16. W. A. JEFFERS and J. Q. GRIFFITH, JR., in *The Rat in Laboratory Investigation* (Eds. E. J. FARRIS and J. Q. GRIFFITH, JR.), p. 196. J. P. Lippincott, Philadephia (1949).
17. G. BRAY, *Analyt. Biochem.* **1**, 279 (1960).
18. L. D. BERGELSON, V. A. VAVER, N. V. PROKAZOVA, A. N. USHAKOV and G. A. POPKOVA, *Biochim. biophys. Acta* **116**, 511 (1966).
19. R. H. ROTH and N. J. GIARMAN, *Biochem. Pharmac.* **17**, 735 (1968).

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N^6-(Δ^2-isopentenyl) adenosine interference with methionine metabolism in axenic cultures of mammalian cells

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N^6-(Δ^2-ISOPENTENYL) ADENOSINE (IPA) is located adjacent to the presumed anticodon in t-$RNA^{Ser,Tyr}$ of yeast, plants and mammalian tissues.[1] It has biological activity as a cytokinin,[2] is toxic to some cultured mammalian cells,[3] dogs and rodents,[4] and has exhibited therapeutic activity in one case of human promyelocytic leukemia.[5] Evidence will be presented herein that IPA interferes with methionine metabolism in a line of cultured mammalian cells.

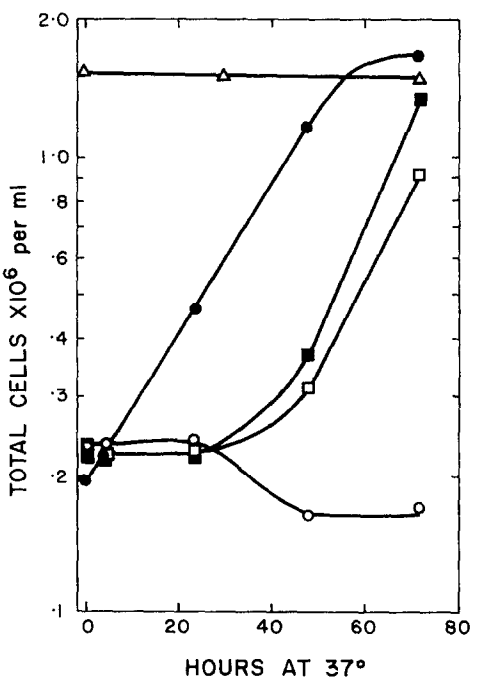

FIG. 1. Growth of RPMI No. 2402 cells in RPMI medium No. 1379 supplemented with 2% by volume calf serum in the presence of the following levels (μM) of IPA: (0), 15; (□) 3; (■), 1·5; (●), zero. The triangles represent a culture in the absence of IPA inoculated with $1{\cdot}5 \times 10^6$ cells per ml.

A cell line (RPMI No. 2402), derived from a small bowel carcinoma of the Syrian hamster,[6] was grown in suspension culture in RPMI medium No. 1379 supplemented with 2% by volume of calf serum, as described in detail elsewhere.[7] Cells were counted in a hemocytometer. Growth was inhibited by IPA (Fig. 1) and it appears that this effect is dose related. In the absence of IPA no lag was observed while 1·5 μM resulted in a 30–40 hr lag. After 72 hr of incubation, the cells were harvested and washed by centrifugation with fresh protein-free medium. The cells were then hydrolyzed in 6 N HCl in sealed tubes under N_2 at 110° for 48 hr and a suitable portion was subjected to quantitative amino acid analysis.[8] The amino acid composition of the cell protein of 10^6 cells revealed major differences only in the methionine content. In Fig. 2 is plotted the methionine content of the cells

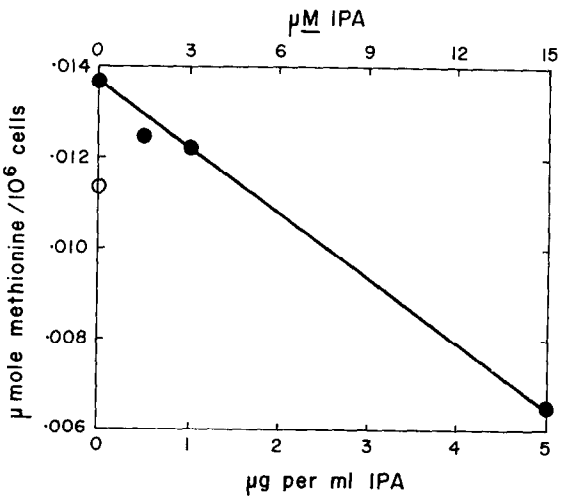

FIG. 2. Methionine content per 10^6 cells harvested after 72 hr of incubation as described in Fig. 1. The open symbol represents the high inoculum culture of Fig. 1.

against the IPA concentration in the growth medium. In order to test if nongrowing cells contain less methionine than actively growing cells, 1·5 × 10^6 cells per ml were inoculated into the medium and incubated for 72 hr. At this cell density, no further cell division is possible with this cell line.[7, 9] The methionine content of 10^6 cells from this culture is also indicated in Fig. 2. Although there is less methionine in these cells than in the ones growing in the absence of IPA, it is significantly greater than in the nondividing cells from the culture incubated with 1·5 μM IPA.

Growth inhibition by IPA could be overcome by the addition of methionine to the growth medium RPMI medium No. 1379 is 200 μM in methionine). Typical results are illustrated in Fig. 3. At these IPA levels, lag periods were again observed. The addition of methionine to essentially double the methionine level in the medium resulted in a reproducible increase in the growth rate and almost doubled the maximum cell density attained. The addition of methionine at the start of the experiment gave results indistinguishable from those illustrated in Fig. 3. Methionine at the level employed had no effect in the absence of IPA. Contrary to the findings of Bloch and Nichol[10] in bacteria, aspartic acid at levels up to 0·03 M did not counteract the toxicity of IPA. Homocysteine was found to be toxic to RPMI No. 2402 cells and therefore could not be tested.

These observations suggest that IPA can interfere with methionine metabolism. This compound has also been shown to inhibit proline hydroxylation in man.[11] In bacteria, IPA interferes with *de novo* pyrimidine biosynthesis[10] and with the methylation of *t*-RNA.[12] It remains to be determined whether any of these are the basis of its chemotherapeutic efficacy or its side effects.

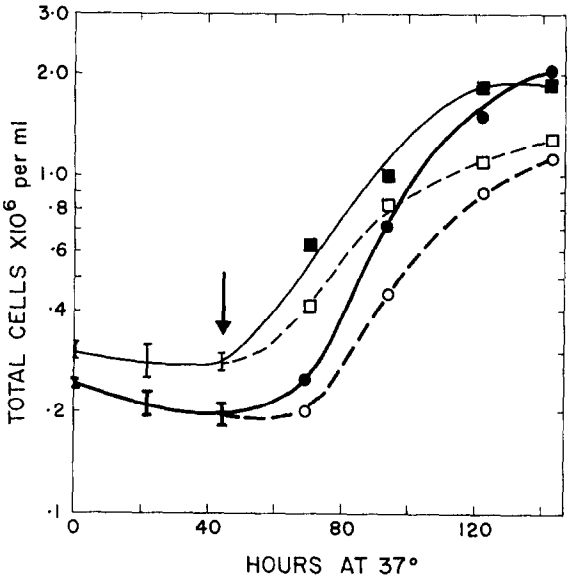

FIG. 3. The ability of methionine to overcome IPA-induced growth inhibition. Duplicate flasks containing 6 μM (squares) and 12 μM (circles) IPA were inoculated with RPMI No. 2402 cells. After 45 hr of incubation, indicated by the arrow, L-methionine to a final concentration of 220 μM was added to one of each of the duplicates and the subsequent cell counts are indicated by the solid symbols. The open symbols represent the cultures in the absence of further additions of methionine.

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REFERENCES

1. R. H. HALL, *Abstr. Seventh Int. Congr. Biochem.*, **1**, 4–4. Tokyo (1967).
2. R. H. HALL and B. I. SAHAI SRIVASTAVA, *Life Sci.* **7**, 7 (1968).
3. J. T. GRACE, JR., M. T. HAKALA, R. H. HALL and J. BLAKESLEE, *Proc. Am. Ass. Cancer Res.* **8**, 87 (1967).
4. D. SUK, D. L. SIMPSON and E. MIHICH, *Proc. Am. Ass. Cancer Res.* **9**, 271 (1968).
5. R. JONES, JR., J. T. GRACE, JR., A. MITTELMAN and M. W. WOODRUFF, *Proc. Am. Ass. Cancer Res.* **9**, 135 (1968).
6. G. E. MOORE, D. MOUNT, G. TARA and N. SCHWARTZ, *J. natn. Cancer Inst.* **31**, 1217 (1963).
7. G. L. TRITSCH, J. A. BELL and G. GRAHL-NIELSEN, *Expl Cell Res.* **48**, 244 (1967).
8. D. H. SPACKMAN, *Fed. Proc.* **22**, 499 (1963).
9. P. F. RAKOWSKI and G. L. TRITSCH, *Expl Cell Res.* **43**, 510 (1966).
10. A. BLOCH and C. A. NICHOL, *Proc. Am. Ass. Cancer Res.* **9**, 22 (1968).
11. G. L. TRITSCH, *Proc. Am. Ass. Cancer Res.* **9**, 280 (1968).
12. E. WAINFAN and E. BOREK, *Molec. Pharmac.* **3**, 595 (1967).