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"GROOTLAMSIEKTE", A SPECIFIC SYNDROME OF PROLONGED GESTATION IN SHEEP CAUSED BY A SHRUB, *SALSOLA TUBERCULATA* (FENZL EX MOQ) SCHINZ VAR. *TOMENTOSA* C. A. SMITH EX AELLEN

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CONTENTS

ABSTRACT

BASSON, P. A., MORGENTHAL, J. C., BILBROUGH, R. B., MARAIS, J. L., KRUGER, S. P. & VAN DER MERWE, J. L. DE B. "Grootlamsiekte", a specific syndrome of prolonged gestation in sheep caused by a shrub, *Salsola tuberculata* (Fenzl ex Moq) Schinz var. *tomentosa* C. A. Smith ex Aellen. *Onderstepoort J. vet. Res.* 36 (1), 59–104.

A specific syndrome of prolonged gestation in sheep in South West Africa was studied and eventually reproduced by artificial feeding of the shrub, *S. tuberculata* var. *tomentosa*. It was determined that the main period of insult occurred during the last 50 days of pregnancy. The trend of both progesterone and cortisol levels resembled those of normal ewes except for a considerable delay during this 50-day period. Apart from the abnormal length of gestation and concomitant features such as retarded udder development, no signs of toxicosis were manifested by the ewes. The most significant features in the postmature lambs were progressive hypophysial, adrenal and thymic atrophy; hypertrophy of the female genitalia, polyfollicularity of the ovaries and Leydig cell hypoplasia; long haircoat, erupted incisors and pigmentation especially of the kidneys and lymph nodes, but no abnormal anatomical features were found. In rats, rations containing the shrub prolonged both gestation and the dioestrous phase of the oestrous cycle. The active ingredient of the plant is not an oestrogenic or anti-oestrogenic substance. It was successfully extracted with alcoholic compounds.

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"GROOTLAMSIEKTE", A SPECIFIC SYNDROME OF PROLONGED GESTATION IN SHEEP

Introduction

"Grootlamsiekte" is the Afrikaans name coined for a specific syndrome in sheep in South West Africa which is characterized by prolonged gestation and foetal postmaturity and its direct translation means "big-lamb-disease". It was first reported by Basson, Nel & Freyer (1959) who investigated outbreaks in Karakul and Karakul-Persian cross-bred sheep in the eastern semi-arid region of Keetmanshoop district. These investigations revealed that the syndrome only occurred during periods of drought and that it must have existed in the area at least since 1955. About eight farms were involved and the incidence varied from approximately 3 to 33 per cent. In one severe outbreak on a farm the mortality, which was mainly due to forced destruction of the animals for humane reasons, reached 73 per cent and included both ewes and lambs. The gestation period was prolonged up to at least 213 days. The ewes almost invariably suffered from dystocia due to the large size of the lambs and they had to be either assisted during parturition or slaughtered when assistance proved useless. Except for poorly developed udders and very distended abdomens no other symptoms were shown up to the time of parturition. Autopsies revealed nothing of significance except that corpora lutea graviditata were absent in most cases. The birth weight of the lambs was increased from an average of approximately 9 pounds for normal lambs to 27 pounds. When delivered alive many of them were lethargic, some showed slight spasms and the neonatal mortality rate was high. The fur of the Karakul pelts was invariably overgrown and therefore either economically worthless or of very little value; the hoofs were long and two or even all the incisor teeth fully erupted. Other notable features of the syndrome in lambs were olive green pigmentation, particularly of, and most intense in the kidneys and lymph nodes and polyfollicular ovaries. With the view to determine the possible cause of the condition specimens were collected and specifically examined for Rift Valley fever, Wesselsbron disease, vibriosis, brucellosis and other bacterial diseases with negative results. Mineral analyses of both water and vegetation did not show any excess or deficiency. Although inbreeding had occurred on a few farms it did not appear to be of major importance as a possible cause. The investigations indicated that in all the outbreaks the sheep were mainly dependent on a *Salsola* sp. for food during drought periods and that this plant was more resistant to drought conditions than other plants.

De Lange, Viljoen & Basson (1960) subsequently confirmed all the previous observations and in addition, described atrophy of the thymus and incomplete *descensus testiculorum* in the lamb as well as abnormal decrease of both amniotic and chorioallantoic fluid and thin semi-dry parchment-like foetal membranes. Microscopical examination of maternal tissues (De Lange, 1961) revealed slight central fatty degeneration of the liver; slight pigmentation of the kidneys, lungs, lymph nodes and spleen; and normal-sized adenohypophysis with alpha cells in various stages of activity and few small degranulated beta cells. The histopathological features observed in the foetus included pigmentation and central fatty degeneration of the liver; suspected intra-epithelial renal haemosiderosis; pigmentation of the lungs; various stages of ovarian follicular development and atresia; large follicles in the thyroid with flattened epithelium and a normal-sized hypophysis with pale degranulated alpha cells and an increased number of heavily granulated beta cells.

Further investigations (Basson, Morgenthal & Bilbrough, 1964; von Maltitz, 1964) indicated that grootlamsiekte was confined to two adjacent areas within the Keetmanshoop and Gibeon districts of South West Africa which have an approximate average annual summer rainfall of 170 mm. This entire area is situated on a low-lying semi-plateau with a thick broken layer of surface limestone and a characteristic vegetation which consists mainly of shrubs and short grasses. One of the differences noticed in the grazing on affected and unaffected adjacent farms was the presence of *Salsola tuberculata* on the former (Basson *et al*, 1964). On some of the farms the condition prevailed only in certain camps, invariably in association with good shrub covering and more particularly with *S. tuberculata*. Whenever barren ewes were transferred from affected to non-affected farms and served subsequently the disease did not manifest itself in them. Similarly, if ewes in the early stages of pregnancy were transferred to non-affected farms the disease failed to maintain itself, whereas removal in late pregnancy resulted in abnormal births for several weeks afterwards. The manifestation of the condition in localized areas and mainly during severe droughts afforded an indication that a plant or nutritional factor might have been playing a major role in the aetiology.

Morgenthal & Basson (1966) reported atrophy of the adrenal cortex and adenohypophysis, mild inactivity of the thyroid, enlargement of the female genitalia and increased hardness of bone in affected lambs. Histochemical studies on the pigment seen in various organs of the lambs indicated that it was lipoproteinaceous and contained sulphydryl and/or disulphide groups as well as aldehydes. The total white blood cell count of the affected ewe was higher than normal during the last week of gestation and the blood picture showed neutrophilia, lymphocytopaenia, eosinopaenia, hyperglycaemia, low potassium and high sodium content, which are probably all related to a stress response. The mean concentration of cortisol in cases of prolonged gestation was 2·52 μg per 100 ml plasma in comparison with 1·34μg/100 ml in normal Karakul sheep 24 hours before parturition.

Materials and Methods

Various aspects of the postmaturity syndrome were studied and attempts made to establish its aetiology.

1. *Symptomatology*

The clinical signs manifested by the ewes were studied in the affected area.

2. *Pathology*

The macroscopical studies and collection of specimens were conducted in the field in South West Africa. Both affected lambs and ewes were sacrificed

for post-mortem examination and tissues for histopathological examination were collected in various fixatives including ten per cent formalin, Susa's fixative, Zenker's solution, Helly's fluid (Cowdry, 1952) and formol sublimate. The ewes were destroyed either before or after signs of parturition or after lambing and some of the lambs were obtained by caesarian section and sacrificed within a few hours after delivery. With the exception of a few cases where weights of fresh, unpreserved organs were obtained, the endocrines and genitalia were weighed after formalin fixation (fixed weights) in the laboratory at Onderstepoort. This was done because of inadequate facilities in the field during the early stages of the studies. In order to determine atrophy or hypertrophy more critically the organ weight (in grams) was calculated as a percentage of the body weight (in pounds). This was called the weight index (WI) of the organ.

Tissue blocks from the fixed organ specimens of both ewes and lambs were embedded in paraffin wax and sections were cut from them with a sliding microtome at 3 to 4 μ thickness. These sections were routinely stained with haematoxylin and eosin (HE), but in addition special staining techniques were used on most tissues. Mallory Azan (MA) (Romeis, 1948), Periodic acid—Schiff (PAS) (Pearse, 1961), Performic acid—Alcian blue—Periodic acid—Schiff—Orange G (PFAAB) (Heath, 1956), Aldehyde Fuchsin—Light green (AFLG) (Kanematsu & Sawyer, 1963) and Aldehyde Fuchsin (Gomori, 1950) were used on sections of the hypophysis and PAS, Schmorl's technique for lipofuscin (Schmorl's) (Pearse, 1961), Kutlik's method for bile (Pearse, 1961), Perl's reaction for ferric iron (BB) (Pearse, 1961), Tirman and Schmeltzer's method for ferrous iron (T & S) (Pearse, 1961), Giemsa, Sudan black, oil red O (ORO) (Anon., 1960), Gomori's methenamine silver impregnation (GMS) (Anon., 1960), Ziehl-Neelsen (ZN) (Cruickshank, 1962), long ZN with Victoria blue (Long ZN) (Winter, 1961), Hueck's method for distinguishing melanins from lipofuscins (Hueck's) (Pearse, 1961), Sudan IV (SIV), alkaline tetrazolium (Alk. tet.) (Pearse, 1961), Pickworth's method for haemoglobin (Pickworth's) (Pearse, 1961), Ralph's method for haemoglobin (Ralph's) (Anon., 1960), microincineration followed by BB, PAS after diastase (PASD), PAS after phenylhydrazine (PASP) (Lillie, 1954), Mallory's method for haemofuscin (M. haem.) (Lillie, 1954), Luxol fast blue (LFB) (Anon., 1960), Gomori's recticulum impregnation (GRI) (Mallory, 1938) and Rubeanic acid technique for copper (RA for Cu) were done on sections of various organs from several cases to determine the nature of the pigment (Table 5). Sections of formalin-preserved livers, kidneys and adrenals obtained by routine freezing technique were also prepared routinely and stained with ORO.

In the hypophysis the acidophils and basophils were not counted individually but their numbers were estimated and according to the quantities divided into various groups. Comparative studies of the results obtained by this method were made between affected and experimental control sheep (*vide infra*) and a classification into normal and abnormal groups with average, increased and decreased numbers of granulated cells was made. Similar studies were undertaken to compare the number of Leydig cells and to evaluate the amount of lipid material in the liver, kidneys and adrenals. Twin lambs were also encountered on several occasions and but for the length of gestation and the occurrence of pigmentation, their data were disregarded in all the other analyses.

3. *Artificial production of grootlamsiekte*

(i) *Introduction of pregnant ewes into the enzootic area:* Twenty-five ewes from an unaffected area, with known dates of conception and in various stages of pregnancy were placed on natural grazing on a farm where the condition had manifested itself. They were confined to these camps for periods varying from 16 to 99 days before they were due to lamb. Within a few hours after birth the lambs were examined, weighed and sacrificed for post-mortem examination. Body and certain organ weights were taken and specimens collected in a similar manner to those described in (2) above.

(ii) *Feeding trials with* S. tuberculata *var.* tomentosa *on sheep at Onderstepoort*: Fourteen mature Merino ewes with regular oestrus cycles, as indicated by vasectomized rams, were selected for this experiment. The ewes were surgically provided with ruminal fistulae by employing the method of Quin, Van der Wath & Myburgh (1938). The experimental animals were hand-served by known fertile rams not less than three weeks post-operatively.

The ewes were then randomly divided into two unequal groups. One group consisting of six ewes received 250 grams of finely ground *S. tuberculata* suspended in one litre of water through the ruminal fistulae daily during the entire gestation period. The plant material was collected in the affected areas in South West Africa. This and subsequent groups fed with *S. tuberculata* in other experiments will henceforth be referred to as the *Salsola* groups. In the second group of eight ewes which served as controls one litre of clean water was administered intraruminally every day throughout the gestation period.

Three hundred millilitre samples of blood were withdrawn from the external jugular vein of each ewe at regular intervals (Fig. 2 to 5). Heparin was used as anticoagulant. The heparinized blood was centrifuged immediately after collection, and the plasma stored at $-15°$ C until analyzed for progesterone and cortisol.

Progesterone was determined according to Short (1958), but quantitated by means of gas chromatography using a Beckman GC-4. The column conditions used were: 6 ft $\times$ 4 mm glass column with 3 per cent SE-30 on 80–100 mesh Gas Chrom Q; 210° C; nitrogen carrier flow 50 ml/min; on-column injection port 300°; flame-ionization detector 270°.

Cortisol was determined by utilizing a method involving solvent partition, thin layer chromatography, the Porter-Silber reaction and spectrophotometry (Van Rensburg, Veterinary Research Institute, Onderstepoort, personal communication, 1967). Steroid values were not corrected for procedural losses.

Eleven additional hand-served pregnant Merino ewes were used similarly for artificial production of postmaturity and collection of specimens for histopathological studies and weight determinations. They were fistulated at approximately two months pregnancy and used for the feeding experiment two months before term. Seven ewes were given half a pound of *Salsola* daily. The four controls were treated similarly as the previous controls. Some of the experimental group, however, did not get their full rations of *Salsola* daily because of a shortage.

All the lambs of the various groups were slaughtered as soon as possible and not later than twelve hours after birth. Some of the ewes were also sacrificed. The carcass and organ weights were determined and specimens preserved in 10 per cent formalin for histopathological studies.

(iii) *Feeding trials with* S. tuberculata *on sheep within the affected area* (1966): Two large camps from adjoining affected farms in South West Africa covering an area of 1,096 hectares were used. They were subdivided into several smaller camps and supplied with paddocks, pens, sheds and water installations. Karakul or Karakul-Persian cross-bred ewes were obtained from either within or outside the affected area. Some were post-term ewes caesarianed previously. Two Karakul rams were procured from one of the affected farms. The ewes were divided into three groups which were treated differently, but all of them were initially put into paddocks and hand-served under supervision when on heat. Those ewes from the affected area were evenly distributed among the groups.

The control (lucerne) group of 25 ewes was fed an average daily ration of 3 lb lucerne hay and 4 oz whole yellow maize. A bone meal and salt lick (2: 1) was supplied *ad lib*. The ewes were constantly kept in paddocks and were supplemented with 2 oz of a 16 per cent protein concentrate daily during the last two months of pregnancy. A second group of 24 ewes (field group) was kept on a similar ration and put on to natural grazing after service. No concentrates or licks were given. The third group of 44 ewes was treated similarly but changed to an adaptation ration of maize meal, lucerne meal and molasses after the first heat period. This was maintained for approximately 18 days and the ewes were only served during the subsequent heat period. Thereafter they were supplied with a daily ration of 1·625 lb dried *S. tuberculata* leaves, 0·625 lb lucerne meal, 1 oz maize meal and 0·75 oz molasses. After a few weeks the *Salsola* leaves were increased to 2 lb and the lucerne meal substituted with 0·5 lb lucerne hay. The ration of some of these ewes was increased to 2·5 or 3 lb *Salsola* and 1 lb lucerne hay during the last fortnight of gestation. As previously stated the experimental group will be referred to as a *Salsola* group.

Most of the ewes in the control and field groups were served between January and March and those in the *Salsola* group between March and May.

The length of gestation and the weights of both ewes and lambs were recorded. All the lambs were sacrificed for pathological studies, but the ewes were saved for further experiments. Specimens were collected as indicated previously and weighed after formalin fixation. The weights of the larger organs were obtained before fixation. The preserved specimens were prepared similarly to those of the natural postmature lambs.

Two of the *Salsola* ewes were subjected to caesarian section at 177 and 184 days, before they showed any signs of parturition, in order to collect specimens for electron-microscopy. This study, however, is still incomplete and does not form part of this report.

(iv) *Determination of the time of insult* (1967): This experiment was conducted in the same camps within the affected area that were used during 1966. A Mettler precision balance was obtained for determination of finer weights especially of the endocrines and genitalia. All the available sheep used in the previous experiment and additional ones purchased within the affected area were used. They were all Karakuls or Karakul-Persian cross-bred sheep varying in age from two-tooth to adult full-mouth. Four Karakul rams were obtained, three of which came from affected farms and one from a farm outside the area. One of the local rams was a postmature survivor. All these rams were tested for fertility and all the sheep were immunized against enterotoxaemia, bluetongue, brucellosis, anthrax and corynebacteriosis before commencement of the experiment. Six teasers were selected for use.

Out of a total of 261 ewes 161 were selected for the experiment. They were initially adapted to handling and feeding by kraaling them overnight in paddocks and given approximately 3 lb of lucerne hay per 50 animals. One pound of maize was added after one week. During this time the teaser rams were put to the ewes for a period of 10 to 15 minutes every evening for five days. Twelve days later the ewes were selected for feed adaptation as soon as they came into oestrus. Feed adaptation was conducted as described for the *Salsola* group in the previous experiment. The ewes were divided into eight groups and each one was served under supervision at the appropriate time. The treatment and final number of ewes in each group which excluded those that had to be discarded because of conception failure or bad feeding, were as follows:—

Group 1: Fourteen control ewes were given lucerne hay throughout the entire gestation period. They were fed twice daily and the total feed per ewe per day was 2·5 lb lucerne meal, 0·625 lb lucerne hay, 2 oz molasses and 2 oz maize. Each ewe received her ration in an individual pen. The residue was given to the entire group in a communal trough. The lucerne hay in the ration, however, was not fed in the pens but given on a total basis in the same paddock.

Group 2: Sixteen ewes were fed on dried *Salsola* leaves throughout the gestation period. Initially 0·25 lb of dried *Salsola* leaves, 0·75 lb lucerne meal and 1 oz of molasses moistened with water were given twice daily to every ewe in separate pens. The amount of *Salsola* was gradually increased and the lucerne meal decreased until a maximum of 2 lb *Salsola* was being fed to every ewe approximately one month after conception. The ewes were given exactly the same amount of feed as those in

the lucerne group, the only difference being that 2 lb *Salsola* and 0·5 lb lucerne meal substituted the 2·5 lb lucerne meal of the lucerne group. The residue together with their lucerne hay ration was given to the entire group in a communal trough. The next five groups were all given *Salsola* in the same daily quantities as for Group 2 but during various periods of gestation.

Group 3: Thirteen ewes during the first 50 days of gestation.

Group 4: Eighteen ewes from 50 to 100 days of gestation.

Group 5: Seventeen ewes from the hundredth day up to parturition.

Group 6: Sixteen ewes during the first 100 days of gestation.

Group 7: Eighteen ewes during the last 100 days of gestation.

Group 8: Nineteen ewes were placed on natural grazing after conception.

The rams used were equally divided among the ewes of each group. The ewes that did not conceive at the first service were served during subsequent heat periods and most of them were served between March and April. The *Salsola* that was fed was picked from October 1966 up to August 1967. The possible variation in the nutritional value of the various rations was not expected to influence the results of the experiment.

The pregnant ewes were weighed at various intervals and the udders were examined regularly. During the lambing season the ewes were observed every day from approximately 6.30 am. to 11.30 pm. in order to calculate the gestation period and to obtain other data as accurately as possible. The various stages and duration of parturition were recorded for each ewe whenever possible. The lambs were cleaned and dried as soon as possible after delivery, subsequently weighed and slaughtered within 12 hours after birth for examination, weighing of all the fresh organs and collection of specimens. The weights of the lambs used in the final statistical analysis were calculated after subtraction of the weights of the forestomachs with their contents. This was done in order to obtain a more reliable weight as some of the lambs had already suckled or swallowed a large volume of foetal fluid. The ewes were weighed after expulsion of the foetal membranes and were only fed after the weighing. A few of them were sacrificed for post mortem examination, organ weight determinations and collection of specimens. The preserved tissues were examined by the methods described previously. Twins were not included in the analyses and histopathological studies.

4. *Feeding trials with* S. tuberculata *on female rats at Onderstepoort*

(i) *Testing the effect of the shrub on the oestrous cycle:* The plant material of *S. tuberculata*, mainly dried leaves and twigs, was collected in the affected area in South West Africa and despatched to the Onderstepoort laboratory where it was finely ground. Extracts with 96 per cent ethanol were prepared from some of the plant material by the continuous extraction of the shrub for 36 hours in a Soxhlet extraction apparatus.

The Onderstepoort strain of the Wistar white laboratory rat was employed and identification was accomplished by means of ear notches. The rats were weighed three times per week and the oestrous cycles followed by daily examination of vaginal smears. This procedure was followed for at least 16 days prior to the commencement of the experiments in order to familiarize the rats with the type of procedure and to select three groups of 12 females each with regular four day cycles and with an average body weight per group which did not vary markedly.

The control group received 40 per cent lucerne meal and the other two groups 20 and 40 per cent respectively of the finely ground *S. tuberculata*. This was mixed with the normal rat ration and feeding was commenced on the day of oestrus of the fifth cycle. It was continued for 15 days, after which the three groups received the normal rat ration used at Onderstepoort for another 34 days when the experiment was terminated. The oestrous cycles were followed by daily examination of vaginal smears.

A similar experiment was conducted where the plant material was substituted by the ethanolic extracts in such a way that each experimental group received the correct amount in their ration to replace the 20 and 40 per cent plant material. The effect of extracts other than that of the alcoholic compounds was also tested in this way.

(ii) *Testing the effect on gestation:* The preliminary procedures of the previous experiment were followed and the rats were divided in three groups of 24 each. The controls received 40 per cent lucerne meal in their ration and the other two groups 20 and 40 per cent *Salsola* throughout pregnancy. Recently proven fertile males were placed with the females on the third day of the fifth cycle. Coitus was confirmed when spermatozoa were detected in the vaginal smears and this was designated Day 1 of pregnancy. The length of gestation was recorded, the litters were counted and weighed immediately after partus and subsequently killed with ether. The adrenals and thymus glands were dissected and weighed on a Mettler precision balance.

(iii) *Testing the effect of the shrub on various organs:* Two groups of 12 rats each were used and the same procedure used in the first experiment was followed. The control group received 40 per cent lucerne meal in their ration and the other group 40 per cent finely ground *Salsola*. This was continued for 15 days and all the rats were then killed by decapitation when everyone in both groups was still in dioestrus. The weights of the various organs were determined on a precision balance.

5. *Analyses of* S. tuberculata

(i) *Determination of oestrogenicity:* Extracts from dried leaves and twigs of the shrub were prepared by using the method of Ostrovsky & Kitts (1963). Five immature female rats were used in each of two groups; the experimental and the control group and each animal in the former group received injections of 0·2 ml of the plant extract. The same volume of

physiological saline solution substituted the plant extract in the control animals. Six hours after injection the rats were killed by ether inhalation and the uteri removed and weighed on a Mettler precision balance.

(ii) *Determination of anti-oestrogenicity:* The methods described by Biely & Kitts (1964) were employed to determine anti-oestrogenicity in the shrub. Seven immature ovariectomized rats were used in each of five groups and every animal received a subcutaneous injection of 0·2 ml of the specific substance, saline or saline mixtures as described in Table 21. The rats were killed by ether inhalation and the uteri weighed, using the same technique that was employed for oestrogenicity determination.

6. *Trace element and vitamin A analyses on sheep*

Liver and some kidney specimens were collected from natural postmature lambs, experimental cases, control lambs and post-term ewes in 10 per cent formalin and absolute alcohol for trace element and vitamin A analyses respectively. The latter was determined by the Carr-Price photometric method (Moore, 1957) and the colorimetric method (Sandell, 1950) was used for the trace element determinations.

7. *Statistical analyses of experiments in South West Africa*

The data on which the most important statistical analyses are based were obtained in South West Africa from the results of Experiments 2 and 3 (iii and iv) above and more specifically of the feeding experiments during 1966 and 1967. Both field groups, however, were excluded. Whereas the 1966 experimental animals were subjected to two feeding treatments, viz. with lucerne hay which acted as controls and *S. tuberculata*, those of 1967 were divided into one lucerne group and six *Salsola* groups fed during various periods of gestation. The mean gestation periods were determined and Scheffe's test applied to test for a difference between the three groups with the highest gestation periods and the four remaining groups. In order to provide sufficient data for the analyses the response to *S. tuberculata* was determined by combining the findings of the *Salsola* group of 1966 with those of the three *Salsola* groups (2, 5 and 7) of 1967 with the longest gestation periods. The combined lucerne groups in both experiments acted as the control group.

Since only a limited number of ewes was slaughtered, only the organ weights of the lambs were used for more thorough statistical analyses. Those of all the twins were regarded as incomparable with single lambs and were disregarded. The means and variances of the organ weights were determined. The lambs from the 1966 and 1967 experimental groups were treated separately as most of the weights of the first experiment were determined after fixation in 10 per cent formalin. After testing for homogeneity of the variances of the *Salsola* and lucerne groups, the differences between the means were tested by the t-test using the hypotheses Ho $= \mu$ (lucerne) $= \mu$ (*Salsola*); σ^2 (lucerne) $= \sigma^2$ (*Salsola*) or Ho $= \mu$ (lucerne) $= \mu$ (*Salsola*); σ^2 (lucerne) $\neq \sigma^2$ (*Salsola*). In Table 23, T1 and T2 indicate respectively which of the above hypotheses were applied. For the hypophyses of the 1966 experiment a corrected fixed weight was used (Tables 9 and 10).

In the controls the relationships of the various organ weights to the body weight were determined and this was used to adjust the organ weights of the *Salsola* groups. These adjusted weights were subsequently used to determine how the organ weights behaved relatively to the lamb weight over the gestation period.

Another method used was to express the organ weight (in grams) as a percentage of the body weight (in pounds). This is called the weight index (*vide supra*). It gives an approximation of the true relationship between organ and lamb weights and could reflect changes such as hypertrophy and atrophy.

Histograms were made to compare the weight indices of the most important endocrines and body weights with the length of gestation. The two different lucerne groups were again treated as one control group with the normal limits of gestation varying from 148 to 152 days. The one case with a gestation period of 153 days, was regarded as unimportant and was not included in the calculations. The width of the histogram represents the number of cases and the mean gestation period was taken as the centre for construction of the histogram. The means of the body weight and weight indices are indicated by the heights of the histograms. The ewes in the combined *Salsola* group were divided into five sub-groups according to the lengths of gestation periods viz. 148 to 152 days (normal range), 153 to 155 days, 156 to 161 days, 162 to 170 days and 171 to 184 days. The number of available weight indices was not always equal to the number of body weights with the result that the means varied slightly. These discrepancies, however, were regarded as unimportant. Cumulative relative frequencies of the gestation periods, body weights and certain weight indices were also determined.

Results

1. *Symptomatology*

It became evident that outbreaks of grootlamsiekte were mainly but not exclusively confined to periods of drought. The condition also manifested itself during various seasons, but more particularly after periods with poor grazing. Both Karakul (Black-head Persian) and Karakul cross-bred sheep were affected. Age did not play any role.

All the observations made by workers referred to in the introduction were confirmed, the most important being the poorly developed udders, distended abdomens and dystocias. These findings, however, were not constant. Speculum examination of the post-term ewes revealed that the cervix was closed and that dilation commenced during the terminal stages just prior to parturition. Prolonged parturition was either due to oversized lambs or apparent uterine inertia.

2. *Pathological studies*

(i) *Macroscopical findings:* (a) Ewes: Sixteen post-term ewes were examined and the most important features were found to be present in the genitalia. Functional and regressed corpora lutea were frequently absent and the ovaries were usually rather inactive with few or no developing follicles. The foetal membranes had an olive green colour and sometimes appeared parchment-like with very small focal disseminated areas of calcification. The amniotic fluid was either depleted or increased in volume and was frequently stained olive green by the meconium. Chorioallantoic fluid was frequently decreased in volume (Table 1). No changes were noticeable in either cotyledons or umbilical cord.

During the prepartal stage the cervix was closed and many of the udders small and nonlactating. Pigmentation of the kidneys was either absent or very mild, and more brownish than olive green when present. The adrenals seemed slightly enlarged. The condition of most of the ewes was good.

TABLE 1.—*Foetal fluids of some natural post-term ewes*

Ewe	Amniotic fluid (ml)	Chorioallantoic fluid (ml)
1.....	Diminished but not measured	None
2.....	300	100
3.....	300	Not measured
4.....	600	Not measured
5.....	638	470
6.....	840	700
7.....	800	Not measured
8.....	1,030	None
9.....	1,065	None
10.....	2,425	None

(b) Lambs: Forty-eight postmature lambs and many borderline cases and apparently normal lambs were examined. All the most important features described previously such as increased birth weight, eruption of one or more pairs of incisors, long haircoat, pigmentation, atrophy of the adrenals, hypophysis and thymus and polyfollicular ovaries were confirmed (Plate 1, Tables 2 and 3). *Descensus testiculorum* was incomplete and the testes frequently present within the inguinal canal. The colour of the pigmented kidneys and lymph nodes varied from a light olive green to almost greenish-black and the carcasses in advanced cases had a slightly brownish discoloration. A striking feature was the mild greenish-brown colour of the bones. The entire genital tract in addition to the ovaries was enlarged in many of the female lambs.

(ii) *Microscopical findings:* (a) Ewes: The microscopical observations described by previous workers were confirmed but appeared to be relatively mild (Table 4). The absence of degenerative, necrotic or inflammatory changes was conspicuous. Medium type of fatty changes were noticed in both liver and kidneys of most cases, the adrenal cortex seemed slightly enlarged, the stroma at the base of the carunculae somewhat increased and the foetal membranes and base of the chorionic villi markedly pigmented. The pigment, which was intra-epithelial, stained positive with Schmorl's technique and was mildly PAS-positive. All the ewes had a small amount of such pigment in both liver and kidney cortex but it was prominent only in exceptional cases. Calcification was noticed in some of the cotyledons and foetal membranes.

The majority of cells in the hypophysis appeared to be acidophils which were either orangeophils or cells of an intermediate type not being either distinctly red or yellowish. Small numbers of granulated basophils were seen in most cases but some contained a conspicuous increase of these cells. In a few cases either somewhat basophilic or granular eosinophilic intranuclear inclusions were noticed in small numbers of both basophils and acidophils [Plate 7 (38 and 39)]. Specific changes in the other endocrines and organs were absent.

(b) Lambs: The most important changes are summarized in Tables 2 and 3 and will be dealt with more or less in order of significance. One of the most striking changes was the pigmentation which will be dealt with under the appropriate organs. For detailed cytological studies Susa's fixative was generally preferred, but formalin fixation was regularly used for general histopathological studies.

Endocrines: Hypophysis: The MA technique gave better differentiation and proved to be the most useful stain and all the others were eventually discarded. Susa's fixative was preferred for finer cytological studies but only formalin-fixed specimens were used for comparative studies and the MA technique. Atrophy and degranulation was one of the most regular changes noticed. The latter was evident from the weights as well as from the microscopical examination [Plate 3 (17)]. The adenohypophysis was mainly or almost exclusively affected, the various cellular elements being small and many either poorly granulated or degranulated. Some of the nuclei were obviously small and crenated, but not invariably so. The distribution of the various types of cells varied and the proportions were inconstant, but the tendency seemed to be more towards an increase in the number of granulated basophils and a decrease in well-granulated carminophils. In extreme cases both seemed to be small and degranulated. In a few exceptional cases the nuclei of a small number of both acidophils and basophils proved to be enlarged, the nucleoli being enormous and light purplish [Plate 3 (14 and 15)].

Adrenal: The microscopical examination confirmed the macroscopical observation of atrophy especially in the cortex [Plate 2 (7)]. The micrometrical measurements of cortex and medulla revealed a considerable decrease in size of the cortex, particularly of the zona fasciculata and zona reticularis. The zona glomerulosa remained either constant in width or possibly slightly enlarged and well demarcated. The ratio of cortex to medulla of 25 cases varied from 0·89 to 0·14 with an average of 0·51. The lipid content of the cortex as determined by staining with ORO was usually either low, absent or less frequently fairly abundant. In one case large eosinophilic, intracytoplasmic globules were found in the medulla.

37826—5

TABLE 2.—*Some endocrinological observations in natural postmature lambs*

No.	Lamb wt (lb)	Adrenals						Thyroid				Hypophysis			
		W I (Fixed)	Cortico-medullary ratio	Lipids			Atrophy	W I (Fixed)	Epithelium	Follicles	Colloid vacuoles	W I (entire hypophysis) (Fixed)	Carminophils: Decrease in number	Basophils: Increase in number	Atrophy
				z. glom.	z. fasc.	z. retic.									
*1	9·0			±	±	±							−	−	+
2	9·0		0·70						Cu–F	M–L–S	±		+	+	
3	9·5		0·45	±	±	±	+		F–Cu	L–M	±				
4	10·0	8·53		±	±	±		15·49	Cu–F	L–M	−	0·85			±
5	10·0	7·76						8·37				0·90	−	+	+
6	11·0														
*7	11·5		0·65	−	−	−	+		F	M	−		+		+
8	11·5		0·81	±	±	±	±		Cu–F	M–S	+		+		±
9	12·0	13·54		±	+	+			Cu–Col–F	M–S	+	1·12	−	−	+
10	12·0	6·88													
11	12·0														
12	12·0	6·09		−	±	±		5·32	F–Cu	M–L–S	−	0·74	+	−	+
13	12·5	2·49										0·43			
14	13·0		0·53	−	−	−	+		F	M	−		±	+	+
*15	13·0		0·68	+	±	±		4·16	Cu–F	S–M	−			+	+
16	13·0		0·31	±	±	±	++	4·39	Low Cu–F	M–S	+		+	−	±
17	13·5		0·69	±	±	±							+		
18	14·0														
19	14·0		0·28				++		F–Cu	S–M	−		−	−	±
20	14·0			++	±	±						0·68			+
21	14·5		0·89	±	−	−	++		F	M–S	±				
22	15·0	5·14		−	−	−	+	8·13	F	M–S	±				
23	15·0	5·18						6·74	F	L–M	−	0·47			+
24	15·0		0·53				+								
25	15·0		0·66	−	−	−	+		F–Cu	M	+		+		+
26	15·5		0·27				++		F	S–M	−		+	+	+
27	16·0		0·69	−	−	−	++		F–Cu	M–S	−		+	+	+
28	16·0														
29	16·3														
30	16·5		0·59	±	±	±	+		F–Cu	M–S	±		+		+
31	17·0			+	−	−						0·75	−	+	+
*32	17·5		0·77				+		F	S–M			+		+

TABLE 2.—*Continued*

No.	Lamb wt (lb)	Adrenals						Thyroid				Hypophysis			
		W I (Fixed)	Cortico-medullary ratio	Lipids			Atrophy	W I (Fixed)	Epithelium	Follicles	Colloid vacuoles	W I (entire hypophysis) (Fixed)	Carminophils: Decrease in number	B sophils: Increase in number	Atrophy
				z. glom.	z. fasc.	z. retic.									
33	17·5	4·25	0·42	++	++	++		5·60	F–Cu	M–L–S	−	0·51	−	+	+
34	18·0		0·34				++		F–Cu	M–L–S	±		−	+	+
35	18·0		0·38				++		Cu–F		−		−	+	+
36	19·5			±	−	−			F–Cu	M–S	−	0·50		+	
37	19·5	2·95	0·46	−	−	−	++	4·48	F	M	−	0·43	+	+	+
38	20·0	3·02	0·40	−	−	−	++	7·60	F	M–S	+	0·68	±	+	
39	20·0		0·55				+		F	L–M	−		±	+	±
40	23·0						+		F	L	−		±	+	±
41	23·0														
42	26·5														
43	27·0		0·50				++		F–Cu	L–M	−				
44			0·19	+++	±	±	++		Cu–F	M–S	−		+	+	
45			0·14	+	−	−	++						+	+	+
46				+	−	−	++		F–low Cu	M	±				
47				−	±	±			Low Cu–F		−				+
48				+	±	±			F–Cu						

* = Ewes that showed signs of parturition

z. glom. = zona glomerulosa

z. fasc. = zona fasciculata

z. retic. = zona reticularis

W I (fixed) = weight index of fixed weights

− = negative

± = extremely mild

± = mild

+ to +++ = increasing order of positive reaction

Cu = cuboidal

Col = columnar

F = flat

M = medium

L = large

S = small

TABLE 3.—*Some histopathological observations on natural postmature lambs*

No.	Lamb weight (lb)	Liver				Kidneys			Testes		Ovaries		
		Lipids	Hyalin degeneration	Pigmentation	Bile-duct proliferation	Lipids	Pigmentation	Suspected nephrosis	W I	Cells of Leydig	W I	Follicles	Corp. fibr. atr.
*1	9·0	±		+	±	±	+	−			1·22	+	
2	9·0	±		±	−	−	+	+		+			
3	9·5	+	±	+		++	+					++	−
4	10·0	±		+			+				1·84		
5	10·0	+	±	+			+				1·35	+	±
6	11·0		+	+	−		+	−				+	
*7	11·5	±		+	−	±	++	+		−			
8	11·5		++	+	−		++	−		+			
9	12·0		−	±	−		±	±			2·29	++	−
10	12·0			±			+				3·16	+	−
11	12·0								18·2				
12	12·0	+	−	+	−	±	++	−			9·53	+	
13	12·5										4·51		
14	13·0	−	+	+	−	−	++	−				++	−
*15	13·0	+	−	+	−	±	+	+			4·96	++	±
16	13·0	+	−	+++	++	+±	+++	−	22·1	+			
17	13·5	±	±	+	−	±	++					−	−
18	14·0										9·10		
19	14·0		−	++			+++					+	−
20	14·0												
21	14·5	±	−	+	±	±	+++	−		−			
22	15·0		±	+	±		+	−			16·75	+++	±
23	15·0										11·04	++	−
24	15·0		±	+	−		++	±			16·63	+	−
25	15·0	±	−	+	−	±	++	−				−	
26	15·5	++	±	+	−	±	+++	±				++	±
27	16·0	−	+	+			++			−			
28	16·0												
29	16·3										5·38	++	
30	16·5	±	±	+	±	±	++	±				++	+
31	17·0	−	±	++	−	−	+++	−		−			
*32	17·5	+	−	+	−	±	++	−		−			
33	17·5	±	−	+	±	±	+++	−	18·9	−			
34	18·0		+	+	±		+++	−		−			
35	18·0	+	−	++		±	++					++	+
36	19·5		−	++	±		+++	−		+			
37	19·5	+	+	+	±	++	+++	−	18·60	±			
38	20·0	++	−	+	±	−	+++	±			6·43	++	+
39	20·0			+			+++					+++	+
40	23·0		+	++	±		+++	−		−			
41	23·0		+	+	−		++++	±			20·75	+++	+
42	26·5		±	++	++		++++	±					
43	27·0			+			+++					+++	++
44		+	+	+	−	+	+						
45			−	+	−		+					++	
46												+	
47													
48				++			++					+	

Corp. fibr. atr.=corpora fibrosa atretica

PLATE 1.—1. Experimentally produced one day old postmature lamb weighing 17·5 lb. It was recovered by caesarian section at 177 days pregnancy when the ewe showed no signs of parturition. The overgrown pelt is evident. 2. The lower jaw of a postmature lamb (field case) with all four pairs of incisors well erupted. The numbers on the teeth represent length in mm. 3. Follicles in an ovary of another postmature lamb (field case) which weighed 16·5 lb. HE × 7·5. 4. Polyfollicular ovaries (4·77 gm) and uterine horns of a 23 lb postmature lamb (field case). 5. Inactive ovaries (0·04 gm) and uterus of a normal lamb.

TABLE 4.—*Histopathological observations and other data on some of the ewes*

Natural post-term ewes	Gestation (days)	Weight post-partus (lb)	Lamb weight (lb)	Signs of parturition	Adrenals						Hypophysis					Liver lipids	Kidney lipids	Regressing corpus luteum
					W I		Cortico-medullary ratio	Lipids			W I		Carminophils	Orangeophils	Basophils			
					Fixed	Fresh		z. glom.	z. fa c.	z. retic.	Fixed	Fresh						
K1	>150						1·03	++	±	±						++		1
K2	>150		19·5				0·60	++	±	±					+	++		
K3	185	89·0	15·0		3·60		0·90				0·72					±	±	
K4	>150	69·0	13·0	—			0·65	++	+	±			+±	+++	+±	+++	++	—
K5	>150	97·0	18·0	—	4·79		1·53	+	±	±	0·83							1
K6	>150	107·0	18·0	—			0·77	—	±	±				++	+	++	+	1
K7	>150	100·0					1·00						++	++	±	—	—	1
K8	>150						1·70											
K9	>150		14·0				0·97	+±	±	±						—		—
K10	>150		17·0					++	±	±			+±	+++	+±	++		1
K11	>150		9·0					++	±	±					++	++	±	—
K12	>150	80·0	14·0	—	5·12		0·80	+++	±	±	1·32		+±	++	±			1
K13	>150	69·0	16·0	—			1·36											—
K14	>150	64·0	11·5	—			1·90									+	+±	—
K15	>150	74·0	15·5			5·00	0·61						±	+++	+	+	±	—
K16	>150	94·0	14·0					±	±	±					+±	++	—	
Experimental post-term ewes																		
M1	159	116·0	11·11	+	3·40	3·16	0·68	—	±	—		0·83	+	++	++	++	—	
M2	161	92·0	9·90	+		3·89	1·00	—	±	—		0·62	+++	++	±	++	+	
M3	157	106·0	8·96	+	3·10	2·98						0·61				+	—	
K4	166	119·5	10·06	+		3·12	1·16	++	±	±		0·60	+	++	++	±	±	1
K5	155	88·0	12·44	+		6·08	1·07	+	±	—		0·87	+	++	—	++	++	1
K6	163	106·0	9·56	+		4·45	1·00	+	±	±		1·15	+	++	+	++	+	
Control ewes																		
K1	151	124·0	10·84	+		2·73	0·94	—	—	—		0·75	±	++	+		±	1
M2	148	113·0	6·93	+	3·58	3·55	0·50	—	—	—		0·91	+	++	+±	+	—	1
M3	149	127·0	8·87	+	3·70	3·36	0·61	—	—	—		0·67	+	++	++	++	—	1
M4	150	111·0	8·80	+	3·65	3·43	0·72	—	—	—		0·87	±	++	+	+±	—	—
M5	148	82·0	9·50	+	3·23	3·07	1·00	—	—	—		0·80	++	++	+	±	—	1
M6	149	105·0	7·61	+	3·20	3·11	1·22	—	—	—		0·95	+++	++	+			1
M7	149	111·0	11·00	+	4·62		0·92	—	—	—			+++	++	+	—	—	1
M8	152	118·0	11·37	+	2·90	2·84	0·77	—	—	—		1·01	+	++	±	±	—	1
M9	149	120·0	9·42	+	3·57	3·46	0·90	—	±	—		0·66	++	++	±	+	—	1

M = Merino.

K = Karakul.

Thyroid: The follicular epithelium was usually either low cuboidal or flat, the colloid stained well but contained a small number of vacuoles (Plate 4). Most of the follicles were either medium-sized or large.

Thymus: The width of the cortex was relatively narrow and lymphocytes present in either fairly small or medium numbers.

Pancreas: Most of these organs proved to be normal, but some were atrophic and slightly oedematous [Plate 4 (24)].

Pineal: No significant changes were found.

Genitalia: One of the most striking features in advanced cases was the extremely large and polyfollicular ovaries which was due to both maturing and atretic follicles [Plate 1 (3)]. On section a maximum of 70 maturing and atretic follicles was counted in one case. No unequivocal evidence of cystic follicles was found although a few with a single layer of flat granulosa cells appeared rather suspicious. Corpora fibrosa atretica with fairly advanced glassy membrane development were noticed in 36 per cent of the cases examined [Plate 2 (6)]. Congestion and intra-follicular haemorrhages were seen in some. Enlargement of the female genital tract with mucous secretion was fairly frequently noticed. However, apart from a high columnar and an actively secretory cervical and uterine epithelium in some of the lambs, no other specific microscopical features were seen. Leydig cells in the testes were either absent or present in small numbers. Other changes in the male genitalia were not found.

Liver: Seventy per cent of the cases had various degrees of liver degeneration of which 44 per cent proved to be fairly mild. The changes almost invariably resembled hyaline globular degeneration, but the specific initial site of development in the lobuli could not be determined [Plate 3 (12)]. The impression was gained that there appeared to be no specific preference for any area and that the distribution was fairly even throughout the lobuli. Some of the nuclei and nucleoli of the parenchyme cells were enlarged. Accumulation of pigment was not such a regular finding as in the kidneys nor did it occur in parallel degrees of severity. It started centrilobularly and was found in the parenchyme cells as well as in the Kupffer cells [Plate 2 (11)]. Enlarged pigment-laden Kupffer cells were frequently rounded off, released and eventually found in the lumens of the central and hepatic veins. Pigment-laden macrophages were also seen within the intima of these vessels. The Kupffer cells were often markedly activated with a progressive accumulation of khaki-brown pigment which stained negative for iron, lipids, copper and bile, but proved to be strongly positive with the Schmorl's technique for lipofuscins. However, in some of the very advanced cases some bile pigment-positive substance was found within the lipofuscin-positive material in conjunction with bile stasis. In 36 per cent of the total number examined, mild or prominent bile duct proliferation was evident [Plate 3 (13)]. Bile epithelium in several cases was very actively secreting a mucoid substance and also contained many fat droplets. Haemosiderin was found in abundance in both Kupffer and parenchyme cells of many of the lambs. Seventy per cent of the livers contained medium, large or more frequently small amounts of fat.

Kidneys: The pigmentation noticed macroscopically proved to be intraepithelially within the cytoplasm of the convoluted renal tubuli [Plates 2 (8 and 9), 6 (34)]. It was of the same colour and histochemical nature as the khaki-brown, Schmorl's-positive pigment of the liver and in the early stages was noticed to start in the centre of the labyrinths close to the cortico-medullary junction. The size and shape of the pigment granules and globules varied considerably and with formalin fixation invariably seemed to lie within a vacuole. Unequivocal signs of degeneration were lacking but 36 per cent of the cases with prominent pigmentation revealed a very marked vesicularity and rarefaction of the epithelial cytoplasm within the cortex. Although a certain degree of vesicularity was present in all cases the moderately or extremely marked degree was absent in those with either mild or extremely severe pigmentation. Some of the smaller pigment globules in the early stages of pigmentation were very mildly PAS-positive, but occasionally very strongly PAS-positive globules were noticed within Bowman's capsule. Overt pigmentation in the renal corpuscles, however, was never noticed. The lipid content varied from negative to prominent, but more than half of the 62 per cent clearly positive cases were only mildly positive for fat. Foci of metastatic calcification in the medulla were seen in exceptional cases.

Lymph nodes: Most of the lymph nodes contained a pigment within the reticulo-endothelial (r.e.) cells of the medulla. The intensity of pigmentation was more marked in the lymph nodes of the body cavities more particularly those of the abdominal cavity. The colour and histochemical nature of this pigment were similar to those of the lipoproteinaceous hepatic and renal pigment [Plate 5 (28)]. In some cases, however, very markedly PAS-positive granules and globules were found in the r.e. cells of the mesenteric lymph nodes and a conspicuously smaller number in some of the other nodes. A number of nodes had a marked increase of neutrophils, more particularly in the medullary cords and sinuses. The cortex was never very prominent, and appeared extremely thin and atrophic in some of the lambs.

Lungs: A very mild mobilization of foam macrophages was noticeable in some of the cases and Schmorl's-positive pigment was often present either in macrophages or free in the alveoli, but the pigmentation invariably remained either mild or very mild.

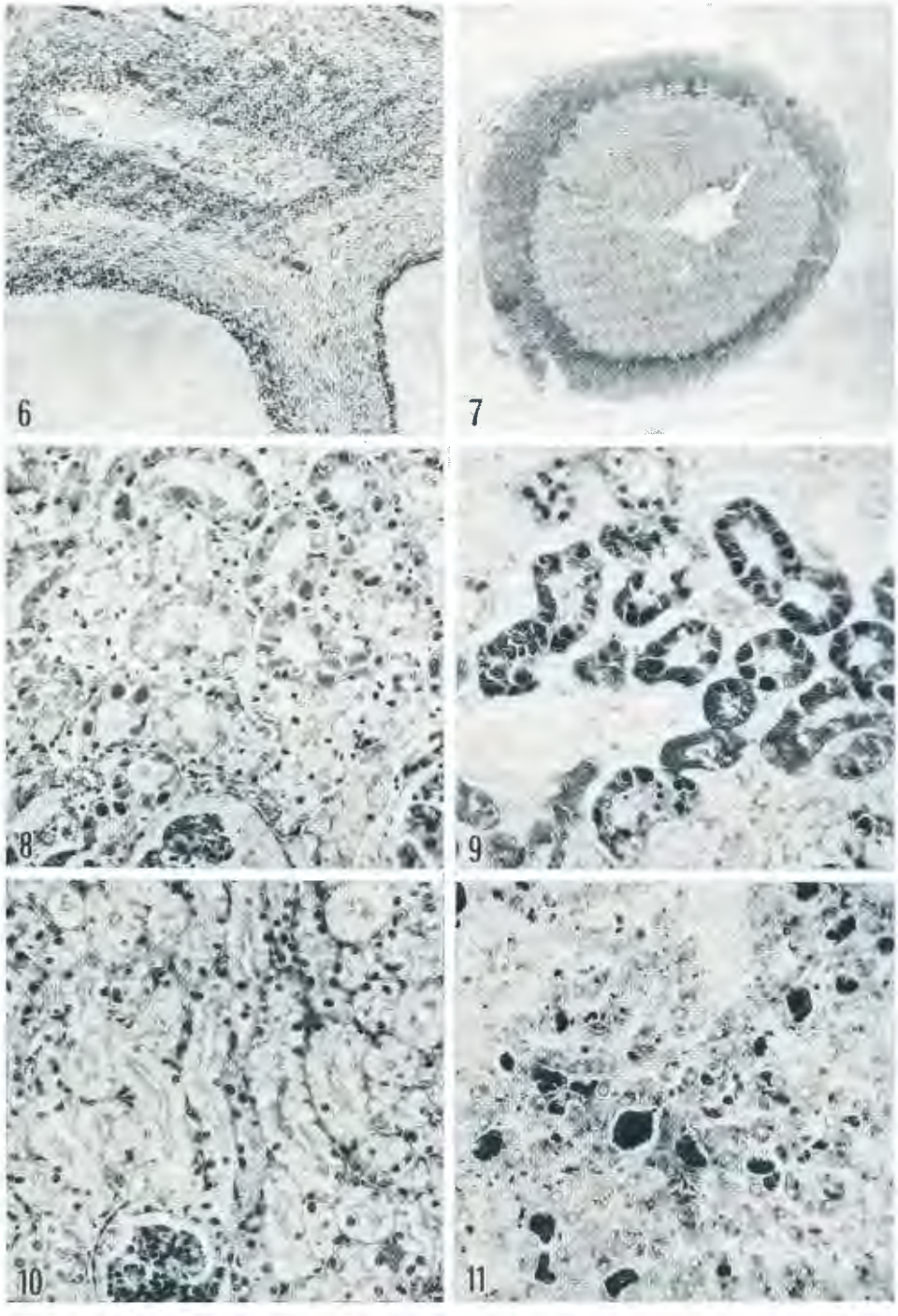

PLATE 2.—6. Larger magnification (× 75) of the ovary demonstrated in Plate 1 (3), showing an advanced corpus fibrosum atreticum with the inner glassy membrane almost completely developed. 7. Cortical atrophy in the adrenal of a field case of postmaturity. HE × 12. 8. Intra-epithelial pigment in the kidney of a 23 lb postmature lamb. HE × 200. 9. Schmorl's special staining technique applied to a kidney section of the same case demonstrated in 8. × 200. 10. Kidney section of an artificial case (lamb) born after 180 days gestation. Extreme rarefaction and vacuolarity are very prominent. Pigment granules are also present. HE × 200. 11. Liver of a 13 lb postmature lamb (field case) with marked pigmentation. Schmorl's × 200.

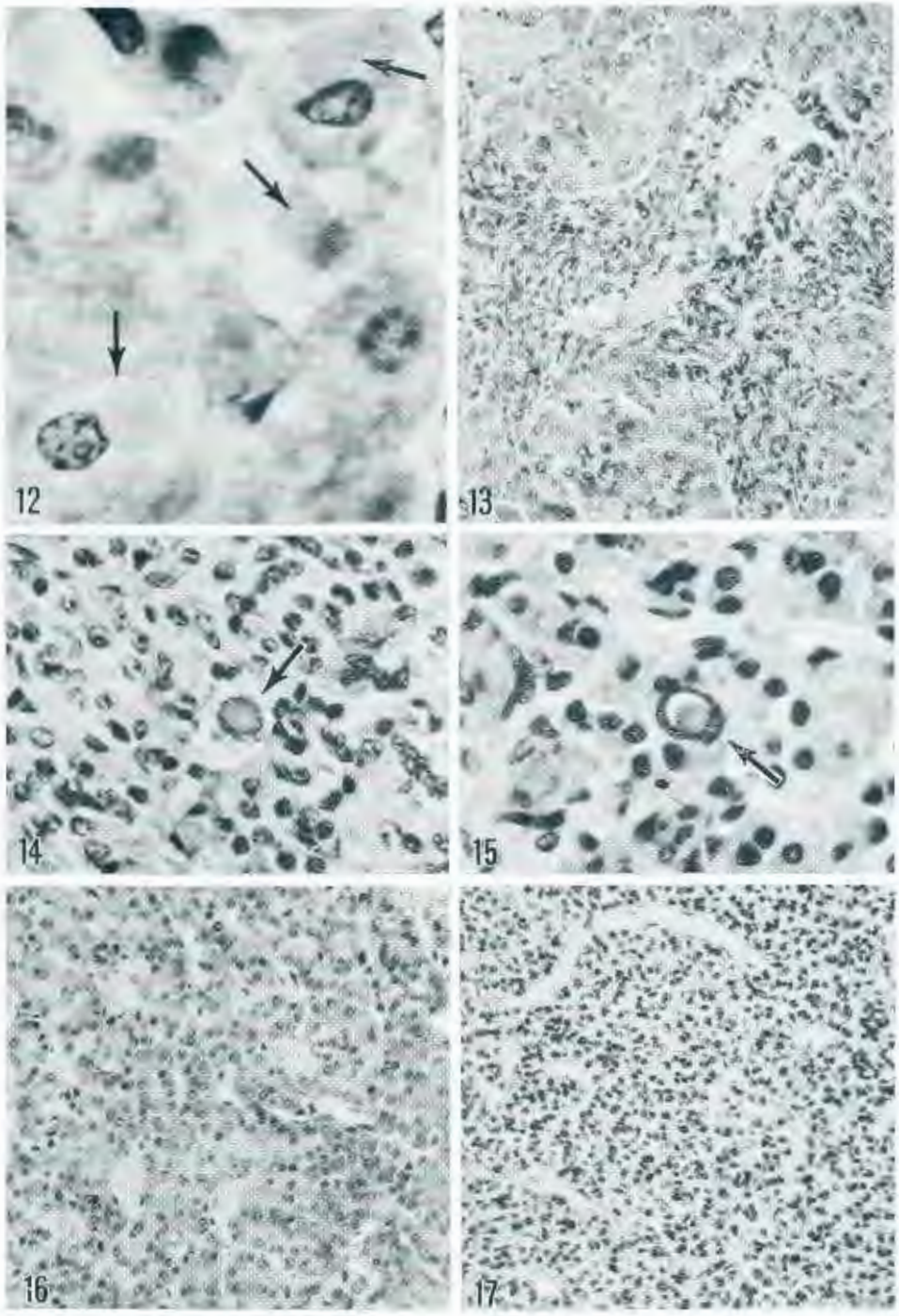

PLATE 3.—12. Hyalin globules (see arrows) in the hepatic cells of a 15 lb artificially produced postmature lamb born after 184 days of gestation. HE × 1530. 13. Bile duct proliferation in the liver of the case demonstrated in Plate 2 (11). HE × 500. 14 & 15. Intranuclear inclusions (arrows) in the adenohypophysis of a 19·5 lb postmature lamb (field case). HE × 500 & 620 respectively. 16. Adenohypophysis of a control (normal) lamb. HE × 200. 17. Adenophypophysis of a postmature lamb (field case) of 12 lb for comparison with a control (16) at the same magnification (HE × 200). Note marked degranulation and atrophy.

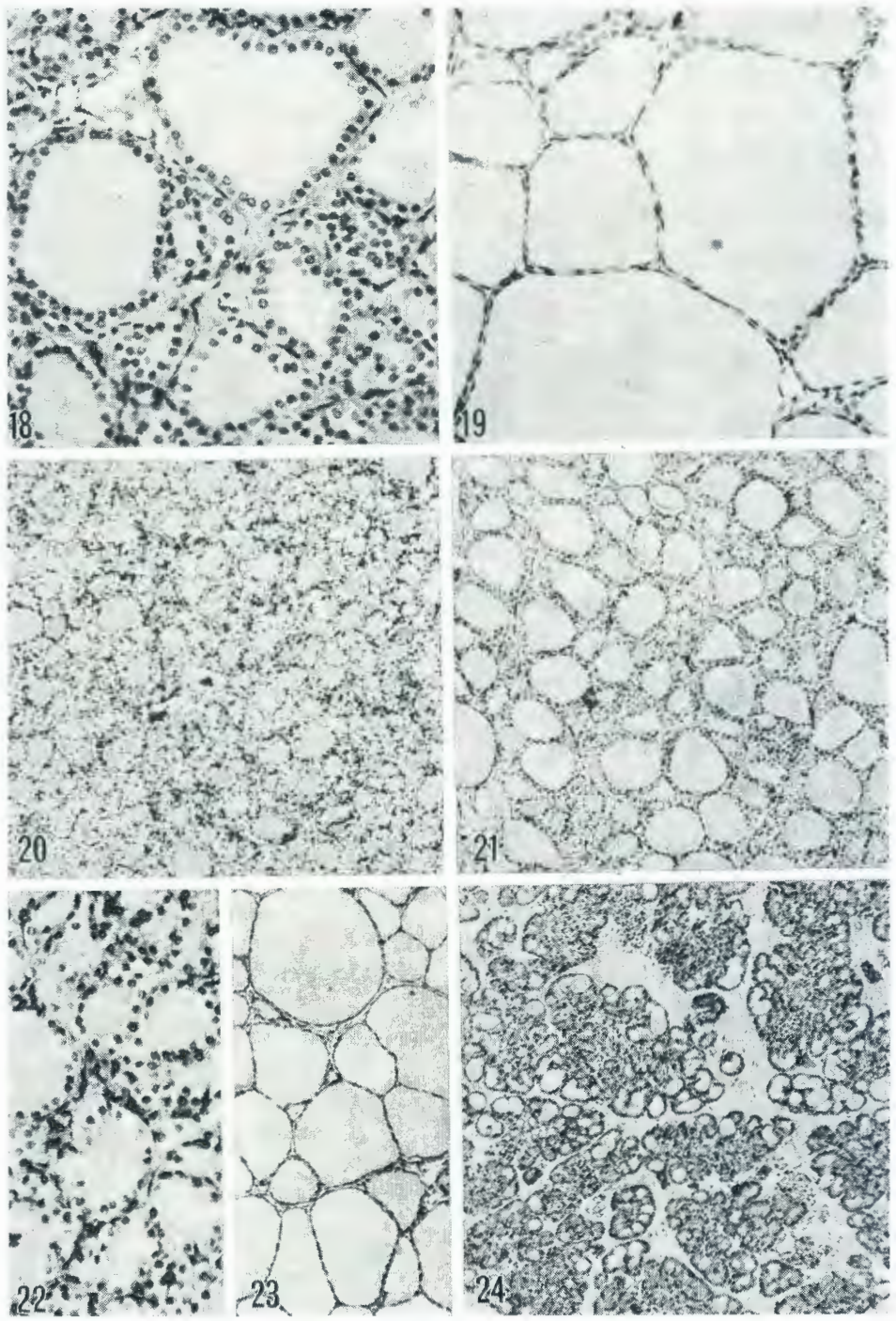

PLATE 4.—18. Normal appearance of thyroid in a control lamb. HE × 200. 19. Thyroid of an experimentally produced postmature lamb (10·5 lb at 164 days gestation) at the same magnification. Note large follicles, flat epithelium and non-vacuolar colloid. HE × 200. 20. Thyroid of another control lamb where the follicles are considerably smaller than in 18. HE × 75. 21. Thyroid of an experimentally produced postmature lamb (9 lb at 161 days gestation) close to normal but with some early stages of abnormal changes. HE × 75. 22. Normal thyroid (control). HE × 200. 23. Abnormal thyroid of a natural postmature lamb of 23 lb. HE × 75. 24. Pancreas of a postmature lamb (field case) showing signs of atrophy. HE × 75.

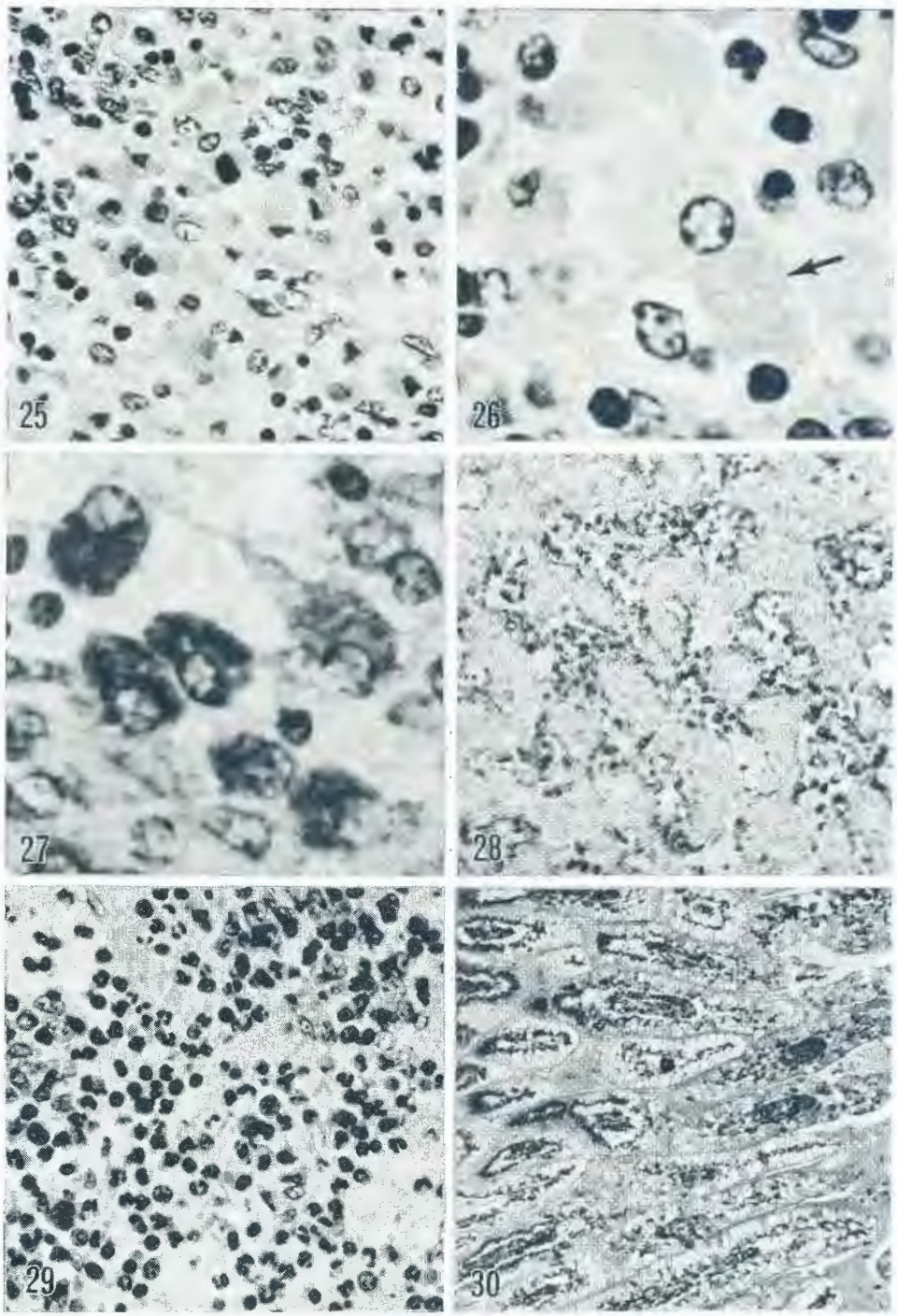

PLATE 5.—25. Mesenteric lymph node of an experimentally produced postmature lamb with pigmentation of the order 1+ showing the accumulation of eosinophilic globules and granules in the r.e. cells (151 days gestation). HE × 500. 26. Section of the same lymph node clearly showing the presence of globules (arrow) and granules within the r.e. cells of the medullary sinuses. HE × 1200. 27. Dark globules in the r.e. cells as demonstrated by the PAS technique in the macroscopically unpigmented mesenteric lymph node of a control lamb. PAS × 1200. 28. Pigment demonstrated in the pigmented mesenteric lymph node of a 16 lb postmature lamb (field case). Schmorl's × 200. 29. Infiltration of neutrophils in the mesenteric lymph node of a control lamb (infrequent finding). HE × 500. 30. Deeply eosinophilic globules probably originating from degenerating goblet cells in the small intestine of a control lamb (exceptional finding). HE × 75.

Other organs: No noteworthy changes were noticed in any of the other organs or tissues except the presence of Schmorl's-positive pigment which, in cases of severe pigmentation, was present to a mild degree in the interstitial cells of the endocrines (even hypophysis), myocardium, voluntary muscle, pancreas, spleen, periosteum, Peyer's patches, testes, intima of some veins and aorta and around some of the vessels in the brain.

Cholesterol clefts were noticed in the neurones of a number of lambs.

The histochemical studies of the pigment are outlined in Table 5.

3. *Artificial production of postmaturity*

(i) *Introduction of pregnant ewes into the enzootic area:* The results are listed in Table 6 and all those findings marked with asterisks indicate possible abnormal tendencies of postmaturity.

(ii) *Feeding experiments on sheep at Onderstepoort:* Two of the *Salsola* ewes had to be discarded because of pregnancy toxaemia. The findings on the other experimental and control sheep are summarized in Tables 4, 7 and 8. The length of gestation was prolonged up to 161 days in the *Salsola* group (Fig. 1), but the difference in lamb weights was not marked. Pigmentation was noticed in a few of the lambs from the *Salsola* group. No unequivocal evidence of atrophy was noticed in either hypophysis or adrenals of the lambs, but the maternal adrenals appeared slightly enlarged. The female genitalia of the *Salsola* lambs were also somewhat heavier than those of the controls.

Progesterone values: The patterns followed by the plasma progesterone of the two groups are illustrated in Fig. 2 and 3. A characteristic feature was the very sharp and sudden progesterone increase of both groups in the latter third of gestation, after it had maintained relatively constant values for a variable length of time during mid-pregnancy. The plasma progesterone of the control ewes reached this relatively constant level between 70 and 80 days after conception, and commenced to increase sharply at 110 days. In the *Salsola* group, after the initial rise, the progesterone level flattened off after approximately 40 to 50 days gestation, then increased at a much slower rate during the ensuing 40 to 50 days. At 110 days gestation a similar but slightly more gradual rise than in the controls was found.

The progesterone of the control ewes reached its highest concentration after 132 days gestation. During the last 10 to 15 days before parturition, the controls' plasma progesterone decreased rapidly from between 1·1 and 1·2 to between 0·35 and 0·57 μg/100 ml plasma. The *Salsola* group, on the other hand, reached its highest level of approximately 1·15 μg/100 ml plasma at about 148 days gestation. It took between 7 and 11 days to fall from its highest concentration to between 0·6 and 0·68 μg/100 ml plasma at parturition.

The progesterone concentration of the control group was significantly higher ($P<0·05$) than that of the *Salsola* group between 120 and 140 days. The converse is true if the progesterone of the controls is compared with the corresponding results of the *Salsola* ewes between 140 and 160 days gestation; the values for the control were significantly less ($P<0·02$) than those of the *Salsola* group.

It was evident that the parturient progesterone levels of the *Salsola* group were higher than those of the controls at parturition ($P<0·05$).

TABLE 5.—*Histochemical nature of pigment in lambs*

Technique	Result	Reaction
R A for Cu	Negative	Negative for copper.
B B	Negative	Negative for ferric iron.
T & S	Negative	Negative for ferrous iron.
Microinc. + B B	Negative	Negative for masked iron.
Pickworth's	Negative	Negative for haemoglobin.
Ralph's	Negative	Negative for haemoglobin.
S IV	Negative	Negative for lipids.
O R O	Negative	Negative for lipids.
Sudan Black	Negative or occasionally slightly positive	Probably indicates presence of lipofuscin.
L F B	Negative	Negative for phospholipids.
M. Haem	Positive	Positive for lipofuscin.
Schmorl's	Positive	Contains either lipofuscin, melanin, argentaffin granules or SH-groups.
Hueck's	Positive	Positive lipofuscin, negative for melanin.
ZN	Negative	Not acid-fast.
Long ZN	Negative or mildly positive	Sometimes slightly acid-fast.
G M S	Negative–Positive	Progressively argyrophilic.
P A S	Progressively ±, +, −	Early stages contain either polysaccharides, muco-proteins or unsaturated lipids.
P A S D	Positive	Negative for glycogen.
P A S P	Negative	Contains aldehydes and/or ketones.
Alk. Tet	Red & Blue	Positive for lipid and protein-bound reducing groups (SS and SH-groups). Early stages more lipid-bound.
Kutlik's Bile	Negative except for a positive centre in a few very advanced cases (liver)	Almost invariably negative for oxidizable bile pigments.
Giemsa	Reddish—mildly bluish	Initially acidophilic and then progressively mildly basophilic.

TABLE 6.—*Observations on lambs from pregnant Karakul ewes introduced into the enzootic area*

No.	Gestation (days)	Days in camp	Weight (lb)	Sex	Teeth. Pairs of incisors erupted	Pigmentation	Hypophysis	Adrenals		Thyroids		Ovaries		Testes
							WI	WI	Cortico-medullary ratio	WI	Epithelium	WI	Follicles	WI
1	148	16	8	♀	(1)	—	1·80	7·26	0·8		Cu–F			
2	151	19	8	♀	1 (3)*	—	1·76	8·22	1·0	5·62	Cu–F	0·62		
3	149	23	9	♂	1*	+	1·72	9·59*	0·9	7·20	Cu			26·01
4	148	22	6	♂	—	±	1·52	9·76	0·67*		Cu–F	0·98		
5	153	36	9½	♀	(1, 3)	±	1·68	6·19		8·00	Cu	0·53		
6	151	34	9	♀	1*	—	1·90	6·28	0·53*	7·67	Cu	0·56		
7	151	35	9	♂	—	—	1·85		0·8	9·56	Cu			24·75
8	154*	36	11	♂	—	—	1·13*	6·86	1·02*	6·22	Cu–F			18·21*
9	149	32	6	♂	—	—	1·97	14·49*		7·51				27·54
10	150	33	5½	♀	(1)	—	1·85		0·82	8·71		0·68		
11	151	33	8	♂	(1)	±	1·71	9·17	0·47*	5·9	Cu–F			24·15
12	148	32	9	♂	—	—	1·72	8·68	0·53*	8·75	Cu–F			23·38
13	150	30	9	♂	(1, 3)	—	2·11	5·82*		7·2	Cu			23·93
14	148	30	6½	♂	—	—	2·06	7·56	0·48*	8·63	Cu			24·83
15	154*	37	8	♀	—		1·78	6·35		9·70	Cu–F	6·82*	+	
16	156*	38	9	♀	—	±	1·54	9·03		4·6	Cu	0·61		
17	151	37	7	♀	(1, 3)	+	1·68	6·48	0·9	7·63	F–Cu*	1·11*	+	
18	150	47	10	♂	(1)	±					Cu			
19	151	57	8½	♂	1*	+								
20	149	62	10	♂		—			0·61*		Cu			
21	153	49	10	♂	(1)	±	1·38	7·26		6·11	Cu			28·48
22	153	52	9	♂	—	±	1·24*	5·73*	1·05*	6·87	Cu			26·10
23	155*	54	9	♀	(1, 2, 3)	+			0·7		Cu			
24	152	66	8	♀	—	±			0·76		Cu			
25	153	99	6½	♀	(1)	±					Cu		+*	
AV.	151·1													

* Indicates abnormal tendencies.

Teeth: Those in brackets indicate partial eruption.

TABLE 7.—*Fresh organ weights (in grams) and other data of lambs from* Salsola*-fed Merino ewes at Onderstepoort*

No. of lambs	Gestation (days)	Weight (lb)	Incisors erupted (pairs)	Pigmentation	Brain	Kidneys	Liver	Spleen	Thymus	Pancreas	Hypophysis	Hypophysis WI	Adrenals	Adrenals WI	Adrenals C/M ratio	Thyroids	Pineal	Ovaries	Ovaries WI	Genitalia (without vagina)	Testes	Testes WI
1	149	6·5	—	—	54·26	16·56	69·05	4·02	6·4511	2·4980	0·1600	2·46	0·5910	9·09	0·6	0·4362	0·0080				1·8481	28·43
2	151	9·1	—	—	57·25	29·81	96·31	11·10	19·7130	5·4000	0·1430	1·55	0·6213	6·75	0·7	0·6208	0·0389				2·6364	28·66
3	153	7·3	(1)	—	57·54	21·34	53·33	4·35	9·7175	2·6875	0·1075	1·47	0·6955	9·53	0·8	0·6590	0·0170				1·8610	25·49
4	154	9·7	(1)	—	54·50	22·11	65·70	4·05	11·1208	4·5060			0·5888	6·07	0·5	0·7302	0·0284	0·4435	4·57	3·7608		
5	154	6·7	—	—	55·70	17·21	54·30	5·11	11·9800	2·3866	0·1350	2·01	0·5156	7·70	0·8	0·6638	0·0153				2·7557	41·13
6	157	9·0	(1)	—	67·00	19·20	83·10	5·90	10·3000	5·0681			0·6057	6·73	0·8	0·6403	0·0212				3·2890	36·54
7	159	11·1	—	—	73·30	40·50	166·50	6·75	8·3320	5·9924	0·1865	1·68	1·2423	11·19	0·8	0·8856	0·0243				4·0209	36·22
8	159	11·4	1, 2	—	57·50	28·40	143·00	5·57	11·3866	4·9180	0·1650	1·45	0·8289	7·27	1·5	1·0089	0·0265	0·5495	4·82	3·8615		
9	160	9·7	—	—	60·04	22·81	93·15	7·82	16·1302	4·8278	0·1478	1·52	1·0478	10·80	1·2	0·6667	0·0238				3·0186	31·12
10	160	8·9	1, 3	—	53·50	21·50	82·40	3·71	10·8000	3·8500			0·5892	6·62	0·8	0·3799	0·0094				2·9485	33·13
11	161	9·9	1, 3	+	56·60	29·70	106·60	6·69	11·2000	4·4112	0·1343	1·35	0·5408	5·46	0·5	0·5976	0·0177				2·8631	28·92
Mean	156·1	9·0			58·83	24·47	92·13	5·92	11·5573	4·2314	0·1474	1·69	0·7152	7·93	0·8	0·6626	0·0210	0·4965	4·70	3·8112	2·8046	32·18

TABLE 8.—*Fresh organ weights (in grams) and other data from control Merino lambs at Onderstepoort*

No. of lambs	Gestation (days)	Weight (lb)	Incisors erupted (pairs)	Pigmentation	Brain	Kidneys	Liver	Spleen	Thymus	Pancreas	Hypophysis	Hypophysis WI	Adrenals	Adrenals WI	Adrenals C/M ratio	Thyroids	Pineal	Ovaries	Ovaries WI	Genitalia (without vagina)	Testes	Testes WI
1	148	6·9	—	—	62·00			4·46	16·3000	4·6206	0·1217	1·76	0·6284	9·10	0·6	0·8510					3·0110	43·63
2	148	9·5	—	—	54·60	23·20	99·80	6·50	20·8000	3·5694	0·1577	1·66	0·6309	6·64	0·8	0·7385	0·0191	0·2305	2·43	2·9578		
3	149	7·6	—	—	61·00			7·64	25·3000	3·5347	0·1426	1·88	0·5626	7·40	0·8		0·0136				2·7767	36·53
4	149	9·4	—	—	62·50	29·80	91·30	6·16	23·5000	3·2471	0·1601	1·70	0·9691	10·31	1·0	1·0742	0·0155				3·5106	37·34
5	149	8·9	—	—	55·00	24·50	96·20	7·97	25·7000	4·1193	0·1250	1·40	0·7079	7·95	0·8	0·6553	0·0238	0·0780	0·88	2·7114		
6	149	11·0	—	—	68·20	24·70	94·20	5·92	26·5000		0·1464	1·33	0·7739	7·04	0·7	0·8469	0·0125				2·9854	27·14
7	150	8·8	(3)	—	57·40	22·30	86·00	9·04	23·7000	3·8852			0·6084	6·91	0·7	0·6884	0·0123	0·1152	1·31	2·9532		
8	150	4·5	—	—	54·24	18·22	68·20	5·15	11·1550	2·7030	0·1094	2·43	0·6575	14·51	0·7	0·4030	0·0226	0·0650	1·44	2·8600		
9	151	Twin	—	—																		
10	152	11·4	(3)	—	59·50	26·60	125·70	7·38	32·8000	4·6040	0·2255	1·98	0·7692	6·75	1·0	1·4592	0·0124				3·2106	28·16
11	152	6·5	—	—	59·50	16·33	58·00	4·98	8·3925				0·4573	7·04		0·6817		0·0560	0·86	2·0103		
12	153	10·4	—	—	70·23	21·58	101·10	7·01	16·3690	3·4816	0·1623	1·56	0·7978	7·67		0·6675	0·0174				3·3850	32·55
Mean	149·9	8·6			60·38	23·03	91·17	6·56	20·9560	3·7517	0·1501	1·74	0·6875	8·30	0·8	0·8066	0·0165	0·1089	1·38	2·6985	3·1466	34·23

$\frac{C}{M}$ = Cortico-medullary.

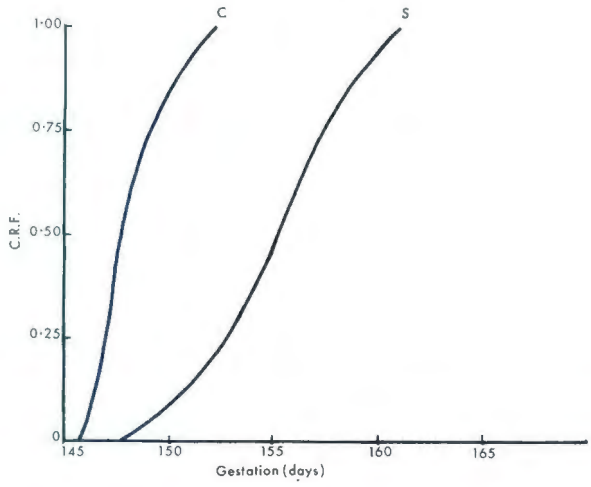

FIG. 1.—Cumulative relative frequencies (C.R.F.) of gestational length in control (C) and *Salsola*-fed ewes (S) at Onderstepoort (free-hand curves).

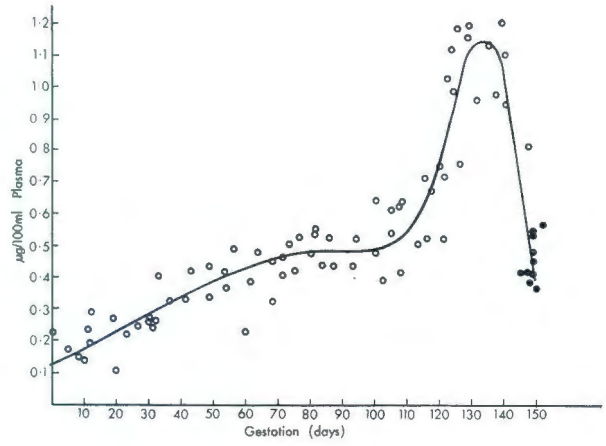

FIG. 2.—Plasma progesterone levels in pregnant control Merino ewes (free-hand curve). The solid black circles indicate the values on the day of parturition.

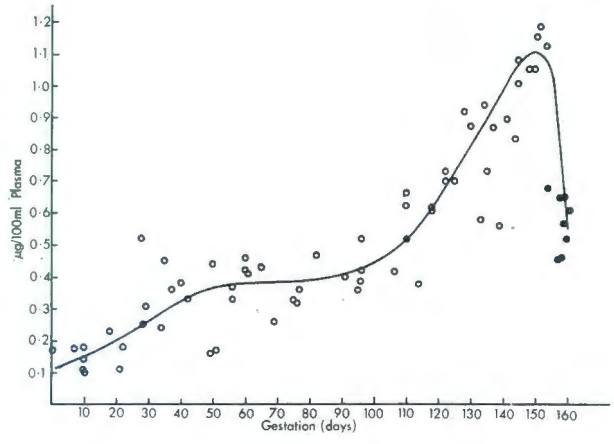

FIG. 3.—Plasma progesterone levels in pregnant Merino ewes fed *S. tuberculata* (free-hand curve). The solid black circles at the end indicate the values on the day of parturition.

Plasma cortisol: The cortisol concentrations in the plasma varied considerably, but it is nevertheless clear that they increased steadily during pregnancy in both groups (Fig. 4 and 5).

In late pregnancy the values declined in all the ewes. This decrease in cortisol values commenced at about 120 days gestation in the control group. It was only about 10 days later when the same phenomenon occurred in the *Salsola* ewes. The lowest values since conception were reached at approximately 138 days and 150 days gestation in the control and *Salsola* ewes respectively. These low values were only prevalent for a few days before the cortisol of both groups increased to reach relatively high levels during parturition. The cortisol levels of the *Salsola* group were significantly higher ($P<0\cdot05$) than those of the controls at partus.

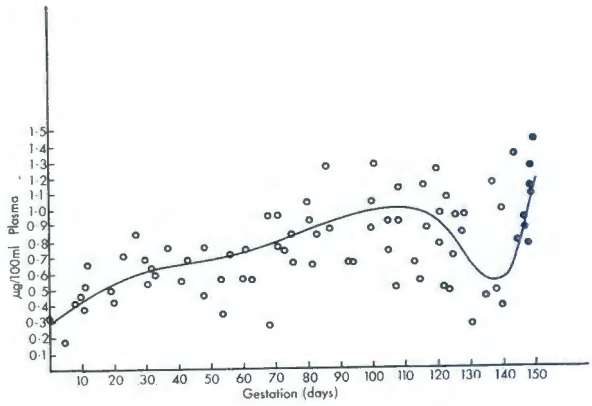

FIG. 4.—Plasma cortisol values in pregnant control Merino ewes (free-hand curve). The solid black circles at the end indicate the values on the day of parturition.

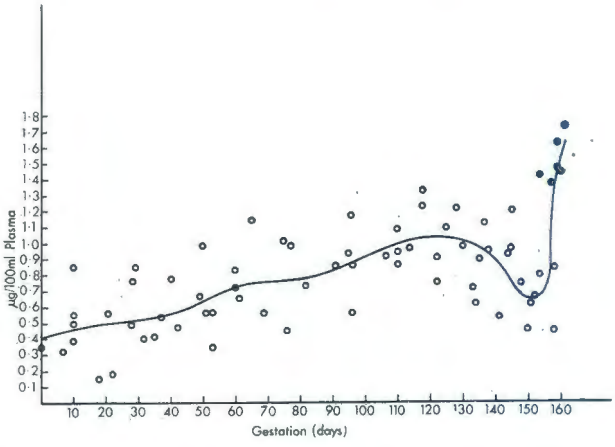

FIG. 5.—Plasma cortisol values in pregnant Merino ewes fed *S. tuberculata* (free-hand curve). The solid black circles at the end indicate the values on the day of parturition.

(iii) *First feeding experiment on sheep in South West Africa* (1966): The length of gestation, weights, and other data from both *Salsola* and control groups are given in Tables 9 to 11.

TABLE 9.—*Weights and other findings of lambs from* Salsola-*fed ewes in South West Africa,* 1966

No.	Gestation (days)	Birth weight (lb)	Incisors erupted (pairs)	Pigmentation	Pelt: weight	Brain: wt.*	Kidneys: wt.*	Liver: wt.*	Spleen: wt.*	Thymus: wt.*	Pancreas: wt.*	Entire hypophysis: wt.	Corrected(*) hypophysis: wt.	Hypophysis: WI	Anterior pituitary: wt.	Adrenals: wt.	Adrenals: WI	C/M ratio	Thyroids: wt.	Pineal: wt.	Ovaries: wt.	Genitalia (without vagina): wt.	Testes: wt.
1.....	150	7·0	—	—	131·9	52·8	19·7	63·2	2·7	8·2	4·00	0·1565	0·1520	2·23	0·1192	0·5168	7·38		0·5884	0·0169			2·3510
2.....	152	10·0	1, 3	±	167·4	52·3	26·7	96·8		12·0	4·00					0·9272	9·27	0·90	0·9216	0·0134	0·0506	3·5410	
3.....	153	8·5	1	—	174·7	55·4	23·9	79·0	5·4	7·7	3·10				0·1112	0·6304	7·42	1·00	0·7618	0·0190			2·7120
4.....	153	9·0	—	—	196·8	53·4	25·1	93·1	4·4	12·3	5·05	0·1056	0·1030	1·17	0·0827	0·6605	7·34	0·70	0·7946	0·0163	0·0621	3·9953	
5.....	153	8·5	1, 3	—	155·9	52·8	23·1	66·3	3·4	15·9	2·79		0·1143	1·34	0·0816	0·8355	9·83	0·70	1·0113	0·0181	0·0329	2·6924	
6.....	153	7·0	1, 3	—	144·0	50·5	14·7	62·2	2·8	9·5	2·30	0·0827	0·0787	1·18	0·0566	0·6309	9·01	0·85	0·5870		0·0430	2·8415	
7.....	154	10·5	1	—	208·0	56·2	25·6	120·2	4·8	15·8	3·85	0·1225	0·1223	1·17	0·0849	0·9567	9·11	1·00	0·9216				2·4801
8.....	154	10·5	1, 3	+	258·0	57·5	28·4	86·7	4·9	13·1	5·00		0·1540	1·47	0·1213	0·8513	8·11	0·70	1·0865	0·0122			3·8900
9.....	155	10·0	1, 3	+	217·7	54·8	25·9	62·0	3·6	13·8	3·90		0·1224	1·22	0·0908	0·7385	7·36	1·10	0·7132	0·0165	0·0508	3·5787	
10.....	157	11·0	1, 3	+	222·7	56·2	29·5	108·7	4·2	20·0	4·30	0·1207	0·1158	1·10	0·0961	1·0206	9·28	0·90	0·9114	0·0149	0·0804	4·6755	
11.....	157	10·0	1	+	234·5	58·4	26·9	81·3	4·9	12·8	4·40	0·1451	0·1395	1·45	0·1118	0·7154	7·15	1·10	0·7225	0·0123	0·0649	3·6467	
12.....	159	10·5	1, 3	±	237·9	58·7	29·1	127·1	4·3	15·1	3·90	0·2162	0·2093	2·06	0·1803	0·8369	7·97	0·90	1·1306				2·7512
13.....	160	10·5	1, 3	+	212·5	61·5	27·5	113·7	5·3	20·6	3·00	0·0825	0·0772	0·79	0·0600	0·7268	6·92	0·70	1·1431	0·0192			
14.....	160	10·0	1, 3	+	220·8	56·5	26·6	116·0	6·0	28·0	3·15	0·1146	0·1083	1·15	0·0805	0·7951	7·95	0·90	1·5557	0·0250	0·0524	4·5077	
15.....	160	12·0	1	++	231·2	54·2	41·2	153·2	5·1	15·5	5·52	0·1368	0·1338	1·14	0·1127	0·9727	8·11	0·90	1·5754	0·0148	0·0694	4·7481	
16.....	161	9·5	1, 2, 3	+	202·4	57·2	21·6	80·9	4·0	13·8	4·25	0·1370	0·1320	1·44	0·1050	0·7247	7·63	1·00	0·5825	0·0299	0·0712	3·1999	
17.....	162	9·0	1, 3	±	181·3	54·6	29·7	81·6	5·5	9·0	3·59	0·1356	0·1282	1·51	0·0916	0·6774	7·52	0·70	0·6096	0·0264			2·8461
18.....	164	10·5	1, 3	+	212·9	55·4	33·8	103·6	4·7		4·23	0·1086	0·1054	1·03	0·0752	0·8700	8·29	0·90	1·6426	0·0129			3·0208
19.....	164	11·0	1, 3	++	178·2	55·7	18·9	66·0	3·4	11·3	3·35		0·0790	0·72	0·0540	0·5252	4·77	0·80	0·8307	0·0226	0·0385	4·0767	
20.....	166	7·0	1, 3	+	149·0	55·2	14·3	40·4	2·3	7·5	2·13	0·0642	0·0612	0·92	0·0375	0·5302	7·57	0·75	0·7986	0·0181			2·3430
21.....	177	17·5	1, 3	+++	282·0	74·4	50·0	95·9	5·2	21·0	4·60	0·1127	0·1054	0·64	0·0697	0·3823	2·18	0·40	1·6130	0·0304			2·8050
22.....	180	13·0	1, 3	+++	207·2	63·4	48·4	120·3	3·5	4·0	5·35	0·1065	0·1013	0·82	0·0690			1·00	0·6260		0·0470	3·7887	
23.....	184	15·0	1, 3	+++	260·8	70·1	41·2	94·1	4·5	13·1	4·53	0·1284	0·1222	0·86	0·0778	0·4922	3·28	0·55	0·6801	0·0386			3·3815
24.....	147	Twins																					
25.....	156	Twins																					
26.....	158	Twins																					
27.....	159	Twins																					
28.....	167	Twins																					
29.....	191	Twins																					

Wt = weight.

* Represents fresh weights. The others are fixed weights.

(*) Corrected hypophysial weight = mean weight of hypophysis with median eminence and hypophysis without median eminence.

TABLE 10.—*Weights and other findings from the control lambs in South West Africa*, 1966

No.	Gestation (days)	Birth weight (lb)	Incisors erupted (pairs)	Pigmentation	Pelt: weight	Brain: wt.*	Kidneys: wt.*	Liver: wt.*	Spleen: wt.*	Thymus: wt.*	Pancreas: wt.*	Entire hypophysis: wt.	Corrected(*) hypophysis: wt.	Hypophysis: WI	Anterior pituitary: wt.	Adrenals: wt.	Adrenals: WI	C/M ratio	Thyroids: wt.	Pineal: wt.	Ovaries: wt.	Genitalia (without vagina): wt.	Testes: wt.
1.....	148	9·50	—	—	186·0	53·6	26·2	106·3	5·4	19·2	3·55		0·2035		0·1810	0·8514	8·96	1·0	0·6823	0·0116			2·7644
2.....	148	9·00	(1)	—	163·0	54·6	19·1	78·5	2·8	21·8	3·00	0·1626	0·1548	1·81	0·1166	0·7916	8·80		0·7071				2·3696
3.....	149	12·00	(1, 3)	—	266·0	60·4	25·7	115·6	5·3	24·1	3·16	0·1844	0·1774	1·54	0·1440	0·7037	5·86	0·90	1·1170	0·0188			3·0764
4.....	150	10·00	(1, 2, 3)	—	202·1	57·9	26·0	91·8	4·7	16·8	3·77	0·1443	0·1392	1·44	0·1068	0·6084	6·08	1·00	0·7007	0·0203			2·6595
5.....	150	11·00	(1)	—	233·0	58·8	29·2	129·7	5·2	19·9	3·50	0·1673	0·1601	1·52	0·1272	0·6720	6·11	0·90	0·6764	0 0170			2·6311
6.....	150	11·00	—	—	235·0	57·8	24·7	94·9	7·8	24·4	3·40	0·1535	0·1475	1·39	0·1152	0·6813	6·19	1·00	0·7750	0·0142			2·5068
7.....	150	11·00	—	—	247·0	58·0	31·5	107·7	6·3	25·1	4·09	0·1447	0·1388	1·32	0·1204	0·8302	7·55	1·00	0·7430	0·0174	0·0394		
8.....	150	9·50	(1, 3)	—	191·0	57·7	21·6	85·0	5·2	28·9	2·46	0·1128	0·1071	1·19	0·0810	0·7595	8·00	0·80	0·6592	0·0110	0·0406	3·813	
9.....	150	10·50	1	—	221·1	57·8	25·3	82·7	5·5	20·1	3·51	0·1922	0·1836	1·83	0·1461	0·6876	6·55	1·10	0·9305	0·0216			2·7144
10.....	151	11·00	(1)	—	234·0	60·6	26·8	101·9	7·9	21·6	4·25	0·1640	0·1572	1·49	0·1304	0·7410	6·74	0·90	0·6456	0·0131			3·0111
11.....	151	11·50	(1)	—	217·0	60·8	27·0	124·1	6·1	17·5	4·00	0·1560	0·1506	1·36	0·1212	0·9673	8·41	0·80	0·8840	0·0195			2·5508
12.....	151	10·50	(1, 2, 3)	—	203·5	60·5	21·4	101·6	4·1	20·7	2·63	0·1370	0·1327	1·31	0·1053	0·8145	7·76	0·90	0·7492	0·0115	0·0552	3·0874	
13.....	152	10·50	(1)	—	213·0	57·3	23·3	92·2	4·0	19·6	3·22	0·1773	0·1708	1·69	0·1318	0·5174	4·93	0·80	0·6773	0·0154			2·2760
14.....	152	10·75	(1)	—	227·0	64·2	29·6	106·4	6·7	24·8	4·33	0·1720	0·1657	1·60	0·1334	0·8314	7·33	0·90	0·8243	0·0193			2·4693
15.....	153	11·0	(1)	—	201·0	58·6	23·6	110·1	6·2	27·5	3·92												

* Represents fresh weights. The others are fixed weights.

(*) Corrected hypophysial weight = mean weight of hypophysis with median eminence and hypophysis without median eminence.

Six ewes from the lucerne group did not conceive, one conceived inconveniently late and another aborted at 135 days. In the field group three failed to conceive and one aborted at 103 days, whereas seven failed to conceive in the *Salsola* group and one died of pregnancy toxaemia at 141 days. All these animals were discarded from the various groups including an additional seven from the *Salsola* group which were bad feeders. The number of ewes in each group was accordingly reduced to 17, 20 and 29 respectively for the lucerne, field and *Salsola* groups.

The condition of the ewes in the lucerne group at the time of parturition was very good and superior to all the others whereas that of the *Salsola* group was poor and the worst of the three groups. This was evidently due to compulsory *Salsola* feeding and inadequate supplementation.

The average gestation in both control and field groups was 150·35 days as compared to the 161·93 days of the *Salsola* group. Two dystocias (11·77 per cent) were encountered in the lucerne group in comparison with 29·6 per cent of dystocias in the *Salsola* group and one dystocia at 156 days in the field group. Six twins were born in the *Salsola* group but none of the three ewes that returned to the rams for a second time, i.e. after they had been on *Salsola* for 18 days had any twins. This evidently indicates that the high number of twins in the *Salsola* group was mere coincidence.

As in the natural cases of prolonged gestation, those produced by feeding *S. tuberculata* did not show any other signs of ill-effects except prolonged parturition and a high percentage of dystocias. The udder usually became turgid only 2 to 4 days prior to lambing, but some of the post-term ewes had poor udder development and milk yield at parturition. The udder of one parturient ewe was practically agalactic.

TABLE 11.—*Results of the feeding experiments in South West Africa*, 1966

Item	Lucerne group	Field group	*Salsola* group
No. of ewes.....	17	20	29
No. of lambs.....	18	21	35
No. of twins.....	1	1	6
Average gestation (days)	150·35	150·35	161·93
Average birthweight (lb)	10·36	8·87	8·97
Maximum birthweight...	12·00	11·00	17·50
No. of dystocias (%)...	11·77	5·0	29·6
No. of dead lambs (%)..	11·11	4·4	15·1
Pelt:—			
Average weight (gm)..	207·85	168·71	173·63
Short hair (%).....	14	19	11·4
Medium short hair (%)	30	62	8·6
Medium hair (%).....	42	19	24·3
Medium long hair (%)	14	—	22·9
Long hair (%).....	—	—	24·3
Very long hair (%)...	—	—	8·6
Incisors erupted:—			
None (%).....	33	25	11·4
1 pair incomplete (%)	50	32	11·3
2-3 pairs incomplete (%)	17	43	8·6
1 pair complete (%)..	—	—	34·3
2 pairs complete (%).	—	—	31·4

(iv) *Determination of the period of insult in South West Africa* (1967): In comparison with the previous experiment all the ewes in this experiment were in very good condition at the time of parturition.

The gestation period, live weights and some organ weights of the various groups are given separately in Tables 12 to 16. A significant increase in length of gestation occurred only in the groups fed *S. tuberculata* during any period of time which included the last 50 days of gestation (Groups 2, 5 and 7). Although the mean length of gestation of the other *Salsola* groups was slightly longer than that of the control it was apparently not significant. One ewe in Group 6, however, had a gestation period of 155 days. The average birth weight of the lambs from ewes fed *S. tuberculata* during any period including the second 50 days of gestation (Groups 2, 4 and 6) was heavier than that of the other groups. The heaviest lamb of 14·31 lb was obtained from a ewe in Group 4 with a gestation period of 150 days. None of the field group had gestations exceeding 153 days and their data and specimens were subsequently not used for further analyses or study. Similarly all the data of the twins were discarded in further analyses.

Parturition was prolonged in several post-term ewes. This was evidently due to inadequate lubrication which resulted from the decrease in volume of foetal fluid, abnormal postures, overgrowth of hair, oversized lambs and seemingly also due to uterine inertia in some of the ewes. The first and second stages of parturition lasted from 10 minutes up to 12 hours and even more than a day in some. No uterine contractions were noticed in one of the ewes which had to be caesarianed, but the foetal membranes broke four days prior to the operation. The twin lambs were normal and still alive at the time of the operation. Considerable blood loss was noticed at parturition in some of the post-term ewes and it was evident that proper lysis of the cotyledons had not occurred in these ewes. As in the previous experiment the udders of the post-term ewes only became swollen a few days prior to lambing. A few of the ewes lost some weight during their post-term period. Many of the postmature lambs were weak and lethargic.

(v) *Pathology:* Macroscopical findings of all the experimental sheep: As there appeared to be no evidence of differences in the microscopical findings of the various experimental groups at Onderstepoort and in South West Africa the animals are all dealt with together as collective control and *Salsola* groups.

Ewes: All the macroscopical observations on natural post-term ewes were confirmed but for a few exceptions (Table 4). As practically all the ewes were allowed to lamb on their own no measurements were made of the volume of the foetal fluids. One case which underwent a caesarian section at 184 days of gestation and which showed no signs of parturition prior to the operation, had a very small volume of amniotic fluid and the cotyledons showed no signs of lysis. Pigmentation of the foetal membranes was present in all the advanced cases of gestational prolongation and renal pigmentation.

TABLE 12 (a).—*Group* 1 (*lucerne control*). *Lambs: Large organs: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Brain (gm)	Liver (gm)	Kidneys (gm)	Spleen (gm)	Heart (gm)	Lungs (gm)	Wet pelt (lb)	Bile (ml)	Pairs of incisors erupted	Pigmentation
A116..	150	104·5	10·53	55·25	101·75	30·50	6·7816	33·00	80·60	1·47	0·05	1, (2)	—
A131..	151	141·0	11·50	58·30	104·00	25·90	9·2180	36·10	74·70	1·50	—	(1)	—
A140..	149	101·0	10·28	59·90	104·10	25·20	5·3902	31·20	57·20	1·81	0·30	(1, 2)	1±
E189..	151	109·5	10·66	61·50	108·30	26·85	5·6762	37·60	68·35	1·84	0·10	(1, 2)	—
I235...	151	124·0	10·84	52·60	102·60	25·90	4·5655	33·30	67·90	1·75	0·50	(1, 2)	—
L378..	148	112·5	10·81	58·30	138·40	31·50	7·6588	41·10	65·20	1·38	0·10	(1)	+
L379..	149	111·5	8·97	48·60	99·10	20·60	4·8662	28 50	55·10	1·41	0·50	(1, 2)	—
L380..	148	117·5	8·94	52·10	91·65	24·20	6·9521	29·40	52·20	1·38	0·05	(1, 2)	—
M429..	148	90·0	8·09	—	81·30	21·15	5·0122	26·60	49·50	1·25	0·10	(1)	—
M430..	148	118·0	9·13	52·10	72·50	19·60	5·0181	28·40	50·55	1·53	0·20	(1)	—
N476..	149	104·0	11·25	57·30	101·50	30·80	6·4268	36·20	65·05	1·81	0·50	(1, 2)	1±
N492..	149	105·5	9·47	47·40	92·45	21·95	4·23	30·20	50·30	1·06	—	(1)	—
H231*.	149	124·5	9·66	50·80	95·20	25·55	3·9654	31·60	51·20	1·50	0·30	—	—
H231*.	149	124·5	9·28	55·15	97·90	21·65	4·9257	32·70	52·00	1·75	0·10	—	—
A118*.	146	110·5	8·28	53·60	67·60	20·20	4·7419	24·10	52·70	1·28	0·10	(1, 2)	—
A118*.	146	110 5	4·69	47·70	47·10	11·80	2·1557	17·50	35·40	0·59	0·20	(1)	—

* Twins.
Gest. = gestation.

Pairs of pinpointing or incompletely erupted incisors are indicated in brackets.

TABLE 12 (b).—*Group* 1 (*lucerne control*). *Lambs: Endocrine weights* (*fresh*)

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Hypophysis (gm)	Anterior pituitary (gm)	Adrenals (gm)	Thyroid (gm)	Pancreas (gm)	Thymus (gm)	Pineal (gm)
A116.....	150	104·5	10·53	0·1962	0·1753	0·7377	0·4666	4·1366	22·00	0·0158
A131.....	151	141·0	11·50	0·2082	0·1812	0·7410	0·9809	4·7242	27·90	0·0246
A140.....	149	101·0	10·28	0·1400	0·1142	0·8752	0·7185	3·8701	24·60	0·0166
E189.....	151	109·5	10·66	0·1321	0·1110	0·8000	0·7108	4·5722	22·20	0·0251
I235.....	151	124·0	10·84	0·1400	0·1253	0·6243	0·6092	3·2453	19·65	0·0087
L378.....	148	112·5	10·81	0·1762	0·1417	0·8927	—	3·1559	19·80	0·0104
L379.....	149	111·5	8·97	0·1337	0·1022	0·6131	1·0237	3·4209	25·30	0·0094
L380.....	148	117·5	8·94	0·1626	0·1410	0·8653	0·6640	3·5019	22·50	0·0110
M429.....	148	90·0	8·09	0·1360	0·1200	0·7000	1·1640	3·1062	26·60	0·0091
M430.....	148	118·0	9·13	0·1299	0·1057	0·7083	0·7103	3·0124	35·35	0·0141
N476.....	149	104·0	11·25	0·1704	0·1530	0·7116	0·7500	5·0038	30·60	0·0242
N492.....	149	105·5	9·47	0·1382	0·1173	0·6270	0·7518	3·6033	23·70	—
H231*.....	149	124·5	9·66	0·1887	0·1673	0·8540	0·5335	2·9663	14·60	0·0094
H231*.....	149	124·5	9·28	0·1500	0·1273	0·6868	0·6306	3·0167	20·40	0·0140
A118*.....	146	110·5	8·28	0·1270	0·1028	0·4908	0·5590	2·9767	18·20	0·0145
A118*.....	146	110·5	4·69	0·0790	0·0646	0·5838	0·3104	2·1175	6·80	0·0079

TABLE 12 (c).—*Group* 1 (*lucerne control*). *Lambs: Genitalia: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Testes (gm)	Genitalia (gm)	Uterus (gm)	Cervix (gm)	Ovaria (gm)	Follicles	Udder (gm)
A116.....	150	104·5	10·53	2·5128	—	—	—	—		—
A131.....	151	141·0	11·50	3·5850	—	—	—	—		—
A140.....	149	101·0	10·28	3·0194	—	—	—	—		—
E189.....	151	109·5	10·66	—	3·4873	1·1501	1·8175	0·0634	—	4·9466
I235.....	151	124·0	10·84	2·3711	—	—	—	—		—
L378.....	148	112·5	10·81	3·2094	—	—	—	—		—
L379.....	149	111·5	8·97	—	2·9479	0·9058	1·5370	0·0645	—	4·2167
L380.....	148	117·5	8·94	—	3·3494	1·7835	1·5251	0·0408	—	7·0295
M429.....	148	90·0	8·09	—	3·7298	1·8452	1·8179	0·0667	—	5·2614
M430.....	148	118·0	9·13	—	3·0566	0·9683	1·6270	0·0387	—	5·6545
N476.....	149	104·0	11·25	3·2539	—	—	—	—		—
N492.....	149	105·5	9·47	—	4·1156	1·1785	2·5665	0·0508	—	3·9690
H231*.....	149	124·5	9·66	2·5206	—	—	—	—		—
H231*.....	149	124·5	9·28	2·7462	—	—	—	—		—
A118*.....	146	110·5	8·28	—	1·8805	0·7139	0·7367	0·0714	—	6·3870
A118*.....	146	110·5	4·69	—	1·4522	0·6351	0·4943	0·0400	—	4·5159

TABLE 13 (a).—*Group* 2 (Salsola *fed entire gestation period*). *Lambs: Large organs: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Brain (gm)	Liver (gm)	Kidneys (gm)	Spleen (gm)	Heart (gm)	Lungs (gm)	Wet pelt (lb)	Bile (ml)	Pairs of incisors erupted	Pigmentation
A114..	155	88·0	12·44	65·00	162·95	48·40	6·6443	42·70	127·00	2·16	0·05	1, (2)	1±
A129..	151	118·0	9·25	58·60	101·30	24·80	5·3466	34·60	66·90	1·56	1·70	1, (2, 3)	2++
C168..	152	101·5	10·25	54·35	101·00	20·80	5·3119	33·90	50·05	1·50	0·05	1, (2, 3)	—
C178..	156	117·5	10·75	54·20	105·65	28·70	7·6353	38·10	49·30	1·56	0·20	(1, 2)	1+
F201..	154	119·5	10·22	54·90	112·30	28·25	4·2631	29·45	56·30	1·47	0·20	(1, 2)	—
F204..	155	130·0	10·56	52·70	124·60	20·60	5·0581	37·30	57·70	2·09	1·0	1, (2)	3++
G225..	159	100·0	11·19	62·40	115·80	36·80	5·2578	43·50	66·60	1·50	0·20	1, 2	1+
H233..	151	134·5	11·28	53·20	142·50	26·70	4·9062	38·80	66·20	1·72	0·05	(1, 2)	—
I237...	155	96·5	10·44	57·00	108·70	29·35	5·6145	30·10	59·50	1·59	0·30	(1, 2)	—
I242...	157	94·5	10·06	51·10	90·55	25·45	4·3362	30·30	47·80	1·69	0·05	(1)	2+
L383..	154	82·5	8·47	56·85	96·70	—	4·1949	30·60	62·70	1·22	0·4	(1)	1±
M431..	152	94·5	10·59	56·20	128·30	24·80	5·0943	35·10	82·65	1·56	0·80	1, (2)	—
M432..	154	116·0	10·53	53·20	123·20	30·80	6·1246	37·80	68·10	1·25	0·20	1, (2)	++
M433..	166	119·5	10·06	52·80	94·20	31·00	3·8300	34·90	57·90	1·50	0·10	1, 2	5+++
E190..	153	108·5	12·34	—	—	—	—	—	—	—	—	—	—
L381*.	151	89·0	7·38	47·70	73·95	14·30	3·1136	24·60	50·30	1·06	0·80	(1)	—
L381*.	151	89·0	7·31	37·50	70·15	16·20	3·35	29·45	32·85	0·97	0·30	1, (2)	—

TABLE 13 (b).—*Group* (Salsola *fed entire gestation period*). *Lambs: Endocrine weights (fresh)*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Hypo-physis (gm)	Anterior pituitary (gm)	Adrenals (gm)	Thyroid (gm)	Pancreas (gm)	Thymus (gm)	Pineal (gm)
A114.....	155	88·0	12·44	0·1368	0·1076	1·2400	1·1881	6·3649	27·40	0·0259
A129.....	151	118·0	9·25	0·1490	0·1200	0·8980	0·7090	3·7811	24 00	0·0163
C168.....	152	101·5	10·25	0·1300	0·1085	0·7130	0·7076	3·5757	21·70	—
C178.....	156	117·5	10·75	0·1261	0·1050	0·7149	0·9731	5·3428	21·50	0·0139
F201.....	154	119·5	10·22	0·1360	0·1110	0·7956	0·7140	4·6700	19·20	0·0129
F204.....	155	130·0	10·56	0·1012	0·0773	0·8623	0·6956	4·0950	13·20	0·0095
G225.....	159	100·0	11·19	0·1623	0·1358	0·7073	0·9100	5·4806	14·20	0·0256
H233.....	151	134·5	11·28	0·1182	0·0989	0·8466	0·8186	3·3661	21·50	0·0103
I237.....	155	96·5	10·44	0·1338	0·1129	0·8623	0·7600	4·5181	17·70	0·0113
I242.....	157	94·5	10·06	0·0803	0·0620	0·7170	0·7066	3·9623	18·70	0·0111
L383.....	154	82·5	8·47	0·1470	0·1219	0·7117	0·5954	3·6443	17·30	0·0226
M431.....	152	94·5	10·59	0·1389	0·1149	0·8723	0·5937	4·5904	20·30	0·0095
M432.....	154	116·0	10·53	0·1315	0·1085	1·1849	1·1095	4·3423	24·10	0·0104
M433.....	166	119·5	10·06	0·0968	0·0176	0·7887	1·3465	4·4370	6·86	0·0103
E190.....	153	108·5	12·34	—	—	—	—	—	—	—
L381*.....	151	89·0	7·38	0·0898	0·0735	0·5600	0·7804	2·4074	13·30	0·0129
L381*.....	151	89·0	7·31	0·0752	0·0540	0·5295	0·4783	2·8846	17·00	0·0087

TABLE 13 (c).—*Group* 2 (Salsola *fed entire gestation period*). *Lambs: Genitalia: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Testes (gm)	Genitalia (gm)	Uterus (gm)	Cervix (gm)	Ovaria (gm)	Follicles	Udder (gm)
A114.....	155	88·0	12·44	—	8·2490	3·4761	3·7516	0·0515	—	16·1124
A129.....	151	118·0	9·25	—	4·2342	1·3860	2·1389	0·0684	—	8·3398
C168.....	152	101·5	10·25	3·1346	—	—	—	—		—
C178.....	156	117·5	10·75	—	4·3621	1·5562	1·9459	0·0324	—	6·3119
F201.....	154	119·5	10·22	—	3·0140	1·1565	1·3998	0·0611	—	5·4528
F204.....	155	130·0	10·56	—	2·9737	0·9855	1·4060	0·0600	—	8·3176
G225.....	159	100·0	11·19	3·5926	—	—	—	—		—
H233.....	151	134·5	11·28	3·2948	—	—	—	—		—
I237.....	155	96·5	10·44	—	4·1484	1·2626	1·9654	0·0845	—	7·5251
I242.....	157	94·5	10·06	—	2·9405	1·1018	1·4701	0·0557	—	6·8168
L383.....	154	82·5	8·47	—	2·3105	1·0692	0·8207	0·0487	—	6·9909
M431.....	152	94·5	10·59	2·6227	—	—	—	—		—
M432.....	154	116·0	10·53	—	4·7076	2·4173	1·4297	0·0515	—	8·5178
M433.....	166	119·5	10·06	—	3·8902	1·5713	1·6940	0·0944	+	8·5518
E190.....	153	108·5	12·34	—	—	—	—	—		—
L381*.....	151	89·0	7·38	—	1·8493	0·8744	0·6835	0·0381	—	4·5136
L381*.....	151	89·0	7·31	—	2·6936	1·1256	1·2144	0·0356	—	3·8548

TABLE 14 (a).—*Group* 5 (Salsola *fed from* 100*th day up to parturition*). *Lambs: Large organs: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Brain (gm)	Liver (gm)	Kidneys (gm)	Spleen (gm)	Heart (gm)	Lungs (gm)	Wet pelt (lb)	Bile (ml)	Pairs of incisors erupted	Pigmentation
A102..	156	118·0	11·88	61·3	128·0	38·0	7·4020	38·1	67·5	1·75	0·10	(1)	1+
A130..	152	122·0	10·66	54·4	93·35	21·2	3·5738	30·50	67·35	1·38	0·10	(1)	—
F203..	152	114·5	10·44	54·6	111·55	24·9	5·2526	36·1	56·90	1·38	0·05	1, (2)	1±
F206..	150	139·0	9·94	50·75	107·4	24·75	5·6344	29·4	58·6	1·56	0·20	(1, 2)	1+
G222..	152	127·5	9·44	59·25	101·30	25·20	5·3968	28·95	48·5	1·31	0·90	(1, 2)	1±
I249...	153	106·5	10·50	57·3	103·1	21·10	4·4470	34·05	66·9	1·69	0·30	(1)	—
L390..	152	123·5	8·88	51·60	86·10	21·18	5·4708	29·05	40·09	1·28	1·10	(1, 2, 3)	1+
L391..	149	116·5	9·44	56·6	82·2	20·15	4·9003	27·8	67·6	1·44	0·60	(1, 2)	3++
L392..	153	125·5	10·66	53·8	86·65	23·4	6·3718	34·6	58·35	1·56	0·30	1, (2)	1±
M440..	154	98·0	11·31	56·4	—	27·6	5·3866	38·8	37·7	1·50	0·80	(1)	—
M441..	163	106·0	9·56	56·1	101·1	21·4	6·8866	34·3	33·05	1·38	0·05	1, (2, 3)	2++
M442..	153	102·0	10·22	53·9	103·3	27·55	5·9850	31·9	57·1	1·31	0·10	1, (2)	—
M448..	152	89·0	9·97	60·8	93·8	23·8	4·6070	32·8	58·15	1·47	0·10	1	1±
N486...	150	127·5	9·00	52·8	91·9	21·20	5·0491	28·4	60·00	1·31	0·20	1, (2)	1±
N493...	151	108·0	11·13	54·2	96·4	23·45	4·5541	38·48	73·35	1·63	0·30	(1)	—
G223*.	155	118·5	7·34	50·80	55·80	19·75	2·7629	25·6	43·60	1·19	0·80	1, (2)	—
G223*.	156	108·0	11·84	65·30	143·20	29·10	7·4521	37·4	60·70	1·75	0·10	(1)	—
G221*.	151	143·5	10·16	53·6	89·0	24·7	6·8234	32·55	70·2	1·31	0·05	—	—
G221*.	151	143·5	8·88	52·45	81·05	22·10	5·7145	28·2	53·05	1·31	0·05	(1)	—

TABLE 14 (b).—*Group* 5 (Salsola *fed from* 100*th day up to parturition*). *Lambs: Endocrines: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Hypophysis (gm)	Anterior pituitary (gm)	Adrenals (gm)	Thyroid (gm)	Pancreas (gm)	Thymus (gm)	Pineal (gm)
A102.....	156	118·0	11·88	0·1435	0·1234	1·0507	0·8355	5·9069	17·1	0·0227
A130.....	152	122·0	10·66	0·1300	0·1160	0·5781	0·5900	3·6500	16·75	0·0099
F203.....	152	114·5	10·44	0·1346	0·1141	0·6609	0·7300	3·4628	21·3	0·0256
F206.....	150	139·0	9·94	0·1448	0·1185	0·9059	1·0583	3·7528	21·0	0·0131
G222.....	152	127·5	9·44	0·1387	0·1194	1·0669	1·1014	4·2175	31·10	0·0227
I249.....	153	106·5	10·50	0·1684	0·1482	0·9167	0·7817	4·0457	21·45	0·0110
L390.....	152	123·5	8·88	0·1273	0·1065	1·1028	0·7147	2·9177	22·6	0·0249
L391.....	149	116·5	9·44	0·1157	0·0907	0·9630	1·1025	3·3008	20·6	0·0118
L392.....	153	125·5	10·66	0·1923	0·1730	0·9579	1·1190	5·1056	29·95	—
M440.....	154	98·0	11·37	0·1803	0·1616	0·9647	1·6933	4·2009	20·35	0·0099
M441.....	163	106·0	9·56	0·1093	0·0877	0·5500	0·8474	5·2927	22·3	0·0129
M442.....	153	102·0	10·22	0·1492	0·1233	0·7734	0·8494	3·1614	16·2	0·0100
M448.....	152	89·0	9·97	0·1800	0·1579	0·9100	0·6738	4·6948	17·8	0·0141
N486.....	150	127·5	9·00	0·1457	0·1259	1·0701	0·6692	3·3387	16·3	0·0198
N493.....	151	108·0	11·13	0·1443	0·1231	0·8696	0·9688	4·3139	14·30	0·0108
G223*.....	155	118·5	7·34	0·1094	0·0969	0·6225	0·5085	2·7331	9·7	0·0100
G223*.....	156	108·0	11·84	0·1647	0·1465	0·8014	0·8445	5·4678	20·65	0·0214
G221*.....	151	143·5	10·16	0·1996	0·1787	0·8000	0·7948	4·5727	16·45	0·0119
G221*.....	151	143·5	8·88	0·1718	0·1500	0·5913	0·7865	3·8858	16·5	0·0125

TABLE 14 (c).—*Group* 5 (Salsola *fed from* 100*th day up to parturition*). *Lambs: Genitalia: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Testes (gm)	Genitalia (gm)	Uterus (gm)	Cervix (gm)	Ovaria (gm)	Follicles	Udder (gm)
A102.....	156	118·0	11·88	3·2158	—	—	—	—		—
A130.....	152	122·0	10·66	3·7921	—	—	—	—		—
F203.....	152	114·5	10·44	2·5925	—	—	—	—		—
F206.....	150	139·0	9·94	—	3·5063	1·4067	1·3283	0·1004	1	6·5394
G222.....	152	127·5	9·44	—	4·6085	1·4684	2·4685	0·0513	—	8·3882
I249.....	153	106·5	10·50	—	4·9757	1·8195	2·3076	0·1210	7	11·6184
L390.....	152	123·5	8·88	—	4·1369	1·3887	1·8497	0·0519	—	6·1283
L391.....	149	116·5	9·44	—	4·4121	1·3498	2·3144	0·0660	1	9·1311
L392.....	153	125·5	10·66	—	5·9539	2·6052	2·3859	0·2464	8	9·0257
M440.....	154	98·0	11·37	3·9421	—	—	—	—		—
M441.....	163	106·0	9·56	—	4·0169	1·1047	2·5214	0·0563	—	7·0537
M442.....	153	102·0	10·22	3·2338	—	—	—	—		—
M448.....	152	89·0	9·97	2·8139	—	—	—	—		—
N486.....	150	127·5	9·00	—	2·7822	1·1027	1·1539	0·0563	—	6·4103
N493.....	151	108·0	11·13	3·1607	—	—	—	—		—
G223*.....	155	118·5	7·34	—	—	—	—	—		—
G223*.....	156	108·0	11·84	—	5·4939	1·8243	2·7862	0·0579	—	6·4507
G221*.....	151	143·5	10·16	3·2565	—	—	—	—		—
G221*.....	151	143·5	8·88	—	3·4411	2·2478	1·439	0·0494	—	8·6745

TABLE 15 (a).—*Group* 7 (Salsola *fed from* 50*th day up to parturition*). *Lambs: Large organs: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Brain (gm)	Liver (gm)	Kidneys (gm)	Spleen (gm)	Heart (gm)	Lungs (gm)	Wet pelt (lb)	Bile (ml)	Pairs of incisors erupted	Pigmentation
A105...	151	140·0	11·69	53·70	126·10	23·30	5·0174	38·10	122·50	1·94	0·50	(1)	1+
C182...	151	122·0	8·03	49·30	71·40	21·60	3·8000	27·35	63·95	1·19	0·70	(1, 2)	—
E184...	156	94·0	5·69	49·45	42·20	12·50	2·2254	19·40	36·80	0·69	0·10	—	—
F200...	157	122·0	13·72	53·45	160·00	37·35	7·9113	55·40	73·40	2·25	0·20	1, 2	4++
F212...	170	110·5	9·28	55·60	92·30	24·10	4·4375	34·50	65·70	1·31	0·10	(1)	1±
H228...	152	120·0	12·97	56·10	124·20	29·70	6·7680	44·00	62·10	1·81	1·00	(1, 2)	1+
H234...	154	120·0	9·56	52·20	91·60	21·90	5·7892	33·85	55·70	1·47	0·05	1, (2)	—
I239....	147	100·5	10·06	—	—	—	—	—	—	1·50	0·05	(1)	—
I245....	157	123·0	10·63	53·20	91·90	24·80	4·5531	40·75	54·40	1·50	3·6	1, (2)	2++
L396...	149	111·5	9·00	47·60	107·40	22·10	6·2819	30·80	52·00	1·28	0·05	(1, 2)	—
L398...	156	89·5	11·97	55·75	126·75	28·60	5·5011	41·20	57·50	1·63	0·20	(1), 2	1±
M446..	155	113·5	10·50	53·05	104·30	21·45	5·6502	38·40	46·80	1·81	0·30	1, 2	1±
M447..	152	104·5	10·31	57·40	92·30	29·55	4·8926	39·30	59·20	1·44	1·20	(1)	—
N488...	152	114·0	9·47	56·60	91·60	25·90	4·7427	31·20	46·50	1·34	0·30	1, (2)	1±
E198*..	152	112·5	8·34	52·50	78·70	18·75	4·2186	29·60	72·50	1·28	0·60	(1)	—
E198*..	152	112·5	8·28	50·90	69·25	17·75	3·7963	29·30	72·00	1·22	0·05	(1)	—
H229*..	152	112·0	8·31	51·60	105·10	24·35	3·8371	36·80	55·30	1·06	0·40	(1)	1±
H229*..	152	112·0	8·47	52·15	119·30	25·70	3·6885	33·60	57·35	1·19	3·80	(1)	2+
L397*..	151	110·0	7·56	57·73	81·30	24·85	3·2362	30·00	42·80	0·75	0·10	(1)	—
L397*..	151	110·0	9·81	58·40	95·20	26·40	6·5945	34·30	55·90	1·25	0·10	(1, 2)	—
N482*..	150	111·5	7·38	50·80	65·70	19·60	3·5242	23·60	55·80	1·06	0·70	(1)	2+
N482*..	150	111·5	8·44	56·15	77·05	19·40	3·9276	26·15	55·50	1·06	0·80	(1)	—

TABLE 15 (b).—*Group* 7 (Salsola *fed from* 50*th day up to parturition*). *Lambs: Endocrines: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. po t-partus (lb)	Wt. of lamb—stomach (lb)	Hypo-physis (gm)	Anterior pituitary (gm)	Adrenals (gm)	Thyroid (gm)	Pancreas (gm)	Thymus (gm)	Pineal (gm)
A105.....	151	140·0	11·69	0·1347	0·1111	0·9699	1·2447	4·3831	17·80	0·0048
C182.....	151	112·0	8·03	0·1042	0·0897	0·6319	0·6031	2·8529	13·00	0·0100
E184.....	156	94·0	5·69	0·0986	0·0798	0·5239	0·4577	2·6659	5·80	0·0143
F200.....	157	122·0	13·72	0·1596	0·1385	1·3585	1·0720	5·7935	25·20	0·0101
F212.....	170	110·5	9·28	0·1030	0·0862	0·8026	0·8093	3·8012	13·90	0·0225
H228.....	152	120·0	12·97	0·1626	0·1377	1·1852	0·9323	4·8430	23·40	0·0108
H234.....	154	120·0	9·56	0·1236	0·1112	1·8415	0·4430	3·9300	13·95	0·0190
I239.....	147	100·5	10·06	—	—	—	—	—	—	—
I245.....	157	123·0	10·63	0·1453	0·1221	1·1509	0·7296	4·2697	16·20	0·0108
L396.....	149	111·5	9·00	0·1536	0·1340	0·7631	0·8695	4·4216	17·70	0·0097
L398.....	156	89·5	11·97	0·1463	0·1249	0·9885	0·6678	4·5437	15·80	0·0201
M446.....	155	113·5	10·50	0·1271	0·1154	0·8083	0·7140	3·9612	18·10	0·0126
M447.....	152	104·5	10·31	0·1573	0·1384	0·9587	1·0735	3·5200	17·20	0·0098
N488.....	152	114·0	9·47	0·1195	0·0999	1·1958	0·9330	3·6694	17·80	0·0152
E198*.....	152	112·5	8·34	0·1238	0·1032	0·8042	0·9343	2·3261	11·50	0·0089
E198*.....	152	112·5	8·28	0·1316	0·1035	0·8158	0·5493	3·0717	11·90	0·0098
H229*.....	152	112·0	8·31	0·1162	0·0939	1·0648	0·7824	3·0082	14·90	0·0125
H229*.....	152	112·0	8·47	0·1160	0·0960	0·9465	1·0286	2·7190	16·20	0·0159
L397*.....	151	110·0	7·56	0·1193	0·0996	0·6854	0·5847	2·8067	10·50	0·0131
L397*.....	151	110·0	9·81	0·1794	0·1518	0·7095	0·7585	3·7400	21·10	0·0299
N482*.....	150	111·5	7·38	0·0901	0·0752	0·8540	0·7982	3·5500	12·50	0·0162
N482*.....	150	111·5	8·44	0·1314	0·1161	0·7933	0·7130	3·5386	15·30	0·0153

TABLE 15 (c).—*Group* 7 (Salsola *fed from* 50*th day up to parturition*). *Lambs: Genitalia: Fresh weights*

Ewe No.	Gest. (days)	Ewe wt. post-partus (lb)	Wt. of lamb—stomach (lb)	Testes (gm)	Genitalia (gm)	Uterus (gm)	Cervix (gm)	Ovaria (gm)	Follicles	Udder (gm)
A105.....	151	140·0	11·69	3·3151	—	—	—	—		—
C182.....	151	112·0	8·03	1·8786	—	—	—	—		—
E184.....	156	94·0	5·69	1·9280	—	—	—	—		—
F200.....	157	122·0	13·72	—	5·5000	2·0901	2·3396	0·3287	+22	10·3711
F212.....	170	110·5	9·28	—	3·7364	1·4085	1·6260	0·0514	—	5·3163
H228.....	152	120·0	12·97	3·3951	—	—	—	—		—
H234.....	154	120·0	9·56	—	2·7687	1·1393	1·1373	0·1005	5	6·6272
I239.....	147	100·5	10·06	—	—	—	—	—		—
I245.....	157	123·0	10·63	2·9498	—	—	—	—		—
L396.....	149	111·5	9·00	—	3·3188	1·4256	1·4140	0·0485	—	5·8616
L398.....	156	89·5	11·97	2·7232	—	—	—	—		—
M446.....	155	113·5	10·50	—	2·9018	1·1293	1·2923	0·0477	—	6·3906
M447.....	152	104·5	10·31	2·5566	—	—	—	—		—
N488.....	152	114·0	9·47	—	3·8805	1·2872	1·8164	0·0394	—	6·3945
E198*.....	152	112·5	8·34	—	2·7563	0·8853	1·4396	0·0503	—	6·0214
E198*.....	152	112·5	8·28	—	2·9916	0·9377	1·4233	0·0853	—	7·1481
H229*.....	152	112·0	8·31	2·4745	—	—	—	—		—
H229*.....	152	112·0	8·47	2·1440	—	—	—	—		—
L397*.....	151	110·0	7·56	2·5995	—	—	—	—		—
L397*.....	151	110·0	9·81	3·0135	—	—	—	—		—
N482*.....	150	111·5	7·38	—	3·7860	1·6501	1·4063	0·0483	—	5·4616
N482*.....	150	111·5	8·44	2·4654	—	—	—	—		—

TABLE 16.—*Length of gestation and lamb weights from* 1967 *feeding experiment, South West Africa*

Group 1 (Controls)		Group 2 *Salsola* 150 days		Group 3 *Salsola* first 50 days		Group 4 *Salsola* second 50 days		Group 5 *Salsola* last 50 days		Group 6 *Salsola* first 100 days		Group 7 *Salsola* last 100 days		Group 8 (Field control)	
Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)	Gestation (days)	Lamb weight (lb)
148	10·81	151	9·25	146	10·63	147	8·50	149	9·44	146	7·38	147	10·06	145	6·78
148	8·94	151	11·28	147	9·31	148	10·47	150	9·00	148	9·94	149	9·00	147	8·50
148	8·09	152	10·59	148	8·94	149	13·72	150	9·94	149	11·16	151	11·69	148	10·12
148	9·13	152	10·25	148	9·09	149	8·75	151	11·13	149	8·41	151	8·03	148	8·50
149	11·25	153	12·34	149	9·38	149	9·41	152	9·97	150	10·28	152	9·47	148	10·12
149	9·47	154	10·53	150	9·03	149	9·72	152	8·88	151	10·75	152	10·31	149	8·94
149	8·97	154	8·47	150	9·78	150	11·34	152	9·44	151	11·28	152	12·97	149	9·81
149	10·28	154	10·22	151	12·34	150	12·56	152	10·44	151	13·22	154	9·56	150	6·44
150	10·53	155	12·44	151	11·38	150	11·41	152	10·66	152	11·97	155	10·50	150	10·53
151	11·50	155	10·56	151	10·41	150	14·31	153	10·50	152	10·19	156	5·69	150	11·78
151	10·66	155	10·44	152	11·09	150	10·38	153	10·66	155	10·69	156	11·97	150	8·66
151	10·84	156	10·75	—	—	151	11·00	153	10·22	—	—	157	13·72	150	7·47
—	—	157	10·06	—	—	151	10·59	154	11·37	—	—	157	10·63	150	9·06
—	—	159	11·19	—	—	151	10·28	156	11·88	—	—	170	9·28	150	11·50
—	—	166	10·06	—	—	153	11·81	163	9·56	—	—	—	—	152	10·60
—	—	—	—	—	—	153	9·22	—	—	—	—	—	—	153	9·34
—	—	—	—	—	—	—	—	—	—	—	—	—	—	153	8·94
Mean: 149·3	10·04	154·9	10·56	149·4	10·13	150·0	10·84	152·8	10·21	150·4	10·48	154·2	10·20	149·5	9·24
146*	—	151*	—	151*	—	147*	—	151*	—	147*	—	150*	—	147*	—
149*	—	—	—	152*	—	148*	—	155*	—	147*	—	151*	—	149*	—
—	—	—	—	—	—	—	—	—	—	149*	—	152*	—	—	—
—	—	—	—	—	—	—	—	—	—	150*	—	152*	—	—	—
—	—	—	—	—	—	—	—	—	—	151*	—	—	—	—	—

* Twins.

In a small number of post-term ewes which lambed normally haemorrhage within the carunculae was very conspicuous. This observation had not previously been made on natural post-term ewes. The weights of all the various organs did not show any significant difference between control and post-term *Salsola*-fed ewes, except for the adrenals which were somewhat enlarged in the latter. The pars tuberalis of the hypophysis in one post-term ewe was very thin and probably atrophic.

Lambs: All the macroscopical changes noticed in the post-mature lambs were produced artificially in lambs by feeding *S. tuberculata* to the pregnant ewes for any length of time which included the last 50 days of gestation. This was more evident in the most prolonged cases of gestation and, in general, of a milder nature than in the natural cases. Some of the lambs were overweight but many were not enlarged and were either of normal or below average size (Table 11, Fig. 7). The incidence and intensity of pigmentation were directly correlated with the length of gestation and were less constant and milder in early postmaturity. It became very evident that the first stage of pigmentation commenced in the mesenteric lymph nodes and kidneys (designated as Stage 1 in Tables 12 to 15). Either the one or the other, however, could be affected first in very early cases. Thereafter the pigmentation spread to the periportal, renal, pancreatic and mediastinal nodes (Stage 2) and then to the prescapular and other peripheral nodes (Stage 3). In some of the lambs the bronchial lymph nodes were affected before the mediastinals. Eventually the thymus (Stage 4) and ultimately the entire carcass (Stage 5) were affected and had a khaki-brownish discoloration. Even the bones in these cases were of the same colour [Plate 6 (33)]. The pigmentation in the kidneys and lymph nodes varied from very mild olive (±), mild olive (+), olive (++), dark olive (+++) to almost olive black (+++ to ++++) [Plate 6 (31 and 32)]. Only the medulla of the lymph nodes and the renal cortex were affected, except in rare cases where the inner zone of the renal medulla was mildly greenish and in very mild cases the pigmentation commenced within the cortex at the cortico-medullary junction. In advanced cases of pigmentation the liver was usually of a very dark chocolate brown colour [Plate 6 (32)] and even the lungs had a brownish discoloration. In one case where the mesenteric lymphatics were carefully examined immediately after slaughter it was observed that the lymph had a slight greenish-brown tinge. Some of the controls showed very mild to mild pigmentation but never further advanced than the first stage of pigmentation. The bile content of the gall bladder was fairly increased in the experimental lambs and more frequently, but not constantly, in those cases with prominent pigmentation. The testes of postmature lambs were either descended or partially descended in the scrotum. The bones, especially those of the skull, gave more resistance to fracturing and sawing and were harder in some of the advanced cases. Many of the incisors were erupted, but never more than three pairs and a maximum of two fully erupted pairs. In the control lambs on the other hand, only one showed fairly advanced eruption, the general pattern being one of partial or pinpoint eruption. In the first feeding experiment 33 per cent of the controls revealed no signs of any erupting teeth.

Polyfollicular ovaries were only found in advanced cases of postmaturity and mildly polyfollicular ones rarely in controls.

Exceptional findings like hypoplastic lungs, oedema and haemorrhages, e.g. around the neck due to prolonged and difficult parturition were also encountered particularly in the postmature lambs. Congenital abnormalities such as scoliosis, wryneck, shortleggedness, firmly flexed legs probably due to shortened flexor tendons, cerebellar hypoplasia and microphthalmus were found in both post-term and unaffected *Salsola* groups, but were probably unrelated to the effect of the plant, because the number of *Salsola*-fed ewes, particularly during the 1967 experiment in South West Africa, was larger than that of the lucerne-fed controls.

Microscopical findings of all the experimental groups: Ewes: By studying both control and experimental post-term ewes, the mild changes suspected in the natural post-term ewes became more apparent. The adrenal cortico-medullary ratio of the experimental ewes and the lipid content of the adrenals, liver and kidneys were higher than those of the controls (Table 4). No unequivocal evidence of shifts in the ratio of the various granulated hypophysial cells could be obtained. There was only a suspicion of a small increase in granulated basophils and a decrease in the number of granulated carminophils and it was obvious that the numbers of both experimental and control ewes that were sacrificed for post-mortem examination were inadequate for proper comparative studies. Intranuclear hypophysial inclusions found earlier in the natural post-term ewes were seen in both control and experimental ewes [Plate 7 (38 and 39)]. Haemorrhage in the carunculae [Plate 7 (40)] and stromal proliferation at the base of the chorionic villi were noticed in a few cases [Plate 7 (41)].

Lambs: The most important changes found are shown in Table 17. By examining 37 controls the normal appearance of lamb tissues and organs was studied and compared with those of the experimental groups and known abnormals. In general, the findings in the experimental animal corresponded with those of the natural postmature ones, the only exception being that they were usually milder, especially in the less advanced cases of postmaturity. There was also a variation in the appearance of the tissues of the control lambs. Some of them showed mild changes particularly mild pigmentation, similar to those of the experimental or natural postmature lambs. This caused some initial confusion and made the study and ultimate evaluation of the findings very difficult. The impression was gained that most of the macro- and microscopical changes of postmaturity are secondary to the primary cause of prolonged gestation and that some of the controls showed evidence of foetal distress and postmaturity within the normal range of gestation.

PLATE 7.—35. *Salsola tuberculata* var. *tomentosa*, the shrub used to produce cases of postmaturity. 36. A ewe which was fed on the shrub during her entire gestation period at 177 days of pregnancy. She showed no signs of parturition at this stage. Her side was shorn and prepared for a caesarian section. A 17·5 lb foetus was recovered. 37. Enlarged nucleus (arrow) in the adenohypophysis of a *Salsola*-fed ewe with a gestation period of 159 days. She was killed immediately after parturition. HE × 500. 38. Intranuclear inclusion with eosinophilic granules within the adenohypophysis of a *Salsola*-fed ewe with a gestation period of 166 days. HE × 1200. 39. Another type of homogenous eosinophilic intranuclear inclusion in the adenohypophysis of a ewe (natural case of prolonged gestation). HE × 620. 40. Caruncula of the case described in 38 showing marked haemorrhage. HE × 5·2. 41. Caruncula of the same case (38 and 40) showing increased stroma at the base of the chorionic villi. HE × 7·5.

Endocrines: Hypophysis: The macroscopical observation of atrophy was confirmed microscopically, as demonstrated in Table 17. In the controls there were several cases (21 per cent) with more than the average number of well-granulated basophils and 88 per cent had a large number of well-granulated carminophils. In the advanced postmature lambs, however, there was an increase of well-granulated basophils (26 per cent) and a decrease in well-granulated carminophils (71 per cent).

Adrenals: It was evident that many of the experimentally produced early postmature lambs from ewes with a gestation period varying from 150 to 160 days, had hypertrophied adrenals. The adrenals of the more advanced cases, however, were atrophied. This was elicited by both the cortico-medullary ratio, and lipid content of the cortex (Table 17) and confirmed the macroscopical observations.

Thyroid: The changes in the thyroid glands of the experimentally produced postmature lambs were milder, but similar to those of the natural cases. On the average, the size of the follicles in the controls was either similar to or smaller than that of the experimental lambs; the epithelium being more regularly cuboidal, high cuboidal or columnar and the colloid more vacuolar.

Thymus: Atrophy was present in several experimental cases, especially the more advanced ones.

Pancreas: Unequivocal evidence of atrophy was not seen in any of the cases.

Genitalia: Polyfollicular ovaries were seen in 50 per cent of the advanced experimental cases of postmaturity and in 6 per cent of the controls. The number of Leydig cells gradually, but progressively decreased with increased postmaturity.

Other organs: Liver: As with most of the other changes, hyaline degeneration was shown to be a progressive lesion and became a relatively prominent feature beyond 165 days of gestation [Plate 3 (12)]. One of the controls showed evidence of suspected very mild degeneration. Various stages of bile duct proliferation were seen in more than 40 per cent of the advanced cases. Lipids were more constantly present in the controls and gradually diminished in quantity in the postmature experimental lambs. Pigment-laden macrophages were also found within the hepatic and central veins of the experimental animals. Haemosiderosis was no distinguishing feature, being present both in many controls and experimental lambs.

Kidneys: The great majority of both experimental and control lambs revealed either traces of, or prominent, intraepithelial pigmentation. In those that appeared unpigmented macroscopically the pigmentation was invariably very mild and usually mainly confined to the labyrinths at the cortico-medullary junction. In the controls the staining reaction with various techniques was similar to that of the experimental and natural postmature lambs. The amount of lipids in the cortex decreased gradually in direct relation to the length of gestation. Marked vesiculation of the epithelial cytoplasm [Plate 2 (10)] appeared more frequently in the most prolonged group of lambs, but was never an important or dominant feature, and all the controls were negative.

Lymph nodes: A lipoproteinaceous pigment was demonstrated in both experimental and control lambs but less frequently in the latter and it corresponded in distribution and intensity with the macroscopical evidence of pigmentation. The mesenteric lymph nodes were invariably the first to be affected and also the most intensely pigmented, followed by a group which included the periportal, pancreatic and renal lymph nodes. Eosinophilic granules and globules similar to those found in the natural postmature lambs were seen in both experimentals and controls [Plate 5 (25 and 26)]. The PAS technique was superior to the HE stain for demonstrating these granules which were PAS-positive [Plate 5 (27)]. They were present in most of the lymph nodes, but in much smaller quantities and mildly PAS-positive in the mediastinal and prescapular nodes. The only lymph node other than the mesenteric lymph nodes which occasionally contained fairly large amounts of PAS-positive material proved to be the periportal node. The sequence of events in the development of the PAS-positive material and the progressive accumulation of pigment appears to be as follows:—

Initially the r.e. cells have an increase in cytoplasm which becomes more eosinophilic. Few vesicles may be present. Subsequently small and larger granules and globules develop which become more intensely eosinophilic with HE stain. Eventually the colour gradually changes to brownish red and medium brown. With the Schmorl's technique and PAS the reaction is at first very mildly positive. Then it increases in intensity. The PAS reaction decreases and eventually becomes negative as the colour of the pigment changes to brown. With the Schmorl's technique, on the contrary, it becomes more intense with a change of colour. In the early stages of pigmentation of the mesenteric lymph nodes and to a somewhat milder degree in the periportal nodes, the distribution and progression of pigmentation directly corresponded to the presence of PAS-positive material. A very characteristic anatomical feature of the chain of mesenteric nodes in sheep is the terminal p-shaped one. Macroscopically the head of this node was only pigmented in the more advanced cases. Pigmentation always commenced within the stem and other mesenteric nodes. The distribution of the PAS-positive granules corresponded directly to the presence and progressive development of the macroscopically noticeable pigmentation with the exception that PAS-positive material was sometimes demonstrated in the stem of the p-shaped lymph node before the pigmentation was macroscopically noticeable. Infiltration of neutrophils was sometimes seen in various lymph nodes of both control and experimental lambs [Plate 5 (29)].

Lungs: Pigment was found in some of the advanced cases of postmaturity. Aspirated meconium gave Schmorl's-positive reaction, similar to that of the pigment.

Pancreas: No definite evidence of atrophy was detected microscopically. The difference in colour observed macroscopically appeared to be due to

TABLE 17.—*Some macro- and microscopical findings from all the experimental and postmature lambs in South West Africa and Onderstepoort*

Item	*Controls*		Entire *Salsola* group		*Salsola group* <152 days gestation		*Salsola* group 152–155 days gestation		*Salsola* group >155 days gestation		*Salsola* group >165 days gestation		*Natural* postmature lambs	
	No. examined	% Positive	No. examined	% Positive	No. examined	% Positive	No. examined	% Positive	No. examined	% Positive	No. examined	% Positive	No. examined	% Positive
Hypophysis—														
Large No. of granulated carminophils	34	88	69	70	11	91	25	64	28	71	6	67	26	42
Increased No. of granulated basophils	34	21	69	41	11	27	25	52	28	36	6	33	22	77
W I <1·25: Mostly atrophied	35	6	71	40	11	17	25	16	29	60	6	100	26	100
W I >1·90: Mostly hypertrophied	35	9	71	6	11	17	25	4	29	3	6	0	26	0
Adrenals—														
Presence of lipids*	35	89	74	70	12	83	27	81	30	53	7	29	27	40
$\frac{C}{M}$ ratio >1·0: Mostly hypertrophied	33	6	74	18	11	27	27	15	30	17	7	0	25	0
$\frac{C}{M}$ ratio >0·9	33	24	74	32	11	55	27	29	30	27	7	14	25	0
$\frac{C}{M}$ ratio<0·7: Mostly atrophied	33	6	74	10	11	9	27	4	30	17	7	43	25	72
W I >9·5: Mostly hypertrophied	36	6	75	17	12	25	28	22	32	22	5	0	11	9
W I <6·0: Mostly atrophied	36	8	75	8	12	0	28	4	32	16	5	40	11	55
Thyroids—														
Follicles mainly M–S or S–M	34	85	70	67	11	82	30	73	28	54	7	0	29	66
Epithelium mainly cuboidal or columnar	34	91	70	50	11	45	30	67	28	32	7	0	32	3
Colloid well vacuolated	34	65	70	59	11	73	30	60	28	57	7	14	29	14
Colloid mildly vacuolated	34	21	70	19	11	0	30	27	28	14	7	14	29	21
Colloid not vacuolated	34	15	70	23	11	27	30	13	28	29	7	71	29	66
Ovaries—														
Presence of Graafian and atretic follicles	17	6	38	29	5	40	18	22	10	50	4	50	26	92
Fairly advanced corp. fibr. atr	17	0	38	3	5	0	18	0	10	10	4	0	26	39
W I>0·9: Hypertrophied	10	0	39	18	5	20	18	17	13	15	4	25	15	100
Testes—														
Presence of Leydig cells	19	100	29	97	7	100	15	100	15	93	3	67	13	38
Absence or very few Leydig cells	19	0	29	17	7	0	15	7	15	27	3	67	13	69
W I >30·0: Mostly hypertrophied	19	5	30	40	5	20	13	46	11	36	3	33	4	0
W I <22·50: Mostly atrophied	19	16	30	3	5	0	13	0	11	9	3	33	4	100
Kidneys—														
Macroscopic pigmentation	37	24	72	64	12	50	30	47	30	80	7	100	39	100
Suspected nephrosis	37	0	72	8	12	0	30	0	30	20	7	43	28	36
Presence of lipids*	32	67	67	42	12	17	30	40	29	38	7	29	21	62
Liver—														
Degeneration	35	3	70	20	12	8	31	13	30	30	7	71	33	49
Bile duct proliferation	35	3	70	10	12	0	31	0	30	23	7	43	33	36
Presence of lipids*	33	73	70	40	12	17	31	48	29	31	7	14	23	70

* Excluding very mild positive cases and in the adrenal also those where z. fasc. and z. retic. only are negative.
M=Medium.
S=Small.

granulation of the epithelial cells. Those lambs that had suckled had a pinkish pancreas due to degranulation and the others that did not suckle a whitish pancreas.

In cases showing advanced pigmentation the general distribution pattern of pigment-bearing macrophages was similar to those in the natural postmature lambs.

Intestine: The intestinal epithelium of a few experimental postmature lambs contained numerous large eosinophilic globules [Plate 5 (30)].

4. *Feeding trials with* S. tuberculata *on rats*

(i) *The effect on the oestrous cycle:* The rats of all three groups lost weight during the period when the plant and lucerne meal was included in their normal ration because of the decreased nutritional value, but at the end of the 15 days no significant differences were perceptible in the weights of the two test groups and the controls. The body weights returned to previous values within 4 days after suspension of the mixed rations.

From the commencement of feeding until observations terminated, all 12 control rats exhibited 12 cycles each. The average length of the 144 cycles observed was 4·082 days (Table 18 and Fig. 6). During this period 11 control rats had one five-day cycle each instead of the usual four-day cycles but this is, however, quite normal for this strain of rat. The remaining rats maintained four-day cycles throughout the experiment.

In the first test group fed the ration containing 20 per cent finely ground *S. tuberculata* each of the 12 rats had an average of 9·25 cycles whilst the mean length of the 111 cycles during this period was 5·037 days. This is significantly ($P<0·005$) longer than the average recorded for the control group. The majority of this group's cycles were arrested in the dioestrous stage after the second cycle after *Salsola* was introduced. The period of dioestrus varied from 12 to 16 days before cyclical activity resumed. Although three rats never showed a prolonged period of dioestrus but irregular cycles, it was found that the plant generally required two cycles before it took effect and the oestrous cycle was restored to normal 6·09 days after the plant was withdrawn from the ration.

The change in the normal cycles of the rats was more dramatic in the second test group which was fed a ration containing 40 per cent of the plant. The plant required less than one cycle to show an effect. Four of the 12 rats changed from a four-day cycle to a five-day cycle and the cycles were arrested in dioestrus. From the day the plant was introduced to the rats until vaginal inspection was stopped 49 days later only 51 cycles were observed. These cycles varied in length from 4 to 31 days with a mean length of 10·99 days. It took the rats, on the average, 18·5 days to return to oestrus after withdrawal of the plant material. The cycles which followed were extremely irregular and varied from 4 to 12 days in length.

The results obtained with the ethanolic extracts were remarkably similar to the previous findings, but it could not be reproduced with extracts involving solvents other than the alcoholic compounds.

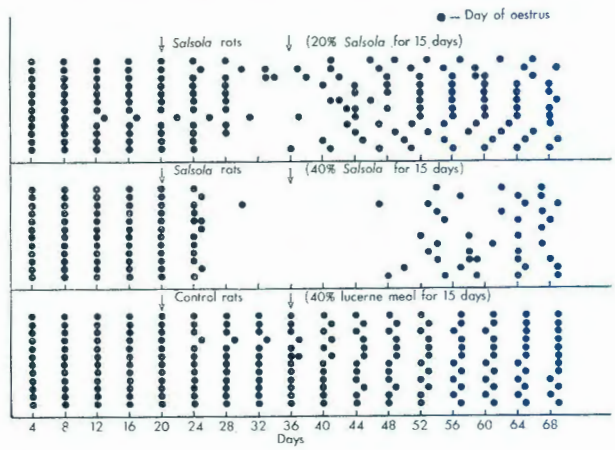

FIG. 6.—Effect of *S. tuberculata* on the oestrous cycle of rats (feeding experiment). The periods between the arrows indicate the duration of feeding with *Salsola* or lucerne meal.

(ii) *The effect on gestation:* No significant differences were found in the sizes of the litters of the three groups, and the same is true for the average body weight per small rat. The gestation periods of the control and the 20 per cent *Salsola* groups were practically similar, although three of the rats in the 20 per cent *Salsola* group were pregnant for 23 days whilst the duration of the longest pregnancy of the controls was 22 days in only two rats. The length

TABLE 18.—*The effect of* S. tuberculata *on the oestrous cycle of the rat (feeding experiment)*

Group	Days exposed to *Salsola*	Days observed*	Average length/cycle (days)	Average number of cycles/rat/ 49 days	Period from withdrawal of *Salsola* until re-appearance of cycles
Control	0	49	4·082	12·00	—
20% *Salsola*	15	49	5·037**	9·25†	6·09 days
40% *Salsola*	15	49	10·99**	3·42†	18·5 days

* Period from when the mixed ration was introduced until end of observations.
** Significantly longer ($P<0·005$) than the oestrus cycles of the control rats.
† Significantly less ($P<0·005$) cycles than control rats.

of gestation of the rats in the 40 per cent *Salsola* group, however, was significantly longer ($P<0.05$) than in both the other groups. Two females in the 40 per cent *Salsola* group had pregnancies lasting 24 days although each had five and six baby rats in the litters respectively; whilst the two rats in the control group with the longest gestation period of 22 days only had one and two small rats in their litters (Table 19). Both the thymus and adrenals of the baby rats in the 40 per cent *Salsola* group weighed significantly less than those in the other two groups ($P<0.05$).

(iii) *The effect on various organs:* The oestrous cycles of the *Salsola* rats were suspended in dioestrus 4·5 days after being fed with the plant material and they were still in dioestrus on Day 15 when all of them were killed. The controls showed normal cycles, but they were also in dioestrus when destroyed for weight determinations. The weights of the uterus, ovaries and hypophysis of the *Salsola* group were significantly lower than the corresponding weights of the same organs of the control rats ($P<0.05$), whilst the adrenals of the *Salsola* group were significantly heavier than those of the controls ($P<0.05$). No significant differences in the weights of the body, spleen, kidneys and liver of the two groups were found (Table 20).

5. *Plant analysis*

(i) *Determination of oestrogenicity in* S. tuberculata: No substance with an oestrogenic effect was extracted from the shrub by the method employed. The *Salsola* group with a mean body weight of 60·15 gm had a mean uterine weight of 0·052 gm as compared to the controls with a mean body and uterine weight of 57·14 gm and 0·070 gm respectively.

(ii) *Determination of anti-oestrogenicity in* S. tuberculata: No biological evidence of an anti-oestrogenic effect was found in the extracts of the plant (Table 21).

TABLE 19.—*The effect of* S. tuberculata *on the length of pregnancy and the litters of the rat (feeding experiment)*

Group	Number of rats per group	Average size of litters	Average weight per small rat (gm)	Average length of gestation (days)	Range of gestation (days)	Day-old rat adrenal (mg)	Day-old rat thymus (mg)	Average weight of mother rats (gm)
Control	24	6·12	5·213	21·725	21–22	2·71	8·16	262
20% *Salsola*	24	5·89	5·096	21·81	21–23	2·69	7·94	251
40% *Salsola*	24	5·76	5·296	22·87**	22–24	2·12*	6·92*	257

* Significantly less ($P<0.005$) than both other groups.
** Significantly longer ($P<0.05$) than in other groups.

TABLE 20.—*The mean weight (in grams) of various organs of* 12 *female rats after being fed with a ration containing* 40 *per cent* S. tuberculata *for* 15 *days*

Group	Body weight	Uterus	Ovaries	Adrenals	Liver	Spleen	Hypophysis	Kidneys
Salsola (40%)	179·6	0·263**	0·034**	0·043†	6·98	0·466	0·007**	1·41
Controls*	183·5	0·648	0·052	0·038	7·25	0·519	0·011	1·54

* Twelve normal female rats fed with ration containing 40 per cent lucerne meal for 15 days and slaughtered in dioestrus.
** Significantly less ($P<0.05$) than corresponding weight of control rats.
† Significantly more ($P<0.05$) than corresponding values of the control rats.

TABLE 21.—*Results of the test on rats for anti-oestrogenicity in* Salsola

	Saline group		Saline + oestradiol group		Saline + oestradiol + *Salsola* extract group		Saline + oestradiol + *Salsola* extract + oestradiol group		*Salsola* extract + oestradiol group	
	Body weight (gm)	Uterine weight (gm)	Body weight (gm)	Uterine weight (gm)	Body weight (gm)	Uterine weight (gm)	Body weight (gm)	Uterine weight (gm)	Body weight (gm)	Uterine weight (gm)
Mean	65·59	0·0540	65·92	0·0870	69·3	0·0857	59·8	0·0701	62	0·0683
SD	±12·1	±0·0113	±16·72	±0·0287	±15·35	±0·0191	±14·96	±0·0227	±15·32	±0·0149

SD = standard deviation.

6. *Trace element and vitamin A analyses*

The results are tabulated in Table 22. No significant constant deficiencies or excesses of copper, cobalt, manganese or vitamin A were present.

7. *Statistical analysis of experiments in South West Africa*

In the feeding experiments conducted to determine the time and duration of the period of insult, the gestation period was significantly increased (P=0·01) in the ewes fed *S. tuberculata* during any period of time which included the last 50 days of gestation. A similar but more prominent increase was noticeable in the combined *Salsola* group which included all the Karakul sheep fed during the period of insult in both experiments in South West Africa (Fig. 8). The lamb weights of this group exceeded the maximum control weight by 8 per cent, and 16 per cent had a subminimum weight as compared to the controls (Fig. 9). A significant parabolic relationship was found between the lamb weight adjusted for the maternal weight and the gestation period (P=0·05), but the quadratic fit was only slightly better than the linear relationship at P = 0·20. The same type of relationship should hold for the *Salsola* group over the initial period, but it was found that the lamb weight increased again after prolonged gestation. This indicated a cubic relationship. Such a relationship fits the data significantly better (P = 0·10) than the quadratic. There is an inflection point at a gestation period of 160 days, but insufficient data in the region >155 days could be a reason why a peak is not found earlier than 160 days. In the combined *Salsola* group, 31·8 per cent of the cases were found within the same limits of gestation as the controls, but the mean gestation period was longer and the mean body weight less than that of the control group (Fig. 7). The weight of the second group with a mean gestation of 153·9 days and the third group at a mean gestation of 157·9 days was respectively less and higher than that of the control. Although the percentage of cases in the subsequent two groups decreased rapidly, indications were present that the increase in body weight was followed by a decrease and an ultimate increase (Fig. 7).

In comparing the means and the variances of the organ weights of the control with those of the *Salsola* group, without correlating them to the body weights, the hypophysis showed a significant decrease in weight, but the adrenal weight was increased during the 1967 experiment and seemingly insignificantly decreased during the 1966 experiment (Table 23). The effect on the brain and liver weights for the two feeding experiments showed opposite tendencies but this can be ignored since the values did not differ significantly. The relationship between the adjusted organ weights and gestation period (Table 24) illustrated more clearly the sequence of events and proved to be similar to the relationship between the weight index and gestation period. The linear relationship with a negative slope of the hypophysis, adrenal and thymus indicates that the growth of these organs relative to the growth of the lamb declined with prolonged gestation. The relative growth of the liver, spleen and thyroid reached a maximum at 154, 155 and 162 days respectively. The kidney weight on the contrary showed a relative increase. By using the weight indices in histograms the weight index of the adrenal (Fig. 10) and hypophysis (Fig. 11) for the first group within normal limits of gestation (148 to 152 days) was respectively higher and lower than that of the control. For the subsequent groups both organs declined in weight index, but more prominently in the case of the hypophysis. In the second and third group, however, the adrenal weight index of the *Salsola* group was still higher than that of the control, but it decreased very markedly in the last group. These findings reflect a consistent progressive atrophy of the hypophysis in contrast with an initial hypertrophy and subsequent gradual but ultimate marked atrophy of the adrenal. The weight index of the testes of the combined *Salsola* group indicated a relative increase up to 155 days of gestation followed by gradual decrease (Table 17). The ovarian weight index on

TABLE 22.—*Trace element and vitamin analyses*

Aminal	Liver				Kidney
	Copper ppm	Cobalt ppm	Manganese ppm	Vitamin A I.U./gm	Copper ppm
Natural postmature lamb	391·6	0·06*	<3·0*		
Natural postmature lamb	368·3				61·5
Natural postmature lamb	279·7	0·20	9·6		42·9
Experimental postmature lamb	558·0	0·15	9·8		
Experimental postmature lamb	240·0	0·16	12·0		
Unaffected lamb	396·3				27·0
Unaffected lamb	336·0	0·20	9·6		
Unaffected lamb	336·0	0·22	12·0		
Unaffected lamb	242·0	0·18	9·5		
Experimental control lamb	270·0	0·18	9·8		
Post-term ewes	135·2	0·17	3·0*		
Post-term ewes	582·8	0·21	10·8		
Post-term ewes	713·3*	0·11	3·6*		
Post-term ewes				375	
Post-term ewes				400	
Post-term ewes				365	

* Copper value rather high, cobalt value somewhat low and manganese values low.

TABLE 23.—*The effect of* S. tuberculata *on the weight of the lamb organs (in grams)*

Organ	1966 Weights*						1967 Fresh Weights					
	Mean		Variance				Mean		Variance			
	Salsola	Lucerne	*Salsola*	Lucerne	C.V.	Test	*Salsola*	Lucerne	*Salsola*	Lucerne	C.V.	Test
Brain.....	57·3	58·6	31·0	6·6	2·3	T2 n.s.	55·0	54·8	12·1	21·4	2·3	T1 n.s.
Kidney.....	28·3	25·4	86·7	10·9	9·2	T2 n.s.	27·0	25·4	37·5	16·6	7·3	T1 n.s.
Liver.....	91·8	101·9	693·1	217·2	7·0	T2 n.s.	105·3	99·8	480·3	256·5	6·6	T1 n.s.
Spleen.....	4·3	5·5	1·0	1·9	8·1	T1††	5·3	6·0	1·2	2·2	7·2	T1 n.s.
Thymus.....	13·6	22·1	29·0	12·6	9·3	T1††	18·9	25·0	25·7	21·1	8·0	T1††
Pancreas.....	3·93	3·52	0·82	0·31	6·2	T2††	4·19	3·78	0·70	0·46	6·4	T1 n.s.
Hypophysis.....	0·1173	0·1563	0·0010	0·0005	7·5	T1††	0·1363	0·1552	0·0005	0·0007	5·7	T1†
Anterior pituitary	0·0893	0·1222	0·0009	0·0005	9·4	T1††	0·1138	0·1323	0·0007	0·0006	7·5	T1†
Adrenal.....	0·7280	0·7469	0·0298	0·0128	7·1	T1 n.s.	0·8722	0·7372	0·0540	0·0086	5·3	T2††
Thyroid.....	0·9481	0·7693	0·1212	0·0172	9·2	T2†	0·8598	0·7772	0·0619	0·0403	9·6	T1 n.s.

T1: t-test with Ho: μ (lucerne) = μ (*Salsola*); σ^2 (lucerne) = σ^2 (*Salsola*).
T2: t-test with Ho: μ (lucerne)=μ (*Salsola*); σ^2 lucerne)$\neq\sigma^2$ (*Salsola*).
n.s.: No significant difference between means of *Salsola* and lucerne groups.
†: P=0·05 significant difference between means of *Salsola* and lucerne groups.
††: P=0·01 significant difference between means of *Salsola* and lucerne groups.
*: Hypophysis, anterior pituitary, adrenal and thyroid weighed after fixation (fixed weights).

the other hand was increased. In contrast to the brain weight index of the control which had a positive linear relationship to the length of gestation those of the *Salsola* group indicated no definite relationship. In the most advanced group of postmaturity indications of a possible negative relationship were found, but unfortunately the number of cases does not permit definite conclusions in this respect (Fig. 12). However, the absence of a well-discernible relationship could be an indication that the organ was affected. Contrary to expectation the relative dry pelt weights did not vary significantly from those of the control but a decrease was appreciable in the most advanced cases of postmaturity (Fig. 13). From the data of the 1967 experiment it was calculated that the control had 8·5 per cent overgrown pelts and none with long hair whereas the affected *Salsola* groups had a total of 30 per cent overgrown pelts and 32 per cent long wool. The cumulative relative frequencies of the adrenal and hypophysial weight indices of natural postmature (P), control (C) and artificial (S) cases are compared in Fig. 14 and 15.

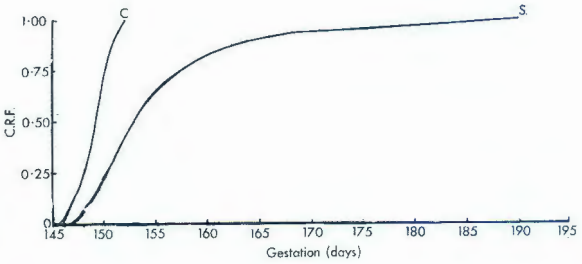

FIG. 8.—Cumulative relative frequencies (CRF) of the length of gestation of all the control Karakul ewes (C) and *Salsola*-fed ewes (S) in South West Africa (free-hand curves).

TABLE 24.—*The relationship between organ weights (adjusted for lamb weights) and period of gestation* (P = 0·05)

Organ	Relationship	Slope (if applicable)
Brain	None	—
Kidney	Linear	Positive
Liver	Quadratic	A maximum at 154 days gestation
Spleen	Quadratic	A maximum at 155 days gestation
Thymus	Linear	Negative
Pancreas	None	—
Hypophysis	Linear	Negative
Anterior pituitary	Linear	Negative
Adrenal	Linear	Negative
Thyroid	Quadratic	A maximum at 162 days gestation

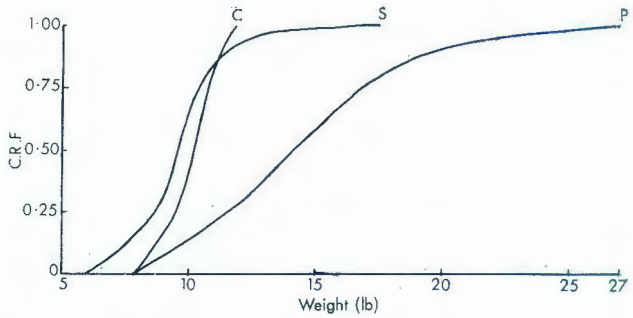

FIG. 9.—Cumulative relative frequencies (CRF) of the weights of control lambs (C), field cases of postmaturity (P) and artificially produced cases of postmaturity (S) in South West Africa (free-hand curves).

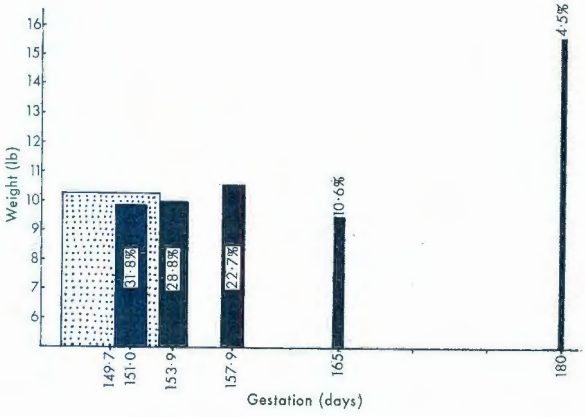

FIG. 7.—Comparative frequency distribution of the body weights of all the control (dotted histogram) and artificially produced postmature lambs (black histograms) in South West Africa.

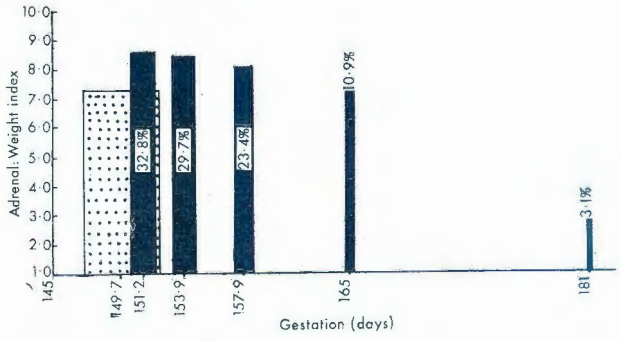

FIG. 10.—Comparative frequency distribution of the adrenal weight indices of control lambs (dotted histogram) and artificially produced postmature lambs (black histograms) in South West Africa.

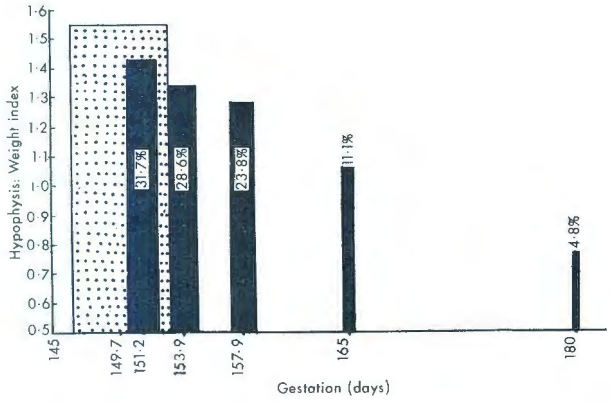

FIG. 11.—Comparative frequency distribution of hypophysial weight indices of control lambs (dotted histogram) and artificially produced cases of postmaturity (black histograms) in South West Africa.

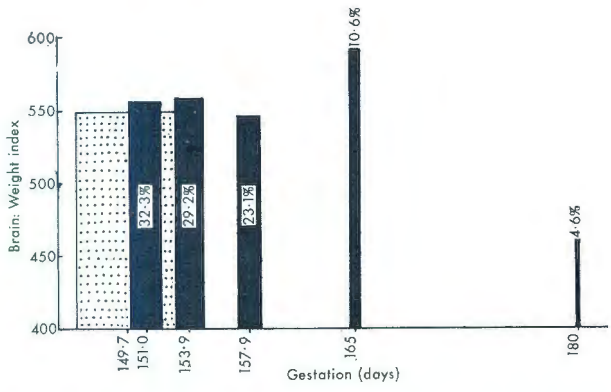

FIG. 12.—Comparative frequency distribution of brain weight indices of control lambs (dotted histogram) and artificially produced postmature lambs (black histograms) in South West Africa.

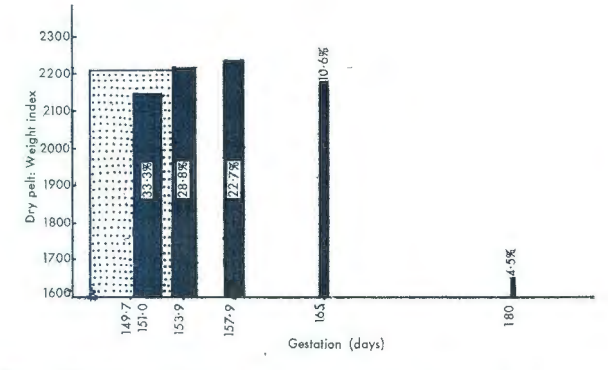

FIG. 13.—Comparative frequency distribution of dry pelt weight indices of control lambs (dotted histogram) and artificially produced postmature lambs (black histograms) in South West Africa.

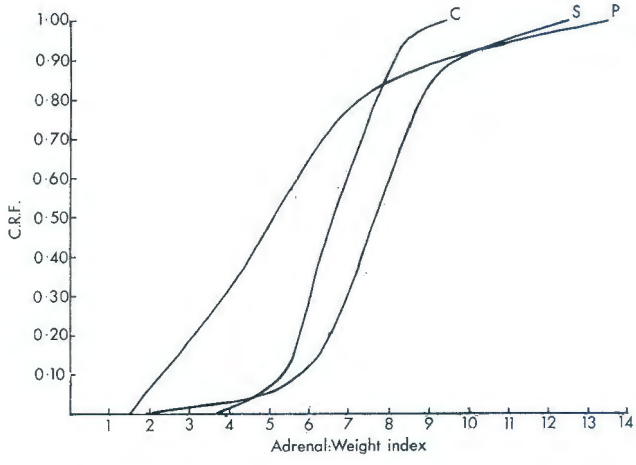

FIG. 14.—Cumulative relative frequencies (CRF) of the adrenal weight indices of control (C), natural postmature (P) and artificial postmature (S) lambs in South West Africa (free-hand curves).

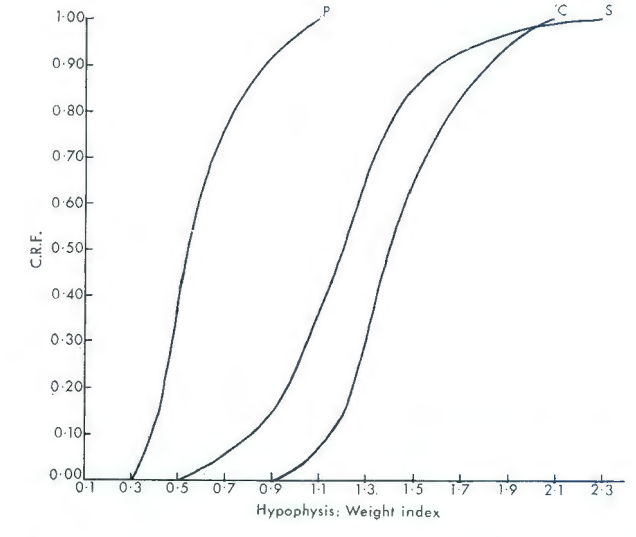

FIG. 15.—Cumulative relative frequencies (CRF) of hypophysial weight indices of control lambs (C), field cases of postmaturity (P) and artificially produced postmature lambs (S) in South West Africa (free-hand curves).

DISCUSSION

Holm (1967) discussed in detail all the various known factors that may cause prolonged gestation. Some of these are genetic factors, environmental influences such as submaintenance rations and high or low environmental temperatures, avitaminosis A, the sex of the foetus and the size of litters. In most dairy breeds the length of gestation is slightly longer for male than female calves. The duration of pregnancy is also inversely related to the number of young in a litter. Binns, Shupe, Keeler & James (1965) described prolonged pregnancy in Idaho, U.S.A. in sheep which is caused by ingestion of the toxic plant *Veratrum californicum* Durand. Cyclopian malformation was a characteristic feature of these postmature lambs, some of which weighed 26 lb after

gestation periods of up to 230 days. Some of these methods used to produce experimental cases of prolonged gestation in laboratory animals include implantation of anterior pituitary and administration of such substances as chorionic gonadotrophin, oestrogens, testosterone and crystalline progesterone to pregnant animals, and foetal adrenalectomy during pregnancy. In the present study of grootlamsiekte, *S. tuberculata* was demonstrated to be the aetiological factor, and the time of insult proved to be confined to the last 50 days of gestation.

Progesterone levels in pregnant cattle declines about 10 days prior to normal term (Short, 1958b) after relatively constant values from mid-pregnancy. The plasma progesterone concentration of prolonged pregnancies of genetic origin in cattle has been described by Holm & Short (1962). Animals that carried post-term foetuses, did not have the decline in plasma progesterone prior to term that occurred in normal individuals.

The progesterone levels during prolonged gestation were maintained at mid-pregnancy levels. A similar pre-term decline was observed in our control sheep except that it started as the downward leg of a peak formed by progesterone values recorded during 120 to 140 days pregnancy. The values constituting the 20 day peak were significantly higher ($P < 0{\cdot}002$) than the results recorded between 75 and 95 days after conception. The decline in the control sheep started between 10 and 15 days prior to parturition. In general, progesterone values increased with advancing gestation; the increase of progesterone values in the *Salsola* group was retarded from approximately 50 days gestation. However, this group ultimately attained normal peak values about 16 days later than usual, which were followed by a rapid decrease and parturition, similar to that in the controls.

The influence of *Salsola* was apparently only necessary during the last 50 days of gestation to induce prolonged pregnancy. During this period plasma concentrations were about doubled in the normal ewes and progesterone increases were most retarded in the *Salsola* group. It thus appears that growth of the placenta may be retarded and/or placental steroid biosynthesis impaired by a principle in *S. tuberculata*. It may also be reasoned that the shrub has a luteotrophic effect in that luteal function was maintained longer than normal.

A similar delay in the occurrence of the decline in cortisol plasma levels was observed. Both control and *Salsola* groups exhibited a more or less corresponding pattern until between 115 and 120 days of gestation when a decline of plasma cortisol of the controls occurred. This drop in cortisol values was only observed from approximately the 135th day in the *Salsola* group, a delay of 15 to 20 days.

This pre-parturient decrease of cortisol plasma levels in ewes is in accordance with the results of Saba (1965), although it occurred in the controls at a somewhat earlier stage than described by him. At term, the cortisol values in our ewes were again increased, but reached significantly higher levels in the post-term ewes.

It is conspicuous that the pattern followed by the progesterone and cortisol plasma values of both groups was very similar up to a certain stage of gestation when the first differences in the values for control and *Salsola* ewes became obvious. This stage, as far as progesterone is concerned, was observed just after 100 days of pregnancy when the progesterone of the controls commenced to increase rapidly in contrast to the much slower rate of incline in the *Salsola* group. This resulted in an interval of about 16 days between the progesterone peaks found in the plasma of the two groups. The discrepancy in the cortisol concentrations of the two groups became evident at 110 days pregnancy. At this stage the cortisol of the *Salsola* group continued to increase whilst the corresponding values of the controls started to drop. The effect of the shrub thus became particularly apparent 100 days after conception and the period of insult was apparently during the last 40 or 50 days of gestation. This was subsequently confirmed by the feeding experiments in 1967 when the shrub induced prolonged pregnancy when fed to ewes during the last 50 days of pregnancy whilst negative results were obtained when the plant was given to the pregnant ewes for the first 100 days only.

The results of the feeding experiments on female rats suggest the presence of a factor, or factors in *S. tuberculata* which have a marked effect on the reproductive functions of this animal. It was shown that both the sexual cycle and pregnancy were affected and the higher the dosage, the longer it took for the rat to return to its normal cycle after withdrawal of the plant. From this study it would appear that the responsible substance is soluble in alcoholic solvents and that it has a luteotrophic effect both on the cycle and gestation. Whether this luteotrophic effect originates from the hypophysis or from a component of the plant itself is, at this stage, unknown. However, the anterior pituitary of *Salsola*-fed rats exhibited conspicuous atrophy, which is probably due to inhibition of hypothalamic releasing factors. These factors are necessary to stimulate the secretion of most adenohypophysial hormones, except in the case of luteotrophic hormone. Its secretion is controlled by a hypothalamic inhibitor which, when removed, allows secretion of luteotrophic hormone from the pituitary at maximal rates.

The effect of the plant was not solely centred upon reproduction and it became evident that most of the endocrines were either primarily or secondarily involved. The uterus and ovaries were also atrophied, but the adrenals of the *Salsola* group were heavier in relation to those of the controls. The small thymus and adrenals of the baby rats born of females in the *Salsola* groups, suggest higher adreno-cortical steroids in the *Salsola* groups than in the controls. This might indicate both luteotrophic and adrenotrophic effects either directly from the plant material or indirectly from the affected hypophysis as indicated above.

Practically all the previously described macro- and microscopical features of postmaturity in lambs discussed by Holm (1967) were present in cases of grootlamsiekte. This specifically concerns the large

skeletal framework, overgrowth of epidermal structures such as hair and hoofs, adrenal and hypophysial atrophy, hydramnios or oligoamnios, Leydig cell hypoplasia, advanced follicular development of the foetal ovaries, external meconium staining, lethargy, poor suckling and high neonatal mortality. Apart from the progesterone levels, other maternal features such as uterine inertia and regression of milk secretion were also confirmed in some of the ewes.

Additional foetal observations in the present study of postmaturity were atrophy of the thymus, inactivity of the thyroid, enlargement of the female foetal genitalia, ultimate mild testicular hypoplasia, liver degeneration and severe pigmentation particularly of the kidneys and lymph nodes. Pancreatic atrophy and nephrosis were observed in a small percentage of cases and hypoplasia of the lungs and cerebellum and other malformations, which are probably unrelated to the problem, only in exceptional cases. The lamb weight was only increased in advanced cases of postmaturity, being near normal or decreased in early postmaturity. In comparison with those of the control lambs, the pelt weights were not much out of proportion with the body weights and ages except in very advanced cases where the pelts actually became lighter

The evidence that the pigment responsible for most of the discoloration of the foetal tissues is lipoproteinaceous and contains incorporated meconial bile pigments, is very convincing. Macroscopical observations indicated that initially pigmentation of the mesenteric lymph nodes and kidneys occurs. The pigment then gradually spreads to the periportal lymph nodes, mediastinals, prescapulars and ultimately affects practically the entire carcass. This development as well as the colour of intestinal lymph indicates that the origin of the pigment is in the contents of the intestinal tract which consists of meconium having a colour that very closely resembles that of the pigmented organs. Special staining techniques further revealed the presence within the pigment, of disulphide and/or sulphydryl groups, aldehydes, lipids and proteins at various stages of its development. The strongly PAS-positive material in the mesenteric lymph nodes and its direct correlation with early pigmentation, is proof that this is the very early form of the pigment. A modification of the pigment takes place in the liver, kidneys, other lymph nodes and tissues where it becomes more lipid-bound. Even in the mesenteric lymph nodes it undergoes a similar change. The negative staining reaction with Kutlik's indicates that it is an altered fixed unconjugated and lipid-soluble bile pigment which is not eliminated by the kidneys.

In the normal breakdown of blood pigments lipid soluble bilirubin-globulin complex is conjugated in the liver with glucoronic acid to form water soluble cholebilirubin. This is passed in the bile into the intestinal tract where it is changed by bacterial degradation to stercobilinogen. Some of this pigment is reabsorbed and is transported to the liver, while the rest is voided with the faeces. Conjugation of bile pigments in the livers of neonates is known to be frequently defective (Keele & Neil, 1961). This inadequate conjugation together with the increasing amounts of such pigments in postmature lambs, in face of a possible inadequate elimination via the placenta, and the absence of bacterial degradation of the pigments in the intestines of foetuses could be significant factors in the pathogenesis of the pigmentation.

Either the pigmentation or hypoxia which is suspected to occur, adversely affects the liver and kidneys with resultant degeneration. The lipid content of both organs decreases except at some of the later stages of pregnancy. Pigmentation of most of the post-term ewes was probably within the normal range for herbivorous animals, but some more advanced cases of pigmentation were apparently related to the increased elimination via the placenta.

The lack of knowledge of the factors governing the normal time of parturition precludes anything but speculation on the endocrine pathogenesis of prolonged gestation. However, it is clear from the observations on grootlamsiekte that the adenohypophysis and adrenals of the foetus play a major role in precipitating parturition. Evidence of progressive and constant hypophysial atrophy and eventual adrenal atrophy in the foetus or neonate in grootlamsiekte is overwhelming. As indicated above it is suspected that *S. tuberculata* inhibits the hypothalamic releasing factors which regulate the secretion of most adenohypophysial hormones. The suppressive effect of the shrub on the oestrus cycle and Leydig cell development further indicate that there is an inhibitory effect on the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH). No microscopical evidence of degenerative or other pathological changes, however, could be found in the brain and such an effect on the hypothalamic centres would apparently be within physiological limits.

Atrophy of the foetal adrenal was preceded by apparent hypertrophy and increased lipid storage. In the ewe and adult rats, on the contrary, there was only enlargement of the adrenals in conjunction with inactivity of the ovaries. However, such adrenal enlargement need not necessarily indicate hyperactivity. The onset of hypophysial atrophy, or even mere inactivity, could cause an initial temporary enlargement of the adrenal cortex due to lipid storage. Unless the responsible circulating substance of the shrub has a more pronounced effect on the foetus the contradictory phenomena about the foetal and maternal adrenals seem to be difficult to explain. On the other hand, an initial adreno-corticotrophic and subsequent suppressive effect on this gland secondarily via the hypophysis should also be considered.

Enlargement of the foetal female genitalia and polyfollicular ovaries, in contrast to the eventual mild testicular hypoplasia, were probably due to the prolonged effect of the placental hormones. Lethargy and high neonatal mortality could be explained by the adrenal atrophy and inactivity. The disturbances in the other endocrines and organs were apparently all secondary to the involvement of specific endocrines such as the hypophysis and adrenals and were not directly due to the shrub. The positive linear relationship of the foetal kidney with gestation

either indicates that it was not affected by the endocrine disturbances or that it was due to the increased pigmentation masking a mild secondary atrophy. The absence of a definite relationship between the adjusted weight of the foetal brain and gestation during prolonged gestation suggests that this organ was probably also affected, but the relationship is not clear. In all these arguments it is assumed that the responsible substance in the plant passes through the placenta to reach the foetal circulation.

The intranuclear inclusions noticed in the hypophysis of both control and post-term ewes are apparently of no significance and may indicate hypertrophy and engulfment of cytoplasm by the enlarged nucleus which frequently becomes indented at one side (Pienaar, Veterinary Research Institute, Onderstepoort, personal communication, 1968). The eosinophilic intra-epithelial globules in the intestines could originate from degenerative goblet cells or mucus.

The neutrophil reaction within the lymph nodes of many normal and postmature lambs could not be correlated with prolonged gestation and may be a reflection of a transient intra-uterine infection.

Summary

Various aspects of a specific syndrome of prolonged gestation and postmaturity in sheep in South West Africa were studied. Clinical, pathological and endocrinological observations were made. The role of a shrub, *S. tuberculata*, which occurs in the affected area was studied in pregnant Karakul and Merino sheep as well as in rats by feeding them with rations containing the shrub. It caused prolonged gestation in sheep and the main period of insult during pregnancy was determined to occur during the last 50 days. In the rat it prolonged both gestation and the dioestrous phase of the oestrous cycle.

In the ewe, apart from prolonged gestation, the most characteristic findings appeared to be adrenal enlargement, retarded udder development or dysgalactia, inactive ovaries, oligoamnios or hydramnios. The trend of both progesterone and cortisol levels resembled those of normal ewes except that they were considerably delayed during the last 50 days of gestation.

The most significant features in the postmature lambs were constant progressive hypophysial atrophy; initial adrenal hyperplasia and eventual atrophy; atrophy of the thymus; inactivity of the thyroid; hypertrophy of the female genitalia and polyfollicularity of the ovaries; a mild delayed testicular hypoplasia and Leydig cell hypoplasia; pigmentation; increase in birth weight, long haircoat and erupted incisors in advanced cases of gestational prolongation; lethargy and high mortality. Evidence is given that the pigment is a lipofuscin with incorporated meconial bile pigments.

The shrub was tested for oestrogenic and anti-oestrogenic substances with negative results, but the potent substance was successfully extracted with alcoholic compounds.

The lack of knowledge at this stage of the investigation prevents complete elucidation of the pathogenesis of the syndrome, but the available evidence indicates that it is triggered by a hypothalamic inhibitor in the shrub which causes secretory dysfunction and ultimate atrophy of the pituitary gland.

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References

ANON., 1960. Manual of histologic and special staining technics. 2nd ed. Washington: Armed Forces Institute of Pathology.

BASSON, P. A., MORGENTHAL, J. C. & BILBROUGH, R. B., 1964. Report on grootlamsiekte. Chief, Vet. Res. Inst., Onderstepoort (unpublished).

BASSON, P. A., NEL, J. A. & FREYER, E., 1959. Report on grootlamsiekte. Director of Agric., Windhoek, S.W.Afr. (unpublished).

BIELY, J. & KITTS, W. D., 1964. The anti-estrogenic activity of certain legumes and grasses. *Can. J. Anim. Sci.* 44, 297–302.

BINNS, W., SHUPE, J. L., KEELER, F. & JAMES, L. F., 1965. Chronologic evaluation of teratogenicity in sheep fed *Veratrum californicum*. *J. Am. vet. med. Ass.*, 147, 839–842.

COWDRY, E. V., 1952. Laboratory Technique in Biology and Medicine. 3rd ed. Baltimore: The Williams and Wilkens Co.

CRUICKSHANK, R., 1962. Mackie and McCartney's handbook of bacteriology. 10th ed. London: E. & S. Livingstone Ltd.

DE LANGE, M., 1961. Prolonged gestation in Karakul ewes in South West Africa. *Proc. 4th Internat. Congress Anim. Reprod. The Hague*, 3, 590–592.

DE LANGE, M., VILJOEN, J. H. B. & BASSON, P. A., 1960. Report on grootlamsiekte. Chief, Vet. Res. Inst., Onderstepoort (unpublished).

GOMORI, G., 1950. Aldehyde-fuchsin: A new stain for elastic tissue. *Am. J. clin. Path.*, 20, 665–666.

HEATH, E. H., 1956. Application of the performic acid—alcian blue—periodic acid—Schiff—orange G stain to sections of pituitary glands from domestic mammals. *Am. J. vet. Res.*, 26, 368–373.

HOLM, L. W., 1967. Prolonged pregnancy. *Adv. vet. Sci.*, 11, 159–205.

HOLM, L. W. & SHORT, R. V., 1962. Progesterone in the peripheral blood of Guernsey and Friesian cows during prolonged gestation. *J. Reprod. Fert.*, 4, 137–141.

KANEMATSU, S. & SAWYER, C. H., 1963. Effects of hypothalamic and hypophysial estrogen implants on pituitary gonadotrophic cells in ovariectomized rabbits. *Endocrinology*, 73, 687–695.

KEELE, C. A. & NEIL, E., 1961. Samson Wright's applied physiology. 10th ed. London: Oxford University Press.

LILLIE, R. D., 1954. Histopathologic technic and practical histochemistry. New York: The Blakiston Company Inc.

MALLORY, F. B., 1938. Pathological technique. Philadelphia: W. B. Saunders Co.

MOORE, T., 1957. Vitamin A. 1st ed. Amsterdam: Elsevier Publishing Co.

MORGENTHAL, J. C. & BASSON, P. A., 1966. Sekere aspekte van die oortydsindroom in Karakoelskape in Suidwes-Afrika. *Geneeskunde*, 8, 93–94.

OSTROVSKY, D. & KITTS, W. D., 1963. The effect of estrogenic plant extracts on the uterus of the laboratory rat. *Can. J. Anim. Sci.*, 43, 106–112.

PEARSE, A. G. EVERSON, 1961. Histochemistry theoretical and applied. 2nd ed. London: J. & A. Churchill Ltd.

QUIN, J. I., VAN DER WATH, J. G. & MYBURGH, S., 1938. Studies on the alimentary tract of Merino sheep in South Africa. IV—Description of experimental technique. *Onderstepoort J. vet. Res.*, 11, 341–360.

ROMEIS, B., 1948. Mikroskopische Technik. München: Leibniz Verlag.

SABA, H., 1965. Plasma cortisol levels in pregnant ewes. *J. agric. Sci., Camb.*, 64, 11–14.

SANDELL, E. B., 1950. Colorimetric determination of traces of metals. 2nd ed. New York: Interscience Publication.

SHORT, R. V., 1958a. Progesterone in peripheral blood. *J. Endocr.*, 16, 415–425.

SHORT, R. V., 1958b. Progesterone in the peripheral blood of pregnant cows. *J. Endocr.*, 16, 426–432.

VAN RENSBURG, S. J., 1967. Gestation in sheep after foetal adrenalectomy and cortisol acetate administration. *J. Endocr.*, 38, 83–84.

VAN RENSBURG, S. J., 1965. Adrenal function and fertility. *Jl S. Afr. vet. med. Ass.*, 36, 491–506.

VON MALTITZ, L., 1964. Report on grootlamsiekte. Director of Agric., Windhoek, S.W.A. (unpublished).

WINTER, H., 1961. An environmental lipofuscin pigmentation of livers. *University of Queensland Papers*, 1, (1) 1–66.