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ALLOXAN INDUCED METABOLIC CHANGES IN LIVER

V. MOHANACHARI, P. NEERAJA, K. INDIRA* AND K. S. SWAMI
Department of Zoology, S.V. University, Tirupati 517 502, *India*

ABSTRACT

Administration of alloxan elevated the glycolytic and Krebs cycle segments, while the protein metabolism, in particular the ammonia metabolism, was impaired. The ammonia detoxification phenomenon tended towards glutamine synthesis rather than urea synthesis in liver.

INTRODUCTION

EXPERIMENTALLY induced diabetes in animals is comparable to Diabetes Mellitus characterized by hyperglycemia, glycosuria, increased protein catabolism, ketosis and acidosis[1]. Alloxan has been shown capable of inducing diabetes by selective necrosis of β-cells of islets of langerhans and by limiting the availability of tissue glutathione[2]. Despite the spectacular advances in understanding the molecular events responsible for the diabetogenic action of alloxan, less attention has been paid on its influence on the 'extra pancreatic tissues'.[3] Hence in the present investigation, this aspect has been focussed as to understand the general metabolism prevailing in alloxanized liver, because of the presence of well documented "insulinase" system in liver[4].

MATERIALS AND METHODS

Healthy adult field mice (*Mus buduga*) of either sex with a weight range of 50–65 g were acclimated to the laboratory conditions at a temperature of $27 \pm 2°$ C, and fed on standard food pellets (from Hindustan Lever Ltd., Bombay) and water *ad libitum*. Alloxan was dissolved in dilute hydrochloric acid solution (1 drop of conc. hydrochloric acid to 100 cc of distilled water) as suggested by Patterson and Lazarow[5]. One set of animals received alloxan 60 mg/kg by subcutaneous injection as per the method of Kass and Waisbren as given by Leyden Webb[2] to get consistent results. The control animals received the same volume of diluted hydrochloric acid solution which was originally utilized for dissolving alloxan. Three hours after injection, they were decapitated and the livers were excised and kept in deep freeze until further use. Ten per cent homogenates with 0·25 M cold sucrose solution for assaying oxido reductase enzymatic systems and ten per cent homogenates with cold distilled water for assaying AMP deaminase, ammonia, urea and glutamine, were prepared. All the homogenates were centrifuged at 2500 g for 20 minutes to remove the cell debris and the clear cell free extracts of the tissues were employed to assay the respective enzymatic activity levels. For estimating the phosphorylase activity, the enzyme source was extracted in EDTA and sodium fluoride buffer (pH 6·5). Phosphorylase (EC 2.4.1.1) activity by the method of Cori, Illingworth and Keller[6], Lactate dehydrogenase (EC. 1.1.1.27) by Nachlas *et al.*[7] as modified by Govindappa and Swami[8], succinate dehydrogenase (EC 1.3.99.1) by Nachlas *et al.*[7] as modified by Pramilamma *et al.*[9]. Malate dehydrogenase (EC. 1.1.1.37) by Nachlas *et al.*[7], Glutamate dehydrogenase (EC. 1.4.1.3) activity as suggested by Lee and Lardy[10] with slight modifications as described by Pramilamma and Swami[11] and AMP-deaminase activity by the method of Weil–Malherebe and Green[12] as modified by Wagelin *et al.*[13] were carried. Ammonia, urea and glutamine content were estimated by adopting the methods given by Bergmeyer[14], Natelson[15] and Colowick and Kaplan[16] respectively and were expressed in μ moles/gm wt. All the enzymatic activities were represented in units of product formed or liberated per mg protein per hour. The protein content in the tissue enzyme extracts was estimated by the method of Lowry *et al.*[17].

RESULTS AND DISCUSSION

The general metabolism in alloxanized liver was assessed by assaying phosphorylase and LDH, the glycolytic enzymes, SDH and MDH of Krebs cycle. Since catabolism of protein was reported earlier[18], the activities of GDH and AMP-deaminase associated with ammonia metabolism were assayed besides the estimation of metabolic products of protein degradation like ammonia, urea and glutamine. An elevation in phosphorylase activity to provide the hexoses to glycolytic and Krebs cycle was witnessed (Table I) as evinced by the stepped up LDH, SDH and MDH activities, which suggests that the administration of alloxan has triggered the feeding of hexoses to glycolytic and Krebs cycle in extra pancreatic tissues like liver as in the present case. The stimulated activity levels of GDH and AMP deaminase indicate the increased oxidative deamination of glutamate and deamination of nucleotides respectively to yield

* For correspondence.

ammonia and thereby contributing to the increased turnover of protein catabolic products. The ammonia content is also found to be high (Table II) in experimental liver which correspondingly agrees with the elevated GDH and AMP deaminase activities.

TABLE I

Alloxan induced enzymatic changes in liver
(*Enzyme activity represented in units/mg protein/hr*)

Sl. No.	Enzyme	Control	Experimental	Per cent difference over control
1.	Phosphorylase (μ moles of iP)	18·27±0·28	22·92±0·36	25·5
2.	LDH (μ moles formazan)	1·69±0·06	3·96±0·09	135·1
3.	SDH (μ moles formazan)	4·64±0·12	7·58±0·72	63·5
4.	MDH (μ moles formazan)	0·62±0·05	1·44±0·10	132·4
5.	GDH (μ moles formazan)	0·78±0·03	2·27±0·04	191·1
6.	AMP-Deaminase (μ moles ammonia)	0·08±0·01	0·15±0·01	80·4

All values are mean of six observations and significant at $P < 0·001$.

TABLE II

Alloxan induced changes in ammonia, urea and glutamine in liver
(μ *moles/gm wet wt of tissue*)

Sl. No.	Protein metabolic product	Control	Experimental	Per cent difference over control
1.	Ammonia	2·59±0·05	3·23±0·05	24·5
2.	Urea	5·86±0·90	2·42±0·09	−58·6
3.	Glutamine	6·30±0·29	8·62±0·78	36·8

All values are mean of six observations and significant at $P < 0·001$.

Since a slightest increase in ammonia concentration is highly toxic to the tissues, the ammonia will be channellized for conversion into less toxic substances like urea or glutamine and therefore an estimation of urea and glutamine was carried out so as to understand the possible operation of detoxification mechanisms involved during alloxanization. The decreased urea content and increased glutamine content (Table II) suggests glutamine production from glutamate. The glutamate is produced from various sources like histidine, hydroxyproline, proline, ornithine and α-keto-glutarate, whereas the utilization of glutamate is only by two ways. Therefore the presence of glutamate can be expected in such a quantity, that can allow both the reactions to proceed to form ammonia *via* GDH and the left over glutamate may be utilized for glutamine synthesis. The presence of such a mechanism involving the production of alkaline metabolites like glutamine during alloxanization appears to have an additional advantage of combating with the ketoacidosis that is formed during diabetes[19]. Interestingly, the urea content in alloxanized liver decreased by 58·6% over the control which supports the earlier report of increased blood urea[20].

Thus the present study elucidates the geared-up glycolytic and Krebs cycle segments, besides a relative dearrangement in the protein metabolism of the liver during alloxan diabetes.

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THE FIRST INDO-PACIFIC SYMPOSIUM ON INVERTEBRATE REPRODUCTION, AURANGABAD, INDIA

The symposium is organized by Indian Society of Invertebrate Reproduction in collaboration with International Society of Invertebrate Reproduction. It will be held at the Department of Zoology, Marathwada University, Aurangabad, Maharashtra 431 004, India, from 22nd January 1981 to 24th January 1981. It is conducted to exchange information and evaluate the present state of knowledge about various aspects of invertebrate reproduction : (1) Gametogenesis, (2) Accessory sex glands, (3) Importance of tissue culture techniques in studying gametogenesis, (4) Neuroendocrine regulations of reproduction, (5) Role of organic reserves in reproduction, (6) Somatic growth *vs.* reproduction, (7) Environmental synchronization of reproduction and (8) Parasites and reproduction. Two copies of abstracts of papers to be presented at the symposium (not exceeding 300 words) should be submitted to the organizer on or before 15th October 1980. The abstracts should clearly bring out the results of the study, or in case of review papers, should identify the lacunae in the knowledge in the area under review. All participants shall pay a symposium fee of US dollars 100 or equivalent. The symposium fee shall include the documents during the symposium and a copy of the proceedings of the symposium. For further details please contact Prof. R. Nagabhushanam, Department of Zoology, Marathwada University, Aurangabad, Maharashtra 431 004, India.

INTERNATIONAL CONFERENCE ON THERMODYNAMICS AND KINETICS OF METALLURGICAL PROCESSES

The Department of Metallurgy, Indian Institute of Science and the Metal Sciences Division of Indian Institute of Metals will be jointly organising the Conference in July 1981. This is the third in the series of Conferences, initiated in 1977. The Conference will cover the following topics : (1) Thermodynamic property and kinetic measurements, (2) Theories of metallic solutions, intermetallics, ionic melts and defect systems, (3) Equilibrium studies on systems involving gas, metal and slag, (4) Kinetics and mass transfer in metallurgical processes and phase transformations, (5) Thermodynamics of phase stabilities, (6) Energetics of surfaces and interfaces, (7) Energetics of thermally activated deformation processes and (8) Vacuum metallurgy processes. For details please contact Dr. G. N. K. Iyengar and Dr. M. Mohan Rao, Conveners, I.C.M.S. 81, Department of Metallurgy, Indian Institute of Science, Bangalore 560 012, Karnataka, India.