ministation of silver nitrate causes accumulation of silver granules in the periventricular glial cells. The increase now observed in the number of the cysteine-rich glial cells in X-ray irradiated animals may be due to enhanced synthesis of the cysteine-rich material during the post-irradiation period in response to the presence of toxic substances arizing in consequence of the irradiation. The lack of lysosomal enzymes in the cysteine-rich periventricular glia[5] is against the possibility that the cysteine-rich granulations arise from a phagocytosed material.

Zusammenfassung. Nach einmaliger Röntgen-Kopfbestrahlung bei Ratten mit 3000 und 4000 R wurde eine statistisch gesicherte Zunahme der Zahl cysteinreicher periventrikulärer Gliazellen im Gehirn festgestellt.

Z. SREBRO

Department of Biology, Institute of Biomorphology, Medical Academy, Kraków (Poland),
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Isolation of a Pharmacologically Active Principle from Ehrlich Carcinoma Tumor Cells

The presence of pharmacologically active substances in tumor tissue has been relatively little investigated. A polypeptide undistinguishable from bradykinin has been isolated from human pulmonary carcinoma[1]. Overproduction of serotonin is an almost uniform finding in patients with carcinoid tumors[2]. Liberation of kalikrein into the blood stream with subsequnet formation of bradykinin has been demonstrated in patients with carcinoid tumor metastases stimulated by epinephrine[3]. GREENBAUM et al.[4] have found kinin forming and destroying enzymes in mice leukemia L 1210 cells.

We report here the isolation of a pharmacologically active principle from mice ascites carcinoma cells. 5×10^8 Ehrlich ascites tumor cells were extracted for each experiment by repeated freezing and thawing in saline. The cell fragments were removed by high speed centrifugation and the supernatant used in the experiments. Similar amounts of Ehrlich ascites tumor cells were extracted with 10% trichloracetic acid. The supernatants were subsequently extracted with ether, the aqueous phase then evaporated to dryness, and the residue redissolved in 10 ml saline and the pH adjusted to 7.2. The saline extracts will

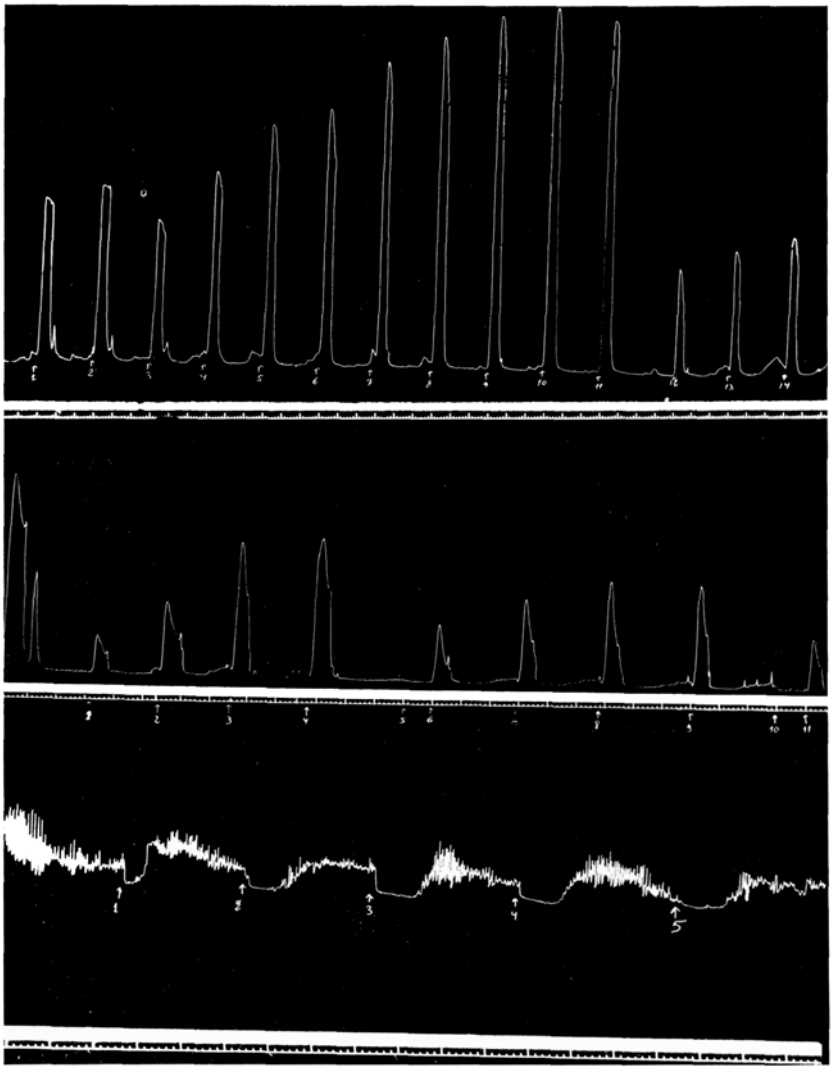

Fig. 1. Effect of Ehrlich ascites carcinoma tumor extract on the isolated guinea-pig ileum.
Experiment 1. Isolated guinea-pig ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1 and 2, 0.01 γ acetylcholine; 3, 0.1 ml; 4, 0.15 ml; 5, 0.2 ml; 6 ,0.25 ml; 7, 0.3 ml; 8, 0.4 ml; 9, 0.5 ml; 10, 0.6 ml; 11, 0.8 ml of TCA extract (TE); 12, partial neutralization of extract by 1 γ atropine 0.6 ml extract after 1 γ atropine; 13, 0.6 ml 7 min after 1 γ atropine; 14, 0.6 ml 10 min after 1 γ atropine.
Experiment 2. Isolated guinea-pig ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1, 0.2 ml; 2, 0.4 ml; 3, 6 and 11, 0.8 ml; 4, 1.2 ml of saline extract (SE); 5, 1 γ atropine; 7, 0.8 ml extract 4 min after 1 γ atropine; 8, 0.8 ml extract 7 min after 1 γ atropine; 9, 0.8 ml extract 10 min after 1 γ atropine; 10, 2 γ atropine.
Experiment 3. Effect of Ehrlich ascites carcinoma tumor extract on the isolated mouse ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1, 0.1 ml; 2, 0.2 ml; 3, 0.4 ml; 4, 0.8 ml of extract (TE); 5, 1 γ atropine + 0.4 ml extract.

be referred to as SE, and the TCA-treated extracts as TE. The contractile activity of the extracted material was tested on the isolated guinea pig and the mouse ileum as well as on the rat uterus.

Cardiovascular effects of the material were tested by i.v. injection in cats anesthetized with sodium pentobarbital (40 mg/kg body wt.). The blood pressure being recorded with a polygraph. It was found that 0.1 ml SE or TE contracted the guinea-pig ileum and the rat uterus and relaxed the mouse ileum (Figure 1). The i.v. injection of 0.1–0.3 ml of SE or TE caused a definite drop of the blood pressure (Figure 2) of the cats. All these effects were partially antagonized by the addition of atropine and/or mepyramine to the incubation medium (10^{-7} g/ml) or by injecting the cats with atropine (1 mg/cat) and mepyramine (200 γ/kg weight). The spasmogenic and hypotensive activity of the extracts was found to be non-tachyphylactic and slightly enhanced after ganglionic blockade with 100 γ hexamethonium. The tumor extracts were further investigated as to their possible identity with other known atropine- and mepyramine-resistant compounds such as 5 hydroxytryptamine (5-HT), some plasma kinins like bradykinin or kallidin as well as the phosphate nucleotides. Figure 3 shows the effect of morphine on the contractile activity of SE, TE and 5-HT on the atropinized and mepyramine treated guinea-pig ileum. It may be seen that 1 γ morphine/ml incubation fluid completely blocked the contractile activity of 5-HT, while enhancing that of SE and TE. Furthermore lysergic acid diethylamide did not antagonize the contractile activity of

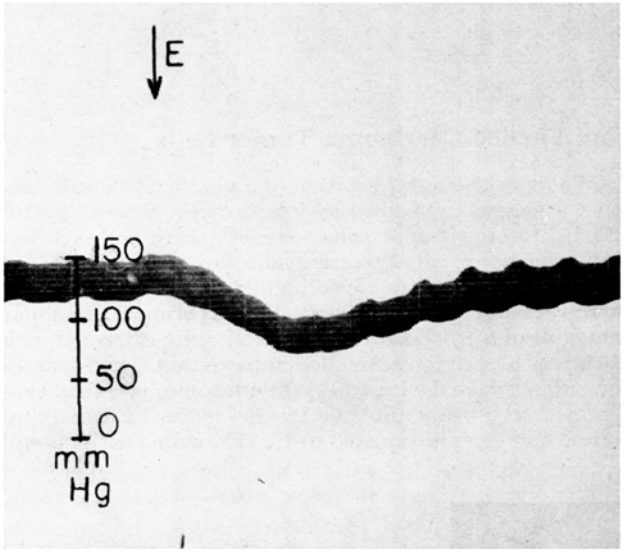

Fig. 2. Effect of Ehrlich ascites carcinoma tumor extract on the cat blood pressure. E, i.v. injection of 0.3 ml extract/kg.

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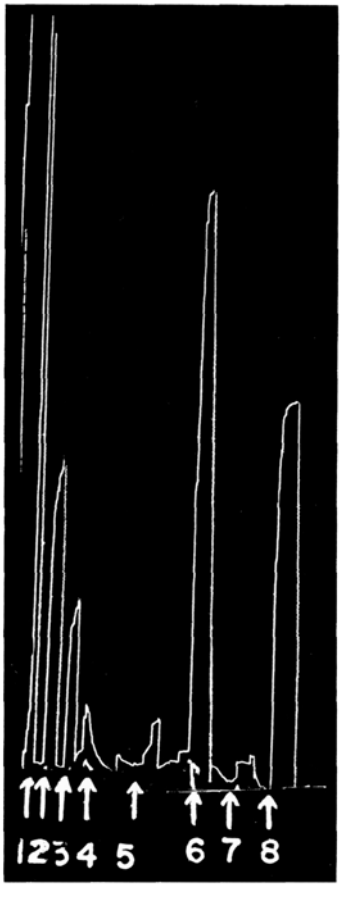

Fig. 3. Effect of Ehrlich ascites carcinoma tumor extract on the isolated guinea-pig lileum suspended in 10 ml of Tyrode solution containing atropine 10^{-7} γ/ml. Time of contact 1 min. 1, serotonin 50 ng; 2, 0.3 ml of TE + mepyramine 1 γ; 3, 6 and 8, 0.3 ml of TE; 4, morphine 10 γ; 5, serotonin 50 ng + morphine 10 γ; 7, serotonin 50 ng.

Fig. 4. Effect of mouse peritoneal cell extract on the isolated guinea-pig ileum suspended in 10 ml of Tyrode solution. Time of contact 1 min. 1, 0.3 ml of mouse peritoneal cell extract followed by atropine 1 γ; 2, 0.3 ml of mouse peritoneal cell extract; 3, atropine 1 γ + mepyramine 1 γ; 4, 0.3 ml of mouse peritoneal cell extract.

Comparative effects of SE, TE, and other pharmacologically active substances as obtained by various experimental procedures

	Guinea-pig ileum	Atropinized and mepyramine treated g.p.i.	Atropinized and mepyramine treated g.p.i. + morphine	Atropinized and mepyramine treated g.p.i. sensitized with chymotrypsin	Mouse ileum	Atropinized and LSD treated rat uterus
SE	+	+	++	++	relaxed	+
TE	+	+	++	++	relaxed	+
Acethylcholine	+	−	−	−	+	−
Histamine	+	−	−	−	−	−
5-HT	+	+	−	+		−
Bradykinin	+	+	+	++	+	+
Kallidin	+	+	+	++	+	+
ATP	+	+	+	+	relaxed	+
ADP	+	+	+	+	relaxed	+
AMP	+	+	+	+	relaxed	+
UTP	+	+	+	+	—	+

+, Contraction; ++, enhanced contraction; −, absence of contraetion.

SE and TE on the rat uterus. These experiments excluded the identity between 5-HT and SE or TE. Incubation of SE and TE with human red cells hemolysates, human plasma, trypsin or chymotrypsin had no effect whatsoever on their contractile activity, showing thus their non-identity with bradykinin and kallidin. Furthermore, it was found that the mouse ileum was contracted by bradykinin yet relaxed by SE and TE (Figure 1). Control experiments with ATP, ADP, AMP and UTP were also performed in order to verify the possible identity of the tumor cells extracts with these nucleotides. SE and TE, when tested on chymotrypsin treated guinea-pig ileum as described by EDERY[5], showed a higher contractile activity in contrast to ATP, ADP and AMP, whose contractile activity were not affected. EDERY has shown that chymotrypsin sensitizes the isolated guinea-pig ileum to various kinins, but not to substance P, eledoisin, angiotensin, adenosine-triphosphate, potassium chloride and barium chloride. The Table summarizes the comparative effects of SE, TE and other pharmacologically active substances as obtained by various experimental procedures.

Addition of papaverine to the atropinized and mepyramine-treated guinea-pig ileum, according to the method of LEVY and MICHEL-BER[6], did not affect the contractile activity of SE and TE, while abolishing the contractile effect of UTP, thus discriminating the tumor extracts from UTP. The possibility that adenine might be involved in the contractile activity of the tumor extracts was also ruled out, since adenine is known to have no effect in rat uterus[7]. SE and TE had the following chemical characteristics: dialysability through cellophane membranes, solubility in 10% trichloracetic acid, resistance to boiling for 20 min in an acid medium, insolubility in ethyl ether at acid pH and progressive loss of activity during storage.

The active principle found in Ehrlich ascites tumor cells was not present in normal peritoneal mice cells. Extracts prepared by the same methods from packed normal peritoneal mice cells showed contractile activity on the guinea-pig ileum, due to contamination with acethylcholine and histamine, since it was completely abolished by atropine and mepyramine addition to the bath (Figure 4). The results obtained in the present investigation and summarized in the Table demonstrate that Ehrlich ascites carcinoma cells contain a pharmacologically active material that does not belong to any of the known compounds so far described. Further experiments will be undertaken in order to clarify the exact chemical nature of the active compound.

Résumé. Les propriétés biologiques d'une nouvelle substance pharmacologique active isolée des cellules de l'ascite d'Ehrlich sont décrites en détail. La substance isolée contracte in vitro l'iléum du cobaye ou l'utérus du rat et relaxe l'ileum de la souris. In vivo, cette substance possède un effet hypotensif chez le chat.

B. SHOHAT and H. JOSHUA

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Electrophoretic Changes of Serum and Virus Particles Type 'C' and 'A' in a Plasmocytoma of Balb/c Mice

HIPA tumor, a mesenteric neoplasm associated with hemorrhagic ascites, was produced in mice of strain BALB/c by i.p. inoculation of a homogenized spleen obtained from a mouse of the same strain given i.p. injections of mineral oil[1].

The original tumor consisted of undifferentiated cells, usually of spindle shape and numerous collagen fibres. During serial cellular transplantation in isologous mice, there was increasing transformation of the cell pattern towards that of plasmocytoma. Plasmocytic elements