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Malignant Conversion of Shope Papillomas and Associated Changes of Serum Ceruloplasmin in Domestic Rabbits (40125)

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Shope papillomas induced in domestic rabbits are converted into squamous cell carcinomas when allowed to grow on skin of the animals for 12 months or more (1). As reported in our previous paper, the papillomas induced in newborn domestic rabbits grew more than 6 months in the majority of animals without sign of regression (2). The malignant conversion began to take place in most of such papillomas after the lapse of 1 year, concomitantly with blueing of the serum in these animals. Bluish serum has also been observed in rabbits bearing Vx2 and Vx7 carcinomas (3). The bluish color of the serum is ascribed to the augmented level of serum ceruloplasmin, a copper-containing blue enzyme. The present paper reports the quantitative and qualitative changes of serum ceruloplasmin, associated with the malignant conversion of Shope papillomas to carcinomas.

Materials and methods. Rabbits. The papillomas were induced in neonatal rabbits with intradermal injection of Shope papilloma virus (SPV). These animals were from the same group as used in the previous report (2). In addition, some domestic rabbits were used for induction of the papillomas, transplantation of Vx2 carinomas, and for mating. Sera obtained from these animals were kept frozen at −20° until use. Fresh sera were also subjected to comparative analysis.

Histological examination. Biopsy specimens were taken from the tumorous tissues periodically and were diagnosed according to the pathological criteria for malignancy.

Quantitative estimation of serum copper level. Concentration of serum copper was measured according to the Bathocuproine method (4) and also by the atomic absorption spectrophotometry (5).

Disc electrophoresis and stainings. Disc electrophoresis of serum was performed in 5% polyacrylamide gel under the conditions described by Ornstein (6). The protein was stained with Amido black 10B and the enzymatic activity was manifested by soaking the gel overnight at 37° in 0.05% *p*-phenylenediamine in 0.1 *M* acetate buffer pH 5.7.

Immunoelectrophoresis. Immunoelectrophoretic analysis was performed in 1.2% agarose gel according to the micromethod (7). Antisera for human ceruloplasmin and for whole rabbit serum were purchased from Hyland (Costa Messa, USA) and Biken (Osaka, Japan), respectively.

Results. Serum copper level in rabbits which encountered malignant conversion of the papillomas. Serum copper level was assayed and comparison was made among the sera of seven tumor-bearing rabbits at 4 months of age and later just before their death due to the malignancy. In these animals, ulceration of papillomas was observed around 12 months after the tumor induction and was followed by the swelling of regional lymph nodes. At autopsy, lung metastasis was observed in all cases. Malignant conversion was confirmed by histological examination of ulcerated portions of the tumor in all animals. As shown in Table I, serum copper level before death was elevated 2–6 times as compared to that at 4 months of age.

Disc electrophoretic pattern of ceruloplasmin activity. A conspicuous single band of enzymatic activity was manifested in the serum at 4 months of age, whereas a faint second band with a larger mobility appeared in the serum from the same animal when ulceration of papilloma was first noticed, and such double band became apparent 4 months later when ulceration was exacerbated (Fig. 1A). Similar findings were observed in the sera from all seven animals examined. The double bands were also observed in rabbits transplanted with Vx2 carcinomas (Fig. 1B). A single band in the normal serum and the double bands in the carcinomatous serum were manifested, irrespective of fresh or frozen serum.

Changes in serum copper level, disc electrophoretic and immunoelectrophoretic pattern of ceruloplasmin in a single rabbit, associated

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TABLE I. SERUM COPPER LEVEL IN RABBITS WITH VIRUS-INDUCED PAPILLOMAS AND AT THE MALIGNANT CONVERSION.

Rabbit No.	Serum copper[a] (μg/dl) At 4 months	Serum copper[a] (μg/dl) Before death	Occurrance (months) of Ulceration	Occurrance (months) of Death
203	91	358	15	17
206	131	353	21	25
208	108	324	17	18
213	97	324	16	18
215	125	301[b]	14	—[c]
233	74	438	12	14
252	108	319	13	16

[a] Determined by the Bathocuproine method.
[b] Measured at 5 months after ulceration was noticed.
[c] Animal surviving.

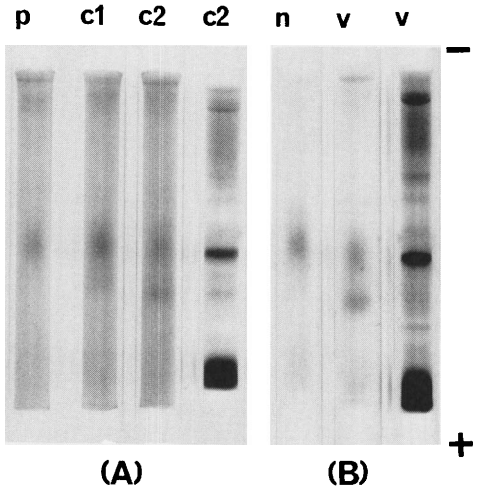

FIG. 1. Disc electrophoretic pattern of ceruloplasmin activity at malignant conversion (A) and at Vx2 transplantation (B). (A), Sera from rabbit No. 215. p: 4 months of age, c1: 14 months of age when ulceration of the tumor was noticed, c2: 5 months thereafter. Three tubes from the left were stained for ceruloplasmin activity and tube 4 for protein. (B), Sera from rabbit No. 322. *n*: normal, v: 2 months after Vx2 transplantation. Two tubes from the left were stained for ceruloplasmin activity and tube 3 for protein. Anode is downward.

with pregnancy, malignant conversion and total resection of tumor. Serum copper level was found to be elevated approximately twice at parturition and four times at malignant conversion as compared with that of the normal serum (Fig. 2). The elevated level in carcinomatous serum dropped to the normal level of 68 μg/dl at 1 month after total resection of the tumor. Ceruloplasmin activity was manifested in disc electrophoresis as a single band in normal serum and at parturition, and as double bands in carcinomatous serum. Immunoelectrophoretic analysis revealed a single precipitation arc in sera from both pregnant and carcinomatous animals, but the arc formed in the latter showed more widely distributed pattern of the precipitate. These results were consistent with those obtained by disc electrophoretic analysis.

Discussion. Elevation of serum copper or ceruloplasmin level is known to occur in diseases such as cancer, inflammation, biliary duct obstruction (8–10). In cancer patients, elevated serum copper level is reportedly high at approximately twice the normal level. In the present work with rabbits, we found the augmentation of serum copper and a possible qualitative change of serum ceruloplasmin accompanying the malignant conversion of Shope papillomas. Ulceration is assumed to be the first macroscopic sign of benign-to-malignant conversion taking place in the tumor system (1). The bluish coloration of the serum, as reported herein, is also indicative of such malignant alteration. Although a direct relationship was not established, it is plausible to assume that the bluish color is due to the elevated level of serum ceruloplasmin, as indicated by elevated copper levels and intense ceruloplasmin activity in the serum specimens.

Physiological augmentation of ceruloplasmin occurs also in association with pregnancy (9). Comparison of such augmented ceruloplasmin was made in a single rabbit at pregnancy and at malignant conversion and a qualitative difference was evident among both sera. The enzymatic activity was manifested as a single band at pregnancy and as double bands at malignant conversion. One might argue that the double band formation could be due to artifacts occurring with storage and that loss of enzymatic activity may have taken place during storage for one component and not for the other. However, fresh sera from carcinomatous rabbit also gave double bands and fresh normal sera a single band, thus excluding such possibilities. Two distinct ceruloplasmin activities could be due to two distinct isozymes of ceruloplasmin, although in the present experiment, we could not exclude the possibility that one band is due to a degraded product or a complex of the native enzyme. The presence of ceruloplasm in isozymes has been reported in human serum (11) but not in rabbit serum. Our

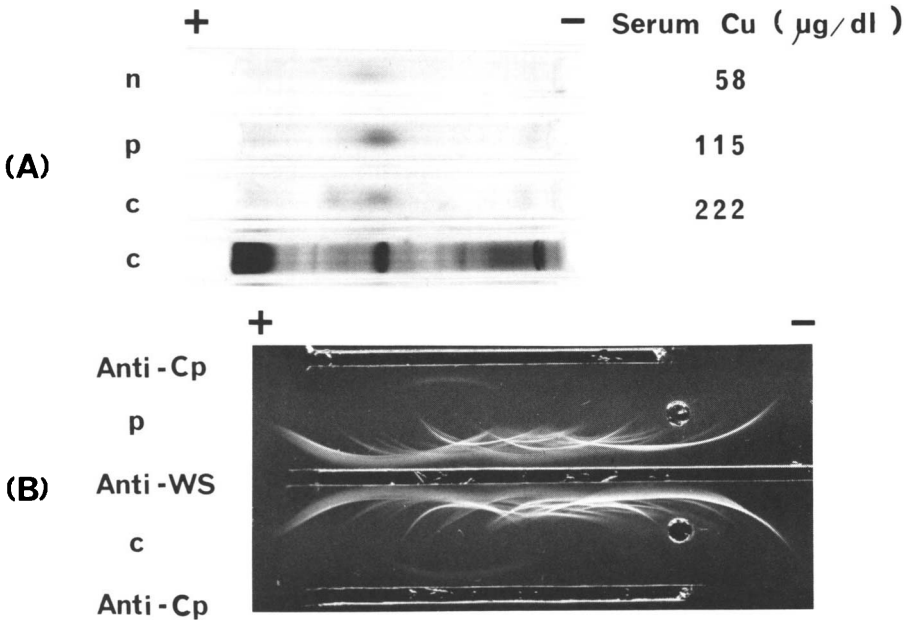

FIG. 2. Disc electrophoretic and immunoelectrophoretic patterns of ceruloplasmin in sera from a single rabbit No. 176 at parturition and at malignant conversion. (A), Disc electrophoretic pattern of enzymatic activity. n: normal, p: at parturition, c: at malignant conversion. Three tubes from the top were stained for enzymatic activity and tube 4 for protein. Each tube is annexed with serum copper level measured by the atomic absorption spectrophotometry. (B), Immunoelectrophoretic pattern of the same sera in (A). Anti-Cp: anti-human ceruloplasmin serum, Anti-WS: anti-rabbit whole serum. Anode is to the left.

preliminary results on heat-stability, optimal pH range and inhibition by NaN_3 have shown substantial differences between the enzymatic activities of the two components, and such finding is consistent with the assumption that these are indeed isozymes. An invariable symptom as such may be utilized as a marker for the malignant conversion of benign tumors.

Summary. Shope papillomas neonatally induced in domestic rabbits were observed to convert into squamous cell carcinomas after the lapse of a year or more. The ulceration of the tumor, the bluish coloration of the serum and the elevation of serum copper level were concomitantly observed in these animals. The serum copper was found to be augmented two to six times higher among animals with malignancies than those with benign papillomas. Disc electrophoretic analysis of the serum showed that ceruloplasmin activity was manifested as single and double bands, before and after the malignant conversion, respectively. The serum at parturition also showed an elevated serum copper level but only a single band of ceruloplasmin appeared in disc electrophoretic analysis.

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