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Effect of Certain Androgenic Steroids and Cortisone on Gastric Ulcerogenesis in Fasting Rats. (24862)

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When intensive corticoid treatment is associated with 4 days fasting in rats, steroid gastric ulcers develop in about 90% of animals. These ulcers are always found in glandular portion of stomach and closely resemble human peptic ulcers. Fasting alone, on the other hand, results in forestomach ulcers, similar to typical lesions of the Shay rat. Robert and Nezamis(1) propose this technic of ulcer production as assay for ulcerogenic property of steroids. Several factors may be responsible for steroid gastric lesions. Most frequently quoted hypotheses concern post-steroid changes in gastric secretion, antiphlogistic role of corticoids and possible decrease of gastric mucus synthesis(1,2). We showed that sulphated mucosubstances are reduced in both gastric tissue and gastric juices of rats treated with cortisone(3). The mechanism of this steroid action is, however, not clear. That corticoids exert a general anti-anabolic effect will be taken into consideration when interpreting any local effect of these hormones. It is known, for example, that the effect of cortisone on tissue healing and on synthesis of collagen is anti-anabolic and that the antiphlogistic role of corticoids is partly explained by this effect(4,5). Some anti-anabolic effects of cortisone may be offset however by certain anabolic androgens(5,6,7), and it seemed interesting to investigate this problem using above mentioned method of producing cortisone ulcers in rats. The present report concerns investigation of possible protective action of some synthetic androgens on development of gastric ulcers in fasting cortisone treated rats.

Materials and methods. Male rats of Wistar strain, of body weight 195 to 220 g were placed in individual cages and fasted 4 days. Some animals received hormonal treatment for 10 days preceding fasting period. 17-ethyl-19-nortestosterone (E.N.T.) (Nilevar, Searle & Co.) was given 5 mg/os daily, crushed tablet mixed with food(5). Testosterone enanthate (T.E.) (Delatestryl, Squibb & Sons) was injected intramuscularly, one 100 mg dose 10 days before beginning of fasting. Cortisone acetate (Merck & Co.) was injected subcutaneously in 10 mg dose once daily, only during fasting period(1). The following 6 groups of rats were used: 1. Pretreated with E.N.T. before fasting; 2. Pretreated with T.E. before fasting; 3. Fasted only; 4. Pretreated with E.N.T. and given cortisone during fasting; 5. Pretreated with T.E. and given cortisone during fasting; 6. Given cortisone during fasting. Isotope was administered 24 hours before killing of rats. Radiosulphur (S^{35} in H_2SO_4, Atomic Energy, Canada) was injected subcutaneously, one mc/kg body weight. The dose was dissolved in 5 ml distilled water together with 40 mg of sodium sulphate(6). Animals were killed with ether, the stomachs dissected, opened along great curvature and the mucosa examined under dissecting microscope (X 10). Stomachs were then studied for S^{35} radioactive mucopolysaccharides following modification(3) of method described previously(8,9).

Livers were dissected for total cholesterol determination(10) and gastrocnemius muscle was removed for study of skeletal muscle electrolytes. Baird Flame Photometer was used for determination of sodium and potassium extracted from the tissue(11). Chlorides were determined by titration(12).

Results. Body weight loss during 4 days fasting period averaged 28, 29, 27, 32, 28 and 33% in 6 groups of rats respectively.

Mortality rate was not affected by E.N.T. but markedly increased in group of rats pretreated with T.E. Cortisone associated with fasting promoted surviving rate of animals whether or not pretreated with E.N.T. and T.E. (Fig. 1). Only rats which survived complete fasting period were examined for gastric lesions.

In rats receiving any type of steroid treatment, ulcers were found only in glandular portion of stomach. Control fasting rats (Group 3) had ulcers mostly in forestomach, sometimes associated with ulcers of glandular part. Macroscopic appearance of these lesions agreed with the description of Robert and Nezamis(1) Fig. 1 shows percent of animals which developed gastric ulcers and where these lesions were localized. Occurrence of ulcers in glandular stomachs was increased in T.E. pretreated rats, fasted only,

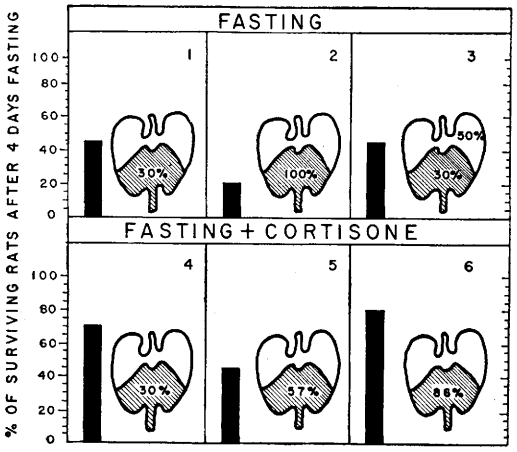

FIG. 1. Percentage of fasting rats surviving (black columns) and of rats with ulcers of glandular stomach (crossed area) and forestomach (open area). Rats of groups 1 and 4 treated with E.N.T. (17-ethyl-19-nortestosterone) and rats of groups 2 and 5 treated with T.E. (testosterone enanthate) prior to fasting and cortisone.

TABLE I. Total Radioactivity of Gastric Tissue in Fasting Rats (cpm/g of Wet Tissue) and % of S^{35} Taken up by Gastric Fraction Containing Sulphated Mucopolysaccharides (S.M.). Mean and range.

Group	Treatment*	No. of rats	cpm/g w.t.	S.M., %
3	None	13	414 (179–552)	73.4 (58.8–86.5)
4	E.N.T. & cortisone	18	314 (197–509)	65.1 (58.0–70.5)
5	T.E. & cortisone	12	267 (179–320)	59.1 (47.9–60.1)
6	Cortisone alone	23	229 (103–320)	44.5 (29.1–49.2)

* Treatment with E.N.T. (17-ethyl-19-nortestosterone) and T.E. (testosterone enanthate) preceded fasting and cortisone.

but this steroid plays some protective action in cortisone treated fasted animals. E.N.T. does not influence occurrence of ulcers in glandular stomachs of fasting rats; it has however very marked protective action in animals treated with cortisone. Cortisone alone results in high incidence of ulcers in glandular stomachs.

Table I presents data on total radioactivity and percentage of S^{35} in fraction considered to contain gastric sulphated mucopolysaccharides (S.M.). This study was performed only in rats of Groups 3, 4, 5 and 6. Rats of Group 3, fasted only, served as controls for fasting animals treated with cortisone alone (Group 6) or pretreated with androgens prior to cortisone and fasting (Groups 4 and 5). Radioactivity of fraction S.M. is reduced in all fasting cortisone treated rats as compared with rats which were fasting only, or which received E.N.T. prior to cortisone and fasting. Pretreatment with T.E. was less effective.

Table II summarizes data on electrolyte content of skeletal muscle and on liver cholesterol.

Discussion. Synthetic steroid 17-ethyl-19-nortestosterone (E.N.T.) is considered a highly anabolic hormone with a low androgenic effect(13). Its protective action against the anti-anabolic effect of cortisone on connective tissue seems to be well demonstrated (5,6,7). Testosterone enanthate (T.E.), on the other hand, is considered a highly androgenic steroid(14). Prolonged duration of ac-

TABLE II. Electrolytes of Skeletal Muscle and Total Cholesterol of Liver in Fasting Rats. Mean and range. 6 groups.

Treatment*	No. of rats	Muscle Cl (meq/100 g fat-free solids)	Muscle Na (meq/100 g fat-free solids)	Muscle K (meq/100 g fat-free solids)	Liver total cholesterol (mg/100 g wet tissue)
E.N.T.	12	10.3 (10.3–11.9)	12.0 (9.5–14.0)	40.3 (36.1–42.8)	316 (226–384)
T.E.	8	10.5 (10.0–11.1)	12.2 (9.6–13.1)	39.1 (35.4–40.5)	253 (258–316)
None	13	10.2 (9.9–10.6)	11.0 (10.0–12.1)	40.7 (37.7–42.2)	284 (222–395)
E.N.T. and cortisone	18	11.0 (8.5–12.3)	11.1 (8.8–12.8)	43.4 (40.3–46.1)	307 (218–450)
T.E. and cortisone	12	9.8 (8.5–11.6)	11.5 (9.3–13.4)	41.4 (38.5–44.2)	311 (216–404)
Cortisone	23	9.7 (9.1–11.8)	10.9 (9.2–12.7)	42.8 (37.0–46.4)	262 (116–337)

* Treatment with E.N.T. (17-ethyl-19-nortestosterone) and T.E. (testosterone enanthate) preceded fasting and cortisone.

tion of an injection makes the use of T.E. especially practical.

E.N.T. and T.E. represent predominantly anabolic and predominantly androgenic steroids respectively. They were tested for ulcerogenic property in fasting rats treated or not treated with cortisone. Because of possible effect of steroid hormones on gastric mucus production, the content of S^{35} sulphated mucosubstances in stomachs of some rats was also studied. As lipid and electrolyte metabolism is knowingly influenced by both fasting and steroid treatment, some supplementary investigations on this subject were also performed.

The high mortality rate in this experiment, as the result of fasting alone or fasting combined with steroids, presents a serious complication, not mentioned by authors proposing steroid ulcerogenesis as assay procedure(1).

It is apparent that cortisone added to fasting, increases survival of rats and that E.N.T. and T.E. have not such a protective action. Steroid treatment does not protect against loss of body weight due to fasting. It is further apparent that pretreatment of rats with E.N.T. gives very marked protection against ulcers in cortisone treated rats. This effect is comparable to that observed in our previous work on E.N.T.(5,6,7,15).

Administration of cortisone during fasting period interferes with uptake of S^{35} by the fraction of gastric tissue, considered to contain sulphated mucopolysaccharides (S.M.). This agrees with previous work on effects of cortisone on metabolism of certain mucopolysaccharides(16) and with our data on post-cortisone utilization of S^{35} by rats' stomachs (3). This significant decrease of S^{35} uptake by S.M. fraction is offset in animals which received E.N.T. prior to cortisone treatment. It is apparent that the anabolic steroid E.N.T. has a somewhat opposite action on S^{35} uptake than cortisone(5,6,7). A possible parallelism between occurrence of ulcers and S^{35} uptake by S.M. fraction of gastric tissue might be considered. This hypothesis, however, needs more precise and detailed study on larger number of animals.

Neither electrolytes of skeletal muscle nor liver cholesterol were significantly affected by steroid treatment or fasting. This may be due to the short duration of experiment.

Summary. The effect of 17-ethyl-19-nortestosterone (E.N.T.) and testosterone enanthate (T.E.) on development of gastric ulcers in fasting cortisone treated rats was studied. Pretreatment of rats with E.N.T. gave a very marked protection against ulcers in cortisone treated rats. It was also noted that S^{35} uptake by sulphated mucopolysaccharides of gastric tissue was significantly reduced in cortisone treated fasting rats as compared with rats fasting only, or those given E.N.T. prior to cortisone. Androgen T.E. had no marked protective action. No

direct parallelism was noted between surviving rate and ulcer occurrence in steroid treated fasting rats.

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Influence of Hypothermia on Secretory Activity of Rabbits' Appendix and on Closed Duodenal Loops.* (24863)

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Experimental observations previously reported demonstrated that rupture of obstructed appendix in rabbits, chimpanzees and man was caused by early development of high intraluminal pressures resulting from rapid rate of appendical secretion(1,2). In a large series of observations upon other species in which activity of appendix was examined, only in chimpanzee and man was there evidence of active secretion. Morton and Sullivan(3) compared closed jejunal loops with similar ones in the ileum. The intra-enteric pressure of jejunal loops (52 cm of water) was 7 times that developed by ileal (8 cm) segments. They concluded that the jejunum secreted more rapidly than the ileum. No observations of duodenal secretory activity were made. Recent observations(4) from this laboratory showed that local gastric cooling in a variety of species, including man, reduced gastric secretory activity and proteolytic digestion. This suggested that the influence of hypothermia upon secretory activity of the obstructed appendix and of closed duodenal loops should be assessed.

Methods. Adult white rabbits weighing 1.5 to 2.0 kg were anesthetized and opened by midline abdominal incision. Appendical closed loops were established by ligating base of appendix with umbilical tape. Closed duodenal loops about 8 inches long were made by occluding the lumen with ligatures at 2 sites to exclude biliary and pancreatic secretions. Integrity of the vascular arcades of these segments was carefully preserved. Rate of appendical secretion was investigated using the simple closed loop described above and in a modified preparation. In the latter, so that secretions could be collected with minimal change in intraluminal pressure, the appendix was cannulated with a plastic tube to which a condom was attached. Duodenal secretions were collected only from unmodified closed loops. In those rabbits that were used for in-

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