or strain differences. Indeed, susceptibility to KTZ may vary in the same manner as susceptibility to chloroquine. The KTZ susceptible strains were derived from Honduras and Indochina [14], whereas the FCR-3 strain (used in this study) is of West African origin. However, because minimal anti-plasmodial effects may still occur with some strains at 1 μg/ml KTZ [15], we recommend that 5-FC be used alone initially. If anti-fungal contamination persists, KTZ can then be added by beginning at 0.5 μg/ml. No anti-plasmodial effect has been reported at this concentration.

Our data also demonstrate that the combination of 5-FC and KTZ is innocuous to two tumor cell lines and thereby suggest that these agents may have a wider applicability in tissue culture work. However, it would be safer to test each cell line before broadening the recommendations for use.

Finally, expense in media preparation is another consideration which unfortunately cannot be ignored. Using the 1988 price list of a popular U.S. chemical supply house as a basis for calculation [16], and assuming the use of 5-FC at a concentration of 50 μg/ml, addition of this reagent would increase the cost of each liter of culture medium by US$ 1.53. Obtaining KTZ from the same supplier would add only US$ 0.01 for each liter (if used at 1 μg/ml). These costs should be balanced against the loss incurred by discarding contaminated medium and plasticware and the labor required to repeat interrupted experiments.

As with all antimicrobials, these agents cannot replace meticulous sterile techniques which remain the mainstay of cell biology laboratory work.

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## Decreased number of asialoglycoprotein receptors in diabetic BB Wistar rat

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*Summary.* The binding of asialoglycoproteins by hepatic binding protein was studied in freshly isolated hepatocytes from genetically diabetic BB Wistar rats. The number of cell surface asialoglycoprotein receptors was dramatically decreased (58,000 ± 38,000 for diabetic rats compared to 267,000 ± 70,000 for normal rats), while the association equilibrium constant was not changed. These results parallel those obtained with streptozotocin-diabetic rats and support the hypothesis that insulin deprivation is responsible for the decrease in the receptor number.
*Key words.* Diabetes; asialoglycoprotein-receptor; hepatocytes.

A transmembrane receptor, the hepatic binding protein (HBP), is responsible for the selective uptake and lysosomal degradation of asialoglycoproteins (ASGP) [1]. Previous reports have shown that in streptozotocin-diabetic rats, less ligand is bound, internalized and degraded due to a decreased amount of cell surface HBP [2, 3]. The toxicity of streptozotocin raises the question of whether this alteration is really a consequence of insulin deprivation.

The purpose of this study was to explore the binding affinity and the quantity of HBP at the cell surface of hepatocytes of genetically diabetic rats. We used livers from BB Wistar rats, in which diabetes is insulin-dependent [4, 5] and shares many features with the type I pathology in human diabetes.

In this report, we show that hepatocytes of BB rats, like those of streptozotocin-diabetic ones, present a de-

creased number of cell surface receptors and also of total cell receptors, while the parameters of ligand binding are not altered.

*Material and methods.* Twelve BB rats which were kindly donated by Prof. Assan (Laboratoire de Diabétologie, CHU Bichat, Paris), were bred and fed ad libitum. They were screened by estimating glycosuria and ketonuria every day. Four of them, which became diabetic, were given single daily s.c. injections of 4–8 units (2 U/100 g) of protamin-zinc-insulin IPZ (Novo, Copenhagen) until four days before sacrifice. At this time, the rats were glycosuric and ketonuric with a glycemia above 25 mM. Five normal Wistar rats, with the same range of body weight, were used as controls.

$75-90 \cdot 10^6$ hepatocytes were obtained from 2.5–3 g liver biopsies using a collagenase perfusion procedure as described by Strom et al.[6]. They were 70–80% viable as judged by 0.06% Trypan blue exclusion.

The ligand used was $^3$H-asialorosomucoid ($^3$H-ASOR). Ligand preparation and binding to cell surface and total cell receptors were performed as previously described[3,7] and outlined in the legend to the figure.

The ligand binding system was characterized by Munson's computerized model[8]; one kind of site and linear progression of the non-specific binding as a function of $^3$H-ASOR concentration. We obtained fitted values for the maximal number of sites (R), the equilibrium association constant ($K_a$) and the non-specific binding (N). The fitted parameters are closely similar to those obtained with an experimental aspecific binding.

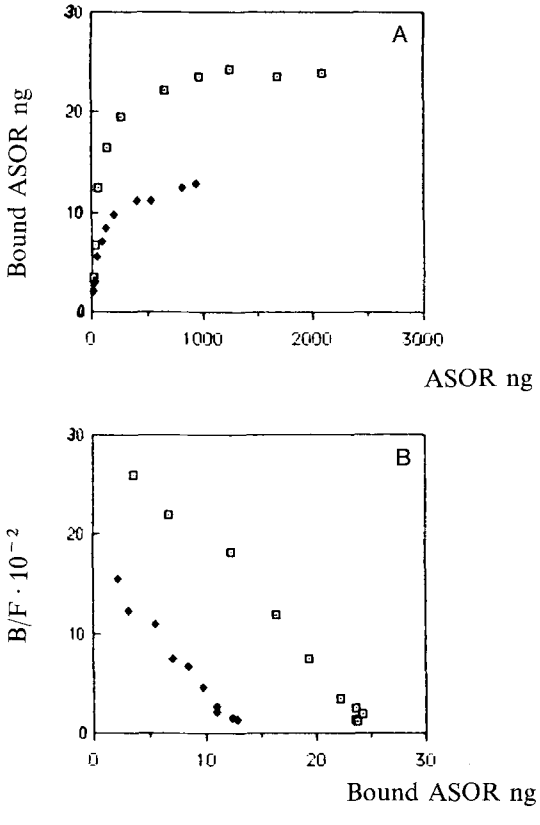
*Results and discussion.* The computerized analysis of ASOR binding equilibrium (bound as a function of Free ASOR) gave the fitted values from the saturation curve (fig., A). The linearity of the Scatchard plot (fig., B) revealed one single kind of site, for both surface membrane and total cell receptors. Normal and diabetic rats did not significantly differ when association equilibrium constants were considered, either for surface membrane or total receptors (table).

On the other hand, when the number of cell surface receptors was considered, a dramatic decrease was observed in diabetic BB rats. For normal Wistar rats, the mean value for surface receptors as measured after equilibrium at 37 °C was 267,000 ± 70,000 per hepatocyte (table), which was not different from the normal value previously found in Sprague-Dawley rats[8]. For diabetic BB Wistar rats the average value for cell surface receptors was 58,000 ± 38,000. Referred to the normal value, it is a 78% mean decrease (fig., B), even more striking than the 60% decrease previously observed in streptozotocin-diabetic rats[2,9].

The number of total cell receptors was 840,000 ± 290,000 for normal and 450,000 ± 300,000 for diabetic rats (table). This corresponds to a 50% decrease for the latter. There was a very large variability between diabetic rats; nevertheless, the significance probability of the decrease was 0.6–0.8.

As observed in streptozotocin-induced diabetes, there is a more dramatic decrease in the number of surface receptors than in the number of total cell receptors. This difference in the degree of deterioration might originate from the existence of two pathological processes. The marked decrease in the cell surface number could be explained by a change in membrane composition and fluidity, as suggested by Nassar[9], while the less significant decrease in the number of total cell receptors could be related to a progressive deficiency of receptor synthesis, as was observed by Jefferson[10] for protein synthesis in the livers of diabetic rats.

These results are in good agreement with the previous observation showing that the decreased number of HBP receptors, which is normalized by insulin treatment[2], is a consequence of insulin deprivation.




Saturation curve A and Scatchard plot B for $^3$H-ASOR binding to cell surface receptors. Cell aliquots, $2 \cdot 10^6$/ml, were incubated for 2 h at + 4 °C in the presence of different concentrations of $^3$H-ASOR. After three washings, cell-associated radioactivity was measured. Two typical experiments from one normal (□) and one diabetic BB rat (◆)[7].

Number of sites per hepatocyte and $K_a$ ($10^9$ $M^{-1}$) for normal and diabetic BB Wistar rats[7]

| | Normal BB rats N = 5 | Diabetic BB rats N = 4 |
|---|---|---|
| Cell surface sites | 267,000 ± 70,000 | 58,000 ± 38,000 |
| $K_a$ cell surface sites | 0.54 ± 0.24 | 0.99 ± 0.25 |
| Total cell sites | 840,000 ± 290,000 | 450,000 ± 323,000 |
| $K_a$ total cell sites | 0.91 ± 0.35 | 1.83 ± 0.86 |

The analogy reported between the diabetes of BB rats and type I human diabetes allows one to predict that similar perturbations might exist in human diabetic livers.

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## Colcemid effects on homologue pairing and crossing over during fetal mouse oogenesis

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*Summary.* Colcemid was administered to gestational day 13 female mice to test effects on homologue pairing, synapsis and recombination of fetal oogenesis. Pairing abnormalities were detected in pachytene oocytes by light and electron microscopy examination of bivalents and synaptonemal complexes. Reduction of total chiasmata per treated diplotene oocyte (22.74) compared to controls (31.07) was found.
*Key words.* Colcemid; meiosis; recombination; oogenesis; nondisjunction.

The organization of events responsible for the accurate pairing of homologous chromosomes and the molecular mechanisms of genetic recombination, particularly in mammals, is not fully understood[1]. Levan in 1939[2], reported that colchicine disrupted prophase in *Allium* and affected crossing over by mechanisms which appeared to be distinct from the well established microtubule inhibitory effects. Subsequent studies attempted to separate out effects on spatial pairing, synapsis and recombination[3,4]. Loidl[5] has recently reported delay or prevention of synapsis at leptotene and defective pairing initiation at zygotene in colchicine-treated *Allium ursinium*. Studies with the pachytene synaptonemal complexes of mouse oocytes and spermatocytes from inversion heterozygotes have suggested that vinblastine and nocodazole, two colchicine-like compounds, revealed specific autosomal and sex bivalent pairing abnormalities[6]. Khawaja and Ellis[7] have also described a colchicine-induced heritable double recessive desynaptic mutation of *Lathyrus*. The present experiments examined effects of demecolcine on pachytene chromomere maps and synaptonemal complexes and diplotene chiasmata frequency in mouse oocytes as measures of perturbation of pairing and recombination during early oogenesis.

*Materials and methods.* Dated gestations were obtained in female Swiss mice (CAMM) by mating proestrous 2–4-month-old females with 4-month-old males of known fertility. Day of vaginal plug was designated day 0 of gestation. Demecolcine (Colcemid, Sigma) was injected as a single dose of 0.2 µg/g of b. wt i.p. to groups of 3 mothers on gestational day 13 when preleptotene oocytes are maximal[8]. Animals were sacrificed on day 17 for examination of pachytene and diplotene oocytes. Fifteen controls were injected with an equal volume of diluent. Sixteen treated pregnant females provided 89 female feti and 178 fetal ovaries were immediately dissected into balanced salt solution. Control females yielded 74 feti and 148 ovaries by the same method. Each ovary was divided into three portions for preparation of oocytes for cytogenetic examination and microscopy of synaptonemal complexes. Pachytene oocyte chromomere maps were prepared by the method of Jagiello and Fang[9], and diplotene oocyte chromomere patterns by the method of Jagiello and Fang[10]. Final identification of pachytene and diplotene bivalents was made by visualizing each bivalent of each cell with a 100X Planapo light and phase objective and simultaneously observing the drawings and photomicrographs (fig. 1 a, b). Chiasmata were identified