Therefore we suggest the preparation of specific antiserum against the acidophilic cells of the human anterior pituitary gland. The therapeutic use of this antiserum might favourably influence some neoplastic processes in humans and prevent the onset or block the growth of metastasis in several malignant tumours like adenocarcinoma of the breast, endometrial cancer and others whose growth hormone-dependence is unpredictable. Also acromegaly and some kinds of hypophysial diabetes may find its elective treatment by the same antiserum.

Zusammenfassung. Die Anwendung von anti-Adenohypophysen-Serum in der Therapie gewisser Wachstumshormon-abhängiger menschlicher Tumoren wird vorgeschlagen. Der Vorschlag einer derartigen immunologischen Intervention basiert auf der bekannten Hormonabhängigkeit oder -empfindlichkeit zahlreicher Tumoren und auf der Tatsache einer langanhaltenden Hemmung Wachstumshormon produzierender Zellen am experimentellen Tier. Viele Tumoren beim Menschen haben wahrscheinlich die Hormon-Abhängigkeit der normalen Gewebe oder Drüsen, von denen sie sich herleiten, beibehalten. Da Mangel an Wachstumshormon keine schädigenden Wirkungen auf andere vitale Funktionen hervorruft, wird die Herstellung eines anti-Menschen-Adenohypophysenserums und dessen Anwendung bei nachgewiesenermassen hormonabhängigen Tumoren oder Metastasen, wie z.B. bei gewissen Formen des Brustkrebses, vorgeschlagen. Eine Beeinflussung der Akromegalie und gewisser Formen von hypophysärer Diabetes durch dasselbe Antiserum kann ebenfalls in Betracht gezogen werden.

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Protein Synthesis in Polymorphonuclear Leucocytes in the Presence of Diphtheria Toxin

Numerous investigations indicate that cells in primary culture, as well as cell lines derived from different mammalian species, maintained the donor animal's sensitivity or resistance to diphteria toxin[1-5]. The important finding that the lethal effect of the toxin is a result of inhibition of protein synthesis in susceptible cells[4,5] enabled an approach for elucidation of the mechanism of toxin resistance of cells. It has been shown that resistance appears to be linked to the cell membrane and to process of macromolecular uptake, and not to the protein synthesizing apparatus of the cells[6]. Although most of the differences in susceptibility to diphteria toxin were observed in cells cultivated in vitro, there is no doubt that the results have implications for the situation in vivo in the host organism. However, the question remains whether all cells of a sensitive mammalian host are sensitive to the action of the toxin or only certain cell types are involved. In studies on the effect of diphtheria toxin in vivo in the sensitive guinea-pig, only the heart and the pancreas showed inhibition of protein synthesis. No such inhibition was observed in the organs of mice, which are toxin-resistant[7]. On the other hand, fibroblasts cultured from guinea-pigs peritoneal exudate were found in preliminary experiments to be resistant to the toxin[6]. In the present work protein synthesis in polymorphonuclear leucocytes of man, guinea-pigs and mice were investigated in the presence of diphteria toxin. Two other cell types were included as controls, namely monkey kidney cells BSC_1 and Ehrlich Ascites tumor cells.

To obtain polymorphonuclear leucocytes from guinea-pigs and mice, the former were injected i.p. with 10 ml of 5% sodium caseinate, the latter with 0.5 ml. Sixteen to 18 h later, 50 ml of saline-heparin (5 U/ml) were introduced into the peritoneal cavity of the guinea-pigs and the exudate was collected by gravity drainage into cellulose-nitrate tubes. The cells from the mice were washed out from the peritoneal cavities with saline-heparin and collected into cellulose-nitrate tubes. The cell suspensions were filtered through perforated stainless steel mesh and harvested by centrifugation for 10 min at 500 rpm in a refrigerated MSE centrifuge. After resuspension of the pellet in Eagle's modified medium supplemented with 10% calf serum, about 10^7 cells/ml were used in the reaction. Differential counts showed that in the cell exudate from guinea-pigs usually 90% were polymorphonuclear leucocytes; in the exudate from mice about 70% were polymorphs.

In order to obtain polymorphonuclear leucocytes from man, 20 ml of venous blood were drawn with siliconized syringe and mixed immediately with 380 ml of cold saline[8]. This suspension was distributed into 8 cellulose-nitrate 50 ml tubes and centrifuged for 10 min at 2000 rpm in a refrigerated MSE centrifuge. The erythrocytes were lysed by resuspension of each sediment in 20 ml of distilled water for about 30 sec, followed by addition of 20 ml of 1.7% solution of sodium chloride. After centrifugation the supernatants were discarded and the sedimented cells, collected into 1 tube with 5 ml of saline, were submitted to osmotic shock for the second time, and treated as above to restore isotonicity. The suspension of cells was centrifuged and, after removal of the supernatant, the cell pellet was resuspended in 5 ml of Eagle's modified medium supplemented with 10% calf serum. Differential counts showed that about 70% of the cells were polymorphonuclears. The suspension used in the reaction contained 10^7 cells/ml.

Monkey kidney cells BSC_1[9,10] were harvested from 4- to 5-day-old monolayer cultures in Roux bottles; the medium was removed and the cells were treated by Versene solution[11] for 15 min at 37 °C. The cell suspension was centrifuged for 5 min at 1000 rpm in a refrigerated MSE centrifuge, and the pellet resuspended in Eagle's medium supplemented with 10% calf serum.

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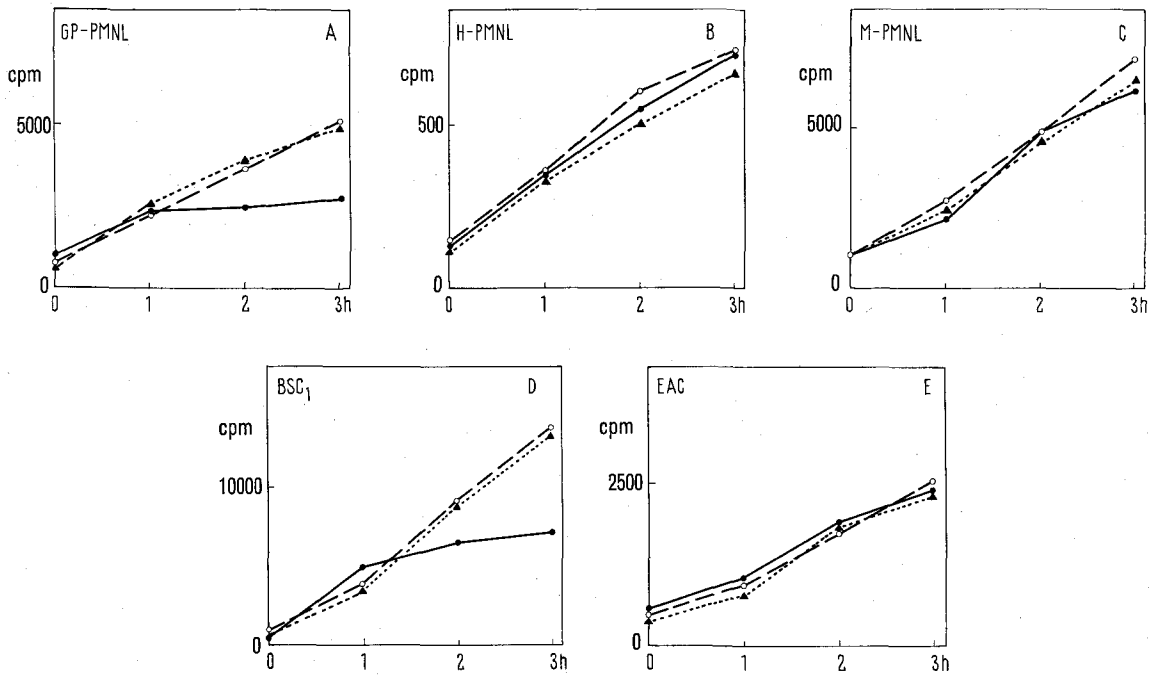

Incorporation of L-leucine-1-C^{14} into protein of polymorphonuclear leucocytes of guinea-pigs (A), man (B) and mice (C), and into monkey kidney cells (D) and Ehrlich Ascites cells (E). ●, in the presence of diphtheria toxin; ○, in the presence of toxin and antitoxin; △, control.

Ehrlich Ascites cells (EAC) of 7 days growth in albino mice were collected from the peritoneal cavities of the animals with salin-heparin (5 U/ml) solution. The cells were centrifuged and the pellet resuspended in the same medium as above. A suspension of 10^6 cells/ml of both BSC_1 and EAC was used in the reaction.

Protein synthesis was measured by incorporation of L-leucine-1-C^{14} into the trichloroacetic acid precipitable fraction of the cells. The reaction mixture contained: cell suspension – 1.5 ml; L-leucine-1-C^{14} 5 μc – 6μmole/ml – 0.1 ml; diphteria toxin 100 Lf/ml[12] (where necessary) – 0.2 ml; antitoxin 200 AU/ml[12] (where necessary) – 0.2 ml. The reaction mixture was incubated in a water-bath shaker at 37 °C. At 1 h intervals, 1 ml of cold 10% trichloroacetic acid was added to 0.5 ml samples of the reaction mixture. The tubes were kept in ice for 10 min and occasionally stirred on a Vortex mixer. After centrifugation for 5 min at 2000 rpm the supernatant was discarded, and the sediment was washed twice by resuspension in 5 ml cold trichloracetic acid and centrifugation. The pellet was dissolved in 2 drops of 5*N* NaOH, then 10 ml of scintillation fluid[13] were added. The radioactivity was measured in a Packard Tricarb scintillation counter.

The results are presented in the Figure. Diphtheria toxin did not affect the incorporation of leucine in human polymorphonuclear leucocytes (B), and in leucocytes from mice (C), in contrast to those from guinea-pigs (A). In the latter, inhibition of protein synthesis started already after 1 h in the presence of toxin. The toxic effect was alleviated completely in the presence of antitoxin. The effect of diphtheria toxin on the monkey kidney cells BSC_1 (D) was similar to that on leucocytes from guinea-pigs. No effect of the toxin was seen in Ehrlich Ascites cells (E). The latter result is in variance with the finding of KATO[14], who observed that diphtheria toxin inhibited protein synthesis in Ehrlich Ascites cells. The discrepancy may be due to the fact that KATO[14] used cells cultivated in vitro.

MESROBEANU et al.[15] concluded that diphtheria toxin was not leucocidal for leucocytes of guinea-pigs, a conclusion drawn after 1 h observation of the cells in the presence of toxin. This seems to be in variance with the results reported here. The discrepancy, however, may be more apparent than real, since STRAUSS and HENDEE[4] have demonstrated that protein synthesis in toxin-treated cells stops several hours before any generalized morphological damage becomes evident.

The fact which emerged from this study, that human polymorphonuclear leucocytes are not sensitive to diphtheria toxin, supports the conclusion that in a toxic sensitive host such as man, only certain organs or cell types are toxin-sensitive. The differences in toxin susceptibility of the same type of cells from man, guinea-pig and mouse probably reflect differences in membrane structures of these cells.

Résumé. La toxine diphthérique n'inhibe pas l'incorporation de la L-leucine-1-C^{14} dans les protéines des leucocytes polymorphonucléaires de l'homme et de la souris; on sait que l'homme est très sensible à la toxine et que la souris est très résistante. La synthèse protéique dans le même type de cellules du cobaye a été fortement inhibée dans les mêmes conditions expérimentales.

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[11] Versene solution – 0.02%, NaCl – 0.8%, KCl – 0.02%, Na_2HPO_4 – 0.115%, KH_2PO_4 – 0.02%, pH 7.2.

[12] Crude diphtheria toxin and antitoxin were kindly provided by Rafa Labs., Jerusalem. Before use, the diphtheria toxin was dialyzed overnight against saline-phosphate buffer 0.01*M*, pH 7.0 at +4 °C.

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