remain constant in the present experiments, if the binding site were specific.

It is to be realized that – on the assumption of non-specificity – the above formula for the approximate calculation of K may loose its full validity when the concentration of the cross-reacting steroids approaches or exceeds the concentration of albumin. Even then, however, the conclusion regarding the presence of a single, strong and non-specific binding site can be fully maintained; this conclusion may be reached even if only the behavior of B/F ratios is considered.

The 3rd experiment (fig. 3) showed that undiluted plasma had a similar effect on the binding of ^{3}H-E_1S to albumin as a high concentration (approximately 10^{-4} mol/l) of DHAS (fig. 1). Since the content of endogenous DHAS in the plasma pool under investigation did not reach this level (being 4×10^{-6} mol/l), other ligands must also have participated in filling up the strong binding site.

On the basis of the above experiments it may be concluded that the binding of E_1S (and very probably of other steroid sulfates) is less strong in vivo (having a lower K) than could be inferred from earlier binding studies in the absence of other plasma constituents[2-4]. This seems to be due to the preferential occupation of the strong binding site by other ligands occurring in blood at high concentrations and with high affinities to albumin (fatty acids and other ligands[5]). Consequently it may be expected that the K value in an individual subject and at a given moment is dependent on the concentration of other ligands existing at that time.

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Alanine and aspartate aminotransferases in normal and denervated skeletal muscle

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Summary. Activities of alanine and aspartate aminotransferases are maintained during the first 5 weeks of growth, but decrease subsequently in normal chick gastrocnemii. In sciatectomized muscles, a 5-fold elevation in these enzymes reveals increased utilization of amino acids as a compensatory metabolic support during denervation atrophy.

Key words. Chicken; skeletal muscle; denervated muscle; alanine aminotransferase; aspartate aminotransferase; denervation atrophy; amino acid utilization.

The significance of motor innervation in skeletal muscle growth and metabolism has been emphasized by many workers[2-4]. During postembryonic growth a switchover occurs from a predominantly glycolytic to an oxidative mode of energy generation in normal muscle[5-7], but the denervated muscle registers a loss of glycolytic and oxidative enzymes[8-11], largely due to poorer availability of glycogen. Asotra[10,11] has recently reported that such a metabolic transformation can be regulated by muscle glucose 6-phosphatase. The loss of intrafibrillar lipids by extrusion rather than intense lipase activity in denervated muscle is also well documented[12]. Thus, during growth and especially denervation atrophy, the utilization of substrates other than glycogen and lipids in muscle metabolism is expected to increase. According to Chang and Goldberg[13,14] and Lehninger[15] the normal muscle can utilize the transamination products of amino acids in glycolytic and oxidative metabolism. Whether transamination activities in skeletal muscle are modified upon denervation is not yet known. In this paper we report on the changes in activity levels of alanine and aspartate aminotransferases, which serve as indices of the utilization of the 2 amino acids, in normal and denervated chick gastrocnemii up to 8 weeks of postembryonic growth.

Materials and methods. 1-day-old chicks of *Gallus domesticus* (white leghorn variety) procured from the Government Poultry Farm, Simla were kept on standard chick mash (Hindustan Lever Ltd, Bombay) and water ad libitum under normal laboratory conditions. Chicks were divided into 2 groups; one group served as the normal controls while members of the second were unilaterally denervated on the 5th day of life by excision of a 5 mm piece of sciatic nerve aseptically after s.c. injection of 0.2 ml local anesthetic Xylocaine (5% v/v). Nebasulf powder was sprinkled over the operated area and the wound stitched. Normal and denervated chicks of both sexes were randomly selected and sacrificed by decapitation 1, 2, 3, 4, 5, 6, 7 and 8 weeks postdenervation. At least 18 chicks were used at each stage of investigation and 3 gastrocnemii pars externa, media and interna excised, cleared of fat and connective tissue and weighed. Muscles were homogenized in 0.1 M phosphate buffer, pH 7.4 with 10 strokes using a hand operated glass-and-teflon homogenizer kept on ice. Homogenate was centrifuged at 14,000 × g for 10 min at 4 °C and suitable aliquots of the supernatant used for the bioassay[16] of alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransferase (EC 2.6.1.1) with DL-alanine and L-aspartate as the respective sub-

Table 1. Alanine aminotransferase activity in mmole pyruvate per g fresh tissue weight per min incubation at 37 °C ± SD

Muscle	Postdenervation period in weeks							
	1	2	3	4	5	6	7	8
Normal								
M. gastrocnemius pars externa	3.082 ± 1.105	2.919 ± 0.979	3.779 ± 0.485	2.835 ± 0.652	2.746 ± 0.557	1.981 ± 0.587	1.109 ± 0.157	1.167 ± 0.239
M. gastrocnemius pars media	1.899 ± 0.278	1.441 ± 0.125	2.855 ± 0.317	0.937 ± 0.062	1.000 ± 0.032	1.102 ± 0.040	0.967 ± 0.186	1.032 ± 0.097
M. gastrocnemius pars interna	4.209 ± 0.745	3.637 ± 0.285	3.545 ± 0.677	3.248 ± 0.372	2.169 ± 0.239	1.542 ± 0.142	1.052 ± 0.267	1.011 ± 0.092
Denervated								
M. gastrocnemius pars externa	3.851 ± 0.913	2.117 ± 1.005	4.812 ± 1.722	5.857 ± 2.583	2.037 ± 0.573	5.278 ± 1.138	6.492 ± 1.691	2.748 ± 0.263
M. gastrocnemius pars media	2.963 ± 0.461	0.976 ± 0.065	2.624 ± 0.788	2.496 ± 0.739	1.804 ± 0.359	1.340 ± 0.105	5.089 ± 2.467	2.256 ± 0.645
M. gastrocnemius pars interna	3.156 ± 0.235	1.166 ± 0.042	2.606 ± 0.885	3.066 ± 1.703	1.952 ± 0.330	1.834 ± 0.272	4.260 ± 1.376	3.420 ± 0.878

Table 2. Aspartate aminotransferase activity in mmole pyruvate per g fresh weight per min incubation at 37 °C ± SD

Muscle	Postdenervation period in weeks							
	1	2	3	4	5	6	7	8
Normal								
M. gastrocnemius pars externa	3.368 ± 0.322	2.642 ± 0.165	4.186 ± 1.046	2.144 ± 0.119	2.776 ± 0.206	1.925 ± 0.630	0.963 ± 0.152	1.167 ± 0.159
M. gastrocnemius pars media	3.652 ± 0.541	2.252 ± 0.189	3.121 ± 0.375	1.583 ± 0.143	2.174 ± 0.342	1.419 ± 0.384	0.849 ± 0.164	1.084 ± 0.193
M. gastrocnemius pars interna	2.395 ± 0.288	2.834 ± 0.223	3.729 ± 0.657	2.440 ± 0.312	2.266 ± 0.230	1.521 ± 0.143	0.935 ± 0.291	1.319 ± 0.218
Denervated								
M. gastrocnemius pars externa	3.903 ± 0.833	3.824 ± 0.655	4.266 ± 0.852	4.800 ± 1.074	1.829 ± 0.559	3.407 ± 0.785	5.215 ± 1.445	3.238 ± 0.560
M. gastrocnemius pars media	3.118 ± 0.579	1.756 ± 0.323	2.603 ± 0.822	2.145 ± 0.556	1.615 ± 0.329	3.289 ± 0.718	4.160 ± 0.921	1.583 ± 0.179
M. gastrocnemius pars interna	3.420 ± 0.305	1.523 ± 0.085	2.688 ± 0.768	2.687 ± 0.813	1.721 ± 0.497	2.037 ± 0.589	4.021 ± 1.366	1.332 ± 0.184

strates and α-ketoglutarate, pH 7.4. The resultant pyruvate was colorimetrically determined using 2,4-dinitrophenylhydrazine; absorbance was recorded at 550 nm in a Carl Zeiss VSU-II spectrophotometer. Enzyme activities were expressed in terms of mmole pyruvate generated per g fresh tissue per min at 37 °C ± SD.

Observations and discussion. Transamination of amino acids provides glucogenic precursors[17] and, conversely, alanine can be synthesized in muscle from blood glucose[18]. Alanine utilization, as revealed by alanine aminotransferase activity, is quite high until the 5th week of postembryonic growth in normal chick gastrocnemii but decreases with further growth (table 1). Denervated pars externa and media show consistently higher enzymic activity which becomes 5–6 times higher than normal by the 7th week. These results suggest that alanine originating from degradation of the branched chain amino acids leucine, isoleucine and valine[19] provides the necessary glycolytic substrate during denervation atrophy when elevated glucose 6-phosphatase restricts both the glycolysis of glycogen-derived intermediates and oxidative metabolism in muscle[11]. Decreased enzymic activity in the 2 muscles at the 2nd week postdenervation corresponds, however, to muscle fiber hypertrophy[11,20]. Subnormal alanine aminotransferase in the denervated pars interna until the 6th week coincides with low glycogen content and phosphorylase activity[11] thus showing a delayed utilization of alanine for glycolysis.

According to Goldberg[21] alanine and aspartate are catabolized to the same extent by normal rat diaphragm. The present results show, however, that normal pars media of chick utilize more but pars interna less aspartate than pars externa during early postembryonic growth (table 2). Supranormal activity levels in pars externa but subnormal aspartate aminotransferase levels in pars media and interna until the 6th week postdenervation, as well as the fact that the transamination rate for alanine is generally higher than that for aspartate in the sciatectomized gastrocnemii show that aspartate is spared during denervation atrophy. In the light of the differential elevations in alanine and aspartate aminotransferases of dystrophic[22] and radiocalcium-treated muscle[23], the present results on denervated gastrocnemii show that an innate capability to utilize amino acids selectively is of pivotal significance in skeletal muscle in the regulation of its energy metabolism during normal growth und under pathological conditions.

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Modulation of 3-hydroxy-3-methyl glutaryl CoA reductase by 2,3-diphosphoglyceric acid

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Summary. Rat liver microsomal 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase was activated by 50% at a concentration of 0.4 mM 2,3-diphosphoglyceric acid (DPG) and by 11-fold at 10 mM DPG. DPG also prevented the inactivation of HMG-CoA reductase by ATP and Mg^{++}. Rat liver microsomal HMG-CoA reductase prepared in the presence of 1 mM DPG was significantly more active than when prepared in the absence of DPG. Activation of the enzyme by DPG and protection of the enzyme against inhibition by ATP and Mg^{++} by DPG were also observed with solubilized HMG-CoA reductase.
Key words. Cholesterol; 2,3 diphosphoglyceric acid; 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase.

Red blood cell 2,3-diphosphoglycerate plays an important role in oxygen transport in mammals, binding to hemoglobin and thus reducing its affinity for oxygen[2]. Noble et al.[3] developed 2 rat strains by genetic selection based upon relative levels of red cell DPG. Starting with outbred hooded rats they produced one set of rats with high cell DPG and another with low DPG. They showed that rats with low red cell DPG had significantly higher plasma cholesterol levels than rats with high red cell DPG. Alterations in red cell DPG were also associated with parallel changes in red cell ATP. There are a number of reports showing regulation of HMG-CoA reductase by ATP and Mg^{++}[4-10]. HMG-CoA reductase is a major regulatory enzyme in the biosynthesis of cholesterol[11-14].

The present study was undertaken in order to determine whether DPG has any direct effect on HMG-CoA reductase and if there is any interrelationship between DPG and ATP with regard to HMG-CoA reductase activity.

Materials and methods. DL-[3-^{14}C]-HMG-CoA (specific activity 18.5 μCi/mol) was purchased from New England Nuclear. Unlabeled HMG-CoA (from PL Biochemicals, Milwaukee, WI) was added to the radioactive substrate to give a specific radioactivity of 1.1 μCi/mol, and this substrate was used in all the experiments. DL-[4-^{3}H] mevalonic acid was purchased from Amersham/Searle. Unlabeled mevalonic acid and 2,3-diphosphoglyceric acid were obtained from the Sigma Chemical Company.

Male Sprague-Dawley rats, 10 weeks of age, were used for all experiments. The rats were maintained ad libitum on a diet of rat chow and tap water. For 2 weeks one group of animals was subjected to the following lighting cycle: dark, 4.00 to 16.00 h local time; light, 16.00 to 4.00 h. For another group of animals this lighting was reversed. The animals were killed at the midpoint of dark phase (10.00 h) for the first group of animals and at the midpoint of the light phase (22.00 h) for the second group of animals.

The livers from these rats were homogenized in 0.04 M phosphate buffer, pH 7.2, containing 0.1 M sucrose, 0.05 M KCl and 0.01 M dithioerythritol. The homogenate was centrifuged twice at 20,000 × g for 12 min each time. The 20,000 × g supernatant was centrifuged at 105,000 × g for 1 h and the pellet suspended in the above buffer and centrifuged at 105,000 × g for a second time. The pellet from the second centrifugation was suspended in the above buffer for use in studying the effect of DPG and other compounds.

In the experiment where the microsomes were prepared in buffer containing DPG, the preparation of the microsomes was as described above except that 1.0 mM DPG was added. In the latter case the liver was divided into 2 portions; one part was homogenized in buffer only and the other part homogenized in the same buffer containing 1.0 mM DPG.

Table 1. Activation of rat liver microsomal HMG-CoA reductase activity by DPG

DPG (mM)	HMG-CoA reductase activity (pmoles mevalonic acid formed/min/mg protein)
None	179
1 mM	633
2.5 mM	933
5 mM	1320
10 mM	2050
20 mM	2110

Microsomes were prepared from the rat liver in buffer without EDTA. Assay conditions were as described under methods. Experiments were done in triplicate.