hours. Thiourea alone was not toxic. The mechanism of death in these animals given both thiourea and alloxan has not been determined.

Conclusion. BAL, in doses of .36-.72 mM/kg completely protected rats against a diabetogenic dose of alloxan (40 mg/kg intravenously) when the protective agent was given immediately preceding the alloxan injection. However, no protection was observed when the disulfhydryl compound was given 5 minutes following the alloxan. Methionine, in doses of 2.9 mM/kg, and thiourea, in doses of 7.5 mM/kg, injected immediately preceding a diabetogenic dose of alloxan did not alter the course of alloxan diabetes.

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The Relation of Pteroylglutamic Acid to Metal-Porphyrin Enzymes.*

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Data was reported recently [1, 2] and interpreted as suggesting that pteroylglutamic acid (PGA) and its conjugates are involved in the synthesis of the porphyrin portions of metal-porphyrin enzymes. This communication offers additional data in support of that hypothesis.

For the experiments recorded here the organism *Streptococcus faecalis* (8043) was grown in the usual pteroylglutamic acid assay medium,[3] but with the addition of either potassium cyanide, hydrogen peroxide, or caffeine. The H_2O_2 was diluted, filtered through a Berkefeld filter and added aseptically in the required quantity to sterile culture tubes containing appropriate volumes of medium. KCN was treated similarly, after neutralization with acetic acid. The caffeine was added before autoclaving. The final concentrations of the inhibitors in the culture tubes were as follows: H_2O_2, 0.012%; KCN, 0.05%; caffeine, 0.5% (Table I). After inoculation, turbidity was determined at various intervals with a Coleman spectrophotometer. Responses of the organism to stimulation with varying quantities of PGA as the limiting factor was determined with and without the added inhibitor. As a control procedure, similar experiments were conducted by varying the amount of Ca pantothenate and keep-

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1 Steinkamp, R., Shukers, C. F., Totter, J. R., and Day, P. L., *Fed. Proc.*, 1947, **6**, 295.

2 Totter, J. R., and Sims, E., *Fed. Proc.*, 1947, **6**, 298.

3 Mitchell, H. K., and Snell, E. E., *Univ. of Texas Pub.*, 1941, No. 4137, 36.

TABLE I.
Growth of *Streptococcus faecalis* in Presence of Inhibitors, with Varying Concentrations of Pteroylglutamic Acid (PGA) or Calcium Pantothenate (CaP).

PGA mγ/ml	CaP γ/ml	Incubation time* hr	Caffeine %	KCN %	H_2O_2 %	Optical density
0.2	0.1	24	0.5	0	0	0
0.5	,,	,,	,,	,,	,,	.04
1.0	,,	,,	,,	,,	,,	.11
5.0	,,	,,	,,	,,	,,	.19
10.0	,,	,,	,,	,,	,,	.25
100.0	,,	,,	,,	,,	,,	.55
10	0.01	48	0.5	0	0	.48
10	0.5	,,	,,	,,	,,	.35
10	1.0	,,	,,	,,	,,	.55
10	5.0	,,	,,	,,	,,	.48
10	0.2	24	0	0.05	0	.36
10	0.5	,,	,,	,,	,,	.46
10	1.0	,,	,,	,,	,,	.48
10	5.0	,,	,,	,,	,,	.43
0.2	0.1	32	0	0.05	0	.01
0.5	,,	,,	,,	,,	,,	.07
1.0	,,	,,	,,	,,	,,	.09
5.0	,,	,,	,,	,,	,,	.51
10.0	,,	,,	,,	,,	,,	.55
100.0	,,	,,	,,	,,	,,	.66
0.2	0.1	48	0	0	0.012	.02
0.5	,,	,,	,,	,,	,,	.00
1.0	,,	,,	,,	,,	,,	.06
5.0	,,	,,	,,	,,	,,	.15
10.0	,,	,,	,,	,,	,,	.17
100.0	,,	,,	,,	,,	,,	.67

* The time required for initiation of growth varies with inoculum size, concentration of inhibitor, and concentration of PGA. The incubation times given were chosen so that growth values were intermediate between no growth and complete growth. In the absence of inhibitor, complete growth (optical density = 0.77) was reached at all concentrations of PGA above 1.0 mγ/ml in 18 hours.

ing the PGA concentration constant.

Table I gives representative values for the growth of the organisms (optical density) at appropriate times. It may be seen that the inhibition induced by KCN, H_2O_2, or caffeine was partially reversed by the addition of large quantities of PGA. On the other hand the addition of Ca pantothenate above the normal requirement for this factor was without beneficial effect. It should be pointed out that in the presence of H_2O_2 low concentrations of PGA eventually permitted nearly complete growth, indicating that there was little or no destruction of the vitamin by the peroxide.

Probably the only biochemical property the three inhibitors have in common is their relation to catalase, peroxidase, cytochrome, cytochrome oxidase, and perhaps other metal-porphyrin systems; it therefore seems logical to conclude that PGA acts by stimulating the production of extra quantities of metal-porphyrin enzymes.

The molarity of KCN required to bring about complete inhibition is of the same order of magnitude as that required to reduce the oxidation potential of hemin to a minimum, as shown by Barron.[4]

Keilin has studied the effect of caffeine on hematins and concluded that the purine forms complexes with porphyrin.[5] According to Cheyney[6] the inhibitory action of caffeine

[4] Barron, E. S. G., *J. Biol. Chem.*, 1937, **121**, 285.

[5] Keilin, J., *Biochem. J.*, 1943, **37**, 281.

[6] Cheyney, R. H., *J. Gen. Physiol.*, 1946, **29**, 63.

on fertilized *Arbacia* egg respiration is mediated through the cytochrome-cytochrome oxidase system. The percentage of caffeine herein found to inhibit *S. faecalis* is very similar to that found by Cheyney to inhibit cell division in *Arbacia*. These considerations would appear to offer strong support to the hypothesis suggested in this and earlier communications.

Summary. *Streptococcus faecalis* was grown in the usual PGA-deficient medium containing graded amounts of PGA, to which either 0.05% potassium cyanide, 0.5% caffeine, or 0.012% hydrogen peroxide was added as an inhibitor. The inhibition induced by any of these three substances was partially reversed by large amounts of PGA. In control experiments where the Ca pantothenate content of the medium was varied but the PGA content kept constant, no such reversal of inhibition was found even with high levels of Ca pantothenate. These data lend additional support to the suggestion that PGA is involved in the synthesis of the porphyrin portions of metal-porphyrin enzymes.

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Habitat of *Endameba buccalis* in Lesions of Periodontoclasia.*

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Many years ago Johns and I[1] showed that *Endameba buccalis* are most numerous at the very bottom of the pyorrhoea pocket. Later Kofoid[2] substantiated, and extended the application of this observation.

Studies of amebae in periodontoclasia have been based largely upon material taken from the lesions around and between teeth *in situ*. Although dental literature contains large numbers of illustrations of sections of teeth, including the periodontal tissues in all stages of periodontoclasia, the amebae present usually have been overlooked. Probably this has resulted from the fact that they are located within the bacterial film on the tooth and are not easily recognized in sections prepared in the usual way. Kofoid and Hinshaw[3] reported the distribution of the amebae found at different levels in relation to the calculus in sections of two incisors removed at biopsy.

More recently, employing an entirely different method, I have been able to ascertain, more accurately, the location and habitat of the parasite in these lesions. The method consists essentially of microscopic study of material removed, under the dissecting microscope, with delicate micro-instruments, from different locations on extracted teeth which have been stained to facilitate indentification of the structures and material present.

Elsewhere I[4] have described a previously unrecognized demonstrable line on extracted teeth which indicates the location of the outer border of the epithelial attachment. It is called the "zone of disintegrating epithelial-attachment cuticle" or zdeac. This line not only indicates the location of the outer border of the epithelial attachment, but it also accurately indicates the location, on the tooth, of the very bottom of the periodontoclasia lesion. With the zdeac as a guide, small particles of the soft bacterial film material can be picked from any selected areas and

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1 Bass, C. C., and Johns, F. M., *J. A. M. A.*, 1915, **64**, 553.

2 Kofoid, C. A., *J. Paras.*, 1929, **15**, 151.

3 Kofoid, C. A., and Hinshaw, H. C., *J. D. Res.*, 1929, **8**, 446.

4 Bass, C. C., *J. D. Res.*, 1946, **25**, 401.