Tableau III. Taux d'anticorps agglutinants anti O et H mesurés au cours de la réponse secondaire

Réponse secondaire anti O et H	T/C	*n*	Axéniques TH		Holoxéniques TH	*n*	*n*	Axéniques TO		Holoxéniques TO	*n*
1ère saignée	T	12/12	6,33	[c]	1,70	8/12	12/12	2,20	NS	1,00	8/12
	–	–	NS		[b]	–	–	NS		[b]	–
	C	8/8	7,60	NS	5,70	8/8	8/8	4,30	NS	5,60	8/8
2ème saignée	T	12/12	5,50	[b]	2,00	5/12	12/12	6,00	[b]	1,60	5/12
	–	–	[b]		[a]	–	–	NS		[a]	–
	C	8/8	8,37	NS	6,70	8/8	8/8	5,70	NS	5,20	8/8
3ème saignée	T	12/12	6,83	NS	4,00	5/12	12/12	6,08	NS	3,20	5/12
	–	–	NS		[a]	–	–	NS		NS	–
	C	8/8	9,00	NS	9,37	8/8	8/8	5,62	NS	4,20	8/8

Les limites de significativité ont été calculées par le test-*t* Student: [a] $p \leq 0.5$; [b] $p \leq 0.01$; [c] $p \leq 0.001$. *n*, nombre d'animaux survivants/nombre total. T, traités. C, témoins.

de cellules disponibles pour une réponse à une stimulation antigénique contrôlée est probablement plus grand que chez les souris holoxéniques soumises à des stimulations nombreuses et incontrôlées. Après irradiation, l'écart s'accroît entre souris holoxéniques et axéniques, quant au nombre de cellules immunologiquement compétentes, non engagées dans une réponse immunitaire.

Sans doute, deux autres facteurs diminuent les réponses spécifiques aux antigènes O et H des souris holoxéniques: a) la compétition d'antigène[5] entre, d'une part, les antigènes O et H, et d'autre part, les antigènes non contrôlés; b) un effet immunodépresseur dû à des produits non définis du métabolisme bactérien[6–10].

Comme avec les rayons X, après immunodépression par les corticoïdes, les souris axéniques recouvrent plus vite une activité immunitaire normale que les souris holoxéniques[2].

En conclusion, il semble confirmé par cette expérience, que les souris axéniques ont une résistance accrue à l'effet immunodépresseur des rayons X.

Summary. Immunodepression induced by a sublethal dose of X Rays is always less in axenic mice than in holoxenic mice. Frequent infections of irradiated holoxenic mice increases the immunodepressive effect of X Rays.

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Normal Human Blood Treated in vitro with Cephalothin. Relationship Between Serologic and Metabolic Abnormalities of Red Blood Cells

Normal human red cells treated in-vitro with cephalothin under suitable experimental conditions give a positive direct antiglobulin (Coombs) test[1,2]. These cells display low acetylcholinesterase (AChE) activity[3,4] and impaired O_2 uptake in the presence of methylene blue[3]. The mechanism by which this drug causes the above effects has not been fully elucidated and still unclear is the relationship between the serologic and the metabolic abnormalities of the altered red blood cells. The aim of the present study is to investigate this relationship.

Blood was drawn with acid-citrate-dextrose from 2 groups each of 7 healthy subjects and was treated with sodium cephalothin (Keflin Lilly) as previously described[2], but for various periods of incubation (first group of subjects) or with solutions of different concentrations (second group of subjects). Blood incubated with saline alone served as a control. At the end of the incubation period erythrocytes were washed 4 times with large volumes of saline. The direct antiglobulin test was carried out using commercial anti-whole-human serum (Ortho) and was scored from $\pm$ to $+++$ depending upon the degree of microscopic agglutination. AChE activity was determined by the manometric Warburg technique according to De Sandre et al.[5] and O_2 uptake in the presence of methylene blue according to Oka and Puranen[6].

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Incubation of whole blood with increasing concentrations of cephalothin at 37 °C causes a concentration-dependent decrease of red cell AChE and O_2 uptake in the presence of methylene blue (Table I). The values of these metabolic activities appear to be a linear function of the final concentration of the drug, the regression equations being respectively $y = 23.3775 - 0.0531\,x$ and $y = 0.5360 - 0.0007\,x$, where y is the considered metabolic activity and x the final concentration of cephalothin. In each case red cell metabolic abnormality was detectable at concentrations of the drug insufficient to give a positive direct Coombs test.

Table II shows the effect of the time of incubation on the metabolic activities investigated and on the development of a positive direct Coombs test. The exposure of red cells to cephalothin required to produce a decrease in their metabolic activities is shorter than that giving rise to serologic abnormality. The values of the former appear to be a linear function of the blood incubation time with cephalothin, the regression equations being $y = 32.1288 - 0.4602\,x$ for AChE and $y = 0.5574 - 0.0053\,x$ for O_2 uptake in the presence of methylene blue.

The available data indicate that red cells exposed to cephalothin develop 2 kinds of detectable abnormalities: a γ-globulin coat and an alteration of the cell membrane. The former is readily revealed by the positivity of the direct antiglobulin test (of the 'γG + complement' type) observed both in patients given cephalothin[1,7,8] and in in vitro studies where the drug is incubated with whole blood[1,2]. An alteration of the cell membrane is suggested by the reduction of AChE[3,4] (an enzyme located in the erythrocyte stroma) and O_2 uptake in the presence of methylene blue[3], which can be considered as an expression of the metabolism of the membrane[6]. Moreover when cephalothin is incubated in vitro with washed normal erythrocytes, these develop a positive direct Coombs test of the 'non-γ' type[1] and become susceptible to acid lysis[9], both phenomena reflecting modifications of some membrane constituents.

The evidence so far obtained suggests that of the two above mentioned abnormalities the membrane alteration is the first modification produced by the drug. The present investigation supports this conclusion since the inhibition of the red cell metabolic activities determined is already apparent when exposure to the drug is not yet sufficient to cause the development of a positive antiglobulin reaction. These in vitro results would seem to conflict with those in vivo and in vitro studies where no metabolic alteration was found to accompany the serologic abnormality. In fact, when cephalothin solutions are incubated with blood from patients with high levels of serum γ-globulins a positive direct Coombs test develops at concentrations of the drug lower than those necessary to cause inhibition of the erythrocyte metabolic acti-

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Table I. Effect of cephalothin concentration on erythrocyte metabolism and development of a positive direct Coombs test

Final concentration of cephalothin (mg/ml)	AChE activity		O_2 uptake in the presence of methylene blue		Direct antiglobulin reaction
	QCO_2	Approximate inhibition (%)	QO_2	Approximate inhibition (%)	
0	30.07 ± 4.10[a]	0	0.595 ± 0.074[a]	0	negative
5	29.68 ± 4.29	1	0.491 ± 0.090	17	negative to ±
10	29.81 ± 4.45	1	0.507 ± 0.092	15	+ to ++
20	23.85 ± 3.84	21	0.439 ± 0.021	26	+++
40	12.70 ± 4.54	58	0.353 ± 0.079	41	+++

Incubation time 180 min. [a] Mean ± one standard deviation.

Table II. Effect of incubation time of normal whole blood with cephalothin (final concentration 40 mg/ml) on erythrocyte metabolism and development of a positive direct Coombs test

Incubation time (min)	AChE activity		O_2 uptake in the presence of methylene blue		Direct antiglobulin reaction
	QCO_2	Approximate inhibition (%)	QO_2	Approximate inhibition (%)	
0	24.48 ± 2.69[a]	0	0.564 ± 0.083[a]	0	negative
30	20.04 ± 2.61	18	0.497 ± 0.031	12	negative
60	20.37 ± 2.86	16	0.488 ± 0.000	13	± to +
120	17.42 ± 3.15	28	0.450 ± 0.044	20	++
180	13.70 ± 1.46	44	0.405 ± 0.000	28	+++

[a] Mean ± one standard deviation.

vities[2]. Furthermore AChE activity and O_2 uptake in the presence of methylene blue of erythrocytes from patients developing a positive direct antiglobulin test during cephalothin therapy have so far been found to be within normal limits[10]. Since the in vitro development of the cephalothin-produced positive direct Coombs test has been shown to be influenced by many variables (e.g. the period and temperature of incubation[1], the concentration of the drug in the reaction mixture[1,2], the level of serum gammaglobulins[2] and particularly of γG globulins[11]), the above discrepancy can be explained if we suppose that under particular conditions (e.g. a high protein concentration in the plasma) a positive direct Coombs test develops when the lesion of the membrane is not sufficient to determine a detectable reduction of its metabolic activities. In spite of this discrepancy, therefore, we believe it can be taken as shown that the primary effect of cephalothin on red blood cells is a lesion of the membrane with subsequent adsorption of some serum proteins and the development of a positive direct Coombs test.

Riassunto. L'incubazione in vitro dell'antibiotico cefalotina con sangue intero determina un'anormalità metabolica degli eritrociti e la positività del test di Coombs diretto. Nel presente lavoro sono stati studiati i rapporti tra i due fenomeni e si è osservato che il primo si produce più precocemente del secondo. In base ad una serie di considerazioni, si conclude che l'effetto primo della cefalotina sugli eritrociti è una lesione della membrana, con successivo adsorbimento di alcune proteine dal plasma.

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Agglutinins from Jerusalem Sage (*Phlomis fruticosa*)

Agglutinins active against human blood group A or B red cells and with little or no activity for group O cells have been obtained from various seeds, such as *Crotalaria striata*[1], *Crotalaria usraemonensis*[2], *Calpurnia aurea*[3], *Sophora japonica*[4], *Coronilla varia*[5], *Bandeiraea simplicifolia* (freshly harvested seeds)[2], and *Caragna frutex* var *latifolia*[2]. The two *Crotalaria* agglutinins react more strongly with A (A_1) cells, the *Calpurnia* agglutinin is about as strong with A as with B, and the others are stronger with B cells.

Ottensooser, Leon and Sato[6] reported such agglutinins in the seeds of *Crotalaria mucronata* and *Crotalaria breviflora*. *Crotalaria mucronata* however is another name for *Crotalaria striata*. *Crotalaria breviflora* seeds were reported by Brilliantine, Aranda, Foster and Allen[7] to contain specific anti-A agglutinins. The discrepancy could be due to variation among different strains of the plant or to variations in agglutinin concentration.

We have found in the seeds of *Phlomis fruticosa* (Jerusalem Sage) of the Natural Order Labiatiae, agglutinins which react strongly with A and B cells and fail to agglutinate O cells (Figure). This is the third specific agglutinin to be found in *Labiatiae*, the others being the anti-A of *Hyptis suaveolens*[8] and the anti-(A + N) of *Moluccella laevis*[9]. We here describe the properties of the *Phlomis* agglutinin.

The methods used were as described previously[10]. Screening tests on tiles against various washed red cells suspended in physiological saline solution showed that the *Phlomis* agglutinin strongly agglutinated AB, A and B cells but not O cells. Titre scores by the tube method were: A_1-78, A_2-64, B-57, 0-nil. A_3 cells gave the typical 'weak-mixed field' appearance as is obtained with human group B or O sera. A_x cells were not agglutinated. The group O polyagglutinable cells 'Ba'[11] were strongly agglutinated. AB serum inhibited the agglutinin. There was no crossreaction with O cells suspended in 30% bovine albumin (Armour) or treated with *Vibrio cholerae* neuraminidase (Behringwerke). Papain-treated O cells were seen under the microscope to be very weakly agglutinated.

Absorption with A_1 cells abolished activity for A_1 and B cells; absorption with B cells left some anti-A.

The agglutinin was completely inhibited by group A secretor saliva, which was a more effective inhibitor of the agglutination of B than of A cells. Group B secretor saliva removed all activity for B leaving strong activity for A cells.

Inhibition tests with 2% aqueous solutions of simple sugars showed that L-fucose, D-glucose, lactose, salicin and N-acetylglucosamine did not inhibit the agglutination. N-acetylgalactosamine removed all activity for both A and B cells; D-galactose removed activity for B but not A cells. N-acetylgalactosamine was a greater inhibitor of agglutination of B than of A cells.

The agglutinins did not show the temperature effects (increased anti-B activity at lower temperatures without enhancement of anti-A) described by Ottensooser and Sato[12] for the anti-(A + B) agglutinin of *Crotalaria striata* (*mucronata*). There were individual differences in the strength of agglutination of the various B cells, possibly analogous to those described by Ottensooser, Leon and Sato[6]. The variation paralleled the strength of reaction with human anti-B and could be shown to be inversely proportional to the H antigen content of the various cells

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