Experimental Study of Acquired Resistance of the Rabbit's Renal Epithelium to Mercuric Chloride

By Warren C. Hunter, M.A., M.D., Portland, Oregon

BIBLIOGRAPHY

Experimental Study of Acquired Resistance of the Rabbit's Renal Epithelium to Merccuric Chloride*†

By Warren C. Hunter, M.A., M.D., Portland, Oregon

In a previous paper I (1) have shown that a high degree of resistance to the injurious effects of uranium in the kidney can be experimentally produced. In a considerable number of rabbits it was found that 54 to 96 times the quantity of poison known to kill original renal tubular cells was insufficient to damage the regenerated epithelium in the same location. After administration of even the largest doses of uranium the metal was still excreted by the kidney and this together with the lack of histologic evidence of cell injury was considered to be proof of an acquired resistance on the part of the new epithelium. In the previous study the literature on experimental chronic nephritis and that portion of the experimental work of Gil y Gil (2) concerned with the pathologic stages of urinary immunity was reviewed in detail and need not be repeated here. Gil y Gil states that the lethal dose of mercuric chloride for rabbits may be determined from the experimental results of Suzuki (3), Barbour (4) and Nakata (5). He says: "Der erste (Suzuki) dieser Autoren stellte fest, das bei Injektion von 0.003 g Sublimat bei einem Kaninchchen von 700 g Gewicht nach 36 Stunden der Tod eintritt. Ein anderes Kaninchchen von 1700 g Gewicht, welches von Suzuki mit 0.005 g Sublimat auch subkutan injiziert wurde, ging nach 3 Tagen zugrunde. Der zweite Autor (Barbour) bezeichnete die tödliche Dosis mit 0.004 g Sublimat subkutan per Kilogramm Körpergewicht des Versuchstieres.

Auf Grund dieser Resultate bezeichnen wir für 2 kg Körpergewicht eines Kaninchens die mitte ren der beiden (0.003 und 0.004 g) Dosen gleich 0.0035 g Sublimat subkutan injiziert als tödliche Dosis." (p. 642)

Reference to these publications shows little basis for such assertions.

Critical Review

Rabbit's Renal Epithelium and Mercuric Chloride 797

Two animals constitute too small a series on which to base definite conclusions regarding the lethal dose of a poison. Furthermore Gil y Gil overlooked an important complicating factor. The smaller rabbit had intravenously 5 cubic centimeters of carmine and the larger animal 7 cubic centimeters of the dye after the subcutaneous injection. Suzuki's protocols show that the former died shortly after the carmine injection and that the latter became so weak within four hours that it was killed. The relatively sudden exitus strongly suggests an embolic death. In my experience the injection of such quantities of carmine has more than once been the cause of death in poisoned rabbits. Forbus (6) has likewise found that rabbits are invariably killed by large injections of carmine. Granting that Suzuki may have been more fortunate than others in this respect the fact remains that a complicating factor was introduced and one cannot be certain whether death was caused by the mercury or the dye.

Barbour's sole purpose was to test the efficacy of an antidote for mercuric chloride. Furthermore he introduced the poison intravenously instead of subcutaneously and the doses were based on 1 kilogram body weight rather than 2 kilos as given in the passage quoted.

Nakata's study was primarily concerned with the pathologic stages of the sublimate kidney in the human. For the purpose of comparison rabbits were given sublimate in quantities corresponding according to body weight with that taken by the human cases. In most instances the dosage in rabbits was considerably greater than 0.0035 gm. per kilogram of body weight.

Gil y Gil did not himself study the pathologic changes in the acute sublimate kidney but quotes the descriptions of Suzuki who found in two rabbits after subcutaneous administration of 0.003 and 0.006 gm sublimate a well marked necrosis of epithelium in the distal and transitional segments of the proximal convoluted tubules. He emphasizes that the amounts capable of producing such damage were administered subcutaneously and that he has given much larger doses intravenously to nearly all rabbits in his series.

The conclusion that renal immunity to merccuric chloride may be experimentally demonstrated is based on the study of nine rabbits. No definite plan of experimental procedure was followed with the exception of time intervals between injections. The irregular manner in which all animals were given both subcutaneous and intravenous injections renders difficult any reliable interpretation of results. Further important information may be gained from the discussion of the microscopic findings in each animal:

Rabbit 1. Necrosis of all except first part of proximal convoluted tubular epithelium; no increase in connective tissue. The author rightfully does not claim that immunity was shown in this case.

Rabbit 2. No demonstrable changes in any tubular cells; hyperemia of glomerulur; glomerular epithelium uninjured. Gil y Gil seeks to explain the marked difference in the kidney of
this animal and the first on the ground that Rabbit 1 received more sublimate in the first two injections than did the second animal. That Rabbit 2 subsequently had a greater amount of poison intravenously without exhibiting kidney lesions led to the conclusion that it was immunized by the first injection since the quantity given was sufficient to produce definite changes in a non-immunized kidney. Here Gil y Gil has quite overlooked such factors as individual differences in susceptibility, variation in absorption of the poison and the fact that the kidney of Rabbit 2 was not examined until twenty days after the last injection, while in the first animal the kidneys were studied two days after the final administration of sublimate. In Rabbit 2 there is no mention of epithelial regeneration yet the animal was considered to be immunized. Again, as in the uranium series, the author fails to state what epithelium becomes resistant. After quoting Menten (7) to the effect that exceedingly small doses of sublimate will produce definite histological alterations in kidney cells within five minutes after injection Gil y Gil allowed twenty days to elapse following an intravenous injection, after which according to his own statement there is rapid and extensive damage, and then considers the kidney immunized.

Rabbit 3. Atrophy of both glomeruli and tubuli in outer zone of cortex; proximal convoluted tubular epithelium well preserved except in some of the third segments where there is obvious necrosis; abundant connective tissue in transitional parts of the proximal convoluted tubules at the junction of cortex and medulla. In this case also Gil y Gil overlooked the very important factor of time. The last sublimate was administered fifty-two days before death and it is highly probable that all of the poison would be excreted long before this time. A poison cannot be assumed to act indefinitely and if cell injury is taken as a criterion of lack of immunity the examination must be made when the injury is at its height.

Rabbit 4. Discussion is unnecessary in this instance since the author does not assert that immunity was produced.

Rabbit 5. Atrophy of cortex with great diminution of epithelium in proximal convoluted tubules which are lined by endothelial-like cells. Most transitional segments of these tubules are difficult to distinguish but appear to have flattened epithelial linings. Evidently Gil y Gil considered this animal immune because of its ability to excrete within three hours about one-third of the last dose of sublimate. The fifteen day interval between the final injection and death is certainly too great for any microscopic evidence of immunity to be of value.

Rabbit 6. Glomeruli unchanged. All tubules intact except the distal divisions of the proximal convoluted tubules where there is cellular regeneration and only slight evidence of injury. Immunity is not mentioned. The protocol shows that the animal lived seventeen days after the last injection and this alone is sufficient to exclude it on the ground that ample time was allowed for complete cellular regeneration.

Rabbit 7. Fresh degenerative changes in the epithelium generally. The quantitative estimation of mercury excreted after the last injection of 0.01 gm. gave 0.004 gm. and for this reason the kidney was believed to be immunized. This in spite of the microscopic evidence of widespread cellular damage.

Rabbit 8. First and second divisions of proximal convoluted tubules uninjured, some necrosis in distal division. Following the final injection of 0.01 gm. between 0.004 and 0.005 gm. was excreted in the urine during the first three hours and the kidney was therefore believed to be immune. When we remember that the selective action of mercury is in the segments of the proximal convoluted tubules, in which the author has described necrosis, it is difficult to believe that any immunity was shown in this instance.

Rabbit 9. No claim is made for immunity.

The author does not state whether or not the intestine was examined in any of the animals. The stain for hyaline droplet degeneration was done in but one instance and was negative.

In order to compare the excretory ability of the kidney in non-immunized and immunized sublimate animals a normal rabbit was given 0.005 gm. of mercuric chloride subcutaneously and the urine collected by catheter for eight hours. In the first four hours there was no excretion while in the second period 0.001 gm. was excreted. From this the author concludes that in non-immunized animal the rate and quantity of excretion is appreciably decreased. However if we compare this result with those obtained in the so-called immune animals it will be found that: Rabbit 5 given 0.01 gm. intravenously excreted about one-third of this amount in three hours; Rabbit 7, with 0.01 gm. subcutaneously excreted one-half the dose, but not until twenty-four hours had elapsed, while in Rabbit 8 which had received 0.05 gm. subcutaneously excreted only about one-twelfth of this amount within three hours. Comparative results are of value only when the dosage and time have been identical.

In summary Gil y Gil states that the experimental results with sublimate corroborate those obtained with uranium. With sublimate it was also possible to build up a remarkable resistance of the tubular apparatus by reason of which the poisons will be, in contrast to non-immunized animals, excreted more rapidly and in greater quantities.

It is obvious that not a single case is convincing and above criticism. In the few instances where immunity was possible the results are questionable on account of the long periods of time elapsing between the last administration of sublimate and the examination of the kidney. The quantitative analyses are of little value as proof of immunity for reasons pointed out above.

AUTHOR'S EXPERIMENTS

In view of Gil y Gil's failure to determine the lethal dose of sublimate and his error in its computation from the work of others it was deemed necessary to establish this point before
proceeding with the immunization experiments.

1. Subcutaneous Lethal Dose

Rabbits of equal weight were grouped in pairs and to each pair the same quantity of poison was administered. The dosage per kilogram of body weight varied from 0.002 gm. to 0.02 gm. Of the fourteen animals receiving from 0.002 to 0.005 gm. per kilo four died (28.5 per cent). These occurred as follows: 0.002 gm. one (17 days), 0.003 gm., two (7 and 8 days), 0.005 gm. one (9 days). The percentage of mortality is not great enough to consider any of these amounts the minimum lethal dose. All of the eight rabbits receiving from 0.006 to 0.012 gm. per kilo survived. Two animals given 0.015 gm. per kilo and one having 0.02 gm. on the same basis died within 2-1 to 3 days. The first figure corresponds very closely to that of Lyon (8) who found that 0.0164 gm. per kilo usually proved fatal within twenty-four hours. On the basis of these results 0.015 gm. per kilo has been taken as the subcutaneous minimum lethal dose of mercuric chloride for rabbits.

2. Intravenous Lethal Dose

The same general plan of the subcutaneous group was followed. Seven animals received 0.001 gm. per kilo; six survived, one died after 17 days. Two rabbits had 0.002 gm. per kilogram; one died within 3 days, the other survived. Both animals given 0.003 gm. per kilo succumbed, one after 15 hours, the other in 9 days. So far as is permissible to draw conclusions from the limited number of these experiments 0.003 gm. per kilo intravenously seems to be the minimum amount that will kill all animals. It will be noted that this is exactly one-fifth the subcutaneous lethal dose.

Immunization Experiments

Material and Methods

Rabbits were used exclusively. The strength of mercuric chloride solution employed was at first 0.001 or 0.002 gm. per cubic centimeter of distilled water. As the dosage was increased the concentration was raised to 0.01 gm. per cc. in order to avoid the injection of such large quantities of fluid as would have been required had the weaker solutions been used.

The subcutaneous immunization series consisted of two groups of three animals each; in one the initial dose was 0.002 gm. per kilo, in the other 0.003 gm. Five rabbits had intravenous injections exclusively, the initial dose of 0.001 gm. per kilo being doubled thereafter. In addition nine animals which had been given sublimates subcutaneously to determine the lethal dose but which failed to succumb were afterward given one or more intravenous administrations.

Intravital staining with carmine, to determine the localization of the sublimates lesions in the tubules, was carried out in one acute and one chronic sublimated animal.

The acute mercuric chloride kidney

In none of the kidneys of animals dying from the effects of small subcutaneous doses of mercuric chloride was there any appreciable necrosis of the tubular epithelium. Three were studied, seven, nine and seventeen days respectively, after administration of the poison. All showed high grade parenchymatous degeneration of the tubular epithelium but little actual necrosis. In the glomeruli aside from slight swelling and sometimes desquamation of epithelium covering the tufts nothing was observed except congestion of the vessels.

With higher doses (0.015-0.02 gm.) definite necrosis of cells in the distal and transitional segments of the proximal convoluted tubules occurred. The affected segments are found here and there in all zones of the cortex with many still escaping injury. At this stage a great number of normal cells have died, but for the most part desquamated, and forming loose casts at the site of injury and in the upper part of the descending limb of Henle. The few nuclei still present are markedly pyknotic. A distinct difference is noted in the appearance of some of the dead cells in the mercuric kidney from that seen in the uranium organ in that their morphology is fairly well preserved and the cytoplasm has a distinctly hyaline appearance. Most cells however break up into finely granular debris. Fat in the necrotic cells is less constant than in uranium nephritis. The epithelium of the first and second divisions of the proximal convoluted tubules shows parenchymatous degeneration. The glomerular vessels are moderately congested; no apparent change is visible in the epithelium of Bowman's capsule, but not infrequently the covering epithelium of the tufts desquamates.
IMMUNIZATION WITH SUBLIMATE—GENERAL CONSIDERATIONS

For reasons already pointed out in the critical review it was not deemed advisable to duplicate exactly the experimental procedure of Gil y Gil. Instead the general plan by which good results were obtained in the uranium series was followed, i.e., adherence to one mode of administration, allowing sufficient time for epithelial regeneration and as complete a recovery of the animals as possible after each injection. Likewise the same criterion of cellular resistance discussed in detail in my previous paper (1), lack of injury to regenerated epithelium after administration of larger quantities of poison than that known to be capable of destroying the original cells, was used. No cases dying within a few minutes after injection or living long enough after the final dose for regeneration to take place are included. The number of animals meeting these requirements is very small.

It is difficult to conceive of a nephrotoxic substance less well suited for the demonstration of local cellular immunity than mercuric chloride. First, there is almost invariably a marked local inflammation and necrosis of tissue at the site of subcutaneous injections. This must mean that some of the poison is not absorbed and is a very undesirable factor when the full effect is wanted in the kidney. Second, intravenous injection of even moderate sized doses is dangerous. A number of animals in the writer's series died from thrombosis of the right ventricle almost immediately after the larger intravenous injections. In these animals the time which the sublimate could have acted on the kidney was so short that no definite fresh epithelial damage could be made out and for this reason it was necessary to exclude several animals from the immune series. Third, any appreciable quantity of mercuric chloride, whether administered subcutaneously or intravenously, produces in rabbits a marked diphtheritic enterocolitis, so severe that clinically the animals seemed to suffer as much from the intestinal lesions as the nephritis. Furthermore an enterocolitis indicates that the metal is excreted through the bowel as well as the kidney and one cannot be certain how much has reached the latter. Fourth, absorption of sublimate after subcutaneous administra-

RATION'S RENAL EPITHELIUM AND MERCURIC CHLORIDE

Rabbits: combined weight 20 gms. The capsules strip with slight difficulty exposing cortical surfaces which are in general pale but show also moderate numbers of petechiae. The surfaces are smooth in places, finely granular in others. On sectioning considerable necrosis is encountered but there is no gross evidence of lime salt deposition. The cortex is pale and at the junction with the medulla there is slight scarring.

Microscopic

Zenker-formalin fixation, hematoxylin and eosin stain. When the number of proximal convoluted tubules showing recent necrosis of epithelium are compared with the same tubules lined by regenerated cells it is apparent that more damage has resulted from the last dose than all previous ones combined. The lumina of the segments lost damaged are packed with desquamated necrotic cells. Regeneration has not yet begun. Casts are very numerous in the Henle's tubules and collecting ducts. None of the casts contain salt crystals. In the cortical zone nearest the medullas many of the transitional divisions of proximal convoluted tubules are lined by regenerated epithelium but this is true to a much lesser extent in the intermediole and outer zones of the cortex. The new cells are flattened or irregular in outline with dark bluish-pink staining cytoplasm and hyperchromatic nuclei and often forming giant cells or containing in the cytoplasm the remains of dead epithelium. In some instances the pinkish-staining debris is disappearing leaving large vacuoles which may contain finely granular brownish pigment. The regenerated cells show no evidence of injury. Sections stained for fat with Scharlach-R disclose a considerable fatty content in casts.

Kidneys: combined weight 10 gms. Weight 1650 gms. Dry gangrene at site of fourth injection. No ascites or hydrothorax. Marked diphtheritic colitis.
administration. No ascites or hydrothorax. Clear yellow urine. Marked diphtheritic co-

first injection and edema at site of last

tubules. Hyaline droplet degeneration is fully

than usual.

staining reaction for hyaline droplet degen-

eration.

rarely there is slight proliferation of Bow-

hemorrhages having the exact appearance

of those frequently observed in the acute

urea. The petechiae seen grossly on the corti-

cal surface are no doubt the rubber num-

erous pure blood casts in the tubules. Hyaline droplet degeneration is fully

as pronounced in the glomerular capillary

walls, as in any acute uremic kidney. Rarely there is slight proliferation of Bow-

man's epithelium and still more rarely small

epithelial crescents. In this case the glo-

meruli certainly cannot be regarded as im-

mune but on the contrary are more damaged

than usual.

Van Gieson's preparations disclose a very

slight increase of connective tissue about the regenerated tu-

bules. In the interstitial tissue are moderate numbers of lympho-

cytes and plasma cells.

Liver: slight fatty degenerative infil-

tration of cells about central veins.

Kidneys: combined weight 20 gms. Cap-

sules not adherent, surfaces smooth except

for a few small dark depressions. No
difficulty encountered in sectioning, no evi-

dence of calcification. The cortex is pale, the

markings are obscured, no fresh ne-
crosis or scar is visible.

Microscopic

Proximal convoluted tubules lined by char-
acteristic appearing regenerated epithelium are

fairly numerous, particularly in the tran-

sitional segments. Although most of the

regenerated tubules contain granular debris there is no evidence of injury in the

lining epithelium. The convoluted tubules

with original epithelium exhibit degenera-
tive changes varying from parenchymatous
dergamation to complete necrosis. In some

of these are coarse deeply pink-staining
droplets which with Mallory's stain take

the deep bluish-black color of hyaline dro-

plet degeneration. Regeneration has not yet

begun in the last injured divisions of the

tubules. Casts of all varieties are abundant.

Sections stained with van Gieson's reveal

no increase in connective tissue.

Glomeruli: On the whole the vessels

are well filled with blood so that the cap-

sular spaces are small. There is no appar-

dent damage to the epithelium covering the
tufts or in Bowman's membrane. The stain

for hyaline droplet degeneration is negative.

Liver: negative.

Rabbit's Renal Epithelium and Mercuric Chloride

The protocols of a few other rabbits

might be added but the degree of

immunity attained is not great enough

to be of any value.

Discussion of Results

Because of the retention of a portion

of the mercury at the site of in-

jection and the excretion by way of the

intestine it was not considered

worth while to attempt to estimate the

quantity excreted by the kidney. That

part of the last dose in each instance

reached the kidney is evidenced by

fresh necrosis of original but not the

reconstituted convoluted tubular cells,

indicating increased resistance on the

part of the latter. The degree of this

resistance is unknown. Had all the

last mercury given reached the kidney

this would be sixteen times the original

dose in the first rabbit and eight times

in the second animal, but we know

from the intestinal and local lesions

that such was not the case.

Tolerance of the whole organism for

mercuric chloride is very limited, in

most instances being only about twice

the subcutaneous lethal dose. This is

in marked contrast to the results ob-
tained with uranium for which a tol-
erance of thirty-two or forty times the

lethal dose was frequently attained.

In only one animal in the subcuta-

neous series was a truly chronic kidney lesion

obtained. The microscopic picture in

this instance was very similar to that

of a chronic uranium kidney, showing
great reduction in the number of origi-
nal proximal convoluted tubular cells, ex-

tensive regeneration, considerable

interstitial scarring and pigment de-
position in the regenerated cells. The

glomerular capsules were thickened,

the Bowman's membrane epithelium

hyperplastic and forming epithelial
crescents and even obliteration of the

capsular space. This animal received

dosage as shown in twenty-six instances.

In the living

functions of the glo-

meruli is not common in either the

acute or subacute sublimate kidney,

occurring in only seven of the thirty-
six animals of the series. In the dam-
age convoluted tubular epithelium

this form of degeneration was ob-

served in twenty-one of the thirty-six

kidneys. Fatty degenerative infla-

tration in living cells and casts was noted

in twenty-six instances. In the living

cells fat is present in about the same

quantity as in the uranium kidney but

in less constant and not so abundant

in casts.

Fatty degenerative infiltration was

observed in the liver only four times,

extensive in one, slight in the others.

The constant relationship to dosage

seen in uranium animals is lacking.

Conclusions

1. Local corrosive action and ir-

regularity of absorption after subcu-

taneous administration, the danger of

thrombosis following intravenous in-

jection and the excretion by the in-

testine resulting in severe enterocolitis,

makes sublimate an undesirable sub-

stance to use in the experimental pro-

ject.
duction of immunity in renal epithelium.

2. In rabbits the amount of mercuric chloride absorbed varies greatly. In some several injections may be given without producing any marked change in the renal epithelium while in others the same quantity causes well marked chronic glomerulotubular lesions very similar to those produced by uranium.

3. A slight degree of acquired immunity for sublimate can be demonstrated in regenerated epithelium and glomeruli.

4. Glomerular injury, as evidenced by necrosis of epithelium, hemorrhages and hyaline droplet degeneration is less frequent than in the uranium kidney.

5. A constant and striking phenomenon in the sublimate kidney is the envelopment of necrotic cells by regenerating epithelium.

6. In the rabbit calcium deposition in casts is inconstant.

REFERENCES


(3) **Suzuki, Tatuzo**: Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen. Fischer, Jena, 1912.


(8) **Lyon, Gezon**: Inflammatory Changes in the Kidney; an Experimental Study of the Action of Some Toxins and Poisons Upon the Kidney and also Upon the Spleen. J. Path. and Bact., 1903, iii: 401-455.