nificantly changed from the normal by adrenalectomy. The absorption of hydrogenated vegetable oil fed as an emulsion with skim milk is significantly decreased following this operative procedure. Evidence for certain changes in fat absorbing mechanisms that are brought about by removal of the adrenals is presented, and it is pointed out that these changes need not result in an altered rate of absorption.

13474

Effect of Some Sulfonamides on Renal Secretion.*

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In studies concerning the correlation between the molecular configuration of organic compounds and the secretory power of the kidney it has been found that the functional behavior of the isolated frog kidney can be influenced in various ways by sulfanilamide and its derivatives. Among these influences is one, which recently came to our attention and which regards a striking and easily observable pH shift in the secretion from an acid towards an alkaline reaction.

Method. The kidney of frogs *(R. pipiens)* was perfused from the aorta or from both the aorta and the abdominal vein with bicarbonate-Ringer aerated by O_2-CO_2 in a mixture providing a pH of about 7.5. The perfusion pressures were 20 to 24 cm when the aorta alone was perfused and 24 and 12 cm respectively, when both vessels were perfused. 0.5 to 0.25 mg % phenol red was added. At this time of the year (October, November) the secretion appearing in the ureter cannulas under these conditions is ordinarily canary-yellow or dark orange-yellow. This indicates an acid reaction of the secretion and a marked concentration of the dye, both being due to the activity of the convoluted tubules. The secretion was taken up drop by drop on white paper and the color shade of each drop noted. This

* This work has been supported by grants from the American Philosophical Society and from the Ella Sachs Plotz Foundation.

The sulfanilamide derivatives were generously furnished by the Calco Chemical Division of the American Cyanamid Company, the Winthrop Chemical Company, Merck & Company, Sharp & Dohme, and Abbott's Dermatological Research Laboratories (through the courtesy of Dr. G. W. Raiziss).

was necessary since the color changes in contact with air by loss of CO_2.

Results. After adding sulfanilamide to the perfusion fluid, the color of the successive drops changes from yellow over a series of orange and red shades to pink, indicating a continuous shift from an acid to an alkaline reaction. This occurs in a few minutes. The process is reversible. Omission of the drug from the perfusion fluid allows the color to change back stepwise to yellow. The entire sequence of changes can be reproduced several times with the same frog preparation. The rate and the intensity of the color changes depend upon the drug concentration. The threshold concentration was found between 5 and 10 mg %.

Several sulfanilamide derivaties have been studied. An effect resembling that of sulfanilamide is brought about by *N^4-acetylsulfanilamide* (Calco), 0.05 and 0.01%, *N^4-sulfanilylsulfanilamide* (Calco), satur. = <0.03%, *N^4-butyrylaminobenzenesulfonamide* (Sharp & Dohme), satur., *N^4-sodium p-sulfamidophenylglycine* (Abbott) = $NaOOC\text{-}CH_2 . NH . C_6H_4 . SO_2NH_2$, 0.025 to 0.1% and *$N^4$-sodium methylenesulfonate of sulfanilamide* (Abbott) = $NaSO_3 . CH_2 . NH . C_6H_4 . SO_2NH_2$, 0.05%. In contrast to the N^4-compounds, the color change fails to appear after perfusing the kidney with the N^1-compounds—*sulfapyridine* (Merck), satur. = <0.05%, *Sulfaguanidine* (Calco), 0.1%, and *Sulfadiazine* (Calco), satur. = <0.01%.† The effect of sulfanilylsulfanilamide is irreversible.

Discussion. It is evident that the capacity of the drugs to turn the reaction of the renal secretion from acid to alkali appears so far to be confined to compounds with an unsubstituted sulfonamide group. The failure of sulfadiazine to act could be due to its low solubility, but sulfapyridine, which is also badly soluble, was perfused without effect at a molarity 5 to 10 times as great as that of sulfanilamide at its threshold concentration.

This behavior of the drugs in relation to the kidney conforms with their behavior in inhibiting carbonic anhydrase as observed by Mann and Keilin.[1] According to these authors, carbonic anhydrase is inhibited by sulfonamides having an unsubstituted N^1 group and not sulfonamides having a substituted N^1 group, irrespective of substitution on the N^4 group. The activity of many other enzymes is

† The substitutes of the amido group in sulfanilamide are called N^1-compounds, the substitutes of the amino group N^4-compounds.

1 Mann, T., and Keilin, D., *Nature*, 1940, **146**, 164.

not affected by sulfanilamide concentrations far exceeding the concentration which abolishes carbonic anhydrase activity.

It is suggestive, therefore, to interpret the pH shift in the renal secretion observed in our experiments as due to the inactivation of carbonic anhydrase. This interpretation would seem consistent with the following facts: First, Davenport[2, 3] has provided evidence that in the parietal cells of the stomach glands, which generally are looked upon as being the site of production of the gastric acid, carbonic anhydrase is present in a concentration even higher than in red cells. Accordingly, the acid secretion of the gastric mucosa is diminished by sulfanilamide. Second, Davenport and Wilhelmi[4] have shown carbonic anhydrase to be present in a significant concentration in the cortex of cat, dog and rat kidneys and Davenport[5] also has shown that carbonic anhydrase is present in the frog kidney.

It seems promising to consider the mechanism of this and other glandular pH shifts on the basis of our experiments. It has been discussed previously, whether the acid reaction of the frog's renal secretion rests upon the reabsorption of HCO_3 or upon the excretion of H. In this regard it is an important fact that, according to Montgomery and Pierce,[6] the acidification of the secretion takes place in a short segment of the distal tubules nearer to their distal than to their proximal end, whereas, according to Walker, Hudson, Findley and Richards,[7] Cl is reabsorbed along the total length of the distal tubules. This is indicative of two independent mechanisms being involved in the normal decrease of OH and of Cl inside the tubules.

This conclusion is corroborated by our further observation that sulfanilamide fails to influence the Cl shift simultaneously with the pH shift. It often happens that the concentration of Cl in the secretion rises during the course of perfusion with Ringer. However, in 15 experiments no correlation whatsoever appeared between the concentration of Cl and the reaction. If the experiment is started with perfusion with Ringer plus sulfanilamide, the Cl concentration is not found to be lower during a subsequent period of perfusion with Ringer alone, although the reaction has become acid. Likewise, if sulfanilamide is added in any one of a series of periods

2 Davenport, H. W., *J. Physiol.*, 1940, **97**, 32.

3 Davenport, H. W., *Am. J. Physiol.*, 1941, **133**, 257.

4 Davenport, H. W., and Wilhelmi, A. E., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 53.

5 Unpublished observations.

6 Montgomery, H., and Pierce, J. A., *Am. J. Physiol.*, 1937, **118**, 144.

7 Walker, A. M., Hudson, C. L., Findley, T., and Richards, A. N., *Am. J. Physiol.*, 1937, **118**, 121.

of perfusion, the sample of secretion corresponding to this period does not show an especially marked increase of Cl concentration.

For all these reasons it seems adequate to assume that the normal pH shift from an alkaline to an acid reaction is due to the localized reabsorption of HCO_3. This will bring about a pH shift from 7.4, corresponding to the normal pH of the blood and of our perfusion fluid, to pH 4.8, equal to our perfusion fluid without HCO_3 and equal to the lowest pH value of frog's urine.[6] This reabsorption can be looked upon as being causally related to the catalyzing effect of carbonic anhydrase.

Summary. Following the addition of sulfanilamide and of sulfonamides having an unsubstituted SO_2NH_2 group to the Ringer-phenol red perfusion fluid of an isolated frog kidney, the reaction of the secretion turns from acid to alkali, as indicated by the color of the indicator. This effect is reversible. Reasons are proposed for the assumption that the change of reaction is due to the inhibitory action of the sulfonamides upon carbonic anhydrase and that this enzyme is involved as a catalyzer in the reabsorption of bicarbonate by the kidney.

13475

Renal Physiology in Infants and Children. II. Inulin Clearances in Newborn Infant with Extrophy of Bladder.

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(Introduced by Jean V. Cooke.)

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Interest in the renal function of newborn infants has been aroused by recent studies which indicate that the glomerular filtration rate is relatively low during the newborn period. The application of a method devised by one of us[1] to 7 apparently normal full-term infants ranging in age from 4 to 9 days suggested glomerular filtration rates between 20 and 40% of the average normal adult values. McCance and Young[2] have since published an extensive study of renal function of newborn infants, in which are included observations on inulin clearances in 3 infants, aged 6 to 13 days, with meningoceles. Urine collections were made by means of catheterization, and the determined inulin clearances were of the order of 43% of the average