ious day-3 females in the present study following treatment with A23187. It has been proposed [19], that exogenous farnesoic acid stimulation of *L. migratoria* CA is equivalent to that by a putative allatotropin [20], the latter possibly being involved in activating rate-limiting steps in the biosynthesis of JH prior to farnesoic acid synthesis. However, the mechanisms of stimulation in both cases remain to be defined, although both allatotropin- and A23187-stimulation probably involve enhancement of rate-limiting steps. Nonetheless, JH biosynthesis by CA from young adult females of *L. migratoria* can be stimulated, as the occurrence of a proportion of CA showing high release rates at this age would suggest; CA from these young females may therefore possess the potential to biosynthesize JH at high rates, although this potential is apparently not expressed continuously. Indeed, it has been suggested [18, 19] that JH production in adult *L. migratoria* is a function of two independent variables – stimulation of the CA by allatotropic substances (effective on a short-term basis) and the development, during early adult life, of responsiveness to allatotropin. The present data indicate that, in the case of gregarious females, the ability to respond to calcium ionophore has developed by day 3 of adult life. In solitarious females, the median rate of JH release in the presence of A23187 was higher than that during initial incubation in control medium at all datapoints. In no case, however, was the difference between 'initial' and 'stimulated' rates significant. Nonetheless, initial median rates of JH release were higher in solitarious than in gregarious females. This suggests that incubation in ionophore resulted in a significant elevation in release rates by CA of gregarious animals only because 'initial' rates were lower in these animals. It should be noted, however, that 'initial' rates were only significantly higher in solitarious females on day 8 (fig.) whereas 'stimulated' rates were significantly higher in gregarious animals on days 3 and 5.

In conclusion, treatment of CA with A23187 significantly stimulates JH III biosynthesis in CA from gregarious but not solitarious *L. migratoria* on days 3, 5 and 8. This difference between the phases reflects a higher 'stimulated' rate of biosynthesis in gregarious females on days 3 and 5 and a higher normal rate of biosynthesis in solitarious females on day 8. The cause of this difference between the phases may be complex and may reflect the interaction of more than one factor.

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Effect of dexamethasone on Fc γ receptor expression in foetal and neonatal rat gut

P. Griffin* and A. E. Wild

Department of Biology, Medical and Biological Sciences Building, University of Southampton, Southampton S09 3TU (England)
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Summary. When injected into 12-day-old suckling rats, dexamethasone caused a precocious disappearance of Fc γ receptors from enterocytes of the proximal small intestine. However, dexamethasone appeared to be necessary for the maintenance or production of such receptors in foetal rat gut cultured in vitro.
Key words. Fc γ receptor; rat gut; dexamethasone; IgG binding; enterocyte.

Transmission of maternal IgG across the gut of the suckling rat is effected in part by Fc γ receptors present on enterocytes of the duodenum and jejunum but absent from the ileum [1]. These receptors bind IgG optimally at pH 6.0 as shown by in vitro assays [2–6], but do not bind IgG at pH 7.2–7.4 and thus are well adapted to the pH prevailing in the lumen of the proximal small intestine [7]. Following binding, receptor-mediated endocytic processes effect the transcytosis of intact IgG across the enterocyte [8]. In vivo, antibody transport ceases after 21 days [9] and this is correlated with an absence of detectable Fc γ receptors on enterocytes obtained from rat gut beyond this age [2, 4, 10, 11]. Antibody transport also takes place across the gut of the late foetal rat [12] and we have recently shown that Fc γ receptors first become detectable on enterocytes in the most proximal region of the small intestine of the 20–21-day-old foetus [11]. Glucocorticoids have been implicated in the termination of Fc γ receptor expression, since in the 21-day-old neonatal rat there is a rise in the level of free plasma corticosterone [13] and injection of cortisone acetate into neonatal rats advances the time of closure of the

gut to antibody [14] and IgG [15] transport by several days; cortisone acetate injection also causes a precocious loss of Fc γ receptors from enterocytes [3, 10]. Organ culture facilitates investigation of the effect on foetal gut of direct exposure to hormones and in such a system dexamethasone has been shown to cause increased levels of lactase activity compared to controls, thus implicating glucocorticoids in the development of foetal gut enzyme activity [16]. Using organ culture, and immunofluorescence to detect specific, acid-pH dependent binding of IgG to gut enterocytes, we have investigated whether dexamethasone has a similar effect in inducing Fc γ receptor expression in foetal rat gut. Since dexamethasone has not previously been used in studies of this nature, we first investigated whether dexamethasone has a similar effect to cortisone acetate in causing a precocious loss of Fc γ receptors from the gut of neonatal rats. For this purpose an erythrocyte-antibody (E-A) rosette assay [2, 3] was used to detect Fc γ receptors on enterocytes, as well as an immunofluorescence assay for comparison with subsequent studies on foetal gut cultures. Two animals from each of two litters of 12-day-old suckling Wistar rats were injected i.p. with a suspension of 5 mg dexamethasone (Sigma) in 0.2 ml sterile 0.85% saline. A control animal from each litter was injected with 0.2 ml sterile saline alone. 48 h later the animals were killed by cervical dislocation, the gut removed, and the small intestine flushed with ice-cold 0.1 M phosphate buffered saline (PBS), pH 7.2; this was then divided into proximal (duodenum and jejunum), junctional, and distal (ileum) regions on the basis of colour differences and enterocytes removed as previously described [3], except that the gut was slit longitudinally rather than being everted prior to incubation separately for 24 h at 4 °C in buffer medium composed of 0.12 M-(N-morpholino)-ethane sulfonic acid, 1 mM EGTA, 0.5 mM $MgCl_2$ and 0.02% sodium azide. Enterocytes were then dislodged from the gut segments by gentle pipetting of the buffer medium, washed three times in PBS pH 7.2 or 6.0 following separation into equal aliquots, and finally re-suspended at 5×10^5 cells/ml in PBS of appropriate pH. E-A rosette assays were also carried out as previously described [3] but with rabbit IgG sensitized sheep red blood cells (SRBC) adjusted to pH 6.0 or 7.2 before mixing with enterocytes. This mixture was centrifuged at 1500 rpm for 3 min and the pellet left for 10 min before being re-suspended and scored for rosettes (binding of five or more SRBC to the enterocyte plasma membrane) by phase contrast microscopy. Controls consisted of unsensitized SRBC or SRBC sensitized with F(ab')2 of rabbit IgG anti-SRBC.

As can be seen from the table, in the saline-injected neonatal rats, close to 50% of enterocytes from the proximal small intestine, but only a very low percentage from the junctional region and none from the ileum, bound indicator SRBC to form rosettes at pH 6.0; no rosettes were formed at pH 7.2. However, rats injected with dexamethasone had no enterocytes taken from any region of the small intestine forming rosettes at pH 6.0, indicating that Fc γ receptors had been caused to disappear prematurely from the proximal small intestine. These results are similar to what has been obtained previously for cortisone acetate injection and monitoring of Fc receptors by E-A rosette formation [3].

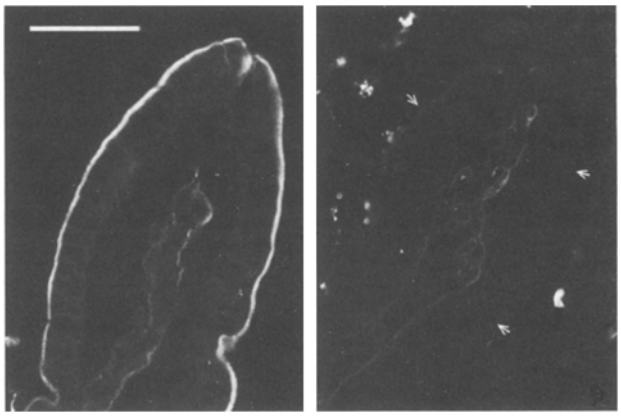
An immunofluorescence assay [11] was also used to monitor Fc γ receptor expression following dexamethasone injection. Two animals from a third litter were treated with dexamethasone and two with saline as previously described and 48 h later duodenum, jejunum and ileum, isolated from the small intestine. From these regions small segments of gut approximately 2 mm long were excised, slit longitudinally, and incubated in PBS, pH 6.0, containing bovine serum albumin (1 mg/ml). The sheets of tissue were then rinsed in PBS, pH 6.0, and transferred to a 2 mg/ml solution of rabbit IgG or F(ab')2 of IgG in PBS at either pH 6.0 or 7.2 and then incubated for 2 h; this was followed by washing for 1 h in PBS of appropriate pH. In order to reduce endocytosis of IgG, all procedures were carried out at 4 °C and all solutions contained 0.02% sodium azide, the latter being known to reduce endocytosis considerably in isolated enterocytes cultured in vitro [17]. Following fixation in a 50:50 ethanol-ether mixture for 30 min, the tissue was processed for the fluorescent antibody technique [18]. De-waxed sections were incubated for 30 min in a 1:50 dilution of FITC-labelled sheep anti-rabbit IgG (Wellcome Labs.) which at this dilution did not cross-react with rat IgG, washed for 30 min in several changes of PBS, mounted in PBS-glycerol, and observed in a Leitz fluorescence microscope. Results were recorded on Ilford HP5 with a 60-s exposure.

As can be seen from figure 1 A, rabbit IgG, as judged by the intensity of fluorescence, became stongly bound at pH 6.0 to the apical brush border of enterocytes on duodenal villi from control saline-injected rats; some weaker binding also appeared to have taken place to the lateral plasma membrane. No such binding to these sites occurred in the case of dexamethasone-injected rats (fig. 1 B). In both cases, however, fluorescence indicative of rabbit IgG was present in the villous core and as isolated specks around villi. It is likely that rabbit IgG had leaked between enterocytes during the incubation period and become trapped in the villous core and also bound non-specifically to mucus. Fc γ receptors are known to be present on the lateral plasma membrane of enterocytes of the proximal small intestine as shown by binding of indicator SRBC [3, 11] and IgG-HRP conjugates [8], as well as on the apical microvilli, which would explain the

% E–A rosette formation with enterocytes from different regions of the small intestine 48 h after injecting 12-day-old suckling rats with saline or dexamethasone

Intestinal region	pH at which assay was performed	Saline-injected animals (n = 2)	Dexamethasone-injected animals (n = 4)
Proximal	6.0	55/48	0
	7.2	0/0	0
Junction	6.0	7/8	0
	7.2	0/0	0
Ileum	6.0	0/0	0
	7.2	0/0	0

* SRBC alone, or sensitized with F(ab')2 of rabbit anti-SRBC, formed no rosettes.



A B

Figure 1. Sections through duodenal villi exposed to rabbit IgG at pH 6.0 from suckling rats injected with saline (A) and dexamethasone (B). Note the absence of binding of rabbit IgG to the enterocyte brush border (arrows) following dexamethasone treatment but its presence, as shown by immunofluorescence, in the villus core. Scale bar = 17 µm.

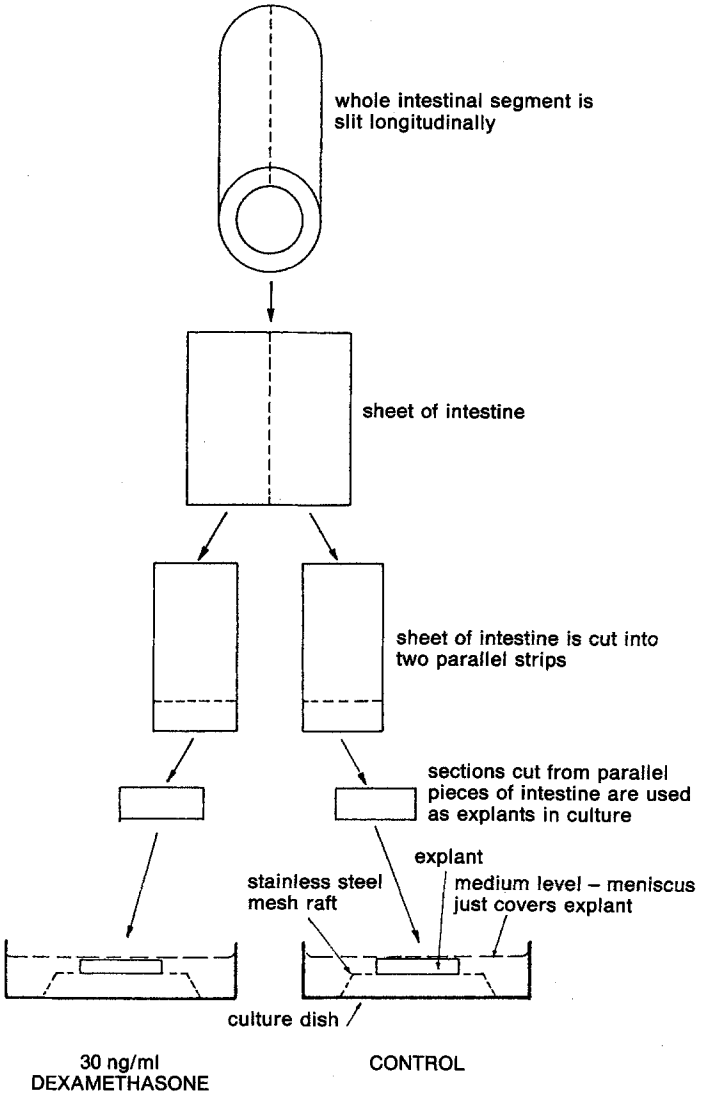


Figure 2. Diagram illustrating method of obtaining and culturing explants of foetal gut.

localisation of IgG at these sites in the saline-injected rats. Similar results to these were found for jejunum from control and experimental rats. No binding occurred to enterocytes at pH 7.2 or to ileal enterocytes at either pH; neither was there binding to enterocytes when F(ab')2 was used as ligand. These results are consistent with dexamethasone having caused precocious loss of acid-pH dependent Fc receptors from enterocytes of the proximal small intestine of suckling rats.

In order to study the effect of dexamethasone on Fc γ receptor expression in foetal rat gut, foetuses at 20 and 21 days gestation (the time when Fc γ receptors are first becoming detectable in foetal rat gut [11]) were removed from their terminally anaesthetized mothers and subjected to laparotomy under sterile conditions. The stomach and intestine were removed, transferred to sterile saline and segments of intestine taken 1 cm distal to the stomach and 1 cm proximal to the caecum. These were slit longitudinally, unrolled to form flat sheets and processed for organ culture as depicted in figure 2. Development of the gut appears to take place in a proximal to distal direction [19] and evidence has been obtained [11] that the same is true with respect to development of Fc γ receptor expression. It was critical therefore, that experimental tissue exposed to dexamethasone and control tissue exposed only to carrier solvent, derived from the same point along the proximal-distal axis of the small intestine. The procedure outlined in figure 2 ensured that this was the case. Dexamethasone was dissolved in ethanol and added to the culture medium (Kennett's medium [20] containing foetal calf serum, NCTC 135, insulin, pyruvate, oxaloacetate, fungizone, L-glutamine and gentamycin, in DMEM) to give a concentration of 30 ng/ml. Cultures were maintained at 37 °C in an atmosphere of 5% CO_2 in air for 48 h with a complete medium change after 24 h. Binding of rabbit IgG to gut enterocytes was then assessed using the immunofluorescence method described for detection of Fc γ receptors on enterocytes of suckling rat gut. The minute size of the cultured tissue precluded assessment by E–A rosette formation. Unlike the situation pertaining in suckling rat gut, the presence of dexamethasone appeared to be a requirement for expression of Fc γ receptors on enterocytes of foetal rat proximal small intestine, as revealed by acid-pH dependent binding of rabbit IgG in cases where the corticosteriod was included in the culture medium (figs 3 A and 3 B), and no binding in its absence (fig. 3 C). Binding was much weaker to foetal enterocytes than to those of sucklings as revealed by intensity of fluorescence, but there was again strong binding to the villous core probably attributable to leakage of IgG between enterocytes. An unexpected finding was that enterocytes from cultured distal small intestine revealed a dexamethasone-dependent binding of rabbit IgG to the apical

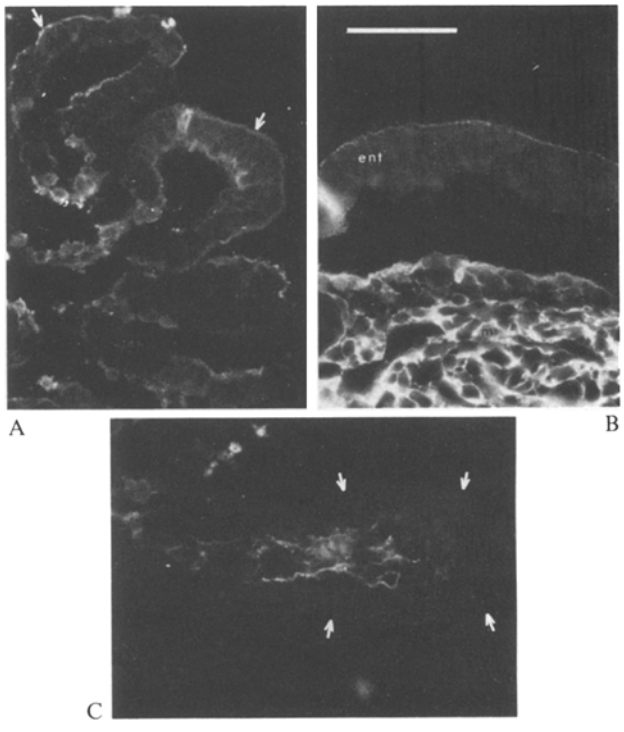


Figure 3. Sections through explants of proximal small intestine of 21-day (A) and 20-day (B) foetal rats cultured in the presence of dexamethasone and treated with rabbit IgG at pH 6.0. Note the binding of rabbit IgG, as shown by immunofluorescence, to the enterocyte (ent) brush border (arrowed in A) and compare this to (C) where explanted tissue from the same region as that shown in (A) was cultured in the absence of dexamethasone and shows no binding of rabbit IgG to the enterocyte brush border (arrowed in C). Mesenchymal tissue (m) in the villus core of the explant of 20-day-old foetal gut shows a high concentration of rabbit IgG and the enterocyte layer has detached, presumably in sectioning; the same is true of the enterocyte layer in (A) where the mesenchymal tissue is disrupted. Scale bar = 17 μm.

surface (data not shown); however, unlike binding of rabbit IgG to proximal enterocytes, this occurred as readily at pH 7.2 as at pH 6.0 and could not therefore be taken as indicative of binding to comparable Fc γ receptors, although as with binding to proximal small intestine it was Fc γ dependent. Actin, present in enterocyte terminal web regions, has also been suggested to be capable of binding IgG in an Fc-dependent manner[21] and could have been exposed during the culture period.

The effect of dexamethasone in causing a precocious loss of Fc γ receptor when administered to suckling rats and an apparent increase or at least maintenance in expression when added to cultured foetal gut, is comparable to the effect of glucocorticoids on lactase activity. Thus administration of corticosteroid to suckling rats causes a precocious decrease in lactase in enterocytes (and a concomitant rise in sucrase and maltase)[22], whilst addition of dexamethasone to cultured foetal rat intestine causes an increase in lactase activity compared to controls[16]. Assessment of Fc γ expression in the method we have used cannot be quantified and we do not know whether Fc γ receptors were present on enterocytes at the start of culture of the tissue and were then maintained in expression by dexamethasone, or whether dexamethasone induced them to appear. Dexamethasone has been suggested to have a protective effect in maintaining enzyme activity in cultured 4-day-old post-natal rat gut with respect to lactase, and a promoting effect with respect to maltase and sucrase[23]. Now that the Fc γ receptor has been isolated from suckling rat jejunal enterocytes, and monoclonal antibodies prepared against it[24], more accurate measurement of hormone-induced expression of Fc γ receptors could be made. Our preliminary findings do however point to their expression being controlled by glucocorticoids in a manner similar to that of certain brush border enzymes, and as part of an overall pattern of development of the gut that adapts to a post-weaning diet[25].

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Proliferation and cell loss of human leukemic cell subpopulations in liquid culture

P. A. Bernabei, F. C. Agostino, R. Bezzini, R. Saccardi, V. Gattei, V. Santini, M. Casini and P. Rossi Ferrini

Div. Ematologia, USL 10/D-Universita' degli Studi, Policlinico di Careggi, I-50134 Firenze (Italy)
Received 28 September 1987; accepted 23 November 1987

Summary. A kinetic study was performed on leukemic blasts from patients with acute myeloid leukemia, separated into 2 subpopulations by a specific density gradient. The growth curve and the [^{3}H]-thymidine uptake were simultaneously analyzed. While cumulative nucleotide uptake fitted with the growth kinetics in the low-density fraction, such a concordance was not found in the high-density subpopulation. That indicated the occurrence of simultaneous growth and loss in the high density fraction, which could not be evaluated by a simple numerical determination.
Key words. Acute myelogenous leukemia; cell subpopulations; cell loss; kinetics in culture.

Leukemic cells harvested from patients with acute myeloid leukemia (AML) may display heterogeneous features as to karyotype[1], phenotype[2] and cytokinetics[3]. According to the difference in size, leukemic cells can be subdivided into two subpopulations which show different cytokinetic patterns: the large size subpopulation, which apparently includes the proliferative component, and the small cell subpopulation, which is quiescent[4] and possibly responsible for the kinetic resistance to chemotherapy[5]. This subdivision has also been supported by sedimentation and elutriation studies, showing that the cells characterized by high or low density display a high or a low labeling index, respectively[6, 7]. This has resulted in most kinetic studies being focused on low-density cells, disregarding the high-density subpopulation.

We considered further study of this topic worthwhile, trying to resolve the in vitro cytokinetics of low- and high-density leukemic cell subpopulations by measuring both cell proliferation and loss. For this purpose we used the method proposed by Olivotto et al.[8], based on the simultaneous determination of the cell number kinetics and of the time-course of the rate of [^{3}H]-thymidine ([^{3}H]-Tdr) incorporation into DNA (R(t)) of the cell population under study. These authors showed that within any cell population, when the mean time spent in S by the cells remains constant, the integral of R(t) as a function of time reflects the total cell number increase if, and only if, the cell loss is negligible, according to the following equation:

$$\int_0^t R(t)\,dt = k \times (N_t - N_0) \qquad [1]$$