Selye, H.: Oncologia *11*: 193–198 (1958)

Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montreal, Canada

Topical Effects of Cortisol upon Walker Tumors *

By Hans SELYE

Numerous publications deal with the effects of corticoids upon the growth of spontaneous and experimental neoplasms. Some investigators consider these steroid hormone effects to be the consequences of systemic, metabolic actions, while others assume that the corticoids can act upon neoplasms directly (for literature, see *Selye* [1], *Selye, Horava* and *Heuser* [2]). Whether a certain hormone will stimulate or inhibit neoplastic growth largely depends upon experimental conditions, but our previous experiments, in which the "granuloma pouch technique" was used for the transplantation of experimental tumors, have demonstrated that glucocorticoids inhibit, while STH enhances, the growth of various transplantable neoplasms [3, 4]. An evaluation of these findings led us to assume that, at least in the case of many experimental tumors, the hormones do not act upon the neoplastic cells themselves, but influence the latter merely by modifying stroma formation and the vascularization within the malignant growths. Yet, the question remained unanswered whether these effects are due to direct (topical) or indirect (systemic) actions of the hormones. The "granuloma pouch technique" is particularly suitable for the study of this problem: it forces the malignant tissue to grow in the form of a thin lining on the wall of a regularly shaped ellipsoid cavity to which accurately measured amounts of hormones can be applied

* This work was supported by Grant No. 22 from the National Institute of Cancer of Canada and by a grant from the Gustavus and Louise Pfeiffer Research Foundation. The author also wishes to thank the Schering Corporation for the supply of cortisol acetate (hydrocortisone acetate) used in these experiments.

evenly by injecting them into the lumen of the neoplastic sac. It is the object of this communication to compare the effect of cortisol, thus directly applied to the neoplasm, with that produced when the same amount of hormone is injected subcutaneously at a distance from the malignant cells.

Materials and Techniques

Sixty female Sprague-Dawley rats, with an average initial body-weight of 101 g. (range 95 to 112 g.), were subdivided into three equal groups. In all animals, a subcutaneous air pouch was produced by injecting 25 ml. of air through a gauge 27 subcutaneous injection needle under the shaved skin of the back, on the first day of the experiment. The animals of *Group I* received no hormone treatment. Those of *Group II* were given cortisol acetate (COL-Ac) in the form of a microcrystal suspension of 3 mg. in 2 ml. of water into the cavity of the sac. The rats of *Group III* received the same dose of the COL-Ac suspension, subcutaneously, under the skin of the belly. Two days later, 0.5 ml. of a Walker tumor suspension was injected into the air sac (in all three groups) and evenly distributed on its inner surface by shaking. This inoculum was prepared by mixing one part of fresh Walker tumor tissue (made into a fine mush in a glass homogenizer) with four parts of 0.9% NaCl solution. After the implantation, 3 mg. of COL-Ac in 2 ml. of water were administered daily, in Group II into the cavity of the pouch, in Group III subcutaneously on the belly, just as in the case of the preparatory injection.

The animals of all three groups were killed with chloroform 16 days after the tumor implantation at which time the exudate that had accumulated in some pouches was measured, and specimens from the tumorous walls of the pouches were fixed in Susa solution for subsequent examination of paraffin-embedded, hematoxylin-eosin-stained sections.

Results

Even mere inspection of the animals at autopsy showed an enormous difference in the development of the tumor pouches in the three groups. The rats that received no COL-Ac treatment (Group I) had enormous neoplastic pouches, those in which COL-Ac was given topically had very small pouches, and those in which COL-Ac was injected subcutaneously at a distance from the tumor had pouches of intermediate size.

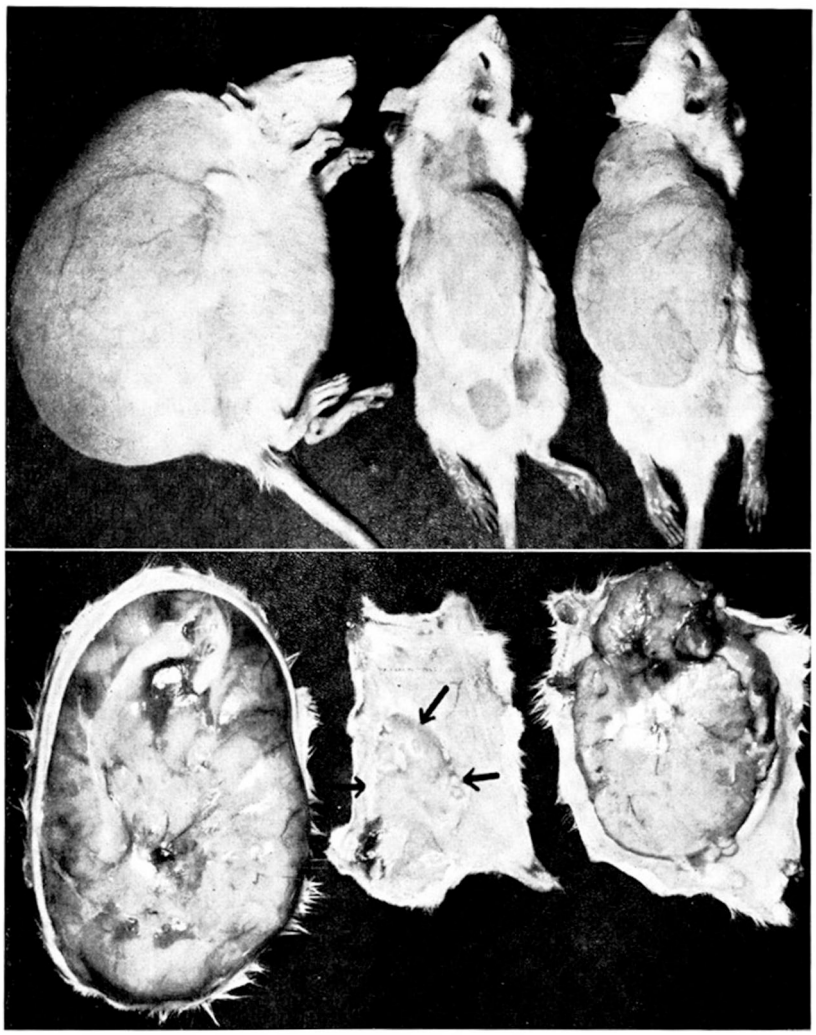

Fig. 1. Top: external appearance of untreated control (left) rat in which COL-Ac was injected directly into the tumour-bearing granuloma pouch (middle), and rat in which the same amount of COL-Ac was given subcutaneously, at a distance from the neoplasm. *Bottom:* dissected tumor-bearing pouches (ventral aspect) of the rats shown in the top row. In the control, as well as in the rat in which COL-Ac was given at a distance from the neoplasm, the wall of the pouch consists of a solid, thick layer of tumor tissue and is greatly distended by fluid. By contrast, in the animal which received COL-Ac directly into the pouch, only minute foci of neoplastic tissue (arrows) are distinguishable, and the pouch is empty.

Upon dissecting the pouches, it became evident that, in the first group, the lining of the pouch consisted of a thick layer of tumor tissue, and the cavity contained a mean of 63 ml. (range 58 to 117 ml.) of tumoral exudate. In the second group, there were only inconspicuous little nodules of beginning tumor formation in the walls of the sacs; the cavities of the pouches were filled by air only, with no trace of fluid. In the third group, the cavities contained a mean of 5 ml. (range 0 to 10 ml.) of tumoral fluid; the lining was covered by a complete shell of Walker tumor tissue, although the neoplastic pouch was less thick than in the rats of Group I (fig. 1).

Histologic study revealed no significant qualitative change in the neoplastic cells that could have been attributed to the hormone treatment; it merely confirmed that the amount of viable tumoral tissue is much more markedly reduced following topical than following systemic administration of COL-Ac.

Discussion

It is evident from these observations that under our experimental conditions, COL-Ac exerted a definite inhibitory effect upon the growth of the transplanted Walker tumor itself as well as upon the formation of tumoral fluid. This inhibitory action was considerably more pronounced in the rats in which COL-Ac was applied topically to the neoplastic tissue than in those in which the steroid was administered subcutaneously, at a distance from the neoplasm. Since the total amount of COL-Ac given was the same in the two last-mentioned groups, it may be concluded that the antineoplastic effect of this glucocorticoid is due to a direct effect and not to the systemic, metabolic changes that are elicited by COL-Ac.

It should be emphasized, however, that our observations do not prove any direct action upon the neoplastic cells themselves. In fact, it is highly probable from previous observations [1, 2] that antiphlogistic hormones inhibit neoplastic growth mainly—if not exclusively—owing to their ability to retard stroma formation and vascularization in developing tumoral foci.

Summary

Experiments are described in which an ellipsoid neoplastic shell is produced by the injection of a Walker tumor suspension into a sub-

cutaneous air space (prepared underneath the dorsal skin of the rat with the so-called "granuloma pouch technique"). The injection of a given amount of cortisol acetate (COL-Ac) into the cavity of such tumoral sacs exerts an extraordinarily pronounced retarding effect upon the development of tumor transplants and completely suppresses the formation of tumoral fluid. When the same amount of COL-Ac is administered subcutaneously, at a distance from the neoplasms, its ability to retard tumor growth and tumoral fluid formation is considerably less pronounced.

It is concluded that the antitumoral effect of glucocorticoids is due to a topical action rather than to the systemic metabolic effects of these hormones.

Zusammenfassung

Rattenversuche zeigen, daß man eine ellipsoide neoplastische Schale durch die Injektion einer Walkertumorsuspension in einem subkutanen Luftsack (Granulombeuteltechnik) hervorrufen kann. Wenn eine gewisse Menge von Cortisolacetat (COL-Ac) in das Lumen eines derartigen neoplastischen Sackes injiziert wird, so wird das Tumorwachstum außerordentlich stark unterdrückt und die Bildung von Tumorflüssigkeit vollkommen verhindert. Wird dieselbe Menge von COL-Ac subkutan entfernt vom Neoplasma injiziert, ist die Fähigkeit des Hormons, das Tumorwachstum und die Bildung von Flüssigkeit in den Tumoren zu unterdrücken, weit weniger ausgesprochen.

Der antitumorale Effekt von Glukokortikoiden beruht anscheinend auf einer lokalen Wirkung und ist nicht durch die allgemeinen Stoffwechselwirkungen dieser Hormone bedingt.

Résumé

Des expériences sur le rat montrent qu'en introduisant une suspension de tumeur de Walker dans une poche d'air (préparée sous la peau dorsale avec la technique de la «poche de granulôme»), on obtient une coquille éllipsoïdale de tissu néoplasique. L'injection d'une certaine quantité de cortisol, sous forme de son acétate (COL-Ac), dans la cavité de ce sac tumoral retarde, d'une manière très prononcée, le développement du néoplasme transplanté et supprime complètement la formation de liquide tumoral. Par contre, la même quantité

de COL-Ac est beaucoup moins efficace, si elle est injectée, par voie sous-cutanée, loin du néoplasme.

On conclut que l'effet anti-tumoral des glucocorticoïdes est dû, non à l'effet systémique de ces derniers sur le métabolisme en général, mais à une action topicale.

Riassunto

Esperimenti con topi dimostrano la possibilità di ottenere una buccia ellipsoidale di tessuto neoplastico mediante iniezione di sospensione di tumore di Walker in un sacco d'aria subcutaneo (tecnica della «tasca di granuloma»). Iniettando una certa quantità di acetato di cortisol (COL-Ac) nella cavità di tale sacco tumorale si inibisce lo sviluppo tumorale in maniera molto forte e si impedisce completamente la formazione di liquido tumorale. Viene iniettata la stessa quantità di COL-Ac subcutaneamente in distanza del tumore si osserva una efficacia molto minore.

Pare che l'effetto antitumorale di glucocorticoidi basi su una azione locale e non su azioni generali del metabolismo di questi ormoni.

Bibliography

1. *Selye, H.:* Stress. (Acta. Inc., Med. Publ., Montreal 1950.)

2. *Selye, H.; Horava, A.* and *Heuser, G.:* Annual Reports on Stress. (Acta. Inc., Med. Publ., Montreal 1951 to 1956.)

3. *Selye, H.:* Stress and abnormal growth. Proc. First Canad. Cancer Conf. Honey Harbour, June 16 to 19, 1954; p. 254. (Acad. Press Inc., New York 1955.)

4. *Selye, H.:* Experimentelle Studien über die Wirkung von Adaptationshormonen (STH. Cortisol) auf transplantierbare Geschwülste. Z. Krebsforsch. *60:* 316 (1955).

Author's address: Dr. H. Selye, Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, *Montreal* (Canada)