Histologic Findings in Rats Subjected to Prolonged Administration of Para-Aminobenzoic Acid. (18228)

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The biologic importance of para-aminobenzoic acid (PABA) has been recognized only recently. Its first important clinical use, stemming from the work of Snyder and his colleagues(1), was for the treatment of epidemic typhus fever(2). During World War II most of the other major rickettsial diseases were shown to be favorably modified by the drug. More recently PABA has been found to benefit a variety of chronic disorders, including scleroderma(3), dermatomyositis(3), certain forms of lupus erythematosus(4), lymphoblastoma cutis, and dermatitis herpetiformis. In contrast to the relatively brief treatment necessary in the rickettsial infections, these chronic conditions may require the continuation of therapy with PABA for weeks, months, and perhaps indefinitely. While previous studies have indicated that PABA has a low degree of toxicity in acute or short-term experiments(5), these recent clinical applications raise the question of possible greater toxicity in prolonged administration of the drug. Our interest in the problem was enhanced by the observation at autopsy of severe retrogressive changes in the hepatic cells in one of many patients treated with PABA for lupus erythematosus(4). For these reasons an investigation was undertaken to determine the toxicity of PABA in long-term feeding experiments with rats. The gross and microscopic findings of that study form the basis of the present report.

Method. Young inbred albino rats weighing 50 to 70 g were divided into 3 groups of 10 animals each. All were fed pulverized Purina fox chow checkers and trimmings from green vegetables. Drinking water was accessible at all times. Males and females were not segregated, except for short periods following the birth of young. At such times the female and her litter were caged separately until after weaning. The rats of Group I served as controls. Group II received the sodium salt of PABA (sodium para-aminobenzoate) and Group III, the corresponding potassium salt. The drugs were administered in the diet by adding to pulverized chow pellets a measured amount of the respective powdered compound or of a stock 10% solution of drug. Sufficient water was added to give the mash a moist, porridge-like consistency for each of the 3 groups. The food-drug mixture was prepared fresh every day and the animals offered an amount barely in excess of their average total intake. The quantity of drug employed was adjusted to provide a daily dosage of 2 g per kilo of body weight. PABA levels were determined on samples of blood drawn from the heart at various intervals after feeding. The Marshall and Litchfield(6) method for sulfonamides was adapted for this purpose. From time to time urine specimens were pooled from each group and tested with Benedict's reagent for reducing substance. Two animals of each group were killed by etherization after intervals of 1, 2, and 3 months respectively. The remainder were sacrified after the drug had been ingested for 6 months. On each

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animal a complete autopsy was performed immediately after death, and tissues were fixed in 10% formalin. Specimens of liver were fixed also in absolute alcohol for glycogen stains. All were imbedded in paraffin, and sections of brain, pituitary gland, heart, lungs, salivary glands, thyroid gland, thymus, spleen, stomach, intestines, pancreas, liver, adrenals, kidneys, and testes (or uterus, tubes, and ovaries) were stained with hemalum and eosin. In addition frozen sections of heart, lungs, liver, adrenals, and kidneys were stained for neutral fat with Sudan IV. Sections of alcohol-fixed liver were stained by Best's carmine method for glycogen.

Results. There were observed no significant differences between the treated and untreated animals with respect to body weight, rate of growth, and organ weights. There was likewise no apparent interference with reproduction, as approximately the same number of normal litters were born in each group. That the PABA was adequately absorbed is evident from the blood level determinations, which ranged from 2.0 to 7.0 mg % during the first 6 hours after feeding and from 0.2 to 2.6 mg % up to 24 hours later. Urine of the treated animals occasionally revealed traces of reducing substance.

Histologically, the only significant changes noted in the treated animals were in the thyroid glands. These alterations consisted of reduction in the amount of intrafollicular colloid and decrease in the intensity of its eosinophilic staining reaction. There was an associated increase in the number and height of the epithelial cells lining the affected acini. In general, the involvement was most marked in the central regions of the gland, but similar changes of a lesser degree were present in peripheral portions. These effects were maximal after the administration of PABA for 2 months; thereafter they became progressively less conspicuous, although still present in the animals treated for 6 months.

Aside from the changes in the thyroid glands, no significant abnormalities were observed in the sections of any tissue or organ, whether stained routinely or by the special methods for fat and glycogen.

Discussion. The "goitrogenic" action of PABA has been well documented previously, and it seems necessary, therefore, only to point out that the thyroid changes in these animals conformed to those of earlier investigations(7-9). The possibility of a hepatotoxic action of PABA is of particular interest and of obvious clinical importance. Scott and Robbins(10) reported acute necrosis of the liver in dogs given massive oral doses of the drug (2 g per kilo at one time.) In this connection, it appears significant that the liver is believed to be the primary site of "detoxification" of PABA as well as other aromatic amines(11). While much PABA is eliminated in the "free" form, some undergoes "detoxification" in the liver by acetylation of the amino group and some by conjugation with glucuronic acid(11). The presence of the latter conjugate has been almost uniformly observed in the urine of patients under treatment with large doses of PABA(12). The finding of traces of reducing substance in the urine from these rats is presumably due to the same compound. Moreover it has been suggested that the glucuronic acid utilized in this conjugation process is derived from hepatic glycogen(13). With these points in mind it was anticipated that marked diminution in liver glycogen, and perhaps other hepatic changes, might be produced. However, no hepatic lesions were observed.

Preliminary studies of the effects of PABA upon chronically starved rats have likewise revealed no evidence of hepatic damage, although a marked depletion of liver glycogen was observed in both treated and untreated starved animals.

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Summary. 1. In doses greatly exceeding the usual therapeutic range for man, para-aminobenzoic acid is a relatively non-toxic substance for the rat. 2. Aside from histologic alterations in the thyroid gland, no significant pathologic changes were detected in the treated animals or in their offspring. 3. The thyroid changes consisted of reduction in the amount and eosinophilia of intrafollicular colloid, associated with epithelial hypertrophy and hyperplasia. These findings were maximal after 2 months of treatment. 4. No hepatotoxic effect was detected.

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Electrical Injury of the Cochlea of the Guinea Pig.*† (18229)

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The original purpose of the present experiments was to polarize electrically the sense organ of the inner ear in the expectation that the thresholds of the aural microphonic and of the action potentials and perhaps also their maximum amplitudes would be modified. Any such modifications would be useful tools for analysis of the origin of the microphonic and of the excitation of the primary neurons by the sensory cells. The method seemed practical because of the success of Riesco and collaborators(1) in drilling small holes into the cochlea of the guinea pig and thereby placing electrodes in known positions relative to the basilar membrane. Our expectations were fulfilled and the results will be presented elsewhere. The present report describes injurious effects produced incidentally by electro-coagulation and electrolysis in our exploratory experiments.

Methods. Holes were drilled as described elsewhere(2) into at least 2 and sometimes 3 turns in the cochlea and another electrode was regularly placed on the round window. No. 38 enamel-insulated silver wire was substituted for the original copper wires. The usual positions of electrodes and their attachment by dental cement to the edge of the opening into the acoustic bulla is shown in Fig. 1. Frequently, however, the electrode in turn 1 was introduced into the scala tympani instead of scala vestibuli by placing it on the opposite side of the dark band that marks the position of the stria vascularis. The thresholds of the aural microphonic at frequencies 200, 1000, 2500 and 8000 cps and also of the action potential in response to a standard filtered click were measured(2). The par-

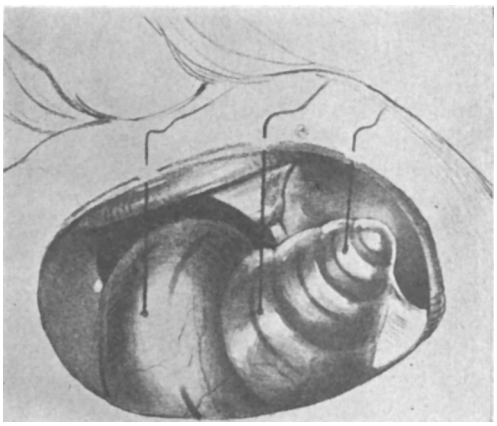

FIG. 1.

Three silver wires (black lines) are placed as electrodes in the first, second and fourth turns of the cochlea of a guinea pig. The wires are held in place by dental cement attaching them to the cut edge of the bulla. The dark band that marks the position of the stria vascularis is also shown.

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