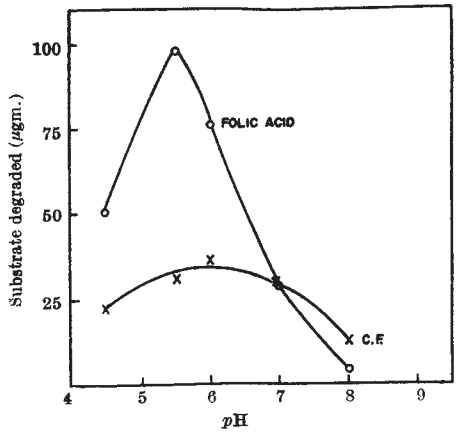

Fig. 1

L-glutamic acid, inhibited by N-formylglutamic acid and apparently does not need any of the above co-factors required by the rat liver system. Results in Table 2 show that L-glutamic acid and N-formylglutamic acid produce negligible effects on the system cleaving folic acid or citrovorum factor in rat liver. *A*-methopterin selectively inhibits the folic acid degradation but citrovorum factor is still unaffected. Furthermore, the folic acid degrading system can be largely separated from the activity towards citrovorum factor by ammonium sulphate fractionation of extracts made from acetone-dried powder (Table 3). The relation of *p*H to activity in presence of the two substrates is illustrated in Fig. 1. The two curves show marked differences. The *p*H optimum for folic acid is at *p*H 5·5 and for citrovorum factor at *p*H 6. Above *p*H 6 the activity in presence of folic acid falls rapidly. This may be a possible explanation for the higher rate of degradation of citrovorum factor obtained by Dinning *et al.*[4] in rat liver homogenates, for their experiments were carried out at *p*H 7·4.

The findings reported here indicate that, in rat liver, separate enzyme systems catalyse the conversion of folic acid and citrovorum factor into a diazotizable amine. The present results do not show whether these conversions proceed through the formation of tetrahydrofolic acid, as has been demonstrated for the system reacting with citrovorum factor in hog liver[5,6]. Differences between the co-factor requirements and the lack of effect produced by L-glutamic acid indicate, however, that the enzymes catalysing the cleavage of citrovorum factor in the two animal species may not be identical.

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A Possible Intermediate in the Biosynthesis of Tryptophan : 1-Deoxy-1-N-o-Carboxyphenyl-Ribulose

During an investigation of compounds formed by washed cell suspensions of mutant strains of *Aerobacter aerogenes* blocked at various stages in tryptophan synthesis, two strains (*N*3–33 and *N*2–18) were found to form a compound which showed an intense blue-violet fluorescence under ultra-violet light, but the absorption spectrum of which did not correspond to any known compound. Neither strain of *A. aerogenes* would grow in the minimal medium described by Davis and Mingioli[1] unless either tryptophan or indole was added and they were therefore presumably unable to convert anthranilic acid to indole.

Cell suspensions were incubated with a mixture of glucose (0·1 *M*), ammonium chloride (0·005 *M*), magnesium sulphate (10^{-4} *M*) and phosphate buffer (0·1 *M*, *p*H 7·7) as described by Gibson, Jones and Teltscher[2]. After removal of the cells, spectrophotometric examination of the supernatant fluid showed the presence of a compound with a spectrum similar in some respects to that of anthranilic acid (Fig. 1). The compound was readily extracted by ethyl acetate after the supernatants had been acidified, but concentration of these extracts to dryness resulted

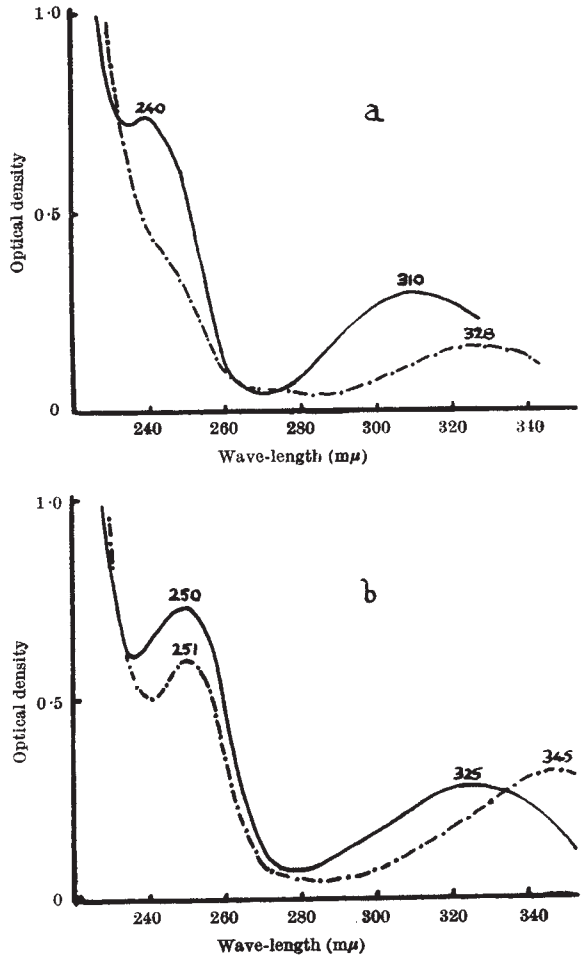
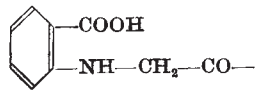
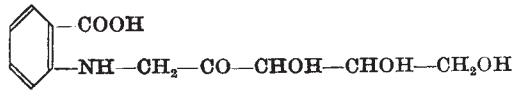


Fig. 1. Ultra-violet absorption spectra of: (*a*) anthranilic acid (10^{-4} *M*) 0·1 *M* phosphate; (*b*) experimental sample with 0·1 *M* phosphate as blank. ———, Spectrum at *p*H 7·7; – – –, spectrum at *p*H ~ 2

in decomposition of the compound. It has not been found possible to isolate the pure compound, but its identity has been established by the absorption spectrum, paper chromatography and by the formation of the crystalline 2,4-dinitrophenylhydrazone. The examination of the absorption spectra of a number of anthranilic acid derivatives indicated that the spectrum was consistent with the structure



The compound appears to be 1-deoxy-1-N-*o*-carboxyphenyl-ribulose.



This compound was suggested[3] as a possible intermediate in the conversion of anthranilic acid to indole and is the Amadori rearrangement product of the riboside or arabinoside of anthranilic acid. It has been prepared from both the D-riboside and D-arabinoside and although not yet isolated was readily detected on paper chromatograms by reagents such as 2,3,5-triphenyltetrazolium chloride. Concentrates of the natural and synthetic compounds readily reduced alkaline methylene blue at room temperature, a property of Amadori rearrangement products[4]. The natural and synthetic products had the same R_F value in two solvent systems and gave the same colour reactions on paper chromatograms (Table 1). The absorption spectra of the natural and synthetic compounds in ethanol and in phosphate buffer at various *p*H levels were identical.

Table 1. Tests on Paper Chromatograms showing Identical Results with the Natural and Synthetic Product

Reagent	Result	Inference	Ref.
Ammonium molybdate	green-blue changing rapidly to dark blue	sugar present	6
Aniline-acetate	purple-brown	pentose	7
Phloroglucinol	dark green	keto-pentose	8
Anthrone	purple	keto-pentose	9
Alkaline 2,3,5,-triphenyl tetrazolium chloride	immediate intense red colour without heating	evidence for Amadori rearrangement products	10 4

The 2,4-dinitrophenylhydrazone of the natural product was obtained by extraction of the original compound first into ethyl acetate and then into phosphate buffer (0·1 *M*, *p*H 7·7). An acid solution of 2,4-dinitrophenylhydrazine was then added and allowed to react overnight at room temperature. The product was recrystallized from alcohol and acetone. The 2,4-dinitrophenylhydrazones of the synthetic compound and of the natural product had the same melting point (213–215°) separately and mixed. The derivatives also seemed identical on paper chromatograms. Analysis of the 2,4-dinitrophenylhydrazone of the natural product gave values for carbon, hydrogen, nitrogen and oxygen consistent with the structure proposed.

Concentrates of deoxy-carboxyphenyl-ribulose when added to minimal medium supported the growth of various mutant strains of *A. aerogenes* and *Escherichia coli* which would utilize anthranilic acid and such strains were cross-fed by strains *N*3–33 and *N*2–18 when tested by the technique described by Gibson and Jones[5]. The formation of the compound by cell suspensions was stimulated by aeration and was detectable at 1 hr. by measurement of the absorption peak at 325 mμ. For the isolation of the compound, the cell suspensions and substrates were incubated with aeration for 24 hr. Cell suspensions of *A. aerogenes* *N*3–33 in phosphate buffer (0·1 *M*, *p*H 7·7) readily converted anthranilic acid to deoxy-carboxyphenyl-ribulose in the presence of glucose. Details of the experiments establishing the identity of the compound will be published elsewhere.

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Mode of Action of Chelates in protecting against *Clostridium perfringens* Toxin

It was reported previously that ethylenediamine tetraacetic acid was capable of protecting mice against the toxin of *Clostridium perfringens*[1]. The protection thus afforded was shown to be probably due to the removal of a metallic ion or ions necessary for the activity of the toxin from the area where the toxin was acting. Inasmuch as the metallic ion or ions necessary for the action of the toxin apparently were higher in the preferential chelation series than calcium, the calcium chelate was shown to be able to protect when given systemically[2]. In this report, studies on the duration of the protection afforded by calcium ethylenediamine tetraacetic acid are presented and their implications are discussed.

The toxin, calcium ethylenediamine tetraacetic acid, and methods of administration of both of these were the same as described previously[2]. 2·5, 5 and 30 per cent solutions of calcium ethylenediamine tetraacetic acid were injected intradermally into mice; at various time-intervals thereafter the mice were injected at a different site with toxin and the deaths occurring within a 48-hr. period were recorded. The results of three typical experiments are presented in Table 1.

Table 1. Duration of Effect of Calcium Ethylenediamine Tetraacetic Acid in protecting Mice against *Cl. perfringens* Toxin

Calcium ethylenediamine tetraacetic acid injected (per cent)	Time (hr.) after injection of calcium ethylenediamine tetraacetic acid that toxin was injected. Groups of ten mice were used for each time-interval and the number that died is recorded									
	0	½	1	2	3	4	6	9	12	15
2·5	0	5	9	10	—	—	—	—	—	—
5	0	—	—	5	—	10	10	—	—	—
30	0	—	—	—	0	—	3	6	9	10