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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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.

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Table I. Effect of ibenzmethyzin hydrochloride on H.Ep. No. 3 in conditioned rats

Dose: mg/kg s.c. × 8	C/T	% survivors	% weight change
100	4.4	50	− 32
50	1.7	80	− 22
25	1.5	100	− 5
Controls		90	+ 7

Table II. Effect of ibenzmethyzin hydrochloride on H.Ad. No. 1 in hamsters

Dose: mg/kg s.c. 14	C/T	% survivors	% weight change
60	2.7	20	− 20
30	2.3	80	− 6
15	0.9	80	+ 13
Controls		100	+ 33

In the case of H.Ad. No. 1, activity was also seen at a toxic dose of 60 mg/kg subcutaneously with 20% of the hamsters surviving and a weight loss of 20%. However, in addition, activity was also seen at a dose of 30 mg/kg subcutaneously where a slight weight loss occurred but where the majority (80%) of the animals survived. At a well tolerated dose of 15 mg/kg subcutaneously, where weight gain in addition to appreciable survival was noted, the substance was inactive.

Zusammenfassung. Ibenzmethyzinhydrochlorid zeigte tumorhemmende Wirkung gegen Human Epithelioma No. 3 in Ratten, die mittels Bestrahlung und Cortison konditioniert waren, und in Hamstern gegen Human Adenoma No. 1.

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An Attempt to Produce Antibodies to Oxytocin and Vasopressin

An attempt was made to produce antibodies to the neurohypophyseal hormones. In view of the fact that a bradykinin-albumin conjugate had been found to possess antigenic properties[1], we sought to conjugate oxytocin and lysine-vasopressin with a molecule of considerably larger size in order to investigate whether such compounds could induce antibody-production and whether the antibodies so produced would or would not inactivate the corresponding free hormones.

The oxytocin-albumin conjugate and the lysine-vasopressin-albumin conjugate were obtained by condensing oxytocin or lysine-vasopressin with rabbit serum albumin in the presence of a water-soluble carbodiimide: A solution of 40,000 I.U. of oxytocin or 20,000 I.U. of lysine-vasopressin in 50 ml dilute acetic acid was adjusted to pH 1.0 with methanesulphonic acid. The solution was concentrated to half its volume in vacuo at 25 °C to eliminate the acetic acid. The pH was then adjusted to 4.0 with 2*N* sodium hydroxide, and 40 mg of rabbit serum albumin was added. When the albumin had completely dissolved the pH was raised to 6.5 by further addition of 2*N* sodium hydroxide, and 400 mg of 1-cyclohexyl-3(2-morpholinyl-(4)-ethyl)-carbodiimide metho-*p*-toluene-sulphonate dissolved in 1 ml water was added. The mixture was stirred for 1 h at room temperature and dialysed for 48 h at 10 °C against distilled water in a Visking bag. The contents of the dialysis bag were lyophilized, yielding 25 to 50 mg of a white fluffy powder.

Oxytocin-albumin conjugate or lysine-vasopressin-albumin conjugate obtained as described above was administered to rabbits according to the following procedure in order to induce antibody formation: An injection of the antigen in Freund's complete adjuvans was administered subcutaneously and three weeks later a second injection was given by the intraperitoneal route. The dose of antigen per injection ranged from 2 to 6 mg, and a second course of injections was given to animals responding to the first course.

To demonstrate the presence or absence of antibodies to the antigens in question in the serum, a gel-diffusion test (double diffusion in two dimensions) for the detection of precipitating antibodies[2,3] was used. The presence of specific antibodies could be demonstrated, as is evident from Figures 1 and 2. Serial dilution tests, as shown in Figures 3 and 4, revealed that the antibody to oxytocin-albumin conjugate still gave precipitation patterns in a dilution of 1:16, whereas the titre for the antibody to vasopressin-albumin conjugate was somewhat lower (1:8).

To ascertain the specificity of the antibodies in question a certain number of control experiments was carried out. These are summarized in Table I. Oxytocin-albumin conjugate (OCO) and lysine-vasopressin-albumin conjugate (VCO) were selectively precipitated. No precipitation patterns were observed on testing the oxytocin or lysine-vasopressin conjugates on control sera from rabbits immunized with the following: rabbit serum albumin reacted with carbodiimide (AC), bovine serum albumin (BSA) or homologous serum albumin (RSA). Sera containing antibodies to the conjugates did not precipitate oxytocin (450 I.U./ml) or lysine-vasopressin (96 I.U./ml).

Antibodies to oxytocin-albumin conjugate, as well as to vasopressin-albumin conjugate, could also be demonstrated by the passive hemagglutination technique with tannic acid treated and with the antigen coated sheep red blood cells[4].

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