ness of these antisera in stimulation of DNA synthesis might be related to the methods of immunization and the sources of the antigens employed. A steep dose response curve for DNA stimulation by ALG with some reduction of response at the highest ALG concentration has also been reported[14] using guinea-pig anti-rabbit-lymphocyte serum on lymph node cell cultures.

The narrow limits of stimulating activity expressed by ALG probably relate to an inherent complement-independent cytotoxic activity. The low number of viable leukocytes found in the presence of high concentrations of ALG indicated that these concentrations did not simply inhibit DNA per se, but instead destroyed the viability of the cells. This is probably why the non-specific stimulation of DNA synthesis by PHA is also prevented in high concentrations of ALG. After dilution of the ALG to a point that cell viability as measured by dye exclusion remained at 45% or above, PHA was again active in the presence of ALG. This confirms the findings of LUNDGREN et al.[15], SIMONS et al.[8], and WOODRUFF et al.[13] that the stimulatory effect of PHA may be inhibited by ALG. However, the inhibition may reflect cell death or injury rather than a more specific biochemical effect.

In the case of ALS, the lack of ^{3}H-thymidine uptake at the 2 highest concentrations was associated with a cell viability which ,while reduced, was as good or better than that at the peak DNA response to ALG alone. This suggests that either the dye exclusion test is an inadequate measure of cell injury or an additional mechanism is involved in the failure of response to the highest concentrations of ALS.

Peak DNA synthesis occurred with only 2.1 mg of ALG protein as compared to 3.75 mg of ALS protein, suggesting a higher specific activity of ALG. Whereas the ALS contains all of the serum proteins, the ALG is the purified 7S IgG fraction, which is known to contain the specific antilymphocyte activity. Since the stimulation of DNA synthesis occurs in such a narrow dilution range of ALS or ALG, this in vitro technique appears not to be an ideal assay for antilymphocyte activity[16].

Résumé. La faculté qu'ont le sérum (ALS) antithymocyte du cheval et son dérivé IgG (ALG) de stimuler la synthèse du DNA avec les leukocytes périphériques humains a été contrôlée in vitro. Sauf déviation même faible de dosage, l'ALS et l'ALG stimulent la synthèse du DNA. Une forte concentration de ALG inhibe l'effet stimulant de la phytohémagglutinine. Cela peut contribuer en partie à la mort de la cellule ou à sa dégradation à cause de la grande concentration de l'ALS ou de l'ALG.

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Immunological Blockade of the Adenohypophysis and its Possible Application in Prophylaxis and Therapy of Neoplasia

Introduction. Immunological intervention provides one method to block organ function. Since antisera to organs can be prepared, it should be possible, therefore, to inhibit specifically or to modulate function by varying doses of antisera or immunoglobulins. In particular, we wish to discuss the inhibition of the adenohypophysis. This gland, besides secreting stimulating factors for various target glands, such as adrenocorticotropic hormone (ACTH), thyrotropic hormone (TTH) and gonadotropic hormones (GH), is also secreting daily a large amount of somatotropic or growth hormone (STH). We wish to propose in this paper that STH is probably involved in the growth of many types of tumours and that consequently blocking its production should lead to tumour growth inhibition.

The hormone dependence or the sensitivity to hormones of many malignant or benign tumours in humans is well known. In the attempt to control growth of many tumours, hormones are therefore used extensively. Hormones have been and are administered even in very high doses, the so-called 'pharmacological dosage' in many types of neoplasia such as the localized or systemic tumours of the lymphatic and haematopoietic systems, carcinoma of the breast, carcinoma of the prostate, myelomatosis and many others. However, no serious attempt has been made to try to interfere in a specific manner with the function of the hypophysis which receives the inhibiting or secretory stimuli both from the periphery and from the hypothalamus and which regulates the entire endocrine system.

Role of growth hormone. STH is a phylogenetically old, polypeptide molecule, which, like prolactin, is present even in primitive species. It has probably acquired new functions in higher organisms but preserved its former functions present in the lower species. One of these acquired functions in mammals is presumably that of controlling cell proliferation either alone or in chronological synergism with other hormones. These actions of STH become evident when the target cells, as for example in the epiphyseal cartilage of the long bones, appear during growth in postnatal life. It is therefore important to establish in man which tumour cells have acquired or maintained the characteristics of STH dependence of certain tissues for their growth. This concept of hormone dependence may be useful for adopting the appropriate hormone therapy for certain tumours.

STH has a structural similarity with another hormone, prolactin, which is seemingly secreted by the same or very similar so-called acidophilic cells of the adenohypophysis. Both of them have a 'trophic' action as shown in many experimental systems. Another main point to be considered is that the acidophilic cells secreting somatotropic hormone, constitute to a large extent the cell population of the adenohypophysis. The reason for this is unknown. It is

also not clear why such a large amount of STH is secreted by the anterior hypophysis since there is no indication for a specific fundamental function being performed by it in the adult age. In fact, the effect of hypophysectomy in adult mammals indicates that severe metabolic disfunctions or death of the operated animals can be prevented by replacement with thyroxine and corticosteroids, STH being not needed for maintainance of normal health. Hypophysectomy can even be performed in pregnant primates[1] and humans[2] without consequences, provided thyroxine and corticosteroids are supplied. Also in this case STH is not needed for growth of the embryo and for normal delivery. Endogenous STH is also not required for growth of hypophysectomized fetal lambs[3]. The fact remains that STH-producing cells populate the hypophysis in very large number until old age[4]. It is possible that STH is needed in large amounts because there is no specific target gland to mediate its action, and many tissues, thyroid and adrenal glands included, need its constant trophic action[5–8]. The endocrinological concept that hormones and in particular STH act as 'amplifiers' of functions, strongly suggests that inhibition of growth of STH-dependent tumours might be achieved by blocking the hormone from which tumour cells depend for their rapid and invasive proliferation. STH has also been shown to be very active on many tissues but mainly on the thymus and thymus-derived cells during their formation, and to be required for the maintainance of a functioning immunolymphatic system. These aspects have been discussed extensively in previous papers[9,10].

Endocrine control of tumours. In our view, up to now, endocrine control of tumours has been partially unsuccessful, due to the technical impossibility of precise manipulation of the endocrine system. Without this exact control, damaging side effects or serious consequences may happen to functions which depend on efficient and well balanced hormonal regulation. One is in fact incapable of predicting the effects of a long-lasting inhibition of certain hormones such as STH.

The possibilities available for a therapeutic regulation of hormone-dependent tumours will now be shortly considered.

1. *Ablation of the hypophysis and/or of other endocrine glands by operational techniques or by radioisotopes.* This procedure is being used as 'extrema ratio' in advanced cancer of the breast, when formation of metastasis has occured. The consequences of such a drastic traumatizing procedure are evident. It can only be considered as a palliative method, especially to reduce the pains from bone metastasis. Removal of other endocrine glands has also been performed for retarding growth of several other tumours.

2. *Inhibition of hormones by anti-hormone sera or by specific chemical inhibitors or competitors of hormones.* This approach is still largely theoretical. Aside from the technical difficulties, the main problem remains that when a hormone is neutralized by various means, more hormones of the same type might be produced and released by the corresponding endocrine gland, unless one could provide an inhibitor which selectively damages the respective hormone-producing cells. This might be a specific endocrine gland inhibitor such as alloxan, which destroys the insulin-producing β-cells in the pancreas.

3. *Administration of hormones in high doses.* This method is being widely used, especially with regard to corticosteroids. There are, however, several reasons against its application: the direct toxic action of such high dosages of hormones, the damaging consequences on the respective hypophysial cells secreting the stimulating hormone, the side effects and the fact that more antagonistic hormones will be produced to balance the levels of the hormone administered. Examples of antagonistic hormones are growth hormone and insulin, growth hormone and corticosteroids.

4. *Inhibition of the 'releasing factors' in the hypothalamus by chemical means or by specific antisera.* This possibility has the same contraindications as mentioned under 2. In addition, its application is actually highly hypothetical as too little is known about the chemistry and properties of these factors. However, development of a specific inhibitor of growth hormone releasing factor is urgent. It might lead to a specific block of growth hormone synthesis and consequent block of growth hormone-dependent tumours.

Inhibition of growth hormone production by anti-adeno-hypophysis-serum. The new approach we wish to propose here is based on the possibility of specifically blocking and damaging STH-producing cells in the adenohypophysis by immunological means. This approach is based on two facts:

A) *STH promotes growth of some tumours.* We believe that if any possibility exists to prevent or control tumour onset or growth by interfering with hormones, it is STH which is one of the key-hormones. This view is supported by abundant literature about a) the determining role of STH in onset of some tumours[11–22], b) its role in growth of primary tumours or of metastasis[11–22], c) its direct

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influence on synthesis of other hormones, like oestrogens and corticosteroids, which promote tumour growth[7,8,23–34].

An explanation of the efficacy of growth hormone inhibition for control of tumour growth is problematic but it can be argued along the following lines: a) STH seems not to be required for somatic growth in the embryonic development in mammals[3,35–39]. b) STH is needed for rapid growth of tissues, especially in the postnatal period when the demand for this hormone seems to be critical for somatic growth[40]. c) STH is probably needed for cell duplication of certain tumour cells *whose degree of anaplasia is not very marked*, as in the case of certain mammary carcinomas. In this respect, the behaviour of tumour cells cannot be compared to embryonic cell but to normal cells of a hormone-dependent tissue in the postnatal stage when the demand for growth hormone is maximal. It is therefore quite probable that growth of certain tumours in humans is completely STH-dependent. This seems to be quite logical considering the high degree of hormone-dependence of exocrine glands during their development. Tumours deriving from cells of exocrine glands *may have preserved the original hormone-dependence* of the normal cells from which the tumour derived. As an example of the possible participation of STH in onset of human tumours, it is known that the incidence of mammary carcinoma increases sharply parallel to the striking hormonal changes at the time of menopause, that the incidence of senile-type diabetes after the menopause is high and that the participation of STH in its aetiopathogenesis is proven. Therefore the link between an increased STH production and onset of mammary carcinoma seems to be logical[41].

B) *STH-producing cells can be blocked by specific antisera.* Sheep anti-rat and rabbit anti-mouse adenohypophysis sera have been prepared. These antisera provoke a block or inhibition of STH-producing cells in the rat or mouse adenohypophysis[42,43]. The specificity of these antisera for acidophilic cells has been proven. The fact that these sera are specifically active depends on the new *way of preparation*[42,43]. This antiserum produces durable inhibitory effects for STH-cells after a single or a few inoculations[42,43]. It has no relevant action on other cells of the anterior pituitary gland which secrete the other trophic hormones. For this reason, *inhibition of other endocrine functions aside from that of STH-producing cells is irrelevant.* Eventually inhibition of other trophic cells in the anterior pituitary gland could be easily overcome by treatment with the stimulating factors (ACTH, TTH, GH). This anti-acidophilic cell antiserum has no other apparent side-effects. Its specificity, properties, long-lasting action and lack of side-effects offer an unique possibility to study the role of growth hormone on tumour growth. This study has already given encouraging results. Hyperplastic nodules from which methylcholanthrene-induced rat mammary tumours originate and their growth and transformation to malignancy are dependent on hypophysial function. Treatment of Sprague-Dawley rats with antiadenohypophysis serum at the first stage of tumour-development results in block of growth of the pre-neoplastic or neoplastic nodules[44]. In contrast spontaneous STH-independent mammary carcinoma in C3H mice are insensitive to antihypophysis-serum treatment[45]. This agrees with earlier findings in hypophysectomized mice[28].

Discussion. The degree of hormone-dependence of tumours is probably proportional to their degree of anaplasia. The most anaplastic tumours are presumably hormone-independent in their growth. Many tumours in man originate from the epithelial cells of exocrine glands or epithelial secretory cells, as the carcinoma of the stomach and intestine, cancer of the endometrium, of the mammary gland and also of the epithelial cells in the bronchi. The hormone-dependence of these tissues for the rapid turnover of their cells (typical example are the epithelial cells of the stomach or of the intestinal mucosa) is unequivocal. Rapidity of replication of cells of intestinal mucosa is comparable to the rate of mitotic activity in the thymus cortex, which is hormone-dependent[9,10]. It is therefore conceivable that de-differentiation or transformation to malignancy of cells belonging to these hormone-dependent cells or tissues results in formation of clones of malignant cells, which at least in the first stage of cancerogenesis maintain their hormone-dependence. Later on, increasing anaplasia will render the tumour cells completely independent from hormones, this probably through a process of tumour cells selection. This seems to be the case in cancer of the uterus or in breast cancer in the stage of pre-cancerous epithelial hyperplasia. Therefore the prophylactic action of the anti-adenohypophysis serum should be exerted at the stage of the hyperplastic hormone-dependent epithelial growth, to prevent proliferation of metastatic cells in lymphodes or elsewhere in the body and eventually permit the specific and nonspecific immune defence mechanism to react against the tumour cells.

STH has been shown to be necessary for formation, maturation and maintainance of an efficient immunolymphatic system in ontogenesis and in adult age[9,10]. The thymus-mediated action of STH and the formation of mature immunocompetent lymphocytes is a fundamental prerequisite for maturation of the immune capacity. The mechanism of immunological surveillance of tumorigenesis must therefore be considered in the light of this STH-dependence of the thymus-derived cells.

The viral origin of leukaemogenic processes in man is possible. Since STH regulates thymus activity[9,10] and the thymus is the organ where viruses seem to find the best environmental conditions for proliferation, at least in mice, the interrelation between thymus and growth hormone in human leukaemia must be considered and the use of anti-adenohypophysis serum can be proposed.

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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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