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EFFECT OF AROGH - A POLYHERBAL FORMULATION ON THE MARKER ENZYMES IN ISOPROTERENOL INDUCED MYOCARDIAL INJURY

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ABSTRACT

To study the protective role of Arogh on isoproterenol induced myocardial damage in rats. The effect of Arogh pretreatment on isoproterenol induced myocardial damage was assessed by studying the levels of lipid peroxides and changes in the activity of marker enzymes such as creatine kinase, lactate dehydrogenase and transaminases in serum and heart tissue. In isoproterenol administered rats, a significant decrease was observed in the activity of marker enzymes in the heart with a corresponding increase in their levels in serum. Lipid peroxide levels increased significantly in the serum and heart. In rats pretreated with arogh, the level of lipid peroxides and the activity of marker enzymes were maintained at near normal values. Pretreatment with Arogh offered a protective effect against isoproterenol induced myocardial damage in rats as evidenced by LDH isoenzyme pattern and histopathological studies of heart tissue.

KEYWORDS

Arogh, isoproterenol, myocardial infarction, lipid peroxides, marker enzymes.

INTRODUCTION

Myocardial infarction is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in the necrosis of the myocardium (1). This is followed by numerous pathophysiological and biochemical changes such as lipid peroxidation, hyperglycemia, hyper lipidemia etc..

Experimentally, Isoproterenol, a β-adrenergic agonist is capable of producing gross, microscopic myocardial necrosis and depletion of tissue enzymes in the heart. Isoproterenol treated myocardial infarction serves as a well standardized model to study the beneficial effects of many drugs and cardiac function, since it mimics the clinical conditions of myocardial infarction due to ischemia in humans (2).

According to practitioners of traditional medicine, a combination of herbs exhibits augmented therapeutic efficacy than a single herb. Arogh is one such polyherbal ayurvedic formulation studied for its antioxidant property (3). It is composed of nine plant ingredients - *Nelumbo nucifera, Terminalia chebula, Zingiber officinale, Glycyrrhiza glabra, Hibiscus rosasinensis, Eclipta alba, Rosa damascena, Quercus infectoria* and *Hemidesmus indicus.*

The present communication embodies the protective effect of Arogh pretreatment on myocardial necrosis induced by isoproterenol, with reference to lipid peroxides and marker enzymes in the serum and heart.

MATERIALS AND METHODS

Chemicals and reagents

Arogh, an ayurvedic formulation was gifted by Rumi Herbal Research Institute Private Limited, Chennai. Isoproterenol, α ketoglutarate, Bovine serum albumin, adenosine triphosphate were obtained from sigma chemical company (St.Louis, Mo, USA). All other chemicals used were of highest purity.

Method of preparation of Arogh

Arogh 5 g was added to 150 ml of boiling water and boiling continued for 2 min. The decoction was cooled, filtered and the filtrate 35 ml is considered to represent 5 g of Arogh. This was orally

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administered to rats using an intra-gastric tube.

Animals

Adult male albino rats of Wistar strain weighing 120 g-150 g were used for the study. The rats were fed with commercial pelleted rat chow and given water *ad libitum.* They were maintained under standard laboratory conditions with 12:12 h light : dark cycle. The rats were divided into four groups of six animals each.

Group I - Normal rats

Group II - Administered isoproterenol (20 mg/100 g s.c., twice at an interval of 24 hrs).

Group III - Rats pretreated rats with Arogh p.o (150 mg/100 g for a period of 60 days)

Group IV - Rats pretreated with Arogh p.o (150 mg/100 g for a period of 60 days) + Isoproterenol (20 mg/100 g s.c. twice at an interval of 24 hrs) at the end of the treatment period.

At the end of the experimental period, the animals were anaesthetized with pentobarbital sodium (35 mg/kg, ip). Blood was drawn from the external jugular vein of the rat and serum separated by centrifugation. Serum marker enzymes such as Lactate Dehydrogenase (LDH) (4), Creatine Kinase (CK) (5), Aspartate transaminase (AST), Alanine transaminase (ALT) (6), Lipid peroxides (LPO) (7) and protein (8) were assayed. The animals were sacrificed 60 ± 5 sec after the injection. Heart was dissected out and immediately washed in ice cold saline and a homogenate was prepared in 0.1M Tris HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was collected. This was used for the assay of marker enzymes and lipid peroxides. A portion of the heart tissue was stored in formal saline for histological analysis. The study was approved by the institutional animal ethics committee.

Statistical Analysis

Students 't' test was used for statistical analysis. Values are expressed as the mean ± SEM for 6 animals in each group.

RESULTS

Arogh pretreatment maintained the level of lipid peroxide and the activities of diagnostic marker enzymes in the heart at near normal levels (Table 1). A significant increase was observed in the level of lipid peroxides in isoproterenol treated rats whereas the alteration was minimized to near

Table I. Effect of Arogh pretreatment on isoproterenol induced changes in the activities of heart LDH, CK, AST, ALT and lipid peroxide

Values are expressed as mean ± SEM for 6 animals in each group

Treatment	Lipid peroxide	LDH	CK	AST	ALT
Control	3.45±0.12	114.37±4.21	9.97±0.33	17.6±0.8	42.28±1.47
Isoproterenol 20 mg/100 g s.c.	5.12±0.15***	63.15±1.86***	5.72±0.2***	10.3±0.48***	29.73±1.12***
Arogh 150 mg/100 mg p.o.	3.39±0.18	110.36±3.84	9.54±0.36	19.3±0.84	41.21±1.7
Arogh 150 mg/100 g p.o. + Isoproterenol @ 20 mg/100 g s.c.	3.98±0.25†	102.71±5.7†	8.82±0.32†	16.0±0.60†	40.06±1.8†

The activities of AST and ALT are expressed as n moles of phosphorous liberated / sec / mg protein and CK expressed as μ moles of phosphorous liberated / sec / mg protein. The level of lipid peroxides expressed as TBARS formed/ml/mg protein.

*** $p<0.001$ compared with control animals.

† $p<0.001$ compared with animals that animals that received isoproterenol alone.

normal levels in rats pretreated with Arogh. Isoproterenol treatment alone, showed a significant decrease in the activities of LDH, CK and transaminases (P < 0.001) when compared to control. Pretreatment of rats with Arogh prevented the decrease in the level of these marker enzymes (P < 0.001) by isoproterenol treatment and they were maintained near normal values (Table 1).

Arogh pretreatment *per se* did not alter the activity of the serum marker enzymes such as LDH, CK, AST, ALT or the level of lipid peroxides when compared to control (Table 2). In isoproterenol administered rats, a significant elevation in the level of lipid peroxides (p < 0.001) and activity of marker enzymes (p < 0.001) was observed when compared to control. In Arogh pretreated isoproterenol administered rats, the alterations in the level of lipid peroxides was minimized and the activity of marker enzymes were retained at near normal levels.

Histopathological Studies

The histopathological section of the control group (Fig. 1a) reveals normal architecture of the myocardium, with intact muscle fibres. Isoproterenol administered heart tissue (Fig. 1b) shows hyalinization of muscle fibres, with focal cellular infiltration or necrosis of muscle fibre. Collection of inflammatory exudate is seen in the focal cells. The heart tissue is found to be damaged on isoproterenol administration.

The heart tissue of Arogh pretreated animals (Fig. 1c) show no changes in cardiac structure and is similar to that of the control group. This indicates that Arogh is free of toxicity.

Figure 1d, reveals the architecture of Arogh pretreated isoproterenol administered heart tissue. There is minimal damage, with mild swelling of muscle cells and focal cardiac muscle fibres. Thus Arogh pretreatment retains near normal architecture of the myocardium when compared with isoproterenol administered group.

DISCUSSION

The need for assessing the size of experimental infarction arises while evaluating the drugs for the beneficial effect against myocardial infarction. The serum enzymes viz, transaminases, creatine kinase and lactate dehydrogenase serve as sensitive indices to assess the severity of myocardial infarction (9). In isoproterenol treated rats, the increased activities of serum LDH, CK and transaminases accompanied by their concomitant reduction in the heart homogenate, confirms the onset of myocardial necrosis (10).

In isoproterenol administered rats, the level of lipid

Table 2. Effect of Arogh pretreatment on isoproterenol induced changes in the activities of serum LDH, CK, AST, ALT and lipid peroxide

Values are expressed as mean ± SEM for 6 animals in each group

Treatment	Lipid peroxide	LDH	CK	AST	ALT
Control	2.18±0.09	74.76±2.43	282.83±3.94	26.82±1.11	12.14±0.48
Isoproterenol 20 mg/100 g s.c.	4.36±0.13***	140.25±5.2***	559±2.34***	47.08±1.82***	24.22±0.76***
Arogh 150 mg/ 100 mg p.o.	1.98±0.07	76.37±2.75	285.95±1.12	27.44±1.08	12.72±0.52
Arogh 150 mg/ 100 g p.o. + Isoproterenol @ 20 mg/ 100 g s.c.	2.30±0.08†	83.27±2.86†	293.96±1.71†	29.23±0.80†	13.70±0.45†

The activities of CK, LDH, AST and ALT are expressed as IU/LT. The level of lipid peroxides expressed as TBARS formed/ml/mg protein.

*** p<0.001 compared with control animals.

† p<0.001 compared with animals that animals that received isoproterenol alone.

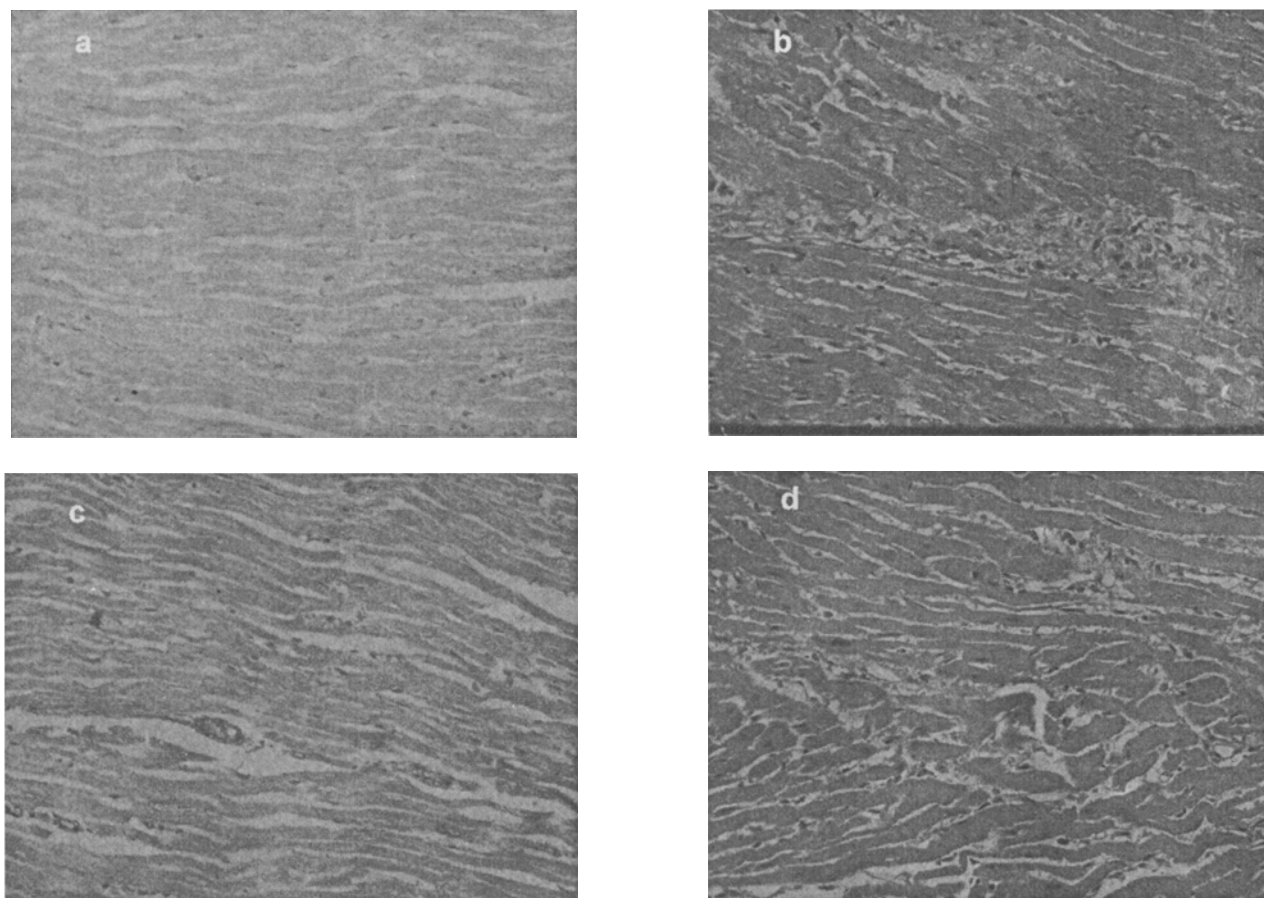

Fig. 1. Histological examination of heart in control and experimental animals (Hematoxylin and Eosin X100)

a. Control animals
b. Isoproterenol administered
c. Control animals treated with Arogh
d. Isoproterenol administered animals pretreated with Arogh

peroxides, were significantly elevated in the serum as well as in the heart homogenate. Free radicals proposed to be generated by isoproterenol, initiate lipid peroxidation of the membrane bound polyunsaturated fatty acids, leading to impairment of the membrane functional and structural integrity (11). Myocardium contains an abundant concentration of many enzymes and once metabolically damaged, releases its content into ECF. The decreased concentration of marker enzymes in the myocardial tissue of isoproterenol administered rats as compared to control, might reflect the consequences of cellular injury due to lipid peroxides (12).

In Arogh pretreated isoproterenol administered rats, the alterations in the level of lipid peroxides and activity of diagnostic marker enzymes were found to be near normal when compared with isoproterenol injected rats. This could be due to the protective effect of the active principles present in the ingredients of Arogh. *T. chebula* has been reported to act directly on the heart muscle. The negative chronotropic, inotropic and hypotensive responses observed with it might protect the myocardium by decreasing its overload (13). This activity is contradictory to the action of isoproterenol which is well known to increase the workload of the myocardium. Flowers of *N. nucifera* (14) and *Z. officinale* (15) are being recommended as cardiotonics in traditional medicines and have been scientifically proved.

T. chebula (16), *H. rosasinensis* (17) and *E. alba*

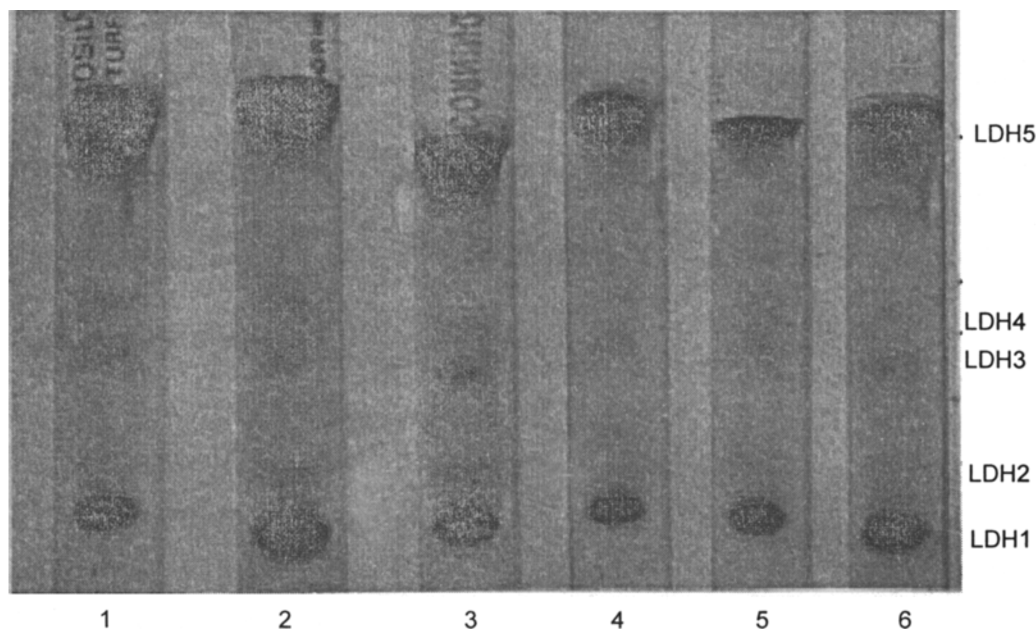

Fig. 2. Electrophoretic pattern of LDH isoenzymes in serum of control and experimental animals

Lane 1	Normal
Lane 2 & 3	Isoproterenol alone administered
Lane 4	Pretreated with Arogh alone
Lane 5 & 6	Isoproterenol + Arogh pretreated

(18) have been proved to have beneficial effects on cardiovascular system. Experimental evidences reveal that *E. alba* (19) is able to suppress the elevated levels of serum transaminases and LDH in liver and other tissues in many experimental animals. The antioxidant, anti inflammatory and free radical scavenging properties of *G. glabra* (20,21), *T. chebula* (22), *H. indicus* (23), *Z. officinale* (24), *E. alba* (25) might synergistically enhance the efficacy of Arogh to scavenge the free radicals, minimize lipid peroxidation, thereby preventing membrane damage and leakage of enzymes.

Electrophoretic pattern of LDH isoenzymes

Figure 2 shows the serum LDH isoenzyme pattern of LDH in control and experimental rats. The myocardial activity of LDH is high enough to make it unlikely that it could be a controlling step for lactate metabolism by the heart. In acute myocardial infarction, an estimate of the infarct size may be formed by measuring the rate of appearance and disappearance of LDH1 in the blood. The LDH isoenzyme pattern of control (Lane 1) shows faint bands which is an indication of intact myocardium. Isoproterenol treated rats show prominent bands (Lane 2 & 3) of the various LDH fraction which could be due to necrosis of the myocardium. Administration of isoproterenol resulted in sharp increase in heart specific LDH1 and LDH5. The increased LDH1 isoenzyme could be due to the molecular adaptation of the myocardium to the mechanical stress of ischaemia (26). It may serve as a compensatory mechanism in the failing heart as LDH5 favours anaerobic metabolism compared with LDH1 (27). Pretreatment with Arogh (Lane 5 & 6) decreased the intensity of elevation. The less prominent bands could be due to the minimal damage to the myocardium which confirms the cardioprotective effect of Arogh. There was no change in LDH isoenzyme pattern of Arogh alone pretreated animals (Lane 4).

The findings of the present study suggest that the polyherbal formulation Arogh may offer protection to the myocardium by preventing the lipid peroxidation of membrane bound polyunsaturated fatty acids, thus ensuring myocardial membrane structural integrity and function.

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REFERENCES

1. Anversa, P. and Sonnerblick, E.H. (1990) Ischemic cardiomyopathy: pathophysiologic mechanisms. Prog. Cardiovasc. Dis. 33, 49-70.
2. Harada, K., Futaka, Y., Miwa, A., Kaneta, S., Fukushima, H. and Ogawa, N. (1993) Effect of KRN 2391, a novel vasodilator, on various experimental anginal models in rats. Jpn. J. Pharmacol. 63, 35.
3. Suchalatha, S., Thirugnanasambantham, P., Maheswaran, E. and Shyamala Devi, C.S. (2004) Role of Arogh, a polyherbal formulation to mitigate oxidative stress in experimental myocardial infarction. Indian J. Exp. Biol. 42(2), 224-226.
4. King, J. (1959). A routine method for the estimation of lactate dehydrogenase activity. J. Med. Lab. Techn. 67, 265-272.
5. Okinaka, S., Kumagai, H., Ebashi, E., Sugaita, M. and Momoi, Y. (1961) Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease. Arch. Neurol. 4, 520-526.
6. Saxena, K.K., Gupta, B., Srivastava, V.K., Saxena, R.S., Singh, R.C. and Prasad, D.N. (1988) Creatine kinase and aspartate transaminase in experimental model to predict the size of cardiac infarct. Indian J. Exp. Biol. 26, 235-236.
7. Okhawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxides in animal tissue with thiobarbituric acid reaction. Anal. Biochem. 95, 351-354.
8. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193, 265.
9. Sheela Sasikumar, C. and Shyamala Devi, C.S. (2000) Protective effect of Abana, a polyherbal formulation, on isoproterenol induced myocardial infarction in rats. Ind. J. Pharmacol. 32, 198-201.
10. Paritha Ithayarasi, A. and Shyamala Devi, C.S. (1997) Effect of á-tocopherol on isoproterenol induced changes in lipid and lipoprotein profile in rats. Ind. J. Pharmacol. 29, 399-404.
11. Farber, J.K. and Young, E.E. (1997) Accelerated phospholipid degradation in anoxic rat hepatocytes. Arch. Biochem. Biophys. 211, 313-320.
12. Ganguly, P.K., Srivastava, M.L., Bora, P.S. and Gupta, M.P. (1980) Lactate dehydrogenase isozymes. Effect of isoproterenol induced myocardial necrosis. Indian J. Exp. Biol. 18, 1443-1445.
13. Srivastava, R.D., Dwivedi, S., Sreenivasan, K.K. and Chandrasekar, C.N. (1991) Cardiovascular effects of Terminalia species of plants. Indian Drugs 29(4), 144-149.
14. Chopra, R.N., Chopra, I.C. and Varma, B.S. (1969) Supplement to Glossary of Indian Medical Plants. CSIR. New Delhi. p.73.
15. Bone, K. and Gupta (1997) Traditional uses, chemical constituents, antiplatelet, anti inflammatory, cardiovascular and antioxidant properties of ginger. Br. J. Phytotherapy. 4(4), 110-120.
16. Inamadar, M.C. and Rajarama Rao, M.S. (1962) Studies on the pharmacology of *T. Chebula.* J. Sci. Industr. Res. 21C, 345.
17. Mishra, M., Shukla, Y.N., Jain, S.P. and Shushil Kumar (1999) Chemistry and pharmacology of some Hibiscus species. J. Medicinal and Aromatic plant Sciences. 21(4), 1169-1186.
18. Gupta, S.C., Bajaj, U.K. and Sharma, V.N. (1976) Cardiovascular effects of *Eclipta alba* Hassk. (Bhringaraja). J. Res. Indian. Med. 11(3), 91-93.
19. Khaksa, G., Zoltaghari, M.E., Dehpour, A.R. and Samadian, T. (1996) Anti inflammatory and antinociceptive activity of disodium glycyrhetinic acid and hemipthalate. Planta Medica. 62(4), 326-328.
20. Takagi, N. and Sanashiro (1996) Health foods containing antioxidative and antiallergy food materials. Japan Kokoi Tokkyo Koho. 070, 1000.
21. Alam, M.I. and Gomes, A. (1998) Viper venom induced inflammation and inhibition of free radical formation by pure compound (2-hydroxy-4 methoxy benzoic acid) isolated and purified from *H.indicus* root extracts. Toxicon 36(1), 207-215.
22. Aeshbaech, R., Loliger, J., Scott, B.C., Murcia, A., Butters, J., Halliwell, B. and Arouma, O.I. (1994) Antioxidant activities of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Fd. Chem. Toxic. 32(1), 31-36.
23. Chandra, T., Sadique, J. and Somasundaram, S. (1987) Anti inflammatory activity of *E. alba.* Fitoterapia. 58(1), 28.
24. Sreeramamurthy, T, Ganga Rao, B, Satyanarayana, T. and Krishna Rao, R.V. (1993) Hepatoprotective activity of *Eclipta alba.* Jour. Res. Edu. Ind. Med. 2, 41-43.
25. Kim, N.J. and Hong, N.D. (1996) Studies on the processing of crude drugs (v) on the constituents and biological activities of glycyrhiza. Korean. J. Pharmacog. 27(3), 196-206.
26. Ballo, J.M. and Messer, Jr. (1968) Lactate dehydrogenase isoenzymes in human hearts having decreased oxygen supply. Biochem. Biophys. Res. Commun. 33, 487-491.
27. Dawson, D.M., Goodfriend, T.L. and Kaplan, N.O. (1964) Lactate dehydrogenases functions of the two types. Science 143, 929-933.