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Effect of salicylates on renal tubular epithelial excretion in children

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EFFECT OF SALICYLATES

ON

RENAL TUBULAR EPITHELIAL EXCRETION IN CHILDREN

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A brisk celluria in response to analgesics, particularly acetylsalicylic acid is an unexplained phenomenon previously documented in adults,[2,7] but not studied in children. Exfoliation of tubular epithelial cells, red blood cells, and white blood cells is of considerable interest in it's potential relationship to the phenomenon of non-inflammatory interstitial nephritis with papillary necrosis that has been clinically associated with prolonged use of analgesics, particularly combinations of salicylates and acetaminophen compounds.

Employing a differential stain developed by Prescott and Brodie[9], easy and accurate quantitation can be made of the excretion of red cells, white cells, and renal tubular epithelial cells. This technique was employed in the evaluation of the renal response to salicylates in children. Data on the response to varying doses of aspirin in children has not been previously reported in the literature.

Even in experienced hands, it is often difficult to distinguish readily, in an unstained urinary sediment, between leukocytes and small renal tubular epithelial cells , especially if degenerate forms are present.

The differential stain developed by Prescott and Brodie[9] is prepared as follows: 300mg. of 2:7 diaminofluorene and 130 mg. of phloxine B are dissolved in 70 ml. of warm 95% ethanol. To this is added 11 gm. of sodium acetate dissolved in 20 ml. of 0.5% acetic acid and 1 ml. of 3% hydrogen peroxide. The mixture is allowed to stand for 48 hours and is ready for use, after filtration is performed.

An aliquot of a timed urine specimen is centrifuged at 1500 r.p.m. for ten minutes. The supernatant is removed carefully with a pipette, two drops of stain added to the sediment, distilled water added to 0.5 ml., and a count is then made of 0.01 ml. in a Neubauer counting chamber.

Employing this stain, the blue oxidation product of 2:7 diaminofluorene is soluble in leukocyte cytoplasm. Initially the cytoplasm is clear, or slightly bluish, whereas the nuclei stain progressively darker blue. The cytoplasm then rapidly darkens, and the whole cell appears uniformly blue-black with some pseudopod formation. The renal tubular epithelial cells are almost identical in size but stain pink and are thus easily distinguished. The erythrocytes stain pink and are much smaller in size. The transitional epithelial cells of the bladder also stain pink , but are easily identified because of their larger size and have a decreased nuclear-cytoplasmic ratio compared to the renal tubular cells. Squamous epithelial cells found in the sediment stain pink but are readily identified by their morphology.

Utilizing the above method, the renal response to salicylates in children was studied. The renal response to aspirin was first studied in five healthy volunteers ranging in age from eight to twelve years and weighing from twenty eight to forty kilograms. These volunteers were carefully questioned to ascertain that there was no history of recent ingestion of aspirin or other medications. Two timed urine collections were obtained daily and examined immediately for a differential count of

renal tubular cells, red cells, and white cells. Quantitative excretion of renal tubular epithelial cells and red cells was remarkably constant for each child, but there was marked variation in the rate of excretion of leukocytes. It was of interest that the base line excretion of renal epithelial cells was about one tenth the value reported by Prescott[2] and about one tenth the value found in our studies of adults. Red cell excretions were approximately one third of adult values. Our only patient with control renal excretion quantitatively equal to that of adults was a fourteen year old boy in well advanced adolescence. This may suggest some endocrine control of cellular desquamation worthy of future investigation.

Aspirin, 1.2 gm. per day in four divided doses was administered for a period of five consecutive days to these five volunteers. As illustrated in Figure 1, at 48 hours there was a threefold increasein the mean excretion of renal tubular cells, but by the fifth day of aspirin administration the renal epithelial cell excretion had fallen well below the control values . The red cell excretion also showed a significant increase to a peak at about 48 hours, but decreased below the control values by the fifth day. The white cell pattern did not change significantly during the testing period. This transient desquamation of renal tubular epithelial cells despite the continued administration of aspirin is very similar to that found in adults by Prescott.[2]

The transientness of the cellular response to aspirin was further studied as shown in figure 2. A nine year old boy weighing 28 kilograms was given 3.6 gms. per day, or 1 grain

per pound per day, in divided doses for an initial period of 36 hours. Within 24 hours, renal and red cell excretion rates increased, reaching a peak shortly after the aspirin was discontinued at 36 hours and then declining during the next 36 hours when no aspirin was administered. When the same dose was readministered at 36 hours the maximum renal cells per hour output was only 126,000 cells per hour as compared with the output during initial administration of 396,000 cells per hour. A similar response, though not of the same magnitude was observed with the red cells. One week later, 3.6 gms. per day were again administered for a period of three days. The maximal renal cell excretion peak occurred within the first 24 hours and reached a peak response of 112,000 renal cells per hour. The peak of the red cell response occurred nearly 24 hours later than the renal cell peak and was similar in magnitude to the red cell excretion rise seen during the secound period of administration.

The mechanism for this transient response has yet to be elucidated. Data on adults suggests that a response of primary magnitude recurs after one month without exposure.[2] Salicylates have been shown to cause desquamation of the gastric mucosa when the salicylates are in direct contact with the mucosa[4]; so a similar mechanism may be involved in the renal celluria that is observed. Enzymatic induction with subsequent alteration of the metabolism of the renal tubular cells may also be a factor that should be considered. Subtle changes in pH with alteration in the buffering of the filtrate presented to the renal tubular epithelial cells may also have a role to play.

The degree of celluria was also found to be related to the dose administered as illustrated in Figure 3. Two boys, each weighing 28 kilograms were utilized in this particular study. The lower pattern is the excretion of renal epithelial cells by an eight year old boy given 1.2 gms. of aspirin per day or about one-third of a grain per pound per day. The maximal renal cell response was 61,000 renal tubular cells per hour. The nine year old boy was given 3.6 gms. per day for 36 hours, the equivalent of 1 grain per pound per day. His control excretion of renal cells was four times that of the younger boy; but upon administration of the aspirin he had a ten-fold increase compared with the lesser response to the lower dose. A similar dose related response was elicited from three other hospitalized children.

Extension of this study to include salicylate compounds such as sodium salicylate and other analgesics has not yet been completed. However it has been reported by other investigators[2] that phenacetin, caffeine, and paracetamol do cause a marked increase in the excretion of renal tubular epithelial cells in adults.

The cellular response was then studied in relation to the renal clearance of salicylates for a given patient. The half life of salicylates in the human body is approximately six hours.[8] However this can be prolonged to as long as 19 hours when the daily dose of salicylates exceeds ten grams per day. It has also been found that alkaline urine causes rapid excretion of salicylate compounds.[6] Almost all of the salicylates ingested at a certain time are excreted within 48 hours.[8]

The rate of urinary excretion of free salicylates is influenced by glomerular filtration rate, the rate of proximal tubular secretion of the salicylate, the rate of urine flow, and the pH of the urine.[8] Salicylates appear in the urine in five different forms: Free Salicylate- comprising 61% of the total, Salicyluric Acid- the glycine conjugate of salicylate and comprising 8% of the total, Salicyl Phenolic Glucuronide - comprising 22%, Salicyl Acyl Glucuronide- comprising 5%, and Gentisic Acid- comprising about 1%.[I]

Utilizing these facts we attempted to discover if the renal tubular epithelial excretion had any relation to the renal clearance of salicylates. The percentage of urinary "free salicylate", meaning salicylate plus it's glycine conjugate, as compared with "total salicylate" values calculated after acid hydrolysis showed no significant variation over a five day period, as illustrated in figure 4. To obtain satisfactory results for "total salicylates" it was necessary to modify Natelson's method of urine salicylate determination by increasing the time of acid hydrolysis to 75 minutes and employing ethylacetate rather than ethylene dichloride for extraction of the salicylate compounds.[5] The pattern of excretion of renal cells was studied in relationship to the filtration, reabsorption, and excretion of salicylates also shown in figure 4. An estimation of "filtered salicylates" was made employing the 24 hour creatinine clearance and the standard assumption that 25% of the total serum salicylate was not protein bound and therefore filterable. Neither in the patient studied nor in another studied similarly did there appear to be any correlation between the

magnitude of the filtered load of salicylate being presented to the renal tubules and the peak of the cellular response.

The clinical significance of renal tubular exfoliation is not clear. The presence of a significant increase in the numbers of renal cells in the urine in response to the administration of aspirin probably indicates a toxic effect on the epithelium. Whether this effect is a causal one in the interstitial nephritis of analgesic abuse can not be determined without administering large doses of aspirin over a considerablle period of time.[6]

Two facts stimulated the research presented in this thesis. The first being there has been no published literature on the effects of aspirin on the renal tubular cell excretion in children, though it is well documented in adults; and secondly, that salicylate poisoning in children is one of the most common if not the commonest type of poisoning by ingestion.

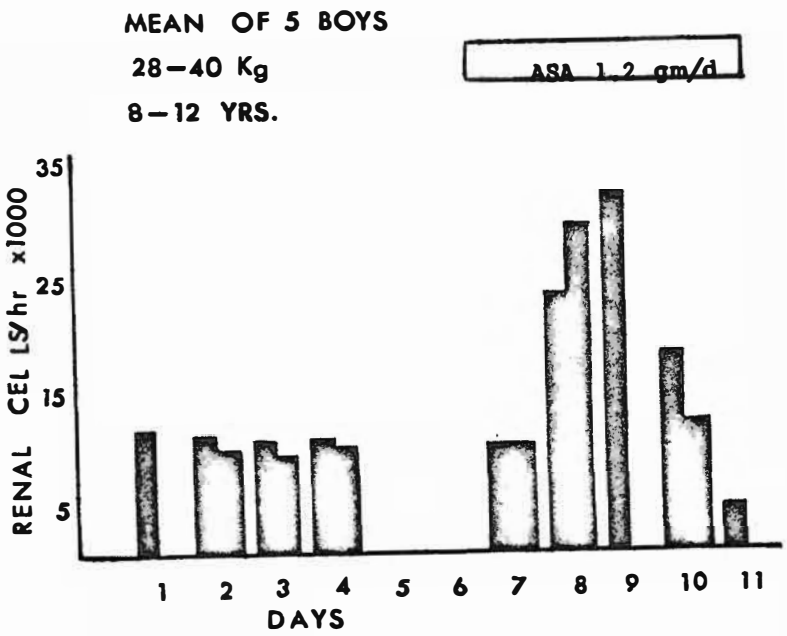

Figure 1

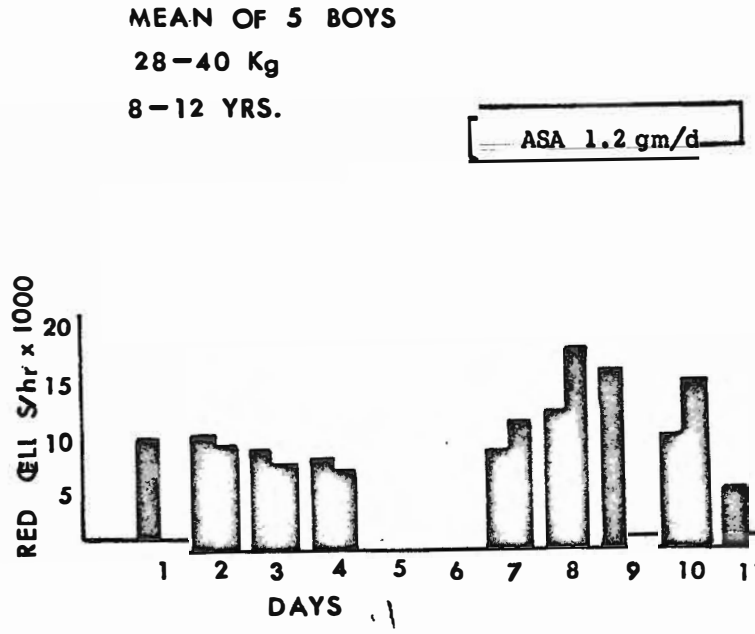

Figure 1a

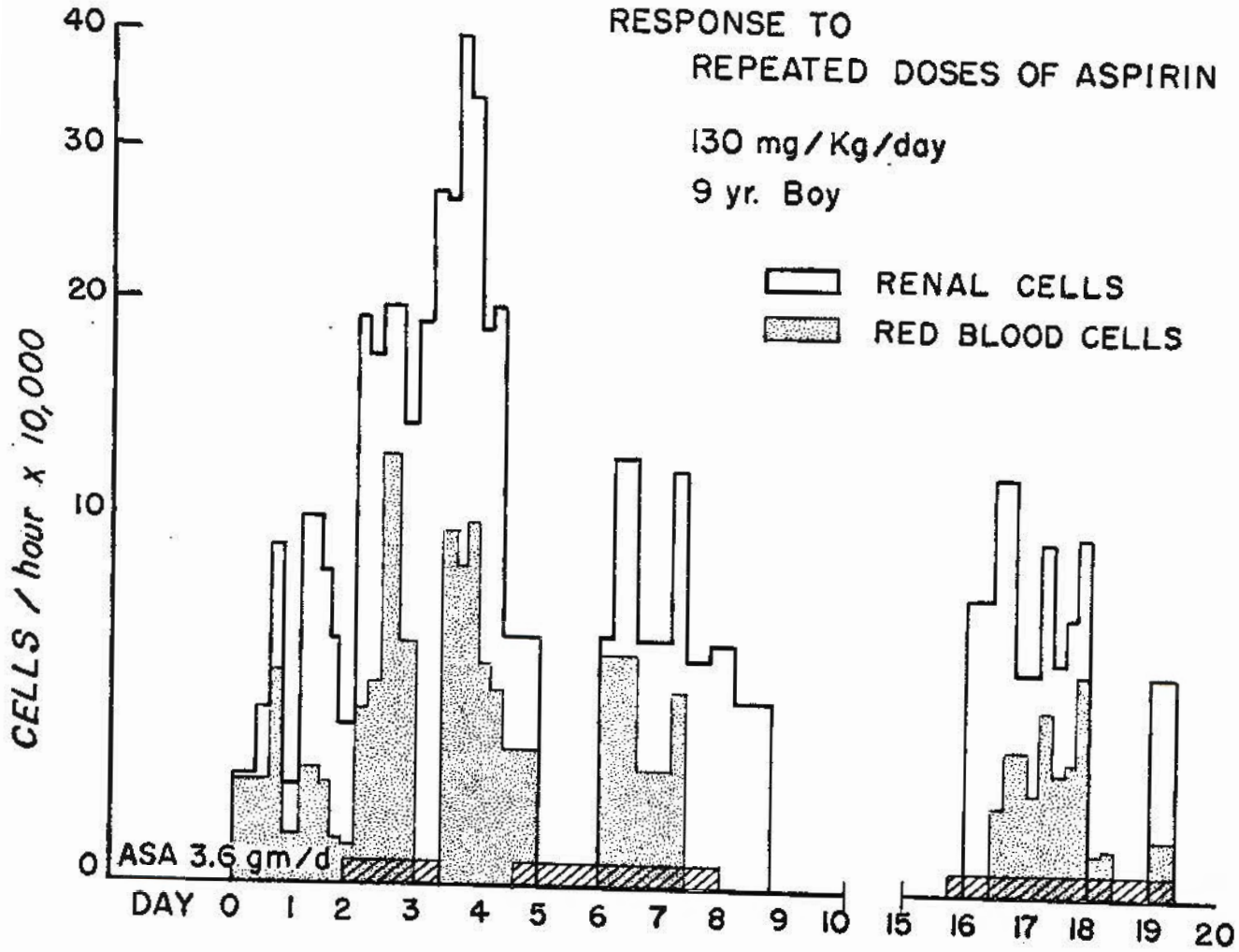
RESPONSE TO
REPEATED DOSES OF ASPIRIN
130 mg / Kg / day
9 yr. Boy
RENAL CELLS
RED BLOOD CELLS
CELLS / hour x 10,000
40
30
20
10
0
ASA 3.6 gm/d
DAY 0 1 2 3 4 5 6 7 8 9 10 15 16 17 18 19 20

Figure 2

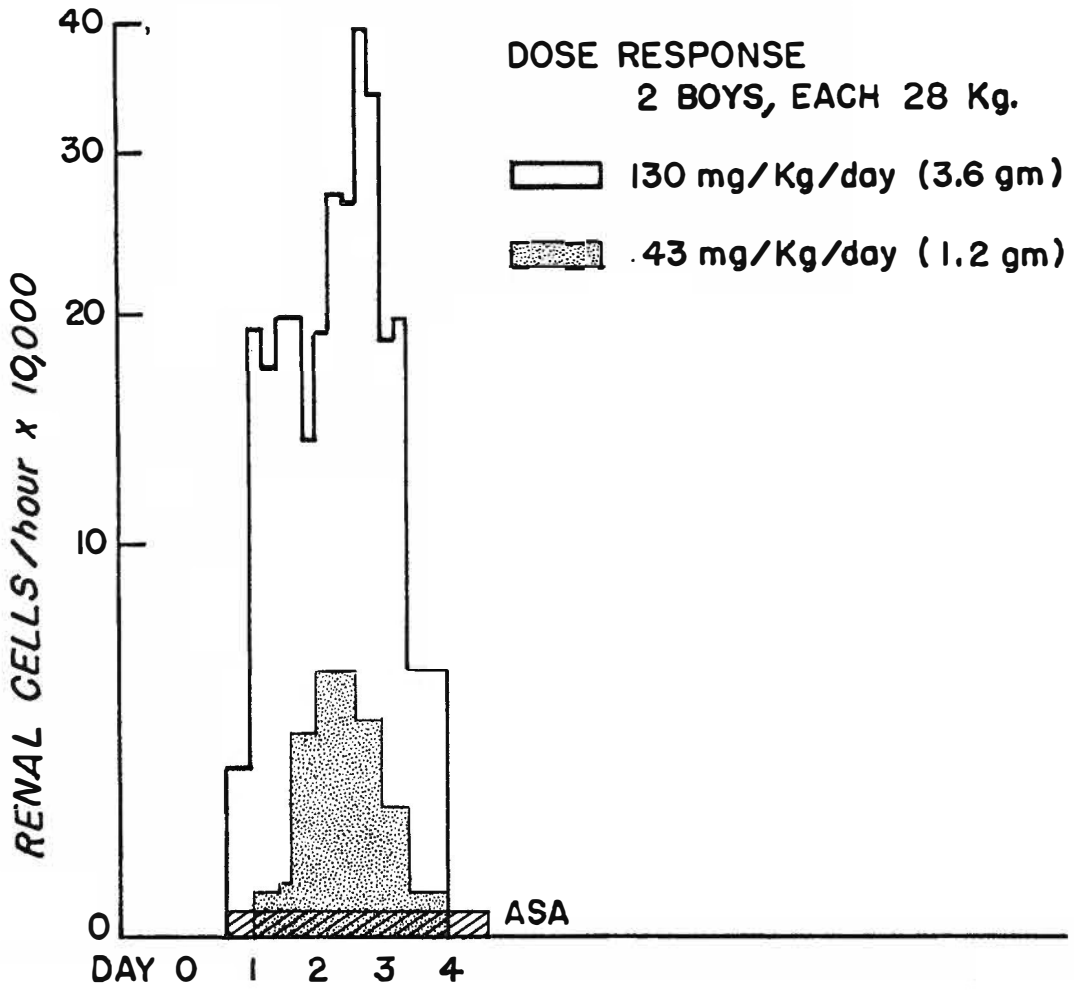
DOSE RESPONSE
2 BOYS, EACH 28 Kg.
130 mg/Kg/day (3.6 gm)
43 mg/Kg/day (1.2 gm)
RENAL CELLS/hour x 10,000
40
30
20
10
0
ASA
DAY 0 1 2 3 4

Figure 3

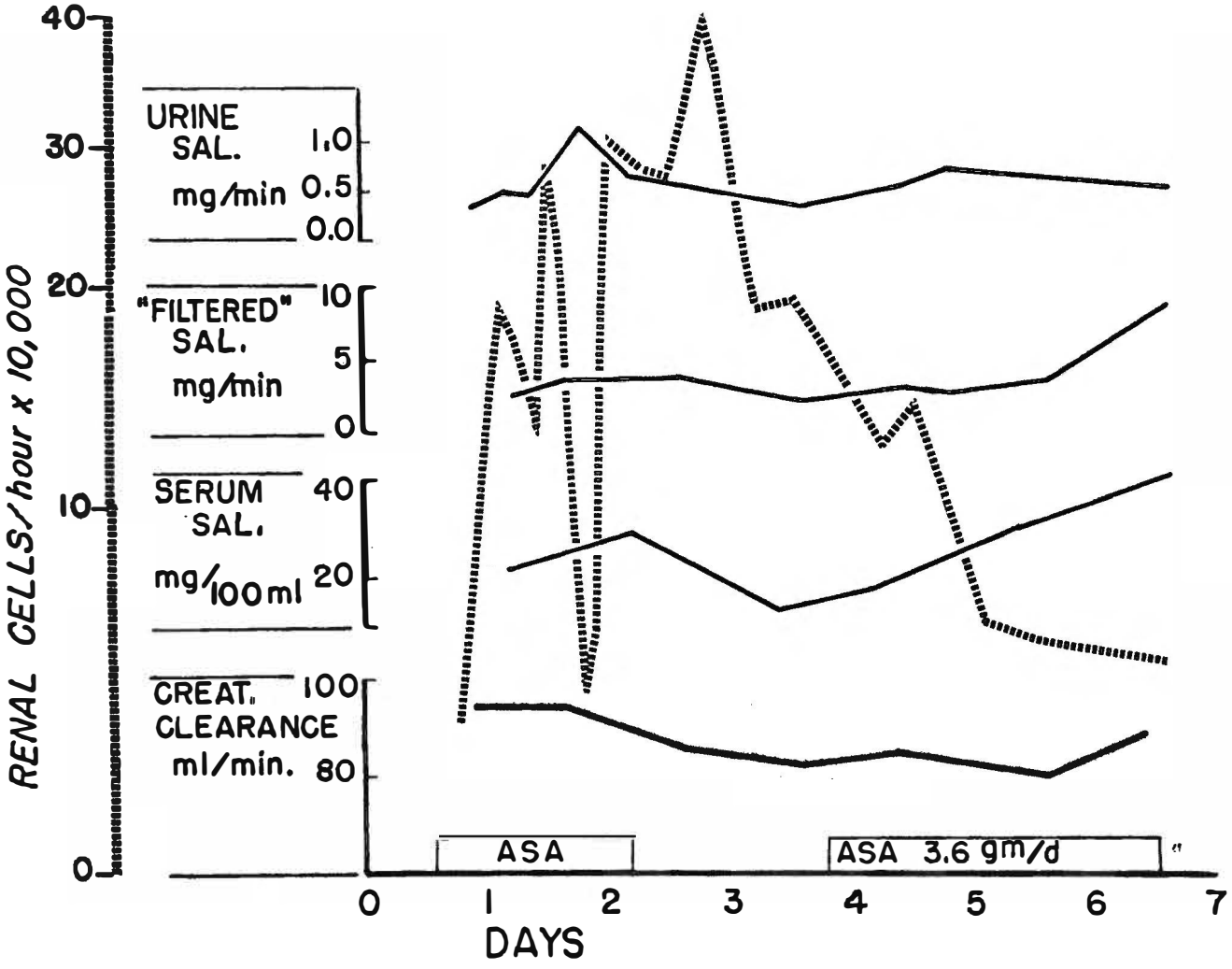
RENAL CELLS/hour x 10,000
40
30
20
10
0
URINE SAL. mg/min
1.0
0.5
0.0
"FILTERED" SAL. mg/min
10
5
0
SERUM SAL. mg/100ml
40
20
CREAT. CLEARANCE ml/min.
100
80
ASA
ASA 3.6 gm/d
0
1
2
3
4
5
6
7
DAYS

Figure 4

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