

Soon after hatching, also, the 3 characteristic e-bands make their appearance. This may be connected with the differentiation of the photoreceptors of the retina, since according to RINGGENBERG<sup>10</sup> the retina of the trout is fully differentiated, over most of its area, shortly after hatching. In *Lebistes reticulatus*, where the eyes of all embryonic stages were tested for e-bands as well as histologically, the appearance of the e-group coincides with the differentiation of rods and cones<sup>11</sup>.

When the embryo and yolk sac of both salmon and trout are tested separately, the most anodal isozymes of group (b) move more rapidly in the latter. This may be due to a possible binding of yolk material to the enzyme in a somewhat analogous fashion to the binding of NAD to alcohol dehydrogenase<sup>12</sup>. The pattern of the yolk sac – with increasing activity from the cathodal to anodal end of group (b) and a faint smear anodally to it – remains the same until the last stage.

b) *Development of organ patterns.* 1. Tail muscle. When the parr marks appear on the postembryo, the full adult complement of bands in groups (a) and (b) is present, with group (b) staining less intensely. However, the relative activity within group (a) is different from the adult. In the postembryo, the anodal bands are more pronounced, whereas in the adult the cathodal bands stain more intensely. Only in the smolt stage of the salmon and the prespawning stage of the trout, the muscle pattern acquires adult expression (Figure 1).

2. Eye. Group (b) and group (e) develop shortly after hatching (group (e) moves faster in the salmon than in the trout). The relative intensities within both groups are the same as for the adult. 2 bands of group (d) appear towards the end of yolk sac resorption. Thus, by the end of the postembryonic phase, the eyes of both salmon and trout possess the adult number and distribution of LDH activity (Figure 2). The pattern remains the same during subsequent development.

3. Heart. Heart extract of both salmon and trout fry (1 week after yolk resorption) yield group (b), but with the exception of the second most rapidly moving band.

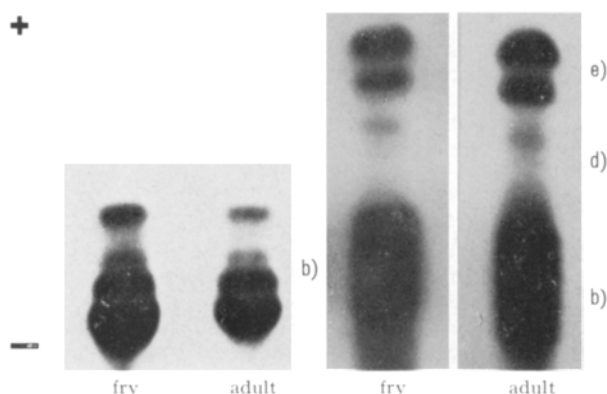


Fig. 2. LDH isozyme pattern of trout heart (left) and salmon eye (right) showing that both organs have acquired an adult pattern already at the fry stage.

The number of bands and distribution of activity are the same as in the adult fish (Figure 2). The pattern remains unchanged in all stages of the subsequent growth period.

4. Brain. 5 bands of equal intensity (group (b)) are resolved in the fry of salmon and trout. Faint and diffuse activity in the region of group (a) and group (d) is also observed. The pattern remains the same throughout the growth period into adulthood.

5. Liver. Liver extracts of salmon and trout fry exhibit, with increasing activity from the cathodal to the anodal end, all 5 bands of group (b). At the position of group (d) diffuse activity is sometimes evident. This early liver pattern resembles closely the isozyme pattern for the yolk sac. Morphological and histological observations show that in teleosts the liver is in close contact with the yolk: in salmonids the contact is particularly close, which makes some authors suggest that the liver is a resorptive organ for yolk<sup>13</sup>. The similarity of both early liver and yolk patterns may reflect the presence of yolk material in the liver. The adult distribution of liver LDH activity, where group (d) is pronounced and group (b) is smeared, is achieved in the salmon at the smolt stage and in the trout at the prespawning stage.

Thus, the developmental LDH isozyme pattern of early organogenesis in the 2 salmonids studied seems to parallel observations in *Xenopus laevis*<sup>5,6</sup>: Once the yolk disappears – either as intracellular platelets, as in *Xenopus*, or concentrated extracellularly in a yolk sac, as in salmonids – the adult number of bands in all organs tested (liver excepted) is established. The presence of yolk may have some influence on gene regulation or directly on enzyme expression. Also, when the yolk is absorbed or immediately afterwards, eye, heart muscle and brain show adult distribution of activity. However, in skeletal muscle and in the digestive tract (intestine in *Xenopus* and liver in salmonids), the adult patterns emerge gradually and are fully established at a very much later stage.

*Zusammenfassung.* Die ontogenetischen Änderungen des Isoenzym-Musters der Laktatdehydrogenase (LDH) von *Salmo salar* und *S. trutta* wurde mittels Stärkegel-Elektrophorese verfolgt. Während der frühen Entwicklungsstadien (Furchung und Gastrulation) sind allein die «Herz-Banden» vorhanden. In allen geprüften Organen wird die adulte Bandenzahl unmittelbar nach der Dottersack-Resorption erreicht. Im selben Stadium weisen Herzmuskel, Auge und Gehirn bereits das adulte Verteilungsmuster auf, während dieses im Skelettmuskel und der Leber erst nach 2–3 Jahren, vor Erreichung der Geschlechtsreife, auftritt.

YVETTE W. KUNZ

Department of Zoology, University College,  
Stillorgan Road, Dublin 4 (Ireland), 20 August 1974.

<sup>10</sup> J. RINGGENBERG, Verh. naturf. Ges. Basel 75, 101 (1964).

<sup>11</sup> Y. W. KUNZ, Rev. Suisse Zool. 78, 761 (1971).

<sup>12</sup> H. URSPRUNG and L. CARLIN, Ann. N.Y. Acad. Sci. 151, 426 (1968).

<sup>13</sup> Y. KUNZ, Rev. Suisse Zool. 71, 445 (1964).

## Metabolic Changes Induced by Galactose

Dietary carbohydrates have been reported to decrease blood cholesterol in man<sup>1,2</sup>, but lipaemia rises with glucose, sucrose and fructose<sup>3–10</sup>. We have studied glucolipidic metabolism after an oral dose of galactose.

*Material and methods.* Blood samples were taken from 9 voluntary healthy subjects after a fasting period of 8 h, and immediately afterwards 100 g of galactose dissolved in water were administered orally. Further blood samples

	Cases	Basal	1/2 hour	1 hour	2 hours	Amounts in
Triglycerides	9	145 ± 50.48	179.55 ± 76.46	177.66 ± 55.14	147.22 ± 55.27	mg/100 ml
Free fatty acids	9	0.42 ± 0.15	0.30 ± 0.16	0.26 ± 0.17	0.33 ± 0.17	mEq/l
Total cholesterol	8-9	166.00 ± 28.96	188.88 ± 49.49	183.50 ± 48.99	178.50 ± 57.82	mg/100 ml
Blood sugar	9	89.47 ± 14.8	100.57 ± 12.82	91.57 ± 12.73	85.70 ± 15.42	mg/100 ml
Insuline	9	30.11 ± 13.27	49.88 ± 14.32	47.66 ± 16.85	32.44 ± 9.11	μU/ml

were taken 1/2, 1 and 2 h later. The following were estimated in each sample: free fatty acids<sup>11-13</sup>, total cholesterol<sup>14,15</sup>, triglycerides<sup>16</sup>, blood sugar<sup>17,18</sup> and serum insulin<sup>19</sup>. The total cholesterol in the basal sample and in the 1-h and 2-h samples could only be checked in 8 patients. For statistical treatment, the results were analyzed by Student's *t*-test.

*Comments.* The results are shown in the Table. There were no significant variations in serum-cholesterol, serum-triglycerides and glucose concentrations. The free fatty acids decreased significantly after 1 h (*p* 0.05). Serum insulin increased significantly after 1/2 h (*p* < 0.01) and continued to be high after 1 h (*p* < 0.05). After 2 h the basal concentrations were restored.

From the foregoing it may be deduced that galactose does not exert any influence on the serum cholesterol, triglycerides or glucose concentrations. It does, however, provide an effective stimulus for the secretion of insulin, resulting in an appreciable antilipolytic effect as demonstrated by the decrease of free fatty acids, a fact which points to the possible existence of pancreatic receptors for galactose or to the production by the gut of some substance which increases the blood sugar content when galactose is present.

*Resumen.* La administracion de 100 g de galactosa a 9 voluntarios sanos por via oral, demostro ser capaz de elevar la insulinenia, sin modificar la glucosa en sangre. Secundariamente se aprecio un descenso de los acidos grasos libres. La galactosa puede actuar directamente sobre el pancreas o a traves del intestino.

E. JOVER SANZ, S. DURAN GONZALEZ,  
J. M. MANSO MARTINEZ,  
R. VELASCO ALONSO and  
C. PARADINAS JIMENEZ

*Departamento de Medicina Interna,  
Medical Faculty of Valladolid (Spain), 27 July 1974.*

- <sup>1</sup> A. KEYS, F. GRANDE and J. T. ANDERSON, *Proc. Soc. exp. Biol. Med.* **106**, 555 (1961).
- <sup>2</sup> R. KUYKEN, N. A. PIKAAR, H. POLMAN and F. A. SCHIPPERS, *Voeding* **23**, 447 (1962).
- <sup>3</sup> M. WINITZ, J. GRAFF and D. A. SEEDMAN, *Arch. Biochem. Biophys.* **108**, 576 (1964).
- <sup>4</sup> A. M. PLESHKOV, *Terapevticheskii Arkhiv* **35**, 66 (1963).
- <sup>5</sup> I. MACDONALD and D. M. BRAITHWAITE, *Clin. Sci.* **27**, 23 (1964).
- <sup>6</sup> J. T. ANDERSON, F. GRANDE, Y. MATSUMOTO and A. KEYS, *J. Nutr.* **79**, 349 (1963).
- <sup>7</sup> A. ANTONIS and I. BERSOHN, *Lancet* **1**, 998 (1960).
- <sup>8</sup> A. ANTONIS and I. BERSOHN, *Lancet* **1**, 3 (1961).
- <sup>9</sup> P. T. KUO and D. R. BASSET, *Ann. intern. Med.* **79**, 1199 (1965).
- <sup>10</sup> E. A. NIKKILA and K. OJALA, *Life Sci.* **4**, 937 (1965).
- <sup>11</sup> W. G. DUNCOMBE, *Biochem. J.* **88**, 7 (1963).
- <sup>12</sup> P. J. N. HOWORTH, *Clin. chim. Acta* **14**, 69 (1966).
- <sup>13</sup> A. ROVESCALLI, *Attual. Lab.* **11**, 33 (1965).
- <sup>14</sup> D. WATSON, *Clin. chim. Acta* **5**, 637 (1960).
- <sup>15</sup> N. ZOLLNER, *Dt. med. Wschr.* **84**, 386 (1959).
- <sup>16</sup> S. LARTILLOT and CH. VOGEL, *Feuill. Biol.* **11**, 39 (1970).
- <sup>17</sup> F. H. SCHMIDT, *Klin. Wschr.* **39**, 1244 (1961).
- <sup>18</sup> H. E. RENSCHLER, *Dt. med. Wschr.* **90**, 2349 (1965).
- <sup>19</sup> C. N. HALES and P. J. RANDLE, *Biochem. J.* **88**, 137 (1963).

## Calcium in Myonuclei: Electron Microprobe X-Ray Analysis

Our previous work with electron microprobe X-ray analysis of embedded thin sections of skeletal muscle and myocardium has shown that calcium can be detected and localized at a subcellular level and that, indeed, the highest intracellular concentration of the element is in the nucleus<sup>1,2</sup>. Here we report further studies of the calcium distribution, in particular within the nucleus, and studies of the changes occurring in conditions known to increase intracellular calcium<sup>3</sup>. We have been especially concerned in this recent work to see how the observed distributions are affected by different methods of specimen preparation.

Three types of preparation were studied. a) Frogs' sartorius muscles were fixed and embedded in various ways (some are shown in Table I). b) Hearts of rats, either untreated (controls) or injected with isoproterenol (100 mg/kg i.p. 6 h before sacrifice), were fixed in a mixture of 1% osmium and 2% pyroantimonate ( $K_2H_2Sb_2 \cdot 4H_2O$ )<sup>3</sup>. The embedding was either conventional in

Epon 812 or in a polymerizable mixture of 50% glutaraldehyde and urea in which dehydration is omitted<sup>4</sup>. c) Biopsies were taken from the heart of an open-chested dog during slow and fast heart rates induced by electrical pacing<sup>5</sup>. The tissue was fixed in a mixture of osmium plus pyroantimonate, as in b), dehydrated, and embedded in Epon 812.

- <sup>1</sup> R. YAROM and J. A. CHANDLER, *J. Histochem. Cytochem.* **22**, 147 (1974).
- <sup>2</sup> R. YAROM, P. D. PETERS, M. SCRIPPS and S. ROGEL, *Histochemistry* **38**, 143 (1974).
- <sup>3</sup> R. YAROM, D. BEN-ISHAY and O. ZINDER, *J. molec. Cell Cardiol.* **4**, 559 (1972).
- <sup>4</sup> D. C. PEASE and R. G. PETERSON, *J. Ultrastruct. Res.* **41**, 133 (1972).
- <sup>5</sup> The open chest dogs' hearts experiments were done in the Hadassah laboratory of cardiac physiology as part of a study on myocardial calcium fluxes now in preparation with the collaboration of Dr. J. KEDEM and P. S. ROGEL.