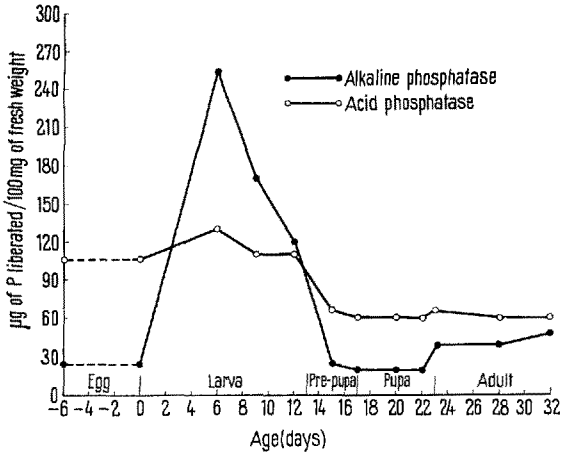
as compared to its correspondingly lower activity in the pupae and the adults (Figure). The highest acid phosphatase activity has also been reported in the egg stage of the stable fly and the house fly by ASHRAFI[6] and BARKER and ALEXANDER[7] respectively. It appears from these findings that increased acid phosphatase activity might be related to rapid cell division, especially during morphogenic changes in the embryonic development and during the larval growth of insects. At the time of pupation in *T. confusum*, a drop in the activity of this enzyme occurs, which seems to be in agreement with the fact that the insect ceases to grow during this period while it is undergoing an internal reorganization of the tissues necessitated by adult development. On adult emergence in *T. confusum*, no major variation in the level of acid phosphatase activity occurs.



Phosphatase activity during the growth and metamorphosis of *T. confusum* Duval. Each point on the curves represents an average of 5 determinations. Dotted lines in the egg period indicate that only one stage during this period was analysed for phosphatase activity.

Alkaline phosphatase: While it is interesting to remark a tremendous increase in the activity of alkaline phosphatase activity in 7-day-old *T. confusum* larvae, the embryonic as well as the pupal stage seems to be deficient in the activity of this enzyme (Figure). Our results mostly seem to be in conformity with the findings of BARKER and ALEXANDER[7] in *Musca domestica*. It therefore seems suggestive that alkaline phosphatase might play an important role in the phenomena concerning growth rather than morphogenesis. Alkaline phosphatase was also thought by YAO[8] to be associated with growth in insects. Adult emergence in *T. confusum* was characterized by a slight rise in the activity of alkaline phosphatase[9].

Résumé. Nous avons mesuré l'activité des phosphatases acide et alcaline au cours de la croissance et de la métamorphose de *T. confusum*. L'activité de la phosphatase acide est à son maximum au cours de l'embryogénèse et du stade larvaire. Il est donc possible que cette enzyme soit reliée aux phénomènes de la différentiation cellulaire et de la croissance. L'activité de la phosphatase alcaline est, au contraire, très élevée au cours de la croissance larvaire. Elle serait donc associée à la croissance seule.

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Department of Biochemistry, School of Medicine, Laval University, Quebec (Canada), October 3, 1963.

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Effect of Adrenalectomy and Diet on the Activity of β-Galactosidase in the Small Intestine During the Postnatal Development of the Rat

Recently the development of β-galactosidase in the small intestine during postnatal development in the mouse (MAIO and RICKENBERG[1]), calf (HUBER et al.[2]) and rat (ALVAREZ and SÁS[3], DOELL and KRETCHMER[4,5]) has again attracted attention. The activity of this enzyme is high at birth and falls rapidly during the weaning period. The reason for this loss of activity, however, is far from clear, in spite of efforts to show a correlation between changes of activity and changes in diet or in supply of lactose (ALVAREZ and SÁS[3], DOELL and KRETCHMER[4]).

An attempt was therefore made to clarify the factors affecting the postnatal loss of β-galactosidase activity. Since the proximodistal distribution of some enzymes is different during the suckling period than later in life (non-specific esterase, KOLDOVSKÝ et al.[6]; alkaline phosphatase, KOLDOVSKÝ et al.[6], VERNE and HEBERT[7], MOOG[8]), this distribution was determined for β-galactosidase by the method of LEDERBERG[9] (see Table I). From Table I it is evident that between day 15 and 20 postnatally, β-galactosidase activity decreases both in the proximal and distal part of the small intestine. It can also be seen that activity is several times higher in the distal part than in the proximal part in rats 15–18 days old. In 20-day-old animals there is no significant difference between activity in the proximal and distal parts of the intestine. The distribution of β-galactosidase activity along the small intestine is thus very near to the distribution of non-specific esterase (KOLDOVSKÝ et al.[6]) and alkaline phosphatase

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(KOLDOVSKÝ et al.[6], VERNE and HERBERT[7], MOOG[8]), e.g. a higher activity was found in the distal part. It is interesting that, on the contrary, lipase (ROKOS et al.[10], YEZUITOVA et al.[11]) and invertase (YEZUITOVA et al.[11]) showed a higher activity in the proximal part of the small intestine of suckling rats.

Next, the role of the adrenal gland was studied. Their removal early postnatally diminishes the usual increase in glucose absorption (KOLDOVSKÝ et al.[12]) while cortisone application accelerates the rise in glucose absorption (KOLDOVSKÝ et al.[12], FALTOVÁ et al.[13]), pancreatic lipolytic activity (ROKOS et al.[14]) duodenal alkaline phosphatase activity (HALLIDAY[15]) and intestinal invertase activity in rats (DOELL and KRETCHMER[16]). Table II shows that the fall of β-galactosidase activity during postnatal development is also under the control of the adrenals. Adrenalectomy slows down the decrease in the activity of the enzyme studied in the proximal and mainly in the distal part. It should be mentioned that the general appearance and weight of the adrenalectomized and sham-operated animals were almost identical. Thus this is a special instance, where the absence of the adrenals in infant rats maintains a high activity of an enzyme when usually such a procedure prevents the normal postnatal rise in function, e.g. glucose absorption (KOLDOVSKÝ et al.[12]). This is evidently related to the anomalous behaviour of β-galactosidase, the activity of which in the small

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Table I. Fed rats (Wistar strain) were sacrificed by decapitation. The small intestine was washed with ice-cool 0.15 *M* KCl, the duodenum was discarded, the whole length of the small intestine was divided into three equal parts by length and the proximal and distal parts were used for the estimation of enzyme activity. An homogenate (1:150) of the intestine was made in ice-cold 0.15 *M* KCl using a Potter-Elvehjem Teflon homogenizer. β-galactosidase activity was estimated using *o*-nitrophenylgalactoside as substrate (LEDERBERG[9]) in a medium containing 0.5 ml 0.3 *M* sodium acetate buffer (pH 3.5), 0.5 ml of the homogenate and 0.5 ml 0.003 *M* *o*-nitrophenyl galactoside. The reaction was terminated after 20 min of incubation at 37°C by the addition of 1 ml potassium carbonate. The *o*-nitrophenol liberated was measured photometrically at 420 mμ. Activity was expressed in μmoles of *o*-nitrophenol × 10^3 liberated in 20 min by 1 mg wet weight of the intestine. The *t*-test was used. p^1 denotes the value of statistical significance between the groups, p^2 between the proximal and distal parts of the small intestine in the same group

Age in days	No. of animals	p^1	Activity of proxim. part mean	S.E.	p^1	Activity of distal part mean	S.E.	p^2
15	8	not significant	40.0	4.7	not significant	274	38	0.001
16	4	0.001	33.0	1.6	0.001	258	2.6	0.001
18	3		13.8	1.45		129	7.3	0.001
20	5	not significant	13.0	1.47	0.001	11.1	1.52	not significant

Table II. Rats were adrenalectomized on day 15 postnatally, controls were sham operated. The animals lived with the mother having free access to physiological saline. For estimation of enzyme activity and other details see Table I

Age in days	Group	No. of animals	p^1	Activity of proximal part mean	S.E.	p^1	Activity of distal part mean	S.E.	p^2
19	adrenalectomized	8	0.02	23	1.0	0.001	133	1.9	0.001
19	sham operated	4		13	3.4		21	6.3	not significant

Table III. From day 14 postnatally the animals (in the presence of the mother animal) were divided into two groups. One received a lactose diet, the second was fed a diet containing galactose plus glucose. The composition of the diets was the following: margarine 500 g, casein 250 g, cod liver oil 10 g, $CaCO_3$ 2 g, Na_2HPO_4 2.7 g, $MgCl_2$ 0.1 g, thiamin 4 mg, riboflavin 6 mg, pyridoxine 5 mg, Ca-panthothenate 40 mg, nicotinamide 40 mg, inositol 200 mg, biotin 20 mg, folic acid 2 g. The whole mixture was divided by weight into two equal parts. The first was supplemented with 75 g lactose, and the other with 37.5 g glucose and 37.5 galactose. For details see Table I

Age in days	Diet	No. of animals	p^1	Activity of proximal part mean	S.E.	p^1	Activity of distal part mean	S.E.	p^2
20	lactose	7	0.02	28.0	2.1	0.001	65.4	7.9	0.001
20	glucose + galactose	6		22.7	1.35		22.7	3.3	not significant

intestine decreases postnatally, while other enzyme activities increase. At the present no explanation for this phenomenon can be offered.

Finally it might be assumed that continued lactose feeding would prevent the fall in β-galactosidase activity. It is evident from Table III that activity was higher in the proximal and distal part of the intestine in rats having access to a lactose diet than in rats fed with a diet containing, instead of lactose, a corresponding amount of glucose and galactose. Following adrenalectomy on day 15 or feeding the lactose diet from day 14 prevents the usual postnatal change in enzyme distribution along the intestine. Thus the proximo-distal gradient is preserved while in the control animals activity becomes the same proximally and distally. It thus appears that the decrease of β-galactosidase activity in the small intestine during the postnatal development is influenced at least by two factors. One seems to be the adrenals, the other the content of lactose in the diet. The same seems to apply to the development of glucose absorption in the small intestine of the rat (FALTOVÁ et al.[17]). The effect of lactose in the diet on the β-galactosidase activity raises the question whether the activity of this enzyme during this period of postnatal development is not regulated by the substrate in the diet, as was described, for instance, for liver tryptophanpyrrolase in adult animals (CHYTIL[18]).

Zusammenfassung. Die β-Galaktosidaseaktivität im Rattendünndarm nimmt nachgeburtlich zwischen dem 15. und 20. Tag ab. Wird am 15. Tag adrenalektomiert oder wird Laktosediät verabreicht, so erhöht sich die β-Galaktosidaseaktivität am 19. Tag um den mehrfachen Betrag als bei den Kontrollratten.

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Über ein gebundenes Gibberellin aus *Phaseolus coccineus* L.[1]

Unreife, reife und gekeimte Samen sowie grüne Fruchtschalen (Hülsen) von *Phaseolus coccineus* L. var. *coccineus* cv. «Preisgewinner» wurden auf Gibberelline untersucht. Hierzu wurden die angesäuerten, wässrigen Konzentrate der methanolischen Auszüge von jeweils 1 kg Pflanzenmaterial mit Essigester sowie anschliessend mit *n*-Butanol extrahiert und deren Rückstände dünnschichtchromatographisch untersucht[1]. Dabei diente diese Methode nicht nur zur Identifizierung bzw. Charakterisierung der vorkommenden Gibberelline, sondern auch zur mikropräparativen Reinigung und Gewinnung der nachgewiesenen Substanzen für die biologische Testung. Für den Nachweis der Gibberellinwirksamkeit der so gewonnenen, dünnschichtchromatographisch einheitlichen Präparate wurde der Zwergerbsentest[2] herangezogen.

Im einzelnen liessen sich ausser den für unreife Bohnensamen bereits beschriebenen[3] Gibberellinen A_1, A_5, A_6 und A_8 weitere in Tabelle I angeführte gibberellinartige Substanzen nachweisen. Hierbei wurden nur solche berücksichtigt, die sowohl biologische Wirksamkeit als auch eine für Gibberelline charakteristische Nachweisfluoreszenz zeigen.

Tabelle I. In *Phaseolus coccineus* L. nachgewiesene «Gibberelline»

Pflanzenteil	Extrakt	Nachgewiesene «Gibberelline»[4]
Hülsen	Essigester	A_1, A_3, A_5, A_6, A_8, «Phaseolus β, γ, δ»
	n-Butanol	A_8, «Phaseolus ε»
Unreife Samen	Essigester	A_1, A_5, A_6, A_8, «Phaseolus α, β»
	n-Butanol	A_3, «Phaseolus β»
Reife Samen	Essigester	Kein Gibberellin nachweisbar
	n-Butanol	«Phaseolus ε»
Gekeimte Samen	Essigester	A_1, A_5, A_6, A_8, «Phaseolus α, β»

Die mit bekannten Gibberellinen nicht identischen Substanzen «Phaseolus α–ε» sind durch die in Tabelle II angeführten $R_{Standard}$-Werte charakterisiert. Während «Phaseolus α» im Dünnschichtchromatogramm mit allen geprüften Entwicklungsgemischen zwischen A_8 und A_3 nachzuweisen ist, besitzen alle übrigen neuen Substanzen kleinere Rf-Werte als A_8.

Arbeiten zur präparativen Isolierung der nachgewiesenen Gibberelline sind in Angriff genommen worden. Nach ersten Ergebnissen, die bei Aufarbeitung von 770 kg grünen Hülsen gewonnen wurden, handelt es sich bei «Phaseolus ε» um ein «gebundenes Gibberellin» mit eindeutig gesicherter biologischer Wirksamkeit. Es verhält sich polar (vgl. Tabelle II) und bleibt bei der Dünnschichtelektrophorese[5], im Gegensatz zu den bekannten, anodisch wandernden Gibberellinen, am Startpunkt zurück, was auf seinen neutralen Charakter hinweist. Nach Hydrolyse mit 2 *n* H_2SO_4 (5 h unter Rückfluss) liess sich

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[3] C. A. WEST und B. O. PHINNEY, J. Amer. chem. Soc. *81*, 2424 (1959). – J. MACMILLAN, J. C. SEATON und P. J. SUTER, Tetrahedron *11*, 60 (1960); *18*, 349 (1962).

[4] Als vorläufige Bezeichnung für die unbekannten, gibberellinwirksamen Substanzen werden bis zu ihrer endgültigen Charakterisierung, die erst nach präparativer Isolierung möglich ist, griechische Buchstaben in Verbindung mit dem jeweiligen Gattungsnamen verwendet.

[5] Kieselgel-G, Puffer nach Theorell-Stenhagen, 150 V, 10–20 mA, pH 7,0 bzw. pH 12,0. Über diese und weitere Ergebnisse der Dünnschichtelektrophorese auf dem Gibberellingebiet wird demnächst an anderer Stelle ausführlich berichtet.