THE PRODUCTION OF MALIGNANT TUMOURS BY CADMIUM IN THE RAT

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IN the search for the mechanism by which cobalt exerts its carcinogenic action, biochemical and tissue culture studies were made on this and other divalent metals (Cd, Ni, Cu, Hg, Zn, Be and Mn) to see (a) if any of the divalent metals which showed a similar metabolic action to that of cobalt was also carcinogenic (b) if the similar metabolic action of the carcinogenic metals was really related to the process of carcinogenesis.

The earlier work had shown that the respiration of mitochondria from rat liver and muscle was strongly inhibited by cobalt, the oxidations of pyruvate and α-ketoglutarate being particularly susceptible. Sanadi, Langley and White (1959) had demonstrated an inhibition by cadmium of the enzymatic oxidation of α-ketoglutarate ; as with cobalt (Dingle, Heath, Webb and Daniel, 1962) this effect appeared to be mediated through the formation of a complex with dihydro-lipoic acid (Webb, 1962). It seemed possible that the inhibition of respiration might be a mode of carcinogenesis. It was therefore logical to test cadmium for carcinogenicity by injection of the powdered metal into rat muscle as was done with cobalt (Heath, 1956). A high incidence of tumours at the injection site was obtained, and a preliminary report of the results has already appeared (Heath, Daniel, Dingle and Webb, 1962).

In the present paper these tumours are described in detail and compared with the cobalt-induced tumours previously investigated.

MATERIALS AND METHODS

Two series of rats were injected on the same day ; in each series ten females of the hooded strain, age 2–3 months, were used. In Series I, 0·014 g. and in Series II 0·028 g. of cadmium metal powder (Hopkin and Williams, Ltd.) was shaken into suspension with 0·4 ml. of fowl serum and injected into the right thigh muscle of each animal from the medial aspect, approximately parallel with the femur and directed towards the hip. On microscopic examination the particles of metal were found to be of most varied shape including small and large spheres and ellipsoids, pyramids, rods and completely irregular forms. Dimensions ranged from 1·7 μ diameter (for spheres) to 85 $\mu \times 50 \mu$ for ellipsoids and rods, and 220 $\mu \times 50 \mu \times 50 \mu$ for the other shapes. Most of the particles were single.

RESULTS

These are summarised in Tables I and II.

In every animal of both series injection of the cadmium was followed by an immediate severe local reaction, and after 3 days the injected thigh muscles were hard, swollen and tender. Histological examination of two animals of Series

II that were killed at 5 and 12 days respectively (Rats 7173 and 7168) showed that there was much necrosis with some attempt at repair ; the granulation tissue was very cellular and vascular (Fig. 1). This immediate severe reaction was in complete contrast to the early response to cobalt, which causes no clinical evidence of extensive damage, although microscopic examination reveals some necrosis. In the remaining cadmium-injected animals the reaction gradually subsided leaving the thigh muscles with contractures and much wasting ; the tumours subsequently developed in the wasted region.

Incidence

Nine of the ten rats of Series I and six of the eight (two were killed early) of Series II developed malignant tumours. The first positive malignant change was observed in a rat (No. 7158) of Series I which was killed at 13 weeks (Fig.2). Thereafter animals with tumours were killed at periods of $20\frac{1}{2}$ weeks to 56 weeks after injection ; the major dimensions of the tumours ranged from $2 \times 1\frac{1}{2} \times 1\frac{1}{2}$ cm. to $4\frac{1}{2} \times 4\frac{1}{2} \times 4$ cm. The remaining three rats were killed at 84 weeks with no apparent tumours ; both macroscopically and microscopically these animals all showed varying degrees of muscle wasting, but no evidence of malignant change.

Six animals of Series I and two of Series II had metastases in varying sites which included inguinal, prevertebral and axillary lymph nodes and lung. In one rat of Series I (No. 7159) there was a second primary tumour in the pelvic cavity.

Gross appearance of tumours

All but two tumours were firm, but the degree of hardness varied from tumour to tumour and indeed within the substance of the same tumour ; one was exceptionally hard. Some tumours contained localised gritty regions ; five showed some necrosis and haemorrhage. In two rats the bones of the leg showed thickening, some roughening of the surface and slight erosion.

Histological appearance of tumours

Primary tumours.—All the tumours had regions of rhabdomyosarcoma (Tables I and II), but six out of nine of Series I and three out of six of Series II also showed some areas of fibrosarcoma (Fig. 3). The tumour in rat 7169 had as additional components malignant synovioma, haemangioma and myxosarcoma, and the second primary in rat 7159 was composed of osteochondrosarcoma (Fig. 4) and rhabdomyosarcoma.

A distinctive feature of some of the tumours arising at the injection site was a great vascularity similar to, but less extensive than the definite haemangioma of the rat 7169.

In the two rats where the tumour had eroded the bone there was no evidence of malignant change in the bone surrounding the eroded region.

The degree of differentiation in the rhabdomyosarcomata varied both from animal to animal, and within a single tumour (Fig. 5–7). Some were much better differentiated than those produced by cobalt (Heath, 1956).

Metastases.—Six rats of Series I and two of Series II had metastatic deposits. All of the metastases from the primary tumours at the injection site in the leg were to lymph nodes (left and right inguinal, left and right axillary and preverte-

TABLE I—*Series I.*—0·014g. Cd/Rat

Rat No.	Time to post-mortem (weeks)	Size and consistency of tumour	Type of tumour	Degree of differentiation	Sites of metastases	Histological type of metastases	Comments
7158	13	—	Rhabdomyosarcoma	Good	None	—	Very early tumour including evidence of pre-malignant change
7159	37	3×4×4 cm. Firm to hard Soft to firm	Rhabdomyosarcoma Fibrosarcoma	Poor to moderate Poor to good	R axillary, R inguinal } lymph nodes	Poorly differentiated rhabdomyosarcoma	Second primary in pelvis—osteochondrosarcoma with metastasis in lung
7160	37	3½×3×4 cm. Very soft to soft	Rhabdomyosarcoma	Poor	None	—	Haemorrhagic area in tumour
7161	48	4×3×4 cm. Fairly soft	Rhabdomyosarcoma Fibrosarcoma	Very poor	None	—	Rudimentary muscle fibre formation
7162	84	None	—	—	—	—	—
7163	20½	3×3×3 cm. Very hard : some fluid present	Rhabdomyosarcoma Fibrosarcoma	Moderate to very good Fair to moderate	Prevertebral, R & L axillary, R & L inguinal } lymph nodes	Undifferentiated	One area of very good differentiation—almost rhabdomyoma
7164	39	3×2×2½ cm. Firm	Rhabdomyosarcoma Fibrosarcoma	Poor Moderate to good	Prevertebral, R & L inguinal } lymph nodes	Poorly differentiated rhabdomyosarcoma	Some parts of rhabdomyosarcoma very vascular
7165	39	2½×2½×2½ cm. Firm	Rhabdomyosarcoma Fibrosarcoma	Poor Good	Prevertebral, R & L axillary, R & L inguinal } lymph nodes	Poorly differentiated rhabdomyosarcoma	Lymph node invasion myoblastic
7166	51	3×1½×2½ cm. Soft ; haemorrhagic	Rhabdomyosarcoma	Poor	Prevertebral, R & L axillary, R & L inguinal } lymph nodes Lung	Moderately differentiated rhabdomyosarcoma	Lymph node invasion myoblastic
7167	56	4×4×4 cm. Soft, with some "gritty" areas	Rhabdomyosarcoma Fibrosarcoma	Poor Poor	Prevertebral lymph nodes	Poorly differentiated rhabdo- and fibrosarcoma	Erosion of femur

TABLE II.—*Series II*—0·028g. Cd/Rat

Rat No.	Time to post-mortem (weeks)	Size and consistency of tumour	Type of tumour	Degree of differentiation	Sites of metastases	Histological type of metastases	Comments
7168	12 days	—	—	—	—	—	Necrotic muscle with very cellular and vascular granulation tissue with some degeneration
7169	32	2×2½×2 cm. Firm but not hard	Rhabdomyosarcoma Fibrosarcoma Malignant synovioma Cavernous haemangioma Myxosarcoma	Poorly differentiated rhabdomyosarcoma Well differentiated fibrosarcoma	Prevertebral lymph nodes	Differentiated rhabdomyosarcoma	A very mixed tumour
7170	24	4×2½×2 cm. Firm and gritty	Rhabdomyosarcoma	Moderate to good	None	—	Cystic degeneration of part of tumour
7171	46	3×5×3 cm. Soft and gelatinous	Rhabdomyosarcoma Fibrosarcoma	Poor to medium	None	—	Fibrosarcoma a very minor component
7172	84	None	—	—	—	—	Normal muscle with some scar tissue—no sign of proliferation
7173	5 days	None	—	—	—	—	Necrotic muscle with very cellular and vascular granulation tissue
7174	23	4½×4½×4 cm. Medium firm	Rhabdomyosarcoma	Very good	None	—	Solid tumour measured 2½×2½×2½ cm. Remainder fluid (cystic degeneration)
7175	29	3×3×2½ cm. Firm, gristly	Rhabdomyosarcoma	Poor to moderate	R & L axillary, R & L inguinal, Prevertebral } lymph nodes	Very well differentiated rhabdomyosarcoma	Some regions of alveolar rhabdomyosarcoma. Some necrosis of bone
7176	32	2×1½×1½ cm. Fairly soft to firm	Rhabdomyosarcoma Fibrosarcoma	Both poorly differentiated	None	—	Tumour fairly vascular
7177	84	None	—	—	—	—	Atrophied. Much scar tissue. No proliferation

bral). The rat with a primary tumour in the pelvis as well as one at the injection site in the thigh had a tumour in the lung in addition to metastases in the right axillary and inguinal lymph nodes ; histologically the lung tumour had the same characteristics as the pelvic primary, from which it was probably a metastasis.

Metastases in the lymph nodes consisted mainly of spindle shaped cells, but in one rat of Series I and two of Series II, some degree of differentiation was found in the metastases (Fig. 8).

DISCUSSION

The two experimental carcinogens, metallic cadmium and metallic cobalt, when injected into rat muscle have in common the ability to produce rhabdomyosarcomata from the muscle tissue itself, as well as fibrosarcomata from the associated connective tissue. Two tumours induced by cadmium had distinctive features : one was an osteochondro-sarcoma and the second, a very mixed tumour, contained not only the usual rhabdomyo- and fibrosarcoma, but also myxosarcoma, cavernous haemangioma and malignant synovioma.

In general the two series of cadmium-induced rhabdomyosarcomata showed a greater degree of differentiation than the corresponding cobalt-induced tumours ; the cadmium tumours that were the first to appear seemed better differentiated than those which developed later.

The high incidence of metastasis in the cadmium-injected rats was in complete contrast to the virtual absence of metastasis in the cobalt-injected animals ; this difference may well have been due to the much greater initial damage produced by cadmium in the tissue at the injection site. In the cadmium-treated rats killed at 5 and 12 days it was seen that the granulation tissue was already very vascular, and it is possible that a concomitant increase in lymphatic drainage may have caused the high incidence of lymphatic metastasis.

Recent experiments strongly suggest that the inhibition of respiration mentioned above is not the prime cause of the carcinogenic action. Thus copper, which like cobalt and cadmium inhibits ketoacid oxidation by mitochondria, is not carcinogenic in our experiments (Heath, 1963), whereas beryllium, which does not inhibit mitochondrial respiration (Heath, Daniel and Webb, 1962), has been shown by others to be carcinogenic for rat bone (Barnes, Denz and Sissons, 1950) and lung (Schepers *et al.*, 1957), although we have been unable to produce tumours with this metal under our conditions (Heath, 1963).

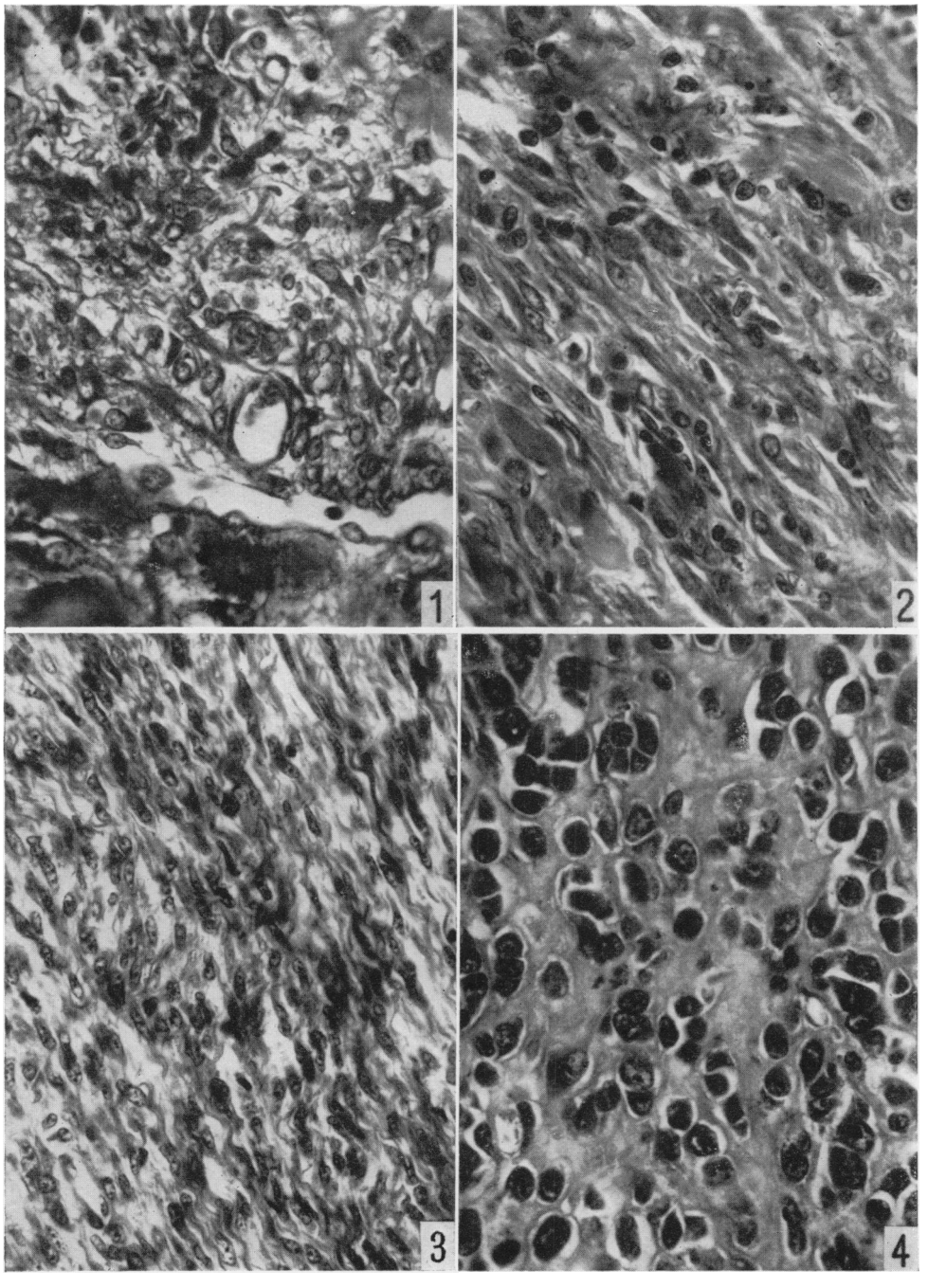
SUMMARY

Powdered metallic cadmium on intramuscular injection into rats produced tumours, a high proportion of which were rhabdomyosarcomata. In general

EXPLANATION OF PLATES

All stained with Azan. ×450.

FIG. 1.—Rat 7168. Vascular granulation tissue.
FIG. 2.—Rat 7158. Early tumour, poorly differentiated rhabdomyosarcoma.
FIG. 3.—Rat 7165. Fibrosarcomatous region of tumour.
FIG. 4.—Rat 7159. Osteochondrosarcoma.
FIG. 5.—Rat 7163. Rhabdomyosarcoma, poorly differentiated region.
FIG. 6.—Rat 7163. Rhabdomyosarcoma, moderately differentiated region.
FIG. 7.—Rat 7163. Rhabdomyosarcoma, well differentiated region. Note striations.
FIG. 8.—Rat 7175. Metastasis in lymph node. Well differentiated rhabdomyosarcoma.



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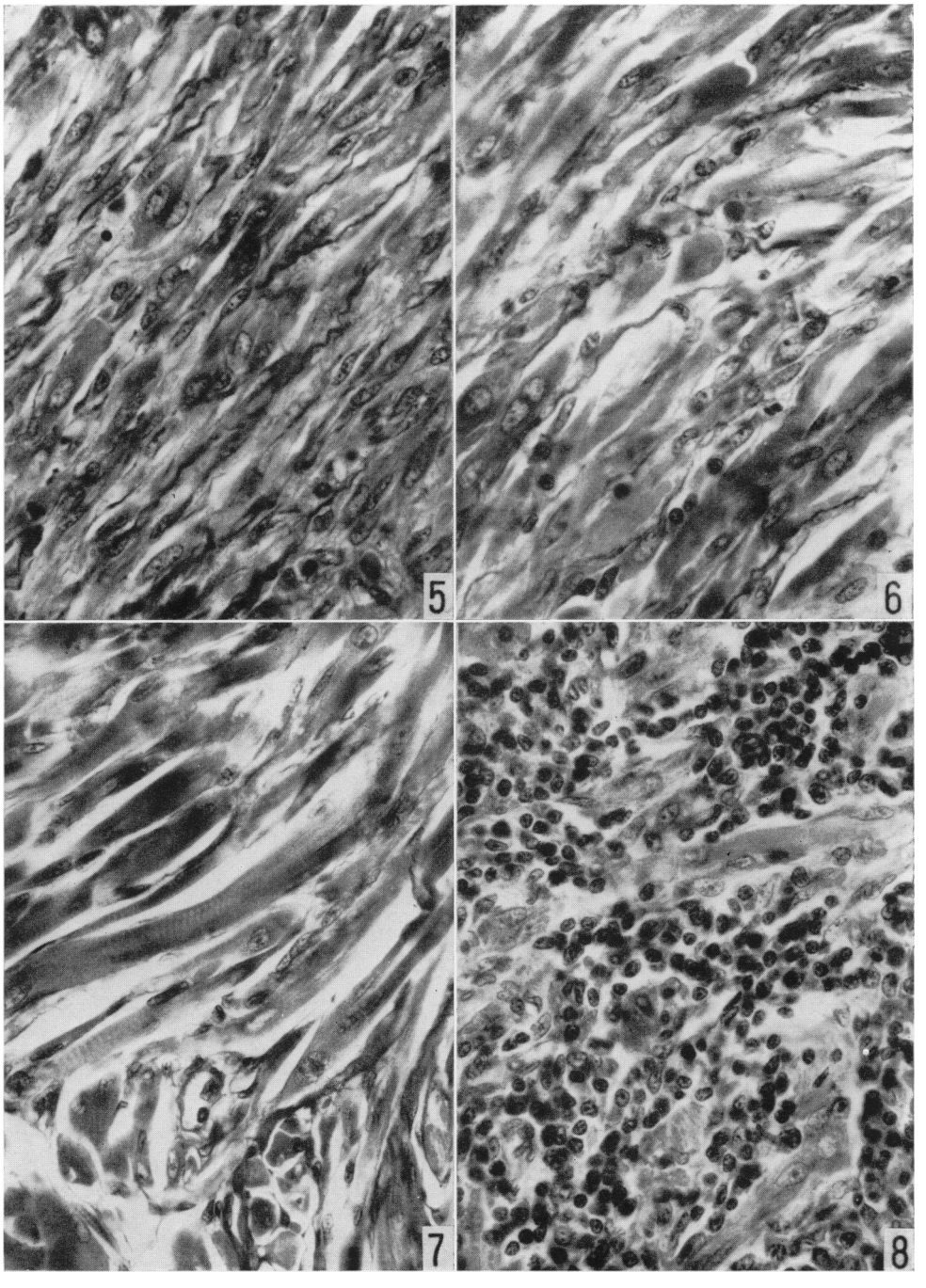

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these tumours were better differentiated than those produced by a similar injection of powdered metallic cobalt, and showed a much greater tendency to metastasize.

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