in the susceptibility of a test animal to infection by any of a variety of infectious agents, as evidenced by rapid weight loss, death and extensive histopathological lesions. The ready applicability of the method is illustrated in this paper by experiments that utilized four microbiological agents: poliomyelitis virus, a Coxsackie virus, *Candida albicans,* and *Blastomyces dermatitidis.* The experimental studies that employed these agents and the findings for other viruses, bacteria and fungi will be reported in detail elsewhere.

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Cortisone-Induced Metastases of Adenocarcinoma in Mice.* (19544)

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In more than 200 routine transplantations of a mammary adenocarcinoma in C3H mice no metastases were found(1). Local growth of this transplantable tumor was inhibited by cortisone as was also adenocarcinoma in another strain(2). But a new and unexpected phenomenon not yet described, to our knowledge, has been found in our work with cortisone: inhibition of local tumoral growth by cortisone was accompanied by the appearance of multiple metastases. Our finding offers the opportunity to study the mechanism underlying production of metastases and the influence steroids may have on the spread of metastases in the body and on resistance against malignant growth. This is why a more detailed study with transplantable adenocarcinoma in C3H mice has been undertaken.

Material and methods. Male and female C3H mice about 2 months old, weighing 16 to 17 g in the first experiment, and 19 to 20 g in the other 2 were used. A 0.1 ml saline suspension of mammary adenocarcinoma K7‡ was injected into the left flank. Treatment

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‡ This adenocarcinoma appeared spontaneously in our C3H mice and was maintained, previous to the present work, by 51 transfers in 200 animals. For our technic see 1,3.

TABLE I. Weight of local growth and incidence of metastases in 20 C3H mice killed 24 to 41 days after inoculation of adenocarcinoma K7.

Group	Wt of local growth, avg and range (g)			Metastases incidence			
	24 days	37 days	41 days	24 days	37 days	41 days	Total
Not treated	5.6 (4.5-7.3)	7.9 (6.4-9.6)	15.7 (12.5-19)	0/3	0/3	0/2	0/8
Treated with cortisone	.6 (.5-.7)	.6 (.5-.6)	.7 (.7-.7)	0/4	0/6	2/2	2/12

TABLE II. Weight of Local Growth and Incidence of Metastases in 20 C3H Mice Which Died Spontaneously 36 to 76 Days after Inoculation.*

Group	Wt of local growth, avg and range (g)				Metastases incidence				
	36 days	43 days	68 days	76 days	36 days	43 days	68 days	76 days	Total
Not treated	6.6 (3.3-8.8)				0/7				0/7
Treated with cortisone	1 (.5-1.2)	.9 (.3-1.3)	1.2	1.8 (1.8-1.9)	2/4	4/6	1/1	1/2	8/13

* Two treated animals are still alive; not considered in Table II.

with cortisone began 8 to 9 days after inoculation when local growth of the tumor had reached about 7 mm in diameter; 0.5 mg of cortisone in 0.1 ml of saline were injected daily intramuscularly in the right thigh. In all experiments a group inoculated in the same way with the tumor but not injected with cortisone served as control. Some animals both of the experimental and control groups were killed at 24, 37, and 41 days after inoculation with the tumor suspension. The rest were left to survive until natural death. The animals were weighed every 3 days and growth of the tumors was recorded simultaneously. After death the weight of the tumor at the site of inoculation and the presence of metastases was registered. Tumors and metastases and organs (heart, liver, kidney, spleen, lymph nodes, adrenals, left thigh muscles) were fixed in 10% formalin and stained with hematoxylin and eosin.

Results. Increase of local tumoral growth was, in the treated animals, much less conspicuous than in the untreated ones; increase took place in treated animals only during the first days and remained practically stationary. At the end of the experiment (Tables I and II) the difference in weight of local growth between treated and untreated animals was remarkable. Average weight of the local growth in untreated animals was 5.6 to 15.7 g at 24 to 41 days after inoculation; in one animal growth reached at 41 days 19 g (Table I); average weight in treated animals was of 0.6 to 1.0 g, reaching 1.3 g in one animal (which died at 43 days), and 1.9 g in one animal (which died at 76 days; Table II).

A most conspicuous aspect in Tables I and II is the fact that there was not a single animal with metastases among the 15 untreated animals; on the contrary, there were 10 animals with metastases among the 25 animals treated with cortisone. This difference was not due to the longer survival of treated animals (Table II) as shown when considering only animals which were killed or died at 36 to 43 days (Tables I and II). There was not a single animal with metastases among the 12 untreated animals (36 to 41 d.); there were, however, 8 animals with metastases among 18 animals (36 to 43 d.) treated with cortisone. The metastases involved axillary, inguinal, mesenteric, and retroperitoneal nodes, the mediastinum, peritoneum, pleura, liver, spleen, kidney, lung, diaphragm, and the muscles of the thigh. Small nodules were present everywhere on the peritoneum except in the region contiguous to the local growth infiltrating the adjacent muscles of the abdominal wall.

Of considerable interest was the experiment

TABLE III. Weight of Local Growth and Incidence of Metastases in 18 C3H Mice Inoculated with a Suspension of Cortisone-Induced Metastases, or with a Suspension of Local Growth; Killed at 38 Days after Inoculation.*

Origin of suspension inoculated	Treatment	Wt of local growth, avg and range (g)	Metastases incidence
Metastases	0	9.4 (6.4-11.7)	0/6
"	Cortisone	.4 (.2-.6)	2/5
Local growth	0	9.1 (7.8-12.1)	0/4
" "	Cortisone	.6 (.2-1.5)	1/3

* Two animals died spontaneously at 36 and 38 days; not considered in Table III.

in which a suspension of cortisone-induced metastases was inoculated subcutaneously (Table III; axillary, inguinal and mesenteric lymphatic nodes were pooled when preparing the suspension). Local growth resulting from the inoculation of metastases was inhibited by cortisone as was local growth resulting from inoculation of the ordinary tumor. Local growth due to the inoculation of cortisone-induced metastases in a group of 6 animals was 6.4 to 11.7 g; when cortisone was given, the weight in a group of 5 animals was 0.2 to 0.6 g only (Table III). There were no metastases from local growth due to inoculation of cortisone-induced metastases; they, however, appeared when cortisone was given.

There were notable *histological* differences between treated and untreated tumors. Local growth in untreated animals consisted of polyhedric epithelial cells of great size; their nuclei were globular, rich in chromatin, with a great number of atypical forms and with mitoses; their cytoplasm was scarce and basophilic. The cells were gathered in irregular nodules, which were separated by a small amount of stroma. There were zones of central necrosis. Neighboring tissues were infiltrated by malignant cells. The tumor may be termed as encephaloid solid cancer, degree 4 of Broder's classification. In local growth of treated animals central necrosis was much more extensive. In the periphery there was a zone of variable thickness in which cells appeared dissociated and with clear necrobiosis such as degenerative changes of the nucleus, picnosis, caryorrhesis, and caryolysis. Numerous globular tumoral cells also were seen whose nuclear structure was apparently normal and whose cytoplasm was abundant and slightly eosinophilic; it was difficult to recognize them as epithelial due to the loss of normal relationship and to their histiocyte-like appearance; in the periphery these cells showed nuclear abnormalities. Mitoses appeared much less frequently than in tumors of untreated animals. The histological condition of the metastases was similar to that of local growth of the treated animals. Degenerative changes of liver and kidney were present in treated and untreated animals; a moderate degree of involution of the lymphoid-macrophage system was present in animals treated with cortisone.

In all groups but with one exception there was, at the end of the experiments, a decrease in *average body weight,* subtracting the weight of tumors. The decrease was 4.2 g in untreated animals, and 3.5 g in treated animals.

Discussion. It is clear that cortisone produced first a delay and later on an arrest of local growth. This effect was due either to a decrease of the mitotic activity, or to a change in the balance of the rate of production and destruction of cells. Both phenomena were evidently present since there was a decrease of mitotic activity and an increase of cellular necrosis. That cortisone induces metastases is, on the other hand, beyond any reasonable doubt. However, cortisone-induced metastases apparently suffer from cortisone the same cellular changes as local growth: both are histologically similar. Neoplastic active cells must be present in cortisone-induced metastases for, when inoculated subcutaneously, they behaved exactly the same as a suspension of a local growth of untreated animals.

Thus it becomes evident that cortisone has 2 different and antagonistic actions on resistance of C3H mice against transplanted adenocarcinoma K7: first, a protective antitumorigenic action through inhibition of local tu-

moral growth; and secondly, an action contrary to protection, through metastasizing or propagation of local growth in the body.

It is but logical to compare the described metastasizing action of cortisone to that of hyaluronidase which is known to favor propagation of infectious agents and tumoral cells (4,5); this action of the enzyme can be potentiated by estrogen, also a steroid hormone (6). There is the coincidence that both cortisone(7) and hyaluronidase(4,8) produce a decrease of mucopolysaccharides in connective tissues, but cortisone also acts on other constituents of the latter(7). The fundamental importance of this substance and of steroid hormones in general for the mesenchym is an established fact(9). All this would mean, though very hypothetically, that through the action of cortisone on connective tissue of the host, the nutrition of the tumor is impaired and at the same time the pathway is modified by which the malignant cells of the local growth can propagate in the body.

Modifications of the lymphatic system also may take part in antitumoral immunity(10).

Implication of the endocrine balance in tumorigenesis is beyond doubt(11). This does not mean that tumorigenic or antitumorigenic action of hormones on cells is always a direct one; however, the latter cannot be discarded. Indeed, a direct action of the steroid on the malignant cell was seemingly not sufficiently warranted by our work: cortisone-induced metastases when inoculated subcutaneously showed as to taking and tumoral development the same behavior as a suspension of local growth of untreated animals (Table III).

The real place of metastases in the complex of malignancy has been amply discussed in recent years(9). In the present paper metastases could be induced by a steroid which inhibits local growth from which these metastases start, and this finding gives new and striking evidence that the phenomenon of metastases is not simply one of the signs of malignancy as such, but a sign of a special phase of malignancy, possibly of malignancy in decay, as in our work, or of malignancy in an earlier phase of evolution, as in prostatic cancer and in goitrogen-induced thyroid tumors(12).

Summary. Local growth of transplantable adenocarcinoma K7 in C3H mice was arrested when cortisone was given. Metastases of transplanted adenocarcinoma were never observed in untreated animals. However, metastases appeared at about 5 to 6 weeks after transplantation in animals treated with cortisone. The longer the animals survived, the greater was the incidence of cortisone-induced metastases. The cortisone-induced metastases also were transplantable and produced local growth. But local growth due to transplantation of metastases did not produce metastases unless cortisone was given to the new host.

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