Induction of Tryptophan Pyrrolase in Rats after Early Injections of Tryptophan or Neonatal Thymectomy

The analogy between the formation of antibodies and the induction of enzymes in higher animals has recently been discussed. Any similarity between these phenomena has been rejected by KRÖGER and GREUER[1] on the basis of their experiments on the effect of X-irradiation and of 6-mercaptopurine on the induction of tryptophan pyrrolase. VAN BEKKUM and NIEUWERKERK[2] have postulated a sort of 'tolerance' phenomenon in enzyme induction, having observed that the induction of tryptophan pyrrolase by tryptophan or cortisone was greatly reduced in rats pre-injected with tryptophan in early life. This observation would be rather difficult to interpret, because L-tryptophan is not a foreign substance, but is a natural component of body and food proteins. Further, it has been shown that tryptophan enhances tryptophan pyrrolase activity not by stimulating the synthesis of this enzyme, but rather by decreasing the rate of its degradation[3], and this makes more difficult a comparison with immune tolerance.

In order to obtain more information on the reported analogy with immune tolerance, we attempted to reproduce the effect of early tryptophan injections, and investigated the induction of tryptophan pyrrolase in neonatally-thymectomized rats.

Rats of the Wistar-Glaxo strain of our animal room were used for the experiments. They were maintained on the stock diet of this laboratory[4]. All rats were weaned at 24 days of age. Male animals were used for the experiments with injections of tryptophan, and rats of both sexes for thymectomy. No differences were observed in the basal level or in the induction of tryptophan pyrrolase in rats of different sexes.

Thymectomy was performed within 24 h of birth. The rats were put in a refrigerator on filter paper at – 12 °C until reflexes were lost. An incision was made through the sternum up to the third rib, and the thymus was removed by suction. The sternum and the skin were closed with a single stitch with silk No. 7.0 and coated with 15% collodion. The operation was performed under a stereomicroscope. Postoperative mortality and cannibalism were low.

At sacrifice the mediastinum content of thymectomized rats was examined histologically, and when fragments of thymus were seen the rats were classified as 'partially thymectomized'. Tryptophan pyrrolase activity was determined by the method of FEIGELSON and GREENGARD[5] as used by VAN BEKKUM and NIEUWERKERK[2]. Kynurenine was estimated by the extinction at 360 nm (maximum absorption peak) in a Beckman DU spectrophotometer; a molar extinction of 4500 as determined with the pure compound (Sigma) was used in the calculations.

Only the experiments with the most intensive pretreatment with tryptophan were repeated. Injection of tryptophan caused a mortality above 50% in young rats. No differences were observed in the response of surviving animals to tryptophan or cortisone induction at the 20th or at the 25th day of age (Table I). The induction of tryptophan pyrrolase by the same agents was also quite normal in thymectomized rats (Table II).

These results do not confirm the experiments supporting the hypothesis of a phenomenon analogous to immune tolerance in the adaptation of tryptophan pyrrolase, as reported by VAN BEKKUM and NIEUWERKERK[2]. The reasons for this discrepancy are not known; these

Table I. Induction of tryptophan pyrrolase in male rats pre-treated with tryptophan

Age at test (days)	Pre-treatment	Enzyme induction	Number of rats	Body weight (g)	Tryptophan pyrrolase activity
20	Saline	–	5	30.4	3.04 ± 0.20
	Saline	Tryptophan	6	29.8	7.77 ± 1.23
	Tryptophan	–	4	20.2	2.45 ± 0.31
	Tryptophan	Tryptophan	5	30.2	7.78 ± 0.96
25	Saline	–	5	42.6	3.90 ± 0.56
	Saline	Tryptophan	5	45.6	14.25 ± 1.63
	Tryptophan	–	5	39.2	3.88 ± 0.52
	Tryptophan	Tryptophan	6	35.2	15.39 ± 1.30
25	Saline	–	6	44.0	3.54 ± 0.44
	Saline	Cortisone	5	47.2	7.06 ± 1.15
	Tryptophan	–	5	40.2	3.19 ± 0.72
	Tryptophan	Cortisone	6	42.8	7.93 ± 0.89

Rats pre-treated with tryptophan received an i.p. injection of L-tryptophan (1 mg/g of body weight) at 3, 5, 7, 9, 11 and 13 days of age. The enzyme was induced by injections of tryptophan (1 mg/g of body weight) or of cortisone acetate (Cortone Merck) (0.01 mg/g of body weight) given i.p. 3.5 h before sacrifice to tryptophan-pretreated rats and their controls. The enzyme activity is expressed as μmoles of kynurenine/h/g wet liver.

Table II. Induction of tryptophan pyrrolase in neonatally thymectomized rats

Enzyme induction	No. and sex of rat	Body weight (g)	Tryptophan pyrrolase activity
Non-operated rats			
–	6 ♂ and 7 ♀	59.3	3.14 ± 0.20
Tryptophan	3 ♂ and 3 ♀	69.0	18.50 ± 0.74
Cortisone	8 ♂ and 8 ♀	59.7	7.69 ± 0.76
Thymectomized rats			
–	7 ♂ and 1 ♀	52.6	2.66 ± 0.44
Tryptophan	5 ♂	57.4	18.75 ± 1.20
Cortisone	7 ♂ and 6 ♀	51.5	7.78 ± 0.67
Partially thymectomized rats			
–	4 ♂ and 5 ♀	60.2	3.26 ± 0.25
Tryptophan	1 ♂ and 2♀	70.7	16.75 ± 1.79
Cortisone	6 ♂ and 4 ♀	54.8	7.81 ± 0.50

Experimental conditions as in Table I, except that the dosage of cortisone was 0.02 mg/g of body weight. The rats were killed at the 30th day of life.

1 H. KRÖGER and B. GREUER, Biochem. Z. *341*, 190 (1965).

2 D. W. VAN BEKKUM and H. T. P. NIEUWERKERK, Science *149*, 548 (1965).

3 R. T. SCHIMKE, E. W. SWEENEY and C. M. BERLIN, J. biol. Chem. *240*, 322 (1965).

4 Composition, in %: whole flour of mixed cereals (wheat, oats, barley, and corn in equal parts) 79, crude casein 8, half-skimmed dried milk 5, dried wheat germ 5, sodium chloride 0.5, calcium lactate 1.5, dried brewer's yeast 1. Supplied in pellets by Laboratorio D.ri Piccioni, Brescia. Ox liver and fresh vegetables were given once a week.

5 P. FEIGELSON and O. GREENGARD, J. biol. Chem. *236*, 153 (1961).

investigators do not give experimental details such as strain, sex and weight of the rats, and the number of experiments performed, thus making more difficult an evaluation and comparison of their results.

The experiments with thymectomized rats exclude further any analogy between enzyme induction and capacity of immune response, the latter being greatly impaired in neonatally-thymectomized rats[6,7].

Riassunto. La somministrazione di triptofano per via endoperitoneale nei primi giorni di vita non influenza l'induzione della triptofano pirrolasi nel fegato di ratto ad opera del triptofano o del cortisone somministrato a 20–25 giorni di età. L'induzione dell'enzima é normale in ratti di 30 giorni timectomizzati alla nascita. Questi risultati non confermano l'esistenza di un fenomeno simile alla tolleranza immunitaria nella sintesi adattativa di questo enzima.

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[6] B. G. Arnason, B. D. Jankovic and B. M. Vaksman, in *The Thymus in Immunobiology* (Eds. R. A. Good and A. E. Gabrielsen; Harper & Row, New York, 1964) p. 492.

[7] We thank Sig. E. Lorenzoni for skilled technical assistance and Merck Sharp & Dohme Italia for a generous gift of Cortone. The work was supported by a grant from Consiglio Nazionale delle Ricerche, Rome.

Effect of D,L-Glyceraldehyde on Bacterial Cells Metabolism

It is only recently that experimental investigations have been directed to the problem of the relationship between energy metabolism and protein synthesis processes. Research on animals and neoplastic cells has been especially concerned with glycolysis, and the oxidation of glucose in relation to the incorporation of labelled aminoacids, the latter being taken as an index of protein synthesis. D-L-glyceraldehyde has proved considerably useful in these studies[1,2] as it inhibits glycolysis at levels which do not affect oxidation. L-glyceraldehyde, in fact, binds the di-hydroxyacetonphosphate with the formation of L-sorbosium-1-phosphate, which is a hexokinase inhibitor[3].

It seemed of interest to extend this study to bacterial cells. In previous investigations we have observed that D-L-glyceraldehyde inhibits the growth of several germs[4]: *E. coli* 817 ISI, *E. coli* B. 806 ISI, *Salmonella typhi*, *Paratyphi C*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Brucella melitensis*, *Serratia marcescens*. This inhibition is shown by a decrease in the growth rate, up to a complete absence of development, which is related to the concentration of D-L-glyceraldehyde and to the type of medium used. The results obtained in studies with *E. coli* in culture are summarized in Table I.

The effect of D-L-glyceraldehyde on the glycolysis and oxidation as well as on the leucine-C^{14} incorporation has been studied with *E. coli* 806 ISI cells cultivated in 1‰ glucose Nutrient Broth, collected in log phase by centrifugation and washed 3 times in H_2O. The Warburg procedure was employed for the glycolysis and oxidation. The leucine-C^{14} incorporation was studied by collecting the cells on Millipore filters and measuring C^{14} content in counts/min with a Nuclear Chicago D47 flowmeter. The results are summarized in Tables II and III.

[1] G. G. Guidotti, A. Fonnesu and E. Ciaranfi, Cancer Res. *24*, 900 (1964).

[2] E. Ciaranfi and A. Fonnesu, Atti Accad. naz. Lincei Rc. Serie VIII *33*, 835 (1962).

[3] H. A. Lardy, V. D. Wiebelhaus and K. M. Mann, J. biol. Chem. *187*, 325 (1950).

[4] C. Cutinelli and F. Galdiero, Atti XIII Congr. naz. Microbiol. Parma-Salsomaggiore (1965).

Table I. Effects of D-L-glyceraldehyde on growth rate of E. coli at 37 °C without shaking

D-L-glyceraldehyde	Media					
	Nutrient broth[a]		G.G.Y.[b]		Glucose minimal medium[c]	
	Growth rate in h^{-1}	%	Growth rate in h^{-1}	%	Growth rate in h^{-1}	%
Control	0.015	100.0	0.021	100.0	0.015	100.0
1.1 m*M*	0.015	100.0	0.022	100.0	0.008	53.3
2.2 m*M*	0.015	100.0	0.019	86.0	0.000	0.0
3.3 m*M*	0.013	86.3	0.006	27.2	–	–
4.4 m*M*	0.010	50.0	0.000	0.0	–	–
5.5 m*M*	0.007	46.6	–	–	–	–

The results are mean values of 5 experiments. [a] Nutrient broth 8‰ + NaCl 5‰. [b] Glucose 10 g ‰, glycine 20 g ‰, extract yeast g 1 ‰. [c] Medium as described by Monod. The growth rate is calculated from Monod: $\mu = \frac{\log x - \log x_0}{t \log 2}$, μ = growth rate; x = density of cells at 't' time; x_0 = density at zero time.