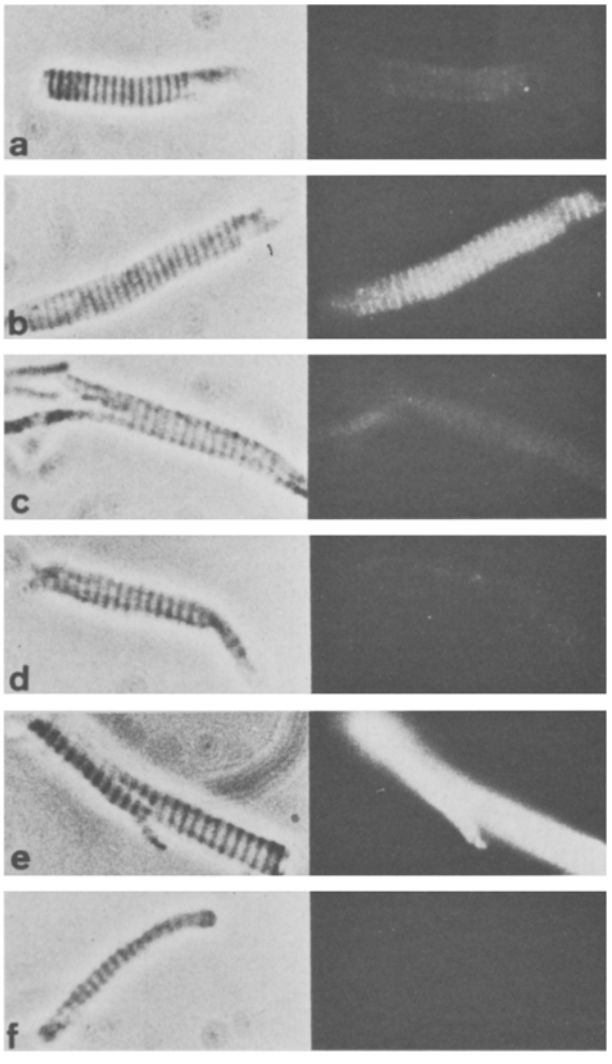

Fig. 3, B. Localization of *a* parvalbumin-like protein, *b* β-actinin, *c* phosphorylase, *d* MM-creatin kinase, and *e* actin in contracted myofibrils after extraction in 5 mM Tris-HCl pH 7.7; *f* control.

are found in the I-band (like actin, the major protein of the thin filament which was chosen for a direct comparison) in the relaxed state, whereas phosphorylase also shows some additional binding in the H-zone[4], MM-creatine kinase was localized mainly in the M-line.

The question was raised whether these components are also bound in strongly contracted myofibrils where the interfilamentary distances are extremely reduced. In this state, the A- and I-bands had disappeared, not allowing a clear localization of the fluorescence, in defined structural regions.

Figure 1, B, shows the localization of the components in contracted myofibrils. Cross-striation is still observed upon incubation with anti-β-actinin and anti-MM-creatin kinase but not with parvalbumin-like protein and phosphorylase.

3. Extraction of the muscle components from relaxed and contracted myofibrils. Mainly 2 procedures for the extraction and isolation of M-line proteins (from myofibrils) are described in the literature: a) a more 'specific' procedure in low salt buffer[9] and b) one under high salt conditions (0.6 M KCl)[10] after extensive washing of the fibrils in relaxing buffer.

The influence of these conditions on the morphology of single fibril is shown in figure 2. It demonstrates a considerable swelling of the still relaxed myofibril in hypotonic, low salt buffer (up to double size) which is immediately reversed by reincubation in relaxing buffer. Extraction (for longer than 2 min) in high salt buffer resulted not only in the extraction of the M-line and A-band, but almost complete destruction of the fibril (figure 2, c). Therefore only low salt conditions were used for extraction and localization. Figure 3, A, shows that creatine kinase and phosphorylase are extracted in 5 mM Tris-HCl pH 7.7 (for 15 min) from relaxed fibrils but not β-actinin or actin. Extraction, however, of contracted fibrils (under conditions used for relaxed myofibrils) resulted in a disappearance of MM-creatine kinase while β-actinin and actin are still visible. Due to the weak fluorescence after incubation with anti-phosphorylase and anti-parvalbumin-like protein sera in contracted fibrils (prior to extraction), no clear result was obtained.

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A differential effect of L-serine on the incorporation of ^{3}H-deoxycytidine and ^{3}H-thymidine into DNA of rat thymocytes[1]

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Summary. The addition of L-serine to short-term cultures of rat thymocytes stimulated the incorporation of ^{3}H-deoxycytidine into DNA, but simultaneously depressed the incorporation of ^{3}H-thymidine into DNA.

Although L-serine is not considered to be an 'essential' amino acid, previous studies have shown that proliferation of certain mammalian cell types can be stimulated by L-serine supplementation[2]. In addition it has been found that the full lymphocytic response to phytohemagglutinin requires an adequate supply of L-serine[3]. Part of the reason for this requirement may be due to an inadequate synthesis of L-serine from glycolytic intermediates[4].

In the process of testing the effect of amino acids on the proliferation of rat thymocytes in vitro, we found that while the addition of L-serine stimulated the incorporation of ^{3}H-deoxycytidine into DNA, the incorporation of ^{3}H-thymidine into nucleic acid was depressed.

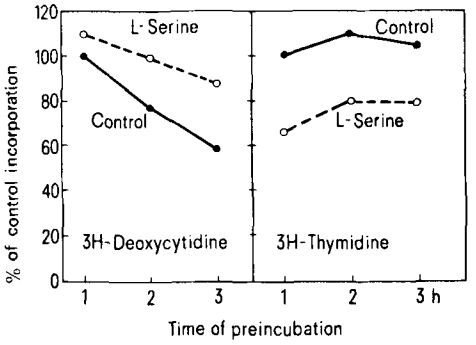
Materials and methods. Sprague-Dawley derived male rats, 35–40 days old, weighing 125–150 g, were purchased from Sasco, Omaha, NE, and were maintained on water and

Rockland Lab Chow ad libitum. The rats were housed in a constant temperature facility with lights on from 6.00 to 18.00 h and were sacrified from 8.00 to 9.00 h. Following decapitation of 3 animals for each experiment, the thymuses were quickly removed, weighed (mean ± SD, 368 ± 100 mg), and placed into ice-cold minimal essential medium (MEM, Joklik-Modified, Grand Island Co., New York) that had been gassed with 10% CO_2/90% O_2 to bring the pH to 7.0–7.2. This medium has no added calcium,serine or glycine. The cells were washed and counted as described[5] and diluted to 4.0×10^8 cells/ml. Preincubations were conducted in duplicate at 4.0×10^7 cells/ml by adding 0.2 ml of the cell suspension to 1.8 ml of MEM containing the appropriate concentration of L-serine (Sigma Chemical Co.). No serum was added. The tubes were gassed, capped and incubated with gentle intermittant shaking at 37 °C. At the appropriate time 0.5 ml of the preincubation cell suspension was pulsed in triplicate from each tube for 1 h with 1.0 μCi of either ^{3}H-deoxycytidine or ^{3}H-thymidine (Schwartz Bioresearch, Inc., 5.0 Ci/mmole and 3.0 Ci/mmole) dissolved in 0.1 ml of MEM. The incubations were terminated, the cells washed and counted by methods previously described[5]. All glassware was siliconized. A significance of differences between means was determined by using the Student's t-test.

Effect of increasing levels of L-serine on incorporation of ^{3}H-deoxycytidine into thymocyte DNA*

L-serine added (mM)	Incorporation** (cpm × 10^{-3})	Percent of control	p-value***
0	6.84 ± 0.25		
0.95	7.75 ± 0.55	113.2	< 0.0023
1.90	8.24 ± 0.41	120.5	< 0.0001
3.80	9.26 ± 0.30	135.4	< 0.0001

* Duplicate tubes of 2.0 ml containing 4.0×10^7 cells/ml were preincubated for 1 h followed by incubating triplicate 0.5 aliquots from each tube with 1.0 μCi of ^{3}H-deoxycytidine. ** Mean values from 6 tubes ± SD. *** Compared to no L-serine added.



Influence of the addition of 3.89 mM L-serine on the incorporation of ^{3}H-deoxycytidine and ^{3}H-thymidine into the DNA of rat thymocytes. Preincubations for the times shown above were conducted as described in the table. Values for the mean incorporation for 6 tubes are compared to that obtained for 6 control tubes following 1 h of preincubation. 1 μCi per tube used for each precursor. Average cpm × 10^{-3} ± SD for controls at 1 h were 7.0 ± 0.27 and 11.6 ± 0.28 for ^{3}H-deoxycytidine and ^{3}H-thymidine, respectively.

Results and discussion. The effect of 3 levels of L-serine addition on the incorporation of ^{3}H-deoxycytidine following a 1-h preincubation is shown in the table. It may be seen that there was a stimulatory influence of L-serine on the incorporation of ^{3}H-deoxycytidine, with the lowest level (0.95 mM or 100 μg/ml) causing a 13% enhancement of isotope uptake into the DNA ($p < 0.0023$). We had shown previously that rat thymocytes incubated in the 90% oxygen tension employed in these studies declined in ^{3}H-deoxycytidine incorporation over a period of hours, but that ^{3}H-thymidine uptake remained stable[5]. For this reason the effect of preincubating the cells with 3.8 mM L-serine for various times was explored. Values in the figure are plotted as a percentage of that obtained with 1 h of preincubation without serine. The figure shows the decline of ^{3}H-deoxycytidine incorporation into DNA with time without a significant change in ^{3}H-thymidine uptake into DNA. In the presence of L-serine, the decline in ^{3}H-deoxycytidine was much less, producing an apparent enhancement of incorporation. In this experiment, however, addition of 3.8 mM L-serine caused only a 10% ($p < 0.006$) enhancement of uptake of ^{3}H-deoxycytidine over the control after a 1-h preincubation period, whereas in the table a 35% stimulation is shown. Although the quantitative effects of L-serine did vary, a significant stimulation of ^{3}H-deoxycytidine incorporation was always observed. After 3 h of preincubating the cells, the incorporation of ^{3}H-deoxycytidine was 50% greater in the presence of L-serine ($p < 0.0001$). On the other hand with L-serine addition ^{3}H-thymidine incorporation was depressed 34% in 1 h and 28 and 25% at 2 and 3 h compared to the controls (all p-values < 0.0004).

Although the effects of L-serine on ^{3}H-deoxycytidine and ^{3}H-thymidine incorporation into DNA could be due to a specific influence on deoxynucleoside degradation or transport across the cell wall, no specificity of this type has been reported. Addition of L-serine could cause an alteration of the pool size and specific activity of the deoxynucleotide precursors for DNA. L-serine which is normally limiting for growth in lymphocyte cultures[2] could supply C-1 units needed for the synthesis of thymidine monophosphate from deoxyuridine monophosphate. Alternatively, it is recognized that the relative incorporation of ^{3}H-deoxycytidine and ^{3}H-thymidine varies with the cellular type in the thymus[6], and L-serine could influence the subpopulations of cells in a differential manner. Resolution of these alternatives awaits further study.

1 This investigation was supported by NIH grants No. CA-10291 and T12CA8021 from the National Cancer Institute.
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