Résumé. Par l'administration du vaccin BCG nous avons observé la réduction des symptômes provoqués chez le rat par l'injection intradermique de l'adjuvant complet de Freund (maladie ou arthrite d'adjuvant). Cet effet peut être attribué à une action directe du BCG sur certaines populations de lymphocytes.

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Foetal Red Cell Macrocytosis Induced by Pyrimethamine; its Teratogenic Role

Pyrimethamine (2,4-diamino-5-p-chlorphenyl-6-ethyl-pyrimidine), administered to pregnant rats, induces a variety of malformations, including cleft palate, brachygnathia and limb defects: syndactylia, oligodactylia and phocomelia[1–4]. Its biochemical effects are known[5]: it inhibits the activity of the dihydrofolate reductase and thus the formation of tetrahydrofolate. This active form of folic acid mediates the transfer of 'one-carbon units' in a series of essential metabolic processes. The megaloblastic anaemia-provoking effect of folate antagonists, e.g. pyrimethamine, via alterations in the cell division is deducible from these key-functions of folic acid[6].

However, the actual teratogenic process of pyrimethamine and other folate antagonists still remains unknown. In this study, we tried to test whether a) pyrimethamine could provoke red cell macrocytosis in the foetus as it does in the adult, and b) whether this induced macrocytosis could cause thrombosis resulting in limb amputations. An analogous process has been described in a rabbit strain[7] (brachydactylia strain), in which the foetal primordial red cells are especially large and numerous and can give rise spontaneously, between the days 15 and 16 of gestation, to thrombosis, oedema, haemorrhages and necrosis of the foetal extremities in the litter.

For studying the drug's influence on the blood, day 14 of gestation was chosen, since the nucleated primordial cells are still numerous at that stage. Other treated foetuses were examined on day 16, in order to determine whether limb haemorrhages were present. Their presence would show that the amputations are not due to a developmental failure of limb buds, but to a necrotic process. In addition, some treated foetuses were examined after birth for the presence of amputations or deformities.

Blood study in 14-day-old foetuses. 3 pregnant Sherman rats were given daily 6 mg pyrimethamine in 2 ml isotonic saline i.p. on days 10 to 13. The foetuses were removed on day 14, blood smears were made and stained by the panoptic method. The diameter of 200 nucleated primordial cells was determined for each foetus (19 treated foetuses from 4 mothers and 16 control foetuses from 4 mothers). A very obvious macrocytosis was observed (Figure): Blood cells having a diameter $\geqslant 12\ \mu m$: approx. 37% in control foetuses and approx. 94% in treated foetuses.

Observations in 16-day-old foetuses. 2 pregnant rats were given daily 6 mg pyrimethamine from day 12 to 15 of gestation; 23 living but growth-retarded foetuses were examined: all presented severe haemorrhages of the 4 limbs and haemorrhagic areas on the snout.

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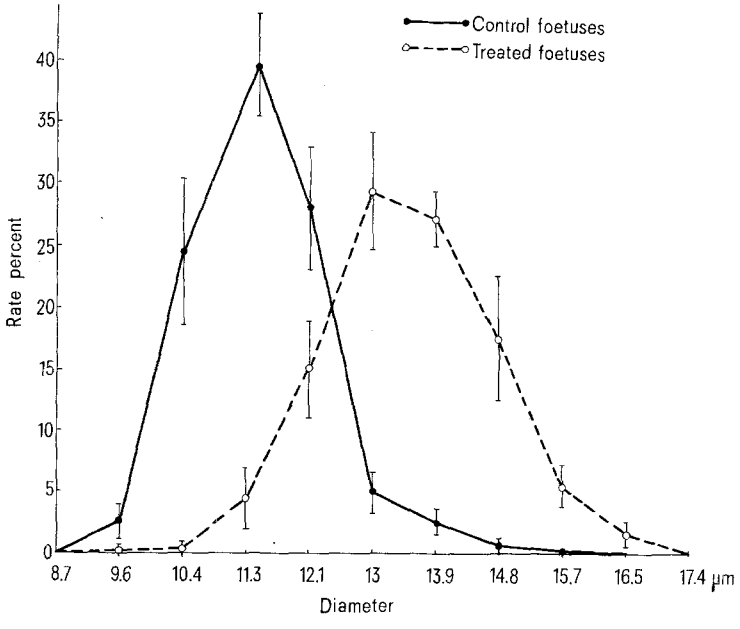

Distribution of nucleated primordial cell diameters in rat foetus treated by pyrimethamine and in controls (day 14 of gestation). Mean ± confidence interval for $p = 0.05$.

Observations in newborns. 2 pregnant rats were treated daily with the same dose from day 12 to 15 of gestation. 22 newborns were examined for external anomalies: all presented limb amputations, micrognathia and microphthalmia, thus confirming the findings of previous authors concerning the teratogenicity of pyrimethamine in rats.

The abnormal primordial cells probably aggregate in the smallest vessels, inducing vascular thrombosis and consequently ischaemia, followed by rupture of the vessel walls with resulting haemorrhages visible on day 16, which lead to necrosis, amputations or deformities. Similar congenital amputations, consecutive to local haemorrhages designated by JOST[8] as 'acroblapsis' have been described after various treatments: pressive hormone injections[8,9], hypertonic mannitol injected in the mother[10], puncture of the amniotic sac[11]; moreover, they occur in a rabbit strain (brachydactylia strain) where they are genetically induced[12].

The present data suggest a new interpretation of the teratogenic process induced by a disturbance of the folate metabolism during pregnancy.

Résumé. La Pyriméthamine induit chez le foetus de Rat de 10 à 13 jours, une importante macrocytose sanguine qui provoque probablement des thromboses génératrices d'ischémie, responsable secondairement des amputations et malformations observées à la naissance.

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The Binding of (^{125}I)-Concanavalin-A to Normal and Endotoxin Stimulated Peritoneal Macrophages

It is known that stimulated macrophages differ from normal macrophages in many respects. Differences in physiology and morphology include behavior in vitro, phagocytic activity, adherence and spreading, metabolic activity, content of hydrolytic enzymes, micobicidal activity, membrane ruffling[1], surface topography[2], electrokinetic properties[3], and the ability to form colonies in soft agar[4]. Some of the above differences may be related to changes in the properties of the plasma membrane. For example, the prominence of acid mucopolysaccharide may be of importance in such phenomena as recognition of foreign material[5], phagocytosis, adherence and spreading[6]. In this respect, a change in the extent of membrane glycoportein could significantly alter the degree of concanavalin-A (con-A) binding. Since con-A binds specifically to glycoportein receptors[7], and macrophages have receptors for con-A[8], it was of interest to determine if normal and endotoxin stimulated macrophages bind con-A to a similar extent. Therefore, the number of glycoprotein surface receptors for con-A can be compaired.

Methods. Mice of the CFW strain, of both sexes, weighing 20–25 g, were used for a source of peritoneal macrophages. Collection of macrophages, cultivation and the con-A binding assay were performed according to the procedures described previously[8,9]. In order to obtain stimulated macrophages, endotoxin (lipopolysaccharide B, Difco) was prepared in imidizol buffered saline and 50 µg of the endotoxin preparation was injected i.p. Macrophages were then harvested 18–24 h later. Morphological examination on glass revealed many large extensively spread macrophages, characteristic of the stimulated macrophage[1].

Determination of deoxyribonucleic acid (DNA) content was done according to the micro-method of BONTING and JONES[10]. This procedure has a range of 0.2–2.0 µg DNA. Extractions were performed directly from the coverslips layered with macrophages. Optical density determinations were made in micro-cuvetts with a Zeiss spectrophotometer at 490 nm.

Results and discussion. Experiments were conducted to determine (^{125}I)-con-A binding at different concentrations. A dose response relationship indicated that for both normal and endotoxin stimulated macrophages, the near maximal binding was obtained between 40 and 80 µg/ml (^{125}I)-con-A (Table I). Since stimulated cells have greater adherence to glass, and the assay system employs only adherent cells[8], different cell populations may be involved. In order to determine if different numbers of adherent cells were involved with the two groups (normal and stimulated), DNA determinations were made from the macrophage preparations and compaired with maximal (^{125}I)-con-A binding ability (Table II). From this table, it is evident that the ratio of (^{125}I)-con-A bound/DNA content is not significantly different for both normal and stimulated macrophages (7.51 vs 7.57×10^3). Therefore, the binding capacity or the number of con-A

Table I. Dose response of (^{125}I)-con-A binding for normal and endotoxin stimulated macrophages

µg/ml (^{125}I)-con-A	cpm $\times 10^3$	
	Normal	Stimulated
20	3100	3350
40	5200	4950
80	6050	6218
160	6900	7100

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