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Brain
chronic lactic acidosis
dihydrolipoyl dehydrogenate
heart
α-ketoglutarate dehydrogenase
kidney
lactate
liver
pyruvate dehydrogenase
skeletal muscles

# Deficiency of Dihydrolipoyl Dehydrogenase (a Component of the Pyruvate and α-Ketoglutarate Dehydrogenase Complexes): A Cause of Congenital Chronic Lactic Acidosis in Infancy

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## Summary

**A male child died at 7 months of age with progressive neurologic deterioration and persistent metabolic acidosis. Investigations during life showed this child to have elevated blood pyruvate, lactate, and α-ketoglutarate as well as elevation of branched chain amino acids and occasional hypoglycemia. Cofactor therapy using either thiamine-HCl (2 g/kg/24 hr) or thiamine tetrahydrofurfuryl disulfide had no measurable effect on the clinical or biochemical status of the patient. Tissue taken postmortem showed normal levels of key gluconeogenic enzymes but a deficiency in the activity of pyruvate dehydrogenase in all tissues tested (liver, brain, kidney, skeletal muscle, and heart). Examination of the individual activities of the pyruvate dehydrogenase complex showed pyruvate decarboxylase ($E_1$) to be normal in liver and other tissues. Dihydrolipoyl dehydrogenase ($E_3$), on the other hand, was deficient in all tissues tested. α-Ketoglutarate dehydrogenase complex, which depends on $E_3$ for its total activity, was also deficient in all tissues tested. The absence of this enzyme is discussed in relation to the clinical and biochemical status of the patient.**

## Speculation

**Congenital lactic acidosis due to a severe defect in the pyruvate dehydrogenase complex for the first time has been shown to be due to the absence of the $E_3$ enzyme of the pyruvate dehydrogenase complex, dihydrolipoyl dehydrogenase. The survival of this infant for 6 weeks of life without hospitalization is suggestive of a reliance on glycolytic rather than oxidative metabolism in the early neonatal period.**

Oxidative decarboxylation is involved in the normal metabolism of pyruvate, α-ketoglutarate, and the branched chain amino acids (leucine, isoleucine, and valine). The oxidative decarboxylation of pyruvate is catalyzed by the so-called pyruvate dehydrogenase enzyme complex. This complex comprises at least five distinct components. Three of these components in sequence catalyze the actual oxidative decarboxylation of pyruvate. These are pyruvate decarboxylase or $E_1$ (EC. 4.1.1.1), dihydrolipoyl transacetylase or $E_2$ (EC. 2.3.1.12), and dihydrolipoyl dehydrogenase or $E_3$ (EC. 1.6.4.3). The total complex comprises these components ($E_1$, $E_2$, $E_3$) in ratios which are tissue specific, e.g., bovine heart 30:60:12 and bovine kidney 20:60:12 (25). Furthermore, the $E_1$ component, an $\alpha_2\beta_2$ subunit tetramer, exists in an active and an inactive form. The interconversion of these is catalyzed by a further two subenzyme components. The conversion of the dephosphorylated active form to the phosphorylated inactive form is catalyzed by specific kinase (pyruvate decarboxylase: ATP transphosphorylase) and the reverse process is catalyzed by a specific phosphatase ($Mg^{++}$- and $Ca^{++}$-dependent pyruvate decarboxylase phosphate phosphatase) (16, 17, 34).

The enzyme complexes responsible for the oxidative decarboxylation of α-ketoglutarate and the branched chain ketoacids are similar to that for pyruvate. However, a regulatory kinase-phosphatase system of the $E_1$ component has not yet been identified.

The total pyruvate dehydrogenase complex comprises six enzyme protein components: the α and β units of $E_1$, $E_2$, $E_3$, specific kinase, and specific phosphatase for $E_1$. Thus, there are six possible hereditary deficiencies. At least eight confirmed cases of $E_1$ deficiency have been described (3–5, 10, 11, 30). Presumptive but unproven diagnoses of $E_2$ (7) and $E_3$ (13) deficiency have been described. We have previously described a case of the specific phosphatase deficiency (23). A specific kinase deficiency remains undescribed.

With specific clinical, biochemical and tissue enzyme data, we present a case in which we confirmed the diagnosis of deficiency of the $E_3$ component in both pyruvate and α-ketoglutarate dehydrogenase complexes, *i.e.*, dihydrolipoyl dehydrogenase deficiency.

## CASE REPORT

The proband was the first born of consanguineous Caucasian first cousins. The 20-year-old mother entered spontaneous labor and delivery at term after an uneventful pregnancy. The 3.1-kg male infant cried immediately after birth but had a congenital laryngeal inspiratory stridor. He developed normally and at 6 weeks of age weighed 4.8 kg. However, at the age of 8 weeks he became acutely sick over a period of 12 hr. At presentation he was pale and mottled in appearance. He exhibited irregular labored respirations with marked inspiratory stridor. He was lethargic and floppy; yet at times became extremely irritable and tense. He had a constant glassy stare. He made purposeless limb movements.

Physical examination revealed an afebrile, well hydrated child with a weight of 4.8 kg, height 56 cm, and head circumference 39 cm. His pulse rate was 100/min, blood pressure 100 mm Hg (systolic). Auscultation revealed no heart murmurs and clear lung fields. His liver was not enlarged. His muscle tone was increased with symmetrical brisk deep tendon reflexes. His gag reflex was present. He did not follow a light source and fundoscopy revealed bilateral optic atrophy. He did not have nystagmus. His anterior fontanelle was open (1.5 × 1.5 cm), and he was normotensive.

Initial investigation revealed a metabolic acidosis with elevated anion gap (pH 7.22; $pCO_2$ 33; bicarbonate 13; sodium

140; potassium 4.0; chloride 109). Cerebrospinal fluid was clear and sterile, as was a blood culture. Hemogram was normal.

Further investigations revealed a persistent elevation of blood pyruvate (0.240–0.370 mM (normal < 0.2 mM)), lactate (2.6–8.7 mM (normal < 2.0 mM)), α-ketoglutarate (0.103–0.147 mM (normal < 0.054 mM)), leucine (0.118–0.344 mM (normal < 0.098 mM)), isoleucine (0.060–0.189 mM (normal < 0.047 mM)), and valine (0.524 mM (normal < 0.199 mM)). Citrate was not elevated (<0.1 mM). During the months he was followed, the blood lactate levels showed wide fluctuations but were persistently above the normal range.

Occasional random plasma samples exhibited low blood glucose values (35–42 mg/100 ml). He underwent caloric deprivation for 8 hr on two occasions. Only on one occasion did he develop hypoglycemia (32 mg/100 ml). A glycemic response was observed with 1 g/kg oral L-alanine (37 mg/100 ml after 45 min).

Therapy was attempted with prolonged courses of thiamine (hydrochloride derivative 1 g/day for 4 weeks and the tetrahydrofurfural disulfide derivative 500 mg/day for 6 weeks). Neither a biochemical nor a clinical improvement was observed (Fig. 1). This was also the case with glutamine supplement (1.5 g/day for 6 weeks) or a high fat diet (120 cal/kg/day, 80% as corn oil).

His neurologic dysfunction was progressive and unremitting. He died after pulmonary aspiration at the age of 7 months at which time his weight was 5.3 kg. An autopsy was performed within 1 hr of death. Tissue samples of liver, muscle, kidney, and brain were obtained and immediately snap frozen in liquid nitrogen and then stored at −70° for later enzymatic analysis.

The major findings at autopsy were in the brain. Myelin loss and cavitation were found in discrete areas of the basal ganglia, thalami, and brain stem. The cerebral cortices appeared to be free of pathology.

## MATERIALS AND METHODS

Lactate (14), pyruvate (6), β-hydroxybutyrate (35), citrate (29), and α-ketoglutarate (2) were measured in whole blood samples extracted 1:1 with 0.6 N perchloric acid by enzyme spectrophotometric methods. Free fatty acids were measured in plasma by the method of Laurrell and Tibbling (15).

Liver, brain, kidney, and cardiac and skeletal muscle were obtained 30 min postmortem at autopsy after informed parental consent. The tissues were frozen in liquid nitrogen and stored at −100° until used for determinations. Control tissues were obtained at autopsy with informed parental consent within 2 hr of death from seven children below the age of 1 year were frozen in liquid $N_2$ and kept at −100° until used for determinations. Of these, four died after a chronic clinical course of debility and malnourishment secondary to congenital heart disease. The other three died acutely of undetermined causes. All determinations were done within 1 year of obtaining the tissues and no deterioration in enzyme activities has been observed on repeated testing during the period of storage. For enzyme determinations, small pieces of tissue were thawed, homogenized in 10 volumes ice-cold 0.25 M sucrose, 5mM Tris-HCl buffer, pH 7.4, and centrifuged at 600 × *g* for 10 min to remove cell debris. Glucose-6-phosphatase was measured by the method of Swanson (30), fructose-1,6-diphosphatase by the method of Pontremoli (20), phosphoenolpyruvate carboxykinase by the method of Roobol and Alleyne (26), and pyruvate carboxylase by the method of Crabtree *et al.* (8).

Pyruvate dehydrogenase was measured by the modified method of Taylor *et al.* (33) as described by Robinson and Sherwood (23). Pyruvate decarboxylase was measured by a modification of the method of Reed and Willms (22) as described by Blass *et al.* (4). Dihydrolipoyl dehydrogenase was measured by the method of Reed and Willms (21), modified in the following manner. Tissue was homogenized in 15 volumes ice-cold 0.01 M phosphate buffer, pH 7.0, and 50-μl aliquots of this were added to identical cuvettes containing 1 ml 0.15 M phosphate buffer, pH 8.1, 0.2 mM NADH at 37°. The decrease in absorbance was measured at 340 nm in a split beam spectrophotometer (Pye Unicam SP 800) on addition of 2.5 mM lipoamide to the test cuvette.

α-Ketoglutarate dehydrogenase was measured by a modification of the pyruvate dehydrogenase method described by Taylor *et al.* (33). Small pieces of tissue (100 mg) were thawed and homogenized in 10 volumes ice-cold buffer containing 10 mM potassium phosphate, 1 mM EDTA, 1 mM dithiothreitol, 1% fatty acid-free bovine serum albumin, pH 7.4. Aliquots of 0.1 ml were then added to the main chamber of 10-ml Erlenmeyer flasks containing 1 ml assay buffer (11 mM potassium phosphate, 1.1 mM EDTA, 12.8 mM $MgCl_2$, 10 mM $CaCl_2$, 1.6% bovine serum albumin (fat free), 1.2 mM dithiothreitol, 0.3 mM α-ketoglutarate labeled with 0.12 μCi α[1-$^{14}$C]ketoglutarate/ml at pH 7.4 and 37°). The reaction was stopped after 2 min by the injection of 1 ml 0.08 M citric acid and incubation was continued for 30 min so that the $^{14}CO_2$ evolved could be collected on hyamine-soaked filter paper inserts held in plastic cups attached to the rubber flask stopper. After this time, the filter strips were removed with forceps and placed in 10 ml toluene-ethanol (9:1 v/v) scintillation fluid, and the $^{14}C$ activity was measured in a liquid scintillation spectrometer. Citrate synthase was measured by the method of Shepherd and Garland (28).

## RESULTS

### BIOCHEMICAL INVESTIGATION DURING PATIENT'S LIFE

The biochemical observations of raised lactate, pyruvate, α-ketoglutarate, and branched chain amino acids, made shortly after admission, were highly suggestive of a lesion involving some aspect of oxidative decarboxylation. For this reason, initial treatment was carried out by oral administration of thiamine hydrochloride, 1 g/day for a period of 1 month. This seemed to have little therapeutic effect, judged either on clinical or a biochemical basis, there being a constantly elevated lactate with an almost daily variation which was not lowered during thiamine treatment (Fig. 1). Fearing malabsorption of the administered thiamine, the vehicle for administration was changed to the more readily absorbed tetrahydrofurfural disul-

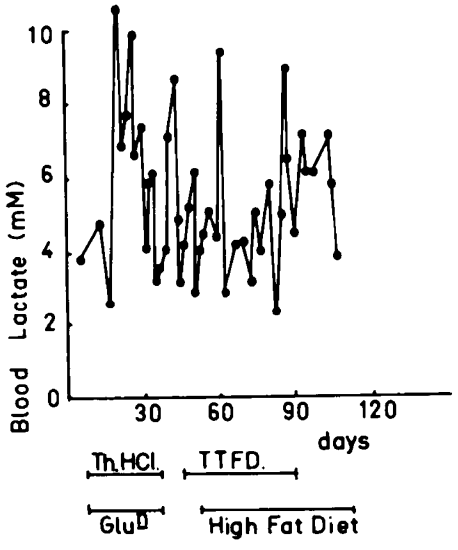


Fig. 1. Variations in blood lactate in the patient over a 4-month period during which different forms of therapy were attempted as described in the text. Th.HCl.: thiamine hydrochloride, 1 g/day; TTFD.: thiamine tetrahydrofurfural disulfide, 500 mg/day; Glu": glutamine, 1.5 g/day; high fat diet: high fat diet, 120 cal/kg/day, 80% as corn oil.

fide (TTFD) (18, 19), 500 mg/day for 6 weeks. This again had little effect on the disease process. Oral glutamine 1–2 g/day for 2 months also had no observable clinical effect.

The tendency towards low blood glucose that was evident even when the infant was receiving intravenous glucose (5 mg/kg/min) was investigated by institution of a fast lasting 8 hours. During this time, the blood glucose fell to 33 mg/100 ml with no change in the blood lactate concentration. Alanine administered at this point elicited a rise in blood glucose concentration to 37 mg/100 ml over a 45-min period after which the test was terminated. Administration of a low carbohydrate high fat diet for a period of 4 weeks did not bring about any clinical or biochemical improvement.

### POSTMORTEM INVESTIGATIONS

As moderate hypoglycemia was a feature of this patient, together with lactic acidosis, measurement of the key enzymes of gluconeogenesis was undertaken in liver. The activities of phosphoenolpyruvate carboxykinase, pyruvate carboxylase, and fructose-1,6-diphosphatase were all in the normal range, whereas glucose-6-phosphatase was slightly below normal but not low enough to be considered as type 1 glycogen storage disease (Table 1).

Total pyruvate dehydrogenase, measured after preincubation with $Ca^{++}$ to convert the enzyme to the active form, showed markedly reduced activity in the patient in every tissue tested compared to controls, giving only 10–20% of normal activity depending on the individual tissue. The most activity was obtained in cardiac muscle, where the pyruvate dehydrogenase activity is at its highest (Table 2). α-Ketoglutarate dehydrogenase activity was found to be deficient in activity in all tissues, the values obtained again being only a small percentage of those observed in control tissue (Table 3). The deficient activities seen in the proband were thought to be unlikely to be due to the patient's chronic debilitated condition, since control tissues from patients with a similar clinical course showed normal activities. On the other hand, citrate synthase activity was normal in those tissues tested (Table 4). In order to examine the activity of the pyruvate dehydrogenase complex further, the activities of the subcomponents were measured in tissues from the patient and control subjects (Table 5). Pyruvate decarboxylase appeared to be normal in liver, heart, kidney, brain, and skeletal muscle. On the other hand, dihydrolipoyl dehydrogenase was deficient in the patient in every tissue, having between 5 and 10% normal activity. We were not able to measure the activity of dihydrolipoyl transacetylase in these tissues.

## DISCUSSION

### OBSERVATIONS DURING LIFE

The consanguinity of the parents was an immediate index of suspicion for inherited metabolic disease. The raised pyruvate, lactate, α-ketoglutarate, and branch chain amino acid concentration observed in this child suggested some problems, either with thiamine as the common denominator or with the oxidative decarboxylases involved in α-ketoacid decarboxylation. On the other hand, there was the presence of moderate hypoglycemia, which might suggest a problem in the gluconeogenic pathway with lactate accumulation occurring as a result of impaired functioning of the Cori cycle. Leigh's subacute necrotizing encephalomyelopathy has been reported as having a distinct neuropathology and nonspecific manifestations including developmental delay, weight loss, weakness, and lactic acidosis in the early years of life. The pathologic findings are symmetrical punctate areas of myelin loss and capillary proliferation in the brain and basal ganglia (18, 32, 36). In some cases there is associated hypoglycemia and documented pyruvate carboxylase deficiency (12), and these cases appear to respond well to glutamine administration (32). Administration of thiamine or thiamine precursors in large doses is successful in some cases,

Table 1. *Activities of gluconeogenic enzymes in liver (micromoles per min per g liver)*[1]

| | Patient (*EK*) | Control subject | | |
|---|---|---|---|---|
| | | Mean | Range | No. |
| G6Pase | 1.01 | 2.55 ± 0.52 | (1.19–4.80) | 7 |
| F16Dpase | 1.03 | 1.40 ± 0.34 | (0.72–3.20) | 7 |
| PEPCK | 1.90 | 1.89 ± 0.45 | (0.69–3.23) | 7 |
| Pyruvate carboxylase | 1.57 | 0.91 ± 0.16 | (0.61–1.66) | 7 |

[1] G6Pase: glucose-6-phosphatase; F16Dpase: fructose-1,6-diphosphatase; PEPCK: phosphoenolpyruvate carboxykinase.

Table 2. *Total $Ca^{++}$-stimulated pyruvate dehydrogenase (nanomoles per min per g tissue)*

| | Patient (*EK*) | Control subjects |
|---|---|---|
| Liver | 107 | 715 |
| | | 790 |
| | | 800 |
| | | 705 |
| | | 698 |
| | | 1084 |
| | | 861 |
| Skeletal muscle | 125 | 1889 |
| | | 1350 |
| | | 1045 |
| | | 1340 |
| | | 1421 |
| Brain | 17 | 578 |
| | | 780 |
| | | 554 |
| | | 606 |
| Kidney | 118 | 800 |
| | | 1111 |
| | | 917 |
| Heart | 733 | 3372 |
| | | 2599 |

Table 3. *α-Ketoglutarate dehydrogenase activity (nanomoles per min per g)*

| | Patient (*EK*) | Control subjects |
|---|---|---|
| Brain | 15.6 | 187 |
| | | 211 |
| Liver | 8.0 | 1325 |
| | | 1558 |
| | | 775 |
| | | 841 |
| Muscle | 39 | 635 |
| | | 859 |
| | | 1180 |
| Kidney | 65 | 1373 |
| | | 2476 |
| | | 2208 |
| | | 2781 |
| Heart | 360 | 3538 |
| | | 2855 |

Table 4. *Citrate synthase activity (micromoles per g per min)*

| | Patient | Control subjects |
|---|---|---|
| Liver | 2.88 | 2.08 |
| | | 2.85 |
| | | 3.69 |
| | | 2.81 |
| | | 8.62 |
| | | 9.07 |
| Brain | 2.86 | 8.47 |
| | | 4.17 |
| | | 6.94 |
| | | 1.84 |
| | | 4.47 |
| Kidney | 5.76 | 5.12 |
| | | 5.70 |
| | | 4.54 |

Table 5. *Activities of pyruvate dehydrogenase components*[1]

| | $E_1$, nmol/min/g | | $E_3$, μmol/min/g | |
|---|---|---|---|---|
| | Patient (*EK*) | Controls | Patient (*EK*) | Controls |
| Liver | 2.28 | 1.17 | 0.260 | 7.41 |
| | | 6.94 | | 14.49 |
| | | 4.54 | | 13.20 |
| | | 1.44 | | 2.10 |
| | | 4.50 | | 7.98 |
| | | | | 6.21 |
| | | | | 4.71 |
| Kidney | 3.61 | 3.81 | 0.117 | 9.05 |
| | | 4.75 | | 5.85 |
| | | 4.62 | | 10.50 |
| | | 4.95 | | 2.62 |
| | | 2.73 | | |
| Brain | 3.75 | 5.71 | 0.080 | 3.75 |
| | | 2.04 | | 3.83 |
| | | 4.74 | | 2.48 |
| | | 5.10 | | |
| Skeletal muscle | 4.36 | 3.34 | 0.080 | 2.64 |
| | | 2.69 | | 2.75 |
| | | 3.48 | | 1.74 |
| | | 2.79 | | 4.79 |
| Heart | 7.19 | 14.78 | 0.353 | 33.6 |
| | | 7.80 | | 3.09 |
| | | 9.62 | | 5.47 |

[1] $E_1$: pyruvate decarboxylase; $E_3$: dihydrolipoyl dehydrogenase.

since in Leigh's disease it has been postulated that thiamine pyrophosphate phosphorylation is defective in the brain and that it is defective synthesis of thiamine triphosphate which is responsible for this disease (19).

The fact that we were not able to elicit any improvement in the patient's condition either with thiamine hydrochloride, thiamine derivatives (TTFD), or glutamine is highly suggestive that the lesion involved was neither a defect in thiamine metabolism nor in pyruvate carboxylase activity. However, the possibility of a pyruvate dehydrogenase defect as described by Cederbaum *et al.* (7) encouraged us to attempt therapy with a high fat/low carbohydrate diet since a greater reliance on oxidative energy derived from fat should lower the dependence on glycolysis and pyruvate oxidation, therefore lessening the rate of lactate production. This did not appear to be successful.

## POSTMORTEM STUDIES

The finding of normal activities of the key gluconeogenic enzymes in the liver of the patient confirmed our suspicion that a primary block did not exist in the gluconeogenic pathway. A primary block, however, was apparent in both pyruvate and α-ketoglutarate dehydrogenases. This coupling of two enzyme deficiencies has been described before by Haworth *et al.* (13). Although they were not able to document this, they proposed that a deficiency existed in the $E_3$ component of pyruvate dehydrogenase, the dihydrolipoyl dehydrogenase. Since this enzyme has been shown, at least in one animal species, to be a common component for both pyruvate and α-ketoglutarate dehydrogenase complexes (27), its absence would cause a double deficiency of the type we have found in this child. We found a deficiency of $E_3$ in every tissue tested, compared with control tissues. The fact that neither pyruvate decarboxylase, the $E_1$ component, nor citrate synthase was deficient emphasized that we were not dealing with any generalized mitochondrial enzyme deficiency.

The major problem that comes to mind in the case of a severe deficiency such as this is how can a child survive for 8 weeks, apparently well, without requiring hospitalization. The two sibs described by Haworth survived 4 and 11 weeks, respectively, without causing enough concern for the parents to seek medical attention. If there is a deficiency in either of the $E_1$ or $E_2$ enzymes of the pyruvate dehydrogenase complex, the resulting pyruvate dehydrogenase defect compromises only the glycolytic source of acetyl-CoA units for the Krebs cycle, and alternative sources of acetyl-CoA may be generated by fatty acid oxidation in most tissues with the exception of the brain. The brain must seek alternative fuel for oxidative metabolism in the form of either ketone bodies or branched chain amino acids. Thus, it is conceivable that with careful management a child with such a deficiency might survive for some years, albeit neurologically compromised. With a deficiency involving $E_3$ and therefore both pyruvate and α-ketoglutarate dehydrogenases (and perhaps the branched chain ketoacid dehydrogenase), not only the input of acetyl-CoA units derived from pyruvate is affected but also the actual functioning of the Krebs cycle, so that no substrate can provide a steady source of reducing power for oxidative phosphorylation. The ability of these children with $E_3$ deficiencies to exist through the first few weeks of life without medical treatment can be explained only by postulating that the newborn infant is highly dependent on glycolysis as a source of high energy phosphate and gets into trouble only when the slow transition to a more oxidative mode of metabolism cannot be achieved. The low levels of pyruvate dehydrogenase which Cremer and Teal (9) observed in the brains of newborn rats and which we (24) observed in several tissues of newborn rhesus monkeys underline the credibility of this hypothesis.

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Alveolar macrophage
lysosomal enzymes
lysosomes
oxidative enzymes
phospholipids

# Maturation of the Rabbit Alveolar Macrophage during Animal Development. II. Biochemical and Enzymatic Studies

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## Summary

**Alveolar macrophages (AMs) obtained from 1-day-old rabbits showed high levels of phospholipid, protein, DNA, and RNA compared to those obtained from 7-day-old, 28-day-old, or adult rabbits. The surfactant material released in alveoli during the perinatal period is extensively phagocytosed by AMs, and appears to be primarily responsible for high phospholipid content of these cells. The high protein, DNA, and RNA levels of AMs may result from phagocytosis of cellular debris.**

**Of the six lysosomal enzymes of AMs studied, acid phosphatase (AP) and lysozyme activities showed a decline in the first postnatal week. In AP this was followed by no significant change. A steady rise in lysozyme activity after the seventh postnatal day was observed. The activities of cathepsin D and DNase did not show significant changes, whereas β-glucuronidase and RNase activities of AMs increased significantly within the first postnatal week.**

**The activities of glucose-6-phosphate dehydrogenase (G-6-PD), 6-phosphogluconate dehydrogenase (6-PGD), lactate de-**