Uridine incorporation and actinomycin binding: mean grain counts (MGC) in 2 normal human bone-marrow specimens

Marrow cells	Case 1		Case 2	
	Uridine (MGC)	Actinomycin (MGC)	Uridine (MGC)	Actinomycin (MGC)
Myeloblasts	114.6	114.7	103	96.4
Promyelocytes	98	107.5	91.1	103.5
Neutr. myelocytes	25	73.8	24.9	72.5
Neutr. metamyelocytes	2.8	65.8	2.3	59.1
Neutr. granulocytes	1.3	61.5	0.9	53.6
Proerythroblasts	154.6	118.6	228	128
Bas. erythroblasts	71.9	70.3	94.7	93.5
Early poly.erythroblasts	10.1	62.7	9.7	55.4
Late poly.erythroblasts	3.2	53.7	3	51.4
Lymphocytes	1.6	39	0.8	44.6

acid (70:30) at 0 °C and then placed in a 5 μCi/ml ^{3}H-actinomycin solution (spec. activity: 6.5 Ci/m*M*) for 1 h at room temperature. They were next treated with 10 μg/ml unlabelled actinomycin and rinsed in running water overnight before preparation for autoradiographic processing in Ilford K2 emulsion, and stained with May-Grünwald-Giemsa. For uridine uptake, bone marrow diluted 1:1 with Hank's medium was incubated at 37 °C for 1 h with 10 μCi/ml 5-T-uridine (spec. activity 3 Ci/m*M*). Preparation of the smears was followed by fixing in acetic Carnoy and autoradiographic processing[1–2]. Grain counts were made for each cell type and in each case on a minimum of 50 cells for myeloblasts and proerythroblasts, or 200 cells for all other types. The mean grain count was then determined. When selecting the autoradiographic preparations, use was made of exposures (20 days) showing significant labelling even on the part of very mature erythroid and myeloid cells.

Results and discussion. The Table gives the mean grain counts for actinomycin D binding and uridine incorporation in myeloid and erythroid cells.

It can be seen that actinomycin D binding values decrease in function of increasing differentiation in both cell lines. This pattern is even more striking in the case of uridine incorporation. Here variations of the order of 100:1 between cells in different stages of differentiation have been noted, in contrast to the approximately 2:1 variations found for actinomycin binding values.

This disparity could be due to large differences in ribosomal RNA synthesis during the various stages of differentiation, while variations in genetic transcription, i.e. messenger RNA synthesis, might be much smaller. The latter is presumably evidenced by the ability of chromatin to bind actinomycin D, while it is clear that uridine incorporation will only permit evaluation of total RNA activity; no distinction between ribosomal RNA synthesis and the lesser messenger RNA and transfer RNA syntheses has so far been possible with this method. In other words, nucleolar organiser DNA would be more active in immature nucleolated cells and this would result in intensive ribosomal RNA synthesis: this high activity would be detectable by measurement of uridine incorporation, but not by that of actinomycin D binding. These findings are in line with recent work on macromolecular synthesis during early embryonic development showing that the synthesis of the various kinds of RNA is not coordinated[4,10].

Riassunto. Nel midollo umano normale, parallelamente alla differenziazione cellulare delle linee mieloide ed eritroide, si osserva, per l'incorporazione dell'uridina nell'ARN, una diminuzione maggiore di quella della fissazione dell'actinomicina-D alla cromatina.

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Immunosuppressive Activity of Azathioprine in Experimental Infection of the Mouse with *Trichinella spiralis* (Nematoda)

Mice infected with the muscle stage of *Trichinella spiralis* characteristically expel the majority of adult worms from the intestine during the second week of the initial infection. This expulsion is due to a primary delayed hypersensitivity (cellular) reaction against the adults and a secondary inflammatory change in the intestinal tissue[1]. This expulsion has been suppressed by various immunosuppressive compounds in various hosts[2]. The effect of azathioprine on *T. spiralis* has been reported only for the guinea pig[2]. As a result, the objectives of this study were to investigate the efficacy of azathioprine against the

[1] J. E. LARSH, N. F. WEATHERLY, H. T. GOULSON and E. F. CHAFFEE, J. Parasit. *58*, 1052 (1972).
[2] J. P. HARLEY, Proc. helminth. Soc. Wash. *40*, 242 (1973).

primary immune and anamnestic responses in the mouse infected with *T. spiralis*.

Materials and methods. Swiss-Webster male albino mice were used throughout. Recovery of larvae from stock mice and infection procedures were according to HARLEY and GALLICCHIO[3]. 20 mice were infected by gavage with 350 *T. spiralis* larvae. After 30 days postinfection, 10 of these mice were desiginated Group I (immunized controls) and 10 Group II (immunized + azathioprine).

Stock mice which had not been infected were divided into Groups III (control, nonimmunized) and IV (control + azathioprine), with 10 mice per group. Each mouse received, by gavage, 350 *T. spiralis* larvae. Groups II and IV were simultaneously treated orally with 3 mg/day of azathioprine (Imuran®, Wellcome Research Laboratories) beginning 2 days preinfection and continuing daily through day 20 postinfection.

All mice were killed 30 days postinfection and the number of muscle larvae determined according to HARLEY and GALLICCHIO[3]. Student's *t*-test was used to determine the statistical significance of the observed differences in numbers of muscle larvae recovered from experimental and control groups. A probability greater than 0.05 was not considered significant.

Results and discussion. The effect of azathioprine on numbers of *T. spiralis* muscle larvae in immunized vs nonimmunized mice is shown in the Table. Statistically, there was no significant ($P > 0.05$) difference in the number of muscle larvae in immunized, nonsuppressed mice (Group I) as compared to nonimmunized, suppressed mice (Group IV). All other differences were significant ($P < 0.05$).

Effect of azathioprine on numbers of *Trichinella spiralis* muscle larvae in immunized vs nonimmunized mice

Group	Mean ± S.D.
Immunized mice + 350 *T. spiralis* larvae (I)	715.832 ± 199.490
Immunized mice + 350 *T. spiralis* larvae + azathioprine (II)	327.191 ± 97.813
Normal mice + 350 *T. spiralis* larvae (III)	499.703 ± 177.797
Normal mice + 350 *T. spiralis* larvae + azathioprine (IV)	945.971 ± 266.164

Thus, azathioprine suppressed the primary immune response of mice infected with *T. spiralis*; however, the anamnestic response was not suppressed. This study gives further evidence that immunity in mice infected with *T. spiralis* is primarily due to delayed (cellular) hypersensitivity. This latter response leads to intestinal inflammation and subsequent expulsion of adult worms. Since the number of muscle larvae was significantly reduced ($P < 0.001$) in immunized, suppressed mice as compared with the immunized nonsuppressed mice, the anamnestic response was not affected.

This again gives further support to the accumulation of evidence that the inflammatory change in the intestine is the final effector mechanism of the delayed hypersensitivity reaction. That this delayed hypersensitivity exists, supports the hypothesis that 'the immunity of mice against the adult worms of T. *spiralis* has a cell-mediated basis'[1]. Finally, this described host-parasite model system can be a useful adjunct to evaluate the immunosuppressive action of other experimental drugs that are designed to inhibit the hypersensitivity phenomena and/ or antibody formation[2].

Zusammenfassung. Die Wirkung von Azathioprin auf die Immunantwort von mit *Trichinella spiralis* infizierten Mäusen ist auf Grund der Anzahl in der Muskulatur aufgefundenen Larven beurteilt worden. Nach einer Erstinfektion zeigen behandelte Tiere gegenüber Kontrollen eine unterdrückte Immunantwort. Eine immunsuppressive Wirkung von Azathioprin bleibt bei einer sekundären Infektion hingegen aus.

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14 January 1974.

[3] J. P. HARLEY and V. GALLICCHIO, Expl. Parasit. *30*, 11 (1971).
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Control of Anticoagulation in Rats

Coumarins are widely used as rat-killers by inducing severe hypocoagulability which results in haemorrhaging, but socalled therapeutic anticoagulation in the rat, as one of the most frequently used laboratory animals, has scarcely been studied. For the laboratory control of the degree of anticoagulation in rats, thromboplastins[1–4] were used, which were not well-defined as to their sensitivity for rat coagulation factors.

Experience in humans has been that calibration of thromboplastins is rather difficult[5]. Sensitivity for clotting factor levels has to be checked as well as the inhibitory influence of inactive clotting factors, called PIVKAs (Proteins in vitamin K-absence; Hemker et al.[6]). We decided to evaluate a rapid and well-standardized assay method for its sensitivity for the mean clotting factor level of the prothrombin complex[7] and the possible influence of PIVKA on the test result in rats under anticoagulation.

Methodology. A) For the construction of a reference curve for blood samples assayed in the Normotest reagent (Nyco, Oslo, Norway) 12 healthy male Wistar rats (aged 4 months, body weight 325–375 g) from an inbred colony[8] fed on a standard diet and with factor II, VII and X levels in the normal range (80–120% assayed against

[1] B. B. COLDWELL and Z. ZAWIDSKA, Blood *32*, 945 (1968).
[2] Z. Z. ZAWIDZKA, B. B. COLDWELL and H. C. GRICE, Experientia *28*, 1482 (1972).
[3] TH. J. MCINTOSH, W. R. WILSON, L. WATERS and J. R. FOUTS, Eur. J. Pharmac. *14*, 176 (1971).
[4] R. NIEDNER, M. KAYSER, N. REUTER, FR. MEYER and W. PERKOW, Arzneimittel-Forsch. *23*, 102 (1973).
[5] E. A. LOELIGER, Thromb. Diath. Haemorrh. *28*, 109 (1972).
[6] H. C. HEMKER, J. J. VELTKAMP, A. HENSEN and E. A. LOELIGER, Nature, Lond. *200*, 589 (1963).
[7] J. J. VELTKAMP, in *Human Blood Coagulation* (University Press, Leiden 1969), p. 381.
[8] H. G. M. GEERTZEN, F. J. G. VAN DER OUDERAA and A. A. H. KASSENAAR, Acta endocr., Copenh. *72*, 197 (1973).