tive indicator of low levels of mumps antibodies. Undiluted serum can be studied for the presence of mumps antibodies and there is no problem of nonspecific inhibition. The levels of antibody 1 year after natural mumps are more stable by the PN method than by HAI or CF tests. This assay procedure is valuable in studying sera where low levels of antibody may be present, as after remote natural disease, or vaccination with attenuated mumps strains.

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Size Distribution and Counting of Liver Nuclei by an Electronic Particle Counter* (33453)

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In order to correlate cellular and subcellular changes in a tissue with the physiological state of the animal, it is necessary to relate the contents of the cell to a so-called "fixed tissue constituent." Recently, DNA has been suggested as a cellular constant (1), but in certain tissues such as liver, heart, and brain, polyploidy occurs (2–4). Polyploidy is the result of an increase in nuclear DNA without cell division, which occurs in liver cells as tetraploidy, octoploidy, 16-ploidy and 32-ploidy (5). Because of this nonconstancy of DNA, it would be advantageous to express the data, on a per cell basis, but the homogenization procedures developed so far to disrupt liver tissue do not leave many of the cells intact. The next best choice appears to be an isolation and determination of the number of nuclei which could then be related to the number of liver cells. Such a calculation necessitates the assumption that binucleated liver cells have twice the cytoplasmic mass of mononucleated cells. Recently, Blobel and Potter (6) suggested a procedure which allows the recovery of more than 90% of the liver nuclei, with very little cytoplasmic contamination. By these procedures, we are able to obtain nuclear preparations that can be readily counted and sized in an electronic particle counter.

Materials and Methods. Male rats (Wistar strain) of various ages were housed in a room with controlled light from 6:00 a.m. to 6:00 p.m. and were fed an 18% casein agar-gel diet (7) between 8:00 a.m. and 12:00 noon. All rats were killed between 12:00 noon and 10:00 p.m. and were perfused with ice-cold 0.9% saline until all traces of blood had been removed from the tissues. The livers were removed and quickly immersed in several volumes of ice-cold 0.25 *M* sucrose in

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TABLE I. Comparison of Nuclear Counting by a Particle Counter and by Visual Determination with a Hemacytometer.[a]

Sample no.	Particle counter (nuclei/ml of sample; $\times 10^6$)	Hemacytometer (nuclei/ml of sample; $\times 10^6$)
1	7.0	7.0
2	8.5	7.8
3	9.3	9.7
4	18.9	20.8
5	28.9	28.6
6	22.0	21.6

[a] Correlation coefficient (r) = 0.95, with a probability of less than 0.01.

TKM (0.05 *M* Tris–HCl, pH 7.6 at 25°; 0.065 *M* KCl; and 0.010 *M* $MgCl_2$). All subsequent operations were performed at temperatures near 0°. Livers were blotted, weighed, pressed through a brass sieve (0.040) mesh, and homogenized with two volumes of ice-cold 0.25 *M* sucrose in TKM. The nuclei in a 1.0-ml sample of the resulting homogenate were isolated and purified by the procedure of Blobel and Potter (6). The nuclear pellet was suspended in 2 ml of 0.25 *M* sucrose TKM and a 1.0-ml sample of this solution was analyzed for RNA, DNA, and protein content by a previously described procedure (8). Another aliquot was diluted 1:250 in isoton (a modified Eagle's medium) and counted in a model B Coulter counter with a 100-μ aperture and 0.5-ml manometer (9). For total nuclear counting optimal settings were found to be at an amplification of ½, an aperture current of 2 and a gain of 100, while an amplification of ¼, an aperture current of 2 and a gain of 50 were used for size distribution measurements on the nuclei. Standardized erythrocytes (9) were utilized to calibrate the Coulter counter for the size distribution studies. Other samples of the nuclear suspension were diluted 1:20 with 3% (v/v) acetic acid containing 0.2% methyl green and counted visually in a hemacytometer.

Results and Discussion. The data in Table I illustrate the close correlation between the number of particles counted by the electronic counter and the number of nuclei visually observed with a hemacytometer. The only major difference is that the particle counter requires less than a minute to count the sample while it takes 5–10 to analyze the same sample with a microscope and hemacytometer. When nuelei are stained with methyl green and examined by light microscopy the preparations are relatively free of any nonnuclear particles and there is marked variations in nuclear size. From his histochemical measurements of liver nuclei, Epstein (5) suggested that the increase in nuclear size is correlated with an elevation in the degree of polyploidy. The data shown in Fig. 1 demonstrate that there is a relationship between the amounts of DNA per nucleus and the size distribution of the nuclear particles.

If all of the liver nuclei were diploid, they would contain about 6.2μg of DNA per nucleus (10) but even in the livers from 25-day-old rats the DNA content of the nucleus was above the diploid level. As the animal grew older both the DNA content and nuclear size of the liver increased as a function of age. Similar results have been observed by histochemical examination of liver nuclei from mice of different ages (5).

While this technique is a valuable tool for counting and sizing of liver nuclei, it is not possible to clearly separate out the various degrees of polyploidy according to precise

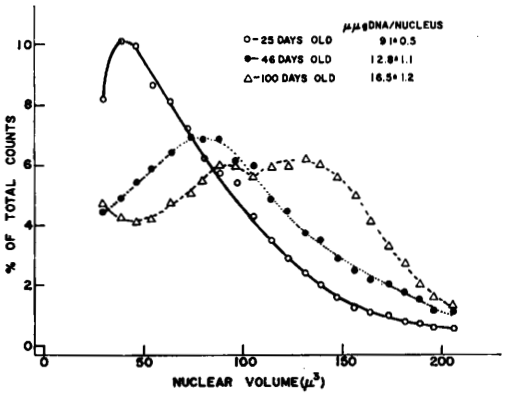

FIG. 1. Size distribution patterns of isolated liver nuclei from rats of various ages: Each point is the mean of six animals; the patterns are correlated with the average DNA content of the total nuclear population; each nuclear DNA determination is the mean ± SEM of six rats.

nuclear volumes. This could be due to: (a) factors which affect the sizing sensitivity of the instrument, or (b) the fact that nuclear size is not completely related to polyploidy, but can be partially changed by other nuclear constituents such as proteins (unpublished data Muramatsu).

Summary. A procedure is described that allows rapid counting and sizing of liver nuclei. A good correlation exists between the degree of liver polyploidy and nuclear size. Both of these parameters increase as a function of age.

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Radioimmunoassay of Serum Levels of Luteinizing Hormone during the Cycle and Early Pregnancy in Ewes* (33454)

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Studies concerning the role of luteinizing hormone (LH) in different reproductive processes in sheep have been limited by the inability to measure accurately this hormone in small aliquots of blood. The recent development of a specific and highly sensitive radioimmunoassay for ovine LH (1) made it possible to determine circulating blood levels of this hormone. This should lead to a better understanding of the endocrinology of the estrous cycle and of early gestation. The present study was conducted (a) to determine the circulating levels of LH during these two periods, and (b) to critically define the time relationships between the onset of estrus and the peak of LH presumably responsible for ovulation.

Materials and Methods. A preliminary experiment was conducted to determine what method was best to obtain maximal yields of serum with a high LH content. Jugular blood was collected from three ovariectomized ewes and 15-ml aliquots were allowed to stand either at room temperature or at 4° for different periods of time. The serum was then obtained by centrifugation, frozen, and stored for subsequent LH assay.

In a second experiment, 10 ewes, which had exhibited at least one normal estrous cycle, were bled daily for 20 days. In addition, 10 ewes which had their estrous cycles synchronized with progestogen impregnated silicone implants (2) were mated at the second estrus following implant removal and were bled once daily for 20 days beginning the day after estrus. Six of these ewes were confirmed to be pregnant by laparotomy and

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