

liche Häufigkeit in den jeweils untersuchten Gefäßen ergab.

Die cytoplasmatische Struktur der lobopodialen Muskelfortsätze macht deutlich, dass auch diese neuromuskuläre Beziehung von Synapsennatur in der glatten Muskulatur des mesenterialen Lymphgefäßes kein fest verankertes Systemgefüge darstellt, das der neuromuskulären Synapse in der motorischen Endplatte der quergestreiften Muskulatur vergleichbar wäre; zumal durch die ausgeprägte Eigenkontraktilität dieses Gefäßes, mit der erhebliche Lumenschwankungen einhergehen, Unterschiede im Dehnungszustand der verschiedenen Strukturelemente in der Gefäßwand auftreten. Demnach ist anzunehmen, dass die hier dargestellten direkten plasmatischen Verbindungen zwischen Axon und Effektor synaptische Strukturen von transitorischem Charakter sind, die auch in Abhängigkeit vom jeweiligen Erregungszustand der Zellen Veränderungen erfahren.

Eine ausführlichere Darstellung der noch laufenden Untersuchungen zur Frage der peripheren vegetativen Synapse im mesenterialen Lymphgefäß wird folgen.

Summary. Electron microscopic investigations of innervation of the mesenteric lymphatic vessels of *Cavia porcellus* demonstrate that, besides membrane contacts between Axon and smooth muscle cell, a local direct plasmatic connection from the nature of synapses between the nerve and the effector cell have to be considered. From this study it has been concluded that also this neuromuscular junction is a transitoric structure and not a static connection comparable to the synapses in the motor end-plate of the striated muscle.

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A Study on the Ontogeny of Kidney in Chick

The epigenesis of the renal organ in birds starts in the intermediate mesoderm¹ at the early stage of the neural plate formation² and in chick in relation to somites 8 to 15³. The importance of pronephros in the formation of the pronephric duct has been a subject of much controversy (KUME⁴, WADDINGTON⁵, CAMBER⁶), though the dependence of the mesonephros on an inductive stimulation from the pronephric duct has been confirmed by BOYDEN⁷, GRUNWALD⁸ and WADDINGTON⁹. The present experiment deals with the further study of ontogeny of renal organs in chicks with special reference to the tubule-cord relationship. For this, chloramphenicol (manufactured by Parke Davis, India, Ltd., sample number 379), a well-known inhibitor of protein synthesis (GALE and FOLKES¹⁰, FLICKINGER¹¹, WEISBERGER et al.¹²) was injected in two dosages into the albumen of fertilized White Leghorn eggs at various stages of incubation so that every treated egg received either 2.5 mg or 5.0 mg of chloramphenicol. Together with this, some control embryos were also examined. Total number of embryos studied was 20 in each type of experiment.

Observations. When chloramphenicol is injected into non-incubated eggs, the nephric duct does not develop in 90% of the cases examined after 24 and 48 h of incubation (Figure 1), while in others a nephric cord develops with no lumen. Pronephric tubules do not develop even when there is a solid nephric cord. The cells in the intermediate mesoderm are small in number and cytolysed in nature. In the experimented embryos incubated for 72 h, a duct connected with the coelome develops but no tubule is found associated with it. When chloramphenicol is administered to 12 h embryos subsequently incubated up to 48 and 72 h, there is no development of the tubules in the majority of cases, though there is a development of the nephric duct in two cases. When the experimented embryos are incubated up to 72 h, the position does not improve; on the other hand the cells become much cytolysed and disorganized. In the third series of experiments, when chloramphenicol acts on 24 h embryos subsequently incubated up to 48 and 72 h, either a solid nephric duct develops or a duct with a narrow lumen at the anterior end

(Figure 2) but which is solid at the posterior end; but in most of them the nephric tubules do not develop. In two or three cases some pronephric tubules with a limited number of cells are formed. In all the 72 h embryos, the cells become cytolysed, particularly in the mesodermal zone, and the nephric duct and tubules are not visible, though the development of the neural tube and that of the notochord are normal.

Discussion. In the case of birds, the pronephros develop after 24 h of incubation though the cells are determined at a still earlier stage¹. In the experiment, chloramphenicol acts on the cells and also on the nephrotomal mass and thus there is no differentiation of the nephric tubules or duct. Embryonic induction, according to FLICKINGER^{11,12} is a stimulus that promotes protein synthesis in the reacting tissue which may have been impaired by chloramphenicol, and thus there is a failure in the formation of the nephric tubules and the duct. In this connection, it may be taken into consideration that chloramphenicol does not impair primary induction¹³ but, as evidenced from the experiment, it inhibits selectively the synthesis of specific protein for kidney development. In those cases where the pronephric duct develops, it is solid in nature or with a small lumen at the anterior end. This duct cannot carry the inducing substance posteriorwards⁴ and there is no induction of mesonephros. The importance of this inducing substance for morphogenesis of the meso-

¹ A. L. ROMANOFF, *The Avian Embryo* (Macmillan Co., New York 1960), p. 784.

² C. H. WADDINGTON, *J. exp. Biol.* 15, 371 (1938).

³ E. T. ABDEL MALEK, *J. Morph.* 86, 599 (1950).

⁴ M. KUME, *Jap. J. Zool.* 9, 487 (1941).

⁵ R. CAMBER, *Bull. Biol.* 82, 214 (1948).

⁶ E. A. BOYDEN, *Proc. Soc. exp. Biol. Med.* 24, 572 (1927).

⁷ P. GRUNWALD, *Arch. Entw. Mech. Org.* 136, 786 (1937).

⁸ E. F. GALE and E. J. P. FOLKES, *Biochem. J.* 53, 493 (1953).

⁹ R. FLICKINGER, *Growth* 23, 251 (1959).

¹⁰ A. S. WEISBERGER, S. ARMENTROUT, and S. WOLFE, *Proc. Nat. Acad. Sci. Wash.* 50, 86 (1963).

¹¹ R. A. FLICKINGER, *Biol. Bull.* 115, 201 (1958).

¹² R. A. FLICKINGER, *Int. Rev. Cytol.* 13, 95 (1962).

¹³ U. B. BLACKWOOD, *J. Embryol. exp. Morph.* 10, 315 (1962).



Fig. 1



Fig. 2

nephros has been confirmed by various authors^{2,6,7}. Besides this, the failure of the ontogeny of mesonephros has another aspect to be considered. Mesonephros can be formed out of the competent mesodermal cells through the inducing process from the neural area and the notochord¹⁴ which become normally developed in almost all the embryos studied in this experiment. Thus the failure of the formation of the mesonephros may be due to two reasons – one the absence of the inducing substance generally conveyed through the pronephric duct, and the other the absence of competence of the intermediate mesoderm, though the cells in this area may get the normal evocation from the chorda and the neural area.

Though, in the majority of the cases, neither the pronephros nor the pronephric duct develop, in some of the experimented embryos a solid duct is formed independently in the absence of tubules. This is in accordance with the findings of KUME⁴ and CAMBER⁵. The pronephric

tubules and the duct develop independently by self-differentiation and the tubules become secondarily attached to the duct.

Zusammenfassung. Chloramphenicol, auf verschiedene Ontogenesestadien des Hühnchens angewendet, hemmt bei den meisten Embryonen die Nierenentwicklung selektiv, während bei einigen ein pronephritischer Gang unabhängig von den Tubuli differenziert wird.

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¹⁴ C. H. WADDINGTON, *Principles of Embryology* (Cambridge University Press, 1956), p. 268.

Physiological Activities of Chondroitin Polysulfate: the Short-Term Effect of Intravenous Injection

Long term administration of synthetic chondroitin polysulfate (ChPS) has reduced serum total lipid and cholesterol and retarded the atherosclerotic process in experimental animals¹. It was postulated that the lipid clearing activity of ChPS was correlated with the sulfur content of this substance¹. Anticoagulant activity of ChPS has also been associated with sulfur content on the basis of infrared spectroscopic evidence².

Physiological changes in the serum components were also demonstrated in hyperlipemic rabbit serum a short time after the intravenous administration of ChPS. The short term effects of ChPS were determined in the following manner.

ChPS was prepared synthetically by sulfation of chondroitin sulfate (ChS) from shark cartilage^{1,2}. Analysis of the ChPS lot used principally in this investigation showed 16.09% sulfur, 2.16% nitrogen and $[\alpha]_D^{20} - 15.0^\circ$ (c 1.0 H₂O). 17 male albino rabbits averaging 2.9 kg were fed 0.1 g cholesterol per kg of body weight daily in a basic

diet. After 6 weeks of cholesterol feeding, an elevation of the serum total cholesterol from approximately 40 mg initially to over 300 mg per 100 ml was attained. At this time single injections of ChPS were administered intravenously into the hyperlipemia-induced rabbits at the rate of 2 to 10 mg per kg of body weight. The blood was drawn at desired intervals to test the serum for turbidity, total and ester cholesterol, and blood coagulation time as reported previously^{1,3}. Horizontal paper electrophoresis was carried out on Whatman no. 1 paper wetted with Veronal buffer, pH 8.6. 25 μ l of serum was applied and a voltage of 350 V (current density 0.4 mA/cm) was used for 5 h. After drying the paper at 90°C, lipoproteins were located by staining the strip with Sudan Black (Merck) in 50% ethanol for 2 1/2 h.

Effects of ChPS on turbidity and total cholesterol in hyperlipemic serum during the short term investigation are shown in the Table. When ChPS was injected intra-

¹ K. MURATA, *Naturwissenschaften* 49, 39 (1962).

² K. MURATA, *Nature*, Lond. 193, 578 (1962).

³ K. MURATA, *J. Gerontol.* 17, 30 (1962).