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RETENTION OF COBALT IN EXPERIMENTALLY INDUCED KIDNEY DISEASE

Studies of ^{60}Co in irradiated and sublimate-poisoned mouse kidney

by

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The inorganic cobalt ion has been considered important in renal erythropoietin production (JACOBSON et coll. 1960) and has been shown to have a curative effect in treating human renal anemia (GARDNER 1953, KASANEN et coll. 1962, 1963). This effect has been assumed in conjunction with the hypothesis that inorganic cobalt stimulates the synthesis of the humoral erythropoietic agent (JACOBSON et coll.). A low erythropoietin level has been demonstrated in patients with renal anemia (GOLDWASSER et coll. 1958, JACOBSON et coll.). Slight or no erythropoietic activity has been reported in urine from anemic and uremic patients (FINNE 1968). The retention of radioactive ^{60}Co has been studied in normal and glomerulonephritic human kidney tissue; a clear-cut difference was observed (LINDGREN & SALMI 1968). In normal kidneys, ^{60}Co was localized in the renal cortex close to the corticomedullar margin.

Autoradiographically, the glomerulonephritic kidneys also presented activity in the cortex; this occurred in irregular paths, however, and occasional large areas in the corticomedullary region were without activity. Radioactive ^{60}Co was

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Table 1

Blood counts in poisoned mice and in control mice

	$HgCl_2$	Controls	P
Hgb	10.9 grampercent	15.5 grampercent	<0.001
Er	6.5 millions/mm³	9.6 millions/mm³	<0.001
MCHC	33 percent	32 percent	
Hcr	34 percent	46 percent	<0.001
Leuc	2550/mm³	2122/mm³	

Table 2

Effect of roentgen irradiation on the cobalt accumulating capacity of mouse kidney

Kidney	Weight of kidney mg	Cpm/mg of kidney tissue	Cpm/whole kidney	P
Irradiated	69	4291	295663	<0.001
Intact	216	4187	903641	<0.001

Table 3

Effect of $HgCl_2$ treatment on the cobalt accumulating capacity of mouse kidney

Animal	Weight of kidney mg	Cpm/mg of kidney tissue	Cpm/whole kidney	P
Treated	216	103	22605	<0.001
Control	220	162	35023	<0.001

localized in the renal tubular cells both in healthy and diseased kidneys; the latter accumulated considerably less activity than normal kidneys. The amount of radioactivity retained by the cortex of normal kidneys was appreciably greater than the amount retained in the medulla.

The present study was undertaken in an attempt to reproduce in experimental animals the effects observed in human patients.

Material and Methods. The experimental material consisted of 200 young white mice of inbred strain. Two kinds of kidney damage were produced: (1) irradiation nephritis, and (2) chronic nephritis by poisoning with corrosive sublimate (mercuric bichloride, $HgCl_2$).

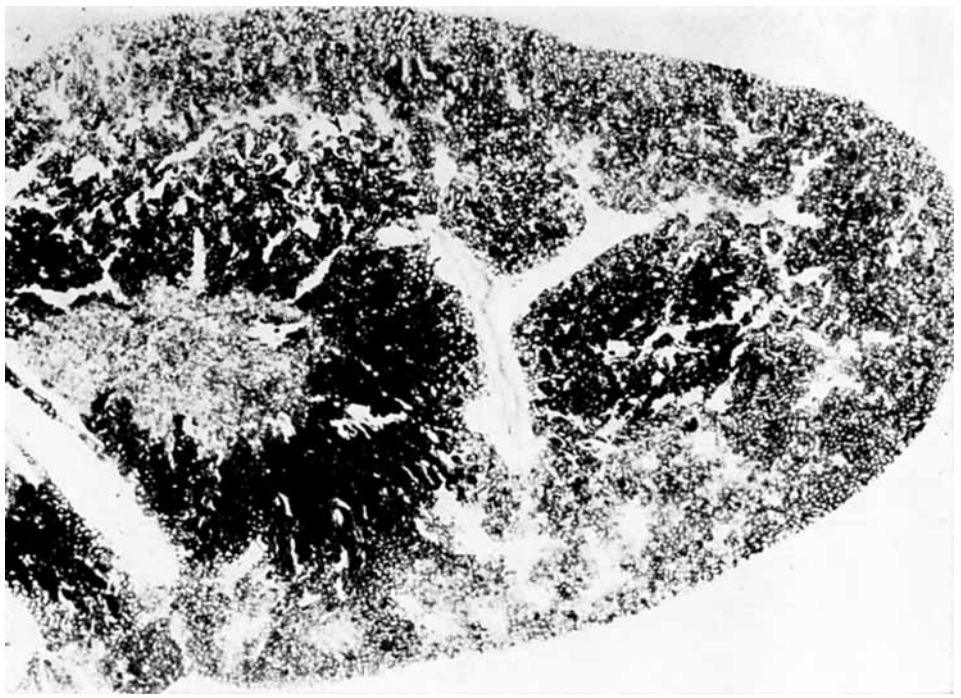

Fig. 1. Autoradiogram of the kidney of a control mouse. Activity of ^{60}Co most abundant at the corticomedullary boundary. ×25.

In irradiation nephritis, one kidney was removed and the remaining kidney was lifted from its bed so that it would receive strictly local irradiation; the latter thus remained attached by its pedicle, through which the circulation continued. The radiation was administered unilaterally, in order to minimize the radiation dose, the mobilized kidney being placed on a 5 mm lead sheet to avoid the effects of total body irradiation. Doses varying from 3 000 to 4 000 R were delivered by a Machlett roentgen tube at 50 kV. The cobalt chloride isotope ($^{60}CoCl_2$, specific activity 50.6 μCi/μg, Amersham, England) in a dose of 0.6 mCi was administered after 21 days.

Chronic nephritis was produced by injecting another series of test animals intramuscularly with daily doses of $HgCl_2$ 2 % in normal saline solution. Progressive doses were given, starting with 0.1 ml on the first day, with an additional 0.1 ml daily until half the number of test mice had died. This occurred at the forty-third day; at this time the surviving animals were also given the cobalt chloride isotope.

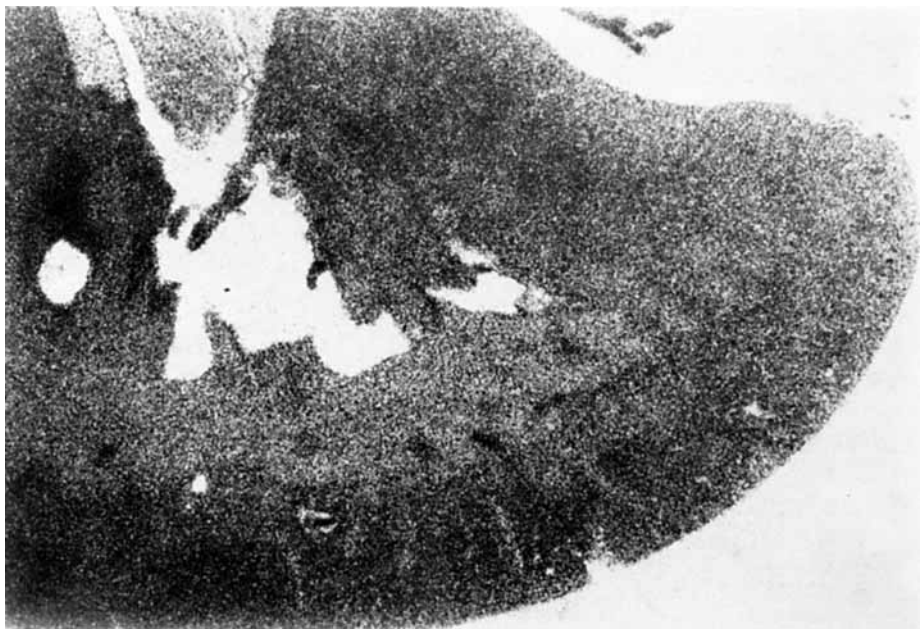

Fig. 2. Autoradiogram of the kidney of a mouse treated with corrosive sublimate until anemia developed. A small amount of ^{60}Co has accumulated in the renal cortex. ×25.

In both experimental series, the animals were sacrificed 24 hours after the isotope injection. A control series that received only the radioactive cobalt chloride was also maintained. Blood counts were made on both the experimental and control series (Table 1). Histologic specimens were obtained of the $HgCl_2$ series in order to exclude the possibility of toxic effects on the bone marrow. The marrow from the femura and vertebrae disclosed microscopically normal active hemopoiesis in all the cell lines.

The stripping film technique with Kodak AR 10 and AR 50 films was employed for autoradiography. The standard alcohol-xylol technique was used in preparation of the histologic samples. This technique washes out water soluble ions, the remaining ^{60}Co being definitely protein-bound.

The tissue specimens were digested in sulpuric acid and the radioactivity was measured in a well-type scintillation counter.

Results

Autoradiography revealed that the greatest activity in normal mouse kidney is concentrated near the corticomedullary zone with wedge-shaped areas towards

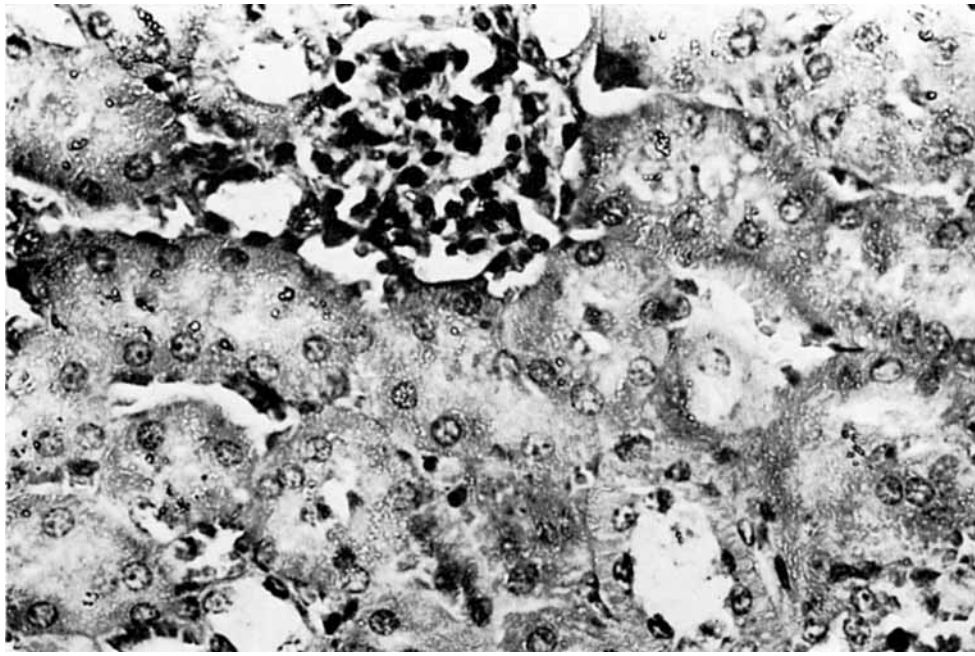

Fig. 3. Detail of the kidney in fig. 2. The glomerulus is well preserved but the tubular cells bear evidence of albuminous degeneration; the cytoplasm is swollen and granular. Van Gieson stain. ×400.

the renal capsule. Activity was absent in the medulla or in the blood vessels near the boundary between the cortex and the medulla. High-resolution autoradiography demonstrated that most of the activity was in the tubuli, and that there was none in the glomeruli.

In the diseased kidneys both in the irradiated and in those affected by the corrosive sublimate, the radioactive cobalt lay in scattered irregular paths in the cortex. The scintillometric measurements (Tables 2 and 3) indicated that a diseased kidney accumulated a smaller amount of cobalt in the cortex than a normal kidney. These measurements confirmed the impression given by the autoradiographic appearances.

The present results, with experimentally induced renal disease, are similar to those arrived at in human subjects dying of chronic glomerulonephritis (LINDGREN & SALMI 1968).

The irradiated kidney accumulates considerably less cobalt than the intact kidney (Table 2). However, the activity per tissue weight unit is similar in both

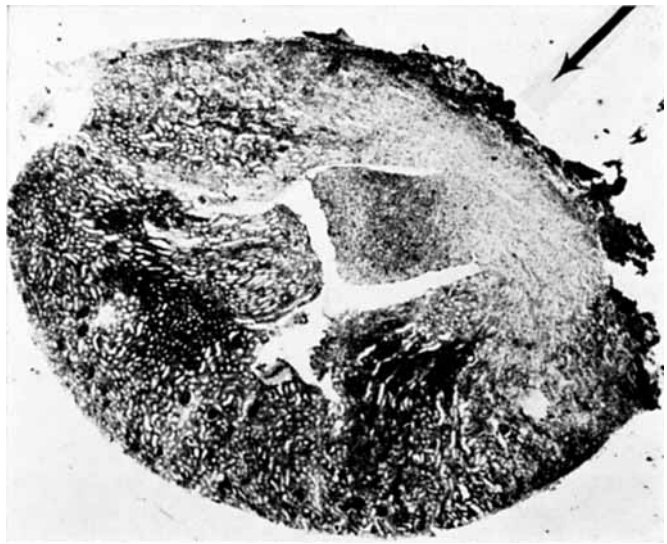

Fig. 4. Roentgenogram from the kidney of a mouse after open irradiation of the kidney with 4 000 R. The kidney is shrunken and the accumulation of ^{60}Co in the tubular cells is diminished. (The arrow indicates the direction of the radiation beam.)

kidneys, the smaller total accumulation probably being due to the shrinkage of the radiated kidney.

It is obvious that corrosive sublimate destroys a great number of the tubuli, as evidenced by high-resolution autoradiography. The tubular damage prevents the storing of cobalt in the renal cortex. The animals develop normochromic anemia with intact bone marrows; the anemia is probably renal in nature and is possibly due to the lack or decrease in the synthesis of renal erythropoietin. The present results indirectly suggest that the site of synthesis of renal erythropoietin may be the tubular cells.

Acknowledgements

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SUMMARY

The accumulation of ^{60}Co in experimentally damaged mouse kidney was studied by means of autoradiography and scintillometry, the damage being induced either by corrosive sublimate poisoning or by open roentgen irradiation. The radioactivity accumulated in the

tubular cells of the cortex both in the diseased and healthy kidneys. The rôle of cobalt in renal anemia and the production of erythropoietin are discussed.

ZUSAMMENFASSUNG

Die Anreicherung von ^{60}Co in experimentell beschädigten Mäusenieren wurde mittels Autoradiographie und Szintillometrie studiert; die Nierenschädigung wurde mittels Verabreichung von Sublimat oder mittels offener Röntgenbestrahlung herbeigeführt. Die Anreicherung der Radioaktivität fand in den tubulären Zellen des Cortex sowohl in den gesunden als auch in den geschädigten Nieren statt. Die Rolle des Cobalts in renaler Anämie und in der Produktion von Erythropoietin wird erörtert.

RÉSUMÉ

Les auteurs ont étudié sur des souris par autoradiographie et par scintillométrie, la fixation de ^{60}Co sur les reins lésés expérimentalement, la lésion étant produite soit par un empoisonnement par le sublimé corrosif soit par une irradiation directe par les rayons de roentgen. La radioactivité s'accumule dans les cellules tubulaires du cortex aussi bien dans les reins lésés que dans les reins sains. Les auteurs étudient le rôle du cobalt dans l'anémie rénale et dans la production d'érythropoiétine.

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