Separation of Bull Spermatozoa carrying X- and Y-Chromosomes by Counter-Streaming Centrifugation

Experiments[1] in which a small fraction (1/20–1/15) of the most easily sedimenting spermatozoa, separated by counter-streaming centrifugation, were used for inseminations gave the unexpected result that the 11 calves obtained from 23 first inseminations were all males. Recent experiments on a larger scale with the same technique have now been performed on semen from 10 different bulls. Whereas in the earlier experiments 1,100 r.p.m. was used, the separation in seven of the present experiments was performed at the same velocity and in eight at 1,000 r.p.m.

The total number of first inseminations with separated spermatozoa was 142, resulting in 63 calves (fertility 44·4 per cent). Compared with the fertility (60·5 per cent) of the corresponding control group (104 pregnancies from 172 first inseminations) this figure is rather low ($\chi^2 = 8{\cdot}26$, $P < 0{\cdot}01$). Of the 63 calves, 36 were females and 27 males (sex ratio 42·9). The sex ratio of this total group does not deviate significantly from the normal one for cattle (106 : 100)[2]. However, a review of the different experiments reveals a considerable variation in fertility, a high proportion of females being apparently associated with high fertility. Therefore the experiments were pooled in two groups, one with fertilities equal to or more than 50 per cent, and the other with fertilities less than 50 per cent. The fertilities for these two groups, 63·8 and 31·0 per cent, respectively, differ significantly ($\chi^2 = 14{\cdot}99$, $P < 0{\cdot}001$).

Of the calves belonging to the first group 28 were females and 9 males (sex ratio 24·3). The corresponding figures of the second group are 8 and 18 (sex ratio 69·2). Also the sex ratios of these two groups differ significantly ($\chi^2 = 12{\cdot}57$, $P < 0{\cdot}001$). This means that the spermatozoa of certain samples are damaged by the counter-streaming centrifugation, and that thereby the female-determining spermatozoa are more sensitive than the male-determining ones. Most probably the factor causing this damage is mechanical stress. This idea is supported by the fact that two groups of experiments which have been pooled according to the r.p.m. used differ significantly as to fertility (pooled samples separated at 1,000 r.p.m. : 53·6 per cent ; pooled samples separated at 1,100 r.p.m. : 35·6 per cent ; $\chi^2 = 4{\cdot}66$, $P = 0{\cdot}05$), the high velocity having decreased the fertility more than the low one.

In the light of the present results, the male predominance in the earlier experiments is more easily understood ; the spermatozoa were submitted to 1,100 r.p.m. in these experiments.

The group of pooled experiments, having a fertility equal to or higher than 50 per cent, shows a sex ratio which deviates significantly from the normal one of cattle ($\chi^2 = 9{\cdot}31$, $P < 0{\cdot}01$). It thus seems possible to select viable-determining spermatozoa by running the counter-streaming centrifuge at a moderate speed.

In another series of experiments the spermatozoa most difficult to sediment were isolated at 1,100–1,200 r.p.m., and used for inseminations. Here also the female-determining spermatozoa have been eliminated more frequently than the male-determining ones in connexion with decreased fertility. No accumulation of male-determining spermatozoa could be detected in this fraction.

A more detailed account of this work will be published in *Acta Agriculturae Scandinavica*.

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Structural Differentiation in the Nucleoid of Mature Vaccinia Virus

It has been known for some time that mature virus particles of the pox group contain a brick-shaped central body, or nucleoid, surrounded by an outer layer of protein[1]. The fact that in pepsin-treated particles this nucleoid cannot be hydrolysed by deoxyribonuclease alone, but only by deoxyribonuclease followed by a further pepsin digestion, has been taken as an indication that the nucleoid itself contains protein as well as nucleic acid[2]. However, when mature vaccinia virus particles are examined with the electron microscope in thin sections after either osmium or ethanol–acetic acid fixation, the nucleoid presents a more or less homogeneous appearance[3].

In order to investigate further the nucleoidal structure of this type of virus, samples from pellets of purified vaccinia virus particles were dehydrated and embedded in methacrylate for thin sectioning, after fixation with buffered osmium-sucrose[4,5] at *p*H 7·6 or potassium permanganate[6] at *p*H 7·6, for about 1 hr.

Examination of the osmium-fixed particles served to give a point of departure for the work and confirmed the observations of Peters[3] on the general structure of the mature virus and the homogeneity of its nucleoid.

On the other hand, permanganate-fixed particles, although showing an overall internal arrangement similar to the foregoing, exhibited in addition two new features of interest. First, the outer zone of the virus, lying between the outer double-limiting membrane and the double membrane surrounding the nucleoid, appeared both much narrower and considerably less electron dense than in material fixed with osmium (Fig. 1) ; it is this zone which is usually considered to consist of the peripheral protein layer of the virus[3]. Secondly, and more important, the brick-shaped nucleoid within its double membrane was found to be strikingly differentiated into an inner and an outer region ; the electron density of the former was great and that of the latter was slight (Fig. 1), being very similar in this respect to the peripheral protein layer of the virus. In view of this similarity, it is possible that the less electron-dense region lying within the double membrane surrounding the nucleoid might represent the protein constituent of this part of the virus, the nucleic acid corresponding perhaps to the very electron-dense