Results. The blood levels of free and bound pantothenic acid after infusion of coenzyme A or calcium pantothenate: (a) During the intravenous administration of coenzyme A, blood levels of free and bound pantothenic acid increased. 20 h after the end of the infusion, the blood concentrations of free and bound pantothenate were still found to be elevated (Figure). (b) During the infusion of calcium pantothenate, the free pantothenic acid in blood rose, whereas the bound pantothenic acid remained unchanged or was within the range of experimental error.

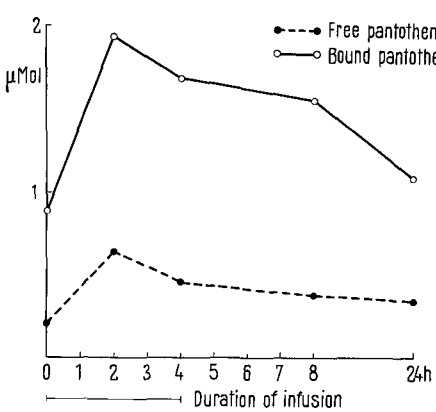
The urinary excretion of pantothenic acid during and after infusion of coenzyme A or calcium pantothenate: (a) The daily urinary excretion of free pantothenic acid was elevated after the administration of coenzyme A and normal again 2 days after the beginning of the infusion. In 3 cases, the extra pantothenate excretion corresponded to 22–24%, and in 1 case to 52% of the infused amount of coenzyme A. No bound pantothenic acid could be detected in urine. (b) For 2 days an extra daily urinary excretion of pantothenic acid was observed after the infusion of calcium pantothenate. It corresponded to 16% and 29% of the calcium pantothenate administered.

Discussion. In humans with normal glomerular filtration rates, an extra urinary excretion of free pantothenic acid is observed after intravenous administration of coenzyme A. This fact and the elevated free blood pantothenate during and after the infusion point to a breakdown of the injected coenzyme A in the organism. On the other hand, the blood level of bound pantothenate is raised after administration of coenzyme A and was found to be still elevated 20 h later.

It is not known whether the enzymatic degradation of coenzyme A to pantothenate by phosphatases and peptidases takes place in the circulating blood in the extravascular extracellular compartment or within the cells.

In 3 out of 4 balance studies with coenzyme A, 20–30% of the dose infused was excreted as pantothenate with the urine. The fate of the remainder is not known. It is assumed that the main part of the coenzyme A infused is adsorbed on the cell surface or is taken up by the cells either as coenzyme A or as one of its breakdown products (dephospho-coenzyme A, phosphopantethein, pantethein, pantothenic acid).

The urinary excretion of infused calcium pantothenate corresponded to 15–30%. This is similar to the results obtained after infusion of coenzyme A and is in good agreement with the observations of SCHMIDT[7,8].



Free and bound (coenzyme A) pantothenic acid in blood before, during, and after intravenous infusion of coenzyme A, α-lipoic acid, diphosphopyridine nucleotide and cocarboxylase. M. S., 1949 ♂; normal glomerular filtration rate.

Zusammenfassung. Bei Versuchspersonen mit normaler glomerulärer Filtration und normaler Leberfunktion wurde nach intravenöser Infusion von Calcium-D-Pantothenat oder Coenzym A die Bilanz der freien und der gebundenen Pantothensäure (Coenzym A) ermittelt. Es konnte gezeigt werden, dass infundiertes Coenzym A teilweise zu freier Pantothensäure abgebaut wird. Ungefähr 70% des infundierten Coenzym A konnten weder im Urin (als Pantothenat) noch im Blut wiedergefunden werden.

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Medizinische Universitätsklinik, Bürgerspital, Basel (Switzerland), December 29, 1965.

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[8] The study was aided by grants from Schweizerischer Nationalfonds and from F. Hoffmann-La Roche & Co. AG, Basel.

Effect of Choline Salicylates and Some Other Analogues of Salicylic Acid on the Replication of EMC Virus in vitro

It has recently been reported that certain simple aromatic phosphonic and carboxylic acids have antiviral activity[1–3]. This communication presents further evidence for the effect of some derivatives of salicylic acid on the replication of encephalomyocarditis (EMC) virus in the secondary mouse embryo tissue culture. Therefore, some aspects of the relationship between the chemical structure of the drugs and their virus inhibitory action are discussed.

The methods used for the assay of antiviral activity of drugs were the same as described hitherto[1–3].

In the Table virus inhibitory action of eleven drugs has been summarized and compared. It can be seen that the antiviral effect of these drugs is connected with the characteristic structure of salicylate. The substitution of oxygen by larger but also bivalent sulphur in the *ortho*-phenolic group enhanced the antiviral activity of the compound (III). On the other hand, the substitution of

[1] A. D. INGLOT, M. KOCHMAN, and P. MASTALERZ, Nature *207*, 784 (1965).

[2] M. KOCHMAN, P. MASTALERZ, and A. D. INGLOT, Nature *207*, 888 (1965).

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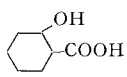
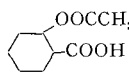
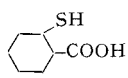
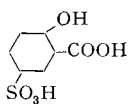
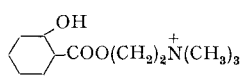
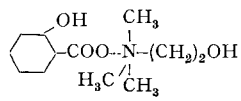
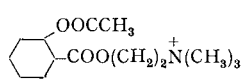
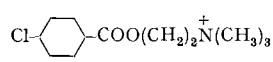
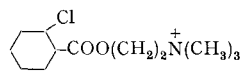
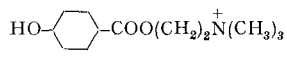
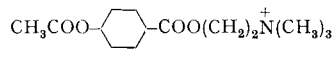
Effects of choline salicylates and some other structural analogues of salicylic acid on replication of EMC virus in mouse ebryo tissue culture

No.	Compound	Formula	Concentraion in m*M*	Protection against CPE[a]	Inhibition of virus yield (% of control)[b]	Inhibition of adsorption and penetration (% of control)[c]	Cytotoxic concentration in m*M*[d]
I	Sodium salicylate		2 1 0.5	> 2 1 0	99.5 90 55	74 62 50	⩾10
II	Sodium acetylsalicylate		2 0.5	> 2 0	94 80	60 –	⩾10
III	Sodium 2-mercaptobenzoate		2 1 0.5	> 2 1.5 0.5	99.6 – 97	82 – 63	⩾10
IV	Sodium sulphosalicylate		5	0.5	74	42	⩾10
V	Choline salicylate A		2 1 0.5	> 2 1.5 1	99.5 98 92	– 54 50	⩾ 5
VI	Choline salicylate B		2 1 0.5	> 2 1.5 1.5	99.9 99 98	– 63 50	⩾ 8
VII	Choline acetoxysalicylate		1	2	96	54	⩾ 5
VIII	Choline *p*-chlorobenzoate		1	0.5	71	22	⩾ 5
IX	Choline *o*-chlorobenzoate		1	0.5	74	42	⩾ 5
X	Choline *p*-hydroxybenzoate		1	0	0	0	⩾ 8
XI	Choline *p*-acetoxybenzoate		1	0	10	0	⩾ 8

[a] Protection against viral cytopathic effects (CPE) was expressed as delay (in days) of degeneration of cell population infected with virus and treated with compound in question in comparison with untreated culture. [b] To measure the inhibition of virus yield the monolayer mouse embryo tissue cultures were infected with approximately 100 plaque forming units (pfu) of virus per culture and were incubated in the presence of the drugs incorporated into the fluid maintenance medium for 48 h at 37 °C. The virus yield was measured by plaque assay. [c] The inhibition of adsorption and penetration of virus was determined by incubation of cell monolayers with these drugs and approximately 50 pfu of virus per culture for 2 h at 37 °C. The drugs were then removed by change of medium, the cultures were washed with saline and were incubated further under methylcellulose medium without inhibitors for 20 h at 37 °C. The results are expressed as % inhibition of virus infectivity of controls treated with saline. [d] Minimal cytotoxic concentrations of the compounds in question leading to the microscopically visible degenerative changes of cells were determined during 48 h incubation period.

the hydroxyl group by chlorine (VIII and IX) or displacement of the hydroxyl group from the position *ortho* to the position *para* (X and XI) resulted in the great reduction of this activity. Moreover, when the strongly electronegative sulphonic group was linked in close proximity to the salicylic group (IV), it also lowered the activity of the compound. Suitable esterification of the carboxyl group can increase the antiviral activity of the drug, since choline salicylates were found to be far more active per molar concentration than sodium salicylate (V, VI and VII). The nature of the linkage of the carboxyl group with choline is not very critical since compounds V and VI are almost equally active. However, compound VI is less toxic to the cells than compound V.

It is interesting to note that choline salicylates have much higher therapeutic activity in vivo than the 'older' salicylates, and they have already been introduced into medical practice as analgesic, antipyrretic and anti-inflammatory agents[4-6].

The results obtained suggest that the virus inhibitory action of the drugs tested so far is intimately connected

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with their antiinflammatory activity. From the biochemical point of view, the ability of these compounds to uncouple oxidative phosphorylation[7] may at least partially explain the inhibition of synthesis of virus[3]. In fact sodium 2-mercaptobenzoate, which is more potent than salicylate in uncoupling oxidative phosphorylation[8], was also proved to have greater antiviral activity. Alternatively, salicylates might become bound to lipoproteins of cell membranes[7], and by the alteration of their properties inhibit the adsorption, penetration and release of virus from cells[3].

The data shown in the Table (column 7) indicate clearly that although salicylate and its close analogues suppress the adsorption and penetration of virus into cells, this effect cannot solely explain the virus inhibitory action of these drugs. Since this action seems to be multiplex and highly unspecific, it is conceivable that salicylates may be active in a variety of cell-virus systems.

Zusammenfassung. Es wird eine 90–99,9%ige Hemmung in vitro der Replikation des EMC-Virus durch 0,5–2 m molare Lösungen folgender Salicylsäurederivate festgestellt: Cholinsalicylat, Cholinacetoxysalicylat und der Natriumsalze von 2-Merkaptobenzoesäure, Salicylsäure sowie Acetylsalicylsäure. Ferner wurde eine etwa 70%ige Hemmung des Natriumsalzes der Sulphosalicylsäure, des Cholin-*p*-chlorobenzoates, sowie des Cholin-*o*-chlorobenzoates beobachtet. Cholin-*p*-oxybenzoat und Cholin-*p*-acetoxybenzoat zeigten hingegen keine Hemmungsaktivität.

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Department of Virology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, and Department of Biochemistry, Medical School of Wroclaw (Poland), December 17, 1965.

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[9] Acknowledgements: We are indebted to Drs. Z. MACHOŃ and T. ZAWISZA from the Department of Synthesis of Drugs, School of Medicine, Wroclaw, for donating of choline salicylates and other derivatives of benzoyl-choline[6].

The Activity of Ibenzmethyzin Hydrochloride Against the Human Transplantable Tumors Human Epithelioma No. 3 and Human Adenoma No. 1

Ibenzmethyzin hydrochloride[1] (N-isopropyl-α-(2-methylhydrazino)-*p*-toluamide hydrochloride) has been reported to be active against transplantable rodent tumors[2–5]. Numerous reports have also appeared concerning its clinical effectiveness, especially for lymphomas[6–9]. The present report extends the experimental data with ibenzmethyzin hydrochloride to include 2 serially propagated human tumors in laboratory animals.

Methods. Solutions of ibenzmethyzin hydrochloride were prepared fresh daily in distilled water and administered in 1.0 ml amounts. A total of 2 experiments were performed for each tumor system, 6 animals per dose.

Human epithelioma No. 3: Wistar female rats weighing 30–40 g were irradiated with a total body dose of 300 r 24 h prior to implantation. The animals were then inoculated intramuscularly with 1.0 ml of minced tumor suspension containing equal parts of tumor and saline supplemented with penicillin G, 500 U/ml, and streptomycin, 500 μg/ml. Cortisone acetate, 60 mg/kg, was administered subcutaneously immediately after and then 2 and 5 days after implantation. Rats were treated with ibenzmethyzin hydrochloride subcutaneously immediately following implantation and then once daily for a total of 8 treatments. The rats were sacrificed 9–10 days after inoculation and the tumors excised and weighed. The average weight of the control tumors (C) was compared with the average weight of the treated tumors (T); an inhibition index (C/T) was calculated and a value of 2.0 or greater was considered to represent an antitumor effect.

Human adenoma No. 1 (H.Ad. No. 1): Golden Syrian hamsters weighing approximately 60 g were employed. After anesthesia with 125 mg/kg aprobarbital (Alurate) intraperitoneally, the everted cheek pouch was implanted with 0.25 ml of a suspension containing 1 part of minced tumor and 2 parts of saline containing penicillin and streptomycin as above. The hamsters were treated by the subcutaneous route immediately after implantation and then once daily for a total of 14 days. On the 21st day the treated and control animals were sacrificed, the tumors excised and weighed and the C/T index determined and evaluated as described for the human epithelioma No. 3 (H.Ep. No. 3) tumor.

Results. The results of experiments with the transplantable human tumors H.Ep. No. 3 and H.Ad. No. 1 are given in Tables I and II, respectively.

For H.Ep. No. 3 an antitumor effect was observed at a dose of 100 mg/kg subcutaneously with 50% of the rats surviving and a weight loss of 32%. A dose of 50 mg/kg subcutaneously and a dose of 25 mg/kg (100% survival) were inactive. In our experience, triethylenemelamine (0.125 mg/kg), 2′-deoxy-5-fluorouridine (25 mg/kg) and 6-mercaptopurine (50 mg/kg) were without effect against H.Ep. No. 3 when administered subcutaneously.

[1] Natulan is a Registered Trade Mark.

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