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THE ACTION OF CORTISONE ON THE GROWTH AND METASTASIS OF TRANSPLANTABLE TUMOURS

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Ever since in 1944 HEILMANN and KENDALL [7] had observed a marked inhibitory effect of cortisone on transplantable lymphoid tumours, this problem has been investigated on a wide scale. Most authors observed a high degree of inhibition of the growth rate in transplanted tumours, and in some cases even the complete regression of the tumour [8, 18, 11, 15]. Others, however noted hardly any, or no, inhibitory effect of cortisone, as compared with the growth rate of various untreated transplanted tumours [6].

Beside the influence of cortisone on tumoral growth, interest has later shifted to its effect on the heterotransplantability of tumours [9, 19, 20), and also to their metastatic behaviour [1, 13].

Despite the numerous contradictory data on the effect of cortisone, the drug is increasingly applied in clinical oncology, and not only in the case of lymphoid tumours. This fact has rendered a prime interest to studying the effect of cortisone on the greatest possible variety of tumour strains.

The purpose of the present experiments was to examine the action of cortisone, both alone and in combination with chemotherapeutics, on various experimental tumours. In the following we shall report on the effect of cortisone on the growth rate and metastasis of M-1 rat sarcoma and Crocker-180 mouse sarcoma.

Materials and Methods

75 albino mice and 50 albino rats of both sexes were used in the experiments. Before starting the experiments proper, the laws governing the growth and metastatic distribution of the two kinds of tumour had been studied in 50 animals each. After subcutaneous transplantation of tumour cells, the mice were administered 0,5 mg, the rats 2 mg of cortisone (11-dehydro-17-hydroxycorticosterone-21-acetate) intraperitoneally, in 0,2 ml of physiological saline. The controls were treated with physiological saline alone. The mice were divided into 6 groups, and the rats into 3 groups. Group 1. 15 mice served as controls ; 2. 15 mice were subjected to cortisone treatment for 7 days, beginning on the day of implantation ; 3. "pre-treated" group of 15 animals, subjected to cortisone treatment started two days before implantation ; 4. "simultaneous" group of 15 animals, subjected to cortisone treatment from the day of implantation ; 5. "post-treated" group of 10 mice ; in these cortisone treatment was started 7 days after implantation ; 6. no tumour cells were implanted into 5 animals ; these served merely for checking the effect of prolonged cortisone treatment.

Of the rats, 1. 10 served as controls ; 2. 20 were treated with cortisone for a week before implantation (pre-treated group) ; 3. in 20 rats cortisone treatment was started on the day of the implantation. Except in mouse group 2., cortisone treatment was carried on until death. The rats were sacrificed when the first controls died, on the 33rd day after implantation.

The weight of the animals as well as the size of the tumour (expressed by the average diameter) was recorded every 3 days. After concluding the experiments, the body weight, the weight of the enucleated tumour and the weight of the various organs were determined. The tumour, the regional and distal lymph nodes, the lungs, the liver, the spleen and the kidneys were worked up for histology.

Results

The implanted tumour took in all the control and experimental rats ; it failed to take in one of the control mice and in 2 mice each in the pre- and post-treated groups. This is well in keeping with the usual percentage of taking of Crocker-180 sarcoma in our mouse strain. Thus, cortisone treatment did not essentially affect the ratio taking.

The implant began to grow simultaneously in the control and in the experimental groups. However, at the first control already the tumours in the cortisone-treated group were found to be somewhat smaller. Intensive growth of the tumours began 8 to 9 days after implantation and, in the case of M-1 rat sarcoma, its rate was far more rapid in the controls than in the animals treated with cortisone (Fig. 1).

The Crocker 180 sarcoma implants showed less marked differences in growth rate. Still, a certain degree of inhibition became apparent after the mean weight of the carefully removed tumours had been established in the various groups. Inhibition was most marked in the pretreated group (Table I).

In the rats, the difference was marked also in the final weight of the tumours. In the cortisone-treated group, the tumours grew to hardly half the size as in the controls (Table I).

During the experiments the animals lost considerable weight. Comparing the final body weight (minus the weight of the tumour), of the animals with their initial weight, the cortisone-treated rats were found to have lost on the average over 40 g more than the controls. In mice the difference amounted to only about 12 per cent (Table I).

As to the weight of the various organs, marked splenic and adrenal atrophy occurred in the cortisone-treated animals, as was also expected.

The animals been sacrificed, the experiments gave no information concerning the effect of cortisone on survival. No essential difference was, however, observed between the survival of control and treated animals in the Crocker-180 sarcoma experiments either, although here the animals were invariably allowed to die spontaneously (Table I).

As to the histological findings, in animals implanted with M-1 sarcoma necrosis was markedly more intensive and widespread in the treated groups

than in the controls, although in this respect quantitative comparison was most difficult and has to rely on estimates. Far more remarkable was the observation that the proliferation of solid masses of tumour cells usual in the controls was absent in the florid, greyish-white, medullary tumours in the cortisone-treated animals. In these, the tumour had completely dissociated in every area ; most of the cells had undergone grave pyknosis (pyknotic dissociation), and, separated from their bonds and rounded off had densely settled. Mitotic forms were rare. Instead, the number of individual cells or cell groups breaking up into smaller chromatin granules and larger nuclear and plasmic fragments (Fig. a) was all the greater in these pyknotic areas. Invasion of the tumour into its own blood vessels was equally rare in treated and untreated animals.

Table I

Effect of Cortisone Treatment on Animals with M-1 and Crocker-180 Sarcoma

	Group	Average body weight at the start	Average body weight at the end of experiments (minus weight of tumour)	Average weight of tumour	Average survival rate
Crocker-180 mouse sarcoma	Control	18,64 ± 1,5	18,52 ± 5,61	9,16 ± 4,99	42,1 ±11,55
	Simultaneous, treated for 7 days only	16,33 ± 1,44	12,97 ± 2,62	9,38 ± 4,13	30,0 ±9,18
	Pre-treated	18,34 ± 2,01	16,22 ± 2,63	5,00 ± 2,39	42,0 ±12,5
	Simultaneous	18,03 ± 2,0	15,72 ± 2,33	6,10 ± 1,97	39,0 ±8,5
	Post-treated	18,62 ± 1,92	17,41 ± 2,63	6,23 ± 3,57	42,0 ± 4,43
M-1 rat sarcoma	Control	146,3 ±15,1	140,88 ±15,0	56,5 ±13,01	31,6 ±1,74
	Pre-treated	147,85 ±14,8	100,13 ±12,7	28,46 ± 9,53	31,0 ± 1,68
	Simultaneous	149,5 ±18,3	104,12 ±12,33	20,48 ±11,85	32,0 ± 1,71

Crocker sarcoma implants yielded essentially the same findings, though their evaluation was even more difficult owing to the character of the tumour.

Histological examination of the organs of cortisone-treated rats with M-1 sarcoma revealed the following. In half of the cases, there were countless, often confluent metastases of various size in the liver, practically diffusely infiltrating the organ (Fig. b). In several cases there were masses of tumour cells in the hepatic vessels, in the central veins and in the vessels of Glisson's triangles (Fig. c). In the metastases mitoses were, however, remarkably rare, and, what is more, in the centre of these tiny metastases — often escaping gross examination — the tumour cells were already disintegrated and necrosed (Fig. d) The lungs presented peculiar finding as far as in many cortisone-treated animals they displayed

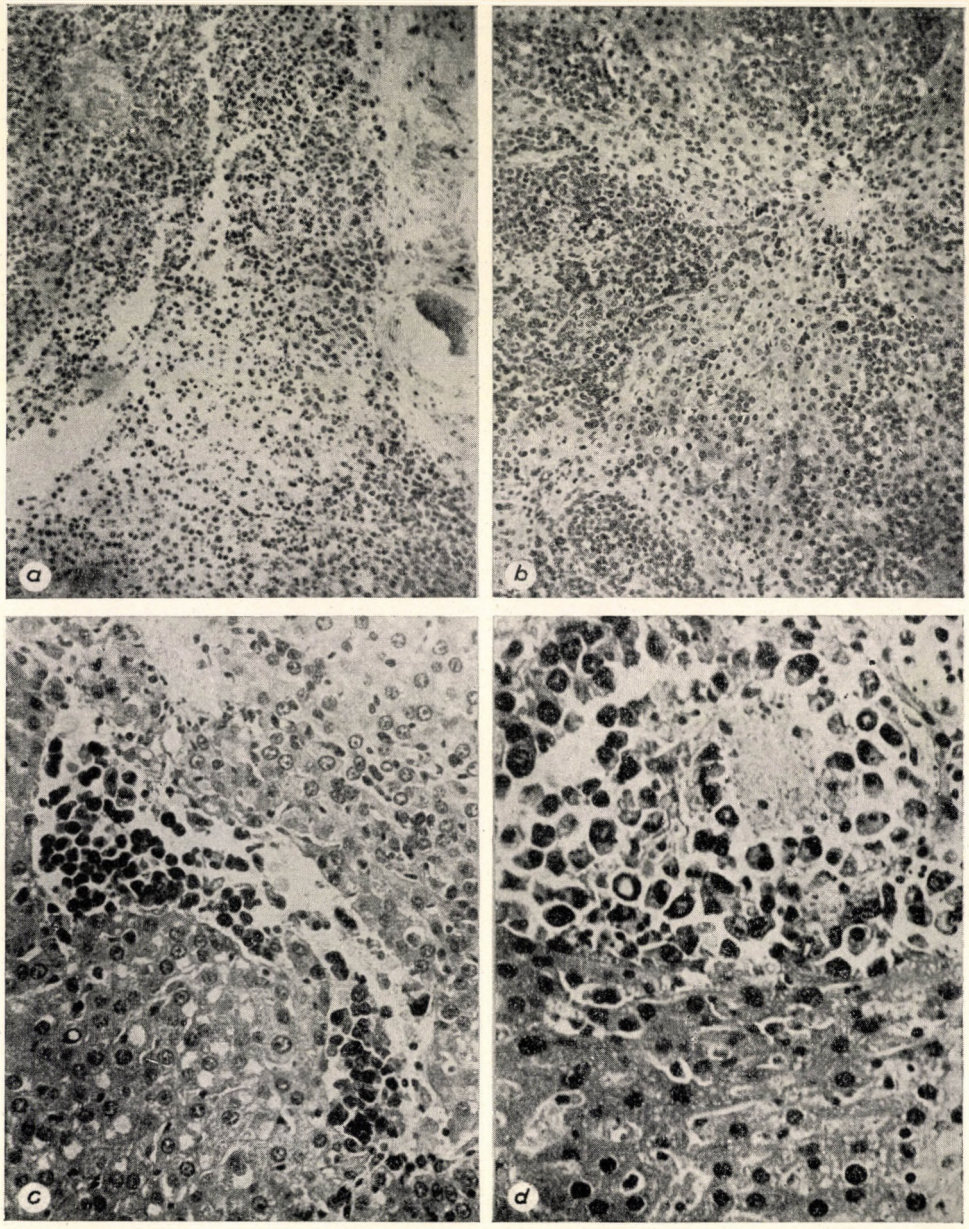

Fig. a. Primary M-1 sarcoma in a rat after prolonged cortisone-treatment ; pyknotic dissociation in the peripheral parts which at gross examination have appeared to be florid.(HE, × 160)
Fig. b. Almost confluent microscopic metastases in the liver of a rat with M-1 sarcoma, after prolonged cortisone treatment. (HE, × 160)
Fig. c. Numerous tumour cells in the hepatic vessels of a rat with M-1 sarcoma, after prolonged cortisone treatments. (HE, × 345)
Fig. d. Central necrosis in the microscopic metastasis in the liver of a rat with M-1 sarcoma, after prolonged cortisone treatment. (HE, × 680)

numerous compact, greyish-white nodes of the size of a grain of pepper ; these had been assumed to be metastases but histologically most of them were found to be abscesses or mycotic granulation. Metastases, if any, had most frequently the character of a microscopically discernible perivascular sheath of cells.

Of the organs of mice with Crocker sarcoma, metastases were seen under the microscope in the lungs only ; these were often most conspicuous at gross examination already and were much more frequent in the cortisone-treated animals than in the controls. In the mice, lung abscess was comparatively rare and mycotic granulation was found only incidentally. In a few animals, however, pulmonary adenoma was observed.

Table II

Metastasis of M-1 Rat Sarcoma

Group	No. of animals	No. of pulmonary metastases	Percentage of pulmonary metastases	No. of liver metastases	Percentage of liver metastases	No. of renal metastases
Old controls	50	4	10%	—	—	—
New controls	10	2		—	—	1
Pre-treated	20	5	25%	10	50%	—
Simultaneous	20	3	15%	10	50%	—

Our findings concerning the induction of metastases have been compiled in Tables II and III. Summing up, liver metastasis was found in 50 per cent of the cortisone-treated rats with M-1 sarcoma, but in none of the controls. Pulmonary metastasis occurred in 20 per cent of the treated and 10 per cent of the control animals. In animals with Crocker-180 sarcoma, there was a remarkable difference in the frequency of pulmonary metastases between cortisone-treated animals (55,6 per cent) and controls (5,4 per cent).

Table III

Metastasis of Crocker-180 Mouse Sarcoma

Group	No. of animals	No. of pulmonary metastases	Percentage of pulmonary metastases	Metastases in other organs
Old controls	42	2	5,4	—
New controls	13	1		—
Simultaneous, treated for 7 days only	15	—	—	—
Pre-treated	13	6	55,6	—
Simultaneous	15	9		—
Post-treated	8	5		—

Discussion

The measure of tumour inhibition observed in the rat experiments was mostly in agreement with the literary data. Apart from leukaemia and lymphoid tumours, where even spontaneous remission had been observed, Selye [15] and Antopol et al. [3] have been the only authors to report a higher rate of tumour inhibition than observed in our experiments. As regards Crocker-180 sarcoma, the rate of tumour inhibition was statistically significant only in the pretreated group.* (Table I). Considering the wide scattering of the values, in experimental oncology a similar degree of tumour inhibition is usually taken as plus-minus. Working with Crocker-180 sarcoma implanted into an Nh strain of mice, Baserga and Shubik [4] observed no inhibition by cortisone.

The mechanism of the tumour inhibition by cortisone is a highly complex and little known process. Antopol et al. [3] emphasized the importance of the effect on the reaction of vessels and connective tissue. The relative deficiency of a stroma in the tumours as compared to the controls was conspicuous in most of our animals subjected to prolonged treatment, particularly in the pre-treated and post-treated groups.

When considering the retardation of growth, the action of cortisone on protein metabolism should not be neglected. It has namely been demonstrated [2, 16] that on cortisone administration, amino acid breakdown is increased and the rate of protein synthesis decreased, as manifested with a general retardation of growth and a negative nitrogen balance. In addition, in our animals a toxic effect due to eventual overdosage had also to be reckoned with ; this was intimated by the substantial losses of body weight.

Agosin et al. [1] and Molomut and al. [13] were the first to point out that cortisone promotes the development of metastases. Kaliss and Borges [12] failed to confirm this ; repeating the experiments by Molomut et al. under identical conditions, they observed no more metastases in cortisone-treated animals than in controls. Last year, however, Baserga and Shubik [4] have again found cortisone to promote the development of metastases in all experimental tumours studied by them, some of which had been induced.

It is felt that the increasing number of experimental data indicating a promotion of metastases by cortisone — without unreservedly applying them to human pathology — gravely cautions against the clinical use of cortisone in oncology. This must all the more be stressed, as unfortunately there are already some clinical observations to substantiate the experimental data. A few years ago, the American Medical Association's Subcommittee on Steroids and Cancer [15] reported that in some cases rapid spreading of the tumour has been observed following cortisone treatment, in spite of the improvement of the patient's

* We are indebted to Dr. L. VEKERDI, member of our Institute for the calculations

general condition; post mortem widespread metastases were found even in the spleen.

It would be of utmost importance to clarify the mechanism by which cortisone promotes the development of metastases, and to reveal the factors which have a role in this effect.

The animals' loss of weight need hardly be reckoned with. Tannenbaum and Silverstone [21] have demonstrated that the number of metastases was reduced by a dietetic restriction of caloric intake.

Some authors hold the survival, the longer life of cortisone-treated animals, responsible for the frequency of metastases [12]. In our experiments, the survival of cortisone-treated mice was not much longer than that of the controls. In the experiments with M-1 sarcoma, the influence of survival — being an indirect factor — has been eliminated by simultaneously sacrificing all animals.

AGOSIN *et al.* [1] attributed to cortisone the increased dissemination of tumour cells, while POMEROY [14], on the contrary, has made cortisone responsible for preparing the soil, favouring the taking of the tumour cells by its destructive effect on lymphoid and reticuloendothelial elements, and by its impairing antigen-antibody reaction. BASERGA and SHUBIK [5] are of the opinion that cortisone acted after the dissemination of tumour cells, i. e. on their taking.

In our opinion, both factors, namely the increased dissemination of tumour cells and the promotion of their settling by preparing the soil may have a share in the increased metastatic dispersion concomitant with cortisone treatment. The fact that cortisone actually increases dissemination of the tumour cells is fairly substantiated by our having found masses of rounded off tumour cells in the treated animals, in particular in the hepatic vessels and sinuses, the aspect of wich hinted at genuine carcinomatous cytaemia. Against this, no cytaemia was ever observed in the controls, and tumour cell emboli were extremely rare. Our findings concerning the primary tumours of cortisone-treated animals were also fully in keeping with our assumption of an increased dissemination. As referred to above marked dissociation was present even in the non-necrosed parts of the tumour. The tumour tissue consisting of rounded off pyknotic cells separated from their bonds may provide an ample source of disseminating tumour cells.

The role of cortisone in the settling of the disseminated tumour cells, in preparing the soil for them, is well illustrated by Pomeroy's experiments [14] mentioned above — altough the mechanism of such action has by no means been clarified. The significance of the "soil" is also indicated by our observations that even in cytaemia, metastases in our animals with M-1 sarcoma have not developed in all organs, but merely in the liver. Besides, cortisone seems to have a particular effect on the liver, as intimated by experiments of Hungarian authors [10]. POMEROY [14] already pointed out that hundreds of metastases were present in the liver of all his cortisone-treated animals, with all kinds of tumours.

It is all the more remarkable that in our experiments with Crocker-180 sarcoma, liver metastases were never observed; instead, cortisone treatment increased the number of metastases in the lung. Whether the cause for thise should be sought for in the comparatively better filtering function of the mice lung, the size of the Crocker sarcoma cells, or in some metabolistic condition, must be decided by future experiments, as also the many unsolved problems pertaining to the mechanism of cortisone effect.

Summary

(i) The effect of prolonged daily administration of cortisone acetate on the growth of M-1 rat sarcoma and Crocker-180 mouse sarcoma, and on the incidence of metastases has been studied experimentally.

(ii) Cortisone treatment has been found to retard the growth of Crocker-180 mouse sarcoma by close to 40 per cent, that of M-1 rat sarcoma by over 50 per cent. At the same time, however, cortisone substantially increased the incidence of metastases. In more than 50 per cent of the animals have metastases been found in the organs, in mice in the lungs, and in rats the lungs and especially in the liver.

(iii) The metastasis promoting action of cortisone is exerted by a combined mechanism. In the experiments cortisone promoted the dissemination of tumour cells, by bringing about pyknotic dissociation in the primary tumour. Cortisone is also known from the literature to favour the settling of tumour cells, in other words to prepare the soil for their reception.

(iv) During the experiments, pulmonary mycosis developed in some animals, particularly in the rats. Due to its significance, this observation will form the subject of another paper.

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ВЛИЯНИЕ КОРТИЗОНА НА РАЗВИТИЕ И МЕТАСТАЗИРОВАНИЕ ПЕРЕВЫВАЕМЫХ ОПУХОЛЕЙ

К. ЛАПИШ и Т. ШАГИ

1. Авторы исследовали в своих опытах влияние длительного ежедневного введения кортизонацетата на рост и метастазирование саркомы крыс М-1 и саркомы мышей Крокер 180.

2. Хроническое введение кортизона замедляет рост мышиной саркомы Крокер 180 почти на 40%, а рост саркомы крыс М-1 выше чем на 50%. Однако, подача кортизона в то же время в значительной степени повысила метастазирования и вызвала у выше 50% животных образование метастаз во внутренних органах, — у мышей легочные метастазы, а у крыс легочные и главным образом печеночные метастазы.

3. Кортизон оказывает свое повышающее метастазирование действие через комбинированный механизм. Согласно исследованиям авторов, кортизон с одной стороны повышает посредством создания пикнотической диссоциации в первичной опухоли разнос опухолевых клеток, а с другой стороны литературные данные указывают на то, что кортизон способствует внедрению опухолевых клеток, и следовательно играет роль подготовителя.

4. В ходе опытов у одной части животных, главным образом на крысах, образовался легочный микоз. Ввиду большого интереса и значения последнего, авторы занимаются легочным микозом в особой статье.

WIRKUNG VON CORTISON AUF DAS WACHSTUM UND DIE METASTASENBILDUNG VON TRANSPLANTIERBAREN GESCHWÜLSTEN

K. LAPIS und T. SÁGI

1. Es wurde die Wirkung von chronischer täglicher Verabfolgung von Cortisonazetat auf das Wachstum und die Metastasenbildung des Rattensarkoms M-1 und des Mäusesarkoms Crocker 180 untersucht.

2. Die chronische Verabfolgung von Cortison verzögert das Wachstum des Mäusesarkoms Crocker 180 um nahezu 40%, das Wachstum des Rattensarkoms M-1 um mehr als 50%. Gleichzeitig wurde aber auch die Metastasenbildung in bedeutendem Masse erhöht, und es bildeten sich bei 50% der Tiere Metastasen in den inneren Organen — bei Mäusen Lungenmetastasen, bei Ratten Lungen- und hauptsächlich Lebermetastasen.

3. Das Cortison übt die steigernde Wirkung auf die Metastasenbildung durch einen kombinierten Mechanismus aus. Laut den Beobachtungen steigert das Cortison durch pyknotische Dissoziation im Primärtumor die Verschleppung der Tumorzellen. Die literarischen Angaben weisen dagegen darauf hin, dass es durch Verbreitung des Bodens die Ansiedlung der Tumorzellen fördert.

4. Im Laufe der Versuche entwickelte sich in einem Teil der Tiere, hauptsächlich bei Ratten Lungenmykose. Damit wollen sich die Verfasser in einer kommenden Arbeit beschäftigen.

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