percentage of IF excreted into the urine within 30 min (after emptying and washing the bladder) were measured and results are reported in table 1 as mean ± S.E. The statistical significance of the difference was evaluated according to Student's t-test.

It appears that IF, once desialylated, remains in the circulation for a very short time and infact its half-life is almost halved. Moreover, it is interesting to note that only a negligible amount has been excreted with the urine, suggesting that the liver uptake may have increased.

For this reason the role of the liver has been tested by using the isolated perfused rabbit liver preparation[25]. The viability of the liver has been greatly improved in comparison to our previous study[10], by using an efficient oxygenator and fresh rabbit blood as perfusion medium. As shown in table 2, only 23–39% of native IF disappears in 15 min from the recirculating perfusate while the loss of desialylated IF is up to 74–85%. This striking difference suggests that the faster disappearance of desialylated IF from the perfusate is attributable to the binding of the asialointerferon to the liver.

Both the results in vivo and in vitro strongly suggest that the mechanism of IF catabolism is equivalent to that reported for several circulating glycoproteins[26]. Desialylation of IF is the preliminary essential step[27] and might occur in microenvironments with sluggish circulation or more likely by membrane-bound sialidase while IF is attached to the cell membrane[28].

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Isoenzymes of creatine phosphokinase in white blood cells*

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Summary. Creatine phosphokinase activity was found in human lymphocytes. Only MM type of the enzyme was detected in lymphocytes and polymorphonuclear leukocytes.

Creatine phosphokinase (CPK) plays an important physiological role in the energy metabolism of skeletal and cardiac muscle, and nerve cells. It catalyzes the reaction: creatine phosphate + ADP = ATP + creatine[2]. CPK exists in multiple forms. It is a dimer, composed of 2 types of subunits, designated M and B. These combine to produce three isozymes, designated MM, MB and BB[3].

MM type is mainly found in skeletal muscle, BB type predominantly in the nervous system and MB type in cardiac muscle[3]. Similarities between contractile muscle and mobile cells are widely recognized. Huxley[4] has recently pointed out that actin- and myosin-like proteins are present in motile non-muscular cells, such as amoeba, acanthamoeba castellanii, and blood platelets. Traniello et al.[5] has examined human polymorphonuclear leukocytes (PMNL) and found that these cells contain cytoplasmic CPK. However, no mention was made as to the isozyme of CPK present in the leukocytes. Meltzer and Guschwan have previously reported BB-type CPK in the platelets of rats and rabbits, although CPK activity was undetectable in human platelets[6]. We therefore investigated which type of CPK was present in lymphocytes and leukocytes. We wish to report that CPK activity is also present in human lymphocytes and that only MM type of CPK is present in lymphocytes and leukocytes.

Materials and methods. Leukocytes and lymphocytes were isolated by a modification of the methods described by Mendelsohn et al.[7] and Boyum[8]. 20 ml of venous blood were drawn from each of 4 normal subjects in heparinized non-sterile Vacutainers. With the Vacutainers oriented at a 45 °C angle, blood samples were allowed to stand for 1 h at 37 °C.

The resulting upper layer of leukocyte-rich plasma was collected and diluted to twice volume with Ca-Mg-free (CMF) Tyrode's solution, pH 7.4. 20 ml, at most, of this diluted plasma were layered over 9 ml of the following solution in a 50 ml conical plastic centrifuge tube (Falcon): 1.8 ml of 50% sodium diatriazoate (Hypaque, Winthrop Labs, New York, New York), 6.35 ml of 9% aqueous Ficoll (molecular weight, approximately 400,000, Sigma Chemical Co., St. Louis, Mo.) and 0.85 ml of water. Centrifugation was performed at 4 °C at 885 g for 15 min. The uppermost plasma layer was removed with a Pasteur pipette. The white fluffy lymphocyte-rich interphase layer originally between the plasma layer and the Ficoll solution was then collected by aspiration in 15 ml conical, plastic centrifuge tubes. Next, the remaining Ficoll layer was carefully removed, leaving a leukocyte-rich layer at the bottom of the tube. This leukocyte-rich layer was resuspended in 2 ml of CMF and collected by aspiration. Each lymphocyte-rich fraction and each leukocyte-rich fraction were washed twice by resuspension in 10 ml CMF and centrifugation at 110 × g for 10 min in order to remove platelets, plasma and Ficoll. After washing, all lymphocyte-rich fractions were combined in one 15 ml

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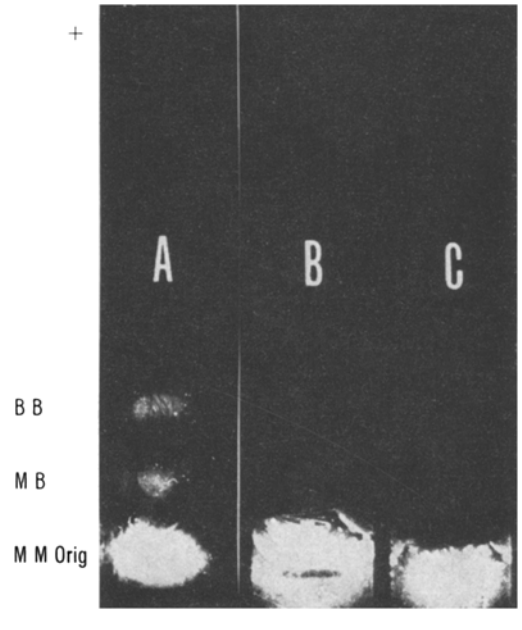
Activity of CPK in the extracts of human lymphocytes, polymorphonuclear leukocytes, human skeletal muscle and brain

	CPK activity (mU/mg protein)	CPK activity* (mU/10^8 cells)
Human lymphocytes	11.3	26
Human PMN	11.4	112
Human muscle	68,000	–
Human brain	6,100	–

*CPK activity was estimated assuming 100% lysis after sonication.

conical plastic centrifuge tube and all leukocyte-rich fractions in another. The total cell content and purity of each suspension were determined by cell counts in a haemocytometer. Only cell suspension of purity $\geq$ 94% were accepted for further study. The average purity of harvested cell suspensions was 96%.

The 2 suspensions were centrifuged at 250 $\times$ g for 10 min and the CMF was removed. Cells were then resuspended in 0.5–0.7 ml of cold Gelman High Resolution Buffer (tris-barbital-sodium barbital, pH 8.8) containing 4 mM mercaptoethanol, and lysed by sonication for 20 sec with a Kontes cell disrupter. Cell debris was removed by centrifugation at 7700 $\times$ g and 4 °C for 10 min. Total CPK activity was measured by the method of Rosalki[9] with reagents obtained from Calbiochem. Protein concentration of the extracts was assayed according to the method of Lowry et al.[10]. Human brain and skeletal muscle from autopsy were homogenized (1:10) in cold isotonic saline solution by means of Tri-R teflon tissue homogenizer. The homogenate was centrifuged at 30,000 $\times$ g for 30 min to obtain supernatant. Three 1 µl aliquots of the extracts of white blood cells and the diluted tissue extracts were electrophoresed on agarose gels (Pfizer Pol-E-film) for 30 min. Then 1 ml of CPK reagent (E.I. du Pont de Nemours & Co., Instrument Products Division, Wilmington, Delaware) was spread over the gel, followed by incubation of the gel at 37 °C for 30 min. The bands of isoenzymes of CPK were detected under UV light.



Electrophoretic mobilities of creatine phosphokinase in the extracts of polymorphonuclear leukocytes and lymphocytes. *A* A mixture of extracts of brain and cardiac muscle of humans. *B* Extracts of human lymphocytes. *C* Extracts of human polymorphonuclear leukocytes.

Results and discussion. As shown in the figure only a single band was present in the extracts of both polymorphonuclear leukocytes and lymphocytes. This band is located at the same position as MM-type CPK. Some non-specific reaction probably occurred at the origin. The lymphocyte and leukocyte band also probably includes some non-specific reaction. However, since 'CPK activity' in the absence of substrate but with the rest of the Rosalki reagents was less than 3% of the CPK activity of lymphocytes and leukocytes when measured spectrophotometrically by the complete Rosalki method and since neither BB band or MB band was detected after electrophoresis, we conclude that the CPK of lymphocytes and leukocytes is MM.

Quantitative estimation of CPK activity in human lymphocytes and polymorphonuclear leukocytes in comparison with that of human brain and skeletal muscle was carried out. The CPK activity per 10^8 cells was 4.3fold greater in leukocytes than in lymphocytes (table). This is probably related to the fact that the relative cytoplasmic space and the average size of leukocytes are greater than those of lymphocytes[11]. The CPK activities per mg protein of the cytoplasm of leukocytes and lymphocytes were comparable. The CPK activity of both of these cell types were extremely low compared with human brain and muscle (table). This does not necessarily rule out that CPK has significant function in the energy metabolism of leukocytes and lymphocytes. Contraction of skeletal muscle involves changes in its length of 20% or more in 50 milliseconds[12]. Conduction velocity of myelinated nerve fibre ranges between 5 and 120 meter per second[13]. In comparison with these rapid physical and electrochemical activities of muscle and nerve fibres, which require rapid replenishment of ATP from creatine phosphate, leukocytes and lymphocytes move by the process of chemotaxis through tissue spaces only 3 times their own length, each minute. Furthermore, the movement of the leukocytes to the target site largely depends on the circulation of blood rather than their own locomotion. Thus, these cells do not require a large pool of reserve energy for movement and the low CPK activity in the cytoplasm of leukocytes may be adequate for the low-energy requirements of these cells.

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