Enfin, ces résultats posent le problème d'une influence de la déficience thyroïdienne sur la maturation du système nerveux s'exerçant déjà pendant la période foetale, même chez une espèce comme le Rat, dont le degré d'immaturité nerveuse est particulièrement grand à la naissance.

Summary. Synaptogenesis is already under way in the cerebellum of the rat immediately after birth. The synaptic contacts are defined by the simultaneous occurrence of 2 membranous thickenings, an intervening gap, and a minimum of 3 presynaptic vesicles. A difference in synapse concentration per area of section for animals born of thyroid-inhibited mothers as compared to normal controls is demonstrated. Synaptogenesis is delayed by thyrodepressive treatment immediately after birth.

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Effect of Decapacitation Factor on the Oxygen Uptake of Rabbit Spermatozoa Recovered from the Uterus

Rabbit spermatozoa must reside in the female reproductive tract for several hours before they can penetrate and fertilize an ovum[1, 2]. This capacitation process may involve removal, probably by enzymic action, of material located on the surface of the spermatozoa; re-exposure of the spermatozoa to seminal plasma leads to loss of capacitation and the process may well involve the restoration of the surface layer or decapacitation factor which normally coats the spermatozoa as they pass through the epididymis[3–6].

Several workers have recovered spermatozoa from the uterus of rabbits and compared their metabolism with those from fresh samples of semen[7–9]. Both the oxygen uptake and glycolytic activity of the spermatozoa appears to be increased after incubation in the uterus and there is also evidence of some switch to pentose shunt activity, as judged by a decrease in the ratio of $^{14}CO_2$ from 6-^{14}C glucose and 1-^{14}C glucose when these are used as substrate for the spermatozoa. The greatest increase in the oxygen uptake of spermatozoa took place between the 4th and 6th h of incubation in utero and since rabbit spermatozoa require about 6 h to become capacitated in the uterus it is tempting to suggest that these metabolic changes may be associated with the process.

One possible criticism of this type of work is that the semen suspensions recovered from the uterus are contamination with leucocytes and other extraneous cells that might influence the results. In the experiments reported in this paper the washed spermatozoa suspensions have been sealed into Millipore tubes before placing the suspension into the uterus of does. This system was also used to study the effect of adding decapacitation factor to the spermatozoa suspension after recovery from the uterus, in expectation that the decapacitation factor might decrease the oxygen uptake of the spermatozoa suspension.

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Oxygen consumption by spermatozoa (µl O_2/h/10^8 spermatozoa)

	Mean ± SE
Experiment 1 ($n = 6$[a])	
Spermatozoa from fresh semen	11.0 ± 3.49
Spermatozoa after incubation in the uterus	25.8 ± 9.42
Spermatozoa after incubation in the uterus, decapacitation factor added[b]	69.6 ± 39.44
Experiment 2 ($n = 3$)	
Spermatozoa from fresh semen	10.0 ± 5.24
Spermatozoa from fresh semen, decapacitation factor added[b]	8.5 ± 4.15
Experiment 3 ($n = 5$)	
Spermatozoa from fresh semen	9.2 ± 3.01
Spermatozoa from fresh semen, bovine serum albumin added[b]	10.7 ± 2.63

[a] n, Number of replicates. [b] Decapacitation factor (10 or 5 mg) or bovine serum albumin (10 mg) were added to the sample chamber of the oxygen electrode.

Materials and methods. Rabbit semen was collected by use of an artificial vagina[10]. The medium for diluting the semen and washing the spermatozoa was calcium-free Krebs Ringer phosphate of pH 7.0[11] containing 3 mg of glucose, 1 mg of streptomycin and 1 mg of penicillin per ml. Washed suspensions were obtained by diluting about 3 ml of semen to 10 ml, centrifuging at 1000 *g* for 10 min, withdrawing the supernatant and resuspending the spermatozoa in a volume of about 6 ml.

Washed suspensions of spermatozoa were introduced into Millipore tubes (20×2 or 1 mm, 0.45 µm pore size), using a Hamilton syringe fitted with a 2″ section of plastic catheter. These were closed with fine glass stoppers or by tying fine silicone bungs in at each end. One tube was then inserted into each uterine horn of an anaesthetized doe by making an incision into the vagina and pushing the tube through the cervix. The doe was then injected with 75 IU of HCG, killed 12 h later and the tubes removed. Up to 4 does were used for each replicate. The spermatozoa suspension was recovered from the millipore tube with a Hamilton syringe and the tube rinsed with diluent. The suspension was then centrifuged at 1000 *g* for 5 min, the supernatant withdrawn and the spermatozoa resuspended in a convenient volume, usually 4 to 6 ml.

Oxygen uptake was measured in a Yellow Springs Model 53 biological oxygen monitor which employs a Clarke type electrode. The final volume in the sample chamber was always adjusted to 3 ml with diluent. Crude decapacitation factor was prepared from rabbit or bull seminal plasma[6,12]. A haemocytometer was used for spermatozoa counts after diluting the suspension with formal saline.

After log transformation the data were subjected to standard analyses of variance with isolation of sums of squares attributable to differences between treatments and differences between ejaculates. The treatment-ejaculate interaction has been used as the error term and the significance of the difference from the control assessed by a *t*-test using the interaction mean square from the analyses of variance as the standard error and the degrees of freedom associated with it.

Results and discussion. Spermatozoa suspensions recovered from the uterus of rabbits in sealed Millipore tubes were free from leucocytes and only rarely contamiated with some erythrocytes. Although the results were variable, there was, usually an increase in the oxygen consumption of spermatozoa recovered from the uterus (Table, experiment 1). However, addition of the decapacitation factor far from decreasing the oxygen uptake, considerably increased it to a level significantly greater than the control ($p < 0.01$) (Table, experiment 1). This seems to be a specific effect on spermatozoa recovered from the uterus and we have found no stimulation of the oxygen uptake of fresh rabbit spermatozoa (Table, experiment 2).

From this experiment it would appear unlikely the mode of action of the decapacitation factor is to bring about some non-specific stabilization of the spermatozoa. Bovine serum albumin, which is known to protect rabbit spermatozoa to some extent from the adverse effect of high dilution[13], also failed to increase the respiration of fresh rabbit spermatozoa (Table, experiment 3). Decapacitation factor itself did not produce any appreciable oxygen uptake when 5 mg was incubated in 3 ml of Ringer solution over 1 h.

Spermatozoa sealed into Millipore tubes apparently become capacitated in the uterus[14]. Whether stimulation of their respiratory activity is directly associated with capacitation, however, remains an open question. If the metabolic changes were a necessary prerequisite for capacitation, the injection of progesterone into the doe might be expected to prevent them, since this treatment has been shown to inhibit capacitation of spermatozoa in the rabbit uterus[15]. The evidence on this point is, however, conflicting; thus MURDOCH and WHITE[9] found that progesterone, if anything, enhanced rather than reduced the effect of the uterus on metabolism of spermatozoa whereas BRACKETT[16] reports no stimulation of metabolism when spermatozoa were placed in the uterus of pseudopregnant does.

Again if the metabolic changes were closely linked with capacitation the presence of seminal plasma in the uterus might also be expected to retard them due to the decapacitation factor. However, essentially similar responses were obtained with washed and unwashed spermatozoa suspensions in the uterus[9] while in the present studies added decapacitation factor further stimulated the oxygen uptake of rabbit spermatozoa after they had been recovered from the uterus.

The mode of action of decapacitation factor in stimulating the metabolism of uterine spermatozoa remains to be investigated. Decapacitation factor is not autoxizeable and the effect would seem to be a specific one dependent on the incubation of spermatozoa in the uterus, since the response was not obtained with fresh rabbit spermatozoa. Perhaps a period in the uterus is necessary to render the spermatozoa more permeable, so that the decapacitation factor can gain entry into the cell. It is also possible that the decapacitation factor may have a stabilizing action on spermatozoa recovered from the uterus although such a stabilizing action on respiration could not be demonstrated with either decapacitation factor or bovine serum albumin when freshly ejaculated spermatozoa were used.

Résumé. L'addition d'un facteur de décapacitation préparé à partir du plasma séminal de lapin et de taureau a généralement produit une augmentation supplémentaire de l'absorption d'oxygène, quand il a été ajouté à la suspension de spermatozoïdes enlevée de l'utérus. Le facteur de décapacitation n'a exercé aucun effet stimulant similaire sur les spermatozoïdes de lapin avant l'incubation dans l'utérus et n'a montré lui-même aucune absorption d'oxygène appréciable.

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