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).

Table I. Incorporation of labelled glutamine into cell cultures

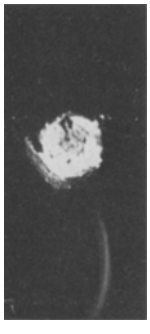
Additions to culture medium		Type and source of cultured cells: Leucocytes — Males (dpm/10^6 cells)	Leucocytes — Contraceptive steroid treated females (dpm/10^6 cells)	Leucocytes — Pregnant females (dpm/10^6 cells)	Lymphocytes — Pregnant females (dpm/10^6 cells)
1. No additions	M	17 (15, 2, 34)	48 (42, 52, 49)	48 (41, 34, 68)	21 (15, 40, 8)
	MS	11 (18, 1, 13)	58 (41, 71, 62)	76 (72, 66, 90)	18 (4, 14, 30, 23)
2. Mestranol	M	63 (48, 56, 85)	73 (91, 41, 86)	94 (71, 98, 113)	25 (17, 44, 13)
	MS	115 (122, 123, 101)	133 (117, 150, 132)	141 (115, 137, 170)	15 (1, 3, 24, 30)
3. Mestranol (labelled glutamine added after incubation completed)	M	7 (1, 9, 10)	9 (15, 1, 12)	4 (2, 9, 2)	—
	MS	5 (8, 5, 3)	13 (6, 25, 9)	8 (5, 11, 7)	—
4. Mestranol + puromycin	MS	—	—	5 (3, 4, 8)	—

Figures in parentheses are the results of individual experiments carried out in duplicate. M, cultured in medium alone; MS, cultured in medium + 15% autologous serum.

plasma layer by centrifugation (150 *g*, 10 min) and lymphocytes were prepared by use of Ficoll-Hypaque gradient[21]. Both were then washed 6 times in Waymouth's MB 752/1 culture medium. Cells (2×10^6/ml) were cultured with 3 μCi, U-^{14}C glutamine or U-^{3}H leucine either in culture medium alone or in medium containing 15% autologous serum. Further additions of mestranol (0.25 μ*M*) or puromycin (1 m*M*)[22] were made to appropriate cultures. Incubation was then carried out at 37 °C in a 5% CO_2/95% air atmosphere for 5 days.

Pure PAM[11] and Freund's complete adjuvant were used for immunizing rabbits and the resultant antiserum was prepared by 50% ammonium sulphate precipitation and DEAE-cellulose fractionation of the serum obtained[23]. The anti-PAM was absorbed 4 times with 4B Sepharose coupled by the cyanogen bromide method[24] to male serum proteins. The specificity of the antiserum was checked by two dimensional immunoelectrophoresis[25] and KRAUSE and RAUNIO's immunodiffusion technique[26].

On completion of the incubation period the cultures were centrifuged and the supernatants treated with NaCl to a concentration of 0.5 *M*. 15% autologous serum or 15% pregnancy serum was then added to the supernatants of the cells cultured in medium alone or male leucocytes respectively. Finally, a predetermined quantity of specific antiserum was added so as to attain the antigen-antibody equivalence point in each case. the precipitates that formed on incubation (37 °C, 1 h) were washed 3 times in either 0.5% glutamine or leucine solution, containing 0.5 *M* NaCl and then counted on a liquid scintillation counter.



Autoradiograph of immunoelectrophoretic pattern prepared with culture fluid from the leucocytes of a pregnant woman. The pattern was developed with specific antiserum to PAM.

Autoradiography was performed as previously described[17]. Leucocytes (5×10^6/ml) from a pregnant woman were cultured ,as indicated above, in the presence of 15% autologous serum, 4 μCi U-^{14}C glutamine and mestranol. The PAM in the supernatant was concentrated by precipitation with ammonium sulphate[11] and then subjected to immunoelectrophoretic analysis against the specific antiserum.

It has already been shown that levels of PAM in the blood are at least partially dependent upon oestrogen concentration, specifically that oral contraceptives and mestranol results in the appearance of the protein in females while stilboestrol causes an increased concentration in males undergoing treatment for prostate cancer[10,13].

Table I summarizes the results obtained from a series of experiments employing labelled glutamine. Leucocytes from both pregnant and contraceptive steroid treated women gave significantly more supernatant counts compared with the male cells, whose levels were similar to those obtained if labelled glutamine was added on completion of the incubation (i.e. background level). This most probably occurred because the female leucocytes were already 'primed' to produce PAM by being in contact with high concentrations of oestrogen in vivo, whereas the male cells naturally had not. However, when leucocytes from all three sources were cultured in the presence of mestranol substantial rises in all the supernatant counts were achieved. This oestrogenic stimulation of PAM production is typical of that obtained in the in vivo studies mentioned above and the fact that the male leucocytes also produced high counts in the supernatants after 5 days culturing correlates with the observation that the glycoprotein becomes immunochemically detectable in vivo approximately 7 days after oestrogen administration[13]. This evidence is substantiated by the results from the tritiated leucine incorporation experiments (Table II), where a similar pattern to that noted with labelled gluta-

[21] A. BÖYUM, Scand. J. clin. Lab. Invest. Suppl. *21*, 97 (1968).
[22] J. J. FERGUSON, J. biol. Chem. *238*, 2754 (1963).
[23] H. A. SOBER and E. A. PETERSON, Fedn. Proc. *17*, 1116 (1958).
[24] J. PORATH, R. AXEN and S. ERNBACK, Nature, Lond. *215*, 1491 (1967).
[25] C. B. LAURELL, Analyt. Biochem. *10*, 358 (1965).
[26] U. KRAUSE and V. RAUNIO, Acta path. microbiol. scand. *71*, 328 (1967).

Table II. Incorporation of labelled leucine into cell cultures

Additions to culture medium		Leucocyte source	
		Males (dpm/10^6 cells)	Contraceptive steroid treated females (dpm/10^6 cells)
1. No additions	M	14	34
	MS	20	58
2. Mestranol	M	68	62
	MS	90	93
3. Mestranol (labelled leucine added after incubation completed)	M	8	7
	MS	11	4

Each experiment was carried out in duplicate. M, cultured in medium alone; MS, cultured in medium + 15% autologous serum.

mine was obtained. Also the autoradiograph (Figure) illustrates that the culture fluid from mestranol treated leucocytes formed a labelled precipitin line against the specific anti-PAM serum. In order to ascertain whether it was the lymphocyte population in the leucocytes which was responsible for PAM production, isolated cells were cultured with and without mestranol. The results shown in Table I provide no indication of the protein's synthesis.

The possibility of protein-protein and protein-labelled amino acid interactions with PAM in the carrier serum could have lead to erroneous identification of labelled protein. For example, labelling of α_2-macroglobulin and lipoprotein has been observed in many cultures of living cells as a result of their capacity to bind enzymes[17,18,20]. These effects however were not considered to have occurred to any significant extent on account of the use of a high molarity of NaCl in both the antibody precipitation and cold amino acid washing stages. Thus, puromycin inhibited cells and unstimulated male leucocytes produced similar counts to the background levels and when NaCl was added to the culture supernatants before the carrier serum, the expected patterns of labelling, indicating PAM synthesis, were again obtained. The fact that the counts were diminished in the serum free cultures is probably an indication that the production of this serum protein, as with others that have been studied[19], is enhanced if serum is added to the culture medium.

The experimental data presented above indicates that this serum α-globulin, commonly associated with pregnancy, can be synthesized by peripheral blood leucocytes.

Résumé. Le sang des femmes enceintes peut contenir plusierus protéines sériques uniques en leur genre. Celle qui se présente le plus souvent est une α-globuline de grand poids moléculaire. Un test in vitro utilisant l'incorporation de ^{14}C-glutamine ou ^{3}H-leucine dans la glycoprotéine a montré que celle-ci peut être synthétisée par des leucocytes.

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Transferrin Behaviour in Primary Haemochromatosis

In Primary Haemochromatosis (PH), a disease due to a congenitally augmented iron absorption, serum transferrin is often reduced to very low levels[1,2], before and independently of liver failure due to late cirrhosis[1]. Mild reductions below normal range have also been observed in some relatives[1,2]. In order to explain these decreases, the existence of a congenitally impaired synthesis of transferrin, i.e. an inborn error of metabolism, has been proposed[2].

However, firstly a decrease of serum transferrin and/or total iron binding capacity (TIBC) is not a constant finding in PH and normal levels have been reported[1,3–7], also in 2 male twins with the disease[6]. Secondly, the stored iron itself seems to affect serum transferrin: 1. a negative correlation between non-hemin liver iron and TIBC exists in normal subjects[8]; 2. in anaemia due to iron deficiency, serum transferrin is increased but it falls to normal after iron stores have been therapeutically reconstituted[9,10]; 3. parenterally iron loading is associated in the rat with a suppression of transferrin synthesis[11]; and 4. in human iron overload secondary to blood transfusions, to excessive iron therapy or chronic haemolysis transferrin or TIBC is lower than in normals[1,3,12].

[1] E. Fiaschi, L. A. Scuro, G. Dobrilla and G. Cartei, *Fisiopatologia e Clinica delle Siderocromatosi* (Pozzi, Roma 1972).
[2] B. Blanc and A. Vannotti, Nature, Lond. *212*, 480 (1966).
[3] T. H. Bothwell and C. A. Finch, *Iron Metabolism* (Churchill, London 1962), p. 366.
[4] L. W. Powell and M. J. Thomas, J. clin. Path. *20*, 896 (1967).
[5] R. Sinniah, Arch. intern. Med. *124*, 455 (1969).
[6] M. Barry, G. Cartei and S. Sherlock, Gut *11*, 891 (1970).
[7] L. A. Scuro, G. Dobrilla, V. Lo Cascio, C. Bosello, F. D'Andrea and A. Innecco, Acta hepato-gastroent. *19*, 90 (1972).
[8] A. Weinfeld, Acta Med. Scand., suppl. 427 (1964).
[9] G. Cartei, G. Previato and A. Innecco, Atti. Soc. Med. Chir. Univ. Padova *58*, 355 (1968).
[10] G. Cartei, A. Meani, L. Okolicsanyi and R. Naccarato, Folia endocrin., Roma *23*, 579 (1970).
[11] R. S. Lane, Br. J. Haemat. *15*, 355 (1968).
[12] M. Barry, P. J. Scheuer, S. Sherlock, C. F. Ross and R. Williams, Lancet, *2*, 481 (1968).