d'acide homovanillique ne sont pas modifiées. Dans l'hypothalamus, au contraire, seule la concentration d'acide homovanillique est significativement diminuée après 30 min.

Discussion. Au niveau du cortex, les variations des concentrations de dopamine sont parallèles à celles de l'histamine[1]: maximales à la 30e min, elles sont normales à la 2e h. Dans le cortex et le neostriatum, l'augmentation des concentrations de dopamine sans modification de celles de l'acide homovanillique est en faveur d'une augmentation de la synthèse de cette amine. On ne peut cependant pas éliminer d'autres hypothèses, en particulier un apport sanguin de dopamine ou une inhibition de l'excrétion d'acide homovanillique qui seraient provoqués par les importantes modifications de volume sanguin et de perméabilité vasculaire observées aux mêmes temps dans les mêmes tissus[1]. De même dans l'hypothalamus, si les résultats observés sont en faveur d'une inhibition de la dégradation de la dopamine, les variations de concentration observées peuvent être dues aux seuls effets vasculaires de l'histamine.

L'augmentation du taux de dopamine dans le neostriatum n'est pas parallèle au développement des effets comportementaux mais plus précoce et plus courte[2]. Cette discordance semblerait donc indiquer qu'il n'y a pas de relations entre les modifications biochimiques observées et les effets comportementaux décrits. Ces derniers pourraient être liés également aux importantes modifications de la vascularisation striée provoquée par l'histamine[1], les variations de dopamine et d'acide homovanillique observées reflétant alors un phénomène différent.

Summary. A dose of 400 mg/kg of histamine chlorhydrate, injected i.p., induces in the rat an increase of synthesis of dopamine in the neostriatum and in the cortex with an inhibition of its degradation in the hypothalamus. However, the kinetics of these effects are not correlated with behavioral changes.

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Some New Data Concerning Effect of Actinomycin D on Early Chick Embryos

A number of workers have studied the effect of actinomycin D, an inhibitor of DNA-dependent RNA synthesis[1,2], on early chick embryos[3–7]. The previous workers generally agree 1. that actinomycin D strongly inhibits the outgrowth of explanted chick blastoderms, brain development, and somite formation and 2. that the pattern of abnormalities produced by actinomycin D is developmental stage dependent. However, all these studies were carried out using chick embryos at the definitive streak stage (stage 4, Hamburger and Hamilton[8]) or older. In addition, there is controversy about the effect of actinomycin D on heart development in early chick embryos. This study was undertaken to investigate thoroughly the effect of actinomycin D on early chick embryos.

Materials and methods. Fertile white Leghorn eggs were incubated at 37.5 °C for varying periods of time to obtain embryos at stages 3–7[8]. The embryos were cultured in vitro using Spratt's[9] technique. Actinomycin D was obtained from Sigma Chemical Co., St. Louis, Mo. A Ringer-agar plus yolk-albumen extract medium[10] was used to culture all explanted embryos. This will be referred to as the basic medium. After the incubation period, all embryos were fixed in Bouin's fluid and preserved in 70% ethanol. For microscopic studies selected embryos were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin-eosin. The results

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Table I. Effect of actinomycin D (0.01–0.02 μg/ml) on heart development in stages 3–4 chick embryos (Hamburger and Hamilton[8])

Average length of streak (mm)	H and H[8] stage	Group	No. of embryos	No. of survivors	Degree of heart development (% of survivors)		
					Not formed	Retarded (non-pulsatile)	Normal (pulsatile)
0.76	3	Control	28	27	0.0	14.8	85.2
		Treated	66	49	79.6[a]	16.3	4.1[a]
1.32	3+	Control	24	22	0.0	13.6	86.4
		Treated	104	85	48.2[a]	41.2[a]	10.6[a]
1.86	4	Control	22	22	0.0	9.1	90.9
		Treated	78	68	7.4	8.8	83.8

[a] For significance of difference between control and treated groups, $P < 0.01$.

Table II. Morphological analysis of stages 3–7 embryos pretreated with actinomycin D (0.02 μg/ml) for 3–4 h and subcultured on the basic medium without actinomycin D for 19–24 h

Stage (s) at pretreatment	Group	No. of embryos at subculturing	Structures showing abnormalities (% of embyos)				
			Forebrain	Midbrain	Hindbrain	Somite	Heart
3	Control	34	11.8	8.8	5.9	17.7	5.9
	Treated	48	20.8	20.8	18.8	25.0	66.7[a]
3+	Control	28	7.1	7.1	7.1	17.9	7.1
	Treated	52	26.9	15.4	15.4	19.2	30.8
4	Control	32	9.4	9.4	6.3	12.5	3.1
	Treated	46	76.1[a]	45.7[a]	8.7	32.6	8.7
5	Control	28	10.7	10.7	3.6	14.3	0.0
	Treated	41	19.5	78.0[a]	19.5	68.3[a]	12.2
6–7	Control	24	4.2	4.2	4.2	12.5	4.2
	Treated	34	8.8	8.8	5.9	70.6[a]	8.8

[a] For significance of difference between control and treated groups, $P < 0.01$.

were analyzed statistically using the test for the significance in proportions[11].

Results. In the first experimental series, embryos at stages 3–4 were grown on the basic medium with or without actinomycin D (0.01–0.02 μg/ml) for 19–21 h. Overall results showed that actinomycin D exerted marked inhibitory effects on the development of early chick embryos as described by previous workers[3–7]. The magnitude of the action of actinomycin D in heart development varied from one stage to another (Table I). In embryos explanted at stage 3, pulsatile hearts developed in 85.2% of surviving controls, but only in 4.1% of surviving actinomycin D-treated embryos. In the latter, pulsatile hearts were small and did not continue beating as long as those of the controls. Embryos explanted at stage 3+ were more resistant to actinomycin D, but still had a low frequency of normal (pulsatile) heart development (10.6% of surviving embryos). However, quite different results were obtained from embryos explanted at stage 4: of the 68 surviving actinomycin D-treated embryos, 57 (83.8%) showed a pulsatile heart, 6 (8.8%) had a non-pulsatile heart or paired heart primordia in retarded stages of fusion, and 5 (7.4%) were acardiac. These results, when compared to those of the controls, were found to be statistically insignificant (Table I).

In the second experimental series, 3–4 blastoderms of the same age group were placed on the basic medium with or without actinomycin D (0.02 μg/ml), kept at room temperature for 8 h, and incubated for 3–4 h at 37.5 °C. Those embryos, which had advanced to the subsequent stage (e.g., from stage 3 to stage 3+), were removed from the medium, washed several times in warm Ringer's solution, and subcultured on the basic medium without actinomycin D for 19–24 h. Great care was taken during handling to avoid unnecessary mechanical stress in the embryos. The type of abnormalities commonly seen in the actinomycin D-treated embryos were: 1. the brain remained widely open and/or was poorly differentiated; 2. somites were few and rather diffuse; 3. the heart was represented by paired primordia or was small and non-pulsatile. The results are summarized in Table II. It can be seen that the highest frequency of abnormalities in the forebrain, midbrain, somites, and heart occurred when embryos were pretreated with actinomycin D at stage 4, stage 5, stages 6–7, and stage 3, respectively.

Discussion. This study shows that the efficacy of actinomycin D on heart development in early chick embryos is stage dependent: if treatment is begun at stage 3, it may yield about 80% of acardiac embryos; if treatment is delayed until stage 4, it has very little effect on heart development. As shown in Table I, actinomycin D can significantly inhibit heart development, only if it is applied to stage 3+ or younger embryos.

This study also shows that the magnitude of the effect of actinomycin D on the development of various structures correlates with the stage at pretreatment (Table II). Actinomycin D has been found to readily penetrate chick embryonic cells[6] and may selectively inhibit DNA-dependent RNA synthesis[2,3]. If this notion is acceptable, then it would be plausible to draw the following conclusion: the synthesis of tissue specific nulcear RNA takes place at particular time(s) in development of early chick embryos. This synthesis occurs at or about stage 3 for the heart, at stage 4 for the forebrain, at or about stage 5 for the midbrain, and at stage 5 and later stages for somites. The hindbrain appears to be rather stable in its development and is not significantly affected by the procedure used in this study (Table II).

Zusammenfassung. Actinomycin D führte beim Hühnerembryo zu einer deutlichen Hemmung der Herzentwicklung nach Inkubation im Nährmedium während der Entwicklungsstadien 3 und 3+. Nach Vorbehandlung der Stadien 3–7 und anschliessender Kultur in actinomycin D-freiem Medium fanden sich Fehlbildungen von Herz, ZNS und Somiten, die in ihrer Häufigkeit von den betroffenen Entwicklungsphasen abhängig sind.

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