4. CCl_4 is known to reduce the activity of drug metabolizing enzymes as soon as 60 min after intragastric application[7]. Hence we measured the oxidation of hexobarbital at various intervals after introduction of the haloalkane. We did not find, however, any decrease in hexobarbital metabolism when compared with control experiments from 0–150 min after CCl_4 treatment. Perhaps this lack of drug enzyme damage is due to the short time of CCl_4 influence during liver perfusion. Therefore the method of introducing gaseous CCl_4 into a liver perfusion system can be regarded as a 'pulse' treatment, allowing the detection of early and reversible functional changes.

Zusammenfassung. Durch kurzzeitige Applikation von gasförmigem Tetrachlorkohlenstoff während Leberperfusion tritt eine rasche und reversibel verlaufende Gallesekretionshemmung ein. Diese Hemmung könnte auf Veränderungen von Transport- oder Permeabilitätseigenschaften der Leberzellmembran durch CCl_4 zurückgeführt werden.

R. KASCHNITZ and K. MITTERMAYER

Institut für Allgemeine und Experimentelle Pathologie der Universität Wien, A-1090 Wien (Austria), 15 December 1969.

An Histochemical Study of the Nerve Supply to the Developing Alimentary Tract

Histochemical techniques for the demonstration of substances involved in nerve transmission processes have been used by several workers in the study of alimentary canal nerve structures in adult mammals and birds. Fibres and nerve cells rich in acetylcholinesterase[1–6] and adrenergic fibres[7–9] have been demonstrated in Auerbach's and Meissner's plexuses and in the muscle layers of the intestinal wall. Very little work, however, has been done in this field as far as embryonic and postnatal development is concerned[10–12].

The aim of the present study was to investigate the differentiation of intrinsic nerve cells as well as the ingrowth of extrinsic nerve fibres, in the developing alimentary tract of the rat, rabbit and chicken using KOELLE's[1] method for the demonstration of acetylcholinesterase activity. Histochemical findings were compared with morphological differences in silver-stained embryos from the early stages of development to birth.

Rat and rabbit embryos from the 11th day[13] and chick embryos from stage 23 H.H.[14] were treated with the method of Koelle-Friedenwald as modified by GEREBTZOFF[15]. The reaction was carried out on in toto dissected alimentary tract and on 20–40 μ thick sections; the samples were pre-incubated with $1 \times 10^{-6} M$ Mipafox to inhibit non-specific cholinesterases. Silver impregnation of embryos at the same stages of development was carried out by means of Cajal-De Castro, Bielschowsky-Gros and Bielschowsky-Boeke techniques.

Nerve fibres from the vagus nerve are found in the oesophagus and stomach wall, from the 13th day on, in rat and rabbit embryos; in the chick, they appear at stage 24–25. In the stomach these fibres are gathered into 2 flattened bundles on the anterior and posterior face (of both proventriculus and gizzard in the chick), but are absent from the greater and lesser curvatures. The fibres show a clear acetylcholinesterase-positive reaction, as do the rare unipolar neuroblasts observed in these organs (Figure 1).

From the 14th day on in the rat, and from the 16th day on in the rabbit, the vagal fibres spread randomly over all the stomach and beyond the pylorus into the duodenal loop. A negative reaction is observed in the remaining parts of the anterior and posterior intestine. Furthermore, Koelle-positive nerve fibres proceed from the coeliac

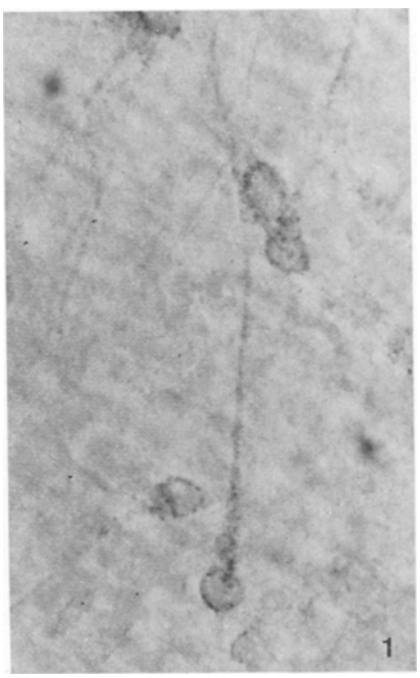

Fig. 1. Monopolar neuroblasts in the wall of the proventriculus show a clear acetylcholinesterasic activity in a small perinuclear area as well as in their processes. Chick embryo at stage 25 H.H. Koelle method. × 600.

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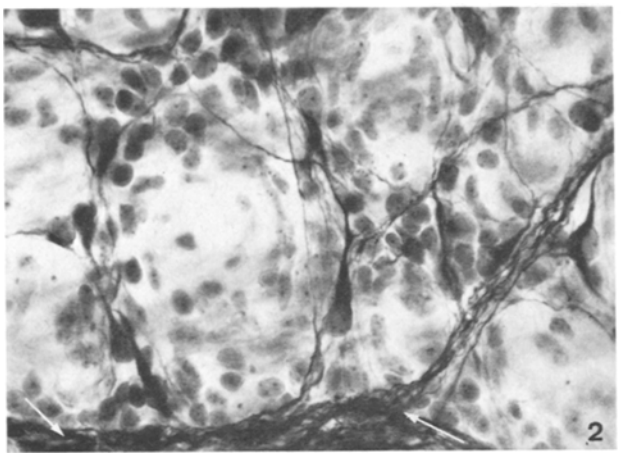

Fig. 2. Vagal nerve fibres (white arrows) and processes of intrinsic neuroblasts are intermingled in a primitive network. A few bipolar neuroblasts are distributed along thicker bundles of nerve fibres. Auerbach's plexus in the stomach of an 18-day rabbit embryo. Bielschowsky-Gros method. × 400.

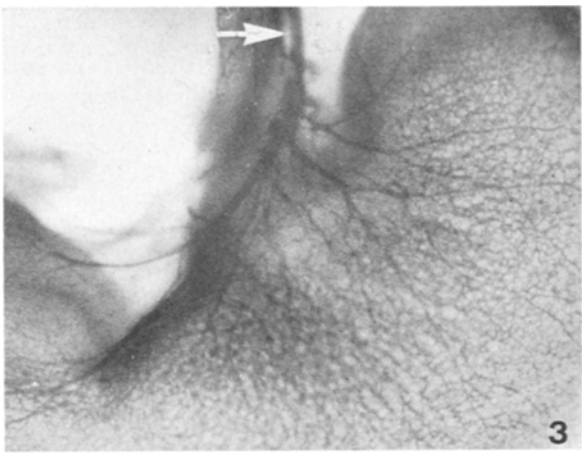

Fig. 3. The branching of the vagal nerve bundles and the meshes of Auerbach's plexus are evidenced by the Koelle reaction on an in toto preparation from a 19-day-old rabbit embryo. White arrow points to the left vagal trunk. × 60.

ganglion along the superior mesenteric artery and reach the duodenal wall while positive fibres from the pelvic nerves reach the wall of the terminal intestine; along the latter fibres silver stainable neuroblasts show also acetylcholinesterase reaction.

From the 15th day (rat) and 17–18th day (rabbit), Koelle-positive fibres and neuroblasts spread from the duodenum and the terminal intestine into the segments in between. The early plexus myentericus is thus outlined below the splanchnopleura along the whole of the alimentary tract.

In the chick embryo, Koelle-positivity is observed in the nervous structures of the anterior intestinal wall down to the origin of the yolk stalk from the stages 29–30. Only from stage 31 on do a few fibres from the coeliac ganglion reach the Remak's ganglion and the wall of the duodenal loop running within the mesentery; Koelle-positive fibres and cells within the wall extend down to the level of the caeca, in part via connecting branches of Remak's ganglion. Koelle-positive fibres and cells do not appear in the terminal intestine until stage 34.

The enzymatic activity of the intramural neuroblasts undergoes variations in intensity in different tracts since its onset. A few strongly positive cells are observed initially, but these gradually increase in number as development proceeds. At birth, the intramural ganglia consist of a proportion of strongly positive cells alongside a few cells with a weak or virtually absent reaction. Such differences in staining intensity may represent expressions of the varying degrees of nerve cell differentiation, which are reflected also by different development of the neurofibrillar network within neuroblasts. In the prenatal period, the density of Koelle-positive fibres and cells is higher in those segments, where they first appeared (oesophagus, stomach and terminal intestine in the case of mammals); in the same segments, the intramural organization of ganglionic nodes and meshes is more complex and more clearly defined (Figures 2 and 3).

Positive fibres and cells belonging to the plexus of Meissner can be demonstrated in the submucosa only around the 4th–5th day after birth in the rat and in the rabbit; in the chick embryo, nerve fibres cross the muscle layer and reach the ventricular submucosa at stage 34 and acetylcholinesterase-positive neuroblasts are distributed along the length of the alimentary canal submucosa at stage 36.

Present observations clearly indicate that the intramural neurons display histochemically detectable acetylcholinesterase activity, since their earliest differentiation stages. The intensity of the Koelle reaction increases as a function of development, though its extent is not the same in all the nerve cells of each ganglion. The distribution of Koelle-positive neuroblasts may be correlated to the appearance of extrinsic fibres showing similar histochemical features. The ingrowth of Koelle-positive extrinsic nerve fibres and the differentiation of acetylcholinesterase containing neurons in Auerbach's plexus follows a different pattern in mammals as compared to the chick. In the rat and rabbit, the first appearance of nervous structures in the stomach and terminal intestine is followed by a progressive merging of the upper and lower nervous populations, whereas in the chick a caudald expansion from the stomach to the terminal intestine takes place. The organization of the plexiform network follows the same spatial and temporal pattern[16].

Riassunto. I neuroblasti dei plessi intramurali del canale alimentare mostrano attività acetilcolinesterasica istochimicamente dimostrabile sin dalle prime fasi di differenziazione. Essi sono riconoscibili nei differenti tratti del canale secondo una successione cronologica sovrapponibile a quella di comparsa di fibre estriseche con identici caratteri istochimici. Le tappe di penetrazione di queste fibre sono differenti nel pollo, a confronto con quanto si osserva nel ratto e nel coniglio.

D. Cantino

Department of Human Anatomy of the University, I-10126 Torino (Italy), 21 December 1969.

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