NOTE

# HISTOCHEMICAL DEMONSTRATION OF BLEOMYCIN

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INTRODUCTION : It is well known that bleomycin is a very potent anticancer agent especially to epidermoid carcinoma, and that it is not effective to adenocarcinoma and sarcoma. ICHIKAWA *et al.* reported that bleomycin was found in higher concentration in the tissue of 20-methylcholanthrene induced epidermoid carcinoma than in the normal skin and than in sarcoma. NOUEL *et al.* reported, in their recent paper, that $^{57}$Co-bleomycin was counted about three times higher in the tumor tissue than in the adjacent non-neoplastic tissue.

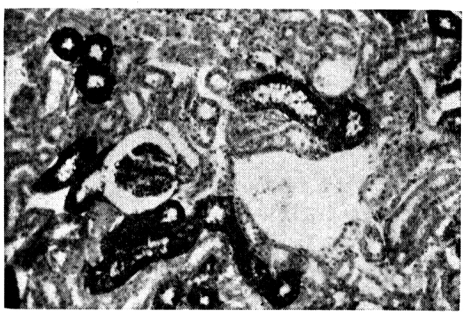

Fig. 1 Kidney of the female mouse, excised 30 minutes after the intramuscular administration of 100 mg/kg of bleomycin (Cu 2.8%).

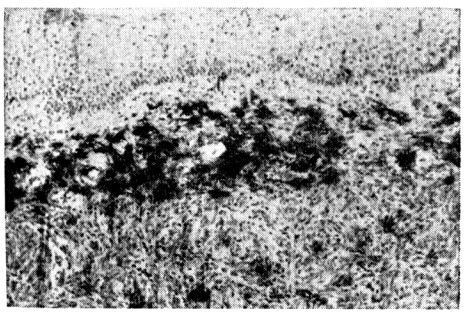

Fig. 2 Uterus of the same mouse on Fig. 1.

These facts suggest that bleomycin has high affinity to the carcinomatous tissues. To confirm this possibility, histochemical studies on the localization of bleomycin were performed.

MATERIALS AND METHOD : Bleomycin (Cu 2.8 %), Cu-free bleomycin or 5-fluorouracil was administered to female mice in 10 mg to 500 mg/kg doses. An excised specimen was fixed and dehydrated in $H_2S$ saturated absolute ethanol for 2 to 12 hours depending on its size and concistency. Paraf-

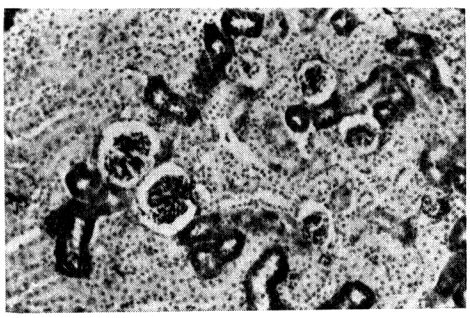

Fig. 3 Kidney, excised 30 minutes after the intramuscular injection of 500 mg/kg of Cu-free bleomycin.

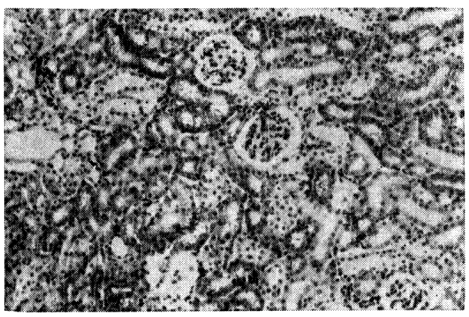

Fig. 4 Kidney, excised 30 minutes after the intramuscular injection of 250 mg/kg of 5-fluorouracil.

fin sections were then made in a routine manner. Staining was done applying TIMM's method for the demonstration of heavy metals.

RESULTS : Bleomycin (Cu 2.8%) was densely stained as brownish black granules or as their accumulation in the glomerules and proximal tubules of the kidney (Fig. 1), and in the subcutaneous tissue of the squamous epithelium of the uterine cervix (Fig. 2). Cu-free bleomycin and 5-fluorouracil were also stained by this method, though the staining was thin (Figs. 3 and 4).

COMMENT : The histological study of antineoplastic agents was carried out mainly by means of autoradiography. But autoradiographic study of bleomycin was technically difficult because, 1) $^{14}C$-bleomycin is unfit to use due to its low specific radioactivity, 2) $^{3}H$-bleomycin is quite unstable, and 3) tissue and/or organ distribution of $^{57}Co$-bleomycin is different from that of unlabelled bleomycin, and $^{57}Co$-bleomycin is not antineoplastic. Therefore, histochemical study of non-radioactive bleomycin is to be preferred.

This method is applicable for the demonstration of Cu-free bleomycin and 5-fluorouracil in tissues. The reason why these nonmetalic compounds were also able to be demonstrated by this method is that these antineoplastic agents chelated copper or some other heavy metals *in vivo*. It is well known that 5-fluorouracil chelates copper. It was previously reported that bleomycin did not chelate copper *in vivo*. However, using column chromatography and thin layer chromatography, we demonstrated and reported that bleomycin also chelates copper *in vivo*. Many anticancer drugs and antibiotics were reported to be metal chelating agents. Thus by this histochemical technic we feel it possible to demonstrate the location of many anticancer agents in the neoplastic tissues.

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