

vaccinia virus multiplication by CACP is approximately the same as that of 6-azauracil riboside⁵. That ratio for benzimidazole, the referential compound is 3, under the same conditions.

CACP, when tested by the plaque method, did not inhibit the multiplication of Newcastle disease virus (NDV) and Western equine encephalomyelitis virus (WEE).

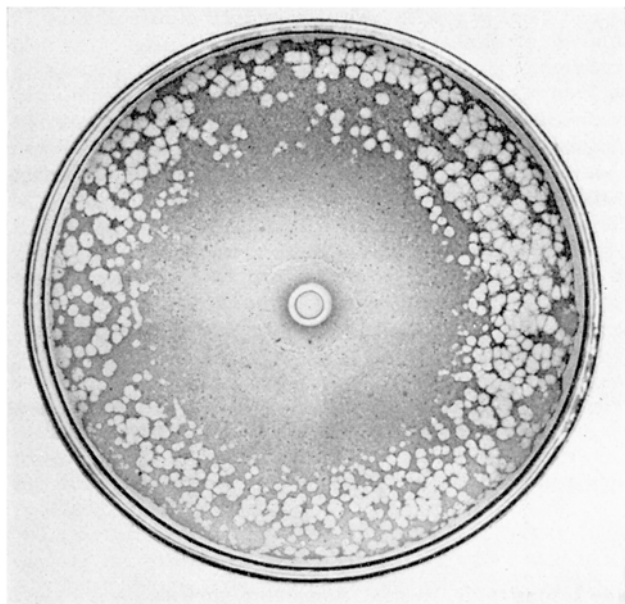


Fig. 2. Inhibitory effect of 2-carboxymethylmercapto-4-amino-5-(*p*-chlorophenyl)-pyrimidine. Monolayer of chick embryo cells was infected with 1000 plaque-forming units of vaccinia virus. After solidifying of the agar overlay, a glass cylinder was mounted in the gel. 0.05 ml of solution 10 mg CACP/ml was pipetted into the cylinder. 5 days after infection. Dish of 10 cm diameter.

Activation of Amino Acids in the Liver in CCl₄ Intoxication

Recent findings on the fatty liver induced in rats by a wide variety of toxic substances show early damage in the extramitochondrial fractions of the cell¹⁻⁵.

With respect to CCl₄ poisoning, an hypothesis has been formulated regarding an inhibition of the hepatic triglyceride-secreting mechanism⁶ probably related to an inhibition of lipoprotein formation. In this connection, a diminished incorporation of amino acids into the liver and plasma proteins^{5,7} as well as into the plasma lipoproteins⁸ has been reported.

The present work concerns a demonstrable inhibition of the first step of the protein synthesis, that is the amino acid activation, which occurs during the early stages of CCl₄ intoxication.

The assay was performed with the method of the amino-acyl hydroxamate formation by using the pH 5 enzyme obtained from liver according to HOAGLAND et al.⁹. Results of our experiments are shown in the Table.

The results presented in this communication suggest that the deficiency in the amino acid activation may

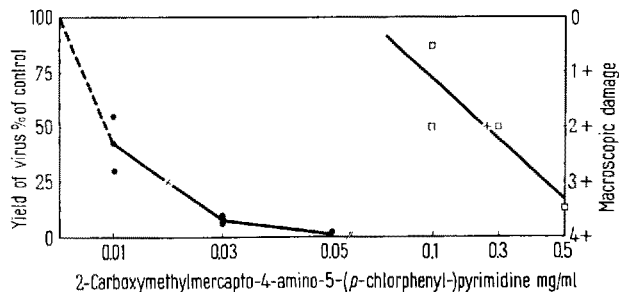


Fig. 3. Relationship between the concentration of CACP and the degree of inhibition of vaccinia virus multiplication and the extent of macroscopic damage to chorioallantoic membranes. • = yield of virus; □ = macroscopic damage. The crosses × and + refer to the 75% virus inhibitory and 2+ toxic concentration respectively.

Zusammenfassung. Mit der Methode der Hemmung der Plaquebildung bei gleichzeitiger Diffusion der antiviralen Stoffe durch den Agar wurde eine hohe Selektivität des 2-Carboxymethylmercapto-4-amino-5(*p*-chlorphenyl)-pyrimidins für die Hemmung des Vaccinevirus festgestellt. Das Verhältnis der toxischen und der virushemmenden Konzentrationen wurde in den Membrankulturen = 15 gefunden. Die Substanz war gegen andere Viren (NDV, WEE) unwirksam.

B. RADA, Z. BUDEŠINSKÝ, and Z. PEŘINA

Institute of Virology, Czechoslovak Academy of Sciences, Mlynská dolina, Bratislava, and Pharmaceutical and Biochemical Research Institute, Prague (Czechoslovakia), September 18, 1962.

⁵ B. RADA, D. BLÁŠKOVIC, F. ŠORM, and J. ŠKODA, *Exper.* 16, 487 (1960).

account for the impairment of the protein and lipoprotein synthesis, as well as for the dramatic morphological changes, which are known to occur in the liver after CCl₄ administration. It is quite likely that such a deficiency may be set in the pathological sequence leading to either necrosis or steatosis, the latter being also related to the

¹ C. OBERLING and C. ROUILLER, *Ann. Anat. Pathol.* 1, 401 (1956).

² D. NEUHART and D. MAIBAUER, *Arch. exp. Path. Pharmacol.* 235, 291 (1959).

³ M. BASST, *Exp. Cell Res.* 20, 313 (1960).

⁴ R. O. RECKNAGEL and B. LOMBARDI, *J. biol. Chem.* 236, 564 (1961).

⁵ E. A. SMUCKLER, O. A. ISERI, and E. P. BENDITT, *J. exp. Med.* 116, 35 (1962).

⁶ R. O. RECKNAGEL, B. LOMBARDI, and M. C. SCHOTZ, *Proc. Soc. exp. Biol. Med.* 104, 608 (1960).

⁷ E. A. SMUCKLER, O. A. ISERI, and E. P. BENDITT, *Biochem. biophys. Res. Commun.* 5, 270 (1961).

⁸ D. S. ROBINSON and A. SEAKINS, *Biochem. J.* 82, 9 P (1962).

⁹ M. B. HOAGLAND, E. B. KELLER, and D. C. ZAMECNIK, *J. biol. Chem.* 218, 345 (1956).

Controls		CCl ₄ treated		
Number of rats	Hydroxamate	Number of rats	h after CCl ₄	Hydroxamate
3	0.55	3	2	0.20
3	0.83	3	4	0.27
3	0.57	3	4	0.41
3	0.56	3	6	0.34
3	0.51	3	24	0.21

Results are expressed as micromoles of hydroxamate calculated on the basis of the molar coefficient¹⁰ from the value ΔE_{430} in the presence of amino acids minus that in the absence of substrates. The enzymes were obtained by precipitating once at pH 5.15 the soluble fractions of liver homogenates in 0.05 M KCl after centrifugation for 60 min at 105 000 $\times g$; from 1 g of liver, 15–18 mg of protein were obtained when dissolving the precipitate in 0.1 M tris-buffer pH 7.5. CCl₄ was administered by stomach tube at a dose of ml 0.75/100 g of body weight of a 1:1 solution in paraffin oil. All food was withdrawn 18 h before the administration (control animals received paraffin oil only).—Conditions of incubation (2 ml): Micromoles: ATP-K (neutralized) 20; MgCl₂ 20; NH₂OH · HCl (with KOH to pH 7.7) 1600; tris-buffer pH 7.7 100; amino acid mixture 24; enzyme: 10 mg of protein. Temperature: 38°C. Time: 60 min. The amino acid mixture contains 2 μ moles each of: alanine, arginine, phenylalanine, glycine, histidine, isoleucine, leucine, lysine, serine, tryptophane, tyrosine, valine.—Under these conditions, the reaction was a linear function of the quantity of enzyme and of the time of at least 2 h.

decrease in fatty acid activation which has been demonstrated in the period when liver fat is rising^{11,12}.

Riassunto. La reazione di attivazione degli aminoacidi è significativamente diminuita nel fegato di ratti nelle prime ore dopo la somministrazione di CCl₄. Questa alterazione si accorda con la dimostrata diminuzione delle sintesi proteiche e può rappresentare uno dei fattori primitivi e principali nella genesi delle modificazioni biochimiche e morfologiche che si osservano in questa condizione patologica.

F. ROSSI and M. ZATTI

Istituto di Patologia Generale della Università di Padova e Centro di Studio «G. Veroni» del C.N.R. per la Fisiopatologia, Padova (Italy), January 4, 1963.

¹⁰ H. BEINERT, D. E. GREEN, P. HELE, H. HIFT, R. W. VAN KORFF, and C. V. RAMAKRISHNAN, J. biol. Chem. 203, 35 (1953).

¹¹ F. ROSSI and M. ZATTI, Exper. 16, 513 (1960).

¹² Acknowledgment. We wish to thank Miss F. VIGNA for technical assistance.

Modification de la vitesse du courant cytoplasmique, induite à distance, par l'irradiation, chez *Nitella flexilis*

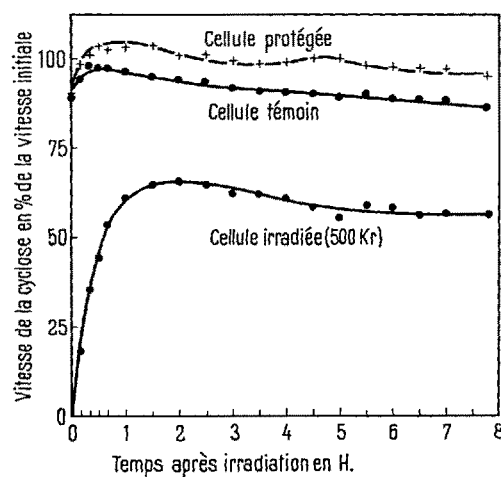
Introduction. La connaissance des effets qu'exerce, immédiatement après l'irradiation, une cellule traitée sur une cellule voisine protégée, relève plutôt de l'interprétation (théorie des poisons: BACQ et ALEXANDER¹) que de l'analyse proprement dite. A part les travaux de KRJUKOVA et KUZIN² montrant que la diffusion de métabolites toxiques à partir des feuilles, chez la Fève irradiée, inhibe la division cellulaire, aucun autre auteur n'a, à notre connaissance, abordé ce problème chez les plantes.

C'est pourquoi nous avons entrepris cette étude tâchant de mettre en évidence, par le biais de la vitesse du courant cytoplasmique, l'influence d'une cellule irradiée sur une cellule non traitée durant la période qui suit l'irradiation. Dans cette note sont exposés les premiers résultats obtenus chez *Nitella flexilis* (L.e.p.) Ag., algue d'eau douce dont les longues cellules internodales sont animées d'une cyclose caractéristique.

Matériel et Méthodes. Cette plante provient d'un étang d'Ardenne (GILLET³) et est cultivée au laboratoire. Avant l'irradiation, deux cellules internodales séparées par les petites cellules de l'entre-noeud sont isolées du tétome en sectionnant les cellules voisines. Elles sont laissées pendant 12 h minimum dans une solution de culture originale (GILLET⁴) puis la vitesse de leur flux protoplasmique respectif est déterminé suivant la technique déjà décrite dans l'article précédent. Au moment de l'irradiation, l'une ou l'autre de ces cellules est entièrement protégée par un écran en plomb de 2 mm d'épaisseur dans l'ouverture duquel s'engage l'extrémité de la cellule traitée. De cette manière, aucun rayon oblique ne peut frapper la cellule protégée. Le générateur de rayons X est un tube Machlett OEG à fenêtre de beryllium fonctionnant avec localisateur sous une tension de 50 kV et

une intensité de 30 mA. Son débit, mesuré dans l'air, à 7,1 cm du foyer est de 99,000 r/min. Aussitôt après l'irradiation, les cellules sont remplacées sous microscope et la vitesse du courant cytoplasmique déterminée à intervalles réguliers et exprimée en % de la vitesse initiale ($V_n = 100 t_0/t_n$). Les expériences ont porté sur 14 couples de cellules et eurent lieu en novembre.

Résultats. La Figure rend compte des résultats obtenus. Chaque point représente la valeur moyenne de 70 observations.



¹ Z. M. BACQ et P. ALEXANDER, *Fundamentals of Radiobiology*, second. édit. (Pergamon Press, Butterworth, London 1961).

² L. M. KRJUKOVA et A. M. KUZIN, *Fiziol. Rasten (russe)* 7, 220 (1960).

³ C. GILLET, *Bull. Soc. Roy. Bot. Belgique* 92, 197 (1960).

⁴ C. GILLET, *Acad. Sci. Belg. Bull. classe des Sci.* 48, 1161 (1962).