Body weight, relative liver weight, liver glycogen and blood glucose values of homozygous and heterozygous whirler male mice

	n	Body wt. (g)	Liver wt. g/100 g body wt.	*n*	Liver glycogen mg/g liver	*n*	Plasma glucose mg/100 ml
Whirler mice	18	23.2	5.9279	18	20.75	16	162.59
S.E.		±0.5	±0.1171		±4.83		±6.48
Hetero. mice	27	27.3	5.6115	26	33.71	25	195.25
S.E.		±0.6	±0.1227		±3.01		±7.56
%Difference Whirler vs. Hetero.		—15.0	+5.6		—34.4		—16.7
P value		<0.001	0.09		0.02		<0.01

KAHAN[10]. The livers were weighed on a Sartorius balance to the nearest 0.1 mg.

In the preliminary study[1] with populations consisting of 8 homozygous and 16 heterozygous male whirler mice, a marked decrease was noted in the blood glucose levels (*P* 0.07) and a significant reduction in liver glycogen. The Table presents the combined results and analyses based on the addition of a second population of 11 heterozygous and 10 homozygous whirler mice. The combined findings, analyzed by standard *t*-test procedures[11] revealed significant decreases in the body weights of the whirler mice which correspond with previous data[1]. The marked increase in relative liver weights of the whirler mice, although not statistically significant, had a low *P* value (*P* 0.09). It should be noted that previous studies have likewise indicated marked and/or significantly heavier relative liver weights in young, mature and aged whirler mice[4,5].

Analysis of the blood glucose levels with the larger population indicated that the −16.7% decrease in the whirler mice was significantly lower. Liver glycogen values were similarly significantly decreased in the homozygous mice. It is apparent that the combined data of the large population reinforce earlier indications of plasma hypoglycemia and diminished liver glycogen content. These alterations may well be a function of the frequent bursts of running activity and excitability of the animal. Exercise[12] has been noted to decrease liver glycogen. The reduction in circulating blood glucose could possibly result from the chronic inability of the whirler mice to satisfy the frequent requests for glucose due to the higher activity and heightened metabolism rates. The latter two parameters have been found to be significantly increased in the homozygous mutant stock[1–5]. The comparative increases in the relative liver weights might represent compensatory measures by the whirler mice to overcome frequent glycogen deficiencies.

Résumé. Des Dosages biochimiques du glucose sanguin et du glycogène hépatique chez des souris mâles homozygotes tourneurs ont montré une diminution nette des taux de glucose sanguin et de glycogène hépatique chez ces animaux comparés à ceux d'animaux heterozygotes issus d'une même portée phenotypiquement normaux.

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Inhibition of Heart Beat Development by Chloramphenicol in Intact and *Cardia bifida* Explanted Chick Embryos

The effects of chloramphenicol (CAP) upon the developing chick embryo have been studied with respect to various parameters. BLACKWOOD[1] demonstrated the gross splanchnopleure abnormalities produced by injection of 0.5 mg CAP into the fertile egg. More recent studies have employed the NEW[2] technique of excising the embryo and culturing it in vitro, ventral side up. Using this technique BILLET et al.[3] confirmed the teratogenic effect of the antibiotic at 200 µg/ml and 300 µg/ml and recorded an absence of hemoglobin production and the open neural tube. NEWBURGH et al.[4] employed the culture technique introduced by SPRATT[5] where the embryos are placed ventral side down upon the culture medium. NEWBURGH[4] found an inhibition of DNA, RNA, and protein synthesis occurred in the presence of 75 µg CAP. Using disaggregated chick embryo hearts cultured in a simplified tissue medium containing CAP, OISHI[6] demonstrated a reduction in numbers of cell nuclei in proportion to the concentration of the antibiotic (8 µg/ml to 800 µg/ml). In order to more clearly demonstrate the effects of chloramphenicol directly upon morphogenesis of the embryonic heart, development of the intrinsic pulsation rate was chosen for study. Both intact embryos and embryos in which the right and left cardiac promordia were forced to develop independently (*cardia bifida*) were used.

Materials and methods. Fertilized eggs of Babcock hybrid stock were incubated at 37.5 °C to provide the necessary stages of development. The embryos were explanted and cultured according to the technique of SPRATT[5] at HAMBURGER and HAMILTON[7] stages 5 through 8. The culture medium was a semisolid Howard Ringer albumin-agar to which was added D-3-chlor-

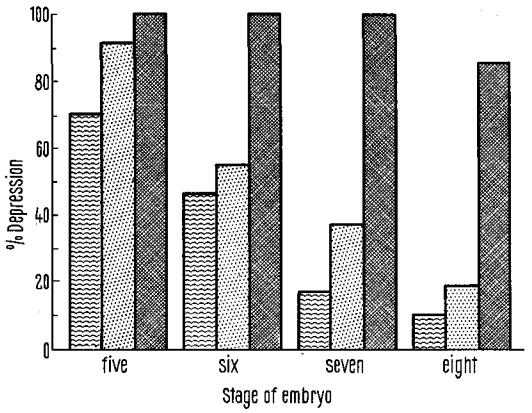

Fig. 1. Per cent depression of intrinsic heart rate of chick embryos cultured in the presence of D-3-chloramphenicol as compared to normal control embryos. Concentrations of CAP: ≋, 125 µg/ml; ⁞⁞, 250 µg/ml; ⊞, 500 µg/ml.

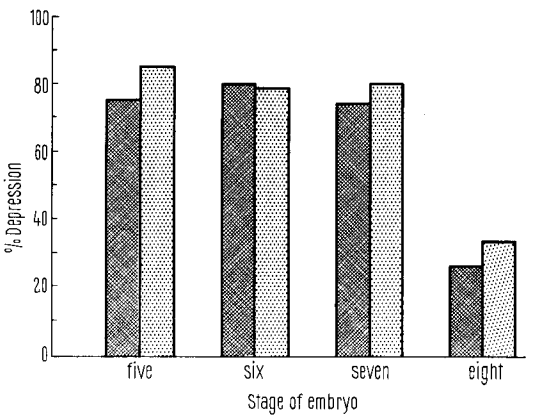

Fig. 2. Per cent depression of intrinsic heart rate of each side of *cardia bifida* chick embryo hearts cultured in the presence of 250 µg/ml D-3-chloramphenicol as compared to control *cardia bifida* embryos. ⊞, left primordium; ⁞⁞, right primordium.

Per cent of embryos cultured at various concentrations of chloramphenicol showing non-pulsatile hearts

	Intact embryos	*Cardia bifida* embryos
Stage 5		
125[a]	43 (28)[b]	–
250	67 (6)	62 (8)
500	100[c] (2)	–
Stage 6		
125	0 (6)	–
250	0 (4)	60 (15)
500	100[c] (1)	–
Stage 7		
125	0 (19)	–
250	0 (7)	58 (26)
500	100 (11)	–
Stage 8		
125	0 (11)	–
250	0 (8)	5 (21)
500	33 (3)	–

[a] Concentration of D-3-chloramphenicol in µg/ml. [b] Numbers in parentheses represent total number of embryos cultured. [c] Grossly teratogenic.

amphenicol in amounts of 125, 250, or 500 µg/ml. All cultures were maintained under semisterile conditions at 37.5 ± 0.5 °C for 24 h. The culture technique of placing the embryo with the ventral side directly in contact with the medium seemed the most effective way of introducing the antibiotic most rapidly into the areas of study – the cardiac primordia. After 24 h of incubation the embryos were transferred to a constant temperature chamber where the average heart rate per min was determined from three 30-sec recordings. *Cardia bifida* embryos were produced by the surgical technique of De Haan[8] where the embryo is separated into 2 equal halves by means of a tungsten needle. These were also cultured according to the method described above. Control embryos were maintained for each stage embryo used in the experimental procedure.

Results and discussion. Figure 1 demonstrates the effect of chloramphenicol upon the intrinsic pulsation rate of the embryonic hearts. It is evident that stage 5 embryos are most susceptable to inhibition by the antibiotic at even the lower concentrations. A concentration of 500 µg/ml appears to be extremely toxic to embryos up to stage 7. When *cardia bifida* embryos were cultured with 250 µg/ml CAP an even more striking inhibition was observed. Figure 2 illustrates the generally greater sensitivity to CAP shown by *cardia bifida* embryos. At all but stage 5 these embryos show a significantly depressed heart rate under the influence of 250 µg/ml CAP when compared to intact embryos. Both heart primordia seem to be equally affected by the inhibitory action of this drug. A further demonstration of the greater sensitivity of the *cardia bifida* embryos comes with a comparison of the number of embryos produced with non-pulsatile hearts (Table).

Inhibition of protein synthesis appears to be the mode of action of chloramphenicol, specifically by blocking RNA binding to ribosomes (Weisberger[9]). From this study it would seem that development of the intrinsic excitation-contraction mechanism of cardiac muscle is highly dependent upon protein synthesis taking place. At the lower concentrations of chloramphenicol used (125 and 250 µg/ml) an apparently morphologically normal heart can develop, but the intrinsic pacemaker and conduction systems are deficient.

Zusammenfassung. Untersuchung über die Wirkung von Chloramphenicol auf die Herzentwicklung von Hühnerembryonen ergibt normale Entwicklung bei kleinen Dosen, hingegen bleibten der Schrittmacher und die Reizleitung insuffizient.

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