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DISTRIBUTION OF CYTOPLASMIC FERROPROTEINS AND IRON IN TUMORS OF THE HUMAN KIDNEY

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The existence of an association between disturbance of iron metabolism and the development of carcinoma of the kidney was postulated by the authors previously [6]. The carcinogenic effect of iron for the kidneys was subsequently confirmed experimentally [7, 15]. Interest in the study of ferroprotein and iron metabolism in carcinoma of the kidney also is due to the functional characteristics of the kidney as the main organ of iron excretion [11]. Transport of iron to the cells is effected by transferrin, whereas ferritin biosynthesis depends directly on the intracellular iron concentration [14].

The aim of this investigation was to study the distribution of cytoplasmic ferroproteins and iron in different regions of tumors of the human kidney.

EXPERIMENTAL METHOD

Adenocarcinoma of the kidney develops from the epithelium of the renal tubules. A characteristic feature of this tumor is that the primary lesion is separated from the renal parenchyma by a quite dense fibrous capsule. In the latter stages of development pairs appear in the capsule and the tumor infiltrates without any definite borders [3]. These morphological features lie at the basis of the subdivision of the kidneys by tumors, with the distinction between the renal parenchyma, the primary node, and tumor tissue infiltrating beyond the capsule of the primary node.

Altogether 19 kidneys with tumors, and 11 definitive and 9 embyonic kidneys were studied, with definitive, adenomatous, and carcinomatous tissues of the prostate gland as the control. Extracts were prepared in Tris-glycine buffer, pH 8.3, under standard conditions: the ratio of tissue to buffer was 1:2 (w/v). Extraction was done once, for repeated extraction gave only a negligible yield of the test proteins. The previously minced tissue was washed thoroughly with physiological saline. Besides the parenchyma and primary node, tumor tissue infiltrating beyond the capsule of the primary node was isolated from 7 of the 19 kidneys with tumors. Standard solutions containing 50 mg/ml of dry substances were prepared from the clarified and freeze-dried extracts: the excess of dry substances (60 mg/ml) was dissolved in the same buffer, incubated for 1 h at 37°C and for 24 h in a refrigerator, and the insoluble residue was separated by centrifugation and its protein concentration determined by Lowry's method, after which the solutions were diluted to a concentration of 50 mg/ml (quantity of soluble protein per gram wet weight of tissue).

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TABLE 1. Concentration of Ferroproteins (in mg/liter) and Detection of KS α_2-Macroglobulin in Embryonic, Definitive, and Neoplastic Kidneys (M ± m)

Test object	Number of tests	Ferritin	Transferrin	KS α_2-macroglobulins
Embryonic kidney	9	12,4±0,2	—	+
Definite kidney	11	37,0±2,9	88,1±13,0	+
Carcinoma of kidney				
parechyma	19	78,1±11,0	222,3±19,0	+
primary node	19	300,8±45,2	196,1±25,8	±
tumor tissue	7	121,2±26,7	134,2±20,9	0
Definitive prostate	6	20,2±2,8	102,1±9,3	0
Adenoma of prostate	11	26,5±4,5	132,0±14,3	0
Carcinoma of prostate	21	16,7±1,6	80,5±6,5	0

Legend. Here and in Table 2: 0) not present, ±) not always present; +) always present.

The methods used to obtain antisera to the test proteins were described by the authors previously [8, 9]. The ferritin concentration was determined by the immunodiffusion method, using a standard test system [12], with a sensitivity of 1 mg/liter. The presence of kidney-specific (KS) α_2-macroglobulin was judged by deviation of the standard test system. The transferrin concentration was determined by radial immunodiffusion [6]. Kidney tissue for histochemical study was fixed in neutral formalin. Sections 5-7 μ thick were stained with hematoxylin and eosin and for Fe^{+++} and Fe^{++} [5].

EXPERIMENTAL RESULTS

The ferritin concentration in the primary tumor node of the kidney was highly significantly higher ($p < 0.001$) than its concentration in the embryonic and definitive kidneys (Table 1). No differences were found in the definitive, adenomatous, and carcinomatous prostate tissues. Incidentally, such significant differences in the ferritin concentration in the tissues were not observed during carcinogenesis in the liver, lung, stomach, ovary, and uterus [2, 10].

The transferrin concentration in the tumor-bearing kidney was higher, but not significantly, than in the normal kidney. KC α_2-macroglobulin was never found in tumor tissue infiltrating beyond the capsule of the primary node, possible evidence of reduction of the specific kidney protein in the tumor cells (the simplification phenomenon). Incidentally, the ferritin concentration in the primary node of each individual kidney with a tumor was always higher than the average values, not only for normal kidneys but also for tissues surrounding the primary node — parenchyma and tumor tissue (Table 2). On this basis it can be postulated that the formation of a clone of cancer cells (primary node) takes place under conditions of ferritin and iron concentrations that are unusually high for the individual kid-

TABLE 2. Concentration of Ferroproteins (in mg/liter) and Intensity of Reaction for Iron (Fe^{+++}) in Different Parts of the Tumor-Bearing Kidney

Patient No.	Parenchyma			Primary node			Tumor tissue		
	ferritin	transferrin	Fe^{3+}	ferritin	transferrin	Fe^{3+}	ferritin	transferrin	Fe^{3+}
1	64	360	0	256	330	++	128	165	+
2	64	360	++	64	280	++	16	280	++
3	128	155	—	1024	165	—	256	75	—
4	32	—	—	512	—	—	32	—	—
5	128	85	++	128	165	++	32	85	+
6	32	53	0	512	130	++++	128	100	++
7	64	360	0	256	100	++	256	100	++

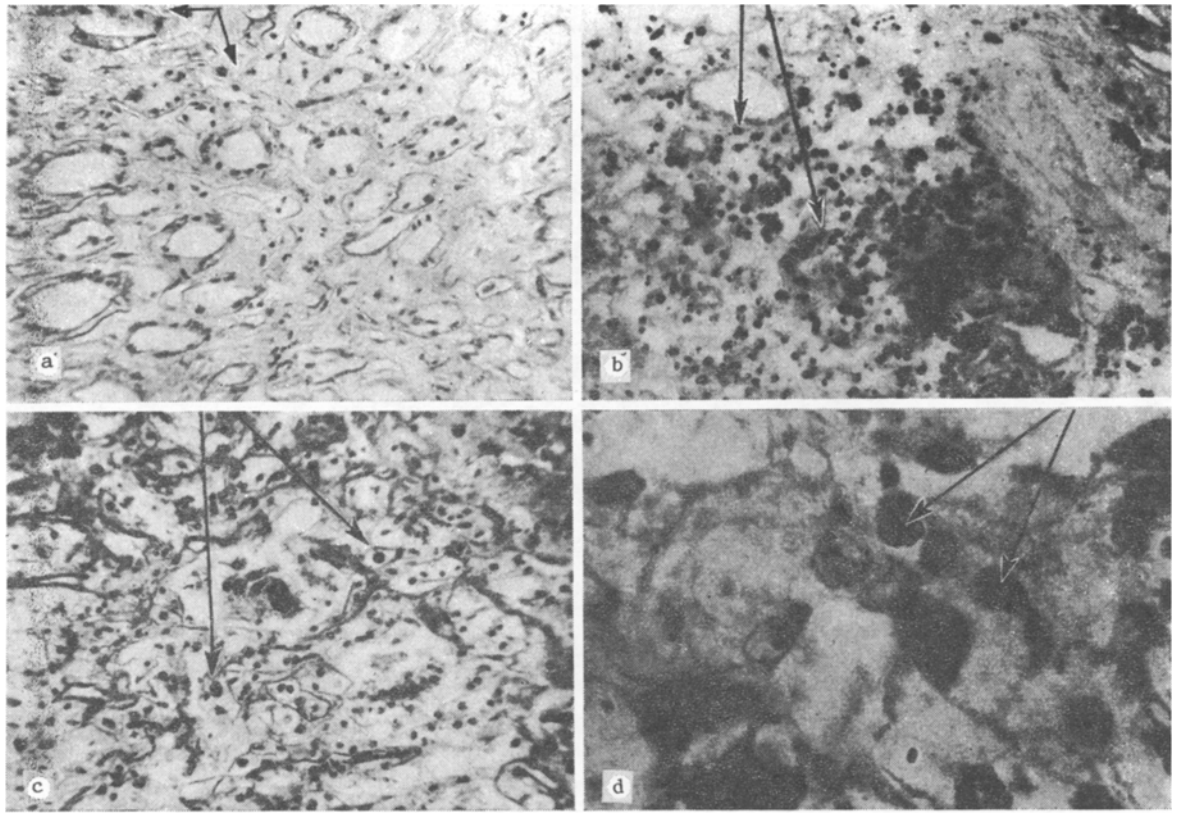

Fig. 1. Distribution of Fe^{+++} in different parts of a kidney affected by adenocarcinoma (Perls' carmine stain). a) Renal parenchyma: single cells of cubical epithelium of proximal renal tubules with Fe^{+++} inclusions (240 ×); b) primary tumor node; tissue packed with Fe^{+++}, epithelial cells of proximal tubules partially preserved and intensively incrusted with Fe^{+++} (140 ×); c) tumor tissue infiltrating beyond the primary node: Fe^{+++} inclusions in single cells (240 ×); d) primary tumor nodes; Fe^{+++} ions located in nuclei (630 ×).

ney, whereas expansive infiltration of such a clone evidently takes place in the presence of more moderate concentrations of these substances.

The histochemical investigations showed that Fe^{+++} ions were located in the parenchyma of the tumor cells in the epithelium of the renal tubules, and incrusted the cytoplasm of cells of the primary node intensively, and at the periphery of the tumor less intensively (Fig. 1). In single cells of the primary node Fe^{+++} ions were found in the nuclei. Fe^{++} ions were virtually not detected in the tumor nodes. In the kidneys of healthy individuals the reaction for Fe^{+++} and Fe^{++} was negative.

Consequently, the differences discovered in the distribution of ferroproteins and iron in kidney, which are evidently regular, for the kidneys excrete up to 1 mg of iron daily, and conditions for iron retention and accumulation can arise in them realistically [11]. The primary tumor node in the kidney has been shown to be a locus of unusually high concentration of ferritin and iron. A high concentration of iron ions has a toxic and carcinogenic action on mammalian kidney cells [15], possibly due to alkylation of guanine in position 7, with conversion of purines into pteridines, which leads to a disturbance of DNA structure [13]. Under these circumstances iron ions are initiators of the formation of highly reactive alkyl compounds in lipid peroxidation reactions [4]. Meanwhile, oxidative processes and oxygen consumption by the kidneys per unit weight exceed those in all other organs [1], and iron ions block the SH-groups of many enzymes (including respiratory enzymes and those of cell division), with their conversion into the inactive form [4].

Ferritin accumulation in the kidneys in carcinoma is probably a protective reaction to the toxic effect of iron, for the apoferritin molecule can bind up to 4000 atoms of iron, converting them into a deposited form, and store them as the hydrated oxide: $Fe_2O_3 \cdot nH_2O$.

Disturbance of iron metabolism in the kidneys can thus evidently be regarded as a primary factor in the carcinogenesis of this organ, and ferritin accumulation in the tumor as a result of this process.

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