Therefore they probably play a minor role, if any, in the response of myxamoebae tested with active extracts.

Extracts of organs of higher organisms were also tested with this assay. Liver, spleen, heart, and kidney extracts were active. Urine and milk, even at dilutions of 1000 attracted myxamoebae. After purification of the extracts by column and paper chromatography[8], it was shown that cyclic 3′,5′-AMP was present. The attractants in human urine evoked a response similar to ca. 5 μM of cyclic 3′,5′-AMP. BUTCHER and SUTHERLAND[13] identified daily levels of 2–7 μM of cyclic 3′,5′-AMP in human urine. Consequently 3′,5′-AMP is the main, if not the only, attractant in urine. In collaboration with F. C. G. VAN DE VEERDONK, University of Utrecht, it was shown that extracts of dark pigmented skin of *Xenopus laevis* contained twice as much attractant as light pigmented skin. The activity of the attractant in 0.5 g darkened skin (wet wt.) was equivalent to ca. 1×10^{-8} g of cyclic 3′,5′-AMP[14]. SUTHERLAND and co-workers studied the production of cyclic AMP after the target cell was activated by a hormone. Interestingly enough I found that extracts of axolotl embryos without hormonal glands (blastula and neurula) are active. More detailed results will be published elsewhere[15].

Comparison of the chemotactic response induced by different concentrations of urine and cyclic 3′,5′-AMP

	Urine dilution × 500		3′,5′-AMP 0.01 μM		Urine dilution × 2000	
	Total positive	% positive	Total positive	% positive	Total positive	% positive
Test 1	25	83	32	73	14	48
Test 2	22	88	25	73	20	59
Test 3	18	60	22	47	7	23

The drops with attractant were placed 3 times with 5 min intervals near (less than 0.45 mm) the responding drops. Observation took place 5 min after the last deposition.

Zusammenfassung. Es wird ein mikrobiologisches Testverfahren beschrieben, mit dem sehr geringe Mengen von zyklischem Adenosin 3′,5′-monophosphat quantitativ nachgewiesen werden können.

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Blood Glucose and Liver Glycogen Content in Male Whirler Mice

Recent studies[1] with relatively small populations of homozygous male whirler versus heterozygous whirler mice have demonstrated marked but not significant reductions in plasma glucose levels (P 0.07), accompanied by significant decreases in liver glycogen. These data suggest differences in the carbohydrate metabolism and utilization processes of the neurological mutant. Previous investigations[2–5] of endocrine and metabolic differences between homozygous male and female whirler mice and their phenotypically 'normal' heterozygous littermates have indicated significant increases in adrenocortical activity and metabolism of the whirlers. The whirler mutation[6] represents a recessive behavioral and neurological factor located in the VIII linkage group. The mice are one of a group of waltzing recessive mutations[7] possessing an extremely nervous, restless and excitable nature. In addition to head-shaking and deafness, the mice display syndromes of rapid clockwise and/or counterclockwise circling locomotor activity. Neurological and labryinthine anomalies have been frequently associated with the waltzing syndrome[7]. The present paper sought to reaffirm and substantiate earlier impressions of hypoglycemia in the whirler mice by comparing groups with larger population sizes.

The original stock of male and female homozygous whirler and phenotypically 'normal' heterozygous mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. After several generations of selective inbreeding, homozygous and heterozygous whirler male littermates were selected for study from matings of phenotypically 'normal' heterozygous females to whirler brother males. Genetically[7], the mutant whirler mice were homozygous for the recessive gene (wi wi). The heterozygous mice had the (wi+) genotype and appeared phenotypically 'normal' in behavior, locomotor activity and hearing. The mice were non-agouti and brown for coat color and hair pigment characteristics[7].

All animals were bred and raised in air-conditioned quarters with room temperatures maintained at 73–75 °F. When breeding units were capable of supplying sufficient numbers of mice for experimentation, homozygous and heterozygous male mice were weaned at 4 weeks of age for subsequent weekly body weight evaluations. The number of male mice was limited to 2 animals per cage to avoid crowding and fighting effects[8]. The cages were stainless steel, having dimensions of $6^1/_2 \times 10 \times 7$ inches and bedded with pine shavings.

At 16 weeks of age, the mice were sacrificed by rapid decapitation 1 h after body weight determinations to reduce undue stress and prolonged handling of the animals. Blood samples were collected in heparinized beakers for plasma glucose determinations[9]. All mice were autopsied rapidly for liver glycogen analyses by the procedure of

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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-