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Riassunto

Da una soluzione di lipovitellina (la lipoproteina del tuorlo dell'uovo di pollo) trattata con spermi vivi di *Arbacia lixula* si ha liberazione di fosfolipidi. Questo esperimento è considerato un modello della reazione corticale di fecondazione dell'uovo di riccio di mare.

On the Incorporation of Radioactive Amino Acids into the Protein Fraction of *Torula utilis*

Torula utilis incorporates labeled carbon supplied in the form of DL-amino acids into its protein fractions by an enzymatic process[1]. It is of interest to learn whether there is a preferential utilization of one optical isomer. When an inert isomer is added to the radioactive racemic mixture prior to incubation with the organism the isomer should decrease by isotope dilution the amount of radioactive protein produced if the isomer is identical with that member of the racemic mixture which the cells select for incorporation into the protein fraction. The results given in the table were obtained by incubating *Torula utilis* with DL-alanine –1– C^{14}. Since there is a decrease in radioactivity of the protein fraction on addition of either the inert L or the D isomer, it must be assumed that the yeast cell utilizes both isomers for incorporation into the protein fraction.

Effect of Addition of Inert Optical Isomers on Incorporation of DL-alanine –1– C^{14} into *Torula utilis* Proteins

Substance Added	Relative Radioactivity of Protein Fraction
Control, standard medium only	100
10 mg inert L-alanine . . .	4·6
10 mg inert L-alanine	4·3
10 mg inert D-alanine . . .	12·8
10 mg inert D-alanine . . .	11·9

Each incubation flask contained 0·2 ml packed cells suspended in 2 ml standard medium (2) and 0·2 mg ($2{\cdot}4 \times 10^{-3}$ mc) of DL-alanine –1– C^{14}. The flasks were incubated at 37°C for 45 min in an oxygen atmosphere. The method of analysis has been described in a previous paper[2].

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Zusammenfassung

Der Eiweissanteil in Gegenwart von radioaktivem DL-Alanin gezüchteter Hefe zeigt eine Aktivitätsverminderung, wenn der Suspension nicht radioaktives D- oder L-Alanin zugefügt wird. Offenbar vermag die Hefezelle beide Isomere auszunützen.

[1] A. H. WEBB, F. FRIEDBERG, and L. M. MARSHALL, Biochim. biophys. Acta *6*, 568 (1951).

[2] F. FRIEDBERG and A. H. WEBB, J. Bact. *58*, 151 (1949).

L-Arginin als Bewegungsregulator des «Venenherzens»

Mit der völligen Isolierung einzelner Teilstücke von Flughautvenen der *Chiroptera* (Fledermäuse und Flughunde) und der Entwicklung des «Venensäckchen-Präparates»[1], haben wir die autochthone Pulsautomatie dieser glatt-muskulären Blutgefässe nachgewiesen. Dann konnte mit Binnendruckreizen der aktive Venenpuls ausgelöst und mit steigendem Druck die Pulsfrequenz erhöht werden. Die intravaskuläre Dehnung ist also ein natürlicher Reiz für die Venenperistaltik[2]. Ferner ermittelten wir eine auffallende Temperaturabhängigkeit der Venenpulsfrequenz, welche über ihren gesamten biokinetischen Temperaturbereich von rund 45°C streng der van-t'Hoff-Arrhenius-Regel gehorcht[3]. Unsere Untersuchungen über die Beteiligung des Sauerstoffs bei der autonomen Venenpulsation[4] wie auch die Temperaturversuche machen es wahrscheinlich, dass der Organisation der aktiven Pulsation ein vermutlich einfacher biochemischer Vorgang zugrunde liegt. Der Temperaturkoeffizient nach VAN T'HOFF Q_{10} entspricht jedenfalls dem, was man sonst von chemischen und enzymatisch gesteuerten Vorgängen her kennt.

Die Prüfung der elektrotonischen Erregbarkeitsänderungen[5] und die Reaktionen der Vene auf weitere elektrische Reize[6] ergaben Kriterien für die Primitivität des kontraktilen Apparates. Auch beim Elektrovenogramm fanden wir ausgesprochen elementare Verhältnisse[7].

Die Untersuchungen der ionalen Einflüsse auf diese Gefässe liessen erkennen, dass die Ionen selbst als Pulsauslöser nicht in Frage kommen können. In der adäquaten Ringerlösung kommt die aktivpulsierende Vene relativ rasch zum diastolischen Stillstand[8]. Mit den bekannten kreislaufaktiven Wirkstoffen, wie Adrenalin, Histamin und Acetylcholin, wurden in physiologischen Konzentrationen nur geringfügige Effekte erzielt[8].

Hingegen konnten wir mit dem dialysablen Anteil des Fledermausserums wie auch anderer Säugerseren meistens und ausgesprochen stark den Puls auslösen bzw. anregen und verstärken[8,9]. Wir haben inzwischen auf der Suche nach diesem vom Blutplasma gelieferten humoralen Auslöser systematisch die freien Aminosäuren geprüft und im L-*Arginin* den spezifischen Reizstoff gefunden. Nach fermentativem Abbau des Arginins mit Arginase, nach Decarboxylierung oder papierchromatographischer Abtrennung des Arginins unterbleibt die anregende Wirkung des zur Badeflüssigkeit (adäquate Ringerlösung) zugetropften Serums. Das L-*Arginin* (synthetisches und das aus dem Serum isolierte *Arginin*) bewirkt in Konzentrationen von 5 bis 100 γ pro cm^3 an der isolierten Flughautvene regelmässig und langfristig drei typische Vorgänge: 1. Frequenzsteigerung, 2. Tonuserhöhung und 3. Amplitudenvertiefung. Die ausführliche Beweisführung für das L-*Arginin* als herzaktive Substanz erscheint zusammen mit unseren Befunden über die Wirkungsweise diverser Herzextrakte in «Helvetica physiologica Acta». Wir halten hier nur das Hauptergebnis fest, dass der von uns früher als eiweissfreier Reizstoff im Serumdialysat eingeengte myotrope Faktor des Venenherzens das L-*Arginin* ist.

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[7] H. MISLIN, Exper. *4*, 28 (1948); Helv. physiol. Acta *8*, C74 (1951).
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[9] H. MISLIN, Z. f. Kreislaufforsch. *42*, 620 (1953).

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H. MISLIN

Zoologische Anstalt der Universität Basel, den 1. September 1953.

Summary

Based on former experiments it is shown that the venous pulse on the isolated vein of the membrane of *Chiroptera* (preparation of the venous sack) is caused by a humoral factor, which is isolated in the serum dialysate of bats and other mammals as L-*arginine*. This amino-acid is responsible for the increasing of amplitude, tonus, and frequency.

Antagonism between Tropolone and Colchicine in Rat Fibroblast Cultures

In extension of previous work on the mechanism of the metaphase arrest by colchicine and on agents antagonizing this effect in cultures of rat fibroblasts[1], we should like to present here a report on the influence of the simplest structural analogue of colchicine, namely tropolone (2-hydroxy-2,4,6-cyclo-heptatrien-1-one)[2], on this system.

In an earlier study it was established that three cycloheptene derivatives, tropolone, its methylether and 4,5-tetramethylene tropolone, did not appear to act as spindle poisons and, in contrast to colchicine, failed to produce any significant metaphase arrest[3]. Further analysis of these observations suggested that tropolone influenced the mitotic phase percentage in a direction opposite to the colchicine effect, and tests were run to determine the action of these two compounds, when applied simultaneously.

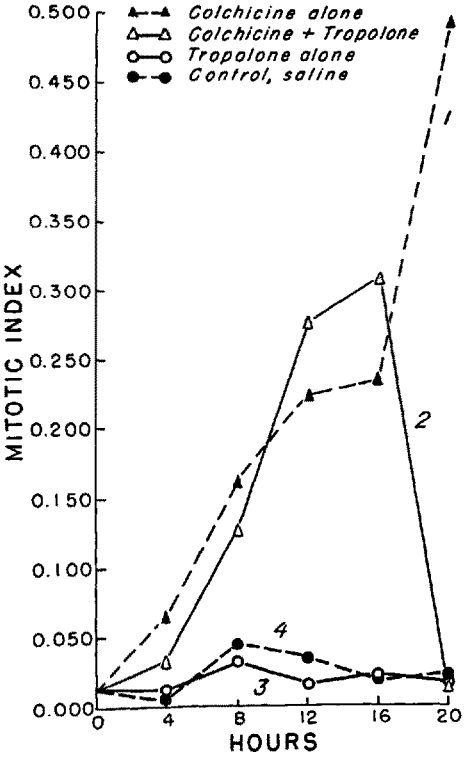
The results indicate that tropolone is a very potent antagonist of colchicine: it favors the formation of the spindle and reverses the metaphase arrest produced by colchicine. This action of tropolone takes effect quite suddenly in dividing rat fibroblasts after 16 h of exposure to the two agents. At this point in the cycle, tropolone, if applied alone, reduces the percentage of metaphase figures and raises the percentage of both pre- and post-metaphase figures, as compared with the untreated cultures. These findings are of special interest because of the structural relationship between colchicine and tropolone.

Fibroblasts, obtained from the subcutaneous areolar connective tissue of 3 to 4 months old male rats, were carried as stock cultures in roller tubes for 10 days and then transferred to MAXIMOW slides, where they were allowed to grow for 5 days before the experiments were begun. The slides for the various experimental groups were carefully matched to insure uniformity of tissue and equal distribution of explants from the different tubes. The experimental series consisted of 4 groups containing:

(1) 2×10^{-6} M colchicine;
(2) 2×10^{-6} M colchicine + 10^{-4} M tropolone;
(3) 10^{-4} M tropolone;
(4) saline (control).

The feeding mixture used in the control experiments (group No. 4) consisted of one volume of human placental serum, one volume of beef serum ultrafiltrate, one volume of chick embryonic extract, and two volumes of SIMMS' balanced saline solution. The latter served, in the experimental groups 1–3, as the solvent of colchicine, tropolone or both. These agents were introduced in quantities sufficient to give the effective final concentrations listed in the Table.

In our previous work on the reversal of the colchicine effect[1] it was noted that the metaphase arrest reached its peak, in terms of the mitotic index, and leveled off, at 20 h after application of the agent. When *meso*-inositol was included at the same time with colchicine, the rising curve broke sharply at 12 h, and by 20 h had descended to a level close to that of the controls. A similar arrangement was followed in the present study involving colchicine and tropolone, whose results are represented graphically in the Figure; one-fifth of the cultures in each group was, at 4 h intervals up to 20 h, fixed in HELLY's fluid. These were later stained with HARRIS' haematoxylin, and cell-counts were made according to the method previously described[1].



Mitotic indices, determined at 4-hour intervals, of rat fibroblast cultures exposed to colchicine and tropolone. (Compare with the Table).

As shown in the Figure, the mitotic index of the group receiving colchicine plus tropolone (No. 2) rises above that of the group receiving colchicine alone (No. 1) between 8 and 16 h after exposure, but descends rapidly thereafter to a point below that of the controls (No. 4), at 20 h. An analysis of the phase percentages of the 4 groups at 20 h (Table) shows that the presence of tropolone has reduced the percentage of metaphases to 72, a figure half way between 99% for the colchicine group,

[1] M. R. MURRAY, H. H. DE LAM (BENITEZ), and E. CHARGAFF, Exper. Cell. Res. *2*, 165 (1951).

[2] We are indebted to Prof. W. v. E. DOERING, Yale University, for this substance.

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[1] M. R. MURRAY, H. H. DE LAM (BENITEZ), and E. CHARGAFF, Exper. Cell. Res. *2*, 165 (1951).