In all these features the action of β-pyrrolo-alanine resembles that of *p*-fluorophenylalanine; cross resistance to these 2 antagonists was accordingly tested for. Strains of *E. coli* B with a 100-fold increased resistance to *p*-fluorophenyl-DL-alanine and a 2000-fold increased resistance to β-pyrrolo-L-alanine, respectively, were obtained by 7 passages through synthetic media containing increasing concentrations of the appropriate antimetabolite.

Cross resistance to β-pyrrolo-L-alanine and *p*-fluorophenyl-DL-alanine. Bacterial growth was determined nephelometrically at 575 nm and is expressed as a % of the growth of control cultures in the absence of inhibitors. Growth of the parent strain was completely inhibited by $2.5 \cdot 10^{-5} M$ β-pyrrolo-L-alanine or $10^{-4} M$ *p*-fluorophenyl-DL-alanine

Inhibitor added	Concentration	Growth of strain resistant to Pyrrolo-alanine	*p*-Fluoro-phenylalanine
β-Pyrrolo-L alanine	$10^{-3} M$	125	0.1
	$10^{-4} M$	95	100
p-Fluorophenyl-DL-alanine	$10^{-3} M$	70	75
	$10^{-4} M$	112	115

The results given in the Table show that cross resistance to the 2 antagonists does, indeed, exist. It therefore appears likely that the mechanism of action of pyrrolo-alanine is closely related to that of *p*-fluorophenylalanine.

If this conclusion is correct, we should expect the heterocyclic analogue to be incorporated into bacterial protein.

Zusammenfassung. β-Pyrrolo-L-alanin, dessen Synthese beschrieben ist, hemmt das Wachstum von *E. coli* B auf synthetischen Nährböden. Die Hemmung wird von L-Phenylalanin und L-Tyrosin vollständig, von L-Tryptophan teilweise, nicht aber von Histidin aufgehoben. Stämme von *E. coli*, die gegen β-Pyrrolo-L-alanin bzw. *p*-Fluorophenyl-DL-alanin resistent sind, zeigen gekreuzte Resistenz.

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The Alkaline Ribonuclease of Rat Serum During Administration of 4-Dimethylaminoazobenzene and Copper Oxyacetate

ZYTKO and CANTERO[1] found that the 'alkaline ribonuclease' of rat serum was somewhat decreased after the animals had been fed for 2 weeks on a diet containing N-dimethylaminoazobenzene (DAB) and fell to 40% of normal when primary liver tumours had been induced. There was, however, a slight increase over normal value in rats bearing Novikoff ascites hepatomas. Elevated values had also been observed in the serum of human cancer patients compared with healthy subjects (ZYTKO and CANTERO[2]). This serum enzyme resembles rat liver alkaline ribonuclease and bovine ribonuclease in having an optimum pH of 7.8 but differs from these in that it is thermolabile. It does not split purine or pyrimidine mononucleotides as does acid ribonuclease. The change in absorption produced by its interaction with nucleic acid is ascribed to a hyperchromic effect due to hindrance of resonance of guanine rings in the polymer molecules (DE LAMIRANDE et al.[3]).

During the past 5 years we have carried out studies on various enzymes and other parameters during the dietary administration of DAB to rats, alone and together with copper oxyacetate. The addition of the copper salt greatly inhibits tumour induction by the azo dye (HOWELL[4], FARE[5]) and modifies various parameters, e.g. decrease in liver succinoxidase and cytochrome oxidase, liver copper storage (FARE and WOODHOUSE[6]), and dye binding in the liver protein (FARE[7]). Also the serum phenylenediamine oxidase activity (copper oxidase) was much diminished by DAB feeding but to a lesser degree when copper oxyacetate was also fed (WOODHOUSE[8]). From his measurements on azo dye binding FARE[7] suggested that the biochemical changes and the partial protection against the carcinogenic activity of the azo dye could be explained as resulting from a competition between the dye and copper for binding sites on protein. It was thought, therefore, that the estimation of the serum alkalinase ribonuclease described by ZYTKO and CANTERO[1] at intervals during the administration of the dye, alone, and together with the copper salt, might give further information.

Materials and methods. 4 groups of 6 male rats were fed, respectively, on the control maize diet, maize plus 0.09% DAB, maize plus 0.5% copper oxyacetate plus 0.09% DAB, and maize plus 0.5% copper oxyacetate. In all cases a diet of proprietary cube was substituted on Saturday and Sunday. Initially the animals were 200–250 g in weight and consumed about 10 g of mixed or basic diet each day, and the weights of the animals were recorded at fortnightly intervals. The enzyme content of blood from the tail vein was determined at intervals of 2–4 weeks over a period of 29 weeks, at which time those which received the DAB only had localized liver tumours, but those also given copper oxyacetate still presented essentially normal livers (FARE[5]). Blood specimens were always taken mid-week in the morning.

The tests were conducted as described by ZYTKO and CANTERO[1], the diluted serum being incubated with the

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Ribonuclease activity of rat sera[a]

Weeks treated	Maize only	Maize and DAB	Maize and copper oxyacetate	Maize, DAB and copper oxyacetate
0	10.40 ± 0.34 (9.96–10.68)	10.0 ± 0.33 (9.72–10.60)	9.92 ± 0.40 (9.44–10.24)	10.24 ± 0.39 (10.00–10.80)
1	9.72 ± 0.45 (9.12–10.40)	9.96 ± 0.47 (9.56–10.50)	9.24 ± 0.43 (8.96–10.16)	9.88 ± 0.32 (9.08–10.44)
3	10.22 ± 0.48 (9.44–10.64)	8.40 ± 0.14 (8.00– 8.76)	7.08 ± 0.42 (6.4 – 7.40)	7.08 ± 0.37 (6.72– 7.52)
7	11.08 ± 0.54 (10.12–11.60)	7.96 ± 0.48 (7.52– 8.32)	6.32 ± 0.36 (5.60– 6.64)	6.32 ± 0.41 (5.68– 6.56)
10	9.60 ± 0.70 (8.80–10.20)	7.56 ± 0.50 (7.00– 8.00)	5.68 ± 0.47 (5.2 – 6.32)	5.92 ± 0.42 (5.32– 6.32)
14	9.52 ± 0.36 (9.20– 9.92)	6.72 ± 0.38 (6.20– 7.20)	5.80 ± 0.36 (5.00– 6.40)	5.44 ± 0.30 (5.20– 6.40)
18	9.80 ± 0.33 (9.20–10.08)	6.0 ± 0.29 (5.64– 6.52)	5.44 ± 0.26 (5.12– 6.36)	4.24 ± 0.29 (3.84– 4.48)
22	9.56 ± 0.35 (9.04–10.40)	5.96 ± 0.25 (5.80– 6.40)	5.20 ± 0.29 (4.80– 5.44)	3.32 ± 0.29 (3.12– 3.68)
24	9.56 ± 0.35 (9.04–10.40)	5.96 ± 0.24 (5.80– 6.40)	5.20 ± 0.29 (4.80– 5.44)	3.32 ± 0.28 (3.12– 3.68)
29	9.92 ± 0.35 (9.24–10.44)	5.76 ± 0.33 (5.16– 6.36)	4.80 ± 0.28 (4.44– 5.20)	3.08 ± 0.28 (2.80– 3.40)

[a] Mean difference in optical density/mg protein, with standard deviation and range.

ribonucleic acid (RNA) substrate at pH 7.8 for 30 min at 37 °C, and the optical density of the clear supernatant, after precipitation of protein by perchloric acid, centrifugation and dilution, was measured at 260 nm. Measurements were also made on supernatants from serum plus substrate, precipitated immediately after mixing. The increase in optical density resulting from the action of the serum enzyme on the RNA was a measure of its activity. Since the enzyme in question has not been isolated it is not possible to give the measurements in standard units of activity. ZYTKO and CANTERO[1] expressed their results in arbitrary units defined in terms of 'the concentration of serum per ml of diluted supernatant required to produce a change in optical density equal to 2.0 during the incubation (30 min at 37 °C)', but they did not give the method of calculation explicitly.

In preliminary tests we found that the values for the changes in optical density due to the enzyme were linear for quantities of the diluted serum between 0.05 ml and 0.20 ml. The values in our experiments are given directly in terms of the change in optical density/mg of serum protein in the test (ZIGMAN and ALLEN[9]). The protein contents of the rat sera were somewhat, but not severely, decreased during the administration of the three diets.

The diluting medium was phosphate buffer pH 7.8, 0.1 *M*. The nucleic acid was prepared and utilized as described by ZYTKO and CANTERO[1], and the same preparation was used throughout the tests. After incubation the nucleic acid was precipitated with 1.0 ml 12% perchloric acid, centrifuged for 10 min, the clear supernatant diluted 1:20 with distilled water, and the optical density measured at 260 nm in 1 cm cuvettes.

Results. The average ribonuclease values for the various groups at the intervals designated are given in the Table. Control series (maize fed): During the whole period the animals on this diet grew normally to 280–300 g. The enzyme values did not alter greatly nor were differences between animals very marked. Azo dye only: After a period of about 2 weeks, during which there was little gain in weight, the animals in this group maintained a slow but steady increase to 250–280 g. The enzyme values decreased slowly during some 10 weeks and then declined more sharply. Copper salt only: This caused a continued depression of enzyme value which was more marked than with DAB alone. The serum, after 10 weeks on copper diet, contained 200 µg Cu%, this being somewhat higher than normal. Azo dye and copper salt: The combined dietary addition depressed the enzyme values to 60% of the controls after 10 weeks and to 30% after 29 weeks.

Discussion. It would seem of some interest that the initial values of the sera of maize-fed rats are higher than ZIGMAN and ALLISON[9] found for rats fed on a diet of good biological value, but very similar to those they obtained with animals fed a protein-deficient diet containing 40% wheat gluten. The standard maize diet used in our experiments, which has been found to be very useful for the studies on the carcinogenic properties of DAB, is also substandard in biological properties.

However, the results obtained in the tests using the diet with only added DAB do not confirm those reported by ZYTKO and CANTERO[1], and, in particular, do not support the idea that the changes in enzyme content have any direct connection with the cancer-inducing properties of the azo dye. These workers suggested that the effects they observed may be due to side reactions of the carcinogen under the conditions of their tests. Also recently the presence of ribonuclease inhibitors which are protein in nature have been demonstrated in various tissues (GIRIJA and SREENIVASAN[10]). The possible role of such substances, if present in serum, would need to be evaluated.

The serum enzyme values in animals receiving copper salt are explicable on the assumption that copper as well as the azo dye binds with the amino acid residues of the enzyme protein[11].

Résumé. On a déterminé l'activité de la ribonucléase alkaline dans le sérum sanguin du rat pendant l'administration per os de diméthylaminoazobenzene et d'oxyacétate de cuivre, séparément ou simultanément, pendant 26 semaines. On a trouvé une activité un peu réduite quand ces substances étaient administrées séparément, mais combinées, elles provoquèrent une diminution plus sensible.

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