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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[27] We thank Dr. J. E. O'Grady and Dr. A. Shephard for obtaining the blood samples. This work was supported by a grant from the Scottish Home and Health Department.

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).

Relationship between serum transferrin levels and iron overload in primary haemochromatosis

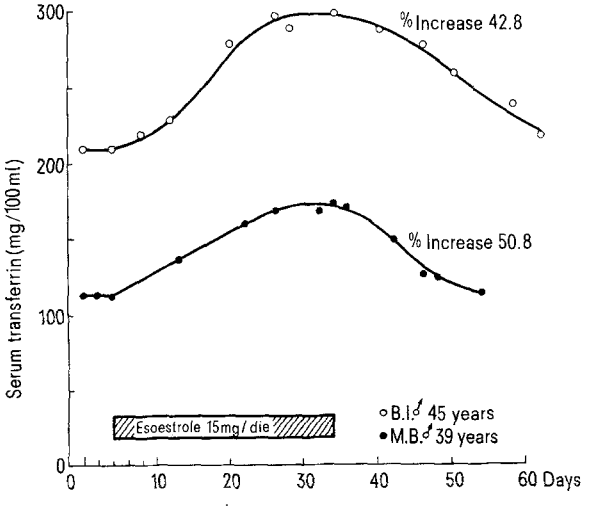
Patients (name, age, sex)	Serum transferrin (mg/100 ml) [a]	Iron overload (g) [b]	Venesection therapy [c]
M.B., 39, ♂ propositus	112–118–110	15	untreated
	310–315	0	treated
	278–290	0.2	6 months after
B.I., 45, ♂ propositus	205–210–220	9	untreated
	295–300	0	treated
	295–300	0.1	4 months after
B.G., 48, ♂ propositus	175–180	13	untreated
	300–310	0	treated
	280–300	0.1	5 months after
M.A., 35, ♂ brother of M.B.	190–195	3.5	untreated
	280–290	0	treated
	285–300	0.1	5 months after
B.E., 29, ♂ brother of B.I.	180–200	2.8	untreated
	300–300	0	treated
	280–285	0.2	5 months after

[a] Immunochemically determined[14]; normal range is from 200 to 320 mg/100 ml (mean ± 2 SD is 262 ± 36)[10]. Values of 3 or 2 successive days are given. [b] ^{59}Fe-DTPA test[6]: values are given in g of iron. [c] Performed by subtracting 800 ml of blood weekly.

We devised a method to investigate whether the reduction of serum transferrin in PH might be secondary to the already accumulated iron overload, instead of representing an inborn defect of protein synthesis. Serum transferrin was studied in untreated PH as well as during and after deironing therapy, and in some relatives.

It has also been observed that in males with normal iron stores and without congenital errors of iron metabolism, the administration of oestrogens caused a reversible increase of serum transferrin[13]. We have investigated such a possible responsiveness in untreated PH.

In 3 patients with PH and their 12 relatives (8 brothers and 4 sisters) serum transferrin[14], iron and TIBC[15], and evaluation of iron stores with the ^{59}Fe-DTPA test[6] were available. In iron-overloaded subjects, deironing therapy was performed by venesection until Fe-overload was removed as judged by serial ^{59}Fe-DTPA tests in all cases and also by liver biopsies in the propositi (hereditary taints). In 2 propositi before venesection, esoestrole was given as previously described in males with normal iron metabolism[13].



Effect of 15 mg/day esoestrole (Estrene 5, Lepetit, Ltd) given by oral route for a month on serum transferrin levels in 2 patients with primary haemochromatosis. In subjects with normal iron metabolism serum transferrin increased by 68% ± 12 (mean ± 2 SD)[13].

Present results show that the initially low serum transferrin levels observed in the propositi were fully corrected by deironing therapy and remained unchanged for about 6 months after the last venesection (Table). A patient (M.B.) was subsequently deprivated of 1.4 g of iron. There was a mild microcytic anaemia and absence of Perls positive material in bone marrow smears and liver biopsy sections, apart from a few scattered granules in hepatic fibrous tissue. Iron deficiency was confirmed by low serum iron (32 µg/100 ml) low transferrin saturation with iron (6.8%) and a 24 h urinary iron excretion induced by desferrioxamine B (Desferal Ciba) of 0.7 mg Fe/24 h (normal range being from 1.2 to 2 mg Fe/24 h[1]). Serum transferrin was rised to 360 mg/100 ml, well in the range of iron deficiency[9,10].

The 12 relatives were clinically healthy and extensive investigation on liver function was normal. Serum irons were consistently normal, while serum transferrin was reduced in 2 subjects (Table). Iron stores were abnormally high only in the 2 subjects (M.A. and B.E.) with reduced serum transferrin. They were subsequently venesected until iron overload was removed; after therapy serum transferrin rose to normal, having steady levels for the following 5 months (Table).

In the propositi, still iron repleted, oestrogen treatment caused a percentage increase of serum transferrin over pretreatment levels of 42.8 and 50.8 respectively (Figure), slightly lower figures than in subjects without siderosis (68% ± 2SD 12)[13]. After suspension of treatment, serum transferrin sank to previous levels (Figure).

In conclusion, deironing therapy was capable of normalizing previously reduced serum transferrin levels in PH both in propositi and in their relatives. Normal

[13] G. CARTEI, A. MEANI and D. CAUSARANO, Int. Nutr. Rep. *2*, 343 (1970).

[14] G. MANCINI, A. Q. CARBONARA and J. F. HEREMANS, Immunochemistry, *2*, 235 (1965).

[15] W. T. CARAWAY, Clin. Chem. *9*, 188 (1963).

levels attained did not change in the following 5 to 6 months. Moreover, in a patient with PH made iron-deficient with removal of further 1.4 g of iron after iron overload was already treated, serum transferrin rose to very high levels, such as are seen in iron-deficient subjects without congenital disorders of iron metabolism[9,10]. Serum transferrin was also reduced in 2 out of 12 relatives, and only in these 2 cases body iron stores were already increased. Venesection therapy removed the iron overload and serum transferrin become normal. Rise of serum transferrin both in controles propositi and in relatives are clearly related to the removal of iron overload and not to venesection per se, since steady levels of transferrin were maintained for months after last subtraction of blood. Among the relatives studied by others[2] and with reduced serum transferrin levels the amount of body stored iron was not evaluated, and effect of venesection not investigated.

Moreover, the responsiveness to oestrogens was not lost in our cases with still untreated PM which supports our hypothesis that in this disease synthesis of transferrin is not congenitally impaired. The smaller rise of transferrin here observed in comparison to that obtained with the same treatment in subjects without iron overload might be due to stored iron itself.

On the basis of the existence of an inverse relationship between body iron stores and serum transferrin[1,3,8,9,11,12], and also considering that serum transferrin may be lower in secondary than in primary haemochromatosis[7], the hypothesis of BLANC and VANNOTTI[2] remains unproved. In our opinion the reduced serum transferrin levels most often observed in PH are due to the already increased body iron stores and eventually to liver cirrhosis.

Riassunto. Nella siderocromatosi primitiva (SP) (propositi e consanguinei ipersiderotici) la transferrinemia (STr) era ridotta. La salassoterapia accrebbe e normalizzò stabilmente la STr. Nei consanguinei non siderotici la STr era normale. Un proposito divenne anemico sideropenico e mostrò iper-STr. In 2 propositi non ancora salassati, gli estrogeni orali (per un mese) accrebbero la STr come in maschi senza SP. La ipo-STr della SP non sembra dovuta al calo congenito di sintesi ma al sovraccarico marziale.

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The Effect of Common Antibiotics on Lymphocyte Transformation

The immunosuppressive effects of several anti-neoplastic antibiotics are well established[1]. The finding that chloramphenicol also has a significant immunosuppressive action[2] has stirred interest in the possibility of immunosuppressive effects induced by more commonly used antibiotics[3].

The decrease in resistance to infections from certain organisms such as *Candida albicans*, sometimes associated with long-term tetracycline treatment, occasioned our interest in further investigating the question of possible immunosuppression associated with commonly used antibiotics. In this study we have investigated the in vitro effects of therapeutic concentrations of tetracycline, penicillin and erythromycin on lymphoblastic responses to phytohemagglutin (PHA). Aspirin was used as a measure of the test system because of its known ability to suppress lymphocyte transformation in therapeutic concentrations in vitro[4].

Materials and methods. Whole blood, aseptically collected from 26 healthy volunteers, was added to the culture medium, RPMI 1640 supplemented with 10% Agamma Calf Serum and 1% L-glutamine (BBL Co., Cokeyville, Md.), in an amount to give a concentration of 2×10^5 lymphocytes per ml. 250 µg PHA (Difco, Detroit, Mich.) was added to each 4 ml culture. 16 µg tetracycline (Achromycin-IV, Lederle), erythromycin (Erythromycin Lactobionate-IV, Abbott), and penicillin (Buffered Sodium Penicillin G-IV, Squibb) in 0.1 ml sterile water, and 0.8 mg acetylsalicyclic acid dissolved in 0.2 ml sterile water were added to triplicate culture sets. After 4 days incubation at 37 °C with 5% CO_2, lymphocyte blastogenesis was measured by adding 0.2 ml ^{14}C-thymidine (0.6 µCi) to each culture. Harvesting of lymphocytes to measure thymidine incorporation was performed 24 h later by serial centrifugations at 1600 rpm for 15 min with decantations of the supernatant. 4 ml of 3% glacial acetic acid was added to each tube to lyse the red cells. The cells were washed with 4 ml isotonic saline followed by 1.0 ml 0.1 *N* NaOH to lyse white cells. Finally 4.5 ml of 6.7% trichloracetic acid were added to each tube to form acid insoluble precipitates with released nuclear material on overnight standing. The precipitates were bleached with 3.0 ml cold methanol, solubilized in 0.5 ml Packard Solulene, washed into a scintillation vial (15 ml of 4% Packard Permafluor in scintillation grade toluene), and counted for 5 min in a liquid scintillation spectrometer.

Results. The values for percent suppression of thymidine uptake, calculated by dividing the average cpm of an antibiotic containing culture set by the control set, are plotted on the graph. 24 tetracycline – 14 erythromycin – and 14 penicillin – containing culture sets, all at a concentration of 4 µg/ml, showed no suppression. Composite values were 101%, 99% and 103% respectively.

The potential effectiveness of the assay method was demonstrated by finding that concentrations of acetylsalicylic acid, analogous to post-treatment levels in humans, suppressed thymidine incorporation by 29% in 10 cultures at a 20 mg/100 ml concentration ($p < 0.02$) and by 65% in 4 additional culture sets at 30 mg/100 ml, values similar to those found by OPELZ et al.[4]. The Achromycin preparation contained ascorbic acid as a stabilizer (1250 mg ascorbic acid per 500 mg tetracycline). Studies with ascorbic acid alone (Ascorbic Acid Injection, Upjohn) in concentrations equal to that in the tetracycline containing cultures, 10 µg/ml, as well as 300 µg/ml, failed to demonstrate any effect on PHA responsiveness.

[1] A. E. GABRIELSEN and R. A. GOOD, Adv. Immun. *6*, 171 (1967).
[2] A. S. WEISENBERGER, T. M. DANIEL and A. GOFFMAN, J. exp. Med. *120*, 183 (1964).
[3] T. MAKINODAN, J. F. ALBRIGHT, E. H. PERKINS and P. NETTESHEIM, Med. Clins N. Am. *49*, 1569 (1965).
[4] G. OPELZ, P. I. TERASAKI, and A. A. HIRATA, Lancet *2*, 478 (1973).