Creatine Phosphokinase Activity in Heart and Skeletal Muscle of Foetal Rabbits.* (25382)

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The evidence which places phosphocreatine and creatine phosphokinase as necessary physiological components of the contraction-relaxation system of muscle is equivocal(1). If it is assumed that phosphocreatine and creatine phosphokinase are necessary for functional contractility, then this relationship should be evident in a close correlation between development of contractility and enzymatic activity in developing foetal muscle. The present investigation suggests that such a close correlation exists in skeletal muscle, but not in heart muscle.

Methods. White New Zealand rabbits purchased from local supplier were used. The rabbits were bred while under observation and foetal ages were calculated from this time. At desired time, animals were given an intravenous injection of sodium pentobarbital (40 mg kg). The foetuses were removed and placed in cracked ice. The hearts and gastrocnemii were removed from 6 to 10 foetuses and pooled. The tissue was removed under a dissecting scope when necessary (14 and 16 day foetuses). Foetal tissue was collected for study only from those rabbits which had from 6 to 10 foetuses. In several instances only 1, 2 or 3 foetuses were present. They appeared larger than usual, and were discarded as well as foetal tissue from rabbits with more than 10 foetuses. The method for creatine phosphokinase extraction and activity determination has been described(2). Creatine and phosphocreatine were determined by the method of Ennor and Rosenberg(3).

Results. Creatine phosphokinase activities of heart and skeletal muscle during foetal development are summarized in Table I. The enzyme was not detected in heart or skeletal muscle at 14 days of foetal development. Enzymatic activity was first detected in heart extracts on 16th gestational day, when no creatine phosphokinase could be detected in skeletal muscle. At 20th day of foetal development, enzymatic activity of heart tissue increased approximately 5 times, but was not demonstrable in skeletal muscle at this late stage of development. Creatine phosphokinase activity was first detected in skeletal muscle on the 23rd day of gestation.

The original assumption was that appearance of creatine phosphokinase activity should correlate with development of contractility. Therefore, additional tests were made on skeletal muscle at 20th and 23rd day of foetal development. These tests revealed that the gastrocnemius muscle of 20 day old rabbit foetus contained creatine, no phosphocreatine, no creatine phosphokinase and did not respond to direct electrical stimulation. At this foetal age when the gastrocnemius gave no evidence of response to electrical stimulation, the same stimulus to the front legs elicited a good quick twitch-response. At 23rd gestational day, the gastrocnemius contained creatine, small amounts of phosphocreatine, first evidence of creatine phosphokinase and responded to direct electrical stimulation with a

TABLE I. Creatine Phosphokinase Activity in Heart and Skeletal Muscle of Foetal Rabbits.

		μM phosphorus transferred from ATP to creatine/g tissue dry wt/min.	
Foetal age, days	No. pooled samples	Gastrocnemius	Heart
14	8	0†	0
16	6	0	32 ± 10*
20	9	0	186 ± 15
23	6	68 ± 8	209 ± 22
30	10	382 ± 22	486 ± 20
Age following birth, days			
30	8	1600 ± 72	750 ± 36
60	9	1690 ± 64	780 ± 44
90	9	1180 ± 56	710 ± 40

* $\sqrt{\frac{\Sigma d^2}{N(N-1)}}$.

† Minimum limit of detectable activity is approximately 8 μM phosphorus transferred/g tissue/min.

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weak slow worm-like contraction followed by an extremely slow incomplete relaxation. At 30 days of gestation, representing tissue collected immediately following birth or from the mother on 30th gestational day, creatine phosphokinase activity of the heart was approximately 60% of the adult level, while enzymatic activity of skeletal muscle was approximately 30% of adult level.

During 30 days following birth, the creatine phosphokinase activity of skeletal muscle rose rapidly, remained high through 60 days and fell to the adult level at 90 days, after which time it remained constant for at least 9 months. Enzymatic activity of heart tissue reached the adult level at 30 days of age and remained stable at this level.

Discussion. While some attempt was made to correlate appearance of phosphocreatine with onset of muscle contraction(4), Herrmann and Cox(5) concluded that in chick embryonic muscle, phosphocreatine appeared at a time when contractility was well developed. However, Koschtojanz and Rjabinowskaja(6) found that phosphocreatine in skeletal muscle of rabbit foetuses was first demonstrable at approximately the time of beginning response to pinprick.

In our investigation, the abrupt appearance of the enzyme creatine phosphokinase in gastrocnemius correlates well with development of contractility as evidenced by response to electrical stimulation. However absence of the enzyme in the beating heart muscle of 14 day rabbit foetuses militates against interpreting this correlation as evidence that the enzyme is necessary for contractility. Dwinnell(7) gave time of apparance of first heart beats in rabbit embryos as 8½ days, a full week before appearance of the enzyme.

Three possibilities should be considered. 1. Phosphocreatine and creatine phosphokinase are necessary for contractility in skeletal muscle but not in heart. This does not seem reasonable, for in all studies involving contractile proteins, those from skeletal muscle function qualitatively in the same manner as proteins of heart(8). 2. Appearance of creatine phosphokinase and phosphocreatine are correlated with development of nervous innervation and control rather than contractility. This problem is presently under investigation. 3. Development of contractility in muscle is dependent only on creatine phosphokinase which is closely associated with actin(9). The presence of actin bound enzyme appears to be involved in the phosphate transfer which takes place during actin polymerization and for formation of the contractile F-actin-myosin(10). The possibility exists that the actin bound creatine phosphokinase may be undetected by the present procedure due to the small amount of foetal tissue available for analysis. The sarcoplasmic creatine phosphokinase(11), in larger amounts, is detectable by our methods, although it may function only indirectly in muscle activity. This problem is presently being investigated and an attempt is underway to develop a more sensitive method of creatine phosphokinase assay.

Summary. Quantitative analysis of creatine phosphokinase in rabbit foetal tissue revealed that the enzyme is first detected in the heart on 16th day of gestation and in the gastrocnemius muscle on 23rd gestational day. There appears to be reasonable correlation between appearance of creatine phosphokinase and development of contractility in skeletal muscle but such a correlation does not exist in heart muscle.

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