animals were shown to contain 70±4% of blood levels of labelled IgG.

The most obvious advantage of this particular method is that no external control is necessary since all measurements within any experiment are from the same animal. Its success depends upon the creation of a good seal between the 2 sides, with minimal damage to local tissues in the process.

Pontamine Sky Blue has a high molecular weight and binds avidly to serum proteins. It has been widely used to provide a simple qualitative estimate of increased vascular permeability, since it is only released from the bloodstream under these conditions. Our results convincingly demonstrated an increased vascular permeability in the inseminated side relative to the control in both the vagina and cervix of most of the animals studied, although the degree varied considerably between individuals. Blood flow changes were not monitored in these experiments. Measurement of ^{125}I-IgG levels allowed a quantitative assessment of IgG transfer to the tissues from the bloodstream following insemination. In all cases the vaginal tissues and vaginal fluids contained elevated levels of labelled IgG, showing that transfer was actually taking place. It is likely, in view of the surprisingly high level of labelled IgG found in the lymph draining from the area, that these figures represent a dynamic balance between IgG release from the blood system into the tissues and its removal by drainage into the lymphatic system, and that they drastically underestimate the actual amounts of IgG transferred.

The differences found in the vaginal samples seem conclusive, but in the cervical and uterine tissues, where contact with spermatozoa is more restricted, increased permeability and leucocytosis occurred on both sides to a similar degree. This suggests that functional isolation of the cervices may not be complete. Virgin and parous rabbits did not differ consistently in their responses: both showed increased vascular permeability and leucocyte invasion of the cervix following insemination.

Thus, we have found that the presence of semen in the rabbit female tract triggers a vascular response which drastically modifies the antibody content in the immediate environment of the spermatozoon. Mating with a vasectomized buck or insemination with seminal plasma[9] (i.e. no spermatozoa) does not elicit a similar response. Considering the present interest in measurement of immunological response of the female tract to spermatozoa, we must emphasize the necessity of also measuring antibody levels in the female tract after mating, since it is these levels that the spermatozoa actually face.

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Adenylate cyclase in the developing rat cerebral cortex and olfactory bulb

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Summary. Activities of adenylate cyclase, measured either in the absence or presence of sodium fluoride and Triton X-100, are determined in cerebral cortex and olfactory bulb homogenate of rats of 1 to 35 days of postnatal age. Differences in properties of the enzyme in the 2 structures are demonstrated.

The involvement of cyclic AMP in the regulation of cell division, growth and differentiation[1-3] through a series of metabolic alterations[4, 5] has been suggested for various tissues. Moreover, in the nervous system, cAMP may play several roles in the control of neuronal function; it has been involved in inhibition of rapid axonal transport of proteins[6] and in modulation of membrane potential, i.e. in transduction step in the primary olfactory neurones[7], in initiation of bursts of action potentials[8] and in variation in duration and depth of inter-burst hyperpolarization[9]. Furthermore, its role in the control of transmitter synthesis and release from nerve endings has been postulated[10]. These effects require ion fluxes through the cell membrane; thus the regulation of these fluxes may be one of the most important actions of cyclic nucleotides.

Regulation of intracellular cAMP concentration is determined by a balance between the activities of 2 enzymes: adenylate cyclase, required for synthesis of cyclic nucleotides from ATP, and phosphodiesterase which catalyzes its hydrolysis to 5'-AMP; it is also influenced by ATPase activity. Adenylate cyclase is a complex enzyme bound to membrane structures; it is formed by physically separate and independent components (receptor, regulatory sites and catalytic units); these components do not develop as a functional unit but may vary independently during maturation of the cell[11, 12]. Changes of cyclic nucleotide metabolism could provide valuable information about changes of tissue functions. Brain development is largely postnatal in the rat; cellular growth[13] and enzyme maturation[14] vary considerably among different regions. The present investigation was undertaken in order to compare the ontogenetic evolution of adenylate cyclase in olfactory bulb and cerebral cortex homogenates; olfactory bulb maturation is characterized by active hyperplasia and moderate cell hypertrophy, the reverse being observed in the cerebral cortex[15].

Material and methods. Experiments are conducted on Wistar rats of our inbred laboratory strain. The offspring are

investigated from birth (day 1) to 35 days of age. Rats are decapitated, cerebral cortex and olfactory bulbs are excised rapidly, weighed and homogenized in 9 vol. of an ice-cold medium containing 2 mM glycyl-glycine buffer pH 7.5, 1 mM $MgSO_4$ and 20 mM NaCl. Homogenates are prepared with a Potter-Elvehjem tissue grinder. The preparations are stored at − 18 °C for 16 h before use.

Adenylate cyclase assay: Adenylate cyclase activity is determined in crude tissue homogenates in the presence of unlabelled substrate under 4 different conditions. Under basal assay conditions. the incubation mixture contains the following final concentrations: 60 mM glycyl-glycine buffer pH 7.5, 3.6 mM $MgSO_4$, 50 mM caffeine, 1.0 mM ATP, 1.0 mM dithiothreitol and 0.1% bovine serum albumin. Fluoride-stimulated activity is determined by the addition of 6.0 mM NaF to this standard medium. Since adenylate cyclase is tightly bound to the cell membranes, the membrane-bound enzyme may have properties different from those of a detergent-solubilized enzyme; solubilization is realized by direct addition to the basal or NaF incubation medium of Triton X-100 at a final concentration of 0.1%. Reaction is initiated by the addition of 0.2 ml of homogenate (approximately 2.0 mg tissue protein) to 4.0 ml of incubation medium; it is allowed to proceed for 20 min in a shaking water-bath at 37 °C. Incubation is terminated by placing the assay tubes in a boiling water bath for 3.5 min. After rapid cooling, protein is sedimented by centrifugation at 18,000 × g for 40 min at 4 °C. The cyclic AMP in the supernatant fluid is extracted and evaluated according to the colorimetric method of Macdonald[16].

Enzyme reaction conducted under these conditions is linear with time for at least 30 min, and is proportional to tissue protein concentration from 0.25 to 1 mg/ml. An ATP-regenerating system consisting of 10 mM creatine phosphate and 100 µg/ml of creatine kinase is verified to be unnecessary under these conditions. In order to consider changes in cellular activity during development, experimental results are reported as pmoles cAMP formed per mg DNA per min. Experiments are performed in quadruplicate and results are the means of at least 2 independent determinations. DNA levels are measured by the diphenylamine reaction[17] adapted for brain tissue[18], using calf thymus DNA as standard. Statistical analysis is performed using Student's t-test.

Results and discussion. 1. General properties of adenylate cyclase in 35-day-old rats: The central nervous system in mammals is characterized by a high basal adenylate cyclase activity[19]. Our results obtained with cerebral cortex homogenate agree with other assays measuring the rate of formation of ^{32}P-cAMP from (α-^{32}P) ATP in adult animals[14, 20-22]. Activity in different regions of the rat brain varies considerably[14,23]; results obtained with olfactory bulb homogenate are significantly lower (16% on a wet weight or 70% on a DNA basis) than those found in cerebral cortex (figure 1). Fluoride increases cyclic AMP formation; this stimulatory effect is generally low in nervous tissue[20], but is higher in olfactory bulb than in cerebral cortex (172 v 130%); fluoride is thought to stimulate the enzyme directly at the catalytic subunit[24]. Adenylate cyclase has been solubilized in order to determine whether the membrane environment is responsible for the differences in activity observed between olfactory bulb and cerebral cortex cyclases. Triton X-100 treatment results in an increase in activity; activation of basal activity is lower in olfactory bulb (82%) than in cerebral cortex (165%). There is a further activation of the solubilized enzyme by NaF; but while the absolute response to fluoride (stimulated activity - basal activity) is somewhat higher, the relative stimulatory effect (expressed as a percentage of the basal level) is lower than observed in original homogenate. This is due to the marked increase in basal activity in the presence of non-ionic detergent.

2. Age-related changes of adenylate cyclase (figure 1). Adenylate cyclase activity is low at birth. The basal activities are enhanced 20-(cerebral cortex) and 28-fold (olfactory bulb) between 1 and 21 days of age. This increase occurs in 2 steps, during the 1st and the 3rd postnatal week. Thereafter, mean basal values slightly decrease (20%), but statistical significance is only demonstrated in olfactory bulb. Adenylate cyclase activity stimulation in response to fluoride appears earlier in olfactory bulb homogenate and

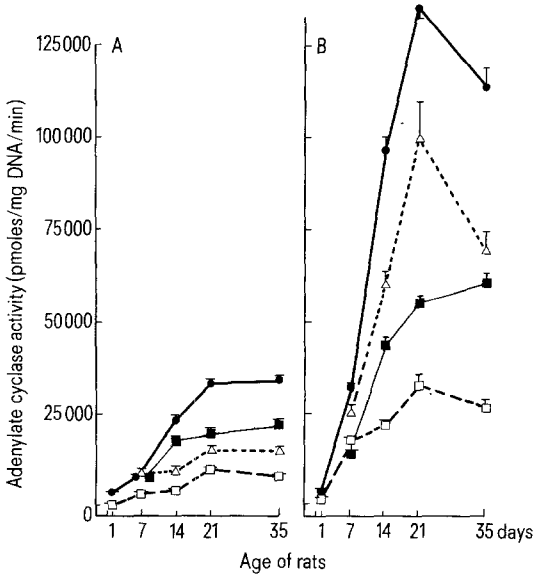

Fig. 1. Postnatal development of adenylate cyclase activity in rat olfactory bulb (A) and cerebral cortex (B) homogenates: (□) basal activity; (■) activity in the presence of 6 mM NaF; (△) activity in the presence of 0.1% Triton-X-100 in assay; (●) activity in the presence of 6 mM NaF + 0.1% Triton X-100. Results, expressed as pmoles of cAMP synthesized per mg DNA in 1 min, are means ± SEM from 6-14 determinations.

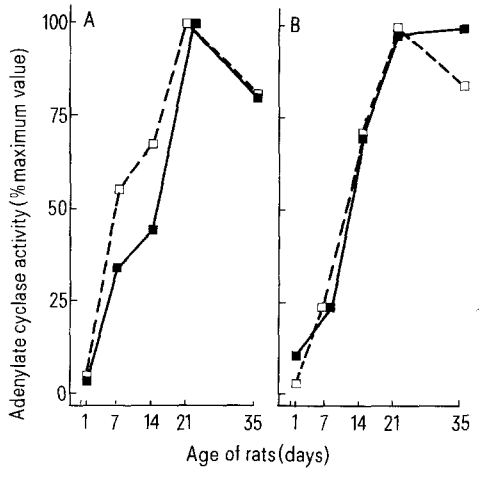

Fig. 2. Changes of adenylate cyclase activity in rats from birth to 35 days of age. *A* Basal activity. *B* Activity stimulated by the addition of 0.1% Triton X-100 + 6 mM NaF in the incubation medium. Open symbols represent evolution in cerebral cortex homogenate, closed symbols evolution in olfactory bulb homogenate. Results are calculated as a percentage of the maximum value noted over the developmental period, activity being expressed as pmoles cAMP synthesized per mg DNA in 1 min.

rapidly increases during the 1st 2 weeks (olfactory bulb) or 3 weeks after birth (cerebral cortex). Thereafter the enzyme activity increases more slowly up to 35 days of age. NaF-induced stimulation is, at all ages, greater in olfactory bulb than in cerebral cortex. These changes could indicate increased numbers of catalytic units during development.

Increased activity with development may also be attributable to alteration of the cell membrane structure or composition[24], but activity increase during development is considerably stimulated in the presence of detergent. In olfactory bulb homogenate, adenylate cyclase activity does not increase proportionaly to the basal activity and the absolute increase in cyclic AMP formation remains relatively constant. In cerebral cortex homogenate, activity progressively increases as a function of age being 3-fold higher on the 21st day than in the absence of detergent; decrease in activity between 21 and 35 days of age is increased (30%). A similar evolution, with higher levels of activity, is observed in response to sodium fluoride in both structures.

The comparison between olfactory bulb and cerebral cortex maturation reveals striking differences and adenylate cyclase activity of cerebral cortex homogenate remains consistently higher (1.73–6.60-fold) than that noted in olfactory bulb homogenate throughout the experimental period and whatever the incubation conditions (figure 1). Qualitatively similar differences with smaller amplitude are observed when enzyme activity is expressed on a wet weight or on a protein basis. However, in figure 2, enzyme activity is expressed as percentage of maximal activities found over the developmental period; the same pattern of evolution in cellular activity can then be observed in the 2 structures.

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The effect of dimethyl sulfoxide on tissue distribution of gentamicin

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Summary. Dimethyl sulfoxide (DMSO) and gentamicin were administered to rats i.p. No significant differences in gentamicin tissue concentrations were found between rats receiving DMSO and gentamicin, and rats receiving gentamicin alone. DMSO does not increase the tissue concentrations of gentamicin.

Aminoglycosides are valuable agents in the therapy of various infections. Some therapeutic failures may be due to insufficient penetration of the agent into body compartments in which infections occur.

Dimethyl sulfoxide (DMSO) has been shown to cross various body membranes[1]. Shortly after administration by various routes DMSO can be detected in almost every body space[2]. In addition, DMSO has been shown to enhance the penetration of many compounds through the intact skin and urinary bladder[3–6] and to increase the brain concentration of various substances when administered i.p. to rats[7].

We have investigated whether the concomitant administration of DMSO and gentamicin to rats would result in higher tissue levels of gentamicin as compared to rats receiving gentamicin alone.

Materials and methods. Charles-River male rats were divided into 3 groups. 1 group of 6 animals received gentamicin 6 mg/kg/day i.p. divided into 2 injections given 12 h apart for 10 days. The other group of 6 animals received the same dosage of gentamicin dissolved in 50% DMSO. (DMSO 0.69 g/kg/day) administered i.p. The 3rd group of animals received the same dosage of DMSO given i.p. and served as a control group. On the 11th day: 9 h after the last injection, the animals were sacrificed. This time was chosen in order to amplify possible differences in gentamicin organ concentrations between the groups. Tissues from the killed animals were removed aseptically, weighed, washed 3 times with phosphate buffered saline incubated in 2.5% trypsin solution (Difco 0152-13) for 2 h at 37 °C and then homogenized. The assay of gentamicin was performed with Oxford-cups on Mueller-Hinton Agar using *B. subtilis* (ATCC 6633) as the test organism. Plates were incubated at 37 °C for 18 h. Standard curves were made by dissolving gentamicin laboratory standard in pooled rat serum. All readings were performed in quintuplicate and then averaged. Statistical analysis was performed according to the Wilcoxon rank-sum-test, between the 2 methods of treatment.