mized dogs. The mean plasma volume was 33% increased in these animals and, therefore, the blood volume showed an 8% increase. This marked increase of the plasma volume is the cause that the 'thyroidectomy anemia' in the dog is more severe when evaluated from peripheral indices than when considered in terms of the total red cell volume. The rate of erythropoiesis measured by the red cell iron turnover rate decreased after thyroid destruction, as it is shown in the Table. The magnitude of the decrease in the hemoglobin synthesis rate was about 45% of the control value. A comparable decrease in plasma iron turnover rate was found. The clearance half time of radioiron from the plasma was prolonged in the thyroidectomized dogs when compared to the normal ones. The thyroidectomized animals had normal erythrocyte life spans as measured with Cr^{51}.

The data presented above are very close with those previously reported by us in adult dogs[1] and suggest that there is no correlation between the time when thyroid insufficiency appears and its effects on the erythropoietic system.

Anemia in the thyroidectomized dog appears to result from a diminished rate of red cell production rather than from an accelerated rate of red cell destruction, as it is suggested by the findings of (1) a normal erythrocyte life span, (2) a prolongation of the clearance half time of radioiron from the plasma beyond the range for the normal dog, and (3) a marked reduction in plasma and red cell iron turnover rates. Therefore, the 'thyroidectomy anemia' in the dog may be included among the 'non-proliferative anemias' and probably due to a decreased marrow stimulation[6].

Resumen. Cachorros radioyodotiroidectomizados a las 3 semanas de vida mostraron un descenso del 32% en la concentración de hemoglobina y el valor hematocrito. El volumen de la masa roja circulante disminuyó un 27% y el volumen plasmático mostró un aumento del 33%. La sobrevida de los eritrocitos no fue afectada por la tiroidectomía. La magnitud de síntesis hemoglobínica disminuyó en un 45%. Estos resultados indican 1) que la influencia de la tiroidectomía sobre el sistema eritropoyético es independiente del período de la vida en que se la realiza, y 2) que la anemia post-tiroidectomía puede ubicarse entre las anemias no proliferativas, siendo su causa probablemente un descenso de la estimulación eritropoyética.

C. E. Bozzini[7], M. E. Barrio Rendo[7] and J. A. Kofoed[7]

Cátedra de Fisiología, Facultad de Odontología, Universidad Nacional de Buenos Aires, and Departamento de Radiobiología, Comisión Nacional de Energía Atómica, Buenos Aires (Argentina), 31 March 1969.

[6] This work was supported in part by Grants No. 1126d and 3209 from the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

[7] Member of the Carrera del Investigador Científico del Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina.

Changes in Protein Metabolism in Proliferating and Non-Proliferating Human Acute Leukaemia Cells Treated with Actinomycin-D

Since actinomycin-D blocks the synthesis of DNA-dependent RNA's, messenger RNA stability can be determined via post-actinomycin variations in protein synthesis. Many mammal cells, including those of the normal human haemopoietic series, display notable protein stability and this fact indicates similar stability on the part of their messenger RNA's[1–15]. A rapid fall to 50% of starting values within 2 h, on the other hand, has been observed when actinomycin-D is added to acute leukaemia blasts[16]; it would seem, therefore, that these blasts are incapable of synthetizing the so-called 'stable' messenger RNA's present in differentiated blood cells[6,12].

The acute leukaemia population contains both a proliferating and a non-proliferating component[17,18], whose protein synthesis behaviour in the presence of actinomycin-D may give evidence of relationships between proliferative activity and RNA metabolism in acute leukaemia blasts. The present paper is concerned with the rate of protein synthesis in proliferating and non-proliferating human acute leukaemia cells incubated with actinomycin-D as a means of assessing the stability of their RNA's.

Materials and methods. Our series consisted of 4 cases of acute myeloblastic leukaemia (cases 1–4), 1 case of acute lymphoblastic leukaemia (No. 5), 1 case of acute

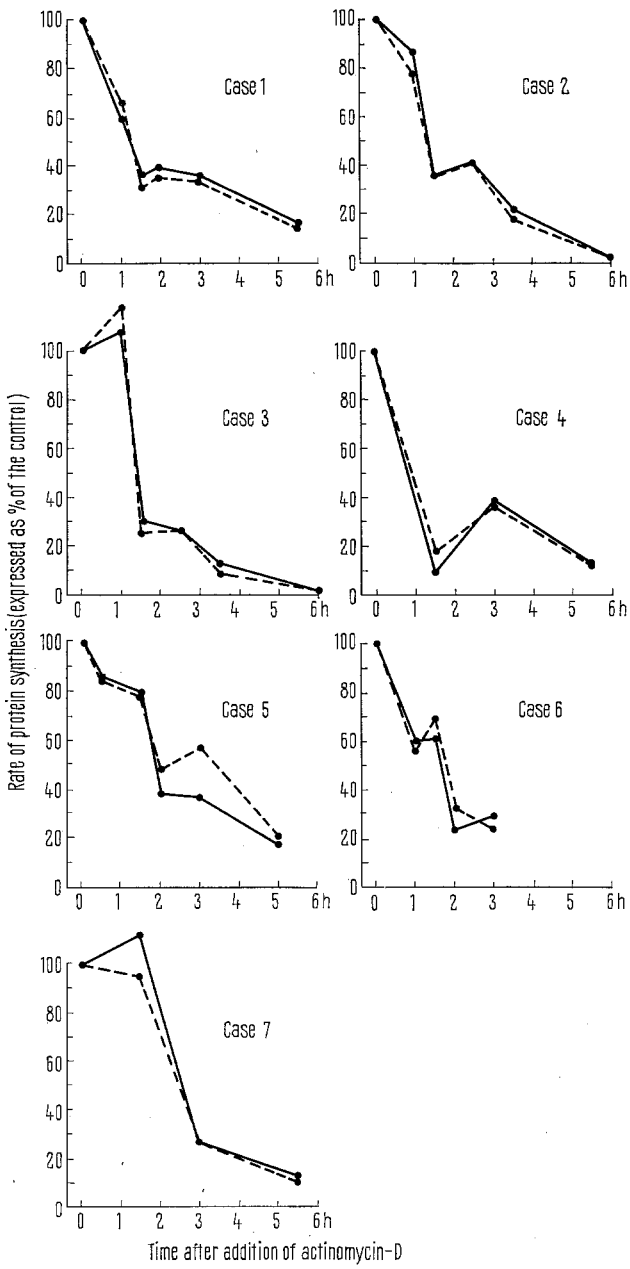
[1] E. H. Davidson, V. G. Allfrey and A. E. Mirsky, Proc. natn. Acad. Sci. *49*, 53 (1963).
[2] A. Ficq, Expl Cell Res. *34*, 581 (1964).
[3] D. L. Kirk, Proc. natn. Acad. Sci. U.S.A. *54*, 1345 (1965).
[4] J. Kruh, Revue fr. Etud. clin. biol. *10*, 271 (1965).
[5] V. Lazda and J. L. Starr, J. Immun. *95*, 254 (1965).
[6] F. Mullinax, G. L. Mullinax and B. Himrod, Am. J. Med. *42*, 302 (1967).
[7] E. Reich, R. M. Franklin, A. J. Shatkin and E. L. Tatum, Science *134*, 556 (1961).
[8] M. Revel and H. H. Hiatt, Proc. natn. Acad. Sci. U.S.A. *51*, 810 (1964).
[9] K. Scherrer, L. Marcand, F. Zajdela, B. Breckenridge and F. Gros, Bull. Soc. Chim. biol. *48*, 1037 (1966).
[10] R. B. Scott and R. A. Malt, Nature *208*, 489 (1965).
[11] S. Tawde, M. D. Scharff and J. W. Uhr, J. Immun. *96*, 1 (1966).
[12] U. Torelli, T. Artusi, G. Grossi, G. Emilia and C. Mauri, Nature *207*, 755 (1965).
[13] U. Torelli, D. Quaglino, T. Artusi, G. Emilia, G. Ferrari and C. Mauri, Expl Cell Res. *42*, 1 (1966).
[14] J. W. Uhr, Science *142*, 1476 (1963).
[15] F. H. Wilt, J. molec. Biol. *12*, 331 (1965).
[16] A. Pileri, R. Bernardelli, L. Brusa, R. P. Tarocco and F. Gavosto, Revue fr. Etud. clin. biol. *12*, 986 (1967).
[17] F. Gavosto, A. Pileri, V. Gabutti and P. Masera, Europ. J. Cancer *3*, 301 (1967).
[18] F. Gavosto, A. Pileri, V. Gabutti and P. Masera, Nature *206*, 188 (1967).

monoblastic leukaemia (No. 6) and 1 case of chronic myeloid leukaemia in terminal blast crisis (No. 7).

In all cases, bone marrow blood was withdrawn in a heparinized syringe and diluted in Hanks fluid (1:1 or 1:4). Horse serum was added to make a final concentration of 20%. Incubation was carried out at 37 °C in a rotating system.

Actinomycin-D inhibition of RNA synthesis was investigated by a 10 min preincubation with the antibiotic (10 μg/ml), followed by the addition of 10 μc/ml uridine-5-H^3 for 1 h.

Changes in protein synthesis were determined by comparison between control cultures and cultures containing actinomycin-D following serial exposures to DL-leucine-4,5-H^3 (specific activity: 10 c/m*M*), each of 1 h, at a final concentration of 10 μc/ml.



Degree of protein synthesis in proliferating (—) and non-proliferating (– – –) acute leukaemia cells.

After dilution in Hanks' fluid, marrow blood was incubated at 37 °C in a rotating system with thymidine-H^3 (concentration: 2 μc/ml) for 1 h to determine its proliferative capacity. After fixing in Carnoy, the smears were immersed in 1:1 distilled water and Ilford K_2 emulsion and left exposed for 10–15 days at 4 °C. After development and fixing, the slides were counterstained with May-Grünwald-Giemsa buffered to pH 7.4. Assignment of blasts to the proliferating and non-proliferating categories was based on diameter and on the thymidine-H^3 evidence of their proliferative activity. In each case, labelling was evaluated on at least 500 cells.

Results. Variations in protein synthesis rate following actinomycin-D-induced RNA synthesis block in acute leukaemia blasts are shown in the graphs. Differences within our series include: from 0–60 min, rapid falls in cases 1, 4 and 6, slight falls in cases 2 and 5, no change in cases 3 and 7; during 60–120 min, a distinct fall from start values in all cases, reaching 50% of control levels by the 2nd hour. These behaviour patterns were the same in both categories in all cases.

Discussion. Actinomycin-D-induced block of DNA-dependent RNA synthesis was always accompanied by decreased protein synthesis, falls to 50% of starting values being generally observed within the 1st and 2nd hours. In some cases, contact with the antibiotic was not immediately followed by decreased values, though massive reduction of protein synthesis was eventually observed.

Behaviour patterns were the same in both the proliferating and the non-profilerating categories in all cases. This means that the transformation of large proliferating into small non-proliferating blasts[19] does not involve meaningful differences in the half-life of their messenger RNA's; this finding is in line with our previous demonstration of similar RNA degradation behaviour in both proliferating and non-proliferating acute leukaemia cells[20].

We may, therefore, draw the conclusion that the transformation of large to small blasts, accompanied by the loss of proliferative activity, must be seen as an abortive expression of cell differentiation due to inability to synthetize the stable RNA's commonly observed during the later stages of haemopoietic cell maturation[21].

Riassunto. Negli elementi blastici di leucemia acuta umana incubati in vitro viene osservato un dimezzamento delle sintesi proteiche fra la prima e la seconda ora di contatto actinomicinico. Uno stesso comportamento metabolico è stato rilevato negli elementi blastici proliferanti e non proliferanti.

A. PILERI, R. BERNARDELLI, L. BRUSA, R. P. TAROCCO and F. GAVOSTO

Haematology Laboratory, General Medical Clinic of the University, 10126 Torino (Italy), 11 March 1969.

[19] F. GAVOSTO, A. PILERI, C. BACHI and L. PEGORARO, Nature *92*, 203 (1964).

[20] A. PILERI, R. BERNARDELLI, L. BRUSA, P. G. DE FILIPPI and F. GAVOSTO, Experientia *24*, 1267 (1968).

[21] This work was supported by the Consiglio Nazionale delle Ricerche.