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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[8] A. JOST, C.r. Acad. Sci., Paris *236*, 1510 (1953).
[9] A. JOST, C.r. Soc. Biol., Paris *144*, 1324 (1950).
[10] C. PETTER, C.r. Soc. Biol., Paris *161*, 1010 (1967).
[11] A. JOST, C. PETTER, G. DUVAL, J. P. MALTIER and J. ROFFI, C.r. Acad. Sci., Paris *259*, 3086 (1964).
[12] H. S. N. GREENE and J. A. SAXTON JR., J. exp. Med. *69*, 301 (1939).

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

[1] G. B. MACKANESS, *Infectious Agents and Host Reactions* (Ed. S. MUDD; Saunders, Philadelphia, Pa. 1970), p. 61.
[2] R. M. ALBRECHT, R. D. HINSDILL, P. L. SANDOK, A. P. MACKENZIE and I. B. SACHS, Expl. Cell Res. *70*, 230 (1972).
[3] J. D. LUTTON and L. KIREMIDJIAN, Expl. Cell Res. *79*, 492 (1973).
[4] HSIU-SAN LIN and C. STEWART, J. Cell Physiol. *83*, 369 (1974).
[5] M. R. RABINOVITCH, *Mononuclear Phagocytes* (Ed. R. VAN FURTH; Blackwell, Oxford 1970), p. 299.
[6] I. CARR, Int. Rev. Cytol. *27*, 283 (1970).
[7] I. J. GOLDSTEIN, C. H. HOLLERMAN and E. E. SMITH, Biochemistry *4*, 876 (1965).
[8] J. D. LUTTON, J. Cell Biol. *56*, 611 (1973).
[9] M. R. RABINOVITCH, Proc. Soc. exp. Biol. Med. *124*, 396 (1967).
[10] S. L. BONTING and M. JONES, Arch. Biochem. Biophys. *66*, 340 (1957).

receptors for normal and stimulated macrophages is similar in the two groups. Apparently, activation of macrophages by endotoxin does not significantly alter the number of available con-A binding sites on the membrane surface. Since con-A binds specifically to a-methyl-mannoside and to a lesser degree a-D-glucose like residues[7], it is not possible to speculate if other glycoprotein receptors are involved. Such alterations in glycoprotein might be expected if the mucopolysaccharide coat of the stimulated macrophage were quanitatively or qualitatively different from that of the normal macrophage.

It is of interest that extensive membrane spreading of normal macrophages does not alter the binding of the agglutinin[8]. Results indicated that the numbers of membrane receptors for con-A were similar for macrophages in the round as in the spread configurations. Such configurations are thought to involve changes in the reserve membrane. A similar condition may exist for the endotoxin stimulated macrophage. This is evident since the amount of con-A bound (or the number of con-A receptors) was the same for both groups even though stimulated macrophages are known to possess greater ruffling (reserve membrane) and a greater ability to spread[1].

Table II. The ratio of (^{125}I)-con-A bound (cpm) to the content of DNA (μg/ml) from normal and endotoxin stimulated macrophages.

(^{125}I) (cpm)	DNA μg/ml	(DNA/^{125}I cpm) × 10^3
Normal		
7321	0.94	7.80
9525	1.19	8.01
7911	1.08	7.35
8003	1.19	7.20
7422	0.98	7.50
7984	0.98	8.13
8682	1.22	7.10
7917	1.14	6.95
		$x = 7.51 \pm 0.51$
Stimulated		
5696	0.92	6.30
5372	0.74	7.28
6117	0.82	7.50
6178	0.75	8.25
7182	0.96	7.50
6131	0.78	7.82
7303	0.90	8.10
6088	0.83	7.35
6016	0.75	8.05
		$x = 7.57 \pm 0.19$

50 μg/ml (^{125}I)-con-A was used in all binding assays. $x \pm$ SE.

Zusammenfassung. Nachweis, dass normale und mit Endotoxin stimulierte Makrophagen des Mäusebauchfells in ihrer Bindungskapazität von Concanavalin-A nicht signifikant divergieren, was darauf hinweist, dass durch ihre Aktivierung die Anzahl von Glykoprotein-Rezeptoren für Con-A nicht ändert.

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Synthesis of a Pregnancy-Associated α-Macroglobulin by Human Leucocytes

The existence of a pregnancy associated serum protein was first noted by SMITHIES[1] in 1959 using starch gel electrophoresis. Since that time many investigators have similarly reported that human pregnancy serum contains one and sometimes more than one additional protein (for example refs.[2–10]).

The serum protein most commonly detectable in late pregnancy serum was recently isolated and characterized[11]. It was shown to be a high molecular weight α-globulin containing 10% carbohydrate. This pregnancy associated α-macroglobulin (PAM) has been quantitated in the blood of subjects in many physiological states and found to be quite widely distributed[8,10,12,13]. It can be readily identified of both males and females receiving oestrogen treatment but it does not appear to be involved in the transport of steroids[14–16]. The biological role of PAM has not as yet been clarified although it has been suggested that it may function in the regulation of the immune response[6].

The sites of synthesis of many individual serum proteins have already been established[17–20] and this report provides strongly suggestive evidence for the synthesis of PAM by peripheral blood leucocytes.

Heparinized blood, obtained from healthy males, contraceptive steroid treated females (Norinyl) and pregnant women (38–40 weeks pregnant), was allowed to sediment at 37 °C for 90 min. Leucocytes were recovered from the

[1] O. SMITHIES, Adv. Protein Chem. *14*, 65 (1959).
[2] J. A. MACLAREN, R. D. THORNES, C. C. ROBY and D. E. REID, Am. J. Obstet. Gynec. *78*, 939 (1959).
[3] J. HIRSCHFELD and U. SÖDERBERG, Nature, Lond. *187*, 332 (1960).
[4] J. F. AFONSO and R. R. DE ALVAREZ, Am. J. Obstet. Gynec., *89*, 204 (1964).
[5] J. C. ROBINSON, W. T. LONDON and J. E. PIERCE, Am. J. Obstet. Gynec. *96*, 226 (1966).
[6] W. H. STIMSON, Lancet, *1*, 684 (1972).
[7] S. A. GALL and S. P. HALBERT, Int. Arch. Allergy *42*, 503 (1972).
[8] B. H. BERNE, Fedn. Proc. *32*, 677 (1973).
[9] B. VON SCHOULTZ and T. STIGBRAND, Acta obstet. gynec. scand. *52*, 51 (1973).
[10] C. H. W. HORNE, A. L. C. MCLAY, H. B. TAVADIA, I. CARMICHAEL, A. C. MALLINSON, A. A. C. YEUNG LAIWAH, M. A. THOMAS and R. N. M. MACSWEEN, Clin. exp. Immun. *13*, 603 (1973).
[11] W. H. STIMSON and L. EUBANK-SCOTT, FEBS Lett. *23*, 298 (1972).
[12] W. H. STIMSON, IRCS Med. Sci. *1973*, 3.
[13] W. H. STIMSON, J. Endocr. *61*, 30 (1974).
[14] W. H. STIMSON, IRCS Med. Sci. *1973*, 10.
[15] R. HOFMANN, H. A. SCHULZE, W. STRAUBE and B. KLAUSCH, Arch. Gynäk. *213*, 86 (1972).
[16] H. BOHN and T. KRANZ, Arch. Gynäk. *215*, 63 (1973).
[17] M. E. PHILLIPS and G. J. THORBECKE, Int. Arch. Allergy *29*, 553 (1966).
[18] V. J. STECHER and G. J. THORBECKE, J. Immun. *99*, 643 (1967).
[19] V. J. STECHER and G. J. THORBECKE, J. Immun. *99*, 653 (1967).
[20] V. J. STECHER and G. J. THORBECKE, J. Immun. *99*, 660 (1967).