considérablement diminué in vitro, dès le 4ème jour d'incubation.

b) Des expériences d'injection de CGP de Dindon dans le réseau vasculaire vitellin d'embryons de Poulet sexuellement stériles âgés de 3 à 6 jours ont démontré que les ébauches gonadiques stériles pouvaient fixer, jusqu'au 5ème jour d'incubation, les gonocytes primordiaux injectés, mais n'en étaient plus capables le 6ème jour[8]. Ce résultat, obtenu in vivo, conduit à repousser l'époque de la disparition du pouvoir attractif de l'épithélium germinatif entre le 5ème et le 6ème jour d'incubation, soit juste avant la première poussée de cordons sexuels, correspondant à une phase de différenciation du cortex gonadique. Ces différentes données étant reportées sur un graphique (Figure), on s'aperçoit que le pouvoir attractif de l'épithélium germinatif s'étend sur une durée de 4 jours environ (entre la 35ème et la 125 ème h d'incubation) alors que la colonisation effective des ébauches gonadiques par les gonocytes ne nécessite que 2 jours, au maximum.

2. *Durée de la réactivité des cellules germinales au stimulus attractif de l'épithélium germinatif.* La réactivité des cellules germinales au stimulus attractif de l'épithélium germinatif se manifeste par la colonisation des ébauches gonadiques (du 2ème au 4ème jour d'incubation) et aussi, sans doute, par la migration intravasculaire qui la précède et qui débute une demi-journée plus tôt (35 h). Toutefois, ici encore, deux catégories de résultats expérimentaux tendent à prouver que cette propriété est bien plus étendue dans le temps qu'il n'y paraît.

a) Les travaux de ROGULSKA[15] ont montré que l'introduction d'un croissant germinal fertile, prélevé au stade de la ligne primitive terminale (18–20 h d'incubation), dans la cavité coelomique d'un embryon de 66 h (25–30 somites) aboutit à la migration rapide (en 1 h, dans certains cas) des CGP du croissant dans les crêtes génitales de l'hôte. Les CGP sont donc capables de répondre au pouvoir attractif de l'épithélium germinatif dès l'époque précoce de leur regroupement dans le croissant germinal extra-embryonnaire (18 h).

b) Les associations de fragments gonadiques en culture in vitro réalisées par DUBOIS[5,14] et rapportées plus haut ont montré que les gonocytes primaires contenus dans les ébauches gonadiques indifférenciées de 5–6 jours étaient encore capables de migrer hors de ces ébauches et, par suite, de réagir au stimulus attractif émanant de jeunes épithéliums germinatifs. D'autres associations faites par le même auteur[5] à partir d'ébauches gonadiques différenciées ont révélé que les gonocytes mâles conservaient leurs propriétés migratrices – et donc leur réactivité au stimulus attractif – jusqu'au 12ème jour d'incubation alors que les gonocytes femelles cessent de répondre à l'attraction de jeunes épithéliums stériles dès le 8ème jour. Là encore, il est important de souligner que ces deux époques de disparition de la réactivité correspondent respectivement chez chacun des sexes au début de la période de multiplication cellulaire intense qui va donner naissance aux ovogonies (8e jour) et aux spermatogonies (12e–13e jour) [SWIFT[16]]. La réactivité des cellules germinales disparaît donc, comme le pouvoir attractif de l'épithélium germinatif, peu de temps avant qu'intervienne une phase de différenciation cellulaire qui les concerne. Elle s'étend sur plusieurs jours (une semaine au minimum) et elle persiste davantage chez le mâle que chez la femelle dont les gonocytes se transforment plus précocement en gonies. Toutes ces données concernant la réactivité des cellules germinales sont également portées sur le graphique (Figure) dont la lecture permet de tirer les conclusions générales suivantes:

1. La durée du pouvoir attractif de l'épithélium germinatif (4 jours) et celles de la réactivité des cellules germinales femelles (7 jours) et mâles (11 jours) à ce pouvoir sont chacune bien supérieures à la durée de la colonisation des ébauches gonadiques (2 jours) qui constitue la manifestation essentielle de ces deux propriétés complémentaires.

2. La réactivité des cellules germinales a pu être décelée un peu avant l'apparition du pouvoir attractif de l'épithélium germinatif (15–18 h plus tôt) et, de toute évidence, elle se perpétue bien au-delà du terme de celui-ci, notamment chez le sexe mâle.

3. Le pouvoir attractif de l'épithélium germinatif et la réactivité des cellules germinales à ce pouvoir cessent chacun au moment où se prépare une phase importante de leur différenciation cellulaire respective.

*Summary.* Experimental results of different authors show that the attractive stimulus of the germinal epithelium for the germ cells in the chick embryo lasts less long than the reactivity of these cells to the same stimulus. However, both of them (stimulus and reactivity) last far longer than the period of the incorporation of germ cells into the primordia of the gonads. Each of them disappears when an important phase of differentiation is about to start in the group of cells concerned.

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## Attempts to Prevent Acquired Immunological Insufficiences in Mice by Bacillus Calmette-Guérin

The antigenicity of tumours induced by viruses, or by physical or chemical agents, the regression of compatible tumours by the action of active immunotherapy[1,2], the immune deficiencies observed in mice[3,4] and in man[5] bearing certain types of malignant tumours raises the question of the correlation between the development of tumours and the spontaneous or acquired depression of immunity.

It is of interest to know what are the states of immune insufficiencies that can be prevented by using active immunotherapy, such as can be produced by BCG inoculations. We have examined whether under certain conditions treatment with BCG can prevent the immunosuppression caused by 6-mercaptopurine, a cytostatic immunosuppressive agent, or by the establishment of a transplanted syngeneic leukaemia.

1st experiment: 40 male adult $F_1$ (DBA/2 × C57Bl/6) mice were divided at random into 8 groups of 5 mice. On day 0 all the mice received $10^9$ sheep red blood cells (SRBC) i.p.; on the 5th day they were killed and the number of haemolytic plaque-forming cells in their spleens assayed by the lysis in agar gel technique of Jerne and Nordin.

On day-15 the animals in groups II, VI, VII and VIII were given 1 mg (dry weight) of living BCG (Institut Pasteur) i.v.

$10^6$ C57Bl/6, E (AkR) leukaemic cells[6] were injected s.c. into the animals in groups V and VIII on day-10, into those in groups IV and VII on day-5, and into those in groups III and VI on day-0.

2nd experiment: 20 adult male $F_1$ (DBA/2 × C57Bl/6) mice were divided at random into 4 groups of 5.

All the animals received $10^9$ SRBC i.p. on day-0. The animals in groups II and IV were given 1 mg living BCG IV on day-15. The animals in groups II and III received daily from day-1 to day-4 inclusive 6-mercaptopurine (24 mg/kg/day) which is $^1/_6$th of the $LD_{50}$ at 6 days[7]. On the 5th day the animals were killed and their spleens assayed for haemolytic plaque forming cells as before.

*Results.* The results of the first experiment are summarized in Table I. It appears that the development of E (AkR) leukaemia causes a great immune insufficiency when the antigenic stimulus (SRBC) was not present until after the leukaemia had been growing for 10 days. This immune insufficiency was notably diminished by pre-treatment with BCG.

In the second experiment (Table II) the treatment with 6-mercaptopurine virtually completely suppressed the response to heterospecific red blood cells, and the suppression was not influenced by pre-treatment with BCG.

These results show that an immunosuppression of the primary response to heterospecific red blood cells could be due to several mechanisms in cancer. The immunosuppression could be the outcome of the effect of a drug given at an opportune moment in relation to giving the primary antigenic stimulus. This was the case in the group of mice treated with 6-mercaptopurine from the day-1 of being stimulated with SRBC until 4 days later. The immune deficiency may result from the growth of the tumour itself, particularly in leukaemia and lymphosarcoma[3].

The E (AkR) leukaemia causes an immunosuppression. It is genetically of C57Bl/6 origin and was first obtained by repeated inoculation of adult C57Bl/6 mice with massive doses of AkR leukaemic cells[6]. The immune suppression appears rather late: during the first 10 days of the evolution of this leukaemia the immune reactions remain normal, but are very much diminished by the 15th day. The mean and median survival time after an injection of $10^9$ cells is about 30 days (limits 27–30 days).

This immunosuppression was still perceptible in animals pretreated with BCG. The mice immunized 5 days after their inoculation with leukaemic cells (group IV) had a response that was equal to the controls who were simply immunized. The mice pre-treated with BCG then receiving the E (AkR) leukaemia, by contrast, had, 5 days later, a response that was only half that observed in mice given BCG but no leukaemic cells. The stimulant action of the BCG was still apparent, because in animals pre-treated with BCG and given E (AkR) leukaemia cells 10 days after this immunization the response was still $^1/_3$ of that in animals given BCG alone and was 5 times higher than in mice which had received the same number of leukaemic cells and were not pre-treated with BCG. The difference in possibility of mounting immunological responses no doubt explains the different patterns of evolution of the leukaemia. In 12 animals, pre-treated with BCG then inoculated with $10^6$ E (AkR) cells, 7 survived, the average survival time in the 5 that died was 56 days, whilst in controls no pretreated with BCG all the animals died after an average survival of 30 days.

The effect of a pre-treatment with BCG is different if the immune depression is induced by a cytostatic drug; the immunosuppression in this instance is just as severe

Table I. Preventive effects of BCG on the immune insufficiency secondary to the development of a syngeneic leukaemia (E (AkR) leukaemia) in $F_1$ (DBA/2 × C57Bl/6) recipients

| Group | Treatment | Day | No. of cells forming plaques of lysis on SRBC 5 days after immunization |
|---|---|---|---|
| I | SRBC | 0 | 26,000 |
| II | BCG | —15 | 76,000 |
| | SRBC | 0 | |
| III | $10^6$ E (AkR) cells | 0 | 28,000 |
| | SRBC | 0 | |
| IV | $10^6$ E (AkR) cells | — 5 | 27,000 |
| | SRBC | 0 | |
| V | $10^6$ E (AkR) cells | —10 | 5,000 |
| | SRBC | 0 | |
| VI | BCG | —15 | 64,000 |
| | $10^6$ E (AkR) cells | 0 | |
| | SRBC | 0 | |
| VII | BCG | —15 | 37,000 |
| | $10^6$ E (AkR) cells | — 5 | |
| | SRBC | 0 | |
| VIII | BCG | —15 | 24,000 |
| | $10^6$ E (AkR) cells | —10 | |
| | SRBC | 0 | |

Table II. Preventive effect of BCG on the immune insufficiency induced by an immunosuppressive chemical: 6-mercaptopurine from day-1 to day-4 inclusive, SRBC on day-0

| Group | Treatment | Day | No. of cells forming plaques of lysis on SRBC 5 days after immunization |
|---|---|---|---|
| I | SRBC | 0 | 27,000 |
| II | BCG | —15 | 4,000 |
| | 6-Mercaptopurine | — 1 to 4 | |
| | SRBC | 0 | |
| III | 6-Mercaptopurine | — 1 to 4 | 4,000 |
| | SRBC | 0 | |
| IV | BCG | —15 | 84,000 |
| | SRBC | 0 | |

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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 |