Peripheral Blood 'Rosette Forming Lymphocytes' in Down's Syndrome

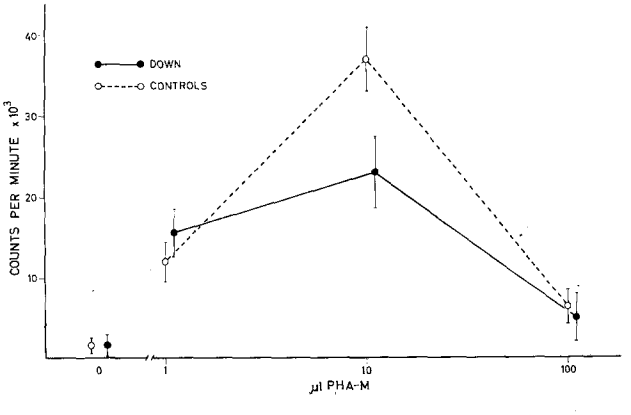
Patients with Down's syndrome have been shown to have a high incidence of infections[1,2], leukemia[3] and thyroid autoantibodies[4–6]. These observations may suggest the existence of an immunological derangement. Although a complete immunological study of these patients is still lacking, immunoglobulin levels appear unbalanced[7] and impaired responses to a variety of antigens have been reported[8]. Phytohemagglutinin (PHA)-responsiveness of peripheral blood lymphocytes has been studied by several investigators with contradictory results[8,9].

In the course of a systematic study of the immune function in Down's syndrome at various ages, the present investigation was designed specifically to evaluate the status of circulating thymus-dependent cells (T-cells) in young adults affected with this disease.

Materials and methods. The study was carried out on peripheral blood lymphocytes from 25 patients with trisomy of the G group, and from 25 mentally retarded, karyotypically normal subjects from the same institution. They were closely matched for sex and age; the age range was between 18 and 30 years.

Lymphomononuclear cells were obtained by separation on Ficoll-Hypaque[10]. Triplicate 1 ml cultures containing 0.25×10^6 cells were suspended in RPMI 1640 containing 20% AB human serum and various amounts (1, 10, 100 µl) of PHA-M added. The degree of proliferation in each culture was determined by the incorporation of (^{3}H)-Thymidine into cellular DNA during the final 18 to 20 h of a 3-day culture period. The percentage of circulating lymphocytes forming 'spontaneous rosettes' with sheep red blood cells (a well known T-cell marker) was estimated according to the method of Ross et al.[11].

Results and discussion. The Figure shows the dose-response curve comparing incorporation of (^{3}H)-Thymidine into DNA of control and Down's syndrome lymphocytes as a function of the dose of PHA. A marked impairment of the maximum response to PHA is quite evident in the lymphocytes from patients with Down's syndrome ($P < 0.001$). The percentage of circulating T-lymphocytes (rosette forming cells) in these patients is also significantly lower than in the control group (Table).



Dose response curves of lymphocytes from Down's syndrome and control subjects to increasing concentrations of PHA-M.

Percentage of circulating rosette forming cells in patients with Down's syndrome and controls

	No. of subjects	Mean ($P < 0.01$)	S.D.	Range
Down's syndrome	25	43.8	10.5	23–55
Control subjects	25	60	8.7	47–72

Our findings of an impaired PHA-responsiveness in adult patients with Down's syndrome are quite in agreement with those of AGARWAL et al.[8] and of RIGAS et al.[12]. Nevertheless some recent studies (to be published) in children with Down's syndrome failed to show any impairment of PHA-responsiveness, when compared to age matched controls.

These observations suggest that thymus-dependent function declines much more rapidly in these patients than in the general population. Although it is tempting to attribute this functional impairment to the observed reduction of circulating T-cells, we were not able to demonstrate a statistically significant correlation between PHA-responsiveness and the percentage of rosette forming lymphocytes. An alternative explanation might be that PHA-responsive and rosette-forming cells represent two different, or at least not completely overlapping, subpopulations of T-lymphocytes.

The increasingly recognized association between susceptibility to infections, malignancy and autoimmunity has recently been interpreted as a consequence of some derangement of the immune function, particularly of the thymus-dependent system[13,14]. Our observations in Down's syndrome add some additional experimental support to this hypothesis.[15]

Riassunto. La reattività alla fitoemoagglutinina e la capacità di formare «rosette» con eritrociti di montone dei linfociti di sangue periferico è risultata significativamente depressa in soggetti adulti affetti da sindrome di Down. Si può ritenere che la funzione timo-dipendente vada incontro in questi pazienti ad un deterioramento assai più rapido che nella popolazione generale.

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Effects of Prostaglandin E_2 on the Uptake of ^{3}H-Thymidine in Pregnant Mice

Despite the widespread clinical use of certain prostaglandins for the termination of pregnancy and the induction of labor, little attention has been directed to the effects of these substances on fetal and maternal metabolism. Prostaglandin E_2 (PGE_2) administered to pregnant rats[1] and mice[2] induced a high incidence of fetal death and resorptions. The mechanism for this lethal action is uncertain. We report here the effects of PGE_2 on the uptake of ^{3}H-thymidine in maternal and fetal tissues of mice.

Materials and methods. Prostaglandin E_2 (50 or 100 µg) was administered s.c. to pregnant Swiss Webster mice from day 8 through 12 of gestation. Another group of mice received the solvent alone and served as controls. The animals were killed on the last day of treatment 1 h after an i.v. administration of 1 µCi/g ^{3}H-thymidine (New England Nuclear; Specific Activity: 6.7 C/m*M*). Selected maternal tissues and the conceptuses were excised under a dissecting microscope and processed subsequently to determine the concentration of radioactivity. The tissue samples were pooled and weighed immediately after removal, dissolved in NCS and neutralized with acetic acid. 10 ml of a scintillation cocktail (5 g PPO; 0.5 g POPOP; 250 ml ethylene glycol monomethyl ether; 750 ml toluene) was added and the samples were counted three times for 10 min in a Beckman LS 150 Liquid Scintillation Counter.

Uptake of ^{3}H-thymidine in pregnant mice treated with prostaglandin E_2

Tissues	Control	Prostaglandin-treated	
		50 µg	100 µg
Maternal			
Brain	303.2 ± 50.4	210.0 ± 11.5	152.5 ± 10.1
P[b]		< 0.05	< 0.01
Lung	387.6 ± 68.8	252.4 ± 54.6	211.3 ± 31.1
P		< 0.05	< 0.05
Liver	653.7 ± 122.5	591.1 ± 101.4	520.2 ± 98.3
P		NS	< 0.05
Kidney	451.5 ± 75.3	303.4 ± 82.7	401.4 ± 90.5
P		< 0.05	NS
Spleen	1881.2 ± 183.4[a]	1555.8 ± 190.3	922.0 ± 167.9
P		< 0.05	< 0.01
Embryos	662.9 ± 81.1	324.6 ± 53.5	
P		< 0.01	

[a] Mean counts per min (cpm) per mg wet tissue weight. Standard error of the mean on the basis of 10 min counts; [b] *P*, significance of difference from the control; NS, not significant.

Results and discussion. A summary of our findings is presented in the Table. The uptake of ^{3}H-thymidine in maternal and embryonic tissues was significantly reduced following pre-treatment with the prostaglandin.

Inhibition of ^{3}H-thymidine uptake in maternal liver, brain and spleen showed a dose dependent relationship. The uptake of ^{3}H-thymidine was markedly reduced in the embryos. However, a correlation between the uptake of ^{3}H-thymidine in the 2 treatment groups was not possible, since all fetuses had been resorbed at the higher dose level.

These preliminary findings suggest that PGE_2 is capable of inhibiting DNA synthesis, either directly or indirectly. This inhibition could account for some of PGE_2 effects on the fetus and placenta during pregnancy[3,4]. Further studies of ^{3}H-thymidine, ^{3}H-uridine, and ^{3}H-leucine at different gestational periods are in progress.

Zusammenfassung. Die Behandlung von trächtigen Mäusen mit Prostaglandin E_2 führt zu einer Verminderung des Einbaus von ^{3}H-Thymidin im mütterlichen und fötalen Gewebe.

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The Response of the Adrenal Gland to Hypoglycaemia in the Conscious Calf

A technique has recently been devised which permits collection of the whole of the effluent blood from the innervated right adrenal gland in the conscious unrestrained calf[1,2]. The present paper describes experiments in which this technique has been employed to investigate the changes in glucocorticoid and catecholamine output from the gland during insulin hypoglycaemia.

Materials and methods. Insulin was injected i.v. at doses of 0.1, 0.5 or 4.0 units/kg 14–24 h after surgery. Adrenal blood flow was estimated gravimetrically and the outputs of steroids and catecholamines were then calculated from the adrenal venous plasma concentrations. Adrenaline and noradrenaline were estimated by a modification of von Euler and Lishajko's fluorimetric technique[3], glucocorticoids by competitive protein binding assay[4] and glucose enzymatically.

Results and discussion. Results of a typical experiment illustrating the characteristic responses to insulin hypoglycaemia at each dose are compared in the Figure.

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