Endogenous Creatinine in Serum and Urine.*† (17837)

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If the renal clearance of endogenous creatinine measured the rate of glomerular filtration, the infusion, injection or ingestion of a large quantity of material would be unnecessary in studies requiring an accurate estimation of glomerular filtration rate. The measurement of glomerular filtration rate with an endogenous substance would be of especial value in long term observations or in studies of fluid and electrolyte distribution and excretion. For this reason, we undertook to devise a method for measuring creatinine in plasma and urine.

Method. The method is essentially a modification of Borsook's(1), adapted for the use of small quantities of material available in studies on infants or small animals. Protein-free serum filtrate is prepared by pipetting 0.5 ml of serum and 1 ml of distilled water into a 13 x 100 mm tube, mixing with a glass rod, then adding 1 ml of 20% trichloroacetic acid with continuous stirring. The tube is stoppered and centrifuged in an angle centrifuge for 5 to 10 minutes. If the urine is protein-free, it is diluted with distilled water to a creatinine concentration between 0.1 and 0.5 mg%. When present, protein is precipitated with trichloroacetic acid.

Two ml of protein-free filtrate or diluted urine are pipetted into 13 x 100 mm tubes and 4 or 5 different aliquots of working standard, containing 0.25 or 0.50 mg% of creatinine in aqu. sol., are pipetted into similar tubes (usually 0.5, 1.0, 1.5 and 2.0 ml) and made up to approximately 2 ml with distilled water. Water (2 ml) serves as the blank. To each tube are added 0.2 ml of saturated oxalic acid and 15-20 mg of Lloyd's reagent. The tubes are stoppered and inverted or shaken occasionally for about 10 minutes. They are then centrifuged for about 5 min. in an angle centrifuge, and the clear supernatant aspirated off through a fine-tipped glass tube and discarded. Five parts of approximately 0.04 M picric acid solution are mixed with one part of 10% sodium hydroxide solution and diluted with 12 parts distilled water. This solution is made the day it is used. Into each tube are pipetted 2 ml of alkaline picrate solution, the original stopper is replaced, and the tube is well shaken to suspend the Lloyd's reagent. The tubes are allowed to stand for at least 10 minutes with occasional shaking and are then centrifuged in an angle centrifuge for about 5 minutes. Part of the supernatant from each tube is transferred with a Wintrobe pipette to a 10 mm cuvette and transmission is measured in a Beckman spectrophotometer (model DU) at 500 mμ against the blank set at 100% transmission. The colors are stable for at least 2 hours at room temperature.

Since blanks or standards diluted with trichloroacetic acid did not differ from the aqueous dilutions, no reagent blank for the serum filtrates was necessary. Plasma, either oxalated or heparinized, and serum showed no difference in creatinine concentration, and hemolysis does not seem to introduce an error. The only important source of error found was protein, which is adsorbed, eluted, and is a chromogen. Care must be taken, therefore, that the serum filtrate and the urine samples are completely protein-free. No chemical interference was found from high concentrations of urea, or from the presence of inulin, thiosulfate, ferrocyanide, or para-aminohippurate in the concentrations obtaining during renal function studies, although para-aminohippurate was found to give a direct Jaffé reaction.

Results and discussion. Recoveries of 0.5

* This investigation was supported by a research grant from the National Heart Institute, United States Public Health Service.

† Presented before the American Physiological Society, *Fed. Proc.*, 1949, v8, 68.

1. Borsook, H., *J. Biol. Chem.*, 1935, v110, 481.

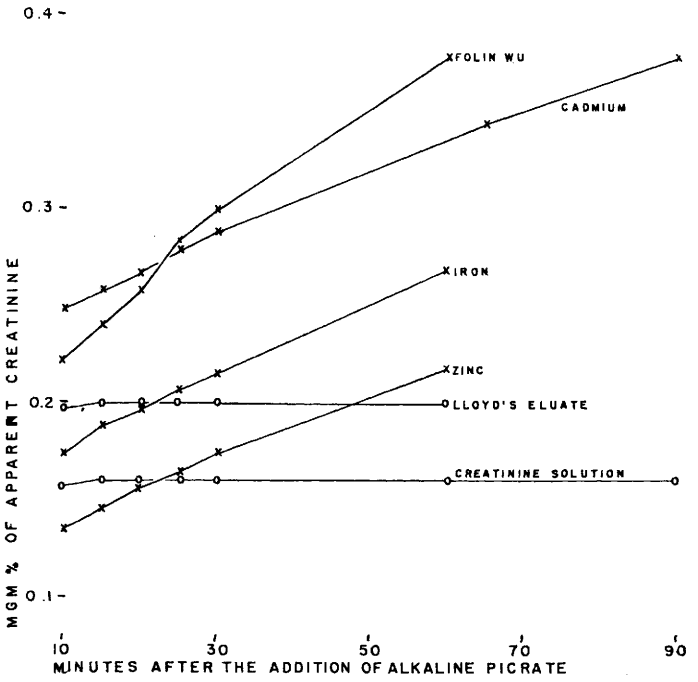

FIG. 1.

The increase in apparent creatinine concentration in Folin Wu, $CdSO_4$, iron, and Somogyi zinc filtrates of a single serum sample compared with the constant value of a Lloyd's eluate of the Folin Wu filtrate. All dilutions are 1:5. A creatinine solution is shown for comparison.

mg or more creatinine from pure solution or added to serum or urine are 98 to 102%. When 2 or more analyses of the same serum or urine are done, the maximum deviation from the average is $\pm 1.5\%$. When serum and urine concentrations are increased by the administration of creatinine by mouth or vein to a level at which the error from non-creatinine chromogens is insignificant, creatinine determinations by this method agree with the direct Jaffé determination. And finally, when creatinine containing C^{14} was adsorbed from solution by this method, no C^{14} could be detected in the supernatant Lloyd's filtrate. The counts were made on dried preparations using a thin window Geiger-Muller tube(2). While the foregoing evidence shows that creatinine is quantitatively recovered by this method, it does not follow that other chromogens are not adsorbed from the plasma filtrate. The evidence on this point depends largely on observations first made by Hayman, *et al.*(3). They noticed that while creatinine solutions treated with alkaline picrate rapidly reached a constant color, plasma filtrates under the same circumstances continued to darken indefinitely, as would a mixed standard of creatinine and glucose. However, if the plasma filtrate or mixed standard were adsorbed on Lloyd's reagent and eluted, the chromogen in the eluate reached a constant color in a short time, as did a creatinine solution. We have repeated and confirmed this observation. Fig. 1 shows the increase in apparent creatinine concentration with time of 4 different protein-free filtrates of the same serum sample; the constant value of the Lloyd's eluate of the Folin Wu filtrate and of a creatinine solution are plotted for comparison.

When recovery of creatinine from the var-

2. *Preparation and Measurement of Isotopic Tracers*, Ann Arbor, Mich., J. W. Edwards, 1946.

3. Hayman, J. M., Jr., Johnston, J. M., and Bender, J. A., *J. Biol. Chem.*, 1935, v108, 675.

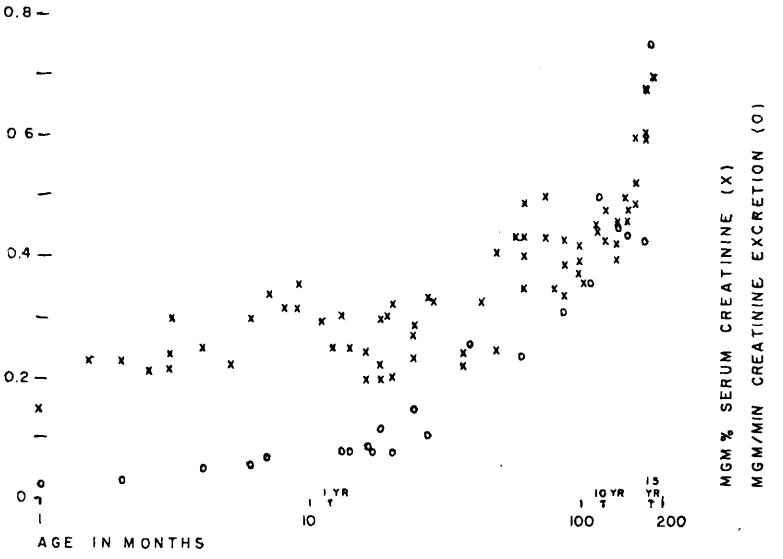

FIG. 2.
The relationship of age to serum creatinine concentration and to the urinary excretion of creatinine.

ious filtrates was compared, trichloroacetic acid, $CdSO_4$(4), and Folin Wu filtrates of the same sample showed the same creatinine concentration and gave 100% recovery of added creatinine; iron filtrates(5) gave concentrations and recoveries 10 to 18% lower. When the total chromogen concentration was considered to be the apparent creatinine concentration of a $CdSO_4$ or Folin Wu filtrate exactly twenty minutes after the addition of alkaline picrate, the normal sera studied showed that about 80% of this was creatinine. The quantity of chromogen recovered as creatinine from urine samples was 90 to 100%, and varied from one sample to another even in a single subject, although none of these urines contained measurable reducing material.

When the clearance of creatinine, as determined by this method, was compared with the inulin clearance in a series of 22 normal infants, children and adults(6), the average ratio of endogenous creatinine to inulin clearance was 1.03 and of exogenous creatinine to inulin clearance was 1.08.

The increase in serum creatinine concentration and in the rate of urinary excretion of creatinine with increasing age is shown in Fig. 2. These data were collected in order to establish the expected values for various ages. Age, rather than weight was selected as the criterion since children with severe malnutrition or obese subjects of similar age and development had closely comparable serum concentrations. However, in children whose growth was severely retarded or precocious, serum levels agreed more closely with apparent than with chronological age. Probably surface area would be the best criterion, if more reliable estimates were available for infants and children. In our experience only subjects with impaired renal function have serum concentrations significantly higher than normal, and we consider an elevated serum creatinine as evidence of such impairment. Three cases of amyotonia congenita are the only subjects in our experience whose serum levels were significantly low, on the basis of either apparent or chronological age. Only a few observations have been made on full-term infants below the age of

4. Fujita, A., and Iwatake, D. *Biochem.*, 1931, v242, 43.

5. Smith, W. W., Finkelstein, N., and Smith, H. W., *J. Biol. Chem.*, 1940, v135, 231.

6. Hare, K., Goldstein, H., Barnett, H. L., McNamara, H., and Hare, R. S., *Fed. Proc.*, 1939, v8, 67.

one month; they indicate that creatinine at birth is at or near the adult level and falls within about two weeks to the level of the month old infant. Premature infants show serum levels which are higher and rates of excretion which are lower than full term infants of the came postnatal age; this is undoubtedly related to the degree of immaturity of the kidney in these infants.

Conclusions. (1) The technic described measures all of the creatinine and only creatinine in blood and urine. (2) There is a gradual rise in the serum concentration and in the rate of urinary excretion of creatinine from one month of age to puberty, when adult levels are reached.

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Dicumarol Labelled with C^{14}.* (17838)

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As a result of the classical work of Link(1), dicumarol has come into widespread clinical use as a therapeutic agent against thrombosis. However, there has been little information available regarding the mechanism whereby dicumarol lowers the prothrombin level in the blood until the recent demonstration by Lupton(2) that after treatment of rats with dicumarol, the livers fail to add prothrombin to blood perfused through them. In an attempt to obtain further information regarding its action, dicumarol labelled with radioactive C^{14} was used in animal experiments.

Methods. Dicumarol, labelled with C^{14} in the methylene bridge, was synthesized using formaldehyde-C^{14}. The labelled dicumarol was dissolved in 0.1 N sodium hydroxide (5 mg per cc) and injected intravenously in mice and rabbits. 0.05 cc of solution (0.25 mg) was given to each mouse, while each rabbit (weight 2 kg) received 2.0 cc. To determine radioactivity in the blood, samples were taken, weighed, dried and the material spread uniformly in a platinum dish. Other tissues were treated similarly. Radioactivity measurements were then made with the end-window type of Geiger-Muller chamber with a scale of 128 counting unit. Correction for self-absorption of C^{14} activity was made. Prothrombin times were determined by the usual methods using 0.02 M calcium chloride solution.

Results. Fourteen mice were each given 0.25 mg of labelled dicumarol of specific activity 120.4 registers/min./mg intravenously (1 register = 128 counts). The first 4 mice were placed in a metabolism apparatus and the expired CO_2 collected and tested for radioactivity. No radioactivity whatsoever could be determined in the expired air and as a consequence, it was not necessary to collect expired air in further experiments. At various times after the injection, the animals were sacrificed and after weighing, the radioactivity determined for lungs, blood, liver, gall-bladder, kidneys, stomach, intestines, intestinal contents, feces and urine. No significant amount of activity could be detected in the lungs. Results for other tissues are shown in Fig. 1. Large amounts of radioactivity were detectable in the liver, intestines, gall-bladder, feces and urine. A trace was found in the kidney and stomach. Activity was determined separately on the intestine and in-

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1. Link, K. P., *Harvey Lectures,* 1943, v39, 162.
2. Lupton, A. M., *J. Pharmacol.,* 1947, v89, 306.