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An Investigation of the Effects of Inoculated and Intralymphatic Vaccinia Virus on Primary and Secondary Deposits of Malignant Melanoma

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Introduction

The therapeutic value of vaccinia virus in malignant melanoma using direct, intradermal inoculation, intravenous injection and injection of regional lymph node metastases has already been reported.[1-5] Results of treatment of intradermal, in-transit metastases have been encouraging.[1,3,4,5,6] This led to the further investigation of vaccinia virus in the treatment of regional metastases. However, there has been little success with regional metastases by direct lymph node inoculation.[5] The use of the intra-lymphatic route of administration has not hitherto been reported. This paper reports experiences with this technique in regional metastases and in the inoculation of intradermal lesions and the primary tumour.

Method

The six patients included in this investigation were selected on the basis of histologically-proven malignant melanomas of the lower extremity, with either local intradermal metastases or regional lymph node involvement. If unvaccinated in the previous five years, vaccination was performed at least 14 days before using the virus therapeutically.

Vaccinia virus was prepared from stock vacccine obtained from the South African Institute for Medical Research. Virus was inoculated on the Chorio-allantoic membrane of 12 day embryonated hens' eggs in which growth was confluent on the third day. Virus was extracted in buffered saline, cleared and tested for sterility. The titre of the undiluted material was 10^7 pock forming units per ml. This was used at a dilution of 1:50 for both the superficial and deep lesions.

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Fig. 1.—A typical malignant melanoma of the right foot of an African patient.

Fig. 2.—A Lymphangiogram of a patient with a malignant melanoma of the right foot showing that the dye has reached the lumbar para-aortic chain of lymph glands. Vaccinia virus was injected intra-lymphatically prior to the injection of the dye.

The superficial lesions were treated as one would a re-vaccination — a drop (approximately 0,02 ml.) of virus suspension was placed on each nodule and inoculated by the multiple pressure technique using approximately 20 pressures. Excess was removed and the area left exposed to the air.

Primary lesions were treated similarly but the amount of virus suspension used was sufficient to cover the lesion and was introduced by multiple puncture.

In those patients treated by the intralymphatic route, cannulation of a large lymphatic vessel on the dorsum of the foot on the side of the lesion was performed, and 0,5 cc. of the 1/50 dilution of the suspension of vaccinia virus was injected. The primary lesion, surrounding the satellite lesions and intradermal in-transit lesions were directly inoculated as above at the same time. A lymphangiogram was performed through the same cannula. The site of cannulation was then covered with a closed dressing and the patients transferred to an isolation ward for the next ten days. Patients were barrier nursed by staff who had been recently vaccinated and precautions were taken in the handling of dressings, bed clothes and other items which may carry virus.

Excision of the primary lesion was followed by a block dissection of regional nodes when indicated, using the lymphangiogram as a guide to lymph node involvement and to the completeness of their clearance. Tissue obtained from the primary lesion and the regional lymph nodes was studied for evidence of persistence of viable virus. The remainder of the excised material was studied histologically to confirm the diagnosis of the primary lesion, to confirm the involvement of regional lymph nodes, and to ascertain the effects of virus therapy. Directly inoculated, in-transit, intradermal lesions were assessed clinically and virologically.

Where vesicle formation had taken place, fluid was collected in capillary tubes and was tested for the presence of vaccinia virus by inoculation into hens' eggs. Lymph node and primary tumour samples were macerated in a Griffiths grinder with buffered saline and tested for vaccinia virus in the same way.

The six cases can be sub-divided into three groups on the basis of the degree of dissemination of disease and the treatment employed (Table 1).

Table I

Degree of Dissemination of Disease and Treatment employed.

Group	Case	Sex	Age	Site of Primary Lesion	Spread	Viral & Related Therapy
I	1	M	57	Sole of R. Foot	Nil	Local inoculation and excision.
	2	M	60	L. heel	Nil	Local inoculation and excision.
II	3	M	60	Sole of R. Foot	Local satellite dermal deposits inguinal node involvement.	Inoculation of primary and satellite lesions, intra-lymphatic injection of virus, local excision of primary lesion and block dissection of inguinal nodes.
	4	F	55	Sole of L. Foot	Inguinal node involvement.	Intra-lymphatic injection of virus, local excision of primary lesion and block dissection of inguinal nodes.
III	5	M	23	Sole of R. Foot	Local dermal satellite and in-transit deposits and inguinal node involvement.	Inoculation of dermal deposits.
	6	M	49	Sole of R. Foot	Local dermal satellite deposits, inguinal node and pulmonary involvement.	Inoculation of dermal deposits.

Group 1 (Cases 1 and 2) had primary lesions only which were treated by direct inoculation and later excised.

Group 2 (Cases 3 and 4) had regional lymph node involvement and were treated by intra-lymphatic injection of virus followed by excision of the primary tumour and block dissection of the involved regional nodes.

Group 3 (Cases 5 and 6) had disseminated disease, a feature of which was numerous intra-dermal metastases. These were treated by direct inoculation.

RESULTS

Vaccinia virus was never recovered from the primary lesions though clinically, considerable necrosis occurred on the surfaces following inoculation of the virus. However, as all these lesions were ulcerated before treatment and were superficially infected, the necrosis can only be regarded as non-specific. There was no obvious clinical response in the regional lymph nodes treated by intra-lymphatic injection. However, all the treated dermal metatases (and up to 20 nodules were treated simultaneously without adverse effect) developed changes of an accelerated vaccination reaction with vesicle formation followed by the development of crusted, superficial ulcers which healed as scars typical of those usually following smallpox vaccination. These scars showed little or no evidence of pigment and in no case was a recurrence in a treated nodule found. In some instances, new tumour nodules appeared in previously untreated areas and these again responded to inoculation.

During vaccinia virus therapy, no systemic disturbance was noted either by the patient or objectively. This is at variance with previously reported experience,[3,4] which suggests systemic disturbance denotes successful treatment with vaccinia virus. These patients however, had either never previously been vaccinated or a considerable period had elapsed since vaccination. Patients in this series were vaccinated prior to treatment, which may have prevented any systemic disturbance. It is noteworthy that in the only other reported series[5] in which patients were vaccinated prior to treatment, no severe systemic reactions were seen although a low grade pyrexia and malaise did sometimes occur. Many of these patients showed satisfactory regression of dermal metastases.

Vaccinia virus was not recovered from tissue obtained from excised primary lesions or from excised inguinal lymph nodes involved with metastatic deposits. It was however easily recoverable from the vesicles which followed inoculation of dermal metastases.

Histologically, the primary tumours, all of which were superficially ulcerated and infected before being inoculated, showed superficial chronic inflammatory infiltration with patchy areas of necrosis but the deeper layers of the tumour appeared normal. It was not possible to determine whether the inflammatory infiltration was due to the vaccinia therapy, bacterial infection or that often associated with even un-ulcerated tumours. Regional lymph nodes submitted for histology did not differ from the normal appearances of metastatic melanoma.

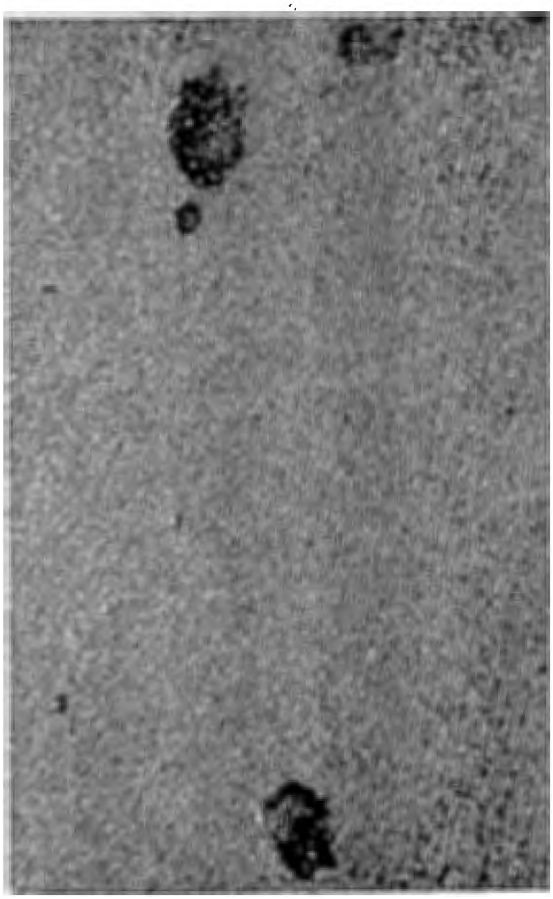

Fig. 3.—Intradermal transit mestastases of a malignant melanoma. The primary tumour has been resected and grafted and a block dissection of lymphatic glands carried out.

DISCUSSION

The results presented here confirm the value of the use of vaccinia virus in the treatment of dermal metastases of malignant melanoma. They are susceptible to inoculated vaccinia virus and disappear completely over a period of from seven to ten days. Long term follow-up has not yet been possible, but no recurrences have taken place in the inoculated areas. The primary tumours seem too large for this to be a successful method of treatment and they did not appear to support the growth of virus. This may be due to the extensive necrosis and borderline viability of many of the cells of the tumour, and possibly to the presence of bacteria in the ulcerated tissue. Thus, wide surgical excision of the primary tumour remains the treatment of choice.

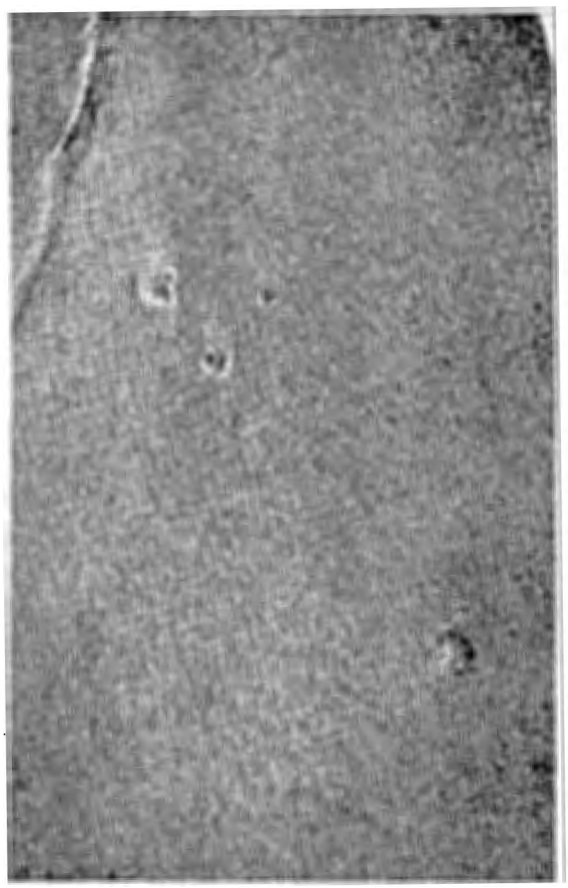

Fig. 4.—The intradermal metastases healing after inoculation. The lowest metastases is still in the stage of vesiculation. All intradermal metastases disappeared after inoculation in this case.

It is also apparent that the virus even in high titre, injected intra-lymphatically is unable to proliferate and hence destroy lymph node metastases. This may be due to the presence of antibody in the circulation and tissue fluids of the patients. It is, however, felt that the danger of treating unvaccinated patients systematically with vaccinia virus at a time when they are suffering from dissiminated malignant disease would be too great a risk.

The method of direct inoculation of even extensive dermal metastases is easily carried out and reliably destroys these deposits in a shorter time than radiotherapy and without the more extensive tissue involvement and side effects of the latter.

It will perhaps be worthwhile to look into the possibility of using less virulent viruses in the inoculation treatment of primary tumours of malignant melanoma, in that this may enable repeated applications at high titre to be performed in the hope that this will produce more satisfactory results.

At present it is felt that the failure to produce either clinical, histological or virological evidence to support the use of vaccinia virus in treating primary or lymph node tumours of malignant melanoma, precludes its use in future patients as this delays the definitive treatment of their disease along generally accepted lines. However, in our service, direct inoculation of dermal metastases with vaccinia virus has become the treatment of choice.

SUMMARY

Primary melanoma and its metastases to lymph nodes and the skin have been treated with vaccinia virus introduced by direct inoculation and intra-lymphatic injection. Existing dermal metastases can be eradicated but the virus could not be recovered from either the primary tumour after inoculation or regional lymph nodes after intra-lymphatic injection.

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REFERENCES

1. BURDICK, K. H., *Arch. of Derm.* 82. 438-439 (1960).
2. BELISSARIO, J. C. and MILTON, G. W., *Austri. Journ. of Derm.* 6. 113 (1961).
3. BURDICK, K. H. and HAWK, W. A., *Cancer (Phil.).* 17. 708-712 (1964).
4. MILTON, G. W. and BROWN, M. M. L., *Austr. and N.Z. Journ. of Surg.* 35. 286-290 (1966).
5. HUNTER-CRAIG, I., NEWTON, K. A., WESTBURY, G., and LACEY, B. W., *Brit. Med. Journ.* 2. 512 (1970).
6. HUNT, J. A. (1970), Personal communication.