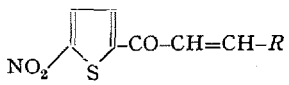
Tableau II. Groupe des naphtols

Substance	Formule	Concentration staphylostatique minimale en γ/ml
6-bromo-2-naphtol		retard à 10
6-tertioamyl-2-naphtol		10
1-chloro-6-tertiobutyl-2-naphtol	VIII	5
1-chloro-6-tertioamyl-2-naphtol	IX	5
1-bromo-6-tertioamyl-2-naphtol		5
1-bromo-6-tertiobutyl-2-naphtol		10
6-(1-méthyl-1-cyclohexyl)-2-naphtol		10
6-(1-éthyl-1-cyclohexyl)-2-naphtol		10
1-chloro-6-(1-méthyl-1-cyclohexyl)-2-naphtol	X	2,5
1-bromo-6-(1-méthyl-1-cyclohexyl)-2-naphtol		10
1-chloro-6-tertiobutyl-2-méthoxy-naphthalène		> 10
1-chloro-6-(1-méthyl-1-cyclohexyl)-2-méthoxynaphthalène		> 10

Tableau III. Groupe des chalcones

Substance	Formule	Concentration staphylostatique minimale en γ/ml
furylidène-(5-nitro-2-acétothiénone)	XI	retard à 10
furylidène-(5-bromo-2-acétothiénone)		retard à 10
benzal-(5-nitro-2-acétothiénone)	XII	10 (retard à 1)
o-méthoxybenzal-(5-nitro-2-acétothiénone)	XIII	10
p-chlorobenzal-(5-nitro-2-acétothiénone)	XIV	1
p-diméthylaminobenzal-(5-nitro-2-acétothiénone)	XV	retard à 10
2-thénylidène-parafluoroacétophénone		retard à 10
2-thénylidène-(5-éthyl-2-acétothiénone)		retard à 10
5-méthyl-2-thénylidène-(5-éthyl-2-acétothiénone)		retard à 10
vératrylidène-parachloroacétophénone		retard à 10



XI; $R =$ 

XII; $R = -C_6H_5$

XIII; $R = -C_6H_4(OCH_3)_{(o)}$

XIV; $R = -C_6H_4Cl_{(p)}$

XV; $R = -C_6H_4\text{-}N(CH_3)_{2(p)}$

Summary

Some substituted derivatives of resorcinol and β-naphthol, and a chalkone derived from thiophene, namely *p-chlorobenzal(5-nitro-2-acetothienone)*, are shown to possess high bacteriostatic activity towards *Micrococcus pyogenes* var. *aureus*; no such activity was found towards *Escherichia coli*, even at higher concentrations.

The Distribution of Mitochondria in Sea Urchin Embryos[1]

It has been reported[2] that in developmental stages of *Psammechinus miliaris*, observed after staining with Nile Blue sulfate, mitochondria are not present in large numbers until the mesenchyme blastula and young gastrula stages, at which time a gradient of the organelles appears, decreasing from the animal to the vegetal pole. The presence of such a gradient was established by counting relative numbers of mitochondria along the animal-vegetal axis. Localized centers of mitochondrial production were observed later in development, suggesting to the authors that the differentiation of an organ rudiment is correlated with the appearance of mitochondrial populations in it. Changes in mitochondrial distribution in embryos treated with lithium or iodosobenzoic acid were interpreted in terms of a double gradient of metabolic types, the animal-vegetal gradient favoring mitochondrial production, the opposite one inhibiting it.

The present studies concern mitochondrial distribution in normal developmental stages of two sea urchins, *Lytechinus pictus* and *Strongylocentrotus purpuratus*. Observations on eggs and embryos with phase contrast, or after vital staining, or after fixation and staining according to methods recommended for the visualization of mitochondria, have led to somewhat different conclusions from those cited above, for this material.

Method. The conditions of observation described by GUSTAFSON and LENICQUE[1], using vital dyes, were duplicated insofar as possible. After staining with Nile Blue sulfate[2] or Janus green[3] (dye concentrations of 0·05–0·1 %), eggs or embryos[4] were flattened under a

[1] Research supported by the National Cancer Institute, National Institutes of Health, United States Public Health Service.

[2] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952).

[1] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952.

[2] Grübler (Leipzig) stain.

[3] Nat. Aniline & Chem. Co., New York: Cert. No. Nj-1.

[4] Cultures maintained at 17°C.

vaseline-ringed cover glass. Specimens in the desired orientation were studied under oil immersion[1]. In attempting counts along the animal-vegetal axis, an eye piece reticule of 10 × 10 squares was employed. With later stages (mesenchyme blastula, gastrula), counts were tried on enlarged photographs of both vitally stained and fixed material.

Preliminary trials with several fixatives and stains indicated that the method recommended by HARMAN[2] for the visualization of mitochondria produced the best results. Large numbers of eggs or embryos can be fixed (Altmann's fluid or osmium tetroxide, 2%) and flattened between cover slips according to the procedure described by TYLER[3]; the specimens were stained with fast green[4] at 60°C and counterstained with safranin[5], cleared and mounted in balsam or Clarite.

Observations on homogenates of eggs or embryos, prepared in 0·78 or 1·1 molar sucrose, made up in phosphate buffer, 0·005 molar, pH 7·5, were made with an oil immersion dark M phase objective.

Results. Phase contrast study of homogenates of *L. pictus* and *S. purpuratus* revealed that, contrary to the report of GUSTAFSON and LENICQUE[6] for *P. miliaris*, there are large numbers of cytoplasmic inclusions corresponding to mitochondria in size, shape and structure, in unfertilized eggs and early stages. In homogenates of mesenchyme blastulae and young gastrulae there is an increase in rodshaped mitochondria, spherical or ovoid forms predominating in the earlier stages. Although GUSTAFSON and LENICQUE[6] give no size range for the mitochondria observed in *P. miliaris*, it is possible that the mitochondrial precursors which they describe, in early stages, as "very small blue units", correspond to smaller mitochondria (0·5–1·0 micra). Particles in this size range have been observed by HARVEY[7], in *Arbacia punctulata*, and in the present studies, in the "clear quarters" of eggs fragmented by centrifugation. It has recently been suggested[8], after a study of electron micrographs of ultra-thin sectioned centrifuged eggs of *Arbacia*, that these "fine granules" may be smaller mitochondria.

Examination of early blastula wall cells, which develop ciliated borders, revealed the presence of larger numbers of cytoplasmic particles corresponding to mitochondria than are found in the cells of more internal regions. Later concentrations of mitochondria in cells of the ciliated bands and gut, suggest a correlation between physiological activity and mitochondrial number. This correspondence, if it exists, however, need not bear a causal relationship to the differentiation of these regions.

The enumeration of mitochondria in intact cells of mesenchyme blastulae or young gastrulae, vitally stained with Nile Blue sulfate or Janus green, has not produced reliable results. Observations made either by focussing through the body wall or at one focal level, revealed approximately the same numbers of cytoplasmic inclusions absorbing the dyes in cells at all levels of the animal-vegetal axis. There are at least three reasons why the difficulties inherent in this method appear to render it incapable of yielding replicable results.

(1) The variability of mitochondria in size (0·5–3·0 or more micra) and shape (spherical, ovoid, rod-shaped, "dumb-bell"-shaped) is well known[1]. When they are stained with a dye (Nile Blue sulfate) which is also taken up by other cellular elements (yolk granules, pigment, fat droplets) in the same size range, it becomes very uncertain which of the dye-absorbing particles are mitochondria. Some observations of centrifuged eggs stained with Nile Blue sulfate, indicate only slight differences in the dye absorption of the different strata of granules. Janus green staining of intact eggs or embryos did not make mitochondrial identification much more reliable.

(2) Embryos stained with Nile Blue sulfate or Janus green have been carefully observed during flattening under the cover glass. Cells in the animal and vegetal regions of mesenchyme blastulae or gastrulae flatten at different rates, the latter having less resistance to pressure, and eventually rupturing sooner than the animal hemisphere cells under continued pressure. As the cells flatten, spreading of granules over a larger area occurs in the more vegetal regions, producing the image of fewer particles per unit area. Visible differences in dye uptake towards animal or vegetal poles in fixed embryos probably are also due to this unequal flattening of cells.

(3) Gradients of other substances in intact embryos, decreasing in a vegetal-animal direction, would tend to obscure mitochondria in more vegetal cells. Large numbers of yolk granules and oil droplets in vegetal cells "mask" other types of inclusions. When embryos are observed under pressure with phase microscope, cells which have been ruptured clearly show large numbers of dark bodies in the cytoplasm which can only be vaguely made out in less flattened cells. Similar observations have been made in vitally stained embryos.

In embryos fixed with Altmann's fluid and stained with fast-green and safranin, mitochondria are more readily identifiable than after vital staining. Observations of cells along the animal-vegetal axis of such embryos, with the highest power oil immersion lens, revealed no significant differences in numbers of mitochondria. As with the vitally stained specimens, however, mitochondrial counts were not replicable from embryo to embryo, for the reasons given above.

As development continues, there is a pronounced and rather rapid increase in mitochondria in the cells of differentiating gut structures (intestine, oesophagus, stomach and proctodeum). Continuing the tendency noted in the earlier ciliated cells, there is also an increase in mitochondria in cells forming the ciliated bands, extending across the body, and the arms of the developing pluteus. At the same time, there is a relative decrease in mitochondria in the cells of the body wall.

A quantitative method for counting mitochondria in homogenates[2] has been adapted for use with developmental stages of sea urchins[3]. Preliminary results with *L. pictus* indicate: (1) Mitochondria in an unfertilized egg occupy about 15% of the volume of the cell; (2) A rapid increase in mitochondria at the mesenchyme blastula stage followed by a decrease in mitochondria in gastrulae and young prism stages (about 25 h at 17°C). It is interesting that it is about this time when GUSTAF-

[1] Zeiss 2 mm Apochromat.

[2] J. HARMAN, Stain Tech. *25*, 69 (1950).

[3] TYLER, Collecting Net *19*, 40 (1946).

[4] Fast green, FCF, Nat. Aniline & Chem. Co., New York. Cert. No. NCf9. 4% in 10% aniline.

[5] Safranine-O, Nat. Aniline & Chem. Co., New York. Cert. No. NS-7. 1 % in 50% ethyl alcohol.

[6] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952).

[7] HARVEY, J. Exp. Zool. *102*, 253 (1946).

[8] LANSING and HILLIER, cited in J. Hist. Cytochem. *1*, 265 (1954).

[1] *Cf.* J. HARMAN, Exp. Cell Res. *1*, 394 (1950).

[2] E. SHELTON, W. C. SCHNEIDER, and M. J. STRIEBICH, Exp. Cell Res. *4*, 32 (1953).

[3] Details of the method and data will be published elsewhere.

SON and LENICQUE[1] noted an overall decrease in mitochondria in *P. miliaris*.

J. R. SHAVER

Division of Biology, California Institute of Technology, Pasadena, March 15, 1955.

Zusammenfassung

Zytologische Untersuchungen an Embryonen von zwei Seeigelarten haben in bezug auf die Lokalisation der Mitochondrien keinen Unterschied zwischen animaler und vegetativer Hemisphäre ergeben. Vorläufige Versuche, mit Hilfe einer quantitativen Methode die Mitochondrien in Homogenaten zu zählen, ergaben eine starke Zunahme auf dem Stadium der Blastula und eine anschliessende Abnahme der Mitochondrienzahl pro Embryo.

[1] T. GUSTAFSON and P. LENICQUE, Exp. Cell Res. *3*, 251 (1952).

Factors Influencing the Fatty Acid Oxidation of Tumor Mitochondria with Special Reference to Changes in Spontaneous Mouse Hepatomas

WEINHOUSE and CHAIKOFF *et al.* gave a conclusive demonstration that slices of tumors are capable of oxidizing fatty acids[1]. Cell-free preparations of a number of tumors did not show this property, however[2]. It is the object of this short communication to report on some of the factors influencing the oxidation of fatty acids by tumor mitochondria *in vitro*, and especially on changes in this respect occuring in mitochondria from spontaneous hepatomas. So far as we are aware, the present data furnish the first positive evidence of fatty acid oxidation by tumor mitochondria.

The hepatomas studied arose in a high incidence in two year old female F_1(♀ C_{57} Black × ♂ C_3He) mice[3] which were kindly put at our disposal by Dr. O. MÜHLBOCK.

The tumors were always divided in two groups, consisting of hepatomas of less than 1 g, which we shall call early stage tumors, and tumors weighing 2–10 g, which are designated here as fully developed tumors. Mitochondria, prepared from both specimens in isotonic (0·25 m) sucrose[4], were washed twice in a volume of cold isotonic sucrose equal to twice the original tissue weight, and the suspension prior to incubation–the preliminary suspension– was made in a (0·13 m) KCl-(0·013 m) phosphate buffer (pH 7·4). After the mitochondrial suspension was pipetted into Warburg flasks containing the proper additions (Table I), only the mitochondria from the early stage tumors showed an oxidation of octanoate with α-oxy caproate and L-malate as "sparkers" of fatty acid oxidation[5].

In a previous publication[6], it was shown that mitochondria prepared from many mouse tumors exhibit a marked "adenosine triphosphatase (ATPase) activity", as measured by the complete inhibition of fatty acid oxidation of normal liver mitochondria after addition of these tumor mitochondria. Most tumor mitochondria showed this high "ATPase activity" irrespective of the fact whether the preliminary suspension was made in isotonic sucrose or in KCl-phosphate buffer. At the same time it was found that no oxidation of octanoate took place by these mitochondria *per se* after addition of NaF (0·01 m) diphosphopyridine nucleotide (10^{-4} m) and coenzyme A (10 γ).

The mitochondria from the fully developed hepatomas also showed a high "ATPase activity", when the preliminary suspension was made in the KCl-phosphate buffer, but, when isotonic sucrose devres as the suspending medium, only a moderate or slight "ATPase activity" was recorded. This was more or less anticipated in view of the apparent lack of octanoate oxidation by the mitochondria from the preliminary KCl-phosphate suspension. After addition of fluoride–a well-known inhibitor of ATPase–a small oxygen consumption resulting from octanoate oxidation could sometimes be seen. Mitochondria from the preliminary sucrose suspension were somewhat variable in their oxidative behaviour, sometimes showing an oxygen consumption with octanoate as the substrate. Isolation of the mitochondria with isotonic sucrose containing 0·001 ethylenediamine tetraacetate (versene) resulted in an enhanced oxidation.

The "ATPase activity" of the mitochondria from the early stage tumors was considered as small or absent, because even the mitochondria from the preliminary KCl-phosphate suspension were capable of oxidizing octanoate; this was confirmed by measurements of the "ATPase activity" in the way indicated.

The absence of "ATPase activity" does not necessarily mean that tumor mitochondria in general are able to effect fatty acid oxidation. Mitochondria prepared from two different transplanted interstitial cell tumors of the mouse testis never showed such ATP splitting activities as would influence the fatty acid oxidation of normal liver mitochondria appreciably. The mitochondria from one of these tumors, prepared either with or without versene, were, however, unable to oxidize fatty acids under the conditions of the present experiments. The mitochondria from the other testis tumors, prepared with sucrose-versene, showed a vivid oxidation of octanoate. Another example may be mentioned of a transplanted granulosa cell tumor of the mouse ovary. Mitochondria prepared from this tumor with isotonic sucrose and suspended in KCl-phosphate buffer had a moderate or marked "ATPase activity". When the preliminary suspension was made in isotonic sucrose and added to normal liver mitochondria, no influence on the fatty acid oxidation of the liver mitochondria could be detected, but the tumor mitochondria *per se* showed no octanoate oxidation. When the whole procedure of preparing the tumor mitochondria was carried out with 0·001 m versene included in the isotonic sucrose solution, a very large oxygen consumption resulted from octanoate oxidation.

Changes in the mitochondrial nitrogen content of the spontaneous hepatomas were also noted. The nitrogen content of the mitochondria per 100 mg fresh tissue of the early stage hepatomas was always found to be higher than the corresponding values of the fully developed tumors, e.g. 0·39 against 0·28 mg N on the average. This may be the result of the greater blood content of the latter. The division of the spontaneous hepatomas into the two groups mentioned was merely

[1] S. WEINHOUSE, R. H. MILLINGTON, and C. E. WENNER, Cancer Res. *11*, 845 (1951). – D. D. CHAPMAN, G. W. BROWN Jr., I. L. CHAIKOFF, W. O. DAUBEN, and N. O. FAUSCH, Cancer Res. *14*, 372 (1954).

[2] C. G. BAKER and A. MEISTER, J. Natl. Cancer Inst. *10*, 1191 (1950).

[3] O. MÜHLBOCK, unpublished.

[4] W. C. SCHNEIDER and G. H. HOGEBOOM, J. Biol. Chem. *183*, 123 (1950).

[5] N. WATERMAN, C. J. BOS, and T. J. BARENDREGT, Enzymologia *15*, 307 (1952). – P. EMMELOT and C. J. BOS, Enzymologia *17*, 13 (1954).

[6] P. EMMELOT and C. J. BOS, Biochim. Biophys. Acta *16*, 620 (1955).