Lack of Cortisone Inhibition of Chromosomal Puffing in *Drosophila melanogaster*

GOODMAN, GOIDL and RICHART[1] have shown that larvae of *Sciara coprophila* will complete normal development without forming the usual large chromosomal puffs when fed cortisone or hydrocortisone. Normal puffing of salivary gland chromosomes is resumed when larvae are transferred back to normal food, so they conclude that cortisone inhibits chromosomal puffing. They also state, somewhat ambiguously, that 'although the formation of large puffs is a convenient marker for the activity of certain genes, it does not appear to be essential for gene action'. Since it is commonly assumed[2,3] that puffed chromosomal regions do indicate the loci of active genes, it seemed worth checking this surprising observation using another species, *Drosophila melanogaster*.

Newly-hatched *Drosophila* larvae were grown germ-free on defined media[4], containing graded doses of cortisone acetate, or of hydrocortisone. Lactic-acetic-orcein squashes of salivary gland chromosomes were made, from either newly-formed pre-pupae, or from 2- to 4-hour-old prepupae. Controls were similarly prepared. No effects of cortisone (or of hydrocortisone) were found on chromosomal puffing, up to levels which were toxic to most larvae (800 mg% of diet). Since pupation was delayed by 2–3 days at this high cortisone concentration, it was possible that the larvae which failed to pupate were the ones not showing puffs. Preparations were therefore made from late third instar larvae, and from larvae which had been grown for the first 2 instars on normal media before beings transferred to cortisone-containing food. In all cases salivary puffing was found, but the puffing patterns were somewhat erratic, as might be expected of larvae which showed a spread of development times[5,6]. GOODMAN et al.[1] fed cortisone acetate at the high rate of about 6 g%, which is nearly an order of magnitude greater than the amount *Drosophila* will tolerate. Failure to repeat their observation may be due to this species difference.

Résumé. L'administration de cortisone (ou d'hydrocortisone) à des larves *Drosophila* n'inhibe pas le bourgeonnement normal des chromosomes de leurs glandes salivaires. Toutefois, la quantité de cortisone que ces larves peuvent tolérer est environ $^1/_{10}$ de celle qui supprime le bourgeonnement chez les larves *Sciara*.

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Ontogenic Development and Anatomical Distribution of a Supernumerary Limb-Inducing Factor

In 1952 BREEDIS showed that *Rana pipiens* renal adenocarcinomas, implanted into the unamputated forelimb of the newt (*Triturus viridescens*), induce the formation of supernumerary limbs[1]. Later both RUBEN and CARLSON have obtained high percentages of supernumerary growth in newts by implantation of pieces of normal frog kidney[2,3], and RUBEN has further shown that frog cartilage and liver stimulate relatively low percentages of accessory structures whereas implants of muscle, skin and sciatic nerve are essentially ineffective[4,5]. The present experiments describe the development of the capacity of frog kidney to induce supernumerary limbs and further explore the anatomical distribution of this inducing capacity.

Methods. Tissues taken from *R. pipiens* or *Hyla versicolor* were implanted into adult newts (*T. viridescens*) from Petersham, Massachusetts. Donor frogs were caught in Minnesota and Michigan. The *R. pipiens* donors were staged according to TAYLOR and KOLLROS[6]. Graft tissues included kidney, tail muscle, urinary bladder and intestine. After the newts were anesthetized in 1:1000 MS 222, 1 mm³ pieces of tissue were placed into tunnels made under the skin of the upper arms in the manner previously described[7]. All limbs were serially sectioned and examined microscopically.

Results. As shown in Table I, the inductive ability of kidney implants progressively increases as the animal matures. From a 47.8% inductive rate at stage VIII, an adult level of inductive ability is reached by stage XIX. Stage XIX represents the beginning of the rapid phase of metamorphosis during which the fully formed front limbs break through the skin window covering them, and the tail begins its rapid resorption.

In view of the numerous references in the literature which emphasize the importance, or at least the prominence of proteolysis in early stages of regeneration, resorbing tail tissue taken from metamorphosing tadpoles was used as implant material (Table II)[8–10]. Resorbing tail tissue is characterized by very high catheptic activity[11]. Tissues from premetamorphic *Rana* tails proved to be an extremely poor stimulus for supernumerary growth whereas tissue from stages of advanced resorption (XX and XXIII) was essentially inactive. These latter stages correspond to those stages in which WEBER noted the highest cathepsin activity in *Xenopus*[11]. Tail tissue from metamorphosing *Hyla* had no inductive ability.

In a further exploration of the distribution of inductive capacity, pieces of urinary bladder were used as implants (Table III). The inductive percentages were as high as

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those of normal frog kidney. After separation of the epithelium from the remainder of the bladder wall, pieces of each component were implanted. The epithelial part was the stronger inducer. Finally, pieces of intestinal wall taken close to the cloaca induced a high percentage of supernumerary growth.

Discussion. The increasing capacity of tadpole kidney tissue to stimulate supernumerary growth as maturation proceeds suggests the differentiation or the quantitative concentration of some component of renal tissue which is presumably responsible for its inductive properties. It is difficult to correlate this increase in inductive ability with any of the known events related to metamorphosis itself for the adult inductive capacity is already attained by stage XIX. This occurs not only before the period of rapid external change of form in metamorphosis, but also before the dramatic shift from an ammonotelic to the ureotelic mode of nitrogen excretion characteristic of the adult[12]. Although little work has been done on the morphology of the frog kidney during metamorphosis, no dramatic changes have been noted. It is quite unlikely, however, that the changing inductive potential would be due to

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Table I. Response of newt limbs to implants of kidney tissue from various developmental stages of *R. pipiens*

Developmental stage of donor (TAYLOR and KOLLROS)[6]	Supernumerary growth		Cartilage proliferation on bone only		No growth		Totals		
	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	% Total growth responses	X^2 p
VIII	7	30.4	4	17.4	12	52.2	23	47.8	< 0.005
IX	2	40.0	1	20.0	2	40.0	5	60.0	< 0.02
XIV	7	43.8	3	18.8	6	37.5	16	62.5	< 0.005
XIX	21	77.8	4	14.8	2	7.4	27	92.6	< 0.9
Adult	235	86.7	13	4.8	23	8.5	271	91.5	–

Table II. Response of newt limbs to implants of tail muscle from metamorphosing *R. pipiens* and *H. versicolor*

Species and developmental stage	Supernumerary growth		Cartilage proliferation on bone only		No growth		Totals	
	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	% Total growth responses
R. pipiens stage X	2	11.1	1	5.6	15	83.3	18	16.7
R. pipiens stage XX	0	0.0	1	5.5	17	94.5	18	5.5
R. pipiens stage XXIII	0	0.0	0	0.0	18	100.0	18	0.0
H. versicolor Tadpole, hind legs present, no fore limbs, tail intact	0	0.0	0	0.0	10	100.0	10	0.0
H. versicolor metamorphosing; tail almost all resorbed, implants from black portion of tail	0	0.0	0	0.0	14	100.0	14	0.0

Table III. Response of newt limbs to implants of various tissues from *R. pipiens*

Tissue	Supernumerary growth		Cartilage proliferation on bone only		No growth		Totals	
	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	(%)	No. of limbs	% Total growth responses
Urinary bladder, stage XXII	7	63.3	0	0.0	4	36.4	11	63.6
Urinary bladder, adult	25	92.6	0	0.0	2	7.4	27	92.6
Urinary bladder Adult, mucosa	7	70.0	0	0.0	3	30.0	10	70.0
Urinary bladder Adult, wall	2	28.6	0	0.0	5	71.4	7	28.6
Intestine Adult, adjacent to cloaca	9	90.0	0	0.0	1	10.0	10	90.0

something peculiar to the kidney alone, for the ability to stimulate supernumerary growth is not confined to renal tissue.

The data on the anatomical distribution of the inducing factor are instructive. From these and previous experiments, some type of a pattern is beginning to emerge. Many of the typical components of extremities (skin, muscle, nerve and resorbing tail) are essentially lacking in inductive ability. Cartilage has moderate ability, but this may be a tissue specific induction. Of the internal organs tested, liver has slight inductive powers, but this capacity is highly developed in kidney, urinary bladder and intestine in the region of the cloaca. On the basis of this distribution as well as the data on the development of inducing capacity in kidney, it would be of interest to further test 2 hypotheses. First, there may be some common developmental association of tissues possessing inductive ability, or second, the fact that this capacity seems to be most concentrated in excretory organs but not altogether lacking in other tissues may indicate a concentration of a substance produced throughout the body. These possibilities are currently being tested[13].

Выводы. Индуктивная способность почки лягушки увеличтвается в течении онтогенеза. У тканей мочевыводящих путей и кишки есть большая индуктивная способность но у других тканей незначительная индуктивная способность.

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Ultrastructural Changes in the Yeast *Candida lipolytica* Caused by Penetration of Hydrocarbons into the Cell

A number of papers have been devoted to the first stages of degradation of hydrocarbons by yeasts or by other microorganisms[1] but no experimentally founded explanation exists with regard to the contact of the microbial enzymes with the hydrocarbon.

In our first paper we described the fact that during cultivation of the yeast *Candida lipolytica* on hexadecane or on gas oil the hydrocarbons pass through the cell wall and are concentrated on the cytoplasmic membrane[2].

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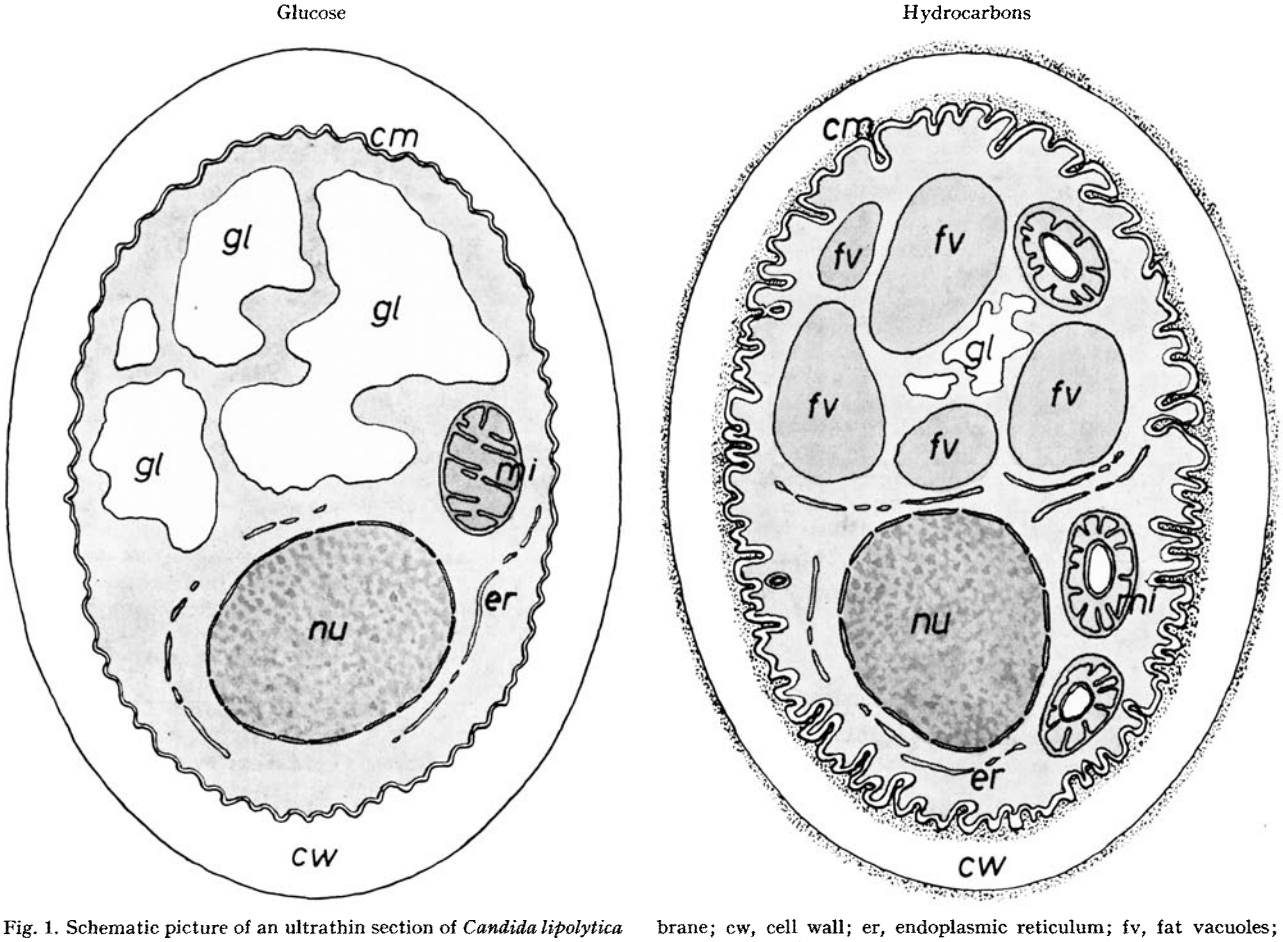

Fig. 1. Schematic picture of an ultrathin section of *Candida lipolytica* grown on glucose or hydrocarbon medium. cm, cytoplasmic membrane; cw, cell wall; er, endoplasmic reticulum; fv, fat vacuoles; gl, glycogen; mi, mitochondria; nu, nucleus.