factors activated by the administered hormone. Perhaps an increased release of growth hormone took place under the influence of adrenaline. Growth hormone is known as a suppressor of liver TAT activity[15].

Our results in intact animals confirm the results by BARTHOLINI et al.[8]. These authors suggested, as one of the possibilities to explain the stimulatory effect of catecholamines on liver TAT activity, a mediation through glucocorticoid hormones, delivered under the influence of catecholamine administration secondarily. We are of the opinion that our results testify strongly in the sense of this suggestion, although a 100% proof is not easy to produce.

Our data concerning plasma glucose and liver glycogen are shown here merely as proofs for full activity of the applied adrenaline.

Table II. Liver tyrosine-α-ketoglutarate transaminase activity in nmoles of product/1 min/1 mg, and plasma glucose in mg/100 ml of adrenalectomized rats (means of 6 values per group ± S.E.)

	Saline ± 90 min	P	Adrenaline + 90 min
TAT	40.3 ± 3.5	< 0.05	27.0 ± 3.6
Glucose	117 ± 4	< 0.02	150 ± 11

Zusammenfassung. Es wird eine Steigerung der Tyrosin-α-Ketoglutarat-Transaminase-Aktivität in der Rattenleber durch Adrenalin bestätigt und eine Verminderung der Transaminase-Aktivität nach Adrenalektomie durch Adrenalin nachgewiesen.

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Effect of Age on the Activity and Citrate Inhibition of Malate Dehydrogenase of the Brain and Heart of Rats

The enzyme, malate dehydrogenase (MDH), is known to exist in two isoenzymic forms, cytoplasmic (cMDH) and mitochondrial (mMDH)[1-3]. The cytoplasmic malate dehydrogenase is essential for gluconeogenesis as it converts the malate to oxaloacetate and then to phosphoenolpyruvate in the cytoplasm[4]. The mitochondrial malate dehydrogenase is required for continuous operation of the Krebs cycle within the mitochondria. The two forms of MDH are NAD^+dependent and are also under the control of two separate genes[5,6]. The present investigation centers around the change in the isoenzymes of MDH of the brain and the heart of young and old rats and a possible control mechanism of the enzyme with citrate, one of the intermediates of the Krebs cycle.

Materials and methods. Animals. The female albino rats used were of Wistar strain taken from the rat colony maintained at 24 ± 2°C. They were fed a commercial rat diet (Anidiet 'A', Chelsea Chemical Laboratory, Poona, India) and were also given gram (*Cicer arietinum*) on alternate days and water ad libitum. The mature adult (22 weeks) and old (96 weeks) rats were killed at a fixed time on successive days.

Preparation of the tissues. The rats were killed by dislocation of the neck. Brain (cerebral hemispheres) and heart (ventricles) were removed immediately, washed in ice-cold sucrose solution (0.25 M) and weighed in a Unimatic CL-41 single pan balance. A 10% homogenate (w/v) of each tissue was prepared in cold 0.25 M sucrose using a Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate was then centrifuged at 800 × g for 20 min in MSE high speed refrigerated centrifuge to remove the nuclei and cell membranes. The supernatant was further centrifuged at 10000 × g and the supernatant

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Activities (Units/g wet wt.) and percent inhibition by citrate (6.66 μM/ml) of cMDH and mMDH of the brain and heart of rats of young and old age

Tissue	Age (weeks)	Cytoplasmic malate dehydrogenase: Units/g wet wt. × 10^4	p	Inhibition (%)	p	Mitochondrial malate dehydrogenase: Units/g wet wt. × 10^3	p	Inhibition (%)	p
Brain	22	3.44	0.01	14.25	0.001	1.30	0.01	42.00	0.20 (NS)
	96	2.77		32.17		0.92		45.82	
Heart	22	2.30	0.05	31.50	0.20 (NS)	4.80	0.05	42.90	0.20 (NS)
	96	2.02		27.12		4.20		43.12	

Each value represents the mean of 4–5 animals. P values of 0.05 or lower were taken as significant signific. NS, notant.

so obtained was used for the assay of cMDH. The pellets were suspended in sucrose (0.25 *M*) and used for the assay of mMDH.

Spectrophotometric assay of MDH. The method of assay for MDH was the same as that of OCHOA[7] with a little modification. The assay mixture included 2.49 ml of water, 0.3 ml of phosphate buffer (0.1 *M*, pH 7.4), 0.01 ml of NADH, 0.1 ml of supernatant and 0.1 ml of oxaloacetate (0.25 m*M* final concentration). The total volume of the assay mixture was 3.0 ml. The concentration of NADH was so adjusted that 0.01 ml gave the O.D. of 0.5 at 340 nm. The reaction was started by adding the oxaloacetate and the decrease in O.D. was recorded at 340 nm in SP-500 spectrophotometer. The percentage inhibition of cMDH and mMDH activities were studied by using citrate concentration of 6.66 μM/ml. This concentration of citrate was decided upon from the inhibition data of preliminary experiments producing the inhibition between 30–50%. The total volume of assay mixture was adjusted by decreasing the volume of water. The activity of MDH was expressed as units/g wet wt. of the tissue. The level of significance between the two sets of data were calculated according to SIEGEL[8].

Results and discussion. The Table shows that the activity of both the isoenzymic forms of malate dehydrogenase, cMDH and mMDH, is significantly greater in the brain and heart of 22-week-old rats as compared to that of the 96-week-old rats. Also, the activity of cMDH is higher in both the tissues than that of the mMDH at both the ages. The activity of mitochondrial malate dehydrogenase was inhibited by citrate to a greater extent than that of the cMDH. However, there was no change in the percentage inhibition of mMDH activity in the brain and heart of 22- and 96-week-old rats. The activity of cMDH was inhibited by citrate at both the ages, but the percentage of inhibition was significantly greater in the brain of 96-week-old rats than that of the 22-week-old rats. There was no significant difference in the percent inhibition of the activity of cMDH of the heart of the 22- and 96-week-old rats.

The greater activity of the cMDH in both the aerobic tissues, brain and heart, indicates that they have efficient glycolytic pathway and that these tissues can tolerate the absence of oxygen during young adulthood (22-week-old) as compared to that of the older rats (96-week-old). This finding is consistent with the earlier observations[9,10]. The activity of mitochondrial malate dehydrogenase is highly inhibited by citrate as compared to that of the cMDH at both the ages of rats. This may suggest that the accumulation of citrate within the cell, which is an intermediate of the Krebs cycle, has some controlling mechanism in the operation of the cycle[11].

Zusammenfassung. Es wurde die cytoplasmatische und mitochondrische Malate Dehydrogenase im Gehirn und Herz junger und alter Ratten spektrophotometrisch untersucht und signifikante Aktivitätsunterschiede gemessen.

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The Effect of Maltose on the Aldosterone Activated Sodium Transport of the Frog Bladder

Little attention has been focused on the metabolism of circulating disaccharides, while the absorption of dietary disaccharides has been studied by many reseachers. Recently some studies of metabolism of circulating disaccharides, especially maltose, were reported[1,2]. They suggested that circulating maltose, unlike lactose and sucrose, might be hydrolyzed by extraintestinal maltases and subsequently metabolized. In the present study, the possibility that maltose was utilized as a substrate after permeating across a cell membrane is discussed.

Materials and methods. The urinary bladder of the frog, *Rana catesbiana*, was used as a model. Frogs were kept at 8°C to keep them in a steady state. After double pithing of the frogs, the bladders were excised and halved. Each hemibladder was mounted in a lucite chamber and incubated for 14 h in Ringer's solution containing penicillin G (1 mg/ml) and streptomycin (1.6 mg/ml) to make the bladder steroid-free. After this overnight incubation, tissue 11-OHCS decreased to approximately 40% of the control[3]. The experiments were carried out at room temperature. The area of the chamber orifice was 3.16 cm^2. To prevent mechanical distortion of the bladder membrane, both orifices of the chambers were covered by nylon mesh and the bladder was sandwiched between 2 discs of nylon mesh. The short-circuit current (SCC) was measured by the method of USSING and ZERAHN[4]. The composition of the Ringer's solution was: NaCl, 111 m*M*; KCl, 3.5 m*M*; $CaCl_2$, 0.9 m*M*; $MgCl_2$, 1.5 m*M*; NaH_2PO_4, 1.9 m*M*; and Na_2HPO_4, 8.1 m*M*. The osmolality was 232 mOsm/L and the pH was 7.4.

After 14 h incubation in glucose-free, antibiotic-containing Ringer's solution, the chambers were washed 3 times with fresh Ringer's solution and then maltose was added to one chamber at the concentration of 10 m*M* as a substrate, while glucose was added to the paired chamber at the concentration of 10 m*M* as the control. After the SCC reached a plateau, D-aldosterone (Sigma Co. Ltd.) was added to the chambers at the concentration of $1 \times 10^{-6} M$.

Before carring out the present experiment, the substrate-dependency of the aldosterone action on the sodium transport was checked. As shown in the Figure A, D-aldosterone increased the SCC when glucose was used as a substrate, but showed no effect on the SCC when the substrate-free Ringer's solution was used. This result is consistent with the data of EDELMAN[5] who used *Bufo marinus*, and indicates that the aldosterone effect on the sodium transport is a substrate dependent action in both species.

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