# EFFECTS OF BIOTIN DEPLETON ON MOUSE LEUKEMIC CELLS IN CULTURE

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SUMMARY. In this investigation it was demonstrated that in mouse leukemic L 5178Y cells grown in vitro biotin deficiency resulted in the impairment of the growth rate and was manifested by a decreased content of L-aspartic acid. In cells devoid of biotin chromosomal disturbances were observed.

The study of Sanford and al. (1) identified biotin for the first time as a vitamin required for maximal growth and survival of L mouse fibroblasts. These results were confirmed by others (2,3) however interrelationships between biotin deficiency and other cell components have not been established so far. Contrary to the results obtained on L cells, from the study of Keränen it seems to be evident that HeLa cells respond to biotin depletion by enhancement of the growth rate and by acquired capability to synthesize the vitamin (4) .

Studies reported here were performed to estimate the effect of biotin depletion on the growth rate of L 5178Y mouse leukemic cells and to test whether these cells acquire capability to synthesize biotin. Since one effect of biotin deficiency in micro-organisms is a decreased synthesis of aspartate (5) present studies were extended to establish whether in L 5178Y cells biotin depletion results in the decrease in the content of L-aspartic acid. Additionnaly chromosome analysis was made to evaluate the possible occurrence of chromosome aberrations in the cells grown in the medium completely devoid of biotin by addition of avidin.



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## MATERIAL AND METHODS

L 5178Y cells were maintained in growth in culture in Fischer's medium (6) containing 2 % serum and passaged by dilution every 96 hours. To delineate the nutritional requirement of L 5178Y cells for biotin the cells were grown in modification of Fischer's medium lacking biotin or in the medium lacking biotin and supplied with avidin (Sigma) to the final medium concentration of 0.02 µg/ml. As estimated by microbiological method this amount of avidin exceeded twice the amount necessary to inactivate the vitamin present in the serum (an average of 0.12 µg/100 ml) . The size of viable cell population at different time intervals after seeding was determined by trypan blue exclusion method. Three parallel cultures were used for each determination. The starting inoculum was identical in all experiments (ca. $2 \times 10^4$ cells/ml) . Samples of cells growing in Fischer's media and in avidin-containing media were harvested for chromosome analysis at various passages. After centrifuging the cells were suspended in hypotonic solution (0.1 % sodium citrate) for 20 minutes at 37°C. They were then centrifuged and fixed in 3:1 methanol-acetic acid fixative. After two changes of fixative, the suspension was kept for 48 hours at - 20°C. One drop of cell suspension was placed on a cold slide, dried and stained with Giemsa.

Biotin and L-aspartic acid were estimated microbiologically by means of the methods described by Barton-Wright (7) . For one determination cells from 10-20 parallel cultures grown in the above indicated media were pooled and washed with 0.85 % NaCl. After hydrolysis with 4 N $H_2SO_4$ for 2 hours at 120°C biotin was determined with L-arabinosus 17-5 ATCC 8014 as test organism. For L-aspartic acid assays cells were hydrolyzed with 6 N HCl for 4 hours at 120°C. L mesenteroides P 60 was used as test organism. D (+) biotin (BDH) and L-aspartic acid Calbiochem were used for standard preparations. Nephelometric readings were made on a Coleman spectrophotometer model 14 at 600 mµ. Each sample was assayed at three or four levels in duplicate.

Protein content was estimated according to Lowry, Rosebrough Farr and Randall (8) and the standard curve was prepared with human serum albumin.

## RESULTS AND DISCUSSION

The effect of biotin depletion upon the growth of L 5178Y cells was estimated by comparison of cell numbers in cultures grown and passaged in complete Fischer's medium, in modification of the Fischer's medium lacking biotin and in the latter medium containing avidin. As can be seen from the growth curves presented in Fig. 1 the impairment of the growth rate of cells passaged in the medium lacking biotin did occur as early as the second passage level. This effect was more drastic for cells passaged in avidin-containing media.

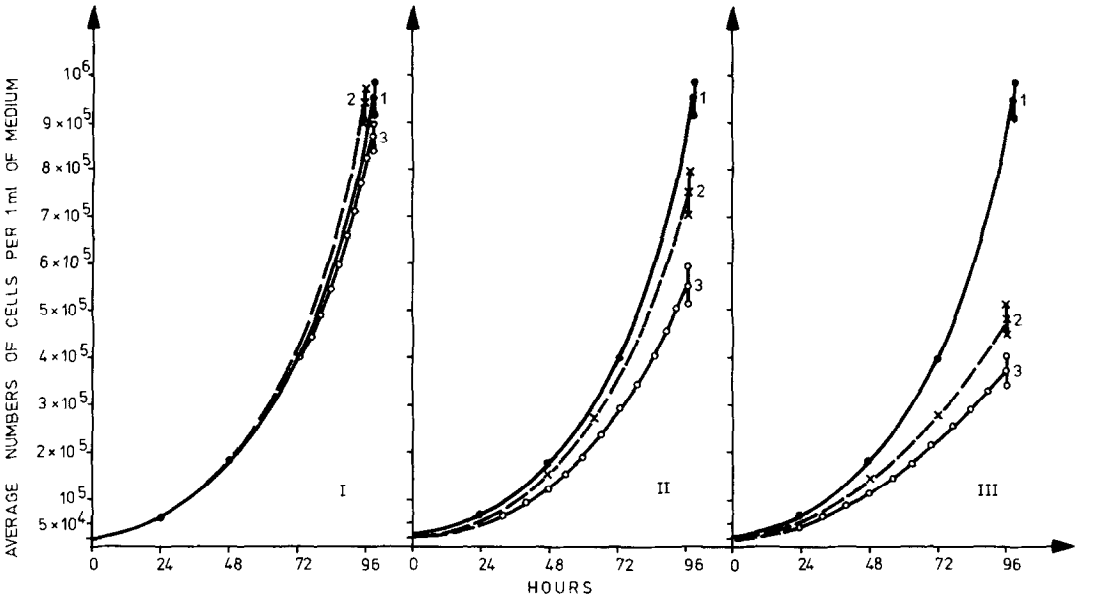


fig. 1

Growth curves of cells cultured in media:
——————— with the vitamin
– – – – – – lacking the vitamin
—o—o—o—o— containing the avidin
in the course of three following passages. Each determination represents the average of three to five cultures. Ranges are indicated by vertical lines.

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The content of the vitamin in cells grown for 96 hours in complete Fischer s medium appeared to be a constant value (Table I) . As can be seen from the same table it diminished to half of this value in cells passaged in media lacking biotin. After the growth through two passages in cells cultivated in avidin-containing media the vitamin was no more detectable.

The effect of biotin depletion upon L-aspartic acid synthesis was estimated by comparison of the content of the amino acid in control cells and in cells grown in avidin-containing media. The results are shown in Table II. As evaluated by statistical analysis the content of L-aspartic acid in the second

Table I. Results of biotin determinations

| Medium | Passage I | Passage II | Passage III |
|---|---|---|---|
| 1. Complete Fischer's medium | 0.28 | 0.31 | 0.27 |
| 2. Biotin-deficient medium | 0.25 | 0.10 | 0.12 |
| 3. Biotin-deficient and supplied with avidin | 0.27 | 0.0 | 0.0 |

Biotin content (mμg/mg) protein was determined microbiologically by L arabinosus 17-5 ATCC 8014 method.

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and third passage was lower ($P < 0.01$) than in the first passage and lower than in control cultures at every passage level.

Chromosome analysis was performed on cell samples taken from control cultures and cultures grown in avidin-containing media. The results are summarized in Table III. They indicate that the impairment of the growth resulting from biotin deficiency was parallel to the appearance of breaks and fragmentation.

Summarizing, experiments reported here show that mouse leukemic cells are dependent in their growth on the presence of biotin. They deny then the possibility presented by Keränen that neoplastic cells in general, like the HeLa cells, acquire the ability to synthesize biotin (4) . Moreover, since the decreased content or absence of biotin in L 5178Y cells was

Table II. Results of L-aspartic acid determinations

| Medium | Passage I | Passage II | Passage III |
|---|---|---|---|
| 1. Complete Fischer's medium | 63±5,5 | 61±6,18 | 64±5,05 |
| 2. Biotin-deficient and supplied with avidin | 62±5,1 | 44±4,85 | 43±8.00 |

L-aspartic acid content (µg/mg) was determined microbiologically with L mesenteroides P 60.

Table III. Chromosome aberrations induced during growth in avidin-containing media

| Metaphase plates scored[x] | Normal metaphases | Aberrant metaphases | |
|---|---|---|---|
| | | Breaks | Fragmentation |
| 100 | 99 | 1 | 0 |
| 100 | 76 | 8 | 16 |

[x]- Metaphases harvested by addition of colchicine

parallel to the decreased content of L-aspartic acid, it seems that one effect of biotin deficiency is the impairment in L 5178Y cells of L-aspartic acid synthesis similarly with the findings dealing with biotin deficiency in micro-organisms.

Chromosomal disturbances observed in cells starved of biotin might be connected at least partly with the decreased synthe-

sis of L-aspartic acid, which in turn, is thought to be involved in the synthesis of purines and pirymidines and then in the metabolic processes leading to the synthesis of nucleic acids and proteins (9) .

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