tion[3,4], have been examined for their inhibitory activity, cytokinins so far have been omitted from such systemic studies. Only the work reported by Gallo et al. in early 1969 disclosed such an effect in N^6-isopentenyladenosine[6]. A recent work by Hecht et al. suggested the possibility of inhibitory activity in various cytokinins from a biochemical basis[1], but an actual comparison of their inhibitory activity has not yet appeared.

This paper disclosed 1 important finding, i.e., cytokinins with a nucleoside structure, including N^6-benzyladenosine and N^6-isopentenyladenosine, substantially inhibited human lymphocyte metabolism stimulated with PHA at concentrations as low as 10^{-6} M. When compared with the effect of dibutyryl cyclic-AMP, almost $^1/_{100}$ of the concentration was sufficient to inhibit blast formation. Bona et al. reported[7] that 5′-deoxy-5′-S-isobutyl-adenosine, a synthetic analogue of S-adenosyl-homocysteine inhibited the mitogen-induced blast formation of human and rabbit lymphocytes. This compound also has the ability to prevent oncogenic transformation of chicken fibroblasts by Rous Sarcoma virus. The effect of time of addition of 5′-deoxy-5′-S-isobutyl-adenosine was identical to that of N^6-benzyladenosine and N^6-isopentenyl adenosine (data not shown). It would be interesting to compare the activities of N^6-benzyladenosine and N^6-isopentenyladenosine with that of 5′-deoxy-5′-S-isobutyladenosine in preventing oncogenic transformations. Biochemical analyses, such as the effect on cyclic-AMP phosphodiesterase on the compounds tested here, is under way.

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On the effects of thiamphenicol and chloramphenicol on nucleic acid and protein synthesis in rabbit bone marrow cells in vivo and in vitro

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Summary. Besides the in vivo effects of thiamphenicol (TAP), this study shows the in vitro effects of TAP and D- and L-threo-chloramphenicol (CAP) on the synthesis of DNA, RNA, protein and hemoglobin in marrow cells and reticulocytes. The experiments make it likely that TAP exerts its action on a stem cell in a proliferating phase.

TAP and CAP are potent inducers of a reversible marrow depression. Furthermore CAP can cause an aplastic anemia. It is generally accepted that the mechanism of the reversible depression is related to the inhibition of protein synthesis in mitochondria[2-5]. Previous results led us to the conclusion that the toxic effects of TAP have their origin at the start of the differentiation of the erythroid cell line[6]. In this report we describe the relationship between the onset of the decrease of cytochrome c oxidase activity (reflecting the mitochondrial protein synthesis) and the activity of marrow cells with respect to the synthesis of DNA, RNA and protein under a TAP regime in vivo. Moreover we included our in vitro studies because of conflicting conclusions about the action of CAP on mammalian protein synthesis as reported in the literature. The inhibitory effects of CAP on reticulocyte protein synthesis, as found by Weisberger[7], could not be reproduced by Zelkowitz[8]. Recently, Agam[9] demonstrated that CAP inhibited platelet protein synthesis in vivo at low doses and in vitro at high doses. A further aim of our study was to shed more light on the meaning of differences between the effects of TAP and CAP on synthetic processes in vitro in relation to the fact that the aplastic anemia has not been reported for TAP. At very high concentrations of the antibiotics, Yunis[10] did indeed observe differences on the synthesis of DNA.

Materials and methods. Chinchilla rabbits were bled on 4

Table 1. Effects of high concentrations of TAP, D-threo-CAP and L-threo-CAP on the in vitro synthesis of DNA, RNA, protein and haemoglobin in marrow cells and on the synthesis of haemoglobin in reticulocytes

Addition	(mg/ml)	DNA	RNA	Protein	Hb (marrow)	Hb (reticulocytes)
TAP	0.5	91±6	102±14	95±6	80±4	100±4
	1.0	91±5	112± 4	85±4	79±6	100±2
D-CAP	0.5	52±7	60± 4	42±7	41±2	96±8
	1.0	27±4	42± 7	21±5	21±9	81±6
L-CAP	0.5	83±2	76± 4	75±2	80±4	88±2
	1.0	47±2	66± 8	44±3	55±8	85±4

Marrow cells and reticulocytes were obtained from phenylhydrazine-treated rabbits. The in vitro synthesis of the different compounds was measured as described in 'materials and methods'. The incorporation values are expressed as percentages of the results from a 2-h incubation without addition of antibiotics (± SEM; n = 3).

consecutive days (30–40 ml/day). Simultaneously the treatment with TAP (as the glycinate) via a dialysis bag s.c. implanted in the neck started and was prolonged to 7 days (dose 250 mg/kg b.wt; days 1–4 2 doses, days 5–7 1 dose). At certain time intervals during and after the TAP treatment a rabbit was bled by heart puncture and the marrow cells were isolated[6]. To measure the rate of synthesis of DNA, RNA and protein, 1 ml of the cells supplemented with 0.25 µCi [2-^{14}C]-thymidine, 5 µCi [5-^{3}H]-uridine or 0.5 µCi [1-^{14}C]-leucine, respectively, was incubated at 37 °C. The reactions were stopped by adding 1 ml 10% $HClO_4$ at 0 °C. The precipitate was washed with 5% $HClO_4$, 80% alcohol, alcohol:ether (3:1) and ether. The precipitate was solubilized in 0.5 ml soluene. The radioactivity was counted in dioxane-PPO-POPOP scintillator in a Nuclear Chicago Liquid Scintillation Counter.

Isolation of reticulocytes. A reticulocytosis was induced in rabbits by i.m. injections of phenylhydrazine. The synthesis of hemoglobin was measured with ^{14}C-leucine as earlier described[6].

Determination of cytochrome c oxidase activity. Marrow cell suspensions were pelleted and frozen. After thawing cytochrome c oxidase activity was measured[11].

Results. The dependence of the rate of synthesis of DNA, RNA and protein on the cell concentrations is shown in figure 1, a, b. The synthetic processes proceed linearly during 2 h of incubation (figure 1, a). Maximal thymidine incorporation per cell was reached at 10^7 cells/ml. The same maximum was found for the leucine incorporation. The maximal ^{3}H-uridine incorporation was at 10^6 cells/ml (figure 1, b). The reason for these differences is unknown. We have chosen a concentration of 5×10^6 cells/ml as the most convenient for the next studies.

The effect of TAP and D- and L-threo-CAP on in vitro synthesis of DNA, RNA and protein. The effects of the antibiotics (added as the free bases) on synthetic processes in marrow cells and reticulocytes from phenylhydrazine-treated rabbits is shown in table 1. TAP up to 1 mg/ml had only minor effects; 0–20% inhibition in the case of DNA and protein synthesis; the synthesis of RNA seems even to be increased. D-threo-CAP at the same concentration did inhibit the synthesis of hemoglobin in reticulocytes with 20%, L-threo-CAP with 15%. The effects on marrow cells were more prominent. At 1 mg/ml D-threo-CAP did inhibit DNA, RNA, protein and hemoglobin synthesis with 73, 58, 79 and 79%, respectively. The inhibition with L-threo-CAP was less. Antibiotic levels comparable to the concentrations added in these incubations are never reached during in vivo treatment[11]; thus it is not to be expected that TAP will have any direct effect on DNA, RNA and cytoplasmic protein synthesis in vivo.

The time course of the effects of TAP-treatment on cytochrome c oxidase activity. TAP administration in vivo led to a decrease in the cytochrome c oxidase activity in marrow cells (table 2). The decrease is progressive and after 7 days only 20% of the original activity remained. After TAP was cleared from the blood (24 h after the last dose), a rapid increase was found. Within 24 h, the activities reached a level of 70% of the starting values.

The time course of the effects of TAP-treatment on the synthesis of DNA, RNA and protein. Compared to marrow cells from untreated rabbits, those from bled rabbits showed an increased rate of synthesis of DNA, RNA and protein (figure 2). The values for the synthesis of RNA even increased with 156%. This stimulation was not found when 1-day-bled rabbits were treated with TAP. On further treatment, the inhibition was more prominent. The decrease in the DNA-, RNA- and protein synthesis started after a 3-day, 5-day and 7-day treatment, respectively. In all cases a fast recovery after the treatment was observed.

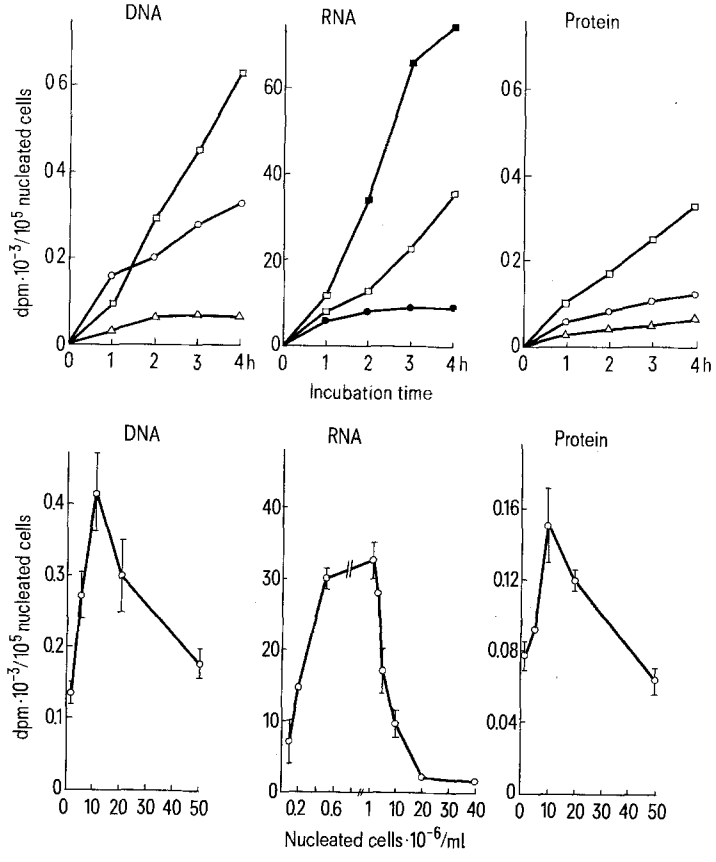

Fig. 1. a: Time course of the incorporation of [2-^{14}C] thymidine, [5-^{3}H] uridine and [1-^{14}C] leucine into DNA, RNA and protein, respectively, at different cell concentrations. Marrow cell suspensions at the concentrations indicated were incubated with radioactive precursors at 37 °C for 4 h. At 1 h intervals samples were taken and the incorporation of precursors was measured as described in 'materials and methods'. ■ = 10^6; □ = 10^7; ● = 1.5×10^7; ○ = 5×10^7; △ = 10^8 nucleated cells per ml. b: The relation between the nucleated marrow cell concentration and the incorporation of radioactive precursors into DNA, RNA and protein. From experiments as described in figure 1, a, the 2-h incubation values were plotted against different cell concentrations (±SEM; n = 3).

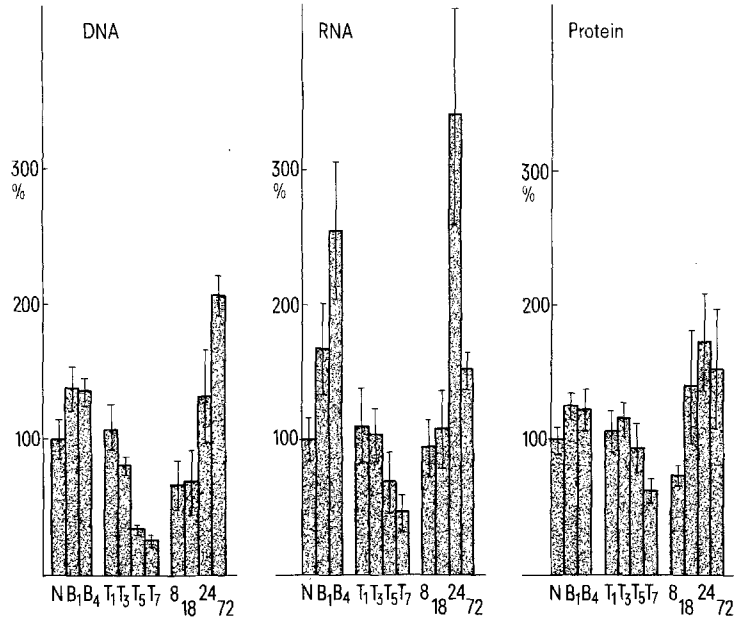

Fig. 2. Effects of TAP-treatment of rabbits on the synthesis of DNA, RNA and protein in marrow cells. (±SEM; n=5-7; 100% values for DNA, RNA and protein synthesis are 502, 11,200 and 178 dpm per 10^5 nucleated cells at 4-h incubation at 37 °C.) For the symbols used legend to table 2.

Table 2. The cytochrome c-oxidase activity in marrow cells from rabbits under different experimental conditions

	Control			During TAP-treatment				After TAP-treatment			
Rabbit	N	B1	B4	T1	T3	T5	T7	8	18	24	72
%	100	120	119	70	53	20	17	33	33	70	117
±SEM	14	24	31	6	25	2	5	12	20	35	41

Values of the reaction constant K($min^{-1} \cdot mg\ protein^{-1}$) of treated rabbits are expressed as percentages of those of normal rabbits (N) (100% value of k=3.0). B1 and B4 values from rabbits bled on 1 and 4 days, respectively. T1-T7 values from rabbits bled on 1-4 days and treated with TAP on 1-7 days. 8-72 values from rabbits bled on 4 days, treated 7 days with TAP being without TAP-treatment during 8-72 h (n=5-7).

Discussion. It is very unlikely that the depression of bone marrow cell activities by TAP in vivo is caused by the mechanism underlying the effects found by others[9,10] with CAP in vitro. Also we find that CAP inhibits protein synthesis in marrow cells but not in reticulocytes. Moreover the general inhibitions in marrow cells cannot be explained by a specific effect on one of these processes. In view of the very high concentrations, and the lipophilic character of the antibiotics, we tend to believe that these effects are caused by a specific interference of the drugs with membrane processes such as transport. Whatever the explanation may be, the inhibitory effects by extreme high concentrations of CAP and TAP in vitro never can be extrapolated to the in vivo situation as Yunis et al.[10] suggested. Furthermore our experiments show a sharp reduction of marrow cell activities after TAP-administration. However, the reduction of cytochrome c oxidase activity precedes that of the synthesis of DNA. This is an indication that inhibition of mitochondrial protein synthesis is the primary effect leading to an inhibition of cell division and becoming manifest as inhibition of DNA synthesis and diminution of young red cells. Bass et al.[12] demonstrated a reduction of cytochrome c oxidase activity in rat embryos. At 80% inhibition, the synthesis of DNA was reduced with 50% in their experiments.

Until now it is unknown if the rest activity, found after 7 days treatment, equals the normal activity of DNA, RNA and protein synthesis in granulocytes and lymphocytes. This may be the case in view of their lower turnover rate. This is subject for further research.

Shortly after TAP levels in the blood became very low, cytochrome c oxidase activity increased. The increase in the cytochrome c oxidase activity from 17% to 33% is apparently sufficient for stimulating the cellular metabolic processes and also DNA, RNA and protein synthesis do increase. As a result, there is also an increment in the proerythroblast and basophilic erythroblast compartments, as was shown earlier[6]. Therefore, we expect that somewhere in the differentiation line of the red cells, just before they become morphologically recognizable, the action of TAP is exerted on a stem cell in a proliferating phase. In these cells, the possibility of a fast reduction of cytochrome c oxidase activity exists. This may lead to unemployment (arrest in the Go phase) instead of differentiation into the proerythroblasts. When mitochondrial protein synthesis is resumed, these cells are reactivated and they differentiate along the red cell line. Further studies are necessary to decide on what kind of stem cell TAP exerts its severe inhibitory effect on erythropoiesis.

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