whether the animals had been pre-treated with BCG or had received no pre-treatment.

This fact, which confirms our previous observations[8,9] tends to show that the effect of BCG is increased by stimulating the multiplication of cells implicated in an immune reaction; this effect can counterbalance the proliferation of the leukaemic cells in the spleen, but was clearly abolished by a cytostatic drug.

It is now necessary to find out the duration of this effect of BCG in the prevention of the immune depression bound to the development of a compatible leukaemia. This idea could have important applications in clinical medicine since it seems that the simple vaccination of infants with BCG has already been demonstrated to have a remarkable effect in diminishing the probability of the development of acute leukaemia in children[10].

The second idea that emerges from these results is the danger in patients stimulated by BCG of subsequent treatment by cytostatics including antiviral products that have cytostatic action. This fear came from the results in experimental animals and, unfortunately, has been found to be true in clinical practice[11].

Résumé. Le BCG donné avant une greffe de cellules leucémiques compatibles atténue l'effet immunodépresseur du développement de cette leucémie; il n'a aucun effet sur l'immunodépression due à un traitement ultérieur par un cytostatique.

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Induction of Chromosome Abnormalities by Reversal of Colcemid Inhibition in Leucocyte Cultures

Several reports have shown that the mitotic inhibitory effect of colchicine and its derivatives can be reversed by washing the cells free of the drug and then growing in fresh drug free media[1-3]. Stubblefield and Kleveez[3] claimed thata higher mitotic yield can be obtained by using this method and that synchronized population of cells could be produced. This mechanism has been investigated mainly in long term monolayer cultures and as yet the effect of colcemid reversal on short term cultures has to be unfolded. We have been investigating this mechanism in human peripheral blood leucocyte cultures and would like to report our findings to date.

Materials and methods. Six (in duplicate) cultures were set up from 3 apparently normal females using a standard method previously described[4].

The test cultures involved the addition of colcemid (CIBA) (0.001 μg/ml) for 2 and 4 h after they had been cultured for 48 h. The cells in all cultures were then washed twice in prewarmed Hank's solution resuspended in medium consisting of 80% Eagle's minimum essential medium and 20% foetal calf serum, incubated for a further 24 h and then harvested in the manner previously described[4].

Cultures 1 and 4 which were set up from the first subject and cultures 2 and 3 which were set up from the second subject were the 2 experiments involving exposure to colcemid for 4 h. Cultures 3 and 4 were not exposed to colcemid but were washed similarly and used as controls to experimental cultures 1 and 2. Culture 5 was exposed to colcemid for 2 h and culture 6 was the control; both cultures being from the third subject. The results are shown in the Table.

Results. 217 cells were examined from the 4 h test culture and of these 99 (45.6%) were hypodiploid. All the cells with 45 chromosomes had a group C chromosome missing. 70% of the cells with less than 45 chromosomes had either 1 or 2 group C chromosomes missing with group E, F and G only minimally affected. An extremely high percentage (54%) of endoreduplicated cells was obtained in culture 1, 20 of (10%) the 200 control cells had 45 or less chromosomes with the losses not being restricted to any one specific group to any appreciable extent.

The metaphase figures from the 2 h experiments were of extremely poor quality. Hence as a result only 40 experimental and 52 control cells were examined.

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Chromosome counts from 6 duplicate cultures

Culture No.	Exposure	Chromosome No. 43	44	45	46	47	48	Tetraploidy	Endoreduplicated	Total cells
1	Colcemid 4 h	19	8	5	10	–	–	4	54	100
2	Colcemid 4 h	45	14	8	41	1	–		8	117
3	Control 4 h	–	5	2	87	–	–	6	–	100
4	Control 4 h	3	8	2	89	–	–	–	–	100
5	Colcemid 2 h	12	5	2	17	–	–	–	4	40
6	Control 2 h	4	2	2	43	–	–	1	–	52

Careful analysis of the cells with abnormal chromosome numbers in both the control and test cultures did not reveal any constant loss of chromosomes belonging to a specific group.

Discussion. Althoug a relatively small number of cells have been examined from the 2 h colcemid treatment; it is apparent that no specific chromosomal abnormalities are induced by the colcemid reversal process. However, nondisjunctional errors are apparent in view of the high percentage of aneuploidy.

The results from the 4 h experiments are more interesting. In addition to specific group C chromosomes being involved in nondisjunction, a high degree of aneuploidy and endoreduplication are evident. KATO and YOSIDA[5] reported random nondisjunctional errors in chinese hamster cells and in addition observed trisomies and tetrasomies. Apart from the fact that we are unable to confirm the randomness of chromosome loss, no trisomies or tetrasomies have been observed. In fact hyperdiploidy with the rare exception of tetraploidy and endoreduplicated cells, were extremely rare.

HERERos et al.[6] investigated the effect of colcemid reversal on blood leucocyte cultures and observed a high incidence of endoreduplicated cells. Their experimental system is not altogether comparable to that of the present report as they cultured their cells for 6 days during which time the cells would have undergone several divisions. In the system used in this report the cells would have undergone a maximum of 2 divisions. Nevertheless we have confirmed in at least 1 experiment (culture 1), the high incidence of endoreduplicated cells in addition to aneuploidy.

The colcemid concentration that permitted the reversed cells to proceed normally through the cell cycle has been reported by ROMSDAHL[2] to vary from cell line to cell line. Gross chromosomal abnormalities (caused by multipolar mitosis) of the kind described by STUBBLEFIELD et al.[7] in chinese hamster cells have not been observed in any of our mitoses examined.

In view of the fact that only a proportion of mitoses show abnormalities one can assume that a certain proportion of arrested cells might even after a 2.h treatment fail to recuperate completely.

The effect of the colchicine and its derivatives on various cell populations is clearly variable; hence, it is important that investigations should be extended to cover a wide range of cell populations in order that a more thorough knowledge of the action of colchicine be aquired[8].

Zusammenfassung. Menschliche Leukozyten, die vorher 2 resp. 4 h der Wirkung von Colcemid ausgesetzt worden waren, ergaben in Zellkulturen 45 resp. über 50% Zellen mit 45 oder weniger Chromosomen im Vergleich zu 10% in Kontrollkulturen ohne Colcemid.

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Über die Wirkung von Na-Diphosphat auf ein Lymphosarkom der Maus

Kürzlich hatte PETERS[1] beschrieben, dass $Na_4P_2O_7$ bei Kaltblütlern eine cytostatische Wirksamkeit besitze. Die Anwendung erfolgte lokal bzw. als Zusatz zum Wasser, in dem die Fische lebten. Uns interessierte nun, ob dieser Befund auf Warmblütler zu übertragen ist. Mäusen mit eben tastbarem Nemeth-Kellner-Lymphosarkom wurde $Na_4P_2O_7$ intratumoral injiziert bzw. in einer anderen Versuchsreihe in DMSO aufgenommen und percutan appliziert. Als Höchstdosis wurden 0,9 mg/20 g Maus vertragen, drei Gruppen wurden mit 0,9 mg, mit 0,6 mg bzw. 0,3 mg je 5 × behandelt. Percutan waren die 0,9 mg kaum verträglich, nach der 3. Applikation musste die Therapie abgebrochen werden. Das Tumorwachstum wurde durch tägliche Messung kontrolliert. Da der Wachstumsimpetus gleichartig war, wurden die Tiere am 10. Tag nach Versuchsbeginn getötet und das durchschnittliche Tumorgewicht bestimmt. In der Tabelle sind die Werte aufgeführt. Daraus geht klar hervor, dass das Nemeth-Kellner-Lymphosarkom der Maus durch $Na_4P_2O_7$ nicht gehemmt wird, wenn es intratumoral appliziert wird. Wird es in DMSO aufgenommen und auf den Tumor aufgetragen, dann wird das Wachstum sogar stimuliert.

Dosis	Applikation	Durchschnittliches Tumorgewicht
0,9 mg/20 g	intratumoral 5 ×	2,61 g
0,6 mg/20 g	intratumoral 5 ×	2,74 g
0,3 mg/20 g	intratumoral 5 ×	2,43 g
0,9 mg	percutan	4,10 g
Kontrollen		2,51 g

Summary. Tests were made to determine whether $Na_4P_2O_7$, which is effective against malignancies in fish, has also an effect on tumours in mice. The Nemeth-Kellner-Lymphosarcoma used did not react to the compound, either after intratumoral or after percutaneous application.

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