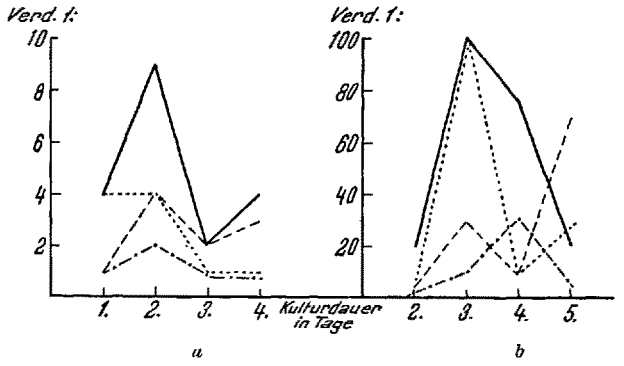
Myzelgewicht von *Aspergillus oryzae* in Gramm
Strombehandelte Kulturen

Kulturdauer Tage	1	2	3	4
Trockengewicht nach Azetonbehandlung .	0,475	0,720	0,273	0,660
Glührückstand	0,028	0,041	0,015	0,022

Kontrollen

Trockengewicht nach Azetonbehandlung	0,785	0,780	0,685	0,610
Glührückstand	0,050	0,042	0,054	0,020

sten Werte mit 10 und 44 mA erreicht. Aus zahlreichen Versuchsserien gleicher Art ist zu entnehmen, dass sich der Unterschied in kleinen Stromstärken nicht grundsätzlich in der Grösse der Aktivität bemerkbar macht. Diese Beobachtung steht in Einklang mit der Anschauung über den Stromeffekt als Ausdruck einer unspezifischen Wirkung kleiner Reize. In allen Versuchen sind – wie früher beschrieben (2, 3) – pH- oder Temperaturschwankungen nicht die Ursache für die unter dem Einfluss schwacher Wechselströme auftretende erhöhte Antibiotikaproduktion.



a) Aspergillus oryzae 18 mA

— Strombehandelte Kultur (Test mit *Staphylococcus aureus*);
... Strombehandelte Kultur (Test mit *Escherichia coli*);
– – Kontrolle (Test mit *Staphylococcus aureus*);
–·– Kontrolle (Test mit *Escherichia coli*).

b) Penicillium notatum

— 44 mA; ... 10 mA;
– – 20 mA; –·– Kontrolle.

Nach Bohdanovicz[1] sowie Kliewe und Neidl[2] zeigen mit Strom vorbehandelte Bakterien, auf ein frisches Nährmedium überimpft, auch ohne weitere Strombehandlung ein schnelleres Wachstum als nichtbehandelte Bakterien. Analoge Versuche mit Sporen verschiedener Schimmelpilze ergaben nicht dasselbe Resultat. In Parallelversuchen wurden einerseits Kulturen angesetzt mit Sporen von strombehandelten Kulturen, ohne dass diese Kulturen weiter unter Stromeinfluss standen, und andererseits mit normalen Sporen desselben Pilzstammes. Beide Kulturen entwickelten sich gleich und zeigten einen durchaus ähnlichen Verlauf der Aktivitätskurve.

[1] Z. Bohdanovicz, Ges. Geb. Hyg. *27*, 745 (1932).
[2] H. Kliewe und G. Neidl, Arch. Hyg. *136*, H. 4, 265 (1952).

Demnach kann angenommen werden, dass es sich bei der beobachteten Wirkung niederer Wechselströme um einen allgemein stimulierenden Effekt auf den Stoffwechsel von Schimmelpilzen handelt, der nur solange anhält, als Strom durch die Kulturen geleitet wird und der nicht zur Bildung von mutierten Sporen führt.

G. Gillissen und S. Carlson

Hygiene-Institut der Universität Mainz, den 26. Juni 1952.

Summary

Low alternating currents between 8 and 50 mA have a stimulating effect on the production of antibiotics by moulds in surface cultures using the medium of Vincent. Small differences of current intensity at low ranges cause no principally different results. The production of all antibiotics formed by the same species of mould is increased under the influence of low alternating currents.

Amino Acid Constituents of Nuclei and Isolated Chromosomes from Normal and Pathological Leucocytes

The study of the amino acid constituents of chromatin in blood cells results from the great interest taken in the relations between protein synthesis and nucleic acids, and in the behaviour of these components in pathological cells. The aim of this research is to find the amino acid components of the nuclei, isolated chromosomes and histone in normal and pathological blood cells. Amino acid constituents of normal and pathological cells have been the object of different author's researches[1], who tested the amino acids with the chromatographic technique or microbiological determinations. Table I summarizes the results.

Material and Technique: The isolation of nuclei, chromosomes and histone was obtained by the technique previously described[2]. The nuclei and isolated chromosomes were examined in the following material: fowl erythrocyte, normal leucocytes and leucocytes of a chemical abscess of horse, normal human leucocytes, lymphocytes of chronic lymphatic leukaemia, leucocytes of chronic myeloid leukaemia, and histone of normal leucocytes. When the free nuclei and isolated chromosomes were obtained, they were lyophilized, and quantities from 10 to 50 mg, hydrolized with five volumes of HCL 6 N for 22–24 h. Hydrochloric acid was removed by distillation to dryness *in vacuo* repeated several times after the addition of water. Approximately 20 γ of aminonitrogen or multiple quantities were used in preparing two dimensional chromatograms. Ascending chromatography on Whatmann N° I of 46 × 56 cm was used. The chromatography cabinets were regulated at a constant level of temperature. Four solvents were used: butanol-acetic acid and phenol; phenol and collidine-lutidine. The butanol-acetic acid was prepared as described by Partridge and Westall[3]. The chamber saturating

[1] R. M. Melampy, J. Biol. Chem. *175*, 589 (1948). – J. N. Davidson and R. A. Lawrie, Biochem. J. *43*, Proc. XXIX (1948). – J. Blumel and H. Kirby, Proc. Natl. Ac. Sci. U.S.A. *34*, 561 (1948). – B. R. Brunish, D. L. Fairley, and J. M. Luck, Nature *168*, 82 (1951). – G. Yasuzumi, G. Miyao, Science *114*, 38 (1951).
[2] E. E. Polli, Exper. *7*, 138 (1951); Chromosoma *4*, 621 (1952).
[3] S. M. Partridge and R. G. Westall, Biochem. J. *42*, 238 (1948).

mixture was water with butanol-acetic acid. The second solvent was phenol (Merk) saturated with water at 23°. In this case, the saturating mixture was composed of water saturated with phenol with an addition of HCN 0·1%, or NH_3 0·1, 0·3, 1%.

In other chromatograms, phenol was used for the first separation and collidine-lutidine for the second one. Room saturating was composed of water with the mixture and addition of diethylamine (Merk) 0·1%.

The larger number of investigations has been made with butanol-acetic acid and phenol. In the first system the front of butanol was kept at about 52 cm, phenol about 32 cm. In the second system phenol was about 42 cm and collidine-lutidine about 43 cm.

The values of R_F were established by pure amino acids (Roche). At the same time, with the chromatograms of the hydrolysates, other chromatograms with amino acid mixtures were also performed. Again, to make more certain, the hydrolysates were run, systematically, with known amino acids added as "markers", controlling the superimposing and the strength of the spots. In other chromatograms different amino acids from the 14 identified were added. In these cases we have obtained distinguishable spots.

The developing substance was ninhydrin (Merk) 0·1% in butanol.

Figure 1 represents one of the chromatograms obtained with isolated chromosomes of normal human leucocytes. The other chromatograms of the normal and pathological material examined do not differ qualitatively from this one.

The following results are obtained: aspartic and glutamic acid, serine, glycine, threonine, alanine, proline, valine give distinguishable spots. The tyrosine gives a lighter but definite spot in several chromatograms. The leucine and phenyl-alanine are not often clearly distinctive, but in a set of chromatograms it is possible

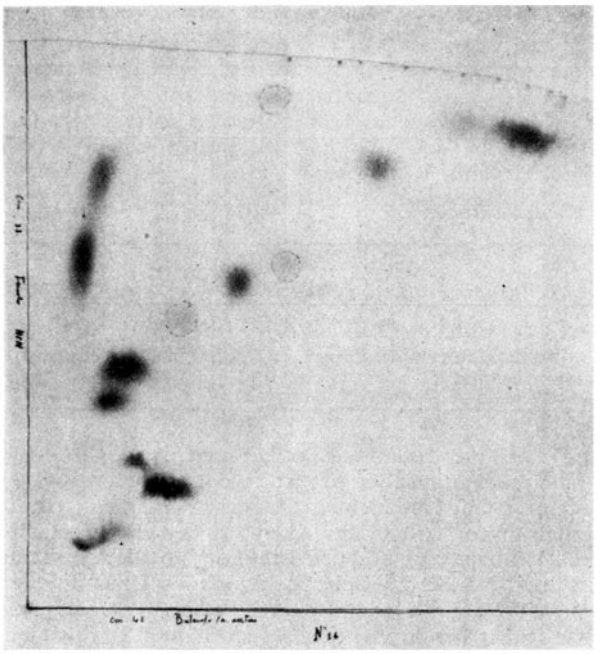


Fig. 1.–Amino acid constituents of isolated chromosomes from normal human leucocytes.

AMINO-ACIDS	CELL COMPONENTS STUDIED				
	NUCLEI OR CHROMOSOMES OF BIRD ERYTHROCYTE	CHROMOSOMES OF HUMAN LEUCOCYTES	THYMONUCLEOHISTONE OF RAT LIVER CELLS	THYMONUCLEOHISTONE OF CALF THYMUS CELLS	RESIDUAL PROTEIN OF CALF THYMUS CELLS
ASPARTIC ACID	DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)
GLUTAMIC ACID	DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)
ALANINE	DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)
ARGININE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
PHENYLALANINE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)		BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
GLYCINE	DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)
HISTIDINE	MELAMPY (1948)		BRUNISH AND COLL.(1951)	HAMER (1951)	
ISO-LEUCINE	MELAMPY (1948) DAVIDSON AND COLL.(1948)		BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
LEUCINE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
LYSINE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
METHIONINE	MELAMPY (1948)		BRUNISH AND COLL.(1951)		
PROLINE	DAVIDSON AND COLL.(1948)		BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)
SERINE	DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	
TAURINE	YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)		
THREONINE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	HAMER (1951)	
TYROSINE	MELAMPY (1948) YASUZUMI AND COLL.(1951)		BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948)	
VALINE	MELAMPY (1948) DAVIDSON AND COLL.(1948) YASUZUMI AND COLL.(1951)	YASUZUMI AND COLL.(1951)	BRUNISH AND COLL.(1951)	DAVIDSON AND COLL.(1948) HAMER (1951)	BLUMEL AND COLL.(1948)

to obtain some papers with the two spots separated. The arginine, lysine, histidine can with difficulty be demonstrated simultaneously in both systems of solvents used. In the butanol-acetic solvent the addition of NH_3 or HCN modifies the spot positions. With HCN it is possible to obtain the chromatograms in which three amino acids are identified, while with NH_3 lysin and arginine are in apposition. Histidine also has a distinguishable spot position regarding the other two amino acids in the chromatograms obtained with the phenol and collidine-lutidine.

Discussion and Conclusions: The comparison between the table listing the previous researches and our data on nuclei, isolated chromosomes and histone allowed us to make interesting comparisons. In all our fractions, under these conditions of hydrolysis, we have found the 14 amino acids already noted, with different frequency, by other authors. These 14 amino acids seem roughly to change in their reciprocal quantitative relations in the hydrolysates of different cases. Proline and tyrosine, as we have found in isolated chromosomes, cannot be dependent on nuclear membrane contamination because of the evidence of the spots. Moreover DAVIDSON and coll.[1], BLUMEL an coll.[2], and BRUNISH and coll.[3], found proline and tyrosine in histone of rat and calf cells and in "residual protein" of calf thymus chromosomes. Besides, it should be noted that histidine was always found when using collidine-lutidine and phenol solvents.

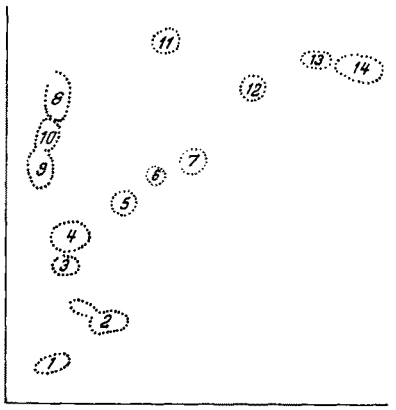


Fig. 2.–*1* aspartic acid; *2* glutamic acid; *3* serine; *4* glycine; *5* threonine; *6* alanine; *7* tyrosine; *8* histidine; *9* lysine; *10* arginine; *11* proline; *12* valine; *13* phenyl-alanine; *14* leucine.

Nuclei and isolated chromosomes of leukaemic cells and horse abscess have amino acids not unlike those of normal cells. Therefore it is concluded that these 14 amino acids are constantly present in the chromosomes of the species studied, both under normal and pathological conditions.

Further researches must be made in this direction; firstly to examine quantitatively the amino acids under different conditions and in different species, and secondly to produce a lesser degree of hydrolysis in order to study a higher plane of molecular organization. The possibility may be still considered that some amino acids contained in minimal quantity may not be revealed by our methods, and that some amino acids may be destroyed during hydrolysis.

Further researches in this direction are being performed.

E. E. POLLI and A. BESTETTI

Institute of Clinical Medecine, University of Milan, April 4, 1952.

Riassunto

Gli Autori hanno compiuto l'analisi cromatografica degli idrolisati di nuclei, cromosomi isolati ed istone di cellule ematiche normali e patologiche. Vennero esaminati: eritrociti di pollo; leucociti (normali e da ascesso) di cavallo; leucociti umani normali; linfociti di leucemia linfatica e leucociti di leucemia mieloide.

I vari componenti cellulari ottenuti mediante il Waring blender e successive centrifugazioni differenziali; vennero idrolizzati con HCL. Con gli idrolisati vennero allestiti cromatogrammi bidimensionali in due sistemi di solventi; butanolo acetico e fenolo, fenolo e collidina-lutidina.

In tutti i cromatogrammi bidimensionali dei due sistemi di solventi si sono potuti mettere in evidenza 14 amino acidi (acido aspartico, acido glutammico, serina, glicina, treonina, alanina, prolina, valina, tirosina, leucina, fenil-alanina, arginina, lisina e istidina).

Quelques effets des inhibiteurs des phosphorylations oxydatives sur des fragments nucléés et énucléés d'organismes unicellulaires

Nous avons décrit récemment[1] les effets exercés par les inhibiteurs des phosphorylations oxydatives (dinitrophénol, acide usnique) sur des amibes intactes et des œufs de Batraciens. Rappelons que, chez ces derniers, l'interruption du couplage entre les oxydations et les phosphorylations conduit à un enrichissement anormal des noyaux en acide ribonucléique. Ce même trouble du métabolisme des acides nucléiques se rencontre dans les hybrides létaux entre Batraciens[1] et dans des œufs de grenouille fécondés par des spermatozoïdes fortement irradiés, puis privés du noyau ovulaire (BRIGGS et ses collaborateurs[2]). Ces faits nous avaient amené à souligner les ressemblances étroites existant entre les effets cytochimiques produits par des altérations nucléaires d'une part, l'inhibition des phosphorylations oxydatives de l'autre; nous y avions vu un argument indirect en faveur de l'idée que le noyau interviendrait dans le couplage entre les oxydations et les phosphorylations[3].

Il nous a paru utile de reprendre cette étude en l'étendant à des fragments nucléés et énucléés d'organismes unicellulaires (*Amoeba proteus* et *Acetabularia mediterranea*). Nous avons constaté, tout d'abord, que le dinitrophénol (10^{-3} *M*) et l'usnate de Na (20 à 40 γ/cm^3) n'exercent pas d'effet appréciable sur la basophilie des deux types de fragments: comme nous l'avons montré précédemment[4], l'énucléation provoque une diminution assez rapide de la teneur en acide ribonucléique des frag-

[1] J. N. DAVIDSON and R. A. LAWRIE, Biochem. J. *43*, Proc. XXIX (1948).

[2] J. BLUMEL and H. Kirby, Proc. Natl. Ac. Sci. U.S.A. *34*, 561 (1948).

[3] B. R. BRUNISH, D. L. FAIRLEY, and J. M. LUCK, Nature *168*, 82 (1951).

[1] J. BRACHET, Exper. *7*, 344 (1951).

[2] R. BRIGGS, E. U. GREEN et T. J. KING, J. exper. Zool. *116*, 455 (1951).

[3] J. BRACHET, Exper. *7*, 344 (1951). – J. BRACHET, Nature *168*, 205 (1951).

[4] J. BRACHET, Exper. *6*, 294 (1950). – N. LINET et J. BRACHET, Bioch. Biophys. Acta *7*, 607 (1951).