Biochemical Pharmacology, 1965, Vol. 14, pp. 41–51. Pergamon Press Ltd., Printed in Great Britain.

METABOLISM OF THE ACCESSORY SEX ORGANS OF THE IMMATURE MALE RAT: CHANGES IN NUCLEIC ACID COMPOSITION AND UPTAKE OF THYMIDINE-^{3}H INDUCED BY CASTRATION AND METHANDROSTENOLONE*

H. SHEPPARD, W. H. TSIEN, P. MAYER and N. HOWIE

Research Department, CIBA Pharmaceutical Company, Summit, N.Y., U.S.A.

(*Received* 8 *June* 1964; *accepted* 16 *September* 1964)

Abstract—The administration of methandrostenolone to both castrated and control immature male rats for seven days resulted in dose-dependent increases in the RNA, DNA, and protein of the levator ani, ventral prostate, and seminal vesicles. The effect of this anabolic agent on these tissues was observable after only two days of treatment and was readily detectable in an increased incorporation of thymidine-^{3}H into the hot acid-soluble fractions containing desoxyribonucleic acid. No effects were noted with the adrenal, thymus, or leg muscle, but a decreased uptake of thymidine-^{3}H was noted in the kidney. The changes in ^{3}H uptake seen after two days of treatment decreased with continued treatment so that after seven days the effect of the drug had almost disappeared. Autoradiograms and general histological examination suggested that the observed changes in the uptake of thymidine-^{3}H paralleled those for rates of mitosis.

TESTOSTERONE and many of its analogues which have been reported to possess different anabolic/androgenic ratios have been examined for their effect on the growth and composition of various accessory sex organs of mature rats,[1, 2] guinea pigs,[3] and mice.[4] Additional studies have been conducted on the effect of some of these agents on the metabolism of protein as measured by the incorporation of labelled amino acids into tissue protein.[5, 6] Burkhart[7, 8] had shown that increased mitosis occurs in the seminal vesicles and ventral prostrates of testosterone-treated castrated rats, thus indicating that an increase in DNA biosynthesis was another metabolic consequence of androgen stimulation.

Inasmuch as thymidine-^{3}H (TdR-^{3}H) has been used successfully by many workers[9] to demonstrate DNA biosynthesis, one should expect to find an increased incorporation of TdR-^{3}H by androgen-stimulated target organs. With this in mind, the experiments to be reported here were undertaken. The steroid used to stimulate the tissues was 17-α-methyl-17β-hydroxy androsta-1, 4-diene-3-one, methandrostenolone,† which was reported by Desaulles *et al.*[10] to have a greater anabolic/androgenic ratio than was observed for testosterone.

METHODS

Dose–response experiment

Twenty-four immature male rats of CIBA strain, weighing approximately 50 g, were divided into six groups of four animals each. One group served as sham-operated

*A preliminary report of this work was made before the 47th annual meeting of the Federation of American Societies for Experimental Biology at Atlantic City, April 1963.

† Dianabol, CIBA Pharmaceutical Co., Summit, N.J.

controls; the remaining groups were castrated. The animals of the five castrated groups were injected s.c. once each morning with 0·0, 0·25, 0·50, 1·0, or 2·0 mg methandrostenolone as a microcrystalline suspension in carboxymethyl cellulose (CMC). All animals were sacrificed by decapitation 24 hr after their seventh injection. The levator ani (LA), seminal vesicles (SV), and ventral prostrate (VP) were removed quickly, trimmed, weighed, frozen over dry ice, and stored in the freezer until ready for analysis.

Time–response experiment

Eighteen immature male rats of the same age as used above were divided into six groups of three animals each and were not castrated. One group, sacrificed immediately, served as zero-time controls; the remaining animals were injected with 4·0 mg methandrostenolone at this time and once every day thereafter. The remaining groups were sacrificed after 1, 2, 3, 4, and 7 days, and the seminal vesicles were removed and stored in the manner described above.

Uptake of thymidime

Thirty-six immature male rats of the CIBA strain were divided into four groups of nine animals each. Two of the groups were castrated and the remaining two served as unoperated controls. Half of both the castrated and unoperated groups were injected s.c. each morning with 4·0 mg of the methandrostenolone suspension in CMC. The remainder of the animals were injected with the CMC vehicle only. Twenty-four hours after the second, fourth, and seventh injection, three animals from each were injected i.p. with 50 μc Td-^{3}H (50 μc/μmole)* per g body weight. Two hours later the animals were sacrificed by decapitation. The tissues were removed, weighed, frozen over dry ice, and stored frozen until ready for use.

The nucleic acids and protein were determined in extracts of the tissues prepared according to the following modified method of Schneider.[11] The tissues were homogenized with 1 ml of 0·6 N perchloric acid and centrifuged. The supernatant was poured off, and the sediment was washed four times with 0·2 N perchloric acid. The final residue was extracted twice with 95% ethanol and three times with a 3:1 mixture of ethanol:ether. The lipid-free residue was suspended in 1 ml of 5% perchloric acid and heated for 15 min at 90° with occasional stirring. This treatment was repeated with 0·5 ml of 5% perchloric acid and heated for 5 min. The residue was then washed once more with 5% perchloric acid. The combined extracts constituted the nucleic acid fraction and were used for the determination of ribonucleic acid [12] and desoxyribonucleic acid.[13] The residue remaining after the hot acid extractions was solubilized in 0·2 N NaOH and the protein content determined by the biuret reaction.[14] All the protein data in the tables refer to that present in the hot perchloric acid-insoluble (HAIS) fraction.

Autoradiography

A group of animals was treated as in the experiment described above. The tissues removed after decapitation were placed in Bouin's fixative for 18 hr. Dehydration and embedding were done according to standard histological procedures.[12] Six-micron sections were cut and picked up with slides which had been previously coated with

* Obtained from Schwarz BioResearch Inc., Orangeburg, N.Y.

0·05% chrome-alum solution. After normal dewaxing and dehydrating procedures,[15] the slices were stained for 6·5 min in hematoxylin solution, followed by 6 min in eosin solution. The latter was double the time usually employed, which gave a more intense strain to compensate for the bleaching of eosin by the subsequent photographic processes. The slices were then rehydrated and coated with Kodak NTB emulsion in the dark under Wratten no. 2 safelight as follows:

1. An adequate amount of emulsion was liquified by heating at 40°–50° for 0·5 hr.
2. A prewarmed 0·75 in. sable-hair brush is used to coat the slides which were also prewarmed to 40°. A single stroke was used, and care and experience minimized the possibility of getting too little or too much emulsion. The slides were warmed on a hot plate for a few seconds to obtain a more even emulsion surface and were then air dried on a flat surface. They were stored in the refrigerator for the desired periods of time in black boxes containing Drierite and sealed with black plastic tape. The autoradiograms were developed for 3 min in Kodak D-72 developer which had been diluted with two parts of water. The slides were then rinsed, fixed in freshly prepared 30% sodium thiosulfate, and washed for 5 min in cold tap water and air dried.

Photographs were taken with Kodak-Panatomic X-35-mm roll film with an exposure time of 1/25 sec, and a Leitz microscope fitted with a blue filter, a Pl-40X objective, and a 10X-Periplan eyepiece.

Counting of radioactive samples

The hot acid-soluble extracts were prepared for counting by placing 0·2 ml aliquots in counting vials and adding 4 ml of absolute ethanol plus 10 ml of toluene containing 0·3% DPO and 0·01% POPOP. Samples were counted in a Tri-Carb liquid scintillation spectrometer. The tritium content was calculated in terms of the disintegration per min per milligram of DNA and is referred to in the test as specific activity of DNA for convenience.

RESULTS

The effect of castration and subsequent administration of 0, 0·25, 0·50, 1·0, and 2·0 mg of methandrostenolone once each day for seven days may be seen in Table 1. Castration had little effect on the milligrams of wet weight per 100 g body weight (relative wet weight) of the levator ani or on its content of RNA, DNA, and HAIS-protein. With the seminal vesicles, castration resulted in a decrease in relative wet weight, RNA, DNA, and HAIS-protein equivalent to 42, 43·6, 32, and 33·2% respectively. The effect of castration was even greater with the ventral prostate, resulting in decreases in relative wet weight, RNA, DNA, and HAIS-protein equivalent to 74·2, 81·4, 42·2, and 74% respectively.

Treatment with methandrostenolone caused dose-dependent increases in all the parameters measured for the three tissues. It was apparent that, while castration caused greater losses of RNA relative to DNA, subsequent treatment with the drug caused greater increases in RNA. Thus the RNA/DNA ratio for the ventral prostate fell from 1·3 to 0·5 after castration but was elevated to 1·53 after drug treatment for seven days. With the seminal vesicles, the RNA/DNA ratio fell slightly from 0·63 to 0·57 after castration but was increased to 1·28 by the drug. The RNA/DNA ratio of the levator ani of the castrated animal showed little change from the 1·33 value for the

sham-operated animal. Treatment with the 2·0-mg dose of drug resulted in an increase in the RNA/DNA ratio to 2·4.

It was observed that different doses of the drug were required to bring the tissues of the castrated animals to the state found for the sham-operated controls. Values for the relative wet weights not significantly different from those of the sham-operated controls were found for the seminal vesicles and ventral prostate after seven days of treatment with 0·25 and 0·50 mg/rat/day respectively. All the other values for these two tissues were significantly different, with a P value $< 0{\cdot}01$.

TABLE 1. THE RNA, DNA AND PROTEIN CONTENT OF THE LEVATOR ANI, SEMINAL VESICLE, AND VENTRAL PROSTATE OF THE IMMATURE MALE RAT AS A RESULT OF CASTRATION AND SUBSEQUENT TREATMENT FOR SEVEN DAYS WITH VARIOUS DOSES OF METHANDROSTENOLONE

		Composition of tissues after the following treatment					
				Castrated + methandrostenolone			
Tissue	Component	Sham-op	Castrated	0·25 mg	0·50 mg	1·0 mg	2·0 mg
	Body wt. (g)	71	72	75	78	79	80
Levator ani	Mg wet wt./100 g body wt.	24·3	26·9*	27·8*	45·5	45·6	57·6
	RNA (μg)	57·9	59·4	76·1	106·8	132·9	166·6
	DNA (μg)	43·5	46·1	42·0	57·9	55·8	69·3
	Protein (μg)	2,040·0	2·180.0	2,560·0	4,230·0	4,640 0	4,980·0
Seminal vesicle	Mg wet wt./100 g body wt.	14·7	8·5	12·9*	30·3	47·7	88·3
	RNA (μg)	72·1	40·6	48·1	148·3	242·0	406·0
	DNA (μg)	104·2	70·2	104·7	159·0	225·8	316·2
	Protein (μg)	870·0	480·0	1,080·0	2,280·0	3·720·0	6,590·0
Ventral Prostate	Mg wet wt./100 g body wt.	47·1	12·0	30·8	48·5*	64·8	86·4
	RNA (μg)	171·6	31·8	95·2	150·6	202·6	340·0
	DNA (μg)	127·4	63·5	106·5	131·2	165·4	221·0
	Protein (μg)	1,920·0	500·0	1,320·0	1,970·0	2,540·0	3,580·0

* Not significantly different from sham-operated controls. All the remaining values for mg wet wt./100 g body weight were significantly different, with a P value $<0{\cdot}01$.

It is seen in Fig. 1 that, with the administration of 4·0 mg methandrostenolone per rat per day to intact rats, an increase in RNA was evident 24 hr after the first injection, and the increase in DNA and protein was not evident until after the second njection. The RNA/DNA ratio changed from 0·5 to 1·24, while the Prot/DNA ratio changed from 7·1 to 23·7 over the seven-day experimental period. In another experiment, qualitatively similar results were obtained with castrated male rats on the same dose of methandrostenolone.

In the next experiment the animals were sacrificed 24 hr after the second, fourth, and seventh injection of methandrostenolone but 2 hr after the i.p administration of TdR-^{3}H. The results of the normal and castrated groups with and without 4·0 mg methandrostenolone are illustrated in Fig. 2. The SV, VP, and LA respond in a qualitatively similar fashion with increases in relative wet weight, RNA, and DNA following treatment with the steroid. It is of interest that the normal animals responded

to methandrostenolone in a manner similar to that of the castrates. While the protein values were not graphed, the results were qualitatively the same as seen for the RNA. It was observed once more that castration had no effect on the LA, a small effect on the SV and a greater effect on the VP. With the VP there was a definite loss of DNA resulting from castration, suggesting an actual loss of cells.

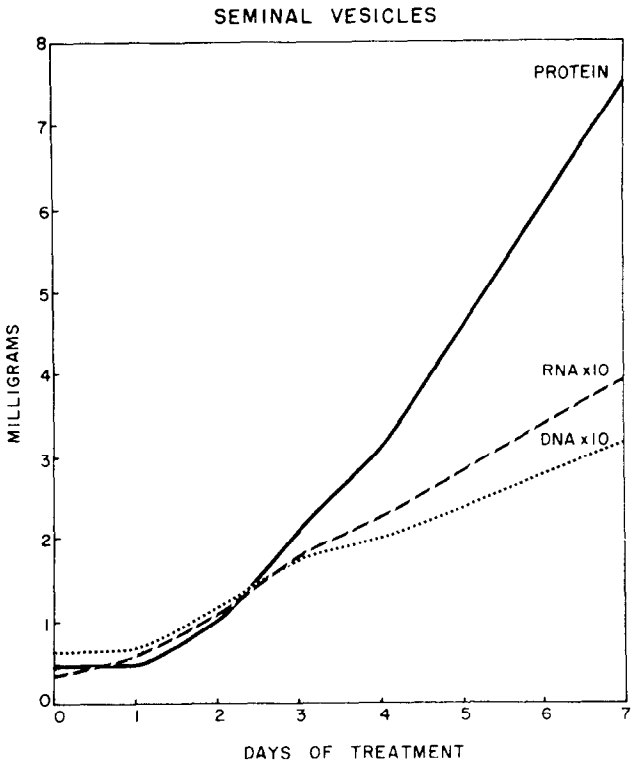


FIG. 1. Changes in the total HAIS-protein, RNA, and DNA of the seminal vesicles of intact immature male rats after the daily administration of 4·0 mg methandrostenolone s.c.

Castration alone did not alter the specific activity of the DNA (dpm/mgDNA) of the LA but did lower this value for the SV and VP. As anticipated, there was a very marked stimulation of the incorporation of TdR-^{3}H into the hot acid-soluble fractions of the tissues from the drug-treated animals. The degree of stimulation then decreased so that by seven days the specific activity of the DNA was at or near the values for the control animals.

Several other tissues were examined to see how their growth and TdR-^{3}H incorporation were affected by castration and treatment with methandrostenolone. The results in Table 2 demonstrated that the drug had essentially no effect on the RNA/DNA ratio of the thymus, adrenal, kidney, or muscle. It is of interest, however, that both the kidney and muscle showed an increased RNA/DNA ratio simply as a result of the growth of the animal. The thymus weight remained essentially unchanged in the drug-treated animals over the entire experimental period, resulting in a fall in relative wet

weight as the animal grew. The thymus of the castrated animal, however, continued to grow in proportion to the whole animal so that the relative wet weight remained constant over the seven-day period. The drug treatment had no effect on the uptake of TdR-^{3}H by the thymus, adrenal, and muscle. With the kidney, however, the specific activity of DNA was depressed on the second day of treatment but was back to normal on the seventh day.

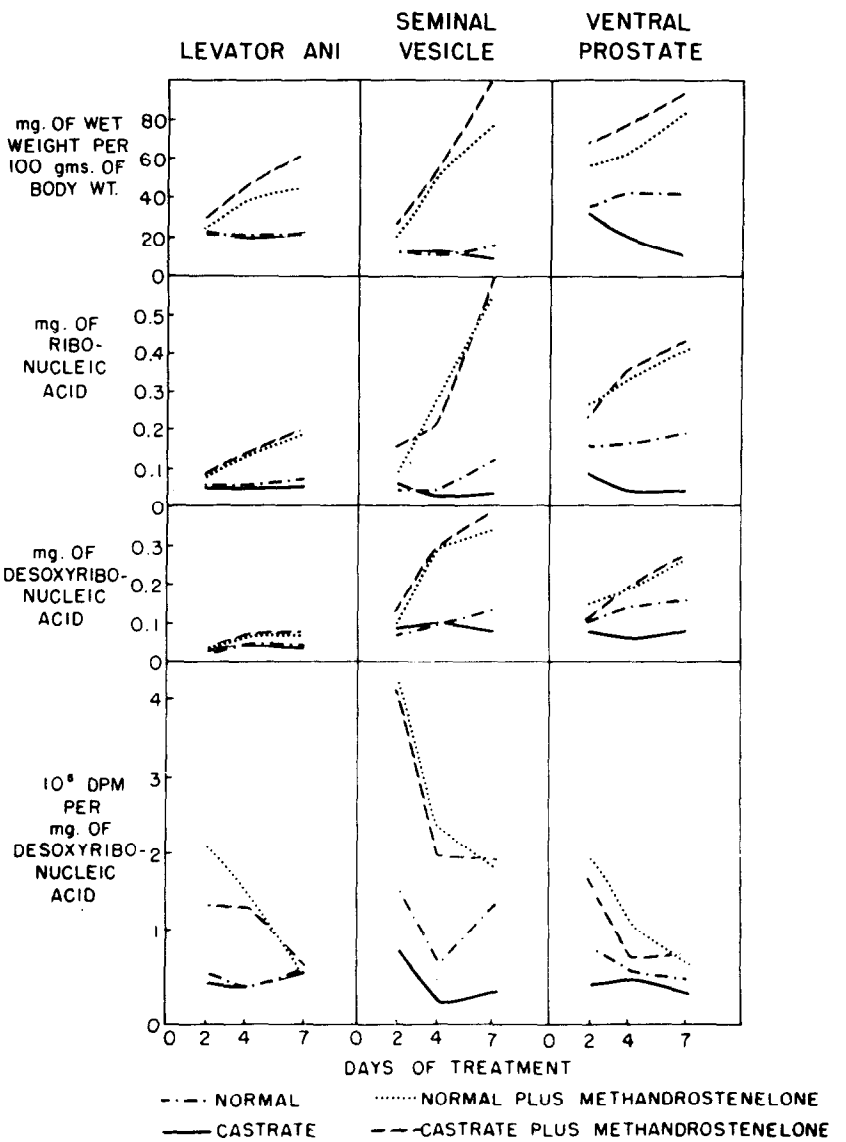
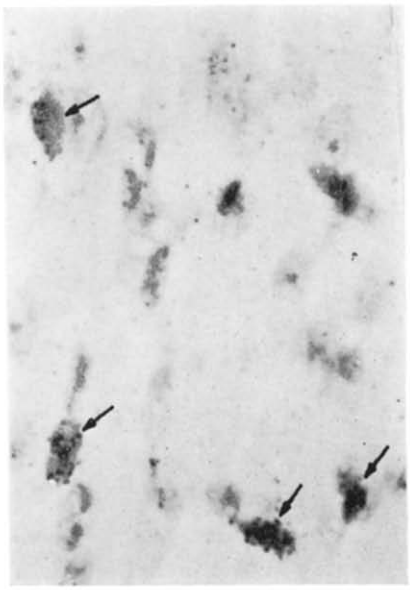
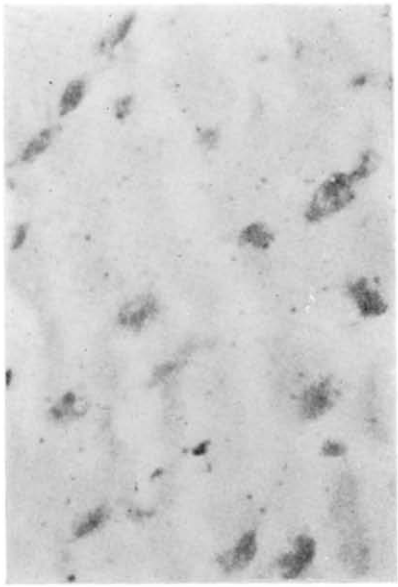


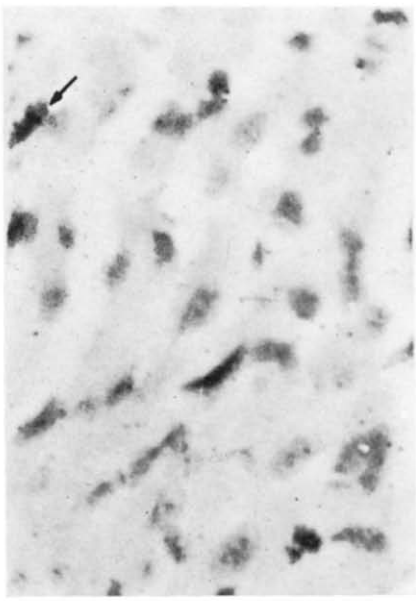
FIG. 2. Changes in relative weight, RNA, DNA, and specific activity (SA) of DNA of the levator ani, seminal vesicles, and ventral prostate of castrated and normal rats with and without the administration of 4·0 mg methandrostenolone for 2, 4, and 7 days.



2 days

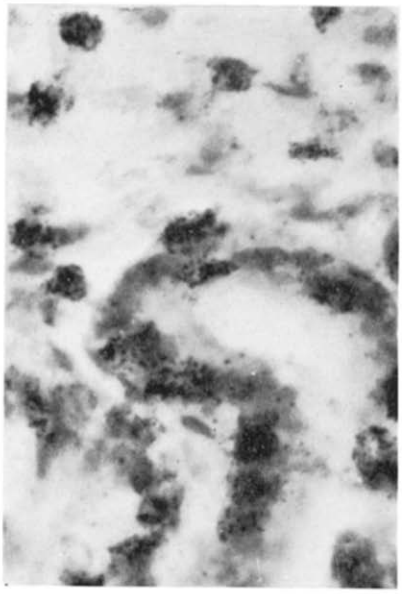


7 days

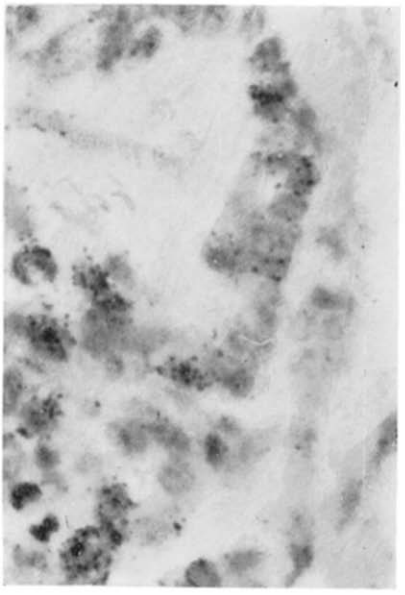


Control

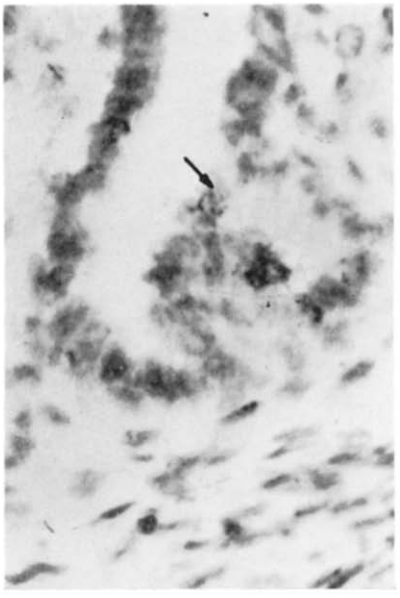
FIG. 3. Autoradiograms of selected areas of slices of the levator ani from castrated control rats and those treated for 2 and 7 days with 4·0 mg methandrostenolone per day.



2 days

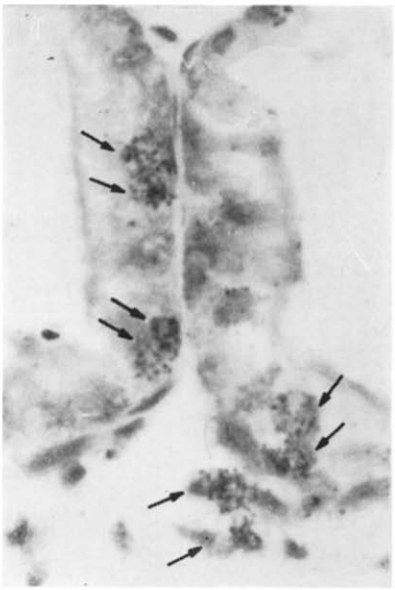


7 days

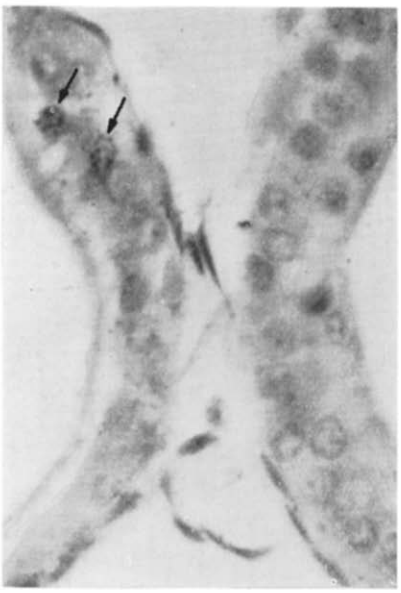


Control

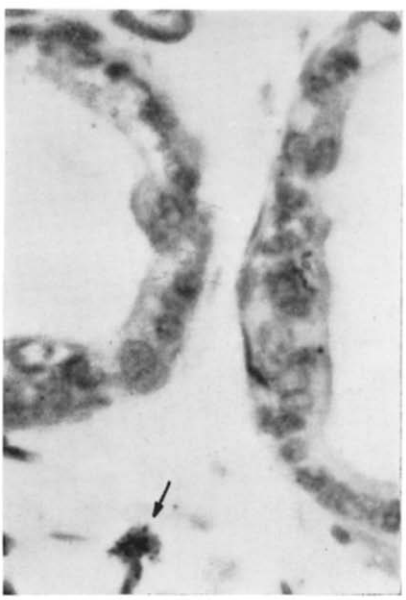
FIG. 4. Autoradiograms of selected areas of slices of the seminal vesicles from castrated control rats and those treated for 2 and 7 days with 0·4 mg methandrostenolone per day.



2 days



7 days



Control

FIG. 5. Autoradiograms of selected areas of slices of the ventral prostate from castrated control rat and those treated for 2 and 7 days with 4·0 mg methandrostenolone per day.

It can be seen in Table 3 that while most of the plasma counts were volatile, indicating extensive decomposition of TdR-^{3}H there was no significant difference between the various groups as far as the evaporated or nonevaporated ^{3}H content was concerned.

TABLE 2. EFFECT OF METHANDROSTENOLONE ON THE RNA/DNA RATIOS AND TDR-^{3}H UPTAKE INTO VARIOUS TISSUES OF THE CASTRATED CONTROL (C) AND TREATED (T) IMMATURE MALE RAT

		RNA/DNA		10^5dpm/mg DNA	
Tissue	Day	Control	Treated	Control	Treated
Thymus	2	0·25	0·25	0·20	0·15
	7	0·25	0·27	0·16	0·18
Adrenal	2	1·60	1·47	0·65	0·55
	7	1·11	1·34	1·31	0·25
Kidney	2	0·73	0·72	0·40	0·13*
	7	0·95	0·01	0·23	0·36
Muscle	2	1·07	1·16	0·31	0·20
	7	1·54	1·94	0·42	0·44

* Significantly different from the castrated control with a P value of $<0·01$.

TABLE 3. PLASMA TRITIUM CONTENT TWO HOURS AFTER THE INTRAPERITONEAL INJECTION OF TDR-^{3}H (50 μC/50 G RAT) INTO NORMAL (N) AND CASTRATED (C) RATS WITH AND WITHOUT TREATMENT WITH 4·0 MG METHANDROSTENOLONE (M) PER DAY FOR 2, 4, AND 7 DAYS

	10^6 dpm/ml plasma					
	2 days		4 days		7 days	
Group	Non-evap	Evap	Non-evap	Evap	Non-evap	Evap
N	1·62	0·056	1·59	1·056	1·61	0·064
N + M	1·63	0·049	1·62	0·069	1·75	0 052
C + M	1·69	0 065	1·62	0·053	1·68	0 055
C	1·82	0·044	1·67	0·046	1·61	0·065

In the autoradiograms in Fig. 3, the castrated control levator ani has only one labeled nucleus. Many similar fields were found to contain none, and others up to two labeled nuclei. After two days of treatment with methandrostenolone, as many as four labeled nuclei could be seen, whereas after seven days no labeled nucleus was visible. It was of interest that the number of nuclei per field and the intensity of staining were lower in the treated slices.

The seminal vesicles in Fig. 4 contained one labeled nucleus in the castrated control slices. Treatment with drug for two days yielded 12 lightly labeled nuclei. The smaller number of labeled nuclei, but not the reduced intensity of labeling, has been observed consistently in those animals treated with drug for seven days. It should be noted that the height of the epithelial cells on the illustrated field of the SV following two days of treatment was only slightly greater than that of the control. After seven days of treatment, however, the height of these cells was much greater. While the epithelial cells in other fields showed greater hypertrophy after two days of treatment, an area

showing only a slight increase in cell size was purposely chosen to indicate that the degree of hypertrophy was not the determining factor in initiating DNA replication. Stimulation of TdR-^{3}H uptake in the smooth muscle and connective-tissue cells was also observed after treatment with drug for two days.

The cells of the ventral prostate seen in Fig. 5 showed only a slight increase in height over those of the controls. Nevertheless, a marked increase in the number of labeled nuclei was seen after two days of treatment. Continued treatment with drug, however, resulted in the appearance of fewer labeled nuclei.

It was clear in the above autoradiograms that all the radioactivity could be found over the nuclei of the labeled cells.

Although no satisfactory pictures of the kidney were obtainable, microscopic examination of the slices suggested that there were fewer labeled nuclei in the proximal tubular cells of the renal cortex from animals treated with drug for two days. No change could be observed in the other areas of the kidney.

No significant differences were noted in the autoradiograms of the adrenal and thymus between the control and treated tissues. The thymus gave very poor autoradiograms, since significantly labeled nuclei could be seen only rarely.

DISCUSSION

The changes observed in the relative weights and composition of the levator ani, seminal vesicles, and ventral prostate of the immature male rat were qualitatively similar to those reported for the mature normal rat[1, 2] and guinea pig[3] treated with steroids other than methandrostenolone. In our studies with immature rats castration for seven days caused no change in relative weight or amount of RNA, DNA, or protein of the levator ani, a small decrease with the seminal vesicles and a larger, decrease with the ventral prostate. The adminstration of methandrostenolone resulted in a dose-dependent reversal of these effects of castration.

The ventral prostate of the sham-operated rat after seven days of the experiment was approximately two to three times heavier than the seminal vesicles. After castration, however, the VP lost weight more rapidly than the SV so that after seven days the VP/SV ratio was only 1·5. From this it may be concluded that the ventral prostate of the immature rat was more dependant than either the seminal vesicle or levator ani on the testicular secretions. Proper replacement of the normal testicular secretion should be possible with some dose of an agent that would be capable of maintaining both the SV and VP at the weights found in the sham-operated control animals. It was obvious that methandrostenolone could not adequately replace the internal secretions of the testis, since different doses were required to maintain the weights at the levels found in the sham-operated controls. In fact, the SV was somewhat more responsive, insofar as it took only 0·25 mg for a maintenance dose as compared with the 0·50 mg required for the VP. This by itself would not be surprising, since methandrostenolone has not been found in testicular secretions. However, unpublished data from our laboratory suggest a similar response to testerone. In addition, it is known from the work of Selye and Albert[16] and others[17] that the SV and VP react differently to a variety of steroids. Androsterone[18] and androstenedione[19] have been reported to have a greater effect on the VP than on the SV. The reports[19] also demonstrated that in the early stages of development of these organs the VP was heavier and increased in weight more rapidly than the SV. As the animal matured, the SV grew more rapidly

and soon became heavier than the VP. These and other observations suggested[20] the possibility that the secretions of the immature testis may include agents other than testosterone. In support of this idea is the observation of Lindner[21] that the androstenedione/testosterone ratio of testicular venous blood was greater than 1 in very young calves, whereas it was less than 1 in older calves and mature bulls. Studies of the androstenedione/testosterone ratio produced by immature and mature rat testis would be necessary to clarify this problem.

The response of the levator ani, seminal vesicles, and ventral prostate to methandrostenolone was relatively rapid, showing increases in RNA and protein as early as two days after administration of the drug. In fact, with the SV, measurable increases in RNA were evident 24 hr after a single injection with methandrostenolone. The DNA levels, however, were not significantly increased after two injections in the LA and only slightly elevated in the SV and VP. In spite of this, however, there occurred a significant stimulation of TdR-^{3}H uptake into the DNA-containing fractions of these tissues. While this observation appears contradictory at first examination, a closer study of the situation shows that this is quite reasonable. Although a definite estimation of mitotic index is difficult to achieve, the report of Burkhart[7] gives a value of about 0·1 for the percentage of cells that were seen in some state of mitosis after treatment with colchicine for 6 hr. With such a low rate of cell division and a mitotic cycle of 6 hr assumed, in 24 hr one would have approximately 0·4% of the cells dividing. This would give a 0·4% increase in total DNA, an increase which is certainly not detectable chemically. In fact, if one considered a statistically significant change to be of the order of 4·0% then one could stimulate DNA replication by a factor of 10 before obtaining a measurable increase in total DNA.

The most unexpected finding was that after the initial increase in the specific activity of the DNA a decrease occurred as the treatment was continued. This was particularly disturbing because with continued treatment the total DNA was increasing. Since the specific activity of DNA may be considered as an index of the proportion of dividing cells, a decrease in such a measurement would signify the division of a smaller *fraction* of the cells. If the cells were to continue to divide at a constant fractional rate, then one would expect the DNA, RNA, and protein to increase geometrically rather than linearly. Thus the decreased specific activity of the DNA was compatible with the observed increases in total DNA.

An important question to be considered is whether the fall in the specific activity of the DNA which resulted from continued treatment with methandrostenolone actually represents changes in DNA replication and cell division. Autoradiography showed that radioactivity was located exclusively over the nucleus of the cell and it could also be seen over various mitotic figures which were present. In addition, the mitotic figures that were observed in the SV and VP were decidedly less frequent after seven days of treatment as compared to two days. It has been reported by Burkhart that the frequency of mitosis increased in the seminal vesicles and ventral prostate of the castrated rat treated with testosterone, but soon began to decrease with continued treatment. This same type of response has also been seen with regenerating liver.[22] The evidence, therefore, supports the concept that in this case, as in so many others, the changes in the uptake of TdR-^{3}H represent changes in DNA replication. However, it is also possible that some increase occurred in the pool size of TdR or its phosphates in the target organs, resulting in a decrease in the specific activity

of TdR-^{3}H. This would then result in a lowered specific activity of the DNA. Some of our own studies indicated a reduced grain count over some seminal vesicle nuclei after seven days of treatment, suggesting a reduced specific activity of the DNA/per cell. This could, of course, be explained as resulting from a reduced specific activity of TdR or one of its phosphates. An increased pool size of TdR has been invoked by Sugino *et al.*[23] to explain a similar fall in specific activity of DNA following recovery from treatment with X rays. It is possible that one is dealing with both a decrease in percentage of mitosis and an increase in thymine pool size.

It is important to recognize that, while the ^{3}H content of the hot perchloric acid-soluble fractions has been referred to as the specific activity of the DNA, no evidence has been presented to show that this is in fact the case. Final proof of such an association must await the isolation of the DNA and its constituent thymine nucleotides. At any rate the assumption that this association exists is based on the work of very many laboratories with many other tissues. Our own autoradiograms certainly place the radioactivity exclusively in the nucleus and associated in the seminal vesicles with the chromatin material in the metaphase as well. The changes in uptake of TdR-^{3}H by the ventral prostate and seminal vesicles correlate fairly well with the changes in the number of mitotic figures that can be seen. Therefore, good presumptive evidence exists for the association of the major portion of the tritium with DNA.

It is apparent that the overall metabolism of the injected TdR-^{3}H is not significantly altered by drug treatment, since (a) the uptake of ^{3}H by tissues such as the adrenal, thymus, and muscle was unaffected, and (b) the blood levels of volatile and non-volatile ^{3}H were not changed. In addition, the work of Sheppard *et al.*[24] showed that these effects of methandrostenolone treatment could be demonstrated even when the thymidine was supplied *in vitro*. Thus, the changes observed as a result of drug treatment must result from alterations of some kind in the responding tissue.

The changes in RNA/DNA ratios are suggestive of changes in cell size, and these are evident in the histological sections and are in agreement with the early observations of Moore *et al.*[25, 26]

The smaller thymus weights in the methandrostenolone-treated animals were certainly expected, but it was of interest that no significant changes in relative amounts of RNA, DNA, and protein could be detected. In addition, by examining the thymus at 2, 4, and 7 days it was demonstrated that the thymus simply stops growing in size. However, there was no change in the specific activity of the DNA, suggesting that there was no change in the fraction of cells that were dividing. It would appear that, in the presence of androgenic substances, the thymus retains fewer of the cells which it produces.

The apparent inhibiting effect of methandrostenolone on the uptake of TdR-^{3}H by the kidney was a completely unexpected finding. While the initial changes during androgen treatment[4] as well as compensatory hypertrophy[27] appear to be in increases in protein and RNA, it could not be readily determined why an inhibition of TdR-^{3}H uptake should be obtained. The effect on the kidney assumes significance when one realizes that a description of the mechanism of action of androgenic agents on DNA replication must explain the stimulation of the accessory sex organs, the inhibition of the kidney, and the lack of effect on the bulk of the body mass.

Acknowledgement—We wish to thank Dr. D. Shoffstall for his assistance in analyzing the histological sections, and Dr. A. A. Renzi for his guidance in designing the experiments.

REFERENCES

1. M. Rabinovitch, L. C. U. Junqueira and H. A. Rothschild, *Science* **114**, 551 (1951).
2. W. W. S. Butler, and A. L. Schade, *Endocrinology* **63**, 271 (1958).
3. C. D. Kochakian, R. Tanaka, J. Hill and D. G. Harrison, *Amer. J. Physiol.* **201**, 1068 (1961).
4. C. D. Kochakian and D. G. Harrison, *Endocrinology*, **70**, 99 (1962).
5. J. D. Wilson, *J. clin. Invest.* **41**, 153 (1962).
6. D. A. Silverman, S. Liao and H. G. Williams-Ashman; *Nature* (*Lond.*) **199**, 808 (1963).
7. E. Z. Burkhart, *Proc. Soc. expl. Biol.* (*N.Y.*) **40**, 137 (1939).
8. E. Z. Burkhart, *J. exp. Zool.* **89**, 135 (1942).
9. Proceedings of a Conference on Autoradiography, *Lab. Invest.* **8**, 59 (1959).
10. P. A. Desaulles, *Helv. med. Acta* **27**, 479 (1960).
11. W. C. Schneider, *J. biol. Chem.* **161**, 293 (1945).
12. G. Ceriotti, *J. biol. Chem.* **214**, 59 (1955).
13. K. Burton, *Biochem. J.* **62**, 315 (1956).
14. S. P. Colowick and N. O. Kaplan, Eds., *Methods in Enzymology* **3**, 450 (1957).
15. *Manual of Histologic and Special Staining Techniques*, 2nd ed. Blakiston, McGraw-Hill, New York (1960).
16. H. Selye and S. Albert, *J. Pharmacol. exp. Ther.* **76**, 137 (1942).
17. L. G. Hershberger, E. G. Shipley and R. K. Meyer, *Proc. Soc. exp. Biol.* (*N.Y.*) **83**, 175 (1953).
18. C. R. Moore and D. Price, *Endocrinology* **21**, 313 (1937).
19. R. C. Callow and R. Deanesley, *Biochem. J.* **29**, 1424 (1935).
20. R. Deanesley and A. S. Parkes, *Biochem. J.* **30**, 291 (1936).
21. H. R. Lindner, *J. Endoc.* **23**, 139 (1961).
22. L. I. Hecht and V. R. Potter, *Cancer Res.* **16**, 998 (1956).
23. Y. Sugino, E. P. Frenkel and R. L. Potter, *Radiat. Res.* **19**, 682 (1963).
24. H. Sheppard, W. H. Tsien, P. Mayer and G. Seymour, *Biochem. biophys. Res. Commun.* **15**, 546 (1964).
25. C. R. Moore, W. Hughes and T. F. Gallagher, *Amer. J. Anat.* **45**, 109 (1930).
26. C. R. Moore, D. Price and T. F. Gallagher, *Amer. J. Anat.* **45**, 71 (1930).
27. A. A. H. Kassenaar, A. Kouivenhoven and A. Querido, *Acta. Endocr.* (*Kbh.*) **39**, 223 (1962).