bound by EDTA-$Na_2$, does not play a role in the processes taking place in the capillary wall.

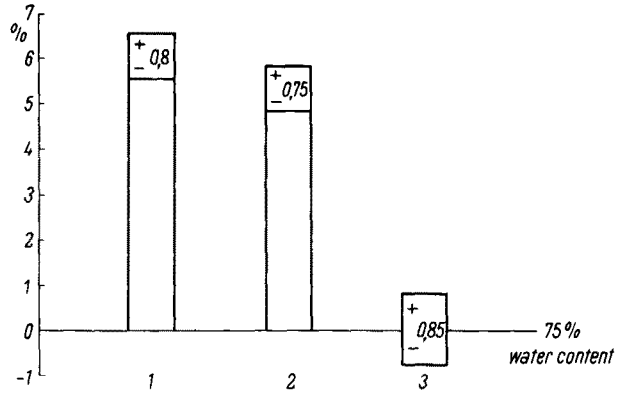


Fig. 3.—The changes in water content of the paw skin of rats. 0% in the diagram indicates the normal water content of skin (75%). Column 1: Histamine (200 $\gamma$, subcut.) (6). Column 2: EDTA-$Na_2$ (370 $\gamma$, subcut.) (12), and EDTA-$Na_2$+Antistin (400 $\gamma$, subcut.), or Synopen or Dibenamine (800 $\gamma$, intraven.) (18). Column 3: Ca-EDTA-$Na_2$ (410 $\gamma$, subcut.) (6), sodium citrate (200 $\gamma$, subcut.) (6) or saline (0·1 ml, subcut.). All substances were dissolved in 0·1 ml saline. The number of animals used in each group is given in brackets.

In 1954 MEYER[6] assumed that EDTA-$Na_2$ might, in some way, influence capillary function. Our observations would seem to corroborate this concept and suggest that the local oedema-producing effect of EDTA-$Na_2$ is due to a binding of the calcium in the interendothelial cement substance of the capillaries.

Thanks are due to Dr. A. KÖRÖSSI and to Dr. S. SAJÓ, Budapest, for their valuable help and suggestions during the course of this study.

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*Zusammenfassung*

Die Lösungen oder Suspensionen von Äthylendiamintetraacetatdinatrium (EDTA-$Na_2$) erzeugen an der Applikationsstelle Ödeme, welche wahrscheinlich der Bindung von Kalzium in der interendothelialen Zementsubstanz der Kapillaren zuzuschreiben sind.

[6] K. MEYER, Connective Tiss. Conf. Fifth Trans. (J. Macy Jr. Found., New York 1954), p. 73.

## The Citric Acid Content of Embryos of *Rana esculenta*

It was previously shown that the mode of determination of the developing crystalline lens in *Rana esculenta* varies with temperature, and that the lactic acid content of gastrulae and neurulae is lower at 12°C than at 25°C[1,2]. Although the effect of temperature on the citric acid content was found to be small, the experimental results may have some value since the literature contains but few data on this subject.

Citric acid content of *Rana esculenta* embryos (µg/100 embryos)

| Stage | 25°C (*n*) | | 12°C (*n*) | | Average (*n*) | |
|---|---|---|---|---|---|---|
| 10 | — | — | 44 | (1) | 44 | (1) |
| 10½ | 42 | (1) | — | — | 42 | (1) |
| 11 | — | — | 46 | (1) | 46 | (1) |
| 11½ | — | — | — | — | — | — |
| 12 | — | — | — | — | — | — |
| 12½ | 67 | (1) | — | — | 67 | (1) |
| 13 | — | — | — | — | — | — |
| 13½ | — | — | — | — | — | — |
| 14 | 44 | (2) | 58 | (1) | 49 | (3) |
| 14½ | — | — | — | — | — | — |
| 15 | — | — | — | — | — | — |
| 15½ | — | — | — | — | — | — |
| 16 | 38 | (2) | 69 | (2) | 54 | (4) |
| 16½ | 50 | (1) | — | — | 50 | (1) |
| 17 | — | — | 50 | (1) | 50 | (1) |
| 17½ | 56 | (2) | 68 | (1) | 60 | (3) |
| 18 | 46 | (2) | 96 | (1) | 63 | (3) |
| 18½ | — | — | — | — | — | — |
| 19 | 71 | (2) | 59 | (1) | 67 | (3) |
| 19½ | 71 | (1) | — | — | 71 | (1) |
| 20 | 63 | (2) | 83 | (1) | 70 | (3) |
| 20½ | 78 | (6) | 81 | (5) | 79 | (11) |
| 21 | 75 | (4) | 73 | (3) | 74 | (7) |
| 21½ | — | — | — | — | — | — |
| 22 | 91 | (4) | 123 | (2) | 100 | (6) |
| 22½ | 107 | (2) | 86 | (1) | 100 | (3) |
| 23 | 94 | (4) | 81 | (3) | 88 | (7) |
| 23½ | 168 | (2) | — | — | 168 | (2) |
| 24 | 155 | (2) | 92 | (3) | 123 | (5) |
| 24½ | 125 | (1) | 68 | (1) | 97 | (2) |
| 25 | 128 | (7) | 67 | (3) | 111 | (10) |

Stages according to SHUMWAY.
(*n*) number of duplicate experiments.

Eggs were reared in tap water either at 12°C or at 25°C. For citric acid determinations, 40 to 200 embryos were ground in 10% trichloroacetic acid and after centrifugation the supernatant was assayed by the method of NATELSON *et al.*[3]. One extraction was sufficient. Within the range from 10 to 40 µg, the standard deviation was 3%. Mostly the amount of citric acid was calculated per embryo in order to compare identical stages from the same batch, reared at different temperatures. In some cases, fresh or dry weight was determined. Stages were indicated according to SHUMWAY[4].

The results are presented in the Table and Figure 1. The quantity of citric acid per embryo varied largely between different batches, probably mainly because of size differences. However, the general trend is clear: after a slow increase between stages 10 and 17, the content rises more rapidly until stage 22, whereafter it increases still more at 25°C, but declines at 12°C. Finally it decreases at both temperatures. Only in one period, a difference between the two temperature groups could thus be found. In some experiments on larvae, reared at room temperature until some days after stage 25 and fed with vegetal material, dry weight was determined. First, 400 larvae were used for a (duplicate) assay: each larva weighed 0·53 mg and contained 0·71 µg (134 mg%) citric acid. The next day another 400 larvae of the same group, which had been starved for one day, had an average weight of 0·46 mg and contained 0·62 µg (111 mg%). Four experiments were made on ovaries of *Rana temporaria*, shortly before

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the laying season; these contained 18 mg% citric acid (fresh weight). It may be concluded that citric acid is synthesized especially in the period between stages 17 (tailbud) and 24, when it increases by nearly 200%.

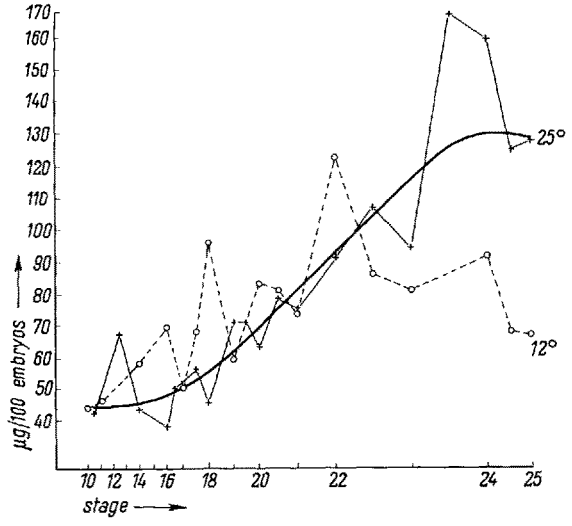


Fig. 1.—Citric acid content of *Rana esculenta* embryos during development at 12°C (broken line) and 25°C. Stages according to SHUMWAY, for *Rana pipiens*

Thereafter it decreases to 0·7 µg per larva, only about 50% more than in the early stages. An effect of temperature is found only between stages 22 and 25, when at 12°C the content remains lower and decreases sooner and more strongly than at 25°C. This might be explained by a difference in the intrinsic, chemical development, in that the cold larvae have reached the lower values of later stages relatively earlier than the warm larvae. Another explanation might be that the metabolism is relatively higher in the cold group, leading to a more rapid exhaustion of the food reserve in the yolk, since the animals were starved. It has been reported that in this species the total amount of oxygen, consumed up to a given larval stage, is about 35% higher at 12 than at 25°C[2]. However, starvation cannot be the sole cause of the decline, since larvae which received plenty of food contained still lower amounts in later stages. A similar decrease was observed by DICKENS[5] in the chick, from about 30 mg% in $3^1/_2$- and 5-day old embryos to 13 mg% at 8 and 12 days of incubation. The citric acid content of frog's ovaries (18 mg%) does not differ much from the values found in the yolk of chicken eggs (15·5 and 13 mg%) by THUNBERG[6] and by TÄUFEL and PAHLOUDEK-FABINI[7].

For a comparison of the other data with those of the literature, calculations must be made on a fresh weight basis. Previously a dry to wet weight ratio of 1:4 was found in embryos of the same species[2]. Assuming a wet weight of the larvae of 2 mg, and, for this approximate calculation, neglecting the losses due to combustion, the following values may be used: starting development with a citric acid content of 20 mg%, a maximum of 75 mg% is reached, followed by a decrease to 35 mg%. These values are generally higher than those which DICKENS found in chick embryos. As compared with animal tissues, the embryonic content is rather high, equalling that of cartilage, in which DICKENS found 35 mg%[5]. It must be concluded that the higher values, encountered in tailbud and early larval stages represent a real embryonic citric acid synthesis.

Some possible explanations for this phenomenon may be indicated. First, as indicated by DICKENS, it might be a consequence of a high carbohydrate metabolism. However, this type of metabolism prevails only in gastrulae and neurulae. Second, it might be a consequence of a high activity of the citric acid cycle which, through oxidative phosphorylation, can provide a high amount of energy in a period of development when the synthesis of specific chemical components is prominent. It should be pointed out that the amount of citric acid actually present only reflects the equilibrium between synthesis and degradation of this acid in the metabolic cycle. Finally, it is known that the citric acid synthesis is mainly located in the mitochondria (SCHNEIDER *et al.*[8]; SZÉKELY[9]). The development and differentiation of mitochondria may thus be highly important. Indeed there is an increase both in number and in complexity of these organellas during neurula and tailbud stages (BOELL and WEBER[10]; EAKIN and LEHMANN[11]). This might be reflected in the rise of citric acid content. The connections between mitochondria and oxidative metabolism seem to make it worth while to compare the curves of respiratory rate and of citric acid during development. Figure 2 represents both on a semilog scale; the data on respiration (at 25°C) were taken from previous experiments on the same species[2]. Some parallel seems to exist between the third part of the respiratory curve and the citric acid curve. Whether this relation has a real basis must be left open, but it seems to suggest some correlation between both functions of mitochondria *in vivo* experiments. Perhaps the differentiation of mitochondrial crests might be involved in both processes.

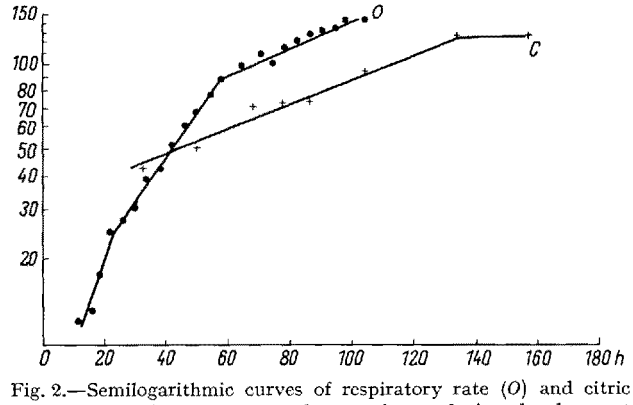


Fig. 2.—Semilogarithmic curves of respiratory rate (*O*) and citric acid content (*C*) of *Rana esculenta* embryos during development at 25°C. Abscissa: time of development. Ordinate: Log. oxygen uptake in µl/h/100 embryos and log. citric acid content in µg/100 embryos

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*Division of Chemical Embryology, Laboratory of Anatomy and Embryology, Municipal University, Amsterdam, July 10, 1958.*

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*Zusammenfassung*

Der Zitronensäuregehalt wurde vom Gastrulastadium bis in larvale Stadien gemessen. Während des Schwanzknospenstadiums und kurz nachher erfolgt eine relativ rasche Zunahme, die möglicherweise in Zusammenhang mit der Entwicklung und Differenzierung von Mitochondrien steht.

## Influenza del $Mg^{++}$ sulla «eptoformazione non ossidativa» nel muscolo scheletrico

In ricerche precedenti[1-3] abbiamo osservato, in estratti enzimatici di muscolo scheletrico di ratto, il processo indicato da BONSIGNORE *et al.*[4] col termine di «eptoformazione non ossidativa», e cioè la formazione di eptosofosfato da esoso-fosfato (G-6-P e F-6-P) attraverso reazioni non appartenenti allo shunt ossidativo. Nelle nostre condizioni, tale eptoformazione veniva aumentata per aggiunta di fruttoso-1,6-difosfato (F-1,6-P).

Per spiegare i dati sperimentali abbiamo ammesso – come suggerito da BONSIGNORE *et al.*[4] – che la velocità del processo eptoformativo dal solo esoso-fosfato fosse «limitata» da tracce di trioso-fosfato presenti nell'estratto enzimatico. Nelle nostre condizioni, il F-1,6-P aggiunto avrebbe agito in quanto aumentava, per scissione aldolasica, la concentrazione del trioso-fosfato.

Nella Tabella seguente sono esposti i risultati di alcune esperienze[5] che suffragano, a nostro parere, questa ipotesi.

Dalla Tabella risulta:

1) Aumentando la concentrazione del F-6-P – nelle prove contenenti questo solo substrato – da 7,5 a 10,0 $\mu M$, la eptoformazione non amuenta. Quindi nelle prove enzimatiche in presenza di «F-6-P+F-1,6-P», l'aumento della concentrazione del F-6-P dovuto alla idrolisi del legame fosforico in 1 dell'estere difosforico, non dovrebbe influire sulla eptoformazione, essendo già ottimale la concentrazione delle 7,5 $\mu M$ di F-6-P aggiunte.

2) Aggiungendo $Mg^{++}$, attivatore della idrolisi fosfatasica in 1 a carico del F-1,6-P, si ha – nelle prove in presenza di ambedue i substrati – una diminuzione della eptoformazione. Questa diminuzione è spiegabile in quanto la attivazione dell'idrolisi fosfatasica da parte degli $Mg^{++}$ sottrarebbe il F-1,6-P alla azione aldolasica. Ne deriverebbe una minore formazione di trioso-fosfato e conseguente minore eptoformazione.

3) L'aggiunta, oltre al $Mg^{++}$, di KF – inibitore della attivazione degli ioni $Mg^{++}$ sull'attività fosfatasica del nostro estratto – ripristina pressochè quantitativamente l'eptoformazione nelle prove in presenza di F-6-P + F-1,6-P.

Tutti questi fatti depongono a favore dell'ipotesi che il F-1,6-P nelle nostre condizioni agisca come donatore di trioso-fosfato, necessario per la eptoformazione, e non come donatore di F-6-P.

Attività eptoformativa di estratti enzimatici di muscolo scheletrico a partire da F-6-P e da F-1,6-P, in assenza e in presenza di $MgCl_2$ (10 $\mu M$) e di $MgCl_2$ (10 $\mu M$) + KF (200 $\mu M$).
Volume finale delle prove: 2,5 ml

| Substrati | Aggiunte | $\mu M$ eptoso formato/ mg N proteico | % F-1,6-P idrolizzato* |
|---|---|---|---|
| 7,5 $\mu M$ F-6-P . . . . | — | 0,18 | — |
| 10 $\mu M$ F-6-P . . . . | — | 0,17 | — |
| 7,5 $\mu M$ F-6-P . . . . | $MgCl_2$ | 0,18 | — |
| 7,5 $\mu M$ F-6-P + 1,8 $\mu M$ F-1,6-P | — | 0,41 | 53% |
| 7,5 $\mu M$ F-6-P + 1,8 $\mu M$ F-1,6-P | $MgCl_2$ | 0,27 | 62% |
| 7,5 $\mu M$ F-6-P + 1,8 $\mu M$ F-1,6-P | $MgCl_2$ + KF | 0,36 | 48% |
| 1,8 $\mu M$ F-1,6-P . . . | — | 0,12 | 50% |
| 1,8 $\mu M$ F-1,6-P . . . | $MgCl_2$ | 0,13 | 62% |

* L'idrolisi del F-1,6-P è stata constatata dosando i fosfati liberi nel mezzo, prima e dopo l'incubazione, con il metodo di BERENBLUM e CHAIN[6]. Tale idrolisi, maggiore in presenza di $Mg^{++}$, si svolgeva a livello del legame fosforico in 1. Infatti il F-6-P non veniva idrolizzato nè in assenza nè in presenza di $Mg^{++}$.
È noto d'altra parte che gli ioni $Mg^{++}$ attivano la F-1,6-P-asi del muscolo scheletrico[7].

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*Summary*

$Mg^{++}$ influence on 'non-oxydative heptoformation' from hexose-phosphate has been studied in enzymatic preparations of rat skeletal muscle. The results give further evidence that F-1,6-P, added to F-6-P, increases the rate of heptoformation inasmuch as it gives, by aldolase action, triosephosphate.

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## Analyses immunoélectrophorétiques des fractions protéiques du sérum humain séparées par électrophorèse en gel d'amidon

Le sérum humain, soumis à une électrophorèse en gel d'amidon se scinde en de nombreux composants (SMITHIES[1]). Ce pouvoir de résolution du milieu, plus élevé que celui du papier ou de la gélose résulte d'un phénomène complexe régissant dans un gel d'amidon, la position des fractions en fin d'électrophorèse.

Dans les modes habituels d'électrophorèse de zone (papier ou gélose) la mobilité en fin d'expérience est due à la résultante du courant électrique et de l'électro-osmose. En gel d'amidon la mobilité habituelle d'une protéine se trouve perturbée par le pouvoir du gel à ralentir les protéines composées de grosses molécules, à l'avantage de celles composées de molécules de plus petites dimensions.

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