monitored by the method described by Bourke *et al.* (1971). A priming dose ($1.4 \mu\text{Ci/kg}$) and sustaining dose ($1.4 \mu\text{Ci/kg}$) of [$1\text{-}^{14}\text{C}$]glucose was commenced and, after a 40 min equilibration period, urine collections and arterial and renal-venous blood samples were withdrawn continuously for six successive clearance periods by using a modification of the technique of Churchill & Malvin (1970).

Total glucose was measured enzymically by using the hexokinase trap. Radioactive glucose was measured by its enzymic conversion into ribulose 5-phosphate and $^{14}\text{CO}_2$ by the method of Costello *et al.* (1972). Glycolysis was calculated from the arteriovenous [^{14}C]glucose difference expressed in $\mu\text{mol/min}$. Similarly net and total gluconeogenesis were calculated.

Glycolysis was demonstrated under conditions of acidosis and alkalosis. Under alkalotic conditions however, significantly more glycolysis was consistently demonstrated. Although no significant difference in net gluconeogenesis between the two groups was observed, gross gluconeogenesis was significantly greater in the chronically alkalotic dogs.

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Concentration of Oestrogens in Jugular-Venous Plasma of Cows during the Oestrous Cycle

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Samples of jugular-venous plasma were collected daily from lactating dairy cows during three 7-day periods each including oestrus, and subsequently during five complete oestrous cycles.

Concentrations of oestradiol and oestrone were determined in samples by radioimmunoassay after extraction with ether and chromatography on ion-exchange resin and lipophilic dextran gel.

Two antiserum preparations, A and B, had been prepared from immunogens in which oestradiol-17 β was conjugated through the 17 β -oxygen, and another antiserum preparation, C, from an immunogen in which 6-oxo-oestradiol-17 β was conjugated at C-6. Antisera A and B cross-reacted considerably with oestrone and oestradiol-17 α whereas antiserum C had very little cross-reaction with oestradiol-17 α relative to the 17 β isomer.

Concentrations of oestradiol were determined in all samples, about half with antiserum A or B and half with antiserum C. Concentrations of oestrone were determined in samples from three complete cycles by means of antiserum B.

Mean concentrations of oestrone were below 1 pg/ml throughout the cycle except for a possible small rise 1–2 days before oestrus.

Oestradiol concentrations measured with antiserum A, B or C were very similar at comparable times of the cycle, and it is tentatively concluded that it was oestradiol-17 β that was being determined. Pooled oestradiol-17 β determinations show two peaks: (1) a pre-oestrus peak with concentrations above basal values for 4 days and a maximum of 5 pg/ml 1 day before oestrus; (2) a post-ovulatory peak with concentrations above basal values for 3 days and a maximum of 7 pg/ml 6 days after oestrus.

The present results are consistent with those obtained by bioassay by Pope *et al.* (1965) and with those of Echternkamp & Hansel (1971) and Wettemann *et al.* (1972), who used radioimmunoassay and uterine protein binding assay.

Whereas the physiological significance of the pre-oestrus oestradiol-17 β secretion is generally accepted, that of post-ovulatory oestradiol-17 β is relatively unknown. The possibility that it has a luteotrophic action will be discussed.

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Isolation of a Fraction from Rat Cerebellum Enriched in Nerve Terminals of One Single Type (Mossy-Fibre Terminals)

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Isolation of synaptosomes has advanced the understanding of the processes of neurotransmission (Whittaker, 1965; De Robertis, 1967). Preparation of synaptosomes from tissue homogenates has the limitation of heterogeneity of terminal types often contaminated by other structures. An improved

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technique has now been developed for the separation of relatively pure specific nerve terminals. Cerebellar mossy-fibre terminals are among the largest nerve terminals in the central nervous system. This anatomical feature was utilized in earlier attempts for their separation (Israel & Whittaker, 1965). Hajós *et al.* (1971) have since reported the isolation, in a relatively well-preserved state, of large glomerular structures from cerebellum consisting of central 'rosettes' of mossy terminals surrounded by synaptic digits of granule-cell dendrites. Contamination by structures of probable molecular-layer origin was still in evidence. The further purification of the 'mossy-terminal fraction' is now described.

Development of the fractionation procedure was guided by electron-microscopic examinations. In addition, the following enzyme activities were determined as markers of subcellular constituents: cytoplasm, lactate dehydrogenase; mitochondria, glutamate dehydrogenase and succinate dehydrogenase; nerve terminals, glutamate decarboxylase and choline acetyltransferase. Protein and DNA were also determined.

Crude 'mossy-terminal fraction' was obtained as described by Hajós *et al.* (1971). All sucrose solutions contained 1 mM-MgSO₄. The cerebellum was dispersed in 0.3 M-sucrose manually with a modified Dounce homogenizer, and the homogenate was centrifuged at 900g for 10 min after filtration through four consecutive nylon sieves (1000, 120, 75 and 50 µm). The resuspended pellet was resolved on a discontinuous sucrose density gradient by centrifugation in a Spinco SW25 rotor at 53000g for 2 h. Mossy terminals were obtained at the 1.2 M/1.4 M-sucrose interphase. This fraction was further purified by centrifugation at 53000g for 2 h on a continuous sucrose density gradient (1.2–1.35 M) layered on top of 2 M-sucrose. Relatively well-preserved mossy-terminal structures were concentrated in a narrow band above the 1.35 M/2 M-sucrose interphase (*M* fraction) and were clearly separated from a particulate layer in the upper half of the gradient. This contained mainly damaged mossy terminals in the lower region and large tissue fragments in the upper regions, which are the contaminants of the initial crude 'mossy-terminal fraction'. Significantly, in the *M* fraction only 1 % of the total protein was recovered, in contrast with about 30 % in the crude 'mossy-terminal preparation'. In five experiments the relative specific activities of the *M* fraction were 2.6 ± 0.35 for glutamate decarboxylase, 1.09 ± 0.135 for choline acetyltransferase, 2.1 ± 0.26 for succinate dehydrogenase, 1.2 ± 0.10 for glutamate dehydrogenase and 0.86 ± 0.17 for lactate dehydrogenase. Electron-microscopic examination showed that mossy-terminal-containing structures constituted the major part of the *M* fraction; very occasionally an endothelial-cell nucleus or molecular-layer fragment was seen.

Preparation time of the *M* fraction can be shortened by separating this fraction directly from the resuspended initial low-speed sediment on a linear sucrose density gradient described above. Both electron-microscopic and biochemical studies suggested that the *M* fraction so obtained is less pure than that prepared by the longer procedure described, but the morphological preservation of the mossy-terminal fragments is better.

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Use of Dansyl Chloride and Micro-Chromatography to Detect and Study the Metabolism of Suspected Transmitter Substances and Amino Acids in Single Neurons

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A micro biochemical method (Neuhoff, 1971; Osborne *et al.*, 1971) was used to study the amine and amino acid contents in two specific types of giant cells in the snail *Helix pomatia*. One type of cell (GSC) is known to contain 5-hydroxytryptamine (serotonin) (Cottrell & Osborne, 1970), whereas the other type of neuron lacks this amine (see Briel *et al.*, 1971). Cell extracts were treated with dansyl chloride, and the dansylated substances were separated on 3 cm × 3 cm polyamide layers. The patterns of amino acids in both were similar. However, the buccal neurons contained significantly more methionine and glycine but less ornithine than the GSC neurons, where the presence of 5-hydroxytryptamine was confirmed and the two additional substances 5-hydroxytryptophan and 5-hydroxyindole were detected. Analysis of the 5-hydroxytryptamine-containing Retzius cells (Rude *et al.*, 1969) of the leech *Hirudo medicinalis* showed that these cells, like the GSC neurons, contained large amounts of glycine and alanine, although they differ in having higher amounts of 5-hydroxytryptamine, tryptophan, 5-hydroxytryptophan and glutamate.

When 5-hydroxy[¹⁴C]tryptophan was perfused through the snail's central ganglia only the GSC