

tion of its esters would produce the same result. With this thought in mind, the series of alkyl and acetyl derivatives of procaine and the acetyl analogue of butamin were prepared and studied.[†]

Our findings confirmed the above assumption in that the N-substituted anesthetics markedly decreased or neutralized completely the antisulfonamide action of these compounds.

Summary. Studies are presented which indicate that substitution of an alkyl group

on the aromatic nitrogen of procaine will markedly reduce or inactivate completely the antisulfonamide action of the local anesthetic. Substitution of an acetyl group in the position mentioned on procaine, as well as butamin, will likewise result in an inactivation of the antisulfonamide property of these compounds.

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Anomalous Offspring and Growth of Wistar Rats Maintained on a Deficient Diet.

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The nutritional deficiency of the maternal diet has been shown to influence the developmental anatomy of rats *in utero*. Gross congenital malformations were induced in approximately one-third of the offspring of rats of the Sprague-Dawley and Baltimore strains reared and maintained on a deficient diet.^{1,2,3,4} However, Wistar rats similarly reared and maintained failed to produce anomalous young (of 21 Wistar female rats, few reached maturity, and only one female had a litter, consisting of one normal young.)³ From these observations it was suggested that a strain difference exists in the reactivity of rats to the deficient diet. We have repeated the

immature Wistar rat experiments of Warkany *et al.*³ noted above, in an attempt to determine if Wistar rats might produce litters with either normal or abnormal offspring. In addition adult Wistar rats have been similarly maintained.

The data, presented below, indicate that adult Wistar rats maintained on the same diet can produce anomalous young and that immature Wistar rats reared and maintained on the same diet grow poorly and generally do not attain sexual maturity.

The experimental diet employed was the following: yellow corn meal, 76 parts; wheat gluten, 20 parts; calcium carbonate C. P., 3 parts; and iodized sodium chloride, 1 part.[†] At least 50 international units of vitamin D in cod liver oil were given each animal by pipette each week. This diet is definitely deficient in vitamins of the B₂ group and is probably deficient in inorganic elements and

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¹ Warkany, J., and Nelson, R., *Anat. Rec.*, 1941, **79**, 83.

² Warkany, J., and Nelson, R., *J. Nutrition*, 1942, **23**, 321.

³ Warkany, J., Nelson, R., and Schraffenberger, E., *Am. J. Dis. Child.*, **64**, 960.

⁴ Warkany, J., and Schraffenberger, E., *J. Nutrition*, 1944, **27**, 477. Riboflavin has been identified as the factor whose absence is responsible for the anomalous offspring.

† Diet is essentially the same as used by Warkany *et al.*^{2,3,4} and Remington⁵ with the exception that the former supplemented the diet with 60 international units of vitamin D as viosterol every tenth day and the latter supplemented the diet with .2% dried yeast.

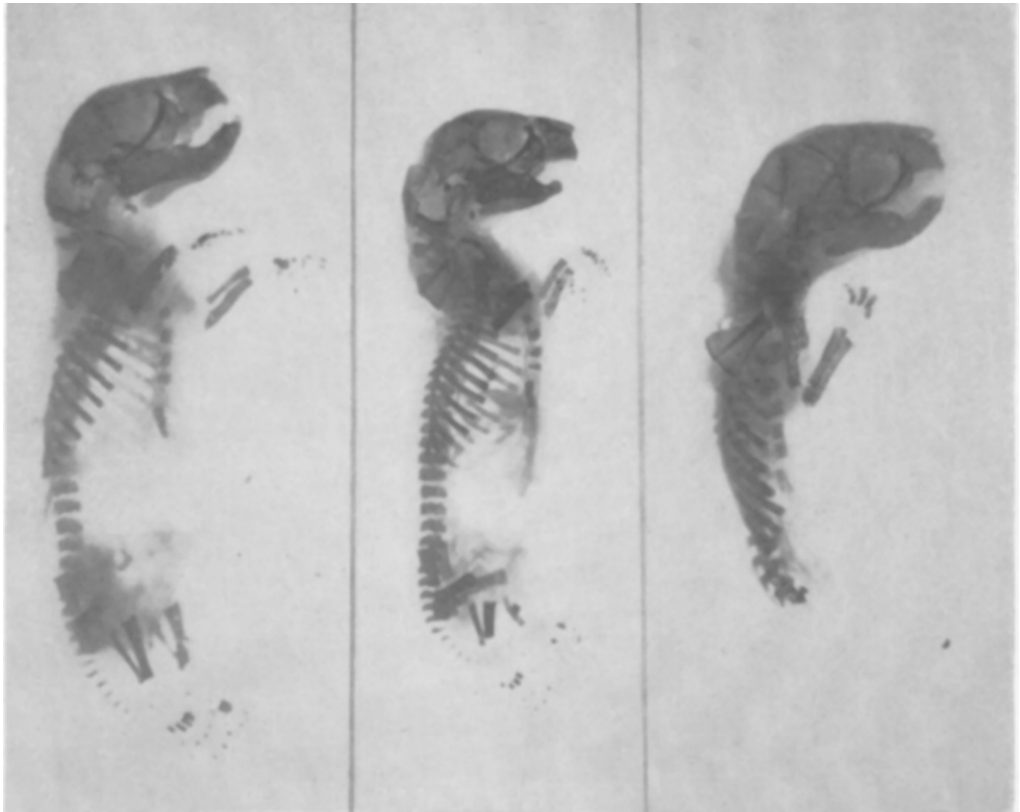


FIG. 1.

FIG. 2.

FIG. 3.

The figures of littermate offspring of a Wistar rat maintained on the deficient diet. All specimens were cleared in potassium hydroxide and stained with alizarin red S.

FIG. 1. Normal offspring.

FIG. 2. Anomalous offspring with some skeletal abnormalities consisting of short mandibles and nubbin-like left tibia. This specimen is smaller than the normal offspring in Fig. 1.

FIG. 3. Anomalous offspring with pronounced anatomical malformations (description in text). Left upper extremity removed.

in the quality of proteins.⁵

Ten adult (ranging from 178 to 273 g) and twenty-one immature (ranging from 43 to 69 g) female Wistar rats were placed on the experimental diet. The breeding males and the 4 adult and 6 immature female control rats, all littermates to the experimental animals, were reared and maintained on the standard dog chow diet supplemented with greens. Diets were administered *ad libitum*.

Results. *Gross malformations in the offspring of Wistar rats maintained on the deficient diet.* The 10 adult experimental Wistar rats maintained on the deficient diet delivered, either normally or by caesarean operation, 17 litters of 67 offspring. Of these 67 progeny,

40 were normal, 23 (36.5% of 63 offspring) exhibited abnormalities and 4 were not classified as they were partially eaten by the mother.

The externally identifiable abnormalities were receding lower jaw, protruding tongue, short limb segments, and varying degrees of syndactylism of the fore and hind feet. Only 12 of the malformed young showed external abnormalities.

The osseous malformations[‡] included the shortening of the mandible and premaxillary bone, varying degrees of shortening to com-

[‡] Skeleton observed in KOH cleared and alizarin red S stained specimens according to method of Noback, C., and Noback, E., *Stain Tech.*, 1944, 19, 51.

⁵ Remington, R., *J. Nutrition*, 1937, 13, 223.

plete absence of the radius, ulna, femur, tibia, fibula, and the bones of the fore and hind feet, the fusion of the ribs and various deformities of the sternum, humerus, maxilla, and scapula. All 23 malformed young showed one or more but never all of these malformations. Except for some minor quantitative differences, the external and osseous malformations observed in these animals are essentially the same as described by Warkany and Nelson¹ for Sprague-Dawley rats.

Two rats had distinctive anomalies. One specimen had an umbilical hernia but it was otherwise normal. The other specimen (Fig. 3), more deformed than any described by Warkany and Nelson,^{1,2,3,4} had a normal skull, upper extremities and cervical, thoracic and lumbar vertebræ. However its ribs were short, and inclined caudally, making acute angles with the vertebral column, while the osseous elements of the sacral and caudal vertebræ and of both lower extremities, including the pelvis, were absent. No demonstrable resorption was noted. Of 50 control offspring prepared for osseous examination, all were normal.

The growth and reproduction of immature rats on the deficient diet. Of the 21 immature Wistar rats reared on the deficient diet, 19 did not attain adult size or mate and, after gaining from 18 to 60 g, they regressed in weight. The remaining 2 of the 21 rats attained weights of 185 and 167 g and mated. Of these 2 rats, one completely resorbed her one litter while the other gave birth to one abnormal and 2 normal young and probably resorbed 5 fetuses (indicated by 8 implantation sights at autopsy).

Similar difficulty in rearing immature Wistar rats to sexual maturity is reported by Warkany *et al.*³ and Remington.⁵ However, immature female rats of either the Sprague-Dawley strain or Baltimore strain can be reared on this diet to attain sexual maturity and to produce litters with anomalous

young.^{1,2,3} On the basis of this difference between Sprague-Dawley and Baltimore strain rats and Wistar strain rats, a strain difference was suggested. As our results show that adult Wistar rats maintained on this diet can produce anomalous offspring, it seems probable that the strain difference of rats reared on this diet is expressed rather in the growth and attainment of sexual maturity than in the production of anomalous progeny.

The adult female Wistar rats fed on the deficient diet exhibited disturbed reproductive activity as evidenced by small and infrequent litters and by prolonged and irregular vaginal smear cycles. The thyroid glands showed some hyperplasia and increase in weight. Aside from the hypertrophied interstitial tissues, no other qualitative differences were observed in the ovaries. The histologically normal uteri were diminished in weight. The pituitary, thymus, and adrenal glands were similar to those of the controls both in weight and histology. The gonadotrophic activity of the hypophyses of control animals and the experimental animals was comparable.^{||}

Summary. Gross anatomical malformations can be induced in approximately one-third of the offspring of Wistar rats, if they are fed a deficient diet after maturity. In agreement with previous reports, immature Wistar rats reared on the same diet grow poorly and give little evidence of reaching adult stature and sexual maturity. It has been demonstrated in the literature, however, that immature female rats of either the Sprague-Dawley or Baltimore strains, reared on this diet, attain sexual maturity and produce litters with anomalous young.

It is suggested that the strain differences of rats reared on this diet is expressed rather in the growth and the attainment of sexual maturity than in the production of anomalous offspring.

[§] Remington⁵ reports similar results but he does not specify the rat strain used.

^{||} The fresh hypophyses were triturated and assayed according to the method of Kupperman, H. S., Elder, W., and Meyer, R., *Endocrinology*, 1941, **29**, 23.