A Method for Demonstrating Acetylcholinesterase and Choline Acetylase in Foetal Brain

The histochemical demonstration of acetylcholinesterase (AChE) in nervous tissue can provide a rough guide to the distribution of neurons which are possibly involved in cholinergic mechanisms. The presence in the tissue of choline acetylase (ChAc) is of much greater significance in this context but unfortunately it cannot, as yet, be detected histochemically. LEWIS et al.[1,2] have, however, described a method in which AChE activity, demonstrated histochemically in a particular area of a tissue section, can be compared with the choline acetylase activity measured biochemically in the corresponding area of an adjacent section. In brief, alternately thin and thick sections are cut from fresh frozen tissue. The thicker sections, still frozen, are stored while the thin sections are stained for AChE. With these latter as a guide, it is subsequently possible to dissect the thick sections into anatomically discrete areas which are then analysed for ChAc. The present report describes modifications which have made the method suitable for analysis of foetal brain tissue.

Method. Foetuses were delivered by Caesarian operation from ewes under methohexitone sodium (Brietal Sodium, Elanco Products Ltd.) and Fluothane (Imperial Chemical Industries) anaesthesia. The cerebellum was dissected out and if the specimens were large they were subdivided with a triple Gillette razor blade. Each sample, orientated in the required plane on a cryostat chuck was frozen solid with carbon dioxide. The chucks, enclosed in small polythene bags, were stored at $-20\,^{\circ}C$ in a Slee-Pearse cryostat until the tissue was sectioned not more than 2 h later, at a temperature of between -15 and $-12\,^{\circ}C$.

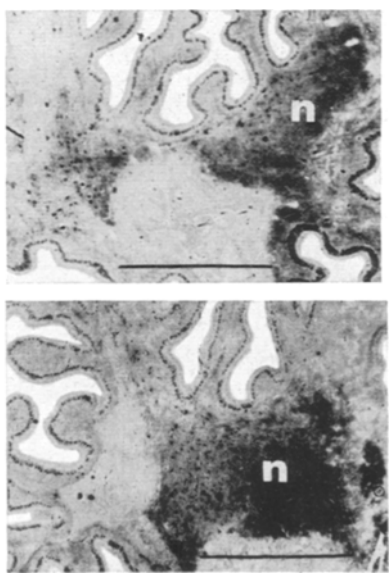
Serial sections were cut in consecutive batches of 5. The first section in each batch was cut 20–40 μ thick and reserved for histochemistry; the next 4 sections, to be analysed for ChAc, were cut 60 μ thick. Sections for histochemistry were lifted off the knife with clean coverslips and were exposed, when dry, to formaldehyde vapour for 2 min. They were pretreated for 30 min in 0.2 M-sodium acetate: acetic acid buffer (pH 5.5), which contained 10^{-4} M-ethopropazine HCl (May and Baker) to inhibit pseudocholinesterases. Sections were stained for AChE activity by LEWIS's modification[3] of KOELLE's technique[4]. The incubation period was either 2 or 4 h; for other details see BISCOE and SILVER[5].

Each section for ChAc analysis was transferred from the knife onto a small square of cooled dialysis tubing. These squares, free from seams and creases, were cut from H.M.I. Visking and included both walls of the tubing. The front wall, on which the section was placed, was termed 'carrier' Visking and the back wall, 'backing' Visking. As soon as a section had been arranged on it, the Visking, with the section upwards, was put onto a perforated plastic grid in a small polystyrene container with a well-fitting lid. A small swab of cotton wool moistened with glass-distilled water was put under the grid to prevent freeze-drying of the section during subsequent storage at $-20\,^{\circ}C$. When it was necessary to store sections over night, the plastic containers were enclosed in air-tight tin boxes.

The sections were analysed for ChAc as soon as possible after the histochemical results were known; this was usually between 6 and 24 h after they were cut. They were removed from the deep freeze and allowed to dry at room temperature. Each Visking square was attached with cellulose tape to a microscope slide and the section examined under a dissecting microscope. Discrete areas corresponding to those shown by the histochemically treated sections to contain high or low AChE activity were cut out of the section with a fine knife made from a razor blade. The cut passed through the section and through the carrier and backing Visking. When the knife was kept at right-angles to the surface of the section the area cut from the backing Visking was the same as that cut from the carrier; preliminary tests without tissue showed that the weight of the carrier and backing Visking cut in this way differed by less than 1%. The backing was peeled off the carrier and the 2 pieces of Visking were put into separate Petri dishes. In areas of high ChAc activity a sample cut from a single section could be analysed but, in general, it was necessary to pool corresponding samples from 2 or 4 sections; when samples were pooled the backing Visking was also pooled.

A Cahn Gram Electrobalance was used for weighing. The backing Visking was put on the pan while the zero point was adjusted and the balance calibrated. The carrier Visking was then substituted for the backing Visking and the weight read. The reading corresponded to the weight of the carrier Visking plus tissue, minus the weight of the backing Visking and was taken, in subsequent calculations, to be the weight of the tissue.

The ChAc activity in the samples was measured by the micromethod of BULL et al.[6]. The incubation medium was that used by HEBB et al.[7] and the acetylcholine



Figs. 1 and 2. Acetylcholinesterase activity in sections of cerebellar nuclei (n) from a lamb of 88 days gestational age. Scale: 2 mm.

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(ACh) produced during incubation was assayed, with appropriate controls, on the rectus abdominis muscle of the frog.

Figure 1 shows AChE activity in the nuclei in the medial vermis of a lamb of 88 days gestation. ChAc activity in the corresponding area was 870 μg ACh/h per g. Figure 2 shows the nuclei further laterally; in this region the ChAc activity was 1600 μg ACh/h per g. Similar experiments on the folia showed that there, the ChAc was probably related to AChE-containing fibres in the white matter rather than to the AChE-containing Purkinje cells which are present in developing cerebellum[8,9].

Résumé. Une méthode est décrite qui permet la mesure de l'activité de la choline acétylase dans des régions localisées de sections en congélation de cerveau embryonnaire, activité qui est comparée à celle de l'acétylcholinesterase dans la même région sur des coupes adjacentes. Des résultats obtenus à l'aide de cette méthode sur le cervelet embryonnaire de mouton sont présentés.

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'Spontaneous' Occurrence of Yaba Tumor in a Monkey Colony

During the last 6 years several hundred rhesus (*Macaca mulatta*), cynomologous (*M. irus*) and stumptail monkeys (*M. speciosa*) were inoculated with Yaba virus in our colony[1,2]. With the exception of 2 instances where animals without record of inoculation developed typical tumors, no signs of contact infection occurred, even when infected and control animals were housed deliberately in the same cage. About 2 years ago, our monkey colony was moved from Buffalo, N.Y. to our Springville campus about 30 miles to the south. Initially, schedules of cleaning and sterilizing cages and rooms could not be as rigorously observed as in the old colony. Occasionally flies and mosquitoes were sighted in the rooms housing monkeys. During this period of time 11 animals without inoculation history developed tumors histologically identical to those produced by Yaba virus. Subcutaneous inoculation of cell-free filtrates from these tumors into normal monkeys produced typical tumors.

Most of the 'spontaneous' tumors occurred on hairless areas of the face, palms, and interdigital areas. Figure 1 shows a monkey with 5 tumors of the face, 1 growing into the orbit. Figure 2 shows an animal with tumor masses growing in the nostril and orbit. Autopsy revealed massive tumor invasion of the paranasal sinuses. Figure 3 demonstrates a small tumor on the mucosal surface of the lip. Figure 4 depicts a large tumor mass occupying the palate and dislocating teeth. The gross anatomy of some of these tumors resembles those published by BURKITT and WRIGHT[3] on BURKITT's lymphoma in African children. Yaba tumors differ, however, in histologic structure, by the electron microscopic finding of oval or brick-shaped particles, often in paranuclear position, resembling pox viruses and by a more benign course, frequently resulting in regression although at times followed by recurrence[1].

The suspicion arose that virus transmission might have occurred with the aid of insect vectors. The colony was thoroughly sprayed with insecticides and strict daily cleaning and weekly autoclaving of cages introduced. The rooms were disinfected weekly with antiseptics and live steam. Strict isolation of the colony was enforced. During the last year no 'spontaneous' tumors have occurred among our monkeys. The original description of Yaba tumors by BEARCROFT and JAMIESON[4] followed an epidemic in a colony of rhesus monkeys housed in open pens in West Africa. If insect vectors are involved in spreading this virus under natural conditions, they seem to be present in Africa as well as in the United States. Tumors found in the oral cavity, paranasal sinuses and orbit may argue against insect vectors. However, as seen on Figure 1, tumor masses may grow from the forehead into the orbit and, as seen on Figures 2 and 3, may originate from the nostrils and lips and grow into the sinuses or produce extensive involvement of the oral cavity. Further studies on the nature of transmission seem to be indicated[5].

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Fig. 1. Five tumors which appeared spontaneously on the face of a monkey. Note a large tumor growing into the orbit.