Difference in the Action of Acriflavine and Hydroxyurea on Synthesis of Macromolecules in a Mammalian Cell System in vitro

Every biochemical activity of a living cell involves one or more of the macromolecular trinity, namely, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or proteins. The biosynthesis of every one of them is a complex, multistep process which requires sequential availability of simple substrates and biologically controlled functioning of a sequence of reactions. Investigations in the last decade have brought out the central place occupied by the DNA molecules in these processes. A disturbance created in the process of DNA synthesis is bound to reflect, sooner or later, in the synthesis of other macromolecules too. The nature and extent of such secondary disturbances would be influenced by the interdependence of these macromolecular events, and their investigation would be helpful to elucidate the mode of action of the agents used. Acriflavine, a diaminoacridine, has been shown to affect DNA synthesis in CHANG's liver cell line[1]. It is also known to affect DNA molecules in microorganisms[2,3]. Hydroxyurea, chemically a much simpler molecule, has been reported to affect DNA synthesis in HeLa S_3 and a Chinese hamster cell line[4], bacteria[5], and cell-free systems[6]. The present communication reports the differential effects of these 2 agents on nucleic acid and protein synthesis in a mammalian cell line cultivated in vitro.

Commercially obtained CHANG's liver cell line, of human origin[7], was cultivated in EAGLE's basal medium[8] supplemented with 15% adult human serum. $1.2 \cdot 10^5$ cells/ml per tube were inoculated into standard Leighton tubes containing coverslips and incubated at 37 °C for 24 h.

Stock solutions of acriflavine and hydroxyurea were freshly prepared in normal saline at different concentrations. Medium from each 24 h incubated tube was replaced with 0.9 ml of the fresh medium and 0.1 ml of the stock solution of desired concentration. The final acriflavine concentration in each set of tubes was 2.5, 5.0 and 10 μg/ml of the medium. The final hydroxyurea concentration in each set of tubes was 25, 50, 125 and 250 μg/ml of the medium. These tubes were incubated at 37 °C for $^1/_2$, 2 and 4 h. The dye-treated tubes were kept in darkness during incubation to avoid photodynamic action[9]. After the required exposure, each tube was rinsed twice with normal saline before addition of 1 ml of the fresh medium.

The control and chemically treated cells were then exposed to labelled precursors, *immediately after* removing the inhibitor and rinsing them with saline. H^3-thymidine was used as a specific precursor for studying DNA synthesis. The concentration was 0.05 μc/ml (specific activity 3 c/m*M*) and the contact time was 1 h. Necessary acid treatment was carried out and the cells fixed and subjected to autoradiographic and photographic procedures, as previously described[10]. For DNA synthesis % labelled nuclei as well as grain distribution per 64 μ^2 (taken as unit area) were registered. Figures 1 and 2 show H^3-thymidine incorporation in the cells exposed for 30 min to different concentrations of acriflavine and hydroxyurea respectively. Figures 3 and 4 show the same in the cells exposed for different periods to either 10 μg/ml acriflavine or 250 μg/ml hydroxyurea.

Since the initial experiments showed that the synthesis of RNA and protein was not affected by exposure to lower concentration of hydroxyurea, only those cells treated with 250 μg/ml hydroxyurea and the cells treated with 10 μg/ml acriflavine were further investigated for their ability to synthesize RNA and protein, using H^3-uridine and C^{14}-phenylalanine as the respective precursors. Concentrations used were: H^3-uridine, 0.1 μc/ml (specific activity 1.22 c/m*M*) and C^{14}-phenylalanine, 1.0 μc/ml (specific activity 1 mc/m*M*). Contact time was 3 h for both the precursors. After the required contact, the cells were subjected to fixation, autoradiographic and photographic procedures as mentioned above. For RNA and protein synthesis, cellwise grain distribution was registered. The results are presented in Figures 5 and 6.

Control values are plotted as 0 h values and each point on the graphs represents a mean of more than 50 samples.

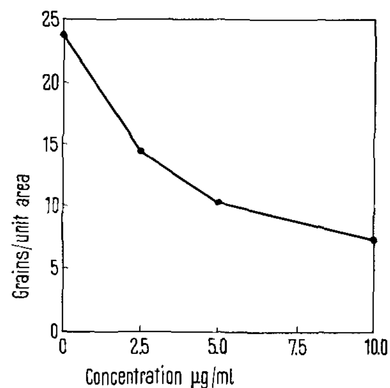


Fig. 1. H^3-thymidine incorporation in cells exposed for 30 min to different concentrations of acriflavine.

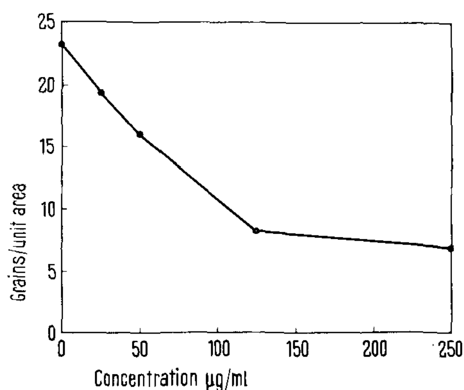


Fig. 2. H^3-thymidine incorporation in cells exposed for 30 min to different concentrations of hydroxyurea.

[1] S. BOSE, B. P. GOTHOSKAR, and K. J. RANADIVE, Expl Cell Res., in press.
[2] S. BRENNER, L. BARNETT, F. H. C. CRICK, and A. ORGEL, J. molec. Biol. *3*, 121 (1961).
[3] E. M. WITKIN, Cold Spring Habor quant. Biol. *12*, 256 (1947).
[4] W. C. MOHLER, Cancer Chemother. Rep. *34*, 1 (1964).
[5] H. S. ROSENKRANZ and J. A. LEVY, Biochim. biophys. Acta *95*, 181 (1965).
[6] C. S. YOUNG and S. HODAS, Science *146*, 1172 (1964).
[7] R. S. CHANG, Proc. Soc. exp. Biol. Med. *87*, 440 (1955).
[8] H. EAGLE, Science *122*, 501 (1955).
[9] N. YAMAMOTO, J. Bact. *75*, 443 (1958).
[10] S. BOSE, W. G. COUTINHO, and K. J. RANADIVE, Ind. J. exp. Biol. *4*, 167 (1964).

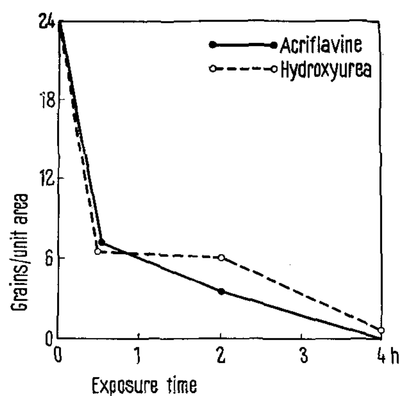


Fig. 3. H^3-thymidine incorporation in cultured cells after acriflavine and hydroxyurea exposure.

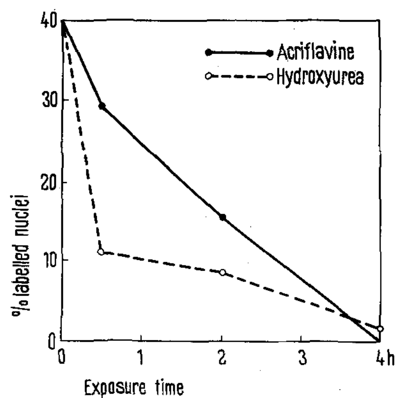


Fig. 4. % labelled nuclei in cultured cells after acriflavine and hydroxyurea exposure.

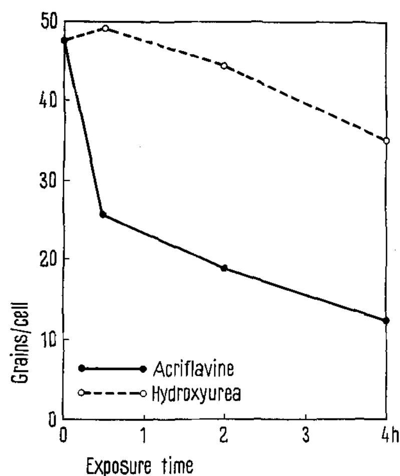


Fig. 5. H^3-uridine incorporation in cultured cells after acriflavine and hydroxyurea exposure.

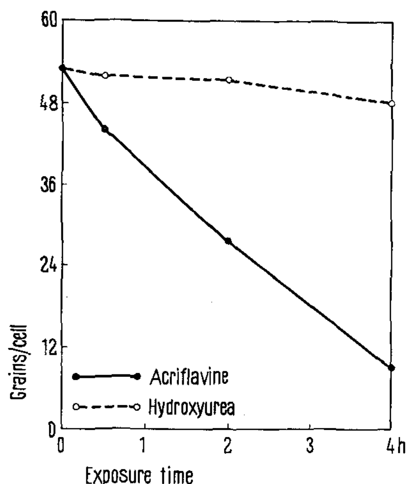


Fig. 6. C^{14}-phenylalanine incorporation in cultured cells after acriflavine and hydroxyurea exposure.

It is evident from Figures 1, 2 and 3 that exposure of the cells to acriflavine or hydroxyurea causes a rapid inhibition of DNA synthesis as seen from the fall in grains per unit area. That the mode of action of these 2 agents on DNA synthesis is different is indicated by the difference in their action on % labelled nuclei (Figure 4). Though both the agents drastically decrease H^3-thymidine incorporation per unit area, only hydroxyurea exposure causes a similar marked reduction in the % labelled nuclei. Acriflavine exposure causes a comparatively slower decrease in the same.

As regards the RNA and protein synthesis, a substantial decrease in both is observed only in the dye-treated cells, not in hydroxyurea-treated cells, in spite of the large amount of the latter agent used. With 4 h exposure to acriflavine, RNA and protein syntheses drop down to about 27% and 16% of the corresponding control values. In the cells treated with hydroxyurea for the same duration, these processes come down to about 75% and 92% of the respective control values.

Acriflavine has been postulated to intercalate with the DNA double helix[11]. Such intercalation has been suggested to obstruct DNA replication by hindering the action of DNA polymerase[12]. The present results support the hypothesis that intercalation at 10 μg/ml concentration immediately affects the priming and/or template activity of the dye-bound DNA molecules for RNA synthesis. With the indispensable requirement of messenger, ribosomal and transfer RNAs for protein synthesis[13], a decrease in the production of these species of RNAs would lead to almost simultaneous decrease in protein synthesis too, as seen in the present case.

On the other hand, exposure of the cells to even 250 μg/ml of hydroxyurea does not seem substantially to affect RNA and protein synthesis, though DNA synthesis shows an inhibition as remarkable as in the dye-treated cells. The results indicate that hydroxyurea-treated cells

[11] L. S. LERMAN, Symp. Molecular Action of Mutagenic and Carcinogenic Agents, J. cell. comp. Physiol. *64*, Suppl. *1*, 1 (1964).

[12] F. J. BOLLUM, in *Progress in Nucleic Acid Research* (Ed., J. N. DAVIDSON and W. E. COHN; Academic Press, New York 1963), vol. 1, p. 1.

[13] F. H. C. CRICK, in *Progress in Nucleic Acid Research* (Ed., J. N. DAVIDSON and W. E. COHN; Academic Press, New York 1963), vol. 1, p. 163.

lose their ability to synthesize DNA, while their capacity to synthesize RNA and protein remains unchanged.

The data presented bring out the difference in acriflavine and hydroxyurea in their selective action on in vitro synthesis of macromolecules in a mammalian cell line[14].

Résumé. L'incorporation autoradiographique des divers précurseurs radioactifs (H^3-thymidine, H^3-uridine, C^{14}-phénylalanine) a été étudiée dans les cellules du foie humain prétraité avec acriflavine ou hydroxyuréa pendant différentes periodes. Les concentrations utilisées ont démontré une inhibition remarquable de la synthèse d'ADN. Mais l'effet des deux substances sur le nombre de cellules synthétisant de l'ADN n'est pas identique. Aussi l'effet des deux agents sur la synthèse d'ARN et de protéine est différent qualitativement.

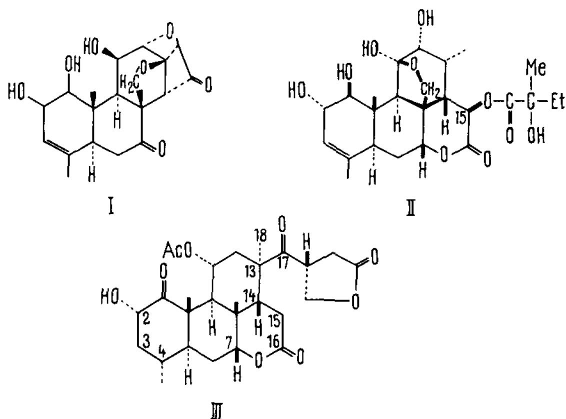
S. Bose, B. P. Gothoskar, and K. J. Ranadive

Indian Cancer Research Centre, Bombay 12 (India), December 10, 1965.

[14] The authors are grateful to Dr. G. R. Gale, Veterans Administration Hospital, Durham, N.C., USA, for a generous gift of hydroxyurea, and to the colleagues at the Tissue Culture Laboratory of the Centre for their cooperation. They are also grateful to Dr. (Mrs.) S. V. Bhide for providing a 'résumé' of the work in French.

Sur la biosynthèse des constituants amers des Simarubacées

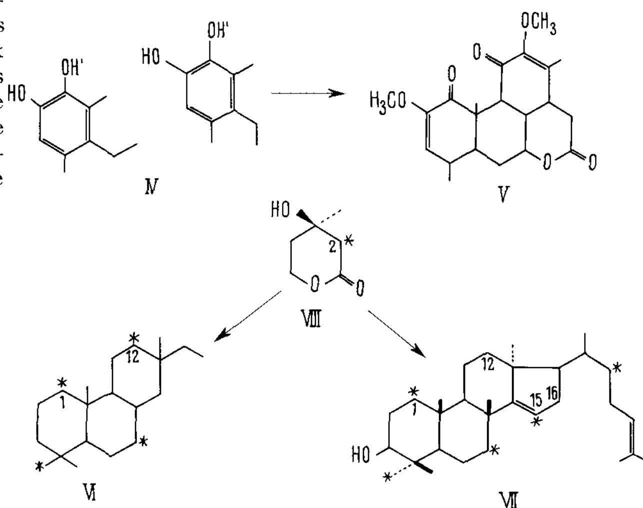
Au cours des dernières années, de nombreux constituants amers ont été isolés à partir de différentes espèces de Simarubacées. Ils possèdent des caractères structuraux communs et forment une nouvelle famille de corps naturels. Les structures des membres de cette famille établies jusqu'à ce jour font apparaître trois squelettes de base différents, en C_{19}, C_{20} et C_{25}, représentés respectivement par la *samadérine C* (I)[1], la *glaucarubine* (II)[2] et le *simarolide* (III)[3]:



La similitude structurale évidente des différents constituants amers des Simarubacées conduit à envisager un même mode de biogenèse pour tous les représentants de cette famille.

Après avoir déterminé en 1962 la structure de la *quassine* (V), Valenta et al.[4] ont envisagé pour ce composé deux voies de biosynthèse: l'une mettant en jeu un couplage oxydatif de deux unités phénoliques en C_{10}, tel que (IV); l'autre postulant le réarrangement d'un diterpène du type *pimarane* (VI).

Plus récemment une troisième hypothèse a été proposée[2,5]: le précurseur biogénétique des constituants amers des Simarubacées serait – comme dans le cas des «triterpénoides» en C_{26} du groupe de la limonine[6] – un triterpène tétracyclique du type *apoeuphol* (VII) qui, par dégradation oxydative plus ou moins poussée, conduirait aux composés en C_{25}, C_{20} et C_{19}. Cette hypothèse est étayée par l'identité de la stéréochimie de ces substances et de celle du précurseur postulé.



Nous rapportons dans la présente note les premiers résultats d'une étude expérimentale de ce problème.

Après incorporation de la lactone DL-[2-^{14}C] mévalonique (VIII) dans de jeunes pousses de *Simaruba glauca*[7],

[1] J. Zylber et J. Polonsky, Bull. Soc. chim. Fr. *2016* (1964).

[2] J. Polonsky, Cl. Fouquey et A. Gaudemer, Bull. Soc. chim. Fr. *1813* et *1827* (1964). – G. Kartha et D. J. Haas, J. Am. chem. Soc. *86*, 3630 (1964).

[3] J. Polonsky, Proc. chem. Soc. *292* (1964). – W. A. C. Brown et G. A. Sim, Proc. chem. Soc. *293* (1964).

[4] Z. Valenta, A. H. Gray, D. E. Orr, S. Papadopoulos et C. Podesva, Tetrahedron *18*, 1433 (1962).

[5] J. B. Son Bredenberg, Chemy Ind. *73* (1964). – D. L. Dreyer, Experientia *20*, 297 (1964).

[6] D. Arigoni, D. H. R. Barton, E. J. Corey, O. Jeger, L. Cagliotti, Sukh Dev, P. G. Ferrini, E. R. Glazier, A. Melera, S. K. Pradhan, K. Schaffner, S. Sternhell, J. F. Templeton et S. Tobinaga, Experientia *16*, 41 (1960).

[7] L'administration d'acide DL-[2-^{14}C] mévalonique à de jeunes pousses d'*Ailanthus altissima* n'a conduit qu'à une faible incorporation (0.005% d'incorporation dans l'ailanthone[11] et la chaparrinone)[11].