

Lipemia and Glomerular Lesions in Rats Fed Diets Containing N-N'-Diacetyl- and 4, 4-4', 4'-Tetramethylbenzidine. (22181)

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While testing a variety of chemical compounds for their ability to induce tumors in Buffalo-strain rats(1), it was observed that both N-N'-diacetylbenzidine and 4,4-4',4'-tetramethylbenzidine caused severe lipemia accompanied by a characteristic renal lesion. The rats given these drugs died from the effects of the drugs before the time when induced tumors ordinarily appear following exposure to a carcinogen.

Methods: The benzidine compounds were dissolved in the propylene glycol portion of the diet incorporated at equal molar levels of 0.25 and 0.27% respectively into diet #222,* an otherwise excellent diet composed mostly of natural foodstuffs. This was fed to 5 groups of rats and 2 groups of inbred mice.

Results. No difference in the effects of the 2 compounds was observed. After 2 to 4 months on the regimen, the rats lost weight rapidly. During the terminal week of life, the loss often amounted to 20-50 g for individual rats. Fifty rats were autopsied. When killed during the terminal period, the blood was pale pink and clotted quickly. Generalized edema with ascites and hydrothorax was usually present and the kidneys were pale brown with slightly granular surfaces.

Frozen sections were made from the brain, liver, and kidney of 10 rats and stained by Sudan III. Large and small globules of bright red material, indicating the presence of fat were found in the vessels. This material was especially abundant in the glomerular capillaries (Fig. 1).

Tissues taken at autopsy were routinely fixed in Zenker-formol, embedded in paraffin,

and stained with hematoxylin and eosin. Selected kidney sections were also treated by the periodic acid-Schiff and the Wilder reticulum method, Masson's trichrome stain, and toluidine blue.

Only an occasional glomerulus was altered in rats killed early, but changes were regularly found in the glomeruli of rats surviving 3-4 months (Fig. 2). The lesion was characterized by vacuoles within the glomerular loops. Bowman's space was often obscured, and there appeared to be adhesions between the layers of Bowman's capsule. The tubular cells were little altered, though many hyaline and waxy casts were present in the

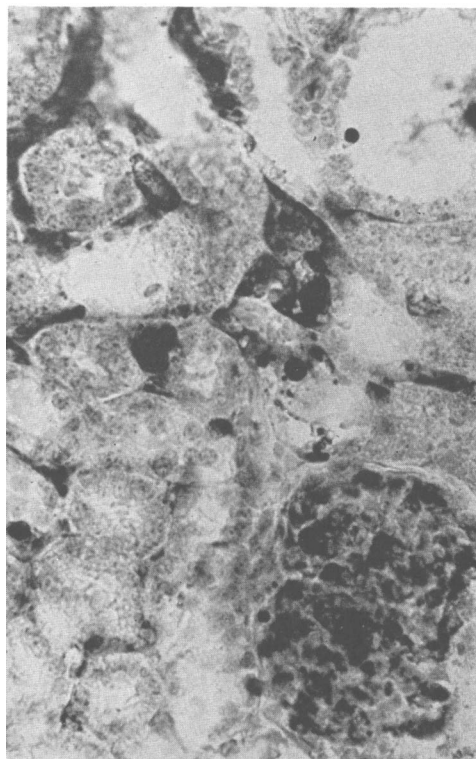


FIG. 1. Glomerular capillaries filled by lipid, which also appears within intertubular vessels. Sudan III. 280 X.

* Composition of diet No. 222: Commercial casein 3, skim milk powder 22.75, whole wheat meal (hard spring) 60.52, debittered brewer's yeast 1, whole liver powder (Wilson's) 1, NaCl 1.4, iron citrate .13, cod-liver oil 2, corn oil 5.2, propylene glycol 3.

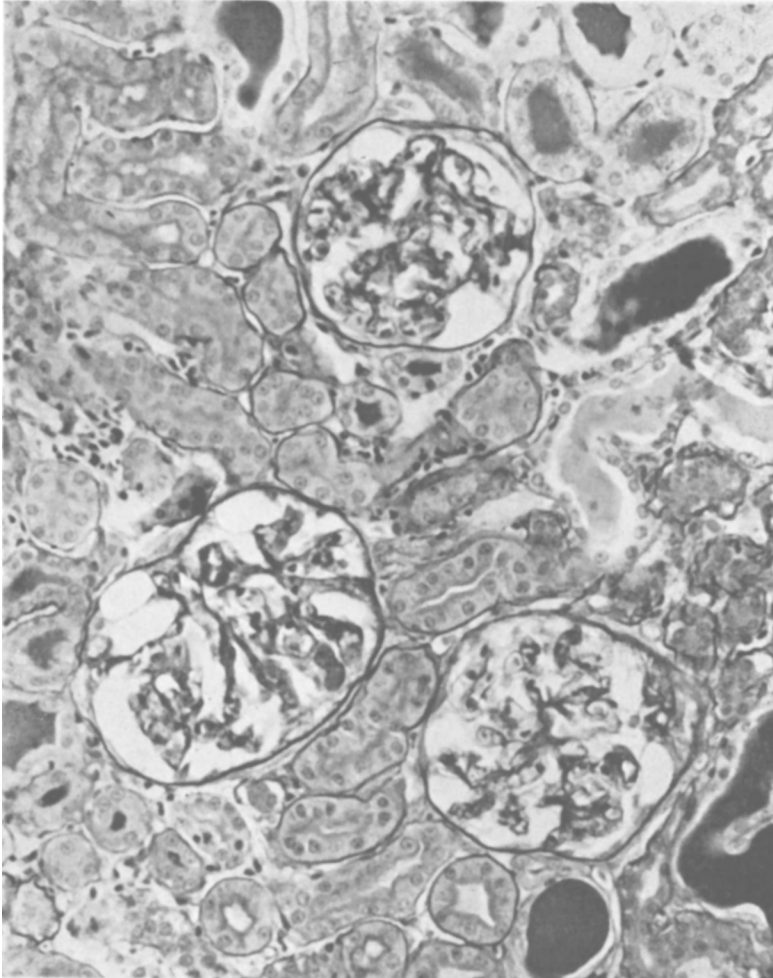


FIG. 2. Three glomeruli, showing small cystic dilatations, and obliteration of Bowman's space. Tubular cells appear normal, but heavily stained casts are present in the lumens. Periodic-acid Schiff. $\times 280$.

tubular lumens. A large number of control animals examined showed none of the changes described.

The mice showed no effects from ingesting the drug; and only those lesions usually found in untreated mice of the strains tested, C3Hb/HeN and A, were observed at autopsy.

Discussion. The toxic effect of the 2 benzidine compounds at the doses given was so severe for rats that the animals failed to survive the period usually required for the induction of tumors. Consequently no appraisal can be made as to the carcinogenicity of these compounds.

Kidney lesions similar to this, to our

knowledge, have never been observed in untreated rats. However, kidney lesions having many of the same morphologic features have been described by Wilens and Stumpf (2) in rabbits treated with cortisone. The initial and constant alteration of the rabbit kidney lesion was a moderate and fairly uniform dilation of almost all glomerular capillaries. The rabbits also had an extreme degree of lipemia, and Wilens and Stumpf suggested that the altered proportions of the blood lipids might cause protracted periods of glomerular capillary stasis. This effect of cortisone has not been found in other species, including man. The dilated capillaries noted

in the rats receiving the drugs appeared to be empty after paraffin embedding, but were shown to be filled with fat in frozen section preparations. The small number of erythrocytes in the blood plasma in the rats and the filling of the lumens with fat may be a reason why the dilated capillaries appeared empty after paraffin embedding.

Summary. Lipemia and a glomerular lesion consisting of many fat filled spaces in the glomerular tuft appeared in from 2-4.5

months in Buffalo-strain rats fed diets containing N-N'-diacetylbenzidine and 4,4'-4'-tetramethylbenzidine. No similar lesions were found in two strains of mice ingesting diets containing these two chemicals.

1. Morris, H. P., Dunn, T. B., and Dubnik, C. S., *J. Nat. Cancer Inst.*, 1948, v9, 225.

2. Wilens, S. L., and Stumpf, H. H., *Am. J. Path.*, 1955, v31, 275.

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Effect of Changes in Environmental Temperature on Stomach Emptying in Rats.* (22182)

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Rats transferred from room temperature to cold environment lose weight during the first few days. One obvious reason seems to be that, in spite of the increased caloric requirements in cold, the animals do not consume more food during this time than they did at room temperature. In search for the cause of this unsatisfactory food intake in experiments reported below we investigated specifically the question whether gastric emptying time is influenced by changes of environmental temperature. Although it has been shown earlier (1) that stimuli for food intake do not originate from the gastro-intestinal tract, everyday experience indicates that disturbances in physiological functions of the digestive organs may interfere with voluntary food intake. An early and important phase of food utilization is emptying of the stomach. We, therefore, considered first the changes in stomach emptying of animals exposed to lower temperature. The effect of changes in environmental temperature on stomach emptying has been discussed earlier(2) but the conclusions were equivocal. We decided, therefore, to investigate this problem directly applying a method for determination of stomach emptying time

which proved to be valuable in our earlier investigations(3). The method is based on determination of N found remaining in the stomach 2 hours after voluntary consumption of a measured quantity of protein.

Methods. Male, Sprague-Dawley rats of 110-220 g weight were used. Stomach emptying time was determined by a method based on the method of Cori(4). However, we found, in preliminary experiments, that stomach emptying time varied widely after forced feeding by stomach tube. Excitement of the animals may be responsible for these variations. Consequently, adult male rats were trained to eat, spontaneously, definite quantities of protein after 24 hours starvation. Animals were maintained at room temperature of 22.2°-0.5°C. After 24 hour fast, with free access to water, each animal was presented with 2,-2,2 g of low fat tuna meat.[†] The N content of each batch of tuna meat was determined and the amount offered was calculated so that approximately 86 or 100 mg was consumed by the animal. Only those animals which consumed all of the tuna without spillage, within 15 minutes, were used for the experiments. After a digestion period of 2

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[†] "Chicken of the Sea" brand Dietetic Tuna was used after draining of the cans.