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EFFECTS OF METHYL-THIOURACIL ON LIVER VITAMIN A, SPERMATOGENESIS AND CALCIUM METABOLISM OF MERINO RAMS.

By W. T. K. HALL, B.V.Sc. (Assistant Veterinary Officer, Animal Health Station, Yeerongpilly) and J. M. HARVEY, M.Sc. (Senior Chemist, Biochemical Section, Chemical Laboratory, Division of Plant Industry).

SUMMARY.

Four groups each of six Merino rams aged 1½-2 years at the beginning of the experiment were fed and treated as follows:—

Group I—Basal ration of bleached oaten chaff and linseed meal.

Group II—Basal ration plus 4-methyl-thiouracil.

Group III—Basal ration plus carotene.

Group IV—Basal ration plus 4-methyl-thiouracil plus carotene.

The dose rate of methyl-thiouracil was approximately 2·5 g. per 100 lb. body weight per day given by mouth.

Semen from all animals was examined every three weeks.

After approximately 100 days of treatment the animals were slaughtered, and thyroid weights, testes weights, serum calcium levels and liver vitamin A concentrations were determined.

The mean weight of the thyroids (16·5 g.) in Groups II and IV was over four times that of the thyroids in Groups I and III.

Serum calcium levels of rams in Groups II and IV were mainly lower (range 4·0–10·6 mg. per 100 ml.) than those of rams in Groups I and III (7·2–11·0 mg. per 100 ml.). The manner in which calcium metabolism was affected, either by the changed activity of the thyroid glands or as a side effect of the methyl-thiouracil treatment, is discussed.

Although there was wide variation in liver vitamin A concentration within the groups (as wide as 55 to 190 micrograms per gram), no differences between groups could be detected.

The lethargic clinical state of the animals and the grossly enlarged thyroids indicated that methyl-thiouracil had produced a hypothyroid state. It was concluded that the hypothyroid condition did not affect the storage of vitamin A in the liver. The hypothyroidism was more severe than that likely to be brought about in the field by starvation and/or by high atmospheric temperature.

No marked differences were apparent in the semen from the different groups.

A record is presented of the very low liver vitamin A concentration of 2 micrograms per gram for a ram which was given a greater aggregate amount of methyl-thiouracil than the other rams but which had been withdrawn from the experiment at an early stage because of small food intake, anaemia and jaundice.

INTRODUCTION.

Temporary infertility in sheep has been observed in north-western Queensland during the hot part of the year and particularly during dry years. Moule (1950) suggested that the so-called "summer sterility" in rams is one of the main causes. In these areas factors that may impair the fertility of rams are low intakes of dietary carotene, protein and phosphate and high atmospheric temperature (Gunn, 1936; Gunn, Sanders and Granger, 1942; Sapsford, 1951).

The thyroid activity of rats was shown by Dempsey and Astwood (1943) to be affected by high temperatures. It was reported by Eveleth, Bolin and Goldsby (1949) that thiouracil treatment of sheep appeared to prevent the conversion of carotene to vitamin A, and Canadell and Valdecases (1947) and Johnson and Baumann (1947) reported the same effect in rats. Best and Taylor (1945) stated: ". . . in hypothyroidism the conversion of carotene to the vitamin is defective; the milk of goats, which ordinarily is pure white, becomes yellow after thyroidectomy, due to the excretion of unchanged carotene."

It has therefore been suggested that in north-western Queensland hot weather depresses the activity of the thyroid and thus reduces the efficiency of conversion of the small amount of available carotene. This could produce a vitamin A deficiency, which in turn may affect the thyroid and so set up an adverse cycle (Moule, 1950). The experiment reported here was designed to test this hypothesis. Facilities for simulating the temperatures of north-western Queensland were not available, so methyl-thiouracil was used to depress the activity of the thyroid.

EXPERIMENTAL.

The objectives were to determine whether depressed thyroid activity affected the conversion of carotene to vitamin A, and whether vitamin A deficiency and hypothyroidism affected spermatogenesis in rams.

Twenty-seven 1½-2-year-old Merino rams were used. They were bred in southern Queensland and were brought to Yeerongpilly on June 8, 1950. To deplete their vitamin A reserves they were allowed no green feed and were fed the lightest coloured oaten hay procurable. During this period of depletion (on July 31) the rams were divided into three groups of seven and one group of six so that an animal of similar weight was in each group.

To provide information for comparison with other rams after treatment, one animal for post-mortem examination was selected at random from each of the three groups of seven animals. Thyroid, testis and epididymis were examined histologically, and liver vitamin A estimations were carried out.

The four groups, each of six animals, were then treated as follows during the experimental period, which began on November 17, 1950:—

Group I—Basal ration.

Group II—Basal ration plus methyl-thiouracil.

Group III—Basal ration plus carotene after the 8th day of the experiment (Nov. 25, 1950).

Group IV—Basal ration plus methyl-thiouracil then plus carotene after the 8th day of experiment.

The basal ration consisted of:—

Bleached oaten chaff ranging from 4·5 per cent. to 7·5 per cent. crude protein fed *ad lib.*

Linseed meal (minimum crude protein 30 per cent.), 4 oz. per sheep per day.

After the 51st day of the experiment ground limestone was mixed with the linseed meal at the rate of 2 oz. per six sheep per week.

METHODS.

General.

The sheep were fed twice a day. The amount of feed taken was about 2½ lb. per sheep per day, the methyl-thiouracil treated sheep eating slightly less than those not given methyl-thiouracil.

At three-weekly intervals the animals were weighed and plasma vitamin A and carotene estimations and semen evaluations were done. Red blood cell counts and serum calcium estimations were made as the clinical states of the animals indicated.*

* Clinical hypocalcaemia in the methyl-thiouracil treated groups was not expected, and consequently serum calcium estimations were not done until late in the experiment.

At autopsy, thyroid and testes were weighed and the liver vitamin A was estimated. Liver, thyroid, testis and epididymis were examined histologically.

Methyl-thiouracil Treatment.

Bogart and Mayer (1946) administered 10 g. thiouracil per day by mouth to produce "summer sterility" in rams. Astwood and Van der Laan (1945) stated that 6-methyl-thiouracil is five times more active in humans than thiouracil. As the drug available to us—4-methyl-thiouracil (British Drug Houses)—was assumed to have the same physiological activity as 6-methyl-thiouracil, a dose of 2 g. 4-methyl-thiouracil per day was used initially.

Since Ely, Olson and Reineke (1948) had shown that thiouracil was almost completely eliminated from the blood stream of calves and goats within 24 hours, the experimental animals were dosed at 9 a.m. and 5 p.m. each day. Dosing was by drenching, because methyl-thiouracil is not palatable to animals.

The initial dose of 2 g. of methyl-thiouracil per day for rams 90-110 lb. was changed after one week because the treated animals had not shown any loss of appetite or any other clinical change. The afternoon dose was increased by 50 per cent. The daily dose rates for the remaining 111 days of the experiment were then:—

Rams 70-90 lb. weight—2·0 g.
Rams 90-110 lb. weight—2·5 g.
Rams 110-130 lb. weight—3·0 g.

During the first three days the drug was given in gelatin capsules, but throughout the rest of the experiment it was administered as a drench containing 1 g. methyl-thiouracil per 5 ml. This drench was a paraffin suspension of finely ground methyl-thiouracil.

Dosing was discontinued or varied at times for clinical reasons as indicated later in the clinical reports.

Carotene Treatment.

Pure beta-carotene was not given until after eight days of methyl-thiouracil treatment in order to give the methyl-thiouracil time to act upon the thyroid. Carotene was then added to the protein supplement each week at the rate of 250 mg. per six sheep per week. This was done by thoroughly mixing into the linseed meal 0·25 g. of pure beta-carotene in 50 ml. of peanut oil containing 0.1 per cent. hydroquinone. The amount of carotene supplement per animal per day was based on the recommendation of the National Research Council, U.S.A. (1944).

Semen Evaluation.

Semen was collected by the electrical stimulation method used by Gunn (1936), and evaluated on volume, density, motility, pH drift and morphology.

Histological Examinations.

Liver and thyroid were fixed in 10 per cent. formalin saline, and testis and epididymis in Bouin's fluid for 18-20 hours. The tissues were stained with haemotoxylin and eosin.

Vitamin A Estimation.

Blood was obtained for vitamin A and carotene estimation by bleeding from the jugular vein with a No. 13 Birmingham Standard Wire gauge needle.

The method of analysis used for blood and liver vitamin A determination was essentially that given by the Association of Vitamin Chemists Inc. (1947).

Fifty millilitres of plasma was saponified with alcoholic potassium hydroxide, the saponified mixture extracted with petroleum ether and the extraction process repeated three times. The bulked ether extracts were washed three times with water, dried over anhydrous sodium sulphate, filtered through a No. 41 Whatman filter paper and evaporated to dryness in a stream of nitrogen. The residue was immediately taken up in moisture-free chloroform made to a convenient volume and an aliquot taken for analysis. Vitamin A was determined by the Carr-Price reaction with antimony trichloride reagent using the Lumetron photoelectric colorimeter and a 620 mμ glass filter. The instrument was calibrated by means of a U.S.P. vitamin A reference standard of crystalline vitamin A acetate in cottonseed oil contained in a special gelatin capsule. Carotene was determined on the petroleum ether extract using a 440 mμ glass filter. The reference standard was carotene (90 per cent. beta, 10 per cent. alpha) obtained from Eastman Kodak Company, Rochester, N.Y.

A similar procedure was adopted for liver analyses. Fifty grams of minced liver was weighed into a flask containing 100 ml. of 10 per cent. potassium hydroxide solution. The extraction after saponification was carried out as for blood. The petroleum ether extracts were made to 200 ml. in a volumetric flask and a convenient volume then taken for analysis as outlined above.

The results of analyses for plasma vitamin A levels were much lower than those recorded in the literature. Duplicate analyses done at the same time were satisfactory, but the results of analyses of samples taken at three-weekly intervals fluctuated widely. Some source of loss of vitamin A was suspected. This loss was ultimately traced to two causes:—

(1) Adsorption of vitamin A by the No. 41 Whatman filter paper used to filter the petroleum ether extract after drying over sodium sulphate.

(2) Adsorption of vitamin A by the sodium sulphate used as a drying agent.

The method of Peirce (1945) eliminates these two sources of error. This procedure has since been adopted and has given satisfactory results.

Because of these findings the blood vitamin A analyses have been excluded. Some loss of vitamin A also occurred in the liver analysis, but the results have been included because they still show that all rams had adequate reserves at the end of the experiment.

RESULTS.

Clinical Observations.

Weight trends in the sheep are presented in Table 1.

Group I—Basal ration.—During the seven months these sheep were in separate groups (pre-treatment and experimental periods) each animal gained between 13 lb. and 28 lb. in body weight. The lowest serum calcium estimation was 7·9 mg. per 100 ml. and blood red cell counts ranged from 6·3 million to 8 million per c.mm.

No clinical abnormalities were noticed.

Group II—Methyl-thiouracil treated.—In this group one animal died from malignant oedema (on the 45th day of the experiment) and two from hypocalcaemia (on the 29th and 51st days). A fourth animal gained only 6 lb.; its serum calcium on 50th day of experiment and at slaughter was low (6·4 mg. per 100 ml.) but the animal showed no symptoms. The remaining two rams gained 21 lb. and 30 lb. in weight; the others made comparable weight gains while not clinically affected. Red cell counts as low as 4·4 million per c.mm. were recorded at the end of the experiment. All animals were lethargic and generally inactive. This was attributed to a hypothyroid state.

Details are:—

Ram 3176.—Gained 27 lb. between July 31 and December 16. On 28th day of experiment it had a flaccid paralysis. The serum calcium was 5·9 mg. per 100 ml., and 15 g. calcium borogluconate was given intravenously. After this the beast was brighter, but on being lifted onto his feet he still could not walk normally. Inability to extend the phalanges caused conspicuous knuckling. Hypocalcaemia was suspected and the animal was killed the next day for post-mortem examination. A sample of serum taken just prior to slaughter contained 6 mg. calcium per 100 ml. and 1.95 mg. magnesium per 100 ml.

Ram 2744.—Gained 21 lb. from July 31 until death on 51st day of experiment, apparently from acute hypocalcaemia (serum calcium 5·9 mg. per 100 ml.).

Ram 2979.—Gained 21 lb. from July 31.

Ram 3021.—Gained 30 lb. from July 31.

Table 1.

LIVEWEIGHTS (IN POUNDS) AND CAUSES OF DEATH DURING THE EXPERIMENT.

Ram No.	Pre-Experimental Period.					Experimental Period.				
	31-7-50.	29-8-50.	25-9-50.	23-10-50.	10-11-50.	1-12-50.	15-12-50.	2-1-51.	23-1-51.	26-2-51.
Group I.—										
3152	96	94	94	102	103	107	103	104	107	110
3172	76	79	83	88	91	93	96	95	99	101
3004	84	91	98	101	103	100	103	105	109	110
3073	98	104	102	114	118	124	121	118	123	126
2743	89	88	91	100	104	105	103	101	105	106
3189	70	72	70	83	87	86	81	80	81	83
Group II.—										
3176	104·5	117	118	122	129	131	131*	..	..	..
2744	81	77	77	89	97	99	102	103†	..	..
2979	84	85	88	92	95	90	91	90	101	105
3021	72	75	79	82	83	88	94	90	103	101
2829	90	92	92	98	100	93	89	81	90	96
2985	91	74	100	113	112	108	95‡	..	..	..
Group III.—										
2820	74	75	67	71	69	69	66	62	62§	..
3065	81	83	89	86	96	96	97	95	102	105
2702	92	102	97	116	119	119	114	113	119	124
3163	91	94	100	103	110	110	110	106	111	114
3000	104	110	111	123	125	126	125	125	133	121
3030	101	103	103	110	116	112	110	113	117	117
Group IV.—										
2902	84	88	86	91	92	81	90	90	99	104
3026	91	95	103	103	112	108	114	115	122	117
2882	102	105	107	115	118	115	92	106	110	113
2671	102	118	119	130	131	132	136	120	132	121
2638	92	86	95	94	94	87	91	94¶	..	..
2983	83	78	84	90	100	100	74	80	88	94

* Killed on 29th day (16th December); hypocalcaemia.

‡ Death from malignant oedema on 45th day.

¶ Death from punctured kidney on 45th day.

† Death from hypocalcaemia on 51st day.

§ Killed 93rd day: abscess of the tongue.

Ram 2829.—During the first 50 days of methyl-thiouracil treatment weight dropped 20 lb.; the serum calcium was then 6·4 mg. per 100 ml. and red blood cell count 5·4 million per c.mm. During the next 50 days the weight improved 11 lb., but red blood cell count remained low at 4·4 million and serum calcium at 6·4 mg. per 100 ml.

Ram 2985.—Gained 16 lb. from July 31 up to the 21st day of experiment, when the appetite was reduced; an injured ear was swollen. On the morning of January 1, 24 days after the onset of this disease, the animal was found dead. Post-mortem examination indicated that malignant oedema was the cause of death.

Group III—Carotene supplement.—In this group only one animal (No. 2820) was clinically abnormal and was dropped from the experiment. There was no evidence of hypocalcaemia in any animal in this group.

Details are:—

Ram 2820.—Throughout the experiment did poorly; it lost 5 lb. from July 31 until treatment commenced and a further 7 lb. afterwards. During the last 50 days of the treatment period, six blood counts ranged from 3 million to 4·2 million red cells per c.mm. A serum sample taken before post-mortem examination contained 11 mg. calcium per 100 ml. Post-mortem examination on 93rd day of experiment showed a small abscess at the base of the tongue. This probably interfered with deglutition, thereby inducing malnutrition, which in turn caused anaemia. This animal will therefore be disregarded when considering the results.

The other five rams gained from 17 lb. to 32 lb. from July 31. Red cell counts ranged from 6·1 million to 8·7 million per c.mm. The lowest serum calcium was 7·2 mg. per 100 ml.

Group IV—Methyl-thiouracil treatment and carotene supplement.—In this group also, one animal (No. 2983) was dropped from the experiment on clinical grounds. Jaundice and severe anaemia developed, apparently due to dosing with methyl-thiouracil. Two other animals (Nos. 2882 and 2671) showed similar but less severe symptoms. No. 2671 was clinically hypocalcaemic. Serum calcium estimations in this group were with one exception low.

Details are:—

Ram 2902.—Gained 20 lb. from July 31. Serum calcium estimations of 7·6 mg. and 8·4 mg. per 100 ml. were recorded on 23rd and 100th days of experiment.

Ram 3026.—Gained 23 lb. from July 31. Red blood cell counts of 7·0, 7·0 and 5·4 million per c.mm. were recorded on 18th, 28th and 104th days of experiment, respectively. The calcium estimation was 4 mg. per 100 ml. on serum taken at slaughter. No symptoms of hypocalcaemia were evident.

Ram 2882.—Gained 3 lb. from July 31. Red blood count was 7 million per c.mm. on 65th day of experiment. The animal was depressed and slightly jaundiced and continued in this condition until 97th day. Dosing with methyl-thiouracil was continued during this period except on 75th and 76th days. Appetite was poor and red cell count dropped to 4 million on the 85th day. Serum calcium was 10·6 mg. per 100 ml. and the red cell count was 5 million on the day of autopsy.

Ram 2671.—Gained 33 lb. from July 31 to 32nd day of experiment. At this stage the red blood cell count was 2.5 million. On 42nd day, in addition to pale mucous membranes and slight jaundice, the beast was lame. The gait suggested tenderness of the lower limb but no injury could be detected. On 44th day the gait was stilted, the back humped and head down. A muscular tremor was obvious and the animal was lying down most of the time. Serum calcium was 5·8 mg. per 100 ml., so 15 g. of calcium borogluconate was given intravenously. No marked improvement could be seen the following day and two further doses of 8 g. and 15 g. were given. After this, gradual recovery took place. Serum calcium was 8.8 mg. per 100 ml. on 68th day. Seventeen days later stiffness and lameness were again apparent and the serum calcium was 4·2 mg. per 100 ml. Another 15 g. of calcium borogluconate was given intravenously. This effected some improvement. From then until autopsy the beast was often unable to get up in the morning, but on being lifted would eat well and appear quite bright. During this last five weeks, calcium (5 g. ground limestone every second day) was given by mouth. Just before post-mortem examination the serum calcium was 6·2 mg. per 100 ml. Five red blood cell counts made after 20th day of experiment ranged from 3 million to 4·5 million per c.mm.

Ram 2983—Fourteen days after treatment commenced, body weight was the same as at the final pre-treatment weighing 21 days earlier. On 15th day of experiment the ram was noticed to be depressed and jaundiced, and soon lost condition. At the second post-treatment weighing 13 days later it was found to have lost 26 lb. Red cell count was 1·2 million on 17th day and a week later 1·1 million. During this week the faeces became fluid, but not pale. After 10 days of sickness, an improvement in general condition was noticed. Between 17th and 28th day, treatment

with methyl-thiouracil was discontinued. It appeared that the symptoms were due to the toxicity of methyl-thiouracil. After this upset the ram was left in the group and treated similarly to the others, but was not considered to be in the experiment. By 82nd day the red cell count had returned to 3·5 million. The dose rate of methyl-thiouracil was increased to 2 g. twice a day from 83rd until 118th day and red cells were counted each week. In chronological order the red blood cell counts during this period were 3·3, 5·2, 6·2, 5·5, 5·5 million per c.mm. The effect of a higher dose rate did not confirm our suspicion about the toxicity of methyl-thiouracil. However, its apparent effect on this animal earlier, and its effect on other animals in the experiment, is evidence that it may cause jaundice and anaemia.

Ram 2638—Gained only 3 lb. from July 31 until 45th day of experiment, when the animal died from a punctured kidney. Apparently this wound was inflicted during electrical stimulation to collect semen.

Semen Value.

Analysis of semen characters showed no significant difference between the groups. The difference between the semen of rams within the groups outweighed any differences there might have been between the groups.

The semen of all rams was poor.

Vitamin A and Carotene Content.

Results of liver vitamin A analyses are recorded in Table 2. Plasma vitamin A levels have not been recorded because, as mentioned under Methods, the technique used in the earlier determinations gave inaccurate results. There was no detectable carotene in the plasma.

Post-mortem Examinations.

The essential data are presented in Table 2. (Four rams, Nos. 2743, 3189, 3000 and 3030, were not autopsied).

The conspicuous feature was the enlarged thyroid glands in all the rams treated with methyl-thiouracil. Their average weight (16·5 g.) was over four times that of thyroids from the non-treated animals (4.0 g.). Macroscopically the enlarged glands were dark burgundy in colour and fleshy in texture.

Microscopically the epithelial cells were hypertrophied, and many were over three times as high as they were wide. In some glands a little colloid was present, but in most it had almost all disappeared. In one very large (27·5 g.) thyroid from ram 3021 a considerable amount of colloid was present in large (1-2 mm.) acini (Fig. 1, a).

The glands from animals not treated with methyl-thiouracil were paler, less vascular and more fibrous. The histology was that of the classical thyroid except that cysts were present in all glands. These cysts varied in size up to

Table 2.

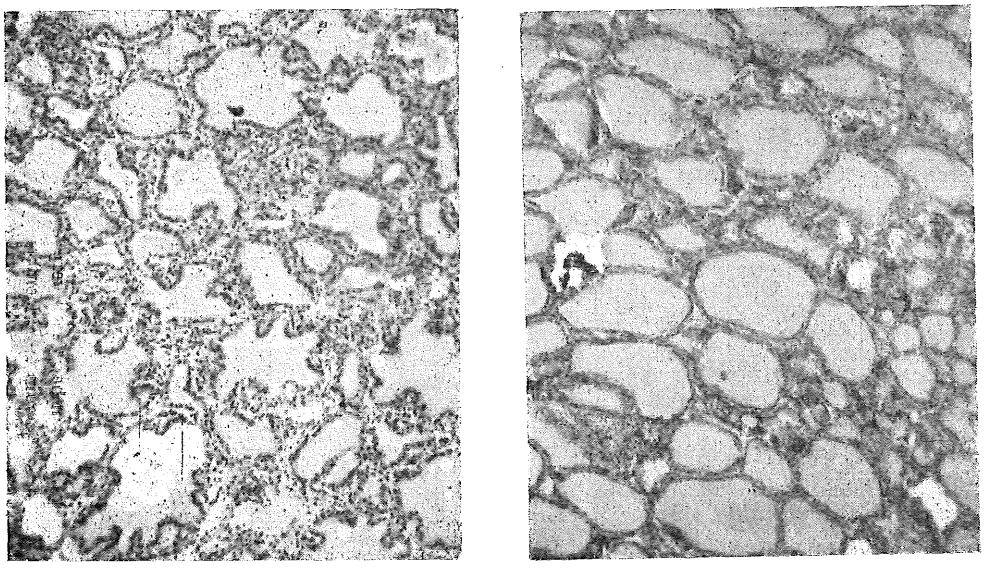
RESULTS OF ESTIMATIONS AND EXAMINATIONS.

Treatment.	Ram No.	Date of Autopsy.	Weight at Autopsy.	Weight of Thyroid.	Weight of Testes.	Serum Calcium.	Liver Vitamin A.
			Lb.	g.	g.	mg./100 ml.	micrograms/g
Pre-experiment control	3159	20–11–50	87	4	433	..	100
	3161	20–11–50	121	6	200	..	200
	2909	20–11–50	115	5·5	300	..	250
Group I.—Control	3152	22–2–51	100	4	390	8·7	65
	3172*	1–3–51	101	4	345	8·4	85
	3004	6–3–51	110	3·5	425	7·9	30
	3073	15–3–51	129	4·5	515	10·3	90
Group II.—Methyl-thiouracil	3176	16–12–50	131	11	380	6	65
	2744	7–1–51	103	23	390	5·9	..
	2979	22–2–51	105	11·6	340	6·5	110
	3021	1–3–51	102	27·5	465	4·3	165
	2829	6–3–51	96	7	285	6·4	150
Group III.—Carotene	2820†	13–2–51	61	3·5	135	11	140
	3065	22–2–51	105	4·5	445	9·5	75
	2702	1–3–51	124	3·5	525	7·2	160
	3163	6–3–51	114	4·6	450	8·9	90
Group IV.—Carotene and methyl-thiouracil	2902	22–2–51	104	13·5	360	8·4	190
	3026	1–3–51	117	16	428	4	55
	2882	6–3–51	113	19	410	10·6	85
	2671	13–3–51	125	19·6	377	6·2	95
Carotene and methyl thiouracil	2983‡	13–3–51	98	24·4	300	7·0	2

* Atrophy of one testicle.

† Abscess on base of tongue.

‡ This ram was discarded from Group IV. on December 15 because during the preceding nine days it had eaten very little and had marked jaundice and severe anaemia. Having recovered to some extent, methyl-thiouracil dosing was started again on December 17. On February 8 the dose rate was increased to 4g. per day, which was continued until post-mortem on March 15.



a *b*

Fig. 1.

EFFECT OF METHYL-THIOURACIL TREATMENT ON THYROID GLAND.

(*a*) Thyroid gland from a ram given methyl-thiouracil. The weight of the gland was 27·5 g.

(*b*) Thyroid epithelium and colloid accumulation in a normal gland weighing 4·5 g. (*Haematoxylin and eosin;* × 80.)

about half the diameter of the gland. They were filled with pale-cream-coloured, homogeneous material of soft, cheesy consistency. Microscopically the material was faintly eosinophilic and enclosed in a thin capsule (Fig. 2). This peculiarity of the normal ovine thyroid has been mentioned by Moutaux (1940), who noticed it in two ewes.

The other constant post-mortem feature of the methyl-thiouracil treated animals was a larger liver darker in colour than those of the other rams. When the liver was minced for vitamin A sampling, this difference was very noticeable. Minced liver from the methyl-thiouracil treated rams was quite fluid and would pour, while liver from the other animals was, when minced, more viscous. Sections from all of the livers were microscopically similar.

Regarding the testes, Table 2 shows that those of methyl-thiouracil treated sheep tended to be lighter in weight.

Histological criteria for determining testicular activity have not been seen in the literature. However, it is reasonable to assume that the quantity of spermatozoa being produced would be indicated by the number of dividing spermatocytes and the presence of all stages of developing spermatozoa in the testes. Further, the degree to which the epididymis is packed with spermatozoa

should also indicate testicular activity, though this would be affected by recent ejaculation. Histological examination could not, except in extreme cases, give any indication of the quality of the spermatozoa being produced. Observations based on these criteria indicated that the testes of the methyl-thiouracil treated animals were slightly less active than those of rams not receiving methyl-thiouracil. This was probably a nutritional effect, as feed intake was depressed in the methyl-thiouracil animals. There is the possibility, however, that it was a thyroid effect, because the histological changes were similar to those described by Maqsood (1951) in rabbits given 0·1 per cent. thiouracil in their feed. This author stated that similar changes occur in rams given thiouracil.

DISCUSSION.

To minimise the carotene intake, a basal ration presumed to be of poor quality was used. The various treatments were begun after five months on the basal ration. It was anticipated that at this stage liver vitamin A reserves would be low in all groups, but the levels found in the pre-experimental controls and in Group I at the end of the experiment showed adequate vitamin A reserves, indicating that the basal ration was not deficient in vitamin A precursors.

The liver vitamin A reserves in rams from Group II and Group IV at the end of the experiment were comparable with those in the controls, thus leading to the conclusion that the hypothyroid state produced in Groups II and IV did not prevent the conversion of carotene to vitamin A.

Fig. 2.

Thyroid Gland, weight 4·5 g., from an Untreated Sheep, illustrating the Nature of the Cyst commonly seen in Normal Sheep.

(*Haematoxylin and eosin;* × 80.)

The total vitamin A reserve in the treated animals was probably greater than indicated in Table 2 because the livers of these animals were enlarged.

No measurable carotene was detected in the plasma of sheep in any group. This is in agreement with observations by Eveleth, Bolin and Goldsby (1949).

No marked differences were apparent in the semen from different groups, but histological examinations indicated decreased testicular activity in the groups receiving methyl-thiouracil. This was probably caused by the lower food intake of the methyl-thiouracil treated groups.

One ram, No. 2983, after receiving 4 g. of methyl-thiouracil per day for three weeks had the very low liver vitamin A concentration of 2 micrograms per gram. This would suggest that methyl-thiouracil, possibly through the hypothyroid state resulting therefrom, affected the storage of vitamin A in the liver. However, all other rams in Groups II and III showed comparable clinical effects of hypothyroidism, but their liver vitamin A reserves were adequate

The effects of hypothyroidism on the conversion of carotene to vitamin A have been studied in rats. Johnson and Baumann (1947) and others found that the hypothyroid state was associated with lowered conversion of carotene to vitamin A. On the other hand, Heimer, Maslow and Sobel (1949) found higher liver vitamin A levels in thyroidectomised and thyroxine-treated animals kept on a vitamin A deficient diet than in controls.

That methyl-thiouracil had produced a hypothyroid state was indicated by the lethargic clinical state of the rams and by the grossly enlarged thyroids. The large, dark, flaccid livers in all of the methyl-thiouracil treated animals and the jaundice and severe anaemia in Nos. 2983, 2882 and 2671 indicate that methyl-thiouracil is to some extent toxic to sheep. This was unexpected; methyl-thiouracil is reputed to be less toxic than thiouracil, and Schultze and Turner (1945) found no toxic effects in the goat when 5 g. thiouracil per day per 100 lb. body weight was given for 30 days.

A feature that claimed our attention was the occurrence of clinical hypocalcaemia in Nos. 3176, 2744 and 2671, all of which were treated with methyl-thiouracil. All serum calcium estimations on the methyl-thiouracil treated animals were appreciably lower than in those not receiving methyl-thiouracil.

Three possible explanations of the hypocalcaemia are:—

(1) Thyroid and parathyroid glands are very closely associated in sheep. The marked hyperplasia of the thyroid may have crowded the parathyroid or may have restricted its blood supply. This could reduce the output of parathyroid hormone and thus lower the serum calcium level.

(2) An alternative is suggested by the statement of Bodansky and Bodansky (1940), after reviewing experimental evidence, that: ". . . the effect of thyroid hormone on calcium and phosphorus metabolism is essentially one of release of these elements from bone." It is therefore possible that in hypothyroid animals calcium and phosphorus would be retained in the bones and serum calcium would drop.

(3) Food intake was reduced in the methyl-thiouracil treated animals, and consequently the calcium intake would also be reduced. As poor oaten chaff is low in calcium, the reduced intake may have been below the critical level.

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