

## Studies on the Photosensitisation of Animals in South Africa.

### VI. The Effect of Surgical Obstruction of the Normal Bile Flow.

By J. I. QUIN, D.V.Sc., Veterinary Research Officer, Onderstepoort.

#### INTRODUCTION.

As pointed out in the first of this series of papers, some considerable difficulty is experienced in producing experimental cases of true geeldikkop in sheep. Although several species of *Tribulus* can definitely be held responsible for outbreaks of the disease in the Karroo areas of the Cape Province, artificial feeding of the plants collected and fed to susceptible sheep on the spot frequently yield no positive results. With fresh or dried plant material despatched to the Onderstepoort Laboratory, no single case of the true disease has yet been produced. Similarly, *Tribulus* either cultivated or growing naturally at Onderstepoort has failed to cause geeldikkop in susceptible Merino sheep.

From experiments conducted with haematoporphyrin, various fluorescent dyestuffs, e.g. eosin, and also two species of the plant *Hypericum*, it was clearly shown that, although the resultant photosensitivity shown by the animals closely simulated that normally seen in true geeldikkop, the other important clinical symptom, viz., the intense generalised icterus normally seen in geeldikkop, was uniformly absent from the experimental cases. The blood serum in geeldikkop is usually of an intense clear yellow colour, giving a strongly positive direct Van den Bergh reaction. This suggests that the icterus is due to some form of obstruction possibly in the liver itself seeing that the bile duct leading to the duodenum is always found patent in affected cases.

In order to ascertain how this icterogenic factor operates, a series of experiments was started in which the bile ducts of sheep were ligated and the subsequent clinical symptoms carefully noted.

Experimental ligation of the bile duct in various animals has frequently been resorted to in connection with problems of digestion, icterus, functions of the gall bladder and the liver. In a recent paper Cameron and Oakley, besides reviewing the extensive literature on the subject, gave a detailed account of their work on rats. They showed that ligation of the common bile duct in the rat was followed by definite pathological changes in the liver. The animals were killed at hourly intervals and sections of the liver examined. Their results were compared with those obtained by other workers on such animals as the guinea-pig, rabbit, dog, cat, pigeons and mice, and from this it was shown that the type of liver response varied fairly widely in the different species of animals. From the available literature, it would seem that no systematic work has been done on the effect of ligation of the bile duct in sheep. For this reason the above-mentioned series of experiments were started on sheep. It was hoped that ligation of the bile duct would throw some light on the occurrence of icterus in natural cases of geeldikkop.

### LIGATION OF THE BILE DUCT IN THE SHEEP.

#### OPERATIVE PROCEDURE.

Under natural conditions it would appear that young Merino sheep are more susceptible to geeldikkop than full-grown ones. For this reason young sheep (under 18 months) were selected for operation as far as these were available. They were usually starved for 24 hours, during which time they were closely shorn along the whole length of the back and also the right flank. Immediately before the operation, a small sample of blood (25 c.c.) was drawn from a jugular vein and collected in a little sodium citrate. This blood was subsequently centrifuged and the percentage red cells ascertained, as also the colour of the plasma. Depending upon the size of the animal, 3 to 4 grams chloral hydrate in 10 per cent. saline solution was injected very slowly into the jugular vein. In this way complete general anaesthesia was usually obtained within 5 or 10 minutes. The right flank, previously shorn, was then shaved clean with a razor, using soap and water to soften the wool. Barium sulphide depilatories were found to be unsatisfactory as they invariably caused hyperaemia and irritation of the sensitive Merino skin. After this, the animal was placed on its left side on the operating table and the feet tied down. The skin over the site of the operation was then flushed down with ether. For the rest, full aseptic precautions were taken during the operation. A laparotomy wound usually 4 to 5 inches long was made parallel to and about 1 inch behind the last rib, the wound being kept open by retractors and artery forceps. By drawing aside the small intestines the visceral surface of the liver, the gall bladder and the extrahepatic bile ducts came into view. Overlying and very close to the vena portae, the ductus choledochus could be made out. This was carefully cleaned from the vena portae for a short distance immediately distal to the junction of the cystic duct. In the first few operations a single silk ligature was placed tightly round the ductus choledochus at this point. However, this procedure soon had to be changed, as it was found that, although clinical icterus made its appearance after a few days, it was of a definitely transitory nature and completely disappeared after 7-10 days. Such animals usually

made an uneventful recovery with very little loss in their general condition. Several such animals were subsequently slaughtered for post-mortem examination, and in each case it was found that the bile duct had reconstructed itself round the ligature, which was found in the lumen of the enlarged portion of the duct. In this way the biliary circulation had become completely re-established. As mentioned by Cameron and Oakley, a similar observation was made by Brodie (1823) following ligation of the common bile duct in cats.

Thus, in order to obviate the bile duct from reconstructing itself, it was decided to place double silk ligatures about  $\frac{1}{4}$  inch from each other and then to sever the duct between the two. In all subsequent operations this was done. On cutting the duct, it was found that the two stumps retracted, leaving a gap of about  $\frac{1}{2}$  inch. In this way the chance for union of the ends was minimised. This was borne out in practice as in no case did the duct become patent again. In addition to ligation and cutting of the ductus choledochus, the cystic duct too was ligated at its entry into the gall bladder and the bile removed from the gall bladder by means of a syringe and needle. This was done in order to prevent bile being forced in large amount into the gall bladder and so possibly causing rupture of its wall. By these means it was attempted to cause as little bile as possible from actually leaving the liver in the extrahepatic bile tracts. The laparotomy wound was closed by three layers of gut sutures applied to the peritoneum, muscles, and skin respectively, and dressed with iodoform and collodion.

All operated animals were placed in a clean stable for 24 hours, during which time only a little green feed was allowed. In practically every case, healing of the wound by first intention took place. From the second day onwards, the animals were regularly kept exposed to sunlight in a paddock daily from 9 a.m. to 4 p.m. In addition, a small volume of blood was collected in citrate every morning and the animals kept under frequent observation. The diet consisted of green lucerne, dry veld hay and a little crushed maize with water *ad lib.*

#### CLINICAL SYMPTOMS FOLLOWING LIGATION OF THE BILE DUCT. PHOTOSENSITISATION ESTABLISHED.

In these experiments a total of 51 animals were operated upon. Without exception the operation was withstood very well, although the period of survival afterwards varied widely. The blood plasma as taken just prior to the operation, was generally found to be water clear, or very faintly yellowish, although not sufficient to yield a positive Van den Bergh reaction.

A description of some of these cases will serve to indicate the type of response obtained.

##### *Angora Goat No. 25303.*

- 12.4.32—ductus choledochus ligated and cut. Cystic duct ligated. Serum clear.  
 13.4.32—animal lively, placed in sun, serum very yellow, no clinical icterus. Serum gives positive direct Van den Bergh reaction.  
 16.4.32—serum intensely yellow, clinical icterus as shown in mouth and conjunctivae.  
 24.4.32—animal dull, icterus intense.



- 25.4.32—marked photosensitisation after one hour in sunlight, scratching ground, marked finching, ears slightly swollen, pits on pressure.
- 16.4.32—ears badly swollen and pendulous, eyelids swollen, eyes partly closed (see photo 1), marked finching (see photo 2), very restless, urine deep yellow brown. (Weather: sky partly overcast, sun bright and hot.)

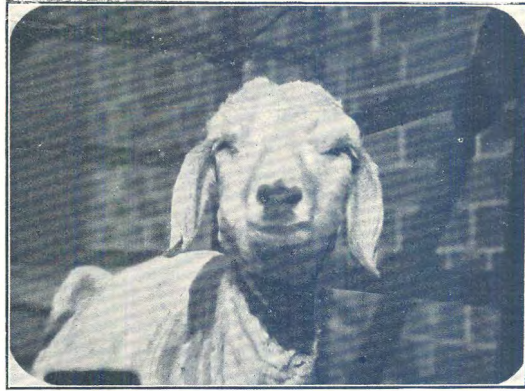


Fig. 1. Goat 25303. Head and ears swollen following ligation of bile duct.

- 27.4.32—condition unchanged, animal feeding fairly well.
- 28.4.32—swelling of ears subsiding, slightly sensitive in sunlight, serum still very yellow.
- 30.4.32—not sensitive, all swellings subsided, serum very yellow, still giving very strong direct Van den Bergh reaction.
- 3.5.32—animal died during night.

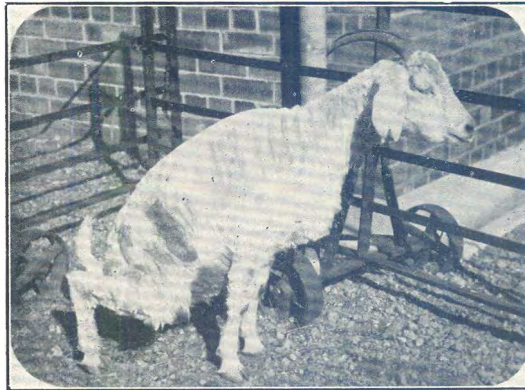


Fig. 2. Goat 25303. Acute photosensitisation following ligation of bile duct.

*Post-mortem Report.*—Emaciation, anaemia, severe generalised icterus, laparotomy wound healed. Bile ducts markedly distended with inspissated bile. Swelling and bile pigmentation of the liver and kidneys. Stasis in fore-stomachs and distal portion of large intestine.

*Merino Sheep No. 33094.*

- 24.6.32—ductus choledochus ligated and cut. Cystic duct ligated. Serum yellowish before operation.
- 25.6.32—animal lively, serum deep yellow, strong direct Van den Bergh test. Conjunctivae yellow.

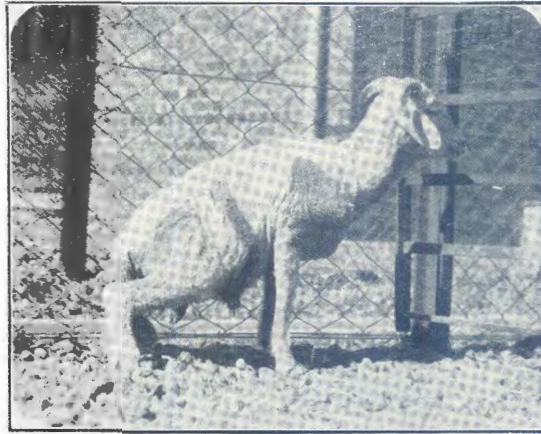


Fig. 3. Sheep 33094. Marked flinching after ligation of bile duct.

- 27.6.32—well marked photosensitisation, serum very yellow.
- 28.6.32—marked clinical icterus, urine yellow. Animal very sensitive in sunlight, crouching, biting its back and frequently sitting down on haunches, scratching the ground. Ears swollen (see photos 3, 4 and 5).
- 4.7.32—swelling subsiding, otherwise no change.
- 7.7.32—still sensitive to sunlight.

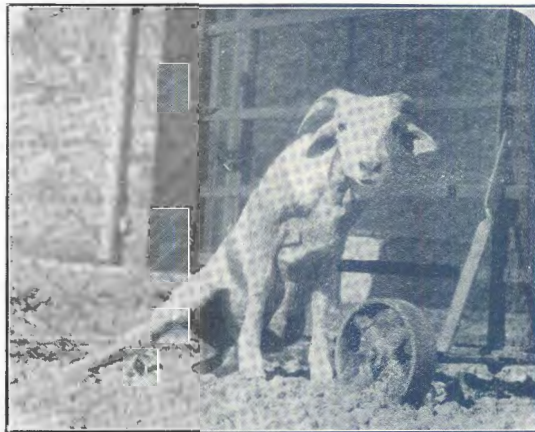


Fig. 4. Sheep 33094. Marked flinching after ligation of bile duct.

- 14.7.32—animal losing condition, although feeding well, marked irritation of the head to sunlight. Serum very yellow. Strong direct Van den Bergh test.
- 21.7.32—condition poor, still sensitive.
- 23.7.32—feet very painful, animal lame, coronets red.
- 30.7.32—markedly sensitive, animal crawling on knees. Eyelids and lips hard.
- 2.8.32—animal in extremis, lying on side, unable to rise. Died at 11 a.m.

*Post-mortem Report.*—Intense generalised icterus, anaemia and general atrophy. Oedema of the subcutis of the head and necrosis of skin. Laparotomy wound healed. Severe bile stasis in liver with marked widening of bile canals which on the surface of the liver appear like enlarged gall bladders. Bile pigmentation of the kidneys. Slightly pasty pellet-shaped faeces in the large intestine.

*Note.*—Throughout the course of the obstruction of the bile ducts the icterus index varied between 30 mg. and 50 mg. bilirubin per litre plasma as determined by the direct van den Bergh reaction.



Fig. 5. Sheep 33094. Head and ears swollen after ligation of bile duct.

*Merino Sheep No. 32577.*

- 18.10.32—ductus choledochus ligated and cut, cystic duct ligated.
- 19.10.32—slight finching noticeable at 4.30 p.m., also slight clinical icterus.
- 20.10.32—finching marked when animal placed in sun.
- 22.10.32—animal very sensitive and finching. Clinical icterus (conjunctivae) definite.
- 24.10.32—animal not sensitive, icterus marked.
- 31.10.32—condition very poor, animal unable to rise.
- 1.11.32—animal in extremis. Killed at 3 p.m.

*Post-mortem Report.*—Cachexia, anaemia, marked generalised icterus, dilatation of bile ducts, atrophy of gall bladder, pigmentation of liver and kidneys, stasis in fore-stomachs.

*Merino Sheep No. 32542.*

- 18.10.32—ductus choledochus ligated and cut. Cystic duct ligated.  
 19.10.32—animal pawing ground, slight flinching.  
 20.10.32—marked flinching, restless, slight clinical icterus, serum intensely yellow. Strong direct Van den Bergh test.  
 22.10.32—definite clinical icterus.  
 24.10.32—very sensitive, restless.  
 31.10.32—marked photosensitisation and flinching as soon as placed out in sunlight. Animal collapsed after 1 hour in sunlight. Placed in stable. In extremis. Killed at 3 p.m.

*Post-mortem Report.*—Cachexia and anaemia, intense generalised icterus, oedema of subcutis of head and ears. Marked bile stasis in liver. Superficial bile duct filled with colourless slightly turbid liquid. Pigmentation of the kidneys. Catarrhal enteritis and stasis in the large intestines.

*Merino Sheep No. 32980.*

- 30.6.32—ductus choledochus ligated and cut. Cystic duct ligated.  
 9 a.m.—before operation, serum water clear.  
 10.40 a.m.—immediately after operation, serum water clear.  
 12 p.m.—serum pale yellowish, faintly positive direct Van den Bergh test. Approximately 0.9 mg. per litre plasma.  
 1 p.m.—serum more yellow, 2 mg. bilirubin per litre.  
 2 p.m.—serum more yellow, 2+ mg. bilirubin per litre.  
 4 p.m.—serum quite yellow, 3 mg. bilirubin per litre.  
 1.7.32—24 hours after operation serum very yellow, 18 mg. bilirubin per litre plasma.  
 2.7.32—48 hours after operation serum deep yellow, 31 mg. per litre plasma, clinical icterus just starting.  
 5.7.32—no photosensitisation yet. Clinical icterus well marked, 55 mg. bilirubin per litre plasma.  
 9.7.32—animal very constipated, still no photosensitisation, icterus well marked.  
 11.7.32—animal died during the night.

*Post-mortem Report.*—Severe generalised icterus, bile stasis in liver with enlarged bile ducts. Intussusception large colon.

**DISCUSSION.**

From the description of a few of the large number of cases in which complete surgical obstruction of the bile flow had been carried out, it is evident that a well marked train of symptoms can be followed in the Merino sheep. Of these the first to be noticed is the very sudden rise of bile pigment in the circulating blood plasma. Thus in sheep 32980, where blood samples were taken at hourly intervals, a faintly positive direct Van den Bergh reaction was given within 80 minutes after operation. This is continued by a progressive rise in the bilirubin content of the plasma, although actual clinical icterus as noticeable on the skin, mouth and other mucous membranes, only follows after the lapse of some days. In the meantime a large



amount of bile pigment is voided in the urine which may be coloured a deep yellow, and also giving a positive direct Van den Bergh reaction. This indicates an effective participation of the kidneys in eliminating some of the increasing amounts of bile pigment. The elimination via the urine is, however, incomplete, seeing that gradually an intense bile staining of all tissues follows, resulting in the well-marked clinical jaundice. Judging from the degree of bile pigmentation of the kidneys (macroscopically and microscopically) it would appear that after some days the kidneys become charged with an excessive amount of bile, all of which cannot be eliminated in the urine. The prolonged bathing of the body tissues in fluids rich in bile pigments then gives rise to the intense jaundice. It is obvious, however, that the liver retains its efficiency for secreting the bile into the normal bile channels, as shown by the tremendous distension of both the intra- and extra-hepatic bile ducts even up to the point of rupture, and also by the comparative ease with which the ligated ductus choledochus is re-established when one ligature only is applied around it. It would appear that no effort is spared on the part of the liver in forcing the increasing amounts of bile into the normal channels. In the liver itself a proliferation of the bile capillaries is frequently noted.

Accompanying the icterus, another symptom which is of unusual interest, is also developed, viz., that of photosensitisation. As far as can be ascertained, this symptom following bile duct obstruction, has not been described hitherto. The onset of photosensitisation varies widely. Thus it may appear on the second or third day after the operation, or it may be delayed for 12 or 14 days, while in rare cases as e.g. in sheep 32980 it may not be observed at all. The majority of animals, however, when closely shorn along the back and head, and kept exposed in sunlight, develop well marked symptoms of photosensitisation within the first week after the operation. That the condition is definitely associated with sunlight is shown by the fact that affected animals continually seek for shade. Furthermore, when the sheep are kept in a stable, no sign of photosensitisation is shown, whereas if they are placed out in the sun, licking of the lips, scratching of the head and ears, marked flinching and restlessness may be observed within a few minutes to a few hours. At times the irritation is so intense that the most abnormal attitudes are adopted, e.g. sitting up on the haunches or dragging the hind legs along, with the abdomen held close to the ground (see photographs). Following on this, oedema of the exposed parts, e.g. ears, face, lips and peri-orbital region rapidly develops, and so causes a peculiar round appearance of the head, with the ears thick and pendulous, and the eyes closed. The height of the oedema is usually reached within 24 hours. Subsequently it gravitates towards the intermandibular space, which becomes bag-like in appearance. With the subsidence of the oedema a change in the affected skin can be noticed. At first it is tough and leathery and of a dull greyish yellow colour, especially so along the shorn back. The base of the horns and the feet round the coronet frequently appear red and painful. The animal may be very sensitive on the feet, or quite lame and disinclined to stand. In no case were those parts of the skin which were fully protected by a covering of wool found to be affected; the demarcation between diseased and



healthy skin corresponding exactly with the shorn and unshorn parts. After some days the affected skin becomes darker and frequently of a greenish tint. At the same time it hardens to the consistence of dried leather and easily cracks. Unless death supervenes too quickly, extensive sloughing of the necrosed layers of the skin and its wool may take place, so exposing the newly formed healthy pink skin. In rare cases the tips of the ears may slough completely.

The actual cause of this photosensitisation has not been ascertained as yet, although it is reasonable to suggest that it is brought about by some fluorescent substance, seeing that it is generally assumed that this phenomenon is only caused by fluorescent bodies. Furthermore, it would appear that following the obstruction of the bile flow, this photosensitising substance which may even be a normal constituent of bile, increases in the circulation as the jaundice becomes more marked. In this connection some functional disturbance of the liver may be of primary importance. In the presence of sunlight and with an unpigmented and exposed skin, the photosensitising principle is rendered capable of producing the symptoms and lesions typical for photosensitisation as, for example, seen with haematoporphyrin.

Another symptom which needs explanation is the marked loss of condition of the animal, in spite of the fact that the appetite remains good after the operation. Furthermore, there are no obvious signs of digestive disturbance except towards the end, when constipation in the caecum and upper portion of the large intestine is frequently noticed. Prior to this, the faeces appear normal, although the bile is prevented from playing its usual part in digestion. With a normally low intake of fats in the food, it would appear that in the sheep the presence of bile in the intestine is of less importance than e.g. in the dog, although such factors as absorption from the intestine, metabolism in the liver and detoxification, may also be profoundly influenced in sheep when the bile flow is obstructed. These factors may actually be responsible for the loss of condition.

From the above description and discussion of the results obtained after simple obstruction of the bile flow in Merino sheep, it is evident that the symptom complex in many respects resembles that seen in true geeldikkop, although in the latter condition the skin lesions are generally far more severe, e.g. as shown by complete loss of ears, total blindness, extensive and deep necrosis of the facial skin causing immobility of the lips, jaws and eyelids. Thus, in an attempt to accentuate the symptoms following simple bile duct obstruction, various other measures were subsequently introduced either simultaneously or after the operation. The following account discloses the results obtained in this series of experiments, the term "bile ducts obstructed" being used to indicate that the above-mentioned operation had been performed, i.e. ductus choledochus ligated and cut and the cystic duct ligated at the neck of the gall bladder:—

1. *Bile ducts obstructed and gall bladder removed.*—This operation was performed on 5 sheep. Four of these sheep died within 7 days after operation without showing photosensitisation, although the icterus was well marked. The remaining one sheep showed typical photosensitisation two days after operation. On the 4th day the ears

were markedly swollen and icterus visible. It died 13 days after operation. Of the 5 sheep, 4 showed a well marked bile peritonitis on post-mortem due to rupture of the superficial bile ducts of the liver and bile seepage from the liver surface where the gall bladder had been removed.

It may thus be concluded that the additional removal of the gall bladder offers no advantages over the ordinary operation, while death from bile peritonitis frequently ensues in such cases.

2. *Partial obstruction of the Vena portae.*—The object in this case was to ascertain whether a decreased supply of portal blood to the liver could possibly lead to functional derangement of the liver resulting in icterus and photosensitisation.

One Merino sheep was operated on through the right flank, and a silk ligature placed loosely round the vena portae about one inch below its entry into the liver. The ligature was adjusted so as to reduce the lumen of the portal vein to about  $\frac{1}{3}$  to  $\frac{1}{4}$  of its normal. The animal made an uninterrupted recovery after the operation. It was placed out in the sun and kept under close observation for 12 days. No symptoms whatever were noted and the serum also remained water clear. The general health and feeding remained good throughout. On the 13th day another laparotomy operation was performed immediately posterior and parallel to the first one. On opening the peritoneal cavity, large masses of calcified omental fat were found. In addition, a large network of newly-formed veins were made out. These veins connected the omentum with the parietal peritoneum and ran in a forward direction. As it was impossible to reach the ligature through these veins, the wound was again closed. Complete recovery again ensued, while the general health remained good. It would appear that due to the partial obstruction in the portal vein, compensation rapidly set in by the formation of new veins from the omentum to the abdominal wall, so ensuring a proper return of portal blood into the systemic circulation.

3. *Partial obstruction of the posterior Vena cava.*—Since obstruction in the portal vein did not yield the required symptoms, it was decided to ascertain the effect of partial occlusion of the posterior vena cava immediately above the liver. This was firstly attempted through an abdominal wound, although this gave great difficulty on account of the close adherence of the liver on to the diaphragm in the region of the great veins and also because of the posterior vena cava actually penetrating through the dorsal part of the liver. Consequently on another sheep, the operation was attempted through the thoracic cavity with the animal under artificial respiration. A silk ligature was tied loosely round the posterior vena cava immediately anterior to the diaphragm and its lumen reduced to about  $\frac{1}{3}$  the normal. The operation was withstood well and the animal quickly recovered. The only symptom noticeable for several weeks after the operation was respiratory distress, the sheep panting vigorously, especially when chased around the paddock. As no further symptoms developed, the animal was killed for post-mortem examination 37 days after the operation. The most important findings included a marked calcification of fat all over the body, severe ascites amounting to 6.5 litres, cardiac dilatation, atrophy and well-marked cirrhosis of the

liver. The digestive tract appeared normal. Thus the result of this experiment was simply what was to be expected in a case of chronic venous stasis in the liver and without any secondary symptoms of icterus or photosensitisation.

4. *Bile ducts obstructed and hepatic artery ligated.*—It was hoped that in addition to the usual results obtained with obstruction of the bile flow, interference with the arterial blood supply to the liver might lead to more severe symptoms of icterus and photosensitisation. Consequently in two sheep the hepatic artery was included in the ligatures round the ductus choledochus and completely obstructed. In one sheep photosensitisation was noticed 5 days after the operation, while the other one never showed this symptom. In both cases, however, icterus was intense. Both animals died, the one after 6 days and the other after 21 days with lesions similar to those found in ordinary bile obstruction cases.

It may thus be stated that the symptoms remain the same whether the hepatic artery is ligated or not.



Fig. 6. Sheep 32645. Head and ears swollen after ligation of bile duct, hepatic artery, splenic artery and vein.

5. *Bile ducts obstructed and spleen removed.*—In order to ascertain what rôle the spleen plays in the production of obstructive jaundice and photosensitisation, various operations were carried out.

In one sheep the spleen was removed at the same time that the bile ducts were obstructed. No photosensitisation was shown by this animal, although the icterus was very marked. Death occurred on the 18th day with marked bile stasis in the liver.

In one sheep the splenic vein was ligated and the bile ducts obstructed. Photosensitisation was well marked within 24 hours after operation. On the third day the animal was flinching badly and the ears were markedly swollen. By the 8th day icterus was marked and the animal still sensitive in sunlight. The serum showed a very strong direct van den Bergh reaction, amounting to 125 mgm. bilirubin per litre. Death occurred on the 18th day. The lesions found included a marked generalised icterus, hard crusts round the lips and nostrils, bile ducts greatly enlarged, swelling and pigmentation of the liver, enlargement and degeneration of the spleen.

In another sheep the bile ducts were obstructed and at the same time the hepatic artery was ligated and also the splenic vein and splenic artery ligated. No symptoms were shown up to the 9th day, when clinical icterus was visible. On the 15th day the animal suddenly became very sensitive to sunlight and the ears and lips very much swollen (see photo 6). On the 16th day the eyes were practically closed and the oedematous conjunctivae protruding. The animal remained very sensitive up to the 21st day, when it died, the main lesions being a generalised icterus, swelling and pigmentation of the liver, marked enlargement of the bile ducts and formation of a diverticulum, swelling of spleen, necrosis of lips and round about eyes. All ligatures were found to be intact.

From these cases it is evident that the symptoms of icterus and photosensitisation may develop without the normal function of the spleen.

6. *Bile ducts obstructed and one kidney removed.*—The object of this experiment was to note whether in the absence of one kidney, the icterus and photosensitisation would be increased, seeing that large amounts of bile pigment are voided in the urine in obstruction icterus cases. In two sheep the right kidney was removed, after ligation of the renal vessels, at the same time that the bile ducts were obstructed. The operation was stood well, the animals soon recovering from the effects. One sheep became very sensitive to sunlight on the third day. The following day the ears were markedly swollen and a slight icterus was noticeable. Thereafter the animal gradually lost its condition while the photosensitisation became less marked. It was killed in extremis 4 weeks after the operation. The carcass was emaciated, bile stasis in the liver, left kidney slightly enlarged and bile pigmented. The other sheep became sensitive on the 4th day after operation. On the 5th day it was flinching badly, the feet were tender and the animal disinclined to move. The ears, face and lips were swollen and a slight icterus was present. The animal remained sensitive up to the 16th day, when it died, the main lesions being marked icterus, slight cirrhosis and bile pigmentation of the liver, slight hypertrophy of the remaining kidney, obstruction in the caecum.

From these two cases it would appear as if the removal of one kidney does not intensify the symptoms obtained with simple biliary obstruction.

7. *Bile ducts obstructed and intestine partially occluded.*—In this series of experiments the object was to ascertain whether obstruction in the intestines at different levels would accentuate the symptoms produced by simple bile duct ligation. The operation was carried out by placing a small aluminium band round the intestine and then by means of pliers bending in the ends to form a ring round the intestine. In this way various degrees of constriction could be produced. In one sheep the ring was placed round the duodenum six inches behind the pyloric sphincter and the lumen reduced to about  $\frac{1}{3}$  the normal. Photosensitisation was first observed 5 days after the operation. The following day the animal showed marked flinching and restlessness with a rapidly developing oedema of face, ears, and



lips (see photos 7 and 8). The next morning the swelling had gravitated and a large bag-like swelling was noticed between the lower jaws (see photo 9). On the 8th day the swelling started to subside. There was no sign of clinical icterus as yet, although the serum was clear yellow. The animal was feeding badly and losing condition. Faeces collected appeared normal. On the 10th day the sheep still showed marked finching as soon as it was placed out in the sun. On the 13th day it collapsed and was killed in extremis. The post-mortem



Fig. 7. Sheep 35353. Marked finching after ligation of bile duct and closure of duodenum.

revealed cachexia, marked necrosis of the skin over the face, ears and lips, swelling of the liver with marked distension of the bile ducts. The ring on the duodenum, although practically occluding the lumen did not provoke a stasis probably because of the fluid consistence of the chyme. In another sheep a ring was placed on the ileum 6 inches above the caecum. The animal died 4 days later after showing symptoms of abdominal pain and great thirst. On post-mortem a large amount of water was found in the rumen and small intestines. Due to rupture of the bile duct above the ligature bile peritonitis had supervened.



Fig. 8. Sheep 35353. Head and ears swollen after ligation of bile duct and closure of duodenum.

Obstruction of the large intestine was produced in two sheep by placing a ring just posterior to the *ansa spiralis*. Both animals became dull after the operation and remained in this state. No photosensitisation was shown by either of them. One sheep died on the 7th day with intense generalised icterus, haemoglobinuria, sub-epicardial haemorrhages, swelling and necrosis of the liver, bile ducts distended, enlargement of the spleen and kidneys, stasis in the colon above the ring. The lesions suggested an acute toxæmia following complete obstruction in the large intestines.

The other sheep died on the 20th day, showing similar lesions to the one above, although less intense.

8. *Bile duct obstruction followed by ultra-violet light irradiation.*—In order to ascertain whether ultra-violet rays were responsible for the photosensitisation, two sheep after being operated, were exposed at a distance of two feet to a quartz mercury vapour lamp 30 minutes daily for 5 days. This procedure had no effect whatever on the animals. On the 6th day the animals were exposed to sunlight,



Fig. 9. Sheep 35353. Swelling gravitated after ligation of bile duct and closure of duodenum.

with the result that both showed well marked photosensitisation within 30 minutes. Both animals died on the 14th day after operation, remaining sensitive to sunlight up to the time of death. In both, lesions of bile stasis, enlargement of the liver and icterus were noticed.

9. *Bile duct obstruction combined with inhalation of arsenuretted hydrogen.*—By allowing operated animals to inhale arsenuretted hydrogen, it was hoped that the well marked haemolysis produced by this poisonous gas might provide the bile forming tissues with extra

amounts of free haemoglobin and so possibly increasing the severity of the jaundice, and the photosensitisation. Two operated sheep were forced to inhale arsenuretted hydrogen for  $\frac{1}{2}$  minute on two consecutive days, starting on the 2nd day after the operation. The sheep were bled immediately after each inhalation and the serum examined spectroscopically. In each case the bands of oxyhaemoglobin were seen. Thereafter the animals were exposed to sunlight. One sheep showed marked flinching and swelling of the head on the 4th day after the operation. This continued up to the 7th day. On the 8th day the swelling had gravitated to the region between the lower jaws, which appeared bag-like and filled with fluid. The eyes remained partly closed. Icterus was intense, even the tears and the saliva assuming a deep yellow colour (see photo 10). The animal was killed the same day. The post-mortem revealed a slight anaemia, generalised icterus, bile pigmentation and swelling of the liver and kidneys, swelling of the spleen. The other sheep showed haemoglobinuria within a few hours after inhalation of the arsenuretted hydrogen. The conjunctivae assumed a dark brown colour. Six days



Fig. 10. Sheep 35353. Head and inter-mandibular region swollen after ligation of bile duct and inhalation of arsenuretted hydrogen.

after the operation the sheep became sensitive to sunlight, showing marked flinching and swelling of the ears. The conjunctivae were intensely yellow and the serum deep brownish yellow. By the 12th day the swellings had subsided, while the skin over the face and ears was hard and dry. On the 14th day marked photosensitisation was again shown, accompanied by some swelling of the ears. The animal was in poor condition and feeding badly as a result of the hardness of the lips (see photos 11, 12 and 13). It was killed on the 20th day after operation. On post-mortem there were lesions of cachexia, anaemia, well marked necrosis of lips, ears and facial skin, enlargement and bile pigmentation of the liver and kidneys, stasis in the fore-stomachs and large intestines.

From the above cases it would appear as if the inhalation of arsenuretted hydrogen did provoke more intense symptoms of icterus and photosensitisation.



10. *Bile ducts obstructed under pure choloform anaesthesia followed by repeated administration of chloroform.*—Seeing that chloroform may act as a powerful liver poison, it was thought that its use as an anaesthetic may cause more pronounced symptoms in cases of bile duct obstruction. Consequently, several sheep were operated under pure chloroform anaesthesia. Thereafter the animals received daily injections subcutaneously of 0.5 c.c. chloroform in liquid paraffin. In one sheep photosensitisation was seen on the 3rd day after operation. On the 6th day there was marked finching and clinical icterus. This continued up to the 16th day, when the ears were noticed to be hard and dry. The animal died on the 20th day with lesions of intense icterus, necrosis of the ears, swelling, pigmentation and degeneration of the liver and kidneys, stasis in the fore-stomachs. The other three animals died within the first week without showing photosensitisation. Thus it appears that chloroform does not greatly intensify the symptoms produced by simple biliary obstruction.



Figure 11.

Fig. 11. Sheep 35351. Necrosis round eyes and lips after ligation of bile duct inhalation of arsenuretted hydrogen.



Figure 12.

Fig. 12. Sheep 35351. Sloughing of skin round eyes and nostrils after ligation of bile duct and inhalation of arsenuretted hydrogen.

11. *Bile ducts obstructed, followed by oral administration of carbon tetrachloride.*—Due to the toxic action of carbon tetrachloride on the liver it was decided to attempt to intensify the liver disturbance by repeatedly dosing it to operated sheep. One sheep was dosed for 4 weeks with a total volume of 228 c.c. carbon tetrachloride dissolved in olive oil, starting with 4 c.c. carbon tetrachloride daily and ending with 20 c.c. daily. The animal became slightly sensitive to sunlight on the 6th day after operation, by which time clinical icterus was also visible. The serum was deep yellow and gave a strongly positive direct Van den Bergh reaction. On the 15th day slight sensitisation was again noticeable. This, however, soon disappeared. Thereafter the animal became dull, and condition was lost until it was killed in extremis on the 31st day.



On post-mortem, the carcass revealed cachexia, anaemia, intense generalised icterus, necrosis of skin round the mouth, marked swelling, degeneration and pigmentation of the liver and kidneys with enlargement of the bile ducts, stasis in the fore-stomachs.

In this case carbon tetrachloride therefore produced no visible effect on the course of the condition, in spite of the large amount administered.



Fig. 13. Sheep 35351. Marked necrosis round eyes and nostrils after ligation of bile duct and inhalation of arsenuretted hydrogen.

12. *Bile ducts obstructed, followed by administration of toluylenediamine and chloroform.*—Since toluylenediamine is known to provoke well marked icterus in the dog, it was decided to test it out on an operated sheep in combination with chloroform. The animal



Fig. 14. Sheep 32638. Marked photosensitisation after ligation of bile duct and dosing with chloroform and toluylenediamine.

received daily doses of 0.5 gm. toluylenediamine and 0.5 c.c. chloroform (in water) by stomach tube. This was continued for a period of 12 days. The animal showed slight icterus on the second day, the urine being deep yellow. On the third day it showed flinching and the ears began to swell up. On the 4th day the sensitisation was very marked, the animal throwing its body into unusual attitudes (see photographs 14 and 15). On the 13th day the skin of the face and over the shorn back was noticed to be hard and cracking. The animal remained sensitive up to the 21st day, while the facial skin changed to a dirty greyish black and covered with hard crusts (see photo 16). The sheep had great difficulty in feeding and drinking, and was killed in extremis on the 23rd day after operation.



Fig. 15. Sheep 32638. Marked flinching (same as Fig. 14).

The post-mortem revealed cachexia, anaemia, necrosis of skin over the head and back, severe icterus, swelling, pigmentation and bile stasis of the liver with diverticula of the superficial bile ducts, swelling and pigmentation of the kidneys, slight swelling of the spleen, stasis in the fore-stomachs, and absence of food in the small intestine.



Fig. 16. Sheep 32638. Necrosis round eyes and nostrils (same sheep as in Fig. 15).

13. *Bile ducts obstructed, followed by administration of phenylhydrazin.*—Phenylhydrazin through its haemolytic action is known to produce icterus in the dog. It was therefore hoped that in the sheep it may increase the symptoms following obstruction of the bile ducts.

Into one operated sheep phenylhydrazin hydrochloride was injected intravenously (in saline solution) in daily doses of 0.1 gm. over a period of 12 days. On the 3rd day the serum was definitely yellow, while haemoglobin bands were faintly visible spectroscopically. On the 5th day the serum assumed a very brown colour, presumably due to the mixture of bile pigments and haemoglobin. The urine, too, was dark brown. The animal became slightly dull and somewhat weak in the legs, but no photosensitisation was shown. On the 9th day clinical icterus was well marked. From the 12th day onwards the serum changed to a pale yellow colour, while photosensitisation was still absent. The condition improved and after 4 weeks the animal was discharged with its serum only faintly yellowish.

14. *Bile ducts obstructed, followed by injections of manganese chloride.*—One operated sheep received daily intravenous injections of 0.1 gm. manganese chloride on three consecutive days. On the 4th day the animal became markedly sensitive to sunlight with flinching and subsequent swelling of the ears. On the following day the swelling of the ears and head had increased (see photo 17), and the animal was intensely irritated. From the 7th day onwards the swellings decreased with clinical icterus definitely visible. By the 14th day the animal was very weak and death followed the same day.



Fig. 17. Sheep 35334. Head and ears swollen after ligation of bile duct and administration of Manganese Chloride.

On post-mortem the carcass showed cachexia, necrosis of the skin over the face and lips, very marked icterus, swelling of the liver with dilatation of the bile ducts, marked swelling of the spleen and kidneys, stasis in the fore-stomachs.

15. *Bile ducts obstructed, followed by injections of B. coli cultures.*—In order to ascertain whether certain types of bacteria normally present in the digestive system, e.g. *B. coli* could, under certain conditions, play a rôle in the production of icterus and photosensitisation, several sheep were injected with 24-hour broth cultures of *B. coli*.

One sheep received 5 c.c. broth culture into the liver via the common bile duct immediately after it was ligated. The animal showed no symptoms until the 3rd day, when it became markedly sensitive to sunlight, finching and very restless. On the 5th day the ears, nose and lips were markedly swollen (see photo 18). On the 7th day the animal was injected intravenously with 10 c.c. 24-hour broth culture of *B. coli*. By this time the animal was showing well marked icterus, while the feet appeared tender and painful. Death ensued that same night.



Fig. 18. Sheep 35323. Head and ears swollen after ligation of bile duct and injection of *B. coli* culture.

On post-mortem there was a well marked oedema of the subcutis of the head and ears, generalised icterus, pulmonary oedema, bile pigmentation of the liver and kidneys.

Another sheep was injected intravenously 5 c.c. broth culture of *B. coli* immediately after the operation. The following day the animal was dull, but the ears were distinctly swollen. On the third day the animal was markedly sensitive, and the ears very much swollen. There was no sign of clinical icterus as yet. On the 7th day the ears and face were still swollen, although the tips of the ears and lips were beginning to harden (see photo 19). There was a soft bag-



Fig. 19. Sheep 35338. Head and ears swollen after ligation of bile duct and injection of *B. coli* culture.



like swelling in the intermandibular space and clinical icterus distinct. Another intravenous injection of 10 c.c. broth culture of *B. coli* was again given. The animal died the following day, the main lesion on post-mortem being a generalised icterus, gelatinous infiltration of the subcutis of the head, ears and intermandibular space, pigmentation of the liver and kidneys and swelling of the spleen.



Fig. 20. Liver showing marked dilatation of superficial bile tracts following ligation of common bile duct and cystic duct.

### SUMMARY.

1. The object in undertaking bile duct obstruction experiments in sheep, was to throw further light on the genesis of icterus and photosensitisation in true geeldikkop as caused by *Tribulus* spp. and also by other plants in South Africa.

2. The operative procedure was described for the ligation and obstruction of the extra-hepatic bile tracts in the sheep.

3. The ensuing clinical symptoms and the blood of operated animals were studied from day to day.

(a) A progressive bilirubinaemia was noted within one hour after operation. Clinical icterus, however, only presented itself several days afterwards and then persisted throughout the course of the condition. Within 24 hours the blood, and later also the urine, showed a strong direct van den Bergh reaction.

(b) Practically every animal, with the head and back closely shorn, when exposed to sunlight, developed symptoms of photosensitisation within one week of the operation. This was shown by the animals flinching, shaking the head and licking the lips. Soon afterwards oedema of the subcutis

of the affected skin set in, e.g. the ears became thick and pendulous and the face swollen. Photosensitisation usually persisted for several days and in some cases lasted throughout the course of the condition. This was followed by hardening of the affected skin and sloughing of the superficial layers. Skin protected by a coat of wool remained normal.

- (c) Animals with the biliary tract obstructed showed a progressive loss of condition up to the point of extreme emaciation.

4. On post-mortem the lesions found were those of intense generalised icterus, enlargement of the liver with marked bile stasis, dilatation of all the biliary tracts above the point of obstruction, enlargement and bile pigmentation of the kidneys, and frequently stasis in the large intestine.

5. Various modifications of the above operation were also attempted. The resultant symptoms and post-mortem lesions, however, were not altered or intensified to any extent.

Thanks are due to Mr. M. Carlisle for assistance rendered and to Mr. T. Meyer for taking the photographs.

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