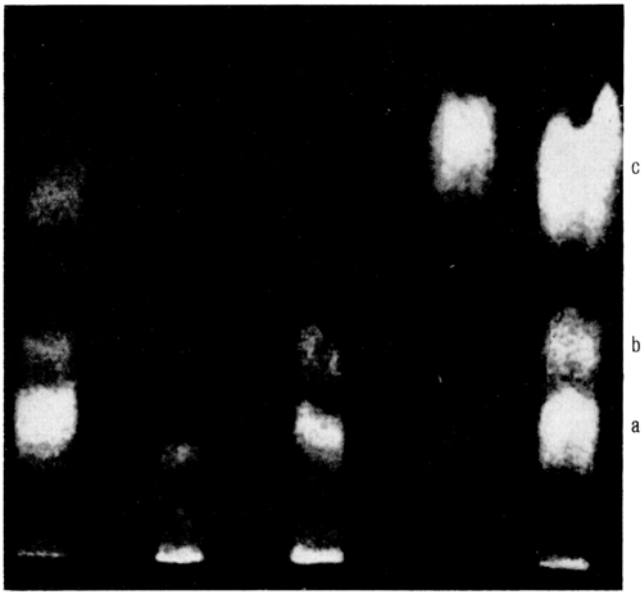

The chromatogram of the incubation mixture of serine aldolase from pigeon liver with tetrahydroaminopterin (0.5% sodium carbonate, ascending technique).
Samples: 1. serine aldolase + tetrahydroaminopterin + serine. 2. sample without enzyme. 3. sample without serine. 4. synthetic N^{5-10}-methylentetrahydroaminopterin. 5. the mixture of sample 1. with the synthetic N^{5-10}-methylentetrahydroaminopterin.
Zones: a) light-green fluorescent decomposition product of tetrahydroaminopterin, probably 2,4,6-triaminopteridine. b) tetrahydroaminopterin. c) N^{5-10}-methylentetrahydroaminopterin.

of tetrahydroaminopterin into N^{5-10}-methylentetrahydroaminopterin was detected by means of paper chromatography of the reaction mixtures. The product showed identical behaviour with that prepared by the non-enzymic hydroxymethylation of tetrahydroaminopterin by low concentrations of formaldehyde. However, the dehydrogenation of the substance mentioned to the N^{5-10}-methylen-tetrahydroaminopterin could not be observed. From the results presented here, it can be supposed that from the enzyme systems of folic acid turnover the hydrogenases are most strongly inhibited by the 4-amino-analogues of folic acid derivates. Even the hydrogenated and N^5 or N^{10} substituted 4-amino-analogues, which are much less active than aminopterin or amethopterin themselves, show the strongest activity on the folic acid hydrogenases. However, in the case of the last-mentioned substances, the inhibition of the hydroxymethyltetrahydrofolic acid dehydrogenase should be considered.

The results of the toxicity determination of the above-mentioned 4-amino-analogues for mice[16] do not agree with the enzyme inhibition observed. Thus another mechanism of action might be supposed; i.e., the interference with the natural folic acid coenzymes in the single carbon transfer reactions. The inhibition of the thymidylate synthetase and the glycinaminoribotide and aminoimidazolcarboxamide ribotide transformylases will be reported later.

Zusammenfassung. Der Einfluss einiger 4-Aminoanalogen der coenzymatisch aktiven Folsäurederivate auf die Enzyme der Folsäureumwandlungen wurde verfolgt. Die stärkste Hemmung zeigen alle 4-Aminoanaloge auf die Hydrogenase der Folsäure, wobei die Hydrierung des Pyrazinringes dieselbe deutlich vermindert. Eine mässige, jedoch deutlich schwächere Hemmung von nonkompetitiver Natur wurde bei der Hydroxymethyltetrahydrofolsäuredehydrogenase, Formiminotransferase und Leukovorincyclodehydrase festgestellt. Weiter wurde die enzymatische Hydroxymethylierung von Tetrahydroaminopterin durch Serinaldolase aus der Taubenleber beobachtet.

VÉRA SLAVÍKOVÁ and K. SLAVÍK

Institute of Hematology and Laboratory of Protein Metabolism, Charles' University (Prague), 8. November 1960.

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Establishment of an Epithelial Cell Strain from Calf Liver in Continuous Culture

Calf liver cells were isolated in September 1958 for various researches in the following way. Fetal calf liver was minced and the fragments were explanted on glass without plasma in a culture medium containing 25% calf serum and 0.5% lactalbumine hydrolysate (N.B.C.) in Hanks balanced salt solution[1]. The cultures were incubated at 37°C. Media were changed every 2–3 days. Subcultures were made after about a month by trypsinizing (0.5% trypsin in a salt solution deficient in Ca^{++} and Mg^{++}). In the beginning growth of the cells was slow, but after the 5th subculture (March 1959) the growth was more regular, quicker and clearly epithelial as a close packed pavement epithelium. Cells from various explants were kept in culture. In the early stages most explants often formed epithelial cells, but after prolonged culture the cells were fibroblastlike. One of the subcultures however grows as a sheet of cells on the glass wall. After establishment of these cells in continuous culture the serum percentage of the medium was reduced gradually to 5%. The doubling time for this strain is two to three days and the mitotic index 2–3%. The shape of the cells from this strain (KaLe) is polygonal (see Fig.). The mean nuclear size, measured in a culture fixed with formol-alcohol-aceticacid and stained with iron hematoxylin, is 16 μ. (The mean nuclear size of liver tissue fixed and stained on the same manner is 7 μ.) There are some 'giants', 6–7%, whose nuclear size varies from 23–42 μ. The number of nucleoli per nucleus varies from 1–7. There is no relation between number of nucleoli and size of the nucleus. The number of chromosomes is 70 ± 2 (normal fetal liver cells 60). The cells on the photograph were fixed and stained with 2% $AgNO_3$[2]. Between the cells a 'cement substance' is clearly visible, which is stained by the silver. (Cultures of cartilage cells, stained with $AgNO_3$, never gave staining of such 'cement substance' although some aneuploid strains grow as epithelial sheets as well.) The mitochondria of the liver cells are mostly spherical, some are filamentous. The plasma of the liver cells fixed with alcohol 70% and stained with the PAS reaction is clearly PAS positive. Physiologically they are thus liver cells. Up to now it was impossible to cultivate this KaLe strain as separate cells in suspension. We tried it with shake and stir cultures, but always groups of cells were found. It is probable that the cells are held together by the cement substance. Biochemical research on this strain will be published elsewhere.

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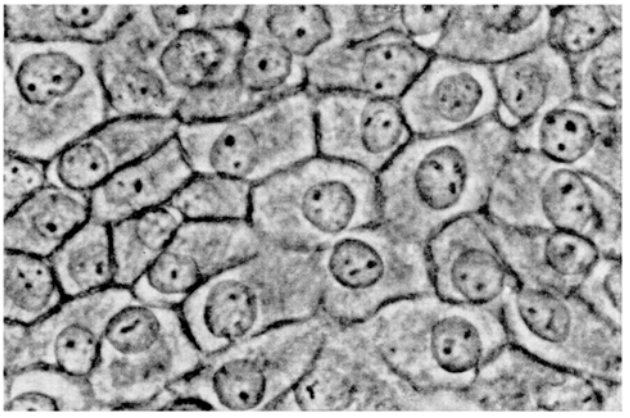

Cells from Ka Le strain magn. 450× fix. col. $AgNO_3$. Phase contrast.

Zusammenfassung. Die Isolierung einer Zell-Linie aus fötaler Kalbsleber wird beschrieben. Die Zellen wachsen in epithelialer Schicht und sind durch Kittsubstanz miteinander verklebt. Nach der fünfzigsten Passage scheinen noch immer spezifische Leberenzyme anwesend zu sein, so dass die Zell-Linie morphologisch und biochemisch als Leberepithel betrachtet werden kann.

ANNA C. M. PIECK and CH. M. A. KUYPER

Laboratory of Cytochemistry of the R. C. University, Nijmegen (The Netherlands), November 18, 1960.

Physical Stress and Emetine Cardiotoxicity

In our previous papers we have reported the toxic patterns and the electrocardiographic changes observed in rabbits subjected to emetine acute intoxication[1], in guinea pigs subjected to emetine subacute intoxication[2], and in guinea pigs receiving courses of emetine chronic treatment similar to those used in human therapy[3]. A case of Wolff-Parkinson-White syndrome was observed in one guinea pig during such a course of treatment[4]. Degenerative and necrotic changes have been found in the myocardium of guinea pigs subjected to emetine subacute poisoning[5]. Emetine has shown inhibitory effects on the frog isolated heart[6], on the guinea pig isolated atria[7] and upon the respiration *in vitro* of guinea pig heart homogenates[8]. From these studies we have concluded that emetine is a drug with specific cardiotoxicity. This conclusion, beside having obvious repercussions in human therapy, provides also a useful basis for producing a pathological condition of the experimental animal heart which may be used in evaluating the cardiac effects of various drugs. So far we have studied the protective effect of fifteen drugs on the mortality, toxic patterns and EKG changes shown by guinea pigs during emetine subacute poisoning. Such an effect was not shown by methionine[2], γ-thyomethyl-α-hydroxybutyric acid[2], potassium chloride[9], diphosphopyridine-nucleotide[10–12], pyridoxine[13], inositol[13], adenosine-triphosphate[11,12], lipoid retina extract[6] or lipoid brain extract[6].

Protection was shown, however, by rybo-flavin-phosphate[11,12], pyridoxine-phosphate[11,12], cocarboxylase[11,12], lipoid diencephalon extract[6], lipoid heart extract[6] and embryonic heart extract[11,12], in increasing order of potency. The negative findings on the protective effect of methionine and γ-thyomethil-α-hydroxybutyric acid were confirmed by our histological studies[5]. Lipoid heart extract also showed its protective effect against emetine cardiotoxicity on the frog isolated heart[6], as did cocarboxylase on the guinea pig isolated atria[7] and embryonic heart extract on the respiration *in vitro* of guinea pig heart homogenates[8]. Emetine subacute poisoning may be useful also in the experimental study of the cardiac effects of various factors, such as physical and psychological stress. In the present paper we report the results of our research on the interactions between emetine cardiotoxicity and swimming exercise[14].

Three groups of guinea pigs were used. One group received 5 mg of emetine/kg/day, subcutaneously[2]. The second group was subjected to swimming exercise without emetine. The third group was subjected to swimming and emetine. In the second group the duration of the daily swimming sessions was 10 min on the first two days, 30 min from the third to the sixth day and 60 min from the seventh to the sixteenth day, as the animals' swimming ability increased. In the third group, from the eleventh day onwards the swimming sessions were followed by the daily injections of emetine (5 mg/kg/s.c., as in the first group). As a first consequence of emetine toxicity, the swimming ability decreased progressively in the third group. Guinea pigs swam in an ordinary bath tub. Water temperature was maintained constant at 30°C. The results are shown in Table I and II.

The combination of heart weight increase and EKG signs of Right Ventricular Strain with compensatory bradycardia, shown by the animals receiving swimming without emetine, is due to the cardiac hypertrophy already described in the rat subjected to swimming[15,16].

The typical effects of emetine subacute poisoning, such as loss of body weight, diarrhea, depression of spontaneous behaviour and EKG changes, appeared earlier and were more pronounced in the animals receiving swimming and emetine than in those receiving only emetine. The former survived a remarkably shorter time than the latter. Moreover, the third group showed EKG signs of coronary insufficiency.

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