Sauerstoff, konstante Temperatur) einer 600-stufigen analytischen Gegenstromverteilung im Kreislaufverfahren unterworfen (Figur 5): Sowohl das Levorin A_2 als auch das Candicidin-Präparat enthalten noch Anteile der Nebenkomponenten. Die Hauptspitzen beider Präparate zeigen gleiche, geringe Abweichungen (Verflachungen) in Bezug auf die entsprechenden theoretischen Kurven. Dieselbe Abweichung zeigte auch die Kurve des Mischpräparates. Die Unterschiede der Verteilungskoeffizienten (K = 1,07 für Levorin A_2, K = 1,02 für Candicidin) dürften somit innerhalb der experimentellen Streuung der Versuchsanordnung liegen.

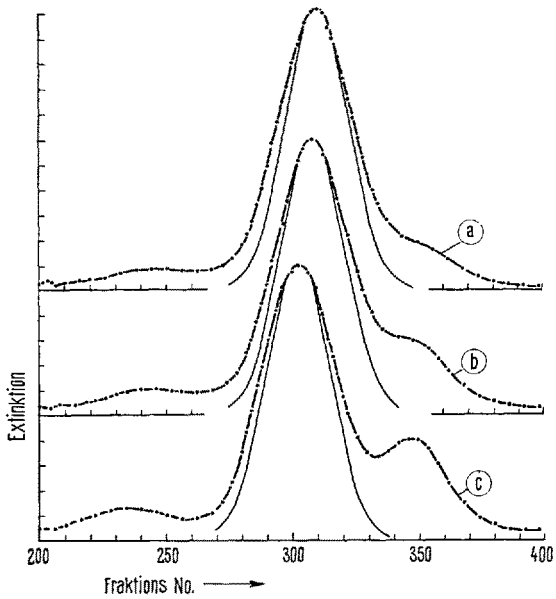

Fig. 5. Craigsche Verteilung über 600 Stufen (25 + 25 ml; Kreislaufverfahren in 200 Element-Apparatur)[8]. -·-·- experimentelle, —— theoretische Kurven. a Levorin A_2 (50 mg); K = 1,07. b Gemisch von Levorin A_2 (25 mg) und Candicidin (stark angereichert) (25 mg); K = 1,05. c Candicidin (stark angereichert); K = 1,02.

Schlussfolgerungen. Die Heptaenantibiotika Levorin A und Candicidin stellen komplexe Gemische sehr ähnlicher Verbindungen dar. Da es sich um labile Präparate biologischen Ursprungs handelt, dürfte ihre qualitative und quantitative Zusammensetzung je nach Herstellungsverfahren und Weiterbehandlung etwas variieren. Die Hauptkomponenten der von uns untersuchten Muster der beiden Antibiotika sind vermutlich identisch, da sie in der verwendeten Testanordnung keine signifikanten Unterschiede zeigen.

Summary. A sample of Russian provenance of the antifungal antibiotic levorin A, containing 30,000 microbiological units of activity/mg, was shown to consist of several components. Three components, designated levorin A_1, A_2 and A_3, were isolated and shown to be distinguishable by their partition coefficients in the system chloroform-methanol-borate buffer pH 8.4 (2:2:1). An enriched sample of the antifungal antibiotic candicidin, prepared from a commercial preparation of American provenance, showed a very similar pattern of distribution as levorin A did in 600 transfers. Levorin A_2, the main component of the levorin A sample, was not distinguishable within the error limits of the applied tests, from the main component of the enriched candicidin sample.

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Zn(II)-Activated Acid Phosphatase in Liver and Metanephros of Developing Chick

Acid phosphatase has been shown to be present in liver, mesonephros, metanephros, and duodenum in developing chick[1–3]. This enzyme from erythrocytes and yeast is activated by magnesium and manganese[4–7], and that from ox kidney by zinc and magnesium[8]. There is, however, no information concerning metallic ion activation of acid phosphatase in tissues of the developing chick. The present communication reports a study on the activation of liver acid phosphatase in the developing chick by Mg(II), Mn(II), and Zn(II). Furthermore, a comparative study has been made on the activation of acid phosphatase by Zn(II) in brain, heart, liver, and metanephros of developing chick to assess its possible relationship with the functional development of each organ.

White Leghorn eggs were used in the present study. The embryos were sacrificed at various developmental stages. The temperature and humidity used for incubation of the eggs, the processes of dissecting the tissues, and the preparation of the tissue homogenates have been previously described[9,10]. Acid phosphatase was assayed in liver, brain, heart, and metanephros homogenates according to the method of LOWRY[11] using *p*-nitrophenyl phosphate as the substrate in 0.05 *M* acetate buffer, pH 5.2, without the addition of magnesium. All samples, blanks, and standards were determined simultaneously in triplicates.

The acid phosphatase activity in the liver and the activation of this enzyme by Mg(II), Mn(II), and by Zn(II) in vitro were determined from the eighth day of incubation to 1 day after hatching (Figure 1). Among the 3 divalent cations studied, the most striking stimulation was found to be by Zn(II), the least by Mg(II), and Mn(II) was intermediate. In order to test the Zn(II) activation of acid phosphatase in tissues of the developing

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Effect of $ZnSO_4$ on acid phosphatase in liver, brain, heart, and metanephros of developing chick

Activation of acid phosphatase activity (%)

$ZnSO_4$ (mM)	Incubation days								Days after hatching							
	8				15				1				8			
	L[a]	B	H	K	L	B	H	K	L	B	H	K	L	B	H	K
0	100	100	100	–	100	100	100	100	100	100	100	100	100	100	100	100
0.001	100	104	107	–	99	117	103	100	102	102	90	102	107	115	94	105
0.01	105	103	109	–	101	118	110	102	107	104	95	101	103	112	94	105
0.1	101	103	107	–	92	110	105	100	96	103	96	98	98	108	92	101
1.0	122	85	86	–	163	94	90	83	172	84	79	95	161	97	89	128
5.0	210	67	75	–	340	81	80	78	324	77	68	121	279	81	68	176

[a] Abbreviations: L, liver; B, brain; H, heart; K, metanephros.

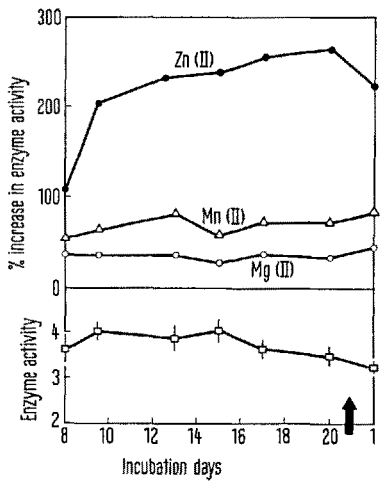

Fig. 1. In vitro effect of 5 mM $ZnSO_4$, $MnCl_2$ and $MgCl_2$ on acid phosphatase activity and its normal development in liver of developing chick. Acid phosphatase activity is expressed as μmoles p-nitrophenol liberated/mg protein/h. Vertical bars represent standard errors of means. Arrow indicates time of hatching. Right side of arrow indicates days after hatching.

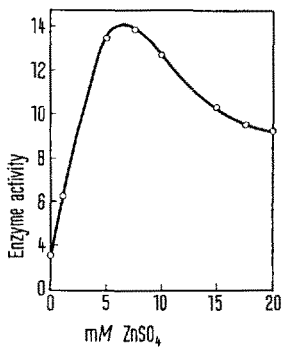

Fig. 2. In vitro effect of various concentrations of $ZnSO_4$ on acid phosphatase activity in liver of day-old chick. Acid phosphatase activity is expressed as μmoles p-nitrophenol liberated/mg protein/h.

chick, various concentrations of Zn(II) were employed in the acid phosphatase assay system. The enzyme activities in liver, brain, heart, and metanephros were determined at 4 developmental stages (Table). A distinctive stimulatory effect of Zn(II) on liver acid phosphatase was observed at the concentration of 1 mM, and an even greater stimulation was obtained at the concentration of 5 mM $ZnSO_4$. A similar result was also observed for the metanephros acid phosphatase 8 days after hatching. Thus, the brain and the heart acid phosphatases were noticeably inhibited at these 2 Zn(II) concentrations. However, $ZnSO_4$ at lower concentrations (0.001–0.01 mM) showed only a slight or no effect on brain, heart, liver, and metanephros acid phosphatase activities. Further increases in Zn(II) concentrations caused diminishing stimulation of the liver acid phosphatase activity, as shown in Figure 2.

It has been shown that the liver of developing chick carries out some of its functions at about the second week of incubation[12,13] and the histological structure or localization of alkaline phosphatase in 17-day liver does not show any difference from that of adult liver[2]. JUNQUEIRA[1] observed no histological difference in 15-day and 17-day metanephros but certain groups of cells are still in differentiation and such process persists up to the twentieth day of incubation. He also found a reduction of acid and alkaline phosphatase activities after the sixteenth day of incubation until hatching and an increase of their activities after hatching. These observations seem to coincide with the present finding in which high activity of Zn(II)-activated acid phosphatase in liver occurred between the seventeenth and twentieth day of incubation and the eighth day after hatching in metanephros. Thus, it appears that the development of Zn(II)-activated acid phosphatase in liver and metanephros follows closely to the functional development of these 2 organs[14].

Résumé. Pendant le développement embryonnaire du poulet, une phosphatase acide activée au Zinc(II) est présente dans le foie et le métanéphros, mais non dans le cerveau et le cœur. L'élévation maximum du taux de cet enzyme se produit dans le foie et dans le métanéphros à des stades différents. Son développement semble être en rapport avec le développement fonctionnel des organes concernés.

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[14] Acknowledgments: This investigation was supported in part by the U.S. Public Health Service Grant No. HD-01856. I thank Miss M. BALZER, Miss K. BERDE and Mrs. M. FOLDVARY for technical assistance.