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

The Clinical Laboratory, Beilinson Medical Center, P.O. Box 85, Petah-Tikva (Israel), 4 January 1971.

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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

first appeared in the 3rd tumor generation and practically made up the entire neoplasm of the 26th generation[2]. Electrophoretic examination of sera of animals bearing the 1st, 2nd and 3rd tumor generation yielded values within the range of normal[1].

During generation 4th to 26th, narrow, steep peaks occurred in the region of α-2-globulins (Figure b), which were suggestive of paraproteins.

From the 5th to the 26th generation, the tumor contained numerous virus-like particles of type C according to BERNHARD[3] (Figure a), which however decreased in number to the 37th generation and were no longer present thereafter[4].

We have now continued similar observations up to the 78th cellular transplantation and have followed the development of the tumor histologically, electron microscopically and by electrophoresis of the serum, as previously described[1,2], by examining the 50th, 53th, 58th, 64th, 70th, 71th, 75th, 78th generation of the tumor.

During the period of observation, the plasmocytic architecture of the neoplasia remained unchanged and the electrophoretic pattern of the serum was normal (Table and Figure d). Virus particles of type C reappeared in small number during the 71th generation. Within the same tumor generation, and at irregular intervals in the subsequent ones, virus particles of type A were noted in the ergastoplasmic cisternae of neoplastic plasma cells. These particles were roundish, measured 75–80 nm and possessed 2 membranes enclosing an electron-lucent center (Figure c).

In contradistinction to viruses of type C, viruses of type A, which are common in experimentally produced plasmocytomas[5], have never proved to be oncogenic[6]. They act as precursors of oncogenic viruses[3,7] or as secretory products of tumor cells, and even as immunoglobulins[8,9]. In the present observation, it was not possible to demonstrate a relation between type A or C and the secretion of immunoglobulins.

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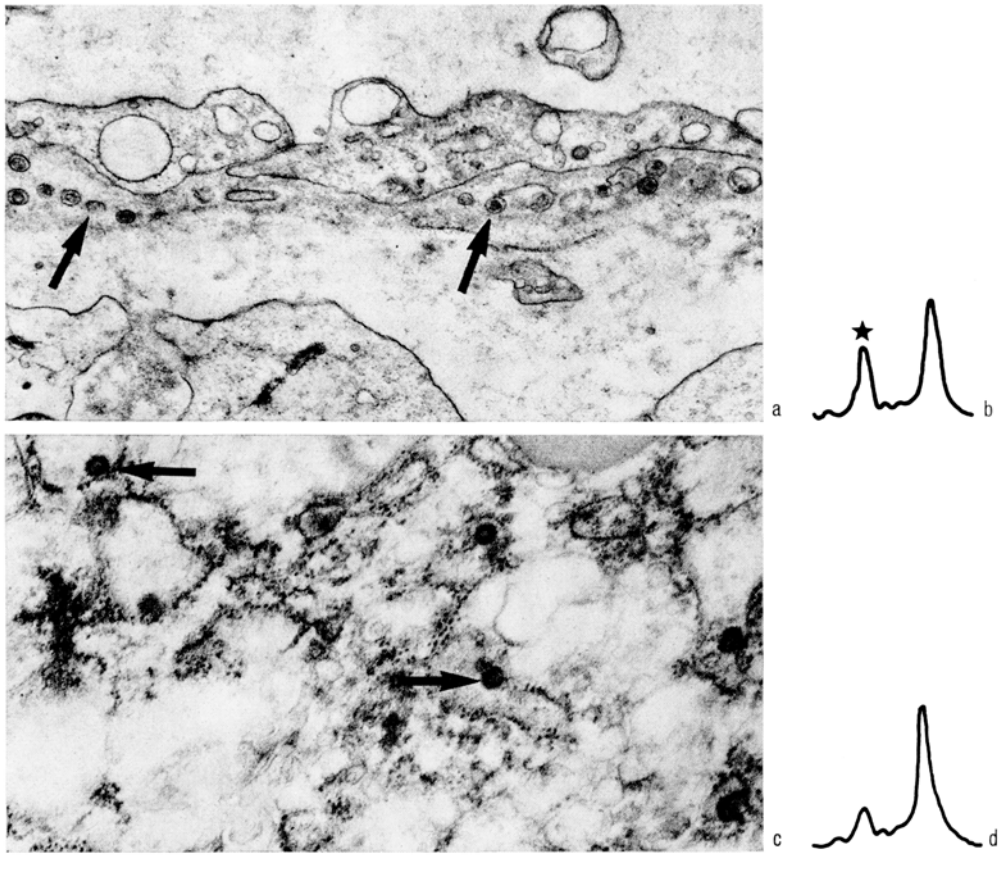

Virus particles and electrophoretic pattern in the course of HIPA tumor evolution. a) 19th tumor generation. Virus particles of type C in the extracellular space (arrows). ×23,077. b) 19th tumor generation. Electrophoretic pattern with narrow, steep peak in the region of α-2-globulins (star). c) 71th tumor generation. Intracisternal virus A particles (arrows). ×41,538. d) 71th tumor generation. Normal electrophoretic pattern.

Morphologic and electrophoretic findings in mice bearing transplanted HIPA tumor

Generations	Histologic form	Virus-like particles A	C	Electrophoretic pattern
1st to 2nd	Undiff. sarcoma	0	0	normal
3rd	Differentation into Plasmocytoma	0	0	normal
4th	Differentation into Plasmocytoma	0	0	α-2-globulin peak
5th to 26th	Differentation into Plasmocytoma	0	+++	α-2-globulin peak
27th to 37th	Plasmocytoma	0	+	normal
38th to 70th	Plasmocytoma	0	0	normal
71th to 78th	Plasmocytoma	+	+	normal

Zusammenfassung. Untersuchungen an einem Plasmocytom von BALB/c-Mäusen ergaben keine Beziehungen zwischen dem Vorkommen von virusähnlichen Partikeln des Typus «A» und «C» und der Sekretion von Immunoglobulin.

G. Pedio and J. R. Rüttner[10]

Institute of Pathological Anatomy, University of Zürich, Kantonspital, CH-8006 Zürich (Switzerland),
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Immunohistochemical Studies on Phenylethanolamine-N-Methyltransferase, Dopa-Decarboxylase and Dopamine-β-hydroxylase

With the use of specific antibodies towards dopamine-β-hydroxylase (DBH) the localization of this enzyme in the adrenal medulla and in the peripheral nervous system was described[1–4]. In addition we also investigated the cellular localization of DBH in the central nervous system[3,4]. We extended these studies, and the localization of other catecholamine synthesizing enzymes was investigated in various tissues. The present paper describes the cellular localization of dopa-decarboxylase (DCA), phenylethanolamine-N-methyltransferase (PNMT) and DBH in peripheral and central tissues of mammals. A preliminary report on the cellular localization of PNMT in the adrenal medulla was previously presented[4].

Male Sprague Dawley rats (body wt. 150–170 g) were used in these studies. In some experiments guinea-pigs, hamsters and mice were also studied.

PNMT was purified from the supernatant fraction of bovine adrenal medulla by the previously reported procedure[5,6]. The ultracentrifuge pattern reveals that the enzyme is homogenous and a molecular weight of 31,000–33,000 was estimated by the meniscus depletion method of Yphantis[7]. The purified enzymes show several bands on disc gel electrophoresis, but all the protein stained bands are enzymatically active, indicating the possible presence of conformational PNMT isoenzymes[6].

DCA was purified from bovine adrenal glands by a recently described procedure[8,9].

DBH was purified from bovine adrenal gland as described previously[10].

Immunization and testing of antibodies was performed as previously described[3,4].

The immunohistofluorescent procedure has previously been described in detail[4]. Cryostate sections from unfixed tissue were used. The sections were usually post-fixed in acetone for 10 min. The indirect method was used for the staining (see[11]), which involved the use of fluoresceine-isothiocyanate (FITC) labelled sheep anti-rabbit immunoglobuline. Before the use of FITC labelled immunoglobuline it was shaken with mouse liver powder or brain powder (Miles Laboratories, USA) at room temperature for 2 h in order to remove as much as possible of the FITC labelled proteins not related to the specific antigens under investigation. Furthermore, the specific antibodies against DCA, PNMT and DBH were incubated with serum from the species studied overnight at room temperature, in order to remove unspecific antibodies. As a specificity test, preimmune serum was used instead of the specific antibody serum.

PNMT. *Adrenal medulla.* In rat and mouse most of the gland cells showed a specific greenish immunofluorescence of moderate intensity which was localized to the cytoplasm of the cells (Figure 1). The outlines of these fluorescent cells were somewhat indistinct, probably due to some diffusion of the PNMT. In guinea-pigs practically all of the gland cells showed a specific immunofluorescence. The cells of the adrenal cortex did not show any specific fluorescence.

Peripheral and central nervous system. No specific immunofluorescence was observed in the sympathetic ganglia or in the CA cell bodies of the central nervous system. Furthermore, no specific immunofluorescence was observed cranial of a transection of the sciatic nerve, which contains many adrenergic axons[12,13]. – In all sections of the brain there appeared a strong unspecific fluorescence in the apical parts of the ependyma outlining the ventricles and in the glial cells, especially the astroglia.

DCA. *Adrenal medulla.* A specific immunofluorescence of weak intensity was observed in the cytoplasm of

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