ment with improper differentiation of the urinary sphincter[2, 3] and regarded it as "hypospadias" resulting from a hypotrophic effect of the hormone.[3] We believe that these abnormal external genitalia may be interpreted best on the basis of an andromimetic effect of the estrogen. The extensive fissure in the clitorine prominence apparently results from more extensive cleavage of the preputial fold than occurs normally. The estrogen also produces a slight enlargement of the clitoris and a feeble differentiation of erectile tissue. In affected animals, the urethral orifice is more posterior to the glans clitoridis than normally. This probably results from the separation of the preputial epithelium from the wall of the distal urethra and from the hypertrophy of the erectile tissue between the glans and the urethral meatus. If this is the correct explanation of the wide separation of the glans clitoridis and the urethral orifice in the treated animals, we question the justification of terming this defect a "hypospadias."

Summary. The administration of estrogen to female rats during the first week of postnatal life produces a defect in the external genital organs characterized by cleavage of the preputial fold, hypertrophy of the glans clitoridis, and a feeble differentiation of erectile tissues. Since these changes resemble, except for extent, those produced by postnatal androgen, it is concluded that estrogen exerts a mild andromimetic effect upon the clitoris of the rat.

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Distribution and Excretion of Sulfapyridine in the Guinea Pig.

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Since the guinea pig is almost exclusively used for experimental tuberculosis therapeutic studies on sulfonamide compounds, it is important to secure information on the distribution and excretion of the most potent anti-mycobacterial sulfapyridine compound in this animal[1]. Our early *in vitro* and *in vivo* studies of the bacteriostatic action of sulfanilamide and "prontosil soluble" on mammalian, avian, and paratubercular acid-fast bacteria[2], confirmed the findings of

1 Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).

2 Birkhaug, K. E., *Beretninger, Chr. Michelsen Institute*, 1939, **9**, 3.

Marshall, Emerson, and Cutting,[3] Proom and Buttle,[4] Whitby,[5] Fuller,[6] and Stewart, Rourke, and Allen,[7] and others, that sulfanilamide is distributed in all the liquids and tissues in the animal organism, more or less proportionally with the water content of the liquids and tissues.

The present study deals with the rate of absorption, distribution and excretion of free and conjugated sulfapyridine in the guinea pig after the oral administration of 0.2 g per kg of the drug suspended in 0.5 cc of a 5% acacia solution. The animal was placed in a small wire basket within a porcelain basin in order to collect urine. A group of 40 normal guinea pigs, having an average weight of 436 g, were employed. At the end of 30 minutes, and 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 48 and 72 hours, an animal was sacrificed with ether. Thus at each stated time interval 3 animals were killed and the average results recorded. Determinations of free and conjugated sulfapyridine were done of the urine voided and removed from the bladder, contents of the stomach, duodenum, jejunum, ileum, colon and rectum, bile, blood, liver, lungs, spleen, pancreas, kidneys, adrenals, testes, muscles of the heart, legs, abdomen and chest, bone-marrow, eyes, spinal cord, brain, skin, and fat, as well as of milk, and of fetal blood.

The methods for estimation of free and total sulfapyridine consist of modifications of the methods suggested by Proom,[8] and Marshall and Litchfield.[9] One millilitre of liquid or one g of tissue (the latter finely macerated with kieselguhr) is diluted with 2 ml distilled water; one ml of 20% aqueous solution of trichloracetic acid is then added, and the mixture filtered through a small filter-paper. To one ml filtrate is added 0.05 ml (one drop) of 0.5% sodium nitrite solution. After standing for 3 minutes to diazotize, 0.1 ml of a 1 M sodium dihydrogen phosphate solution containing 0.5% of ammonium sulfamate ($NaH_2PO_4.H_2O$, 13.8 g; ammonium sulfamate, 0.5 g water, 100 cc) is added in order to destroy excess nitrous acid which forms after diazotization, and to buffer the solution before coupling with dimethyl-a-naphthylamine. After standing for 2 minutes, 0.5 ml of a 1.0% alcoholic solution of dimethyl-a-naphthylamine is also added, and the whole is mixed by gentle shaking. After standing for 15

[3] Marshall, E. K., Jr., Emerson, K., Jr., and Cutting, *J. A. M. A.*, 1937, **108**, 953; *J. Pharm.*, 1937, **61**, 196; *J. A. M. A.*, 1938, **110**, 252.

[4] Proom, H., and Buttle, G. A. H., *Lancet*, 1937, **1**, 661.

[5] Whitby, L. E. H., *Ibid.*, 1937, **1**, 1517.

[6] Fuller, A. T., *Ibid.*, 1937, **1**, 194.

[7] Stewart, J. D., Rourke, G. M., and Allen, J. G., *J. A. M. A.*, 1938, **110**, 1885.

[8] Proom, H., *Lancet*, 1938, **1**, 260.

[9] Marshall, E. K., Jr., and Litchfield, J. T., *Science*, 1938, **88**, 85.

minutes to allow the color to reach its maximum intensity, the color is compared with that obtained by treating in a similar manner standard solutions containing known quantities of sulfanilamide. To determine the total sulfanilamide, one ml of the filtrate is treated with 0.1 ml of 2 N HCl, heated in a boiling water bath for one hour, cooled, and the volume adjusted to one cc. The subsequent procedure is the same as for the estimation of free sulfanilamide. Since sulfanilamide standards were used for comparison, these were converted to sulfapyridine concentrations by multiplying by the factors of 1.25 in the free and 1.5 in the total sulfapyridine determinations, according to the experimental findings of Schmidt and Hughes.

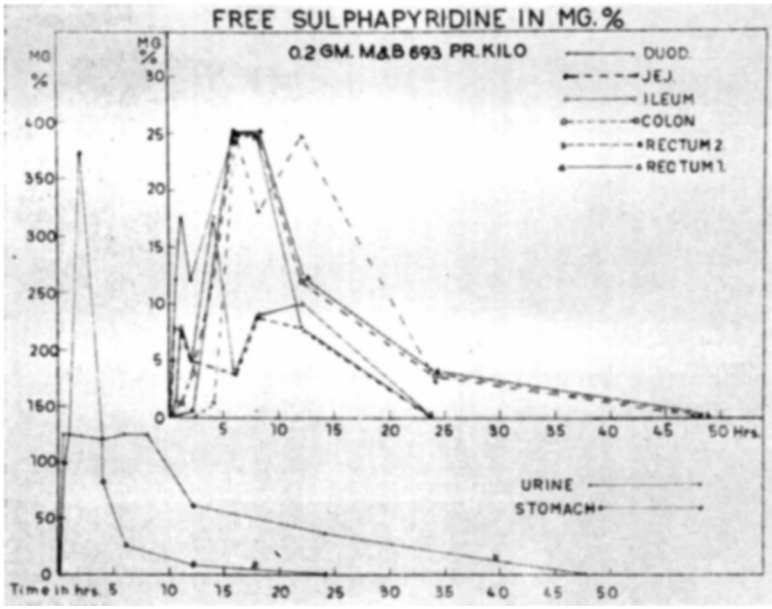

FIG. 1.

Graph 1 shows that of the 200 mg sulfapyridine ingested per kg, 45.7% remain in the stomach after 30 minutes; 33.5% after one hour; 30.25% after 2 hours; 18.75% after 3 hours; 7.1% after 4 hours, and only 1.9% after 12 hours. Apparently the absorption from the stomach is nearly complete in 4 hours after the oral administration of the drug. Very little of the drug passes into the smaller gut (1.08% in one hour; 1.51% in 4 hours, and 0.86% is recovered in 8 hours). It is possible that resorption of the drug takes place from the mucosa in the large gut since the contents in the colon showed 12 mg % of free sulfapyridine after 4 hours, 25 mg % after 8 hours, and 3.7 mg % after 24 hours. Likewise, the lower seg-

ment of rectum showed 22 mg % after 4 hours, 21 mg % after 8 hours, and 3.7 mg % after 24 hours. Approximately 25% of the total sulfapyridine in the gastro-intestinal tract is in the conjugated form.

The maximum urinary excretion of sulfapyridine takes place during the first 8 hours when approximately 50% of the ingested drug is excreted. After 24 hours about 72% of the drug is eliminated in the urine, and less than 1% of the administered drug is excreted between 24 and 48 hours. Presumably is the difference eliminated in the feces. Approximately 30% of the total sulfapyridine excreted in the urine is in the conjugated form.

Graph 2 shows that the bile concentration of free sulfapyridine reaches to 18 mg % and the conjugated form to 2 mg % after one hour, and 25 mg % of the free and 2 mg % of the conjugated form in 4 hours. After 12 hours the curve of free sulfapyridine in the bile declines to 5 mg % and after 24 hours to 0.12 mg %. The blood concentration curve of free sulfapyridine rises sharply after one hour to 10 mg %, while the conjugated form amounts to 3 mg %. The maximum concentration occurs 4 hours after ingestion and amounts to 12 mg % of free and 3 mg % of the conjugated form. After 12 hours the curve of free sulfapyridine declines to 3 mg % and after 24 hours to 0.12 mg %. By estimating that the blood equals 8% of the guinea pig body weight, we observe that of the ingested 0.2 g sulfapyridine per kg, 6.25% is found in the blood after one hour, 7.6% after 4 hours, 5.1% after 8 hours and 0.08% after 24 hours.

Graphs 2, 3 and 4 show the distribution of free sulfapyridine in liquids, organs and tissues. It is quite apparent that the drug is distributed throughout the entire body and that the period of maximum concentration coincides with that in the blood, namely—between the fourth and seventh hour after the ingestion of the drug. The highest concentration curves occur in the bile, blood, skeletal and heart muscles, liver, lungs, kidneys and testes in descending strength in the order mentioned (25-11 mg %). Then follows the salivary glands, pancreas, and eyes with concentrations varying between 4.5 and 2.7 mg %. The lowest concentrations are found in the adrenal glands, bone-marrow, skin and fat (2.0 to 1.2 mg %). Approximately 10-20% of the total sulfapyridine in the above-mentioned liquids, organs and tissues occurs in the conjugated form.

The milk concentration curve was studied in 2 guinea pigs. It was found to coincide with the concentration of free and conjugated sulfapyridine in the blood. The fetal blood concentration curve is available at 8, 18, 24 and 48 hours after the maternal ingestion of

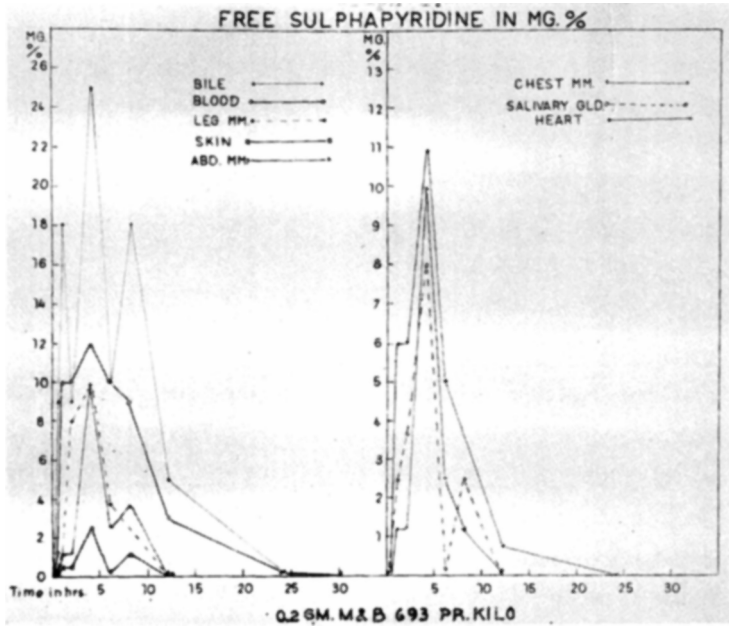
FREE SULPHAPYRIDINE IN MG.%
MG. %
BILE
BLOOD
LEG MM.
SKIN
ABD. MM.
CHEST MM.
SALIVARY GLD.
HEART
Time in hrs.
0.2 GM. M & B 693 PR. KILO

FIG. 2.

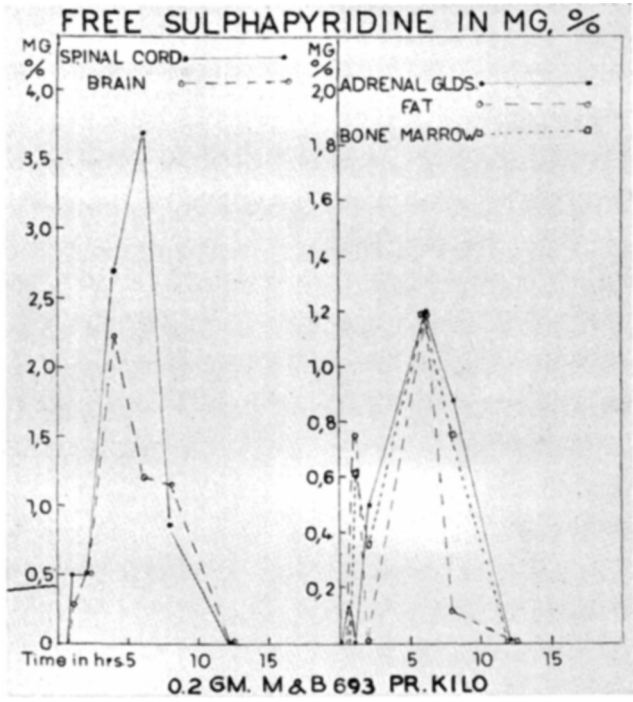
FREE SULPHAPYRIDINE IN MG. %
MG %
SPINAL CORD
BRAIN
ADRENAL GLDS.
FAT
BONE MARROW
Time in hrs.
0.2 GM. M & B 693 PR. KILO

FIG. 3.

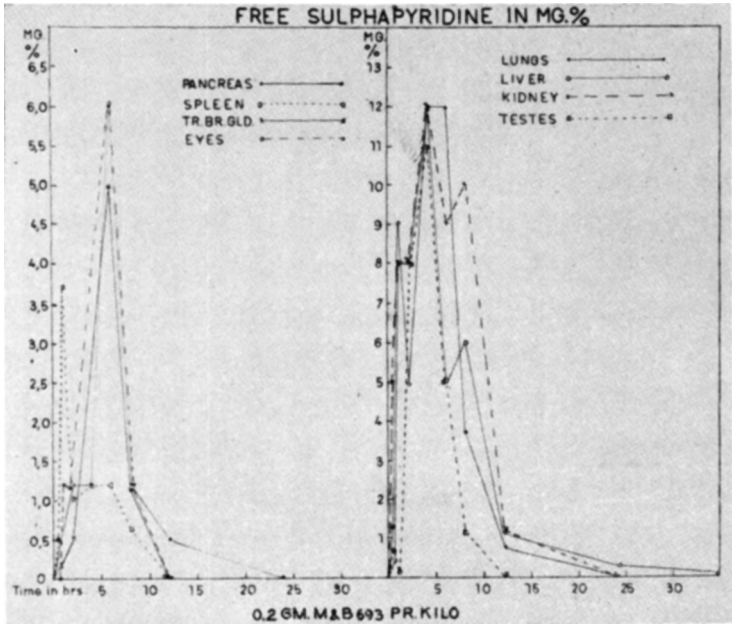

FIG. 4.

0.2 g of the drug per kg, and this is nearly identical with the maternal blood concentrations of free and conjugated sulfapyridine.

Conclusion. The orally administered sulfapyridine is nearly completely absorbed from the stomach after 4 hours, and is distributed to every liquid, tissue and organ in the guinea-pig body, mostly in the free form. About 70% of the ingested drug is excreted in the urine after 36 hours and resorption from the larger gut mucosa may account for the discharge of the balance with the feces.

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Bacteriostatic Effect of Sulfapyridine, Sulfanilamide and Prontosil Rubrum *in vitro* on Mycobacteria.

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In view of the inhibitory action of certain sulfanilamide compounds on the development of an infection of guinea pigs with human and bovine tubercle bacilli,[1] the present study was undertaken to

[1] Birkhaug, K. E., *Brit. Med. J.*, 1939, **2**, 54 (Review of literature).