lation of cell division by PHA does not appear to require induction of delayed hypersensitivity in the rat.

Summary. A method of culturing rat leukocytes with phytohemagglutinin has been described. The variation of mitotic indices of 16 cultures from 48 normal Sprague-Dawley rats has been shown. The mean mitotic index was 1%, which is comparable to values for normal cultures of human leukocytes. Induction of delayed hypersensitivity to PHA did not enhance proliferation in this species.

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Serum Phosphocreatine Kinase in Hereditary Muscular Dystrophy and Cardiac Necrosis of Syrian Golden Hamsters.* (29610)

MONIKA EPPENBERGER, C. W. NIXON, J. R. BAKER AND F. HOMBURGER
Bio-Research Institute, Cambridge, Mass.

Markedly increased serum phosphocreatine kinase activity has been reported by Aebi *et al*(1) and Dreyfus and Schapira(2) in the Duchenne type of muscular dystrophy in children even before the onset of clinical manifestations, and moderate increases in serum enzyme level prevail in heterozygote carriers of the gene which transmits this disease in man(3,4,5). In this paper are reported elevated serum phosphocreatine kinase levels in Syrian hamsters with hereditary muscular dystrophy and cardiac necrosis(6), and changes of serum enzyme levels which occur during the progress of the dystrophy and cardiopathy.

Materials and methods. Sixty dystrophic (BIO 1.50 and BIO 14.6) male and female hamsters divided among 6 age groups, 10 heterozygote F_1 hamsters from a cross between BIO 1.50 and other (non-dystrophic) strains, 50 non-dystrophic hamsters of both sexes and similarly divided as to age, were studied. Ages of the controls ranged from 13 to 458 days and those of the dystrophic animals, from 13 to 230 days. The hybrids were from 129 to 220 days old. The presence or absence of dystrophy was determined by histologic examination of the heart and 6 different muscles of each animal. The histological criteria for diagnosis of dystrophy were presence of rowing nuclei, inequality of fiber

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TABLE I. Serum Phosphocreatine Kinase Activity in Non-dystrophic, Heterozygote F_1 and Dystrophic Hamsters.

Animals	Age, days	No. animals	Enzyme activity* Means†	Range
Non-dystrophic	13–458	50	23.2 (13)	6.5 91 ‡
Heterozygote F_1	129–220	10	23.5 (15)	7.4 53.7
Dystrophic	13– 15	10	107 (19)	79 129
"	20– 25	10	157 (99)	38.3 349
"	26– 70	10	731 (616)	162 2070
"	71–120	10	707 (611)	132 1950
"	121–150	10	221 (95)	84 377
"	151–230	10	182 (175)	12.9 474

* Expressed in μMol/min/1000 ml at 37°C. † Standard deviation in parentheses.
‡ Second highest value was 42.

diameter, central nuclei on transverse section surrounded by halos and presence of coagulation necrosis. The animals were anesthetized with sodium 5-ethyl-5-(1-methylbutyl)-barbiturate (Nembutal) before bleeding by heart puncture. After diluting the serum 1:10 with NaCl 0.9%, the phosphocreatine kinase was assayed by the optical test(7). The results are expressed in international units (IU), *i.e.*, μmoles of substrate metabolized per minute per 1000 ml of serum at 37°C.

Results. The results are shown in Table I and Fig. 1. Typical histologic changes and the gross appearance of advanced disease are shown in Fig. 2. Serum phosphocreatine kinase levels were significantly increased in all dystrophic animals regardless of age. The normal mean activity was 23.2 IU, whereas mean values in the dystrophic group ranged from 107 IU to 730 IU. This increase manifests itself as early as on the 13th day of age, and it is most marked in animals aged from 26 to 120 days. In animals 121 to 230 days old, the levels are less markedly, but still significantly, elevated. The difference in serum enzyme activity of normal and dystrophic animals of all ages was highly significant ($p<0.001$). Within the dystrophic animals, there were significant variations of enzyme activity, animals 13 to 25 days old having significantly ($p<0.02$) lower levels than animals 26-120 days of age. In hybrid animals 129 to 220 days old, the serum phosphocreatine kinase levels are not elevated. It has been ascertained that hemolysis is without influence on the level of phosphocreatine kinase in the serum of hamsters.

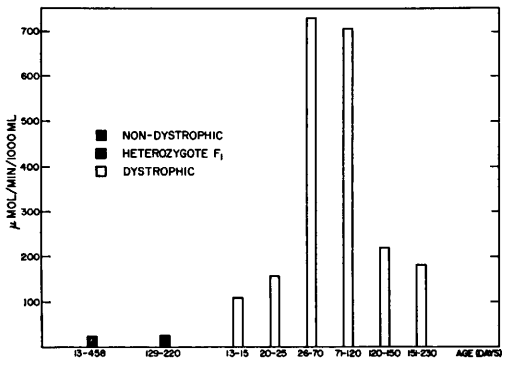

FIG. 1. Serum phosphocreatine kinase activity (arithmetic mean) in non-dystrophic, heterozygote F_1 and dystrophic hamsters.

Discussion. Phosphocreatine kinase catalyzes the phosphorylation of creatine by means of adenosine triphosphate and thus is a key enzyme in muscle metabolism. The principal sources of this enzyme are skeletal muscle, heart and brain. In the Duchenne type of human muscular dystrophy, where a number of serum enzymes(8) are markedly increased, phosphocreatine kinase is the most elevated of all(1,2). Dreyfus *et al*(2,3,4) and Aebi *et al*(5) also reported elevated values of this enzyme in mothers of affected children, whereas Okinaka(9) found elevated serum phosphocreatine kinase values only in some female sibs of the affected boys. Hosenfeld *et al*(10) found a significant elevation of this enzyme in dystrophic mice, but not in heterozygous carriers. This was also confirmed by Nichol *et al* (personal communication). Schapira and Dreyfus(11), on the other hand, noted an increased serum aldolase in dystrophic mice, but failed to find an in-

creased phosphocreatine kinase level.

To study the relation between the dystrophic and myocardial disease processes and the serum phosphocreatine kinase activity, one must review the clinical course of the hamster disorder. This knowledge is based on 426 complete autopsies and about 2,000 muscle biopsies. Primary, hereditary generalized polymyopathy accompanied by cardiac necrosis in an inbred line of Syrian hamsters, caused by a single autosomal recessive gene, manifests itself histologically after the 21st day of life(12,13,14). At the age of about 60 days, clinical manifestations such as abnormal gain appear. The involvement of the heart is less severe in younger animals, but between 180 and 220 days of life the animals die suddenly because of cardiac disease. Nearly always this is preceded by chronic cardiac decompensation and ascites. The histological characteristics of the skeletal muscular dystrophy are quite similar to those in the mice of the dystrophic Bar Harbor strain and in human muscular dystrophy. In addition, there are features resembling closely the lesions described by Denny-Brown(15) as "necrotizing myopathy."

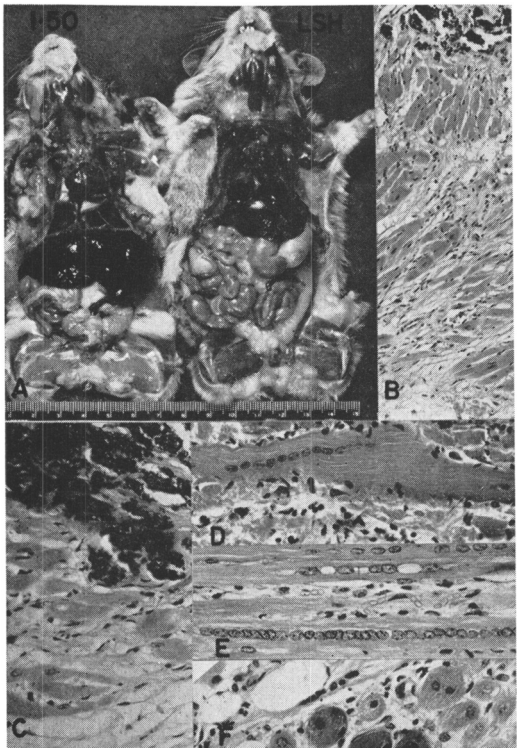

FIG. 2. A. Gross appearance of dystrophic hamster (left) female, strain 1.50, aged 138 days, and a normal control (right) female, strain London School of Hygiene (LSH), aged 150 days. The dystrophic animal was close to death when sacrificed. There were 25 ml of ascites fluid. Notice the smaller body size and smaller muscle masses. There is marked dilatation of heart and severe hepatic congestion.

B. Section of heart, hematoxylin eosin × 80. Female 14.6 hamster, aged 144 days, clinically severely dystrophic. Note focal ischemic fibrosis and focus of calcification (top).

C. Same as B, ×180. von Kóssa, note calcification.

D. Biopsy of back muscle of dystrophic male hamster, 66 days of age. Hematoxylin eosin ×180. Coagulation necrosis and rowing nuclei.

E. Biopsy of back muscle of dystrophic male hamster, 70 days of age. Hematoxylin eosin × 200. Typical internal rowing of nuclei with some of them dropping out.

F. Biopsy of back muscle of dystrophic male hamster, 70 days of age. Hematoxylin eosin × 200. Transverse section showing abnormal position of nuclei, some of them multiple, and necrosis of some fibers; fatty degeneration.

The serum enzyme values are already markedly elevated at the age of from 13 to 25 days at a time when there is no heart disease in any animal and when skeletal muscle is still immature and histologically indistinguishable in normal and dystrophic animals, with the exception of focal necrosis seen occasionally in the dystrophic strain. The early elevation of serum enzyme levels in dystrophic hamsters is analogous to the observation in a case of an 11-month-old human of increased enzyme values before any other symptoms(1). There is a striking correlation between the histologically detectable onset of heart disease and the most marked elevation of the enzyme at around 30 days. After 42 days of age, all animals had cardiac necrosis and elevated serum levels, whereas before 42 days there were no cardiac necroses and relatively lower enzyme levels. The continuous necrotic process in hamster hearts may well account for the increasing enzyme levels, whereas in humans, serum phosphocreatine kinase is only temporarily elevated in the more acute and self-limited necrosis following coronary occlusion(7,9,16). There is a noticeable decline of the elevated enzyme levels (although not to normal levels) with advancing age. In humans, the difference between phosphocreatine kinase levels in sera

of normal and dystrophic individuals is greatest in young subjects and decreases with increasing age(1). Muscle phosphocreatine kinase activity, which is greater in normal than in dystrophic mice, decreases in both groups with increasing age(17). This suggests an exhaustion, with age, of enzyme production which may be accelerated and intensified by the reduction of muscle mass in the course of dystrophy. In addition, in animals with chronic cardiac congestion and ascites, among those 150-230 days old, hemodilution (as apparent from serum protein determinations) accounts for approximately 50% of the observed loss of serum enzyme activity, as compared with that of younger dystrophic animals.

The close resemblance in behavior of serum phosphocreatine kinase in dystrophic humans (preclinical elevation, decline with age), emphasizes the resemblance of metabolic aspects of hereditary hamster dystrophy and the disease in man (Duchene type)(18). On the other hand, mice as well as hamsters differ from man regarding this enzyme, in that the heterozygote F_1 generation shows no enzymatic anomaly in the rodents but is increased in man. This, however, may be due to differences in degree of inbreeding. (No human carrier is really equivalent to an F_1 produced by matings of pure strains, since no cases have been studied of offspring of dystrophic humans.)

These observations have the added significance of permitting preclinical diagnosis of hereditary dystrophy in affected hamsters.

Summary and conclusions. Serum phosphocreatine kinase of Syrian hamsters with hereditary muscular dystrophy is increased in very young animals before any clinical or histological signs of muscle disease can be detected. Maximum increases are found at 26-120 days of age at a time when cardiac necrosis occurs. The serum enzyme levels are not closely correlated with the extent and severity of skeletal muscle disease. Elevated serum phosphocreatine kinase levels are pathognomonic of muscular dystrophy in Syrian hamsters, but fail to reveal heterozygote carriers of the recessive autosomal gene which conditions this disease. With respect to phosphocreatine kinase metabolism, similar anomalies exist in Syrian hamsters as in humans with muscular dystrophy (Duchenne type). In both cases, these enzymyopathies appear before any other manifestation of disease becomes detectable.

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