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A STUDY ON THE PROPERTIES OF RABBIT LDH ISOENZYMES AND ON THE EFFECT OF GINSENG FRACTIONS ON THEM

by

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Five types of lactate dehydrogenase (LDH) have been identified in mammalian tissues by starch-gel electrophoresis (Markert and Møller, 1959), LDH-1 being nearest the anode, and LDH-5 nearest the cathode. It is now well established (Cahn *et al.*, 1962) that each isoenzyme is composed of four polypeptide chains which are assembled from two different polypeptide units, A and B, as designated by Markert (1961) (M and H by Cahn *et al.*, 1962).

LDH isoenzyme 1 through 5, therefore, have the following polypeptide compositions: A_0B_4, A_1B_3, A_2B_2, A_3B_1 and A_4B_0. Since the synthesis of the A and B polypeptides is controlled by two different genes (Markert, 1963), the phenotypic expression of the LDH isoenzyme pattern of any tissue or cell is known to be dependent on the relative activity of these two genes.

The proportions of the LDH isoenzymes have been observed to change during the development of various tissues from rat (Fine, Kaplan and Kuftireck, 1963), chick (Chan *et al.*, 1962: Nebel and Coklin, 1965), rabbit (Vessell, Philip and Bearn, 1962) and human (Fine, Kaplan and Kuftireck, 1963: Latner and Skiller, 1964), which suggests that the two structural genes are under independent but possibly linked control.

Attention has been directed chiefly towards liver and skeletal muscle in which the proportions of the more slowly migrating M-containing isoenzymes increase during development, and towards heart where the proportions of H-containing isoenzymes increase during development.

It is known (Cahn *et al.*, 1962) that these two enzymes differ with respect to their amino acid composition (particularly striking is their difference in histidine content), their stability, and their catalytic characteristics, as indicated by their affinities for pyruvate as well as their reaction with the coenzyme analogues. It is also known that antibodies made in the rabbit against the H-LDH show no cross reaction with the crystalline M-LDH, when tested by double diffusion in agar, by quantitative inhibition, by precipitation of emzyme activity, or by quantitative complement fixation (Kaplan, 1963). The same is true for antibodies against the crystalline M-LDH; the antibodies are absolutely specific and do not cross react with the enzyme from heart, which strongly illustrates that the LDH isoenzyme pattern is tissue- and species-specific in nature.

Even in a given organ, the property and

distribution pattern of the LDH isoenzyme were reported clearly different as in the cortex and medulla of kidney (Lee, 1969), and the different behavior toward pyruvate was demonstrated in rabbits (Chung and Kimm, 1971), mouse (Lee, 1970), which disclosed the fact that the H-LDH isoenzyme activity is more markedly inhibited than the M-LDH isoenzyme by pyruvate and that, under the presence of urea, pyruvate inhibition of the H-LDH isoenzyme decreases, while that of the M-LDH isoenzyme increases.

In seed plant such as soybean, the two isoenzyme showed different behavior during germination (Lee and Kimm, 1970): that is, the cotyledon of soybean contains abundant M-LDH as compared to H-LDH, which is markedly manifested during germination because of anaerobic nature of metabolism of the tissue during the early stage of germination. The total LDH and M-LDH activities in the tissue rapidly increase shortly after germination proceeds, but, decline later as aerobic metabolism begins to take over as is manifested by the gradual increase in the H-LDH.

The present report extends these previous observations on the difference in the properties of H- and M-LDH isoenzymes to another perspectives——developmental difference in rabbit heart and liver LDH isoenzyme, and kinetic difference at higher enzyme concentration; and the effects of the ginseng fractions on the pattern of LDH isoenzyme from the various organs of rabbits are also investigated.

Materials and Methods

1. *Source of Enzymes*:

Adult rabbits were sacrificed by cervical dislocation, and the heart and liver were surgically removed, perfused and frozen unless treated immediately. The same organs were dissected from rabbit fetuses and treated in the same way as with the adult organs.

They were homogenized in a mortar, frozen previously in a deep freezer, with ice-cold 0.25 *M* sucrose solution to obtain 20% (w/v) tissue homogenates. Homogenization of the heart tissue was carried out by grinding it with the addition of sea sand. The homogenates were then centrifuged at $600\times g$ for 20 minutes in order to eliminate the nuclear fraction and cell débris. The resulting supernatants were used as the source of enzyme, and the whole experiment was performed at 4°C. or on ice-bath unless otherwise specified.

For electrophoresis and kinetic studies, the above homogenates, together with the washings of the sediments with an appropriate amount of the sucrose solution, were again subjected to centrifugation at $10,000\times g$ for 10 minutes and the supernatants were used as the source of tissue enzyme preparation.

2. *Crude preparation of H-and M-LDH*:

Based on the property of selective adsorption of M-LDH to the DEAE cellulose, the two LDH isoenzymes were separated after the Bergemeyer's procedure (1963).

A portion of tissue (heart, liver and muscle) supernatant was dialyzed against $2\times10^{-2}M$ phosphate buffer, *p*H 6.0, for two hours. Discarding a negligible amount of precipitated protein on dialylisis, the contents of the dialysis sac was used for fractionation without filtering.

DEAE-cellulose suspension (10%, w/v) in the same phosphate buffer, chilled in the refrigerator previously, was suspended evenly by shaking and pipetted into a centrifuge tube with the equal amount of dialyzed tissue supernatant, mixed with a thin glass rod, and allowed to stand for 10 minutes with occasional stirring, followed by centrifugation at about $4,000\times g$ for 10 minutes and the supernatant was poured off. The sedimented DEAE-cellulose was, washed twice with the buffer, and the combined supernatant and washings served as the crude preparation of M-LDH.

To the DEAE-cellulose sediment, to which H-LDH was adsorbed, phosphate buffer was added, mixed, and centrifuged, and the supernatant was discarded. This removed the M-LDH activity, if any, which may have

been carried down with the cellulose.

To the sediment, sufficient phosphate buffer-NaCl solution ($2\times10^{-1}M$ phosphate; $2\times10^{-1}M$ NaCl, *p*H 6.0) was added, stirred thoroughly and centrifuged for five minutes at $3,000\times g$. The elution procedure was repeated twice and the combined supernatants were used as the crude preparation of H-LDH.

3. *Partial Purification of H-and M-LDH*

H-LDH from rabbit heart and M-LDH from rabbit muscle were partially purified in order to observe kinetic difference of the two at high enzyme concentration.

Following the procedure as employed by Chung and Kimm (1971), about 5.0 fold purification of each isoenzyme was achieved, starting with the supernatant of 20% tissue homogenates. Harvesting the salted-out protein precipitate between 35~60% saturated ammonium sulfate fractions, the enzyme preparation was subjected to dialysis against $2\times10^{-2}M$ phosphate buffer of pH 6.0 and treated batchwise with DEAE-cellulose.

The final specific activities for M- and H-LDH were 4.20 and 3.30 unit/mg. protein and the yields of them were 35 and 8% respectively.

Protein contents during the purifying process were monitored by Lowry *et al*'s phenol method (1951).

4. *Assay of LDH activity*:

Assays of LDH activity were performed at 25°C using the Calbiometer with a fixed wavelength of 340 *n*m., with a thermoregulator. The reaction was initiated by mixing a solution of enzyme with a solution containing sodium lactate, NAD and glycine-NaOH buffer, *p*H 10.0, in the cuvette with various concentrations of pyruvate. The initial linear increases of optical densities due to the reduction of NAD were observed with 10 second intervals. The activity of LDH was calculated by the extinction coefficient of NADH at 340 *n*m. A unit of enzyme activity is defined as a μ mole of NAD reduced during one minute incubation per ml. enzyme preparations.

5. *Electrophoretic analysis*:

Using the Gelman separaphore III, electrophoretic analysis of LDH isoenzyme was performed according to the procedure as proposed by Preston, Briere and Eatsakis (1965), in the Gelman electrophoresis apparatus. Electrophoresis was run with a current of 6 *m*A per strip, applying the sample 1.5 to 2.0 cm. on the cathode side of the center of the cellulose acetate strip, and immediately transferring the strip to the electrophoresis chamber with veronal buffer, *p*H 8.6, ionic strength 0.05.

Upon completion of electrophoresis, as additional fresh cellulose actate strip was floated on the staining solution, containing 1.0 ml. of 1.0 *M* sodium lactate, 3.0 m l. of nitrobluetetrazolium solution (1 mg/ml), 0.3 ml. of phenazine methosulfate solution (1 mg/ml) and 1.0 ml. of NAD (1 mg/ml), and superimposed on the original electrophoretic strip, wet surface down. The superimposed pair of strips was incubated for 30 minutes at 37°C, in the dark moist chamber, which was to carry out Formazan reaction for visualization of LDH activity. The strips was then held in methanol acetic acid solution for approximately 10 minutes. The LDH isoenzymes appeared as discrete blue separate bands.

Interpretation of the isogram was made by densitometric quantitation with a Spinco Analytrol and integrated by a planimeter.

6. *Fractionation of the ginseng constituents and incubation with isoenzymes*:

Commercially available ginseng, a Kum-San product, was purchased and fractionated by means of Gas chromatography and purified by preparatory thin-layer chromatography with silica gel. The purified powders of ginseng including fatty acids, steroids, saponin and alkaloids are prepared and donated to the author by Chung (1970), Section of Chemistry, the Institute of Tabacco Monopoly, Seoul, Korea.

The ginseng fractions were incubated with crude preparations of tissue LDH's in a shaking incubator at room temperature for

10 minutes, and an appropriate amount of each incubation mixture was subjected to electrophoretic analysis.

Results

(1) *Developmental difference in rabbit heart and liver LDH isoenzyme*

Alterations in LDH isoenzyme pattern in livers between fetal and adult rabbits was characterized by diminished activity in the anodal band and intensified staining of activity in the cathodal band (Fig. 1). The opposite

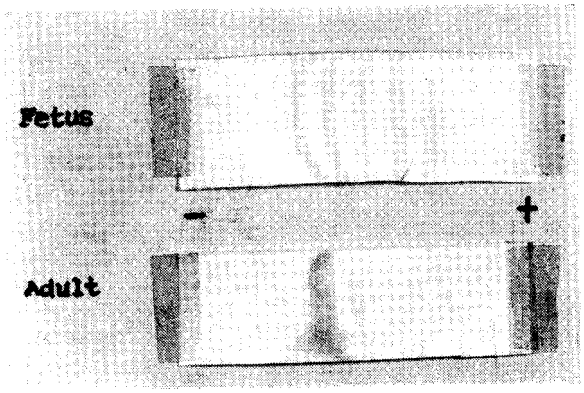

Fig. 1. Comparison of the fetal and adult rabbit liver LDH electrophoretic isoenzymograms, showing developmental difference.

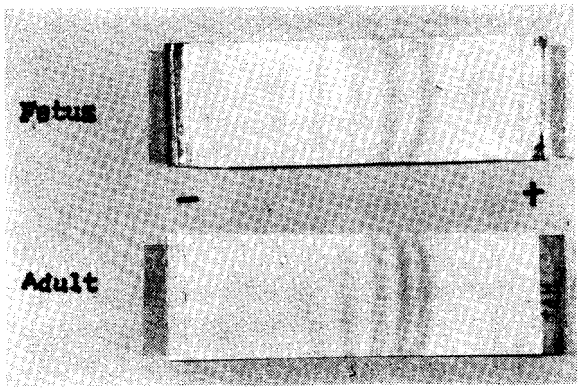

Fig. 2. Comparison of the fetal and adult rabbit heart LDH electrophoretic isograms, showing developmental difference.

phenomena were observed in the heart between the two (Fig. 2). In other words, the results suggest that a shift of the activity from H-LDH to M-LDH occurs in the rabbit liver and the *vice versa* in the rabbit heart during development.

Fig. 2 illustrates that in the rabbit heart five distinct bands, though the intensity was somewhat fainter in cathodal side and stronger in anodal side, were observed. In the adult rabbit heart, however, the tendency, showing the more anodal the stronger the intensity of Formazan band of LDH, was illustrated markedly.

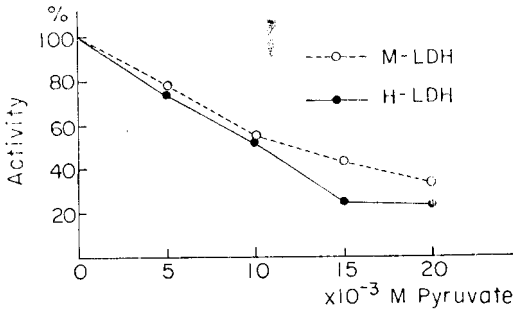

Fig. 3. Pyruvate inhibition of LDH isoenzymes at various concentration of pyruvate, when observed with higher concentration of enzymes.

(2) *Kinetic difference of LDH isoenzyme at higher enzyme concentration*:

Activities of H-LDH and M-LDH were assayed over a range of pyruvate concentrations from 0 to $20\times10^{-3}M$. Isoenzyme concentrations used were 4.20, units/mg. protein for M-LDH and 3.30 units/mg. protein for H-LDH with the other ingredients as specified in method section. But these samples were diluted 5- and 10-fold respectively to see the effect of high, medium and low enzyme concentration on the kinetic behavior of both isoenzymes. At all concentrations of pyruvate used in the present work (more than $15\times10^{-3}M$), both isoenzymes exhibited inhibited activities (Table I and II). There was, however, difference in the way they are inhibited, as depicted in Fig. 3.

The inhibition brought out by pyruvate was more pronounced in the H-LDH as compared to the M-LDH isoenzyme, which was universally true whether higher or lower enzyme concentrations were used.

The pyruvate inhibition, however, appeared to be influenced significantly by the concentration of enzymes used in the kinetic assay.

Table I. Effect of different enzyme concentrations on pyruvate inhibition of H-LDH activity purified from rabbit heart.

Enzyme Concentration	Pyruvate Concentration $\times 10^{-3}$M				
	0	5	10	15	20
Higher	1.59*	1.18	0.82	0.40	0.37
Medium	0.83	0.57	0.39	0.19	0.14
Lower	0.17	0.10	0.043	0.032	0.017

* Figures denote units/ml. sample

Table II. Effect of different enzyme concentrations on pyruvate inhibition of M-LDH activity purified from rabbit muscle.

Enzyme Concentration	Pyruvate Concentration $\times 10^{-3}$M				
	0	5	10	15	20
Higher	3.92*	3.05	2.10	1.68	1.32
Medium	2.13	1.48	1.05	0.80	0.61
Lower	0.46	0.29	0.21	0.13	0.09

* Figures denote units/ml. sample

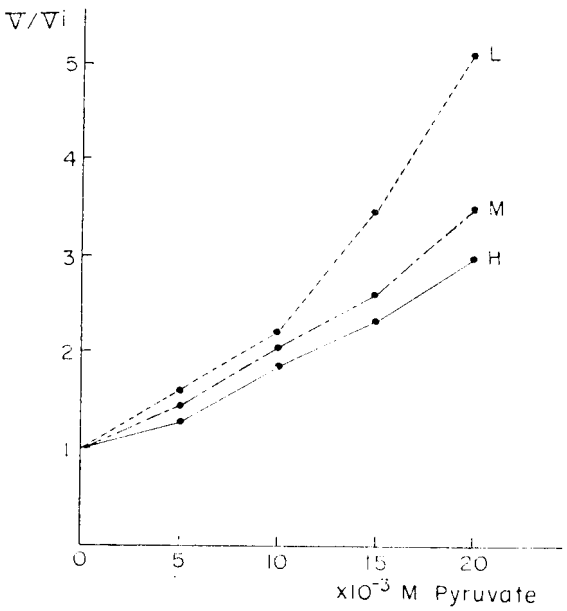

Fig. 4. Effect of increasing pyruvate concentration on the activity of higher (H), medium(M) and lower concentration (L) of H-LDH purified from rabbit heart. V/Vi; a ratio of enzyme activity in the absence of pyruvate to that in the presence of pyruvate.

As tabulated in Table I, and II and illustrated

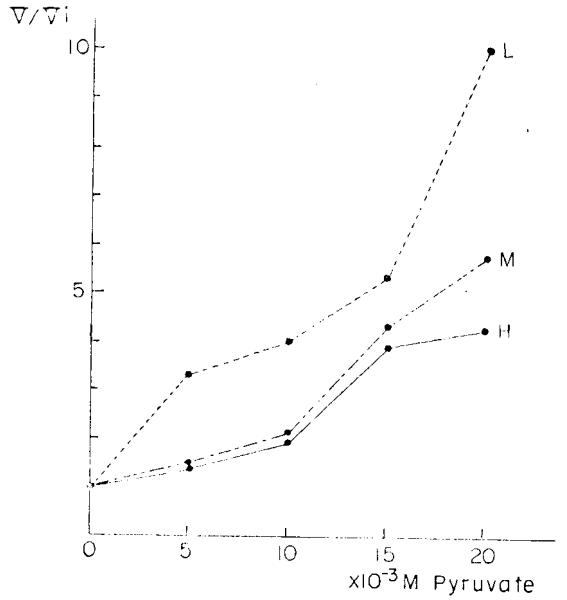

Fig. 5. Effect of increasing pyruvate concentration on the activity of higher (H), medium(M) and lower concentration (L) of M-LDH purified from rabbit psoas muscle. V/Vi; a ratio of enzyme activity in the absence of pyruvate to that in the presence of pyruvate.

in Fig. 4 and 5, pyruvate inhibition increased progressively as both the isoenzymes were diluted, and the magnitude of pyruvate inhibition with increasing isoenzyme dilution was greater for H-LDH (Fig. 4) than for M-LDH (Fig. 5). The degree, as expressed by V/Vi, where V denotes activity without pyruvate and Vi activity with pyruvate, was apparently

Table III. Comparison of the control LDH isoenzyme distribution patterns of rabbit organs.

LDH	1	2	3	4	5
Brain	28.3*	2.8	3.9	41.0	24.0
Heart	25.6	29.3	11.0	19.2	14.9
Lung	73.1	26.9	—	—	—
Liver	—	—	2.6	29.6	67.8
Kidney	26.9	2.6	1.3	27.4	41.8
Muscle	—	—	—	21.5	78.5

* Figures denote percentages of LDH isoenzyme activities.

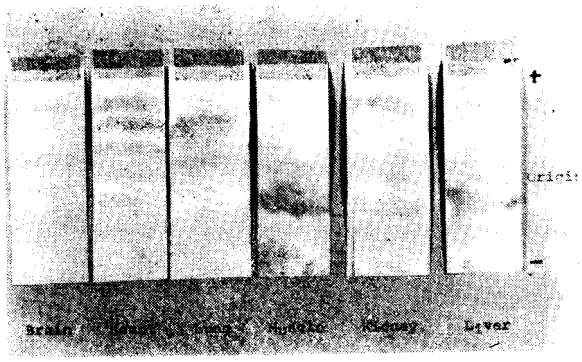

Fig. 6. Electrohoretic LDH isograms of various rabbit organs.

greater in H-LDH than in M-LDH as dilution of the enzyme was greater.

(3) *Effects of the ginseng factions on the rabbit tissue LDH isoenzyme patterns.*

Control patterns of LDH isoenzymes of rabbit organs are illustrated in Fig. 6 and their densitometric analyses are reproduced in Fig. 7, the percentage variations of which are tabulated for comparison in Table Ⅲ. Comparing these patterns with accumulated data (Wilkinson, 1965) there seems to be considerable discrepancies between values obtained for the same tissue by different investigators. These may be partly due to variations in analytic technigue and in the treatment of the specimen before electrophoresis, and also possibly to disproportionate loss of the more labile slow-moving isoenzyme LDH-4 and LDH-5.

Dispite these discrepancies mentioned above, there was observed wide agreement that in

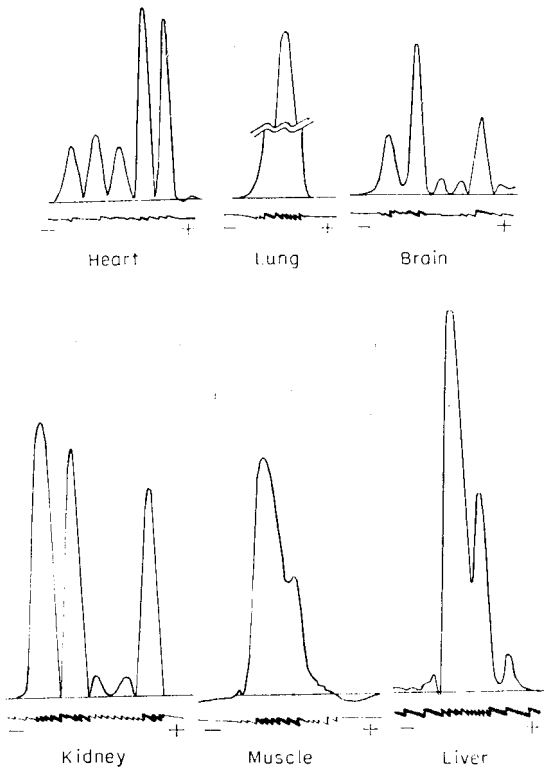

Fig. 7. Desitometric profiles of LDH isoenzymes of various rabbit organs.

heart and lung the fast-moving LDH-1 and -2 predominate, whereas in liver and skeletal muscle the LDH-4 and -5 predominated. The patterns in kidney and brain resembled each other in that proportions of hybrid forms were smaller than the homogeneous tetramer of both LDH-1 and-5.

Fig. 8 discloses the effect of ginseng fractions

Table Ⅳ. Rabbit brain LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	28.3	2.8	3.9	41.0	24.0
Fatty Acids	24.4	19.3	11.7	32.5	12.1
Steroids	23.3	1.9	3.5	27.6	43.7
Alkaloids	27.3	24.3	13.6	18.2	16.6
Saponin	30.9	3.1	4.4	34.4	37.2

Table Ⅴ. Rabbit heart LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	25.6	29.3	11.0	19.2	14.9
Fatty acids	32.3	37.3	18.1	5.6	6.7
Steroids	31.9	31.0	23.1	9.9	4.1
Alkaloids	29.0	29.0	19.0	13.0	10.0
Saponin	26.1	34.8	19.6	13.0	6.5

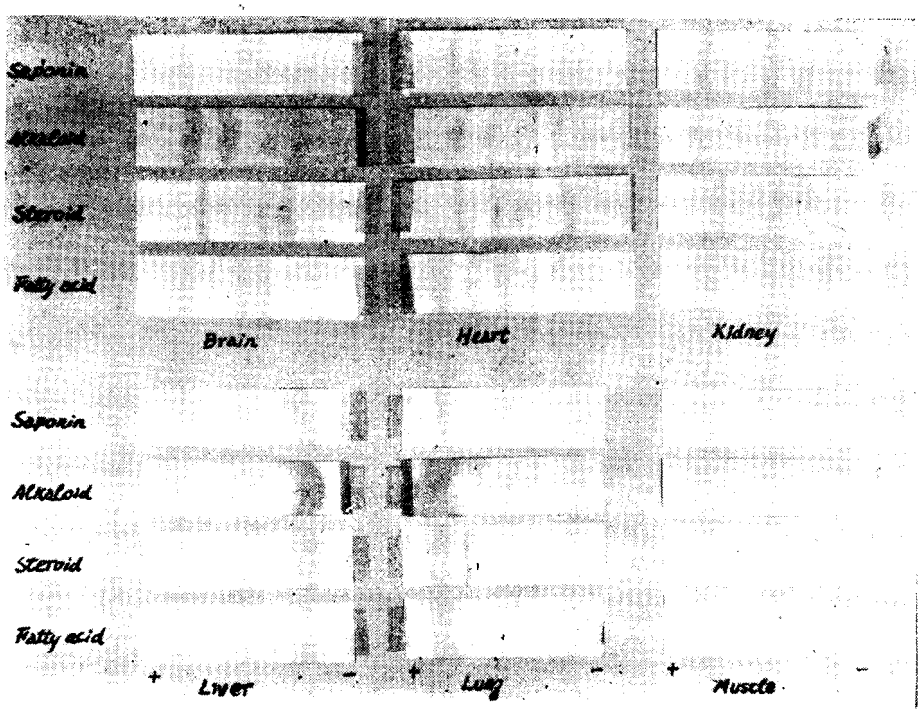

Fig. 8. Comparison of electrophretic LDH isoenzymograms of various rabbit organs as affected by ginseng fractions.

on rabbit organ LDH isoenzymes and Table Ⅳ through Ⅸ summarize the effects observed with the ginseng fractions. Fatty acid fraction of the ginseng seemed to bring accentuated activities in anodal isoenzymes in the rabbit heart and lung, and in cathodal isoenzymes in the liver and mucle, But both in brain and kidney, the hybrid form, LDH-3, showed stronger band as compared to the control.

Similar tendency as with fatty acid was observed with steroid fraction: however, with alkaloid almost exclusively LDH-3 appeared to be elevated among the organs investigated, showing little variations in extreme anodal and cathodal isoenzymes.

Effect of saponin fraction of the ginseng was obscure on brain, heart, lung and kidney. But it was clear that, in liver and muscle, LDH-5 predominated more markedly as compared to the control values.

Table Ⅵ. Rabbit lung LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	73.1	26.9	—	—	—
Fatty acids	80.0	20.0	—	—	—
Steroids	85.6	14.4	—	—	—
Alkaloids	57.5	42.5	—	—	—
Saponin	70.8	29.2	—	—	—

Table Ⅶ. Rabbit liver LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	—	—	2.6	29.6	67.8
Fatty acids	—	—	trace	11.4	88.6
Steroids	—	—	3.9	10.9	85.2
Alkaloids	—	—	trace	23.1	76.9
Saponin	—	—	trace	5.7	94.3

Table Ⅷ. Rabbit kidney LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	26.9	2.6	1.3	27.4	41.8
Fatty acids	18.9	19.9	14.6	24.6	22.2
Steroids	15.8	7.5	2.5	23.3	50.9
Alkaloids	30.3	18.9	8.3	22.7	19.8
Saponin	15.9	12.4	8.0	27.5	36.2

Table Ⅸ. Rabbit muscle LDH isoenzyme pattern expressed in percentages as affected by ginseng fractions.

LDH	1	2	3	4	5
Control	—	—	—	21.5	78.5
Fatty acids	—	—	trace	15.9	84.1
Steroids	—	—	—	13.5	86.5
Alkaloids	—	—	—	18.1	81.9
Saponin	—	—	—	4.5	95.5

Discussion

(1) *On the heart and liver LDH isoenzyme of fetal and adult rabbits*:

The present results indicate that rabbit tissues under *in vivo* conditions of embryonic development exhibit characteristic alterations of their LDH isoenzyme pattern. Similar oberservation was reported by Philip and Vesell (1962) with the chick.

It is quite apparent from the present experiment that, in the embryologic development of rabbit liver, there is a gradual redistribution of total LDH activity from the anodal isoenzymes toward cathodal isoenzymes, giving rise to the adult pattern in the later stage. In the rabbit heart, the reverse series of event appear to take place.

The first observation of such difference as those in the present work was that of Markert and Møller(1959), who detected a number of components in embryonic pig heart than in the adult heart. They rejected the suggestion that the observed differences between the fetal and adult tissue LDH isoenzyme patterns might be resulted from the changes in cell population, because changes in cell population during development do not appear to be sufficiently great to account for such a possibility, and prefered to consider the isoenzyme pattern of a tissue as a parameter of the differentiation status of its cells.

Recent observations, which strongly support this view, are the report of Flexner *et al.* (1960). They observed differences between the brain LDH of adult and new born mice during LDH chromatography on DEAE-cellulose, and between the electrophoretic patterns of the liver enzymes of adult and new-born guinea pigs. The liver enzymes of adult and new-born rats have been shown to differ in their capacities to reduce coenzyme analogues. (Kaplan and Ciotti, 1961a).

It was also observed by Philip and Vesell (1962) that chick tissue lose their characteristic isoenzyme patterns after tissue culture *in vitro*. Muscle, skin, heart, and liver all showed a decrease in the anodic fractions, a finding which was interpreted as indicating a return to the random assortment found in immature tissues (Nebel and Conklin, 1964).

In the rat heart, however, changes during development resemble those occurring in the mouse, since LDH-5 appears first (Kaplan and Ciotti, 1961b), but soon after birth LDH-1 replaces LDH-5 as the most abundant form. Similar changes have been observed during the development of rat brain (Bonavita *et al.*, 1962). It seems, therefore, in the rat and mouse, anaerobic glycolysis leading to the accumulation of lactate appears to be the principal metabolic pathway *in utero.* and it has been suggested that excess lactate may be removed *via* the placenta (Cahn *et al.*, 1962). The same situation may be operating in the rabbit fetal heart LDH isoenzyme as judged

from the present results.

(2) *Different kinetic behavior between H- and M-LDH at various enzyme concentration*:

Intracellularly most of the enzymes exist in such high concentrations that dilution of tissue homogenates several hundred-fold is required before spectrophotometric assay (Srere, 1967). It is, therefore, rather customary to determine kinetics of enzyme including LDH exclusively at highly dilute, and consequently unphysiologic concentrations.

It is now well known that the kinetic behavior of LDH isoenzyme is significantly affected by many factors, such as temperature, *p*H, buffer, ionic strength, and addition of substances including NAD or lactate (Vesell, 1968). It is important to investigate the kinetic behavior of H-LDH and M-LDH at near physiologic enzyme concentrations to determine whether they are different in kinetic nature at such conditions. Different properties obtained so far were based on the observations made on the kinetic differences between the two isoenzymes at low enzyme concetrations (Dawson, Goodfriend, and Kaplan, 1964: Lee, 1970: Chang, 1970: Chung and Kimm, 1971).

The present experiment, though the enzyme concentrations utilized was not sufficiently high enough to resemble physiological intracellular level, was aimed to observe if there is any different behavior between the two isoenzymes at reasonably higher concentrations comparing with those obtained at lower concentration.

Pyruvate inhibition, as reported previously as one of the different properties between the two isoenzymes (Chung and Kimm, 1971), increased progressively as the isoenzymes were diluted; however the magnitude of pyruvate inhibition with increasing enzyme dilution was greater for H-LDH than for M-LDH. With higher concentration of H-LDH and M-LDH, similar results were obtained, though the pyruvate inhibition with the former was a little marked than the latter as reported previously (Chung and Kimm, 1971; Wuntch, Vesell and Chen(1970).

High pyruvate concentrations, therefore, seems to cause less enzyme inhibition with physiologically high level of enzyme concentration. According to Wuntch, Vesell and Chen(1970), an estimated value of 7.5×10^{-7} mole/l for the concentration of LDH-1 in rat renal medulla lies on a level that exhibits no substrate inhibition; and in heart, cellular concentration of 23.4×10^{-7} and 9.7×10^{-7} mole/l were determined for LDH-1 and LDH-2 respectively, assuring that they are on the high level that reveal no substrate inhibition, and that tissue concentrations of LDH-5 in rat muscle are also above those concentrations where pyruvate inhibition occurs. They have gone so far as to suggest that pyruvate inhibition of LDH-1 and -5 is probably not of physiological significance. The present results also seem to support their observation, though the enzyme concentrations are not sufficiently high as those they adopted.

It is, therefore, very interesting to compare the different behavior of LDH at highly dilute and at physiologically higher enzyme concentrations. The fact that substrate inhibition, which is so prominent in assays with low LDH-1 concentrations, is hardly detected at physiologic enzyme concentrations and that substrate inhibition increases progressively as H-LDH is diluted raise objections to the theory that H-LDH and M-LDH are distributed in tissues solely according to the extent of their intracellular inhibition by pyruvate (Dawson, Goodfriend and Kaplan, 1964).

As Wuntch, Vesell and Chen (1970) have pointed out, there could be an additional criticism on this theory; that is, (i) in mammals, the liver is a highly aerobic tissue, exhibiting almost exclusively LDH-5, but according to the theory, liver, as an aerobic tissue, should contain mainly LDH-1. (ii) The theory predicts that concentrations of pyruvate or lactate sufficiently high to inhibit LDH-1 exist in many so-called anaerobic tissues (Dawson, Goodfriend and Kaplan, 1964). (iii) Inhibition of LDH-1 by pyruvate, though

marked at 6° and 25°C, is greatly diminished at physiological temperature of many mammals (Vesell, 1968; Vesell and Pool, 1966).

Presently available explanations as follows for this apparently contradictory theory may explain the situation: a regulatory function of LDH-5, allosteric in nature (Fritz, 1967), an association of isoenzymes in different proportions with various subcellular fractions (Vesell, 1965), predominance of LDH-5 in rapidly dividing cells (Papaconstantinou, 1967), and a conservative metabolic role in which one isoenzyme would be required to maintain critical enzymatic function in a tissue where another isoenzyme is rapidly degraded (Fritz *et al.*, 1969). Finally the reason for the progressive pyruvate inhibition with the dilution of isoenzyme may be attributable to the fact, as postulated by Wuntch, Vesell and Chen (1969), that an abortive ternary complex composed of LDH, pyruvate, and NAD may be responsible for this. At low enzyme concentration the abortive ternary complex may form rapidly, but it may form very slowly at physiologic LDH concentrations.

(3) *Effects of ginseng fractions on the LDH isoenzyme of rabbit organs.*

Fractions of the ginseng seem generally to alter the LDH isoenzyme patterns of the rabbit organs. As seen from Table Ⅳ through Ⅸ and Fig. 8, a generalized effect of the fractions was a shift of the total activity among isoenzymes.

For example, fatty acid and steroid fractions were same in their redistributing effect; that is, LDH-1 is more strongly stained on the isogram in the organs such as heart and lung, than original LDH-1. LDH-5 is extensively stained in the organs such as liver and muscle, than original LDH-5.

Among all fractions, it is known (Kimm, 1972) that the only steroid fraction is effective in activating rabbit tissue LDH, which might be due to the elevations both in LDH-1 and LDH-5 when affected by the fraction.

Alkaloid, however, appeared to be the only fraction that brought elevation in LDH-3 in brain, heart and kidney, although there was a little elevation in LDH-5 in liver and muscle. It seems that this fraction may act to enhance the *in vitro* hybridization of the H- and M-LDH isoenzymes. Saponin fraction, though its effect on LDH-5 in liver and muscle was a little noteworthy as observed with alkaloid, seems to have no significant effect on LDH isoenzymes of brain, heart, lung and kidney.

Ginseng extract may not have any significant effects on glycolysis, particularly in tumor-bearing rats (Chu, 1963), but the present data clearly illustrated the fact that each of the fractions of the ginseng affects the rabbit LDH isoenzyme as to make the distribution pattern shift characteristically, which may reflect the fact that the fractions might be characteristically involved in a given set of metabolic pathway, since H-LDH and M-LDH isoenzyme functions with different kinetic properties.

Conclusion

Heart and liver of the fetal and adult rabbits were investigated with regard to their LDH isoenzyme patterns by means of cellulose acetate electrophoresis. In order to evalute the difference in the kinetic behavior between the H- and M-LDH, the isoenzymes were partially purified respectively from the heart and psoas muscle of the adult rabbit with the use of DEAE-cellulose adsorption and ammonium sulfate fractionation. The partially purified isoenzymes were subjected to kinetic analysis at various concentration of isoenzymes in the presence of wide range of pyruvate concentrations.

Along with the above observation, the ginseng was fractionated into fatty acids, steroids, alkaloids and saponin, which were incubated with the enzyme preparations of the adult rabbit organs, followed by cellulose acetate strip electrophoresis to observe the effect of ginseng fractions.

The conclusions are made as follows:

(1) Comparing the fetal and adult rabbit heart and liver LDH isoenzyme patterns a loss of the rapidly migrating anodal bands in the liver was observed during the development from the fetus into the adult. The reverse change was observed in the rabbit heart.

(2) The kinetic properties of H- and M-LDH determined at their higher concentrations revealed the fact that pyruvate inhibition was progressive as dilution of enzyme was greater in both isoenzymes, though it was pronounced in H-LDH than in M-LDH.

(3) The above results suggest that pyruvate inhibition *in vivo* at physiologically high LDH isoenzyme concentration may differ from results obtained with diluted enzyme concentration *in vitro*, the underlying causes of which were discussed briefly.

(4) Effects of fractions of the ginseng were characteristically reflected in the shifts of LDH isoenzyme activities; fatty acids and steroids fractions being marked in activating the heart and lung LDH-1 and the muscle and liver LDH-5, alkaloid fraction in activating LDH-3 in the brain, heart, and kidney, saponin fraction appeared to have no significant effect in shifting the LDH isoenzyme patterns in brain, heart, lung and kidney, though a little effect was observed in activating LDH-5 in liver and muscle.

—국문초록—

토끼 LDH isoenzyme의 특성과 인삼분획이 미치는 영향에 관한 연구

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Cellulose acetate 전기영동에 의하여 태아 및 성숙 토끼의 심장과 간장 LDH isoenzyme을 분석하는 한편, H- 및 M-LDH 간의 특성차이를 규명키 위하여 성숙토끼의 심장과 요(腰)근에서 각각 H- 및 M-LDH를 DEAE-cellulose 흡착과 황산 암모니움 분획을 실시하여 부분정제하여 각종농도의 isoenzyme 존재하에 pyruvate 억제 실험을 시행하였다.

이와 병행하여 인삼을 fatty acids, steroids, alkaloids 및 saponin으로 분획하고 성숙 토끼 장기의 효소시료(試料)와 incubate하여 역시 cellulose acetate 전기영동을 시행하여 인삼분획이 LDH isoenzyme pattern에 미치는 영향등을 관찰하고 다음과 같은 결론을 얻었다.

1) 태아와 성숙토끼의 심장 및 간장 LDH isoenzyme 활성분포 양상을 비교하면 태아 간장에서 볼 수 있었던 양극 isoenzyme이 성숙 토끼의 간장에서는 없어지며 심장에서는 이와는 반대로 음극 isoenzyme이 없어진다.

2) 고 농도의 효소시료를 사용하여 H- 및 M-LDH의 특성차이를 관찰한 바 pyruvate 억제의 정도는 사용한 효소의 농도를 희석할수록 커지며, 이러한 현상은 H-LDH의 경우가 M-LDH의 경우보다 현저하다.

3) 상기 결과는 효소농도가 고농도인 생리적 조건에서의 pyruvate 억제가 저농도인 시험관 내의 그것과 서로 상이함을 말하는 것임으로 이에대한 간단한 고찰을 시도하였다.

4) 인삼의 각 분획은 토끼 LDH isoenzyme 활성의 분포변화를 전형적으로 가져오는 바, fatty acid와 stroid 분획은 심장과 폐장의 LDH-1 및 근육과 간장의 LDH-5을 그리고 alkaloid 분획은 뇌, 심장 및 신장의 LDH-3의 활성을 부활하며 saponin 분획은 간장과 근육의 LDH-5등의 활성증가를 약간 가져오기는 하나 뇌, 폐장, 심장 및 신장 등에는 별로 의의있는 영향을 미치는 것 같지 않다.

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