tion period. After the second day of treatment, the young whose mothers were left intact gained weight less rapidly than the sucklings of non-castrate controls. On the contrary, the rapid growth rate was continued for 6 days in the young of the castrate mothers which were given 1.0 mg, and for 12 days in the litters of castrate mothers which were given 0.2 mg doses. The suppression of lactation was much more pronounced in intact than in castrate animals. The ovaries, adrenals, and pituitary of those animals that were injected showed a pronounced increase in weight over those of the control mothers.

The premature opening of the vagina which occurred at 12 days after birth in most of the treated litters indicates that large amounts of the estrogen were secreted with the milk and also that this hormone is not readily attacked by any of the enzymes of the rat.

Conclusions. 1. Progestin is not necessary for complete mammary development in cattle. 2. Estrogens will induce mammary development and copious milk secretion in cattle without injection of prolactin. 3. The turgidity of the udder may be used as an indicator of the secretory activity of the non-lactating gland. 4. The titer of estrogen determines its effect on the mammary gland. A low titer induces proliferation of the parenchyma and secretion of milk; a high titer suppresses lactation and brings on involution of the mammary gland.

13218

Renal Carbonic Anhydrase.

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The presence of carbonic anhydrase in the mammalian gastric mucosa has been reported and discussed by Davenport and Fisher[1] and by Davenport.[2, 3] Although the rôle of carbonic anhydrase in the gastric secretion of hydrochloric acid is not yet

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1 Davenport, H. W., and Fisher, R. B., *J. Physiol.*, 1939, **94**, 16P.

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

3 Davenport, H. W., *Am. J. Physiol.*, 1940, **128**, 725.

directly established the presence of this enzyme in high concentration in the cells of a tissue primarily concerned with the secretion of ions is of particular interest. One of the chief functions of the kidney is the tubular transfer of ions, and if carbonic anhydrase in tissues is generally part of the chemical mechanism performing such transfer it might be expected that carbonic anhydrase is present in kidney tissue. This expectation is realized in the experiments described in this paper. The kidneys of cats, dogs and rats have been found to contain concentrations of carbonic anhydrase of the same order of magnitude as have been found in blood and gastric mucosa.

Methods. The animals were anesthetized with ether, and the abdominal aorta was exposed. A cannula was inserted into the aorta distal to the kidneys, and after the aorta proximal to the kidneys had been clamped off 0.9% sodium chloride solution was forced through the kidney blood vessels by gravity. Perfusion was continued until the fluid flowing from the cut renal veins was free of blood. The kidneys were then excised, and slices of them were weighed on a torsion balance, ground in a glass mortar and extracted with water.

Carbonic anhydrase was determined by the method of Meldrum and Roughton.[4] The apparatus was used at 0°C and at atmospheric pressure. The activity was calculated and expressed as enzyme units (E) according to the method of Meldrum and Roughton, but no correction was applied to bring the calculated activity to 15°C. The enzyme unit used in this work is therefore 2 to 3 times smaller than that of Meldrum and Roughton.

Results. Carbonic anhydrase was found in the kidney extracts. The extracts catalyzed the hydration and dehydration of carbon dioxide. The addition of the extracts to the buffer used in the enzyme determination did not change the pH of the buffer as measured by a glass electrode. The end points of the catalyzed and uncatalysed reactions were the same. The activity of the extracts was destroyed by 30 sec boiling, by heating to 65°C for 30 min and by acid and alkali. The activity was inhibited by 0.001 M HCN, by 1 mg % sulfanilamide and by 0.003 M NaSCN.

The activity of the extracts was not caused by the presence of carbonic anhydrase derived from red blood cells remaining in the kidney blood vessels. The perfusion of the kidneys was complete, and no areas of infarction were found. Histological sections of the perfused cat and dog kidneys were made, and a careful micro-

[4] Meldrum, N. U., and Roughton, F. J. W., *J. Physiol.*, 1933, **80**, 113.

scopic search of their blood vessels revealed no red cells. Cat blood contains about 3.7 E per cmm,[2] and dog blood contains from 3.1 to 7.4 E per cmm.[3] Slices of cat kidney contained up to 3.8 E per mg and of dog kidney up to 2.2 E per mg. Had the presence of blood carbonic anhydrase been responsible for the observed activity a large fraction of the slices would have been made up of red cells, and the hemoglobin associated with carbonic anhydrase in the red cells would have been easily detectable. The perfused kidneys were yellow-brown in color and showed no signs of containing any hemoglobin. The extracts ranged from opalescent white to yellow in color while samples of blood from the same animals diluted to the same carbonic anhydrase activity were bright red and obviously contained at least 50 times as much hemoglobin. When tested with the benzidine reaction the kidney extracts were either negative or very faintly positive while the blood samples diluted to the same enzyme concentration were strongly positive. Consequently it can be concluded that carbonic anhydrase is present in the kidney tissue itself.

Fourteen samples of cat kidney cortex obtained from 4 kidneys contained 2.6 ± 0.6 E per mg wet weight, and 13 samples of dog kidney cortex contained 1.8 ± 0.3 E per mg wet weight. There is no carbonic anhydrase in the medulla of either the cat or dog kidney. When rat kidneys were sliced no distinction could be made between medulla and cortex. Ten samples of rat kidney obtained from 6 kidneys contained 1.1 ± 0.4 E per mg wet weight. If there is no carbonic anhydrase in the rat kidney medulla the concentration in the cortex is probably still higher.

Discussion. Davenport[2] showed that the concentration of carbonic anhydrase in aqueous extracts of the gastric mucosa is of the same order of magnitude as that found in the kidney cortex. The concentration is lower than that in red cells, but when it had been shown that the enzyme is confined to the cells of the surface epithelium and to the parietal cells it could be calculated that the concentration of the enzyme in the cells is several times higher than in red blood cells. In this study it has not been demonstrated that carbonic anhydrase is localized in any particular type of cell in the kidney cortex. If it can be shown that the enzyme is in one type of cell and not in another it may be found that the enzyme is actually present in the cells in very high concentration.

The presence of carbonic anhydrase in the kidney makes possible the study of the functions of this enzyme in an organ more accessible and more readily controlled than is the gastric mucosa. At

present the rôle of carbonic anhydrase in the kidney is only the subject of speculation, but the effects on the kidney of sulfanilamide, a powerful inhibitor of carbonic anhydrase, offer a promising line of attack on the problem.

Summary. Carbonic anhydrase is present in significant concentrations in the cortex of cat, dog and rat kidneys.

13219

A Method for Detecting in Human Serum Protective Bodies Against Hemolytic Streptococci.*

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The antigenic structure of the Group A hemolytic streptococci has been greatly clarified by the important work of Lancefield. Antistreptococcal rabbit serum has been used extensively in these studies for determining antigen-antibody relationships. There has been as yet no similar analysis of the antibodies in human serum from streptococcal disease. The passive protection of mice would be a useful approach to such a study. Neufeld[1] used this technic in human streptococcal disease but failed to obtain protection with the one scarlatinal serum tested. The work of Dochez, Avery, and Lancefield,[2] and of Hirst and Lancefield[3] indicates that in using mouse-protection it may be necessary to employ strains of streptococci which kill with 10^{-8} cc of culture. Unfortunately, freshly isolated strains of streptococci do not often kill in dilutions greater than 10^{-4} cc.[4, 5] Recently, however, the use of 50% endpoints in biological titrations has become more and more popular. This method of analysis has been made simple and practicable by Reed and Muench.[6] Accordingly, it appeared worth while to attempt to

* Aided by a grant from the Fluid Research Fund of the Yale University School of Medicine.

1 Neufeld, F., *Z. f. Hyg.*, 1903, **44**, 161.

2 Dochez, A. R., Avery, O. T., and Lancefield, R. C., *J. Exp. Med.*, 1919, **30**, 179.

3 Hirst, G. K., and Lancefield, R. C., *J. Exp. Med.*, 1939, **69**, 425.

4 Wadsworth, A., and Coffey, J. M., *J. Immunol.*, 1935, **29**, 505.

5 Wu, J. P., *J. Immunol.*, 1941, **40**, 179.

6 Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.