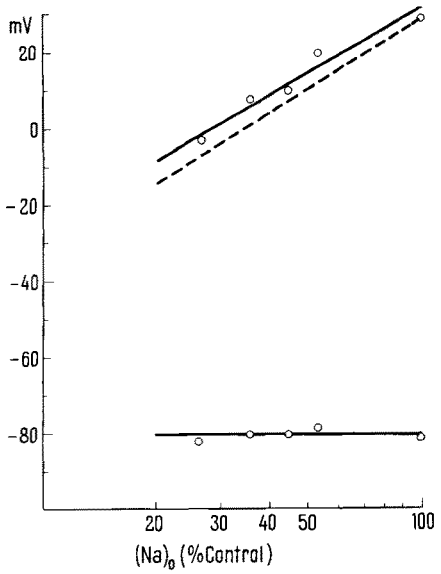

Fig. 2. Action potentials (top) and resting potentials (bottom) as a function of the extracellular sodium concentration. The dashed line is drawn through the mean overshoot of 29 mV with a slope equal to 61.5 mV/tenfold change of $[Na]_0$.

permeable to sodium ions. Clearly, the agreement between results and theory is as good as can be expected, thus justifying the conclusion that the atrium of the guinea pig heart, in contrast to the ventricle, follows the sodium hypothesis.

When the tissue was kept in a sodium-poor solution for more than 1 h, the resting potential dropped by 10 or 20 mV. This same effect has been reported for ventricular fibres of the guinea pig[4,6]; it may be due to a low intracellular sodium concentration, resulting in a decreased rate of sodium extrusion.

Zusammenfassung. Es werden Aktionspotentiale vom Meerschweinchenvorhof in Badelösungen verschiedener Na-Konzentrationen mittels intrazellulärer Elektroden registriert. Während das Aktionspotential des *Ventrikels* relativ unempfindlich gegen Veränderungen der Na-Konzentration bleibt[4,6], lässt sich die «Na-Hypothese» für den *Vorhof* anwenden.

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Heterophile Antigens in Ehrlich Ascites Tumor Cells

The study of the immunological reactions which can be obtained by injections of tumor cells has been, and remains, a matter of the utmost interest for several authors. As concerns the bibliography on this subject, we refer to the previous works made by one of us[1–4], while in the present research we wanted to investigate the possibility of seeking possible heterophile antigens of FORSSMAN[5,6] in common between normal and tumoral cells.

As we have not found in the ample bibliography of the Ehrlich Ascites Tumor[7–16] any reference to heterophile antigens with erythrocytes, either human or animal, we decided to investigate whether immune antisera for this tumor may contain antibodies capable of haemolyzing human and/or animal erythrocytes.

Materials and Methods. For our research we used ten normal rabbits of an average weight of about 2000 g each, divided into three groups. The first group, of four rabbits, was treated with eight intravenous injections of suspensions of living cells of Ehrlich Ascites Tumor, and further by three new call injections of cells, made about one month *after* the first treatment, for a total of eleven intravenous injections to each rabbit, corresponding to 30 millions of living cells. Suspensions of living cells were prepared according to the method described by ROSSI and DI VITA[17,18], while the living cells were tested by the method of NOVELLI[19]. The second group, of three rabbits, was treated by the same technique by injections of erythrocytes of normal albino mice of the same strain used for the transplantations of the Ehrlich Ascites Tumor, while the third group, of three rabbits, was treated by liver homogenate of albino mice. Activity of the immune antisera for Ehrlich Ascites Tumor cells was controlled *in vitro* by the method of LEE, RICHARDS, and KLAUSNER[20].

When immune antisera for Ehrlich Ascites Tumor cells were obtained, we investigated whether they could be haemolytic for human Blood Group 0 erythrocytes, as for mouse, rat, guinea-pig, sheep, ox and horse erythrocytes. 0.5 ml of decomplemented and diluted immune antisera were put with 0.5 ml of 10% suspension of erythrocytes and with 0.5 ml of fresh complement. Test tubes were put at + 37°C, and we read twice, after 30 and 60 min of incubation respectively; for each series of tests we always made a blank with normal rabbit sera. Table I

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Tab. I

Immune antisera Ehrlich Ascites Tumor Title of dilutions	Erythrocytes Mouse	Rat	Guinea-pig	Sheep	Ox	Horse	Human blood group 0
1:2	++++	+++–	+++–	++++	----	----	----
1:10	++++	++––	----	++++	----	----	----
1:20	+++–	+–––	----	++++	----	----	----
1:50	++––	----	----	++++	----	----	----
1:100	+–––	----	----	+++–	----	----	----
1:200	----	----	----	++––	----	----	----
1:300	----	----	----	----	----	----	----

Tab. II

Title of dilutions of antisera proved with sheep erythrocytes	Immune antisera for albino mouse erythrocytes	Immune antisera for albino mouse liver homogenate
1:2	----	----
1:10	----	----
1:50	----	----
1:100	----	----

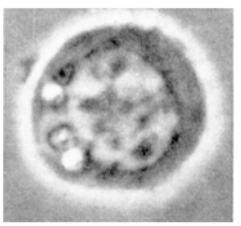
Symbols: ++++ complete haemolysis; +++– 75% haemolysis; ++–– 50% haemolysis; +––– 25% haemolysis; ---- no haemolysis.

shows the results we obtained; titles are the average of single titles. The blanks made with normal rabbit sera did not give any haemolysis.

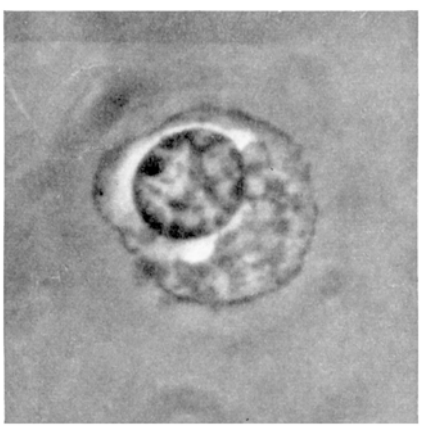
The results we obtained by this series of experiments might permit us to conclude that Ehrlich Ascites Tumor cells contain an antigen in common with erythrocytes of mouse, rat and sheep; excluding the first two, as titles are very low and also because it might depend on a species antigen, we decided to make controls on sheep erythrocytes. Then we studied whether immune antisera of rabbits, treated by liver homogenate and erythrocytes of normal albino mouse, could be haemolytic for sheep erythrocytes, and whether immune antisera for sheep erythrocytes could have a specific toxic action *in vitro* on the cells of Ehrlich Ascites Tumor. Table II shows the results we obtained, proving the immune antisera for mouse erythrocytes and for mouse liver homogenate with sheep erythrocytes, by the same technique mentioned above.

The control, to test whether immune antisera for sheep erythrocytes could show a toxic action *in vitro* on the Ehrlich Ascites Tumor cells, was made by the method described by LEE, RICHARDS and KLAUSNER[20]. Two parts of diluted immune antisera for sheep erythrocytes (ISM, Milano, title 1 : 10000) and two parts of fresh complement were added to one part of 10% suspension of living Ehrlich Ascites Tumor cells, and with four parts of NaCl solution 9%, and mixed. Some drops of suspension of cells were then immediately put on a slide, covered, and cover borders sealed by paraffin; tumor cells were observed by a Zeiss phase microscope during 120 min, to observe their swelling. The same control was made with immune antisera for Ehrlich Ascites Tumor cells. The enclosed microphotographs show respectively Ehrlich Ascites Tumor cell after 120 min of incubation with immune antisera for sheep erythrocytes (a), Ehrlich Ascites Tumor cell after 60 min (b), and after 120 min (c) of incubation with immune antisera for Ehrlich Ascites Tumor cells; the evident swelling of cells as from (b) and (c) confirms the toxic action *in vitro* of the specific immune antisera, while no swelling is observed in the cells treated by immune antisera for sheep erythrocytes.

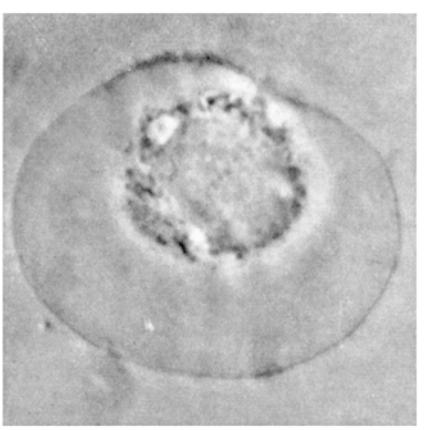
Conclusions. (1) Rabbit immune antisera for Ehrlich Ascites Tumor cells showed an haemolytic activity, at low titles, for mouse, rat and sheep erythrocytes; such titles are not increased by further several call injections. No haemolytic action is shown by such antisera for erythrocytes of guinea-pig, ox, horse and for human erythrocytes of Blood Group 0. (2) Haemolytic activity for sheep erythrocytes is specific of immune antisera for



(a) Ehrlich Ascites Tumor cell incubated for 120 min with immune antisera for sheep erythrocytes.



(b) E.A.T. cell incubated for 60 min with immune antisera for E.A.T. cells.



(c) E.A.T. cell incubated for 120 min with immune antisera for E.A.T. cells.

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 cells[1-6], excepting in bird erythrocytes which according to BRACHET[7] 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 laboratory[8] 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 stained[9] 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 tetrozolium, MTT d'une importance particulière.

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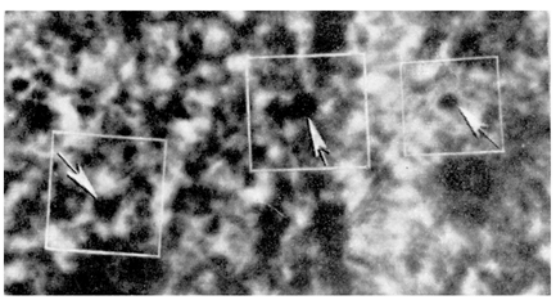
Cell Research Department, Chittaranjan National Cancer Research Centre, Calcutta (India), August 13, 1962.



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 ×100, eyepiece 12.5 ×).

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Occurrence of an Eledoisin-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 μg 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.