

Ehrlich Ascites Tumor cells, while immune antisera for mouse erythrocytes and mouse liver homogenate have no haemolytic action. (3) The haemolytic action on sheep erythrocytes by immune antisera for Ehrlich Ascites Tumor cells does not appear to be due to a complete antigen in common between Ehrlich Ascites Tumor cells and sheep erythrocytes; the reaction in fact is not reversible, as immune antisera for sheep erythrocytes have not shown any toxic action *in vitro* for Ehrlich Ascites Tumor cells. (4) It may be stated that the haemolytic action for mouse, rat and sheep erythrocytes exercised by immune antisera for Ehrlich Ascites Tumor

cells might be due to the presence in the tumoral cells of an antigenic fraction of heterophile type.

Riassunto. Gli autori hanno osservato che i sieri di animali immunizzati con cellule del tumore cancro ascite di Ehrlich sono emolitici per gli eritrociti di topo, ratto e montone e presumono che le cellule del tumore di Ehrlich possano contenere frazioni antigene di tipo eterogenetico.

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Nucleolar Localization of Succinic Dehydrogenase in Human Malignant Cells with MTT

It is known that succinic dehydrogenase is absent in both nuclei and nucleoli of mammalian cells¹⁻⁶, excepting in bird erythrocytes which according to BRACHET⁷ might be due to absence of mitochondria in these erythrocytes. Nucleolar localization of succinic dehydrogenase in normal and malignant stratified epithelia of human cervix were observed in this laboratory⁸ with 2,3,5 triphenyl tetrazolium chloride (TTC) and 2,2'-di-p-nitrophenyl-5, 5'-diphenyl-3, 3'-(3, 3'-dimethoxy-4, 4'-biphenylene) ditetrazolium chloride (Nitro-BT).

The present report is to confirm the nucleolar localization of succinic dehydrogenase with a third important tetrazolium salt, 3-(4,5-dimethyl-thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT).

Frozen sections (8 μ) were cut from fresh unfixed epidermoid carcinomatous tissue of human cervix from two cases. The sections were mounted on clear glass slides and stained⁹ in the incubating media containing MTT. The common controls to ensure the specificity of

the reaction were applied^{4,10}. An additional frozen section was stained by pyronin methylgreen for observing the position and size of the red stained nucleoli.

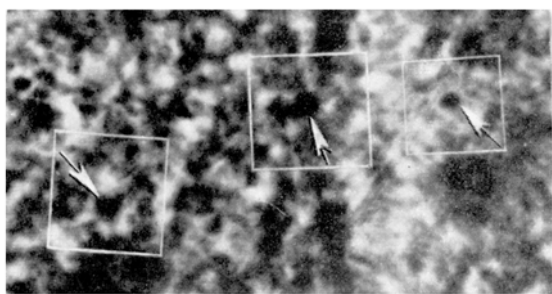
The sections were studied and photographed under oil immersion objective of a light microscope. More than one thousand cells were studied in each case.

Intracellular deposits of black cobalt formazan were observed in the cytoplasm and on the nucleolus-like intranuclear bodies of malignant cells. These stained intranuclear bodies exhibited (Figure) a morphological pattern of nucleoli. The body of the nuclei remained unstained.

Résumé. Les auteurs complètent leurs recherches sur la localisation cytochimique de l'activité de la déshydrogénase succinique dans les nucléoles en utilisant un troisième sel de tétrazolum, MTT d'une importance particulière.

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Frozen section of human epidermoid carcinoma cervix, stained for succinic dehydrogenase activity with MTT. Arrows exhibit nucleolar enzymatic activity in three malignant cells within the enclosed areas. (Objective $\times 100$, eyepiece 12.5 \times).

Occurrence of an Eleldoisin-Like Polypeptide (Physalaemin) in Skin Extracts of *Physalaemus fuscumaculatus*¹

Acetone and methanol extracts of wet or dry skin of *Physalaemus fuscumaculatus*, a South-American amphibian (Tucuman, Argentine), contain a principle which exerts a powerful stimulant action on several smooth-muscle preparations and potently lowers the systemic blood pressure in dogs and rabbits.

The active principle, which we call *physalaemin*, is in all probability a polypeptide. In fact, it was completely inactivated both by chymotrypsin and trypsin digestion (extract corresponding to 0.1 g fresh skin plus 100 μ g chymotrypsin or 1 mg trypsin; incubation for 30 min at pH 7.5–7.7 and 38°C), and acid hydrolysis of active chromatographic spots yielded a mixture of amino acids.

¹ Supported by grants from the Consiglio Nazionale delle Ricerche, Roma, and the Rockefeller Foundation, New York.

¹ P. M. MARCAUSE, Lab. Invest. 6, 137 (1957).

² B. MONIS, M. M. NACHLAS, and A. M. SELIGMAN, Cancer 12, 1238 (1959).

³ M. M. NACHLAS, K. C. TSOU, E. DE SOUZA, C. S. CHENG, and A. M. SELIGMAN, J. Histochem. Cytochem. 5, 427 (1957).

⁴ K. OGAWA and H. M. ZIMMERMAN, J. Histochem. Cytochem. 7, 342 (1959).

⁵ B. PEARSON and V. DEFENDI, Cancer Res. 15, 593 (1955).

⁶ M. WACHSTEIN and E. MEISEL, J. biophys. biochem. Cytol. 1, 484 (1955).

⁷ J. BRACHET, Biochemical Cytology (Academic Press Inc., New York 1957), p. 94.

⁸ P. DE, R. CHATTERJEE, and S. MITRA, J. Histochem. Cytochem. 10, 6 (1962).

⁹ A. G. E. PEARSE, Histochemistry, 2nd Ed. (J. & A. Churchill Ltd., London 1960), p. 910.

¹⁰ R. FRIEDE, J. Histochem. Cytochem. 6, 350 (1958).

Physalaemin may be distinguished in parallel assays from all other known polypeptides, including eledoisin². However, eledoisin is the polypeptide to which physalaemin seems so far to be most similar in its pharmacological properties.

Like eledoisin, physalaemin potently stimulates movements and tonus of the isolated rabbit large intestine (threshold concentration: extract corresponding to 1–2 µg fresh tissue per ml nutrient liquid), the isolated guinea-pig ileum (2–6 µg fresh tissue/ml), and other preparations of gastro-intestinal smooth muscle as well (rat stomach, rat duodenum, frog stomach, etc.); like eledoisin, physalaemin is not very active on the oestrus uterus of the rat.

The blood pressure of the anaesthetized dog and rabbit is potently lowered by the intravenous injection of *Physalaemus* extracts. Intensity and duration of hypotension are proportional to the injected dose of physalaemin and there is no sign of tachyphylaxis. A short-lived but evident fall of blood pressure may be produced in the dog by the intravenous injection of the extract corresponding to 5 µg fresh skin per kg body weight.

The accompanying Table gives some approximate activity equivalencies for the extract corresponding to 1 g fresh *Physalaemus* skin.

Approximate equivalencies for 1 g fresh <i>Physalaemus</i> skin				
	Eledoisin	Substance P	Bradykinin	Histamin
Dog blood pressure	300–400 µg	20000 to 30000 µ	> 20 mg	> 20 mg
Rabbit large intestine	150–200 µg	50000 µ	> 15 mg	> 100 mg
Rat uterus	30–50 µg	50–200 µ	< 1 µg	inhibition
Rat duodenum	100 µg	---	inhibition	---
Guinea-pig ileum	100–200 µg	---	0.5–2 mg	---

Rabbit large intestine, guinea-pig ileum and dog or rabbit blood pressure are particularly suitable for the

quantitative bioassay of physalaemin, owing to their high sensitivity and the excellent dose/response relationship.

Crude *Physalaemus* extracts do not apparently contain other active substances with the possible exception of small amounts of a bradykinin-like polypeptide. A biologically pure physalaemin preparation may be obtained by absorption of the crude *Physalaemus* material dissolved in 95% ethanol on an alkaline alumina column and subsequent elution with descending concentrations of ethanol, followed by ion-exchange chromatography on a column of Amberlite CG-50.

The occurrence of physalaemin is now being investigated in other *Physalaemus* species, as well as in numerous other amphibians gathered throughout the world.

A full report on the pharmacological properties of physalaemin will be published elsewhere. The isolation of the polypeptide and the elucidation of its structure is in progress.

Riassunto. Gli estratti di pelle fresca o secca di *Physalaemus fuscumaculatus* contengono una sostanza attiva di natura polipeptidica, la *physalaemina*, dotata di potente azione ipotensiva e di intensa azione stimolante su alcuni muscoli lisci extravasali. La *physalaemina* è facilmente distinguibile, mediante saggi paralleli, da tutti gli altri polipeptidi biogeni finora noti, compresa la eledoisina che alla *physalaemina* s'accosta per parecchie delle sue azioni farmacologiche.

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² V. ERSPAMER and A. ANASTASI, *Exper.* 18, 58 (1962). – A. ANASTASI and V. ERSPAMER, *Brit. J. Pharmacol.*, in press. – V. ERSPAMER and G. FALCONIERI ERSPAMER, *Brit. J. Pharmacol.*, in press.

Occurrence of Bradykinin-Like Substances in the Amphibian Skin¹

Besides being an enormous store-house of indolealkylamines, phenylalkylamines and imidazolealkylamines, the amphibian skin appears to be an important production and/or storage site of highly active polypeptides.

One of them, physalaemin, has been described in a preceding communication². In this preliminary report, the occurrence of bradykinin-like substances will be briefly described. The term bradykinin-like should be interpreted in a broad sense.

So far about eighty amphibian species, collected in all parts of the world, but especially in South America, have been studied. High, and sometimes enormous, amounts of bradykinin-like compounds appeared to be present in methanol extracts of the skin of the examined *Phyllomedusae* (*Phyl. sawagi*, *Phyl. rhodei*) and *Ranae* (*R. esculenta*, *R. temporaria*, *R. pipiens*, *R. warschewitschii*, *R. japonica*, *R. calesbiana*, *R. nigromaculata*).

The polypeptide nature of the active skin constituents is shown by their rapid and complete inactivation

produced by chymotrypsin incubation, and by some preliminary results obtained following acid hydrolysis of the eluates of active paper chromatographic spots.

The accompanying Table presents the activity, expressed in terms of pure bradykinin, of crude methanol extracts of the *Phyllomedusa* and *Rana* species examined. It should be emphasized that the figures are merely indicative of the relative potency of action possessed by the crude skin extracts on the different test-objects. Bradykinin served only as a standard substance, and very often the response to skin extracts was even qualitatively different from that given to bradykinin. In reality, all or nearly all aspects of the biological activity of crude *Phyllomedusa* or *Rana* extracts are due to a more or less complex mixture of active polypeptides. To elucidate the composition of this mixture, each extract should be studied separately and singularly.

¹ Supported by grants from the Consiglio Nazionale delle Ricerche, Roma, and the Rockefeller Foundation, New York.

² V. ERSPAMER, G. BERTACCINI, and J. M. CEI, *Exper.* 18, 562 (1962).