

## The Nitrogen Retaining Activity in Rats of 19-Nortestosterone 17-(4'-methylbicyclo [2.2.2] oct-2'-ene-1'-carboxylate) (35339)

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Scribner *et al.* (1) described a series of 19-nortestosterone bicyclo [2.2.2] octane- and octene carboxylates having prolonged myotrophic activity. Because of the favorable myotrophic/androgenic ratio and prolonged myotrophic activity, 19-nortestosterone 17-(4'-methylbicyclo [2.2.2] oct-2'-ene-1'-carboxylate) (NMB) was studied for its ability to retain nitrogen in rats in order to confirm its protein anabolic activity.

**Materials and Methods.** Twelve female Simonsen albino rats<sup>1</sup> which were 4-months-old and weighed 270–290 g were placed into individual metabolism cages and fed a controlled quantity of ground Purina Laboratory Chow. Water was given *ad lib*. The amount of food given was adjusted downward over a period of 3 months until the body weight and urinary nitrogen established a plateau. At the plateau level, all 12 rats were consuming 14 g of ground chow/day. The average body weight was approximately 300 g and the urinary nitrogen, expressed in terms of urea, was approximately 325 mg/day. After the plateau was reached, a single injection of 6 mg of NMB was administered subcutaneously in 0.2 ml of a vehicle containing 10% benzyl alcohol and 90% sesame oil to 6 rats (phase I). The remaining 6 rats served as controls. Eleven weeks after the first injection, a crossover experiment was performed in which those animals which formerly served as controls received 6 mg NMB as before and those rats which previously received the test material received the vehicle only (phase II).

The data are grouped into four 2-week periods to facilitate analysis: a 2-week preinjection period and weeks 1 and 2, 3 and 4,

and 5 and 6 postinjection. An analysis of variance was performed using the combined data of each individual nitrogen and body weight determination for the control and NMB-treated group for each of the four periods.

The Laboratory Chow was analyzed for protein content and found to contain 24% protein. Each gram of food therefore contained 38.4 mg nitrogen.

The analytical method used for determining the urinary nitrogen was that of Chaney and Marbach (2) utilizing urease and the Berthelot reaction. The colorimetric determinations were made at 640 m $\mu$  using a Leitz photometer. One 72-hr and two 48-hr urine collections were made each week.

**Results.** NMB elicited a highly significant nitrogen retention during the first 4 weeks immediately following the injection. An average of 37 and 20 mg/day less urea was excreted during the first 2 weeks and second 2 weeks, respectively, in the treated group than in the control group (Fig. 1). The urinary nitrogen pattern was no longer significantly different from the controls during the third 2-week post injection period. The rebound phenomenon which has been reported to occur after treatment with anabolic steroids (3) was not observed following the administration of NMB, for an increase in urinary nitrogen above that of the control group was not seen.

A significant increase in body weight was also seen following the injection of NMB. Two weeks after treatment, the treated group weighed an average of 14 g more than the control group, which difference increased to 19 g at the end of 4 weeks (Fig. 1). It is important to note that this weight differential persisted through the second phase of the study. This is in contrast with reports

<sup>1</sup> Obtained from Simonsen Laboratories, Gilroy, California.

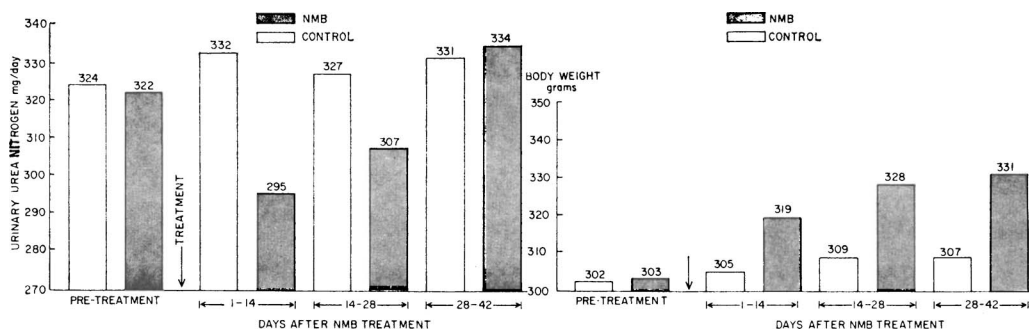


FIG. 1. Urinary urea nitrogen and body weight of rats receiving 1 injection of 6 mg of NMB compared with nontreated group (6 rats/group). Data grouped in successive 2-week periods.

of other investigators (4, 5) who found anabolic agents to elicit only a temporary increase in body weight when administered to castrate male rats fed on a diet on which they were maintained at a constant body weight and nitrogen equilibrium prior to treatment. Although simultaneous electrolyte studies were not carried out, it seems unlikely to credit the increased weight to water retention. Although initially this may be partially true, the fact that the weight-gain was maintained for 19 weeks (including phase II) would rule out a significant electrolyte effect. In an acute test for sodium and potassium retaining activity similar to that described by Kagawa *et al.* (6), a subcutaneously administered 500- $\mu$ g dose of NMB did not elicit electrolyte retention in adrenalectomized rats not receiving a sodium load.

To confirm the observations made in the first part of the study, a second phase involving a crossover procedure was begun 11 weeks after the initial injection, at which

time the urinary nitrogen and body weights of the rats were on a plateau. The rats continued to eat 14 g of ground laboratory chow daily. The group which this time received 6 mg of NMB showed significant nitrogen retention for the 2-week period immediately following the injection, whereas nitrogen retention was not seen during the second 2-week period (Fig. 2). That the rats did not elicit significant retention for 4 weeks as in phase I may be due to the increased age of the rats (3). Figure 2 shows that whereas there was a difference ( $p > 0.05$ ) in weight between the previous control and treated groups (315 and 330 g, respectively), after receiving treatment the weight of the former control group increased to the level of the previously treated group. The experiment was terminated 8 weeks after initiating phase II.

*Summary.* NMB was given in one subcutaneous injection in oil at a dose of 6 mg to female rats on nitrogen and body weight balance. A constant level of food was consumed.

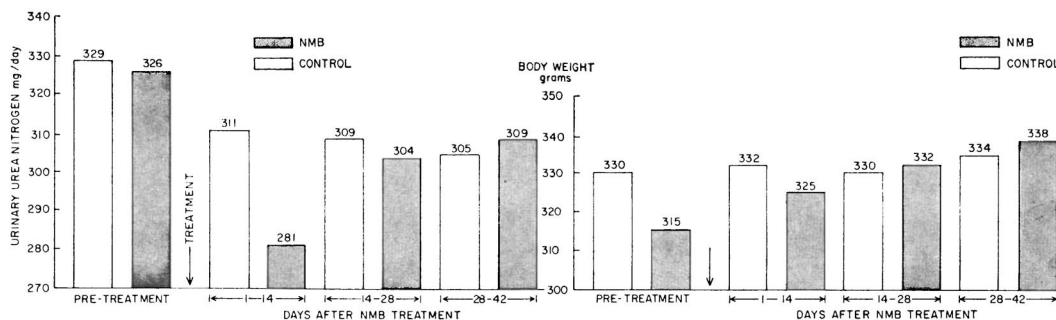


FIG. 2. Urinary urea nitrogen and body weight of rats receiving 1 injection of 6 mg NMB compared with rats which had previous NMB treatment (control group). Data grouped in successive 2-week periods.

A highly significant and prolonged nitrogen retention was obtained. No nitrogen excretion rebound was seen. A significant increase in body weight was produced which was maintained for 19 weeks.

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