

Studies on the Photosensitisation of Animals in South Africa.

IX. The Bile Flow of the Merino Sheep under Various Conditions.

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INTRODUCTION.

In a series of articles (1933, 1934, 1935) which appeared under the above general title, various experiments were described in which the main object was the elucidation of the "geeldipkop" problem in South Africa. From the results obtained one was forced to the conclusion that in the symptom complex of this disease two distinct factors had to be considered. Firstly there was the severe generalised jaundice and secondly the acute photosensitisation of unpigmented animals leading to necrosis and subsequent deformity of the facial skin. In considering the problem from different aspects, it was at one time thought possible that the one symptom could be the direct cause of the other. Thus it was thought that some normal biliary constituents, e.g. the bile pigments themselves when present in excessive amounts in the general circulation, could under certain circumstances give rise to photosensitisation. Alternatively it was thought possible that photosensitising principles present in the plant *Tribulus* or elaborated in the animal body after ingestion of the plant, might be the cause not only of a primary photosensitisation but also of a secondary jaundice through some derangement of the normal liver function. However, numerous experiments conducted in this light have failed to prove—

- (1) that any photosensitising principle may in itself be the cause of jaundice, and
- (2) that normal bile or any of its constituents when present in excessive amounts in the circulation could give rise to symptoms of photosensitisation.

Moreover as far as could be ascertained from the current literature, no conditions are recorded in which these two symptoms have any bearing on each other.

With regard to the photosensitising factor in geeldikkop, Rimington and Quin (1934) were able to show that the plant porphyrin, phylloerythrin was directly responsible for the symptoms of light sensitivity. It is formed in the digestive canal of many animals feeding on chlorophyll containing materials, and after its absorption into the portal circulation is normally excreted by the liver in the bile and thus passed out with the faeces. Should the excretory function of the liver be deranged, so that increased amounts of bile and thus also of phylloerythrin are present in the circulation, symptoms of photosensitisation may appear at any moment, provided the animals have unpigmented skins and are exposed to sunlight (see article 6 of this series). Furthermore, injections of phylloerythrin into healthy sheep provoke acute photosensitisation without any signs of jaundice, thus acting in the same way as other photosensitisors such as haematoporphyrin, certain fluorescent dyes, and hypericin (Quin 1933, 1934). One was thus forced to conclude that the primary disturbance in geeldikkop caused by *Tribulus* or by various other plants responsible for this peculiar symptoms complex, consisted in some derangement in the normal bile flow from the liver whereas the photosensitisation was of a secondary and purely accidental nature.

The main problem therefore resolved itself into attempts at elucidating the icterogenesis caused by *Tribulus* and other materials showing a similar effect. It must however be admitted that so far all attempts have failed in demonstrating an icterogenic principle in these plant materials except in the case of certain species of *Lippia* and to which reference will be made later on. In *Tribulus* for example this factor must be of an extremely labile character seeing that the plant may be harmless at one moment and decidedly dangerous soon afterwards.

Furthermore the liver in geeldikkop cases shows very little morphological change microscopically except for the bile staining. Hence the disturbance appears to be largely functional in that it is not regularly accompanied by any of the wellknown structural alterations.

In view of these difficulties in explaining the action of *Tribulus* on the liver it was decided to make a closer study of the normal bile flow in Merino sheep and to ascertain how this was influenced by different factors. As can be gathered from the large volume of literature published, studies on the bile excretory function of the liver have commanded the attention of many workers in different parts of the world. Not only can bile be regarded as one of the most peculiar excretions of the body but through a variety of causes (some known and others unknown) liver derangement may lead to definite symptoms of which jaundice is perhaps the most striking.

In a comprehensive article by Rich (1930) it is pointed out that the development or non-development of jaundice depends on the balance struck between the amount of bilirubin delivered to the liver and the capacity of the liver to excrete it. Thus with a normally functioning liver, excessive amounts of bile pigments are readily eliminated whereas under certain circumstances great difficulty may be experienced in the excretion even of small amounts of bile. The problems of liver derangement are further complicated by the fact

that no strict relationship seems to exist between the type and extent of liver damage and the degree of jaundice developed. Thus it is peculiar that in many cases of severe fatty changes of the liver or in extensive cirrhosis the icterus may be only very slight or completely absent. On the other hand as pointed out previously, the liver in geeldikkop may show practically no morphological change and yet the icterus may be most intense.

In studying the bile excretion, regular examination of the blood serum is generally regarded as of the greatest importance since Hymans van den Bergh has demonstrated that not only can the bile pigments be determined quantitatively but also the type of jaundice can be ascertained by the diazo reaction. Beyers (1923), in his monograph on urobilinuria and icterus in herbivorous animals, gives a detailed account of these conditions in the various species. It is pointed out that whereas a physiological bilirubinaemia is present in the horse, no bile pigments are normally found in the blood serum of adult bovines, sheep, goats, pigs and dogs. The serum of the horse, which is normally of a yellow colour, contains 1-2 units of bilirubin, whereas in the other species the serum is usually water clear or only slightly tinged.

This indicates that the equine liver treats bilirubin as a substance with a definite threshold value, whereas in most of the other species it appears to be excreted as fast as it arrives in the liver, i.e. treated as a non-threshold body.

EXPERIMENTAL PROCEDURE.

1. *The Blood Serum in Healthy Sheep.*

Clinical jaundice is usually first detected by the yellow discoloration of the visible mucous membrane such as the conjunctiva and sclera of the eye ball and the gums. It is well-known, however, that this discoloration only appears when excessive amounts of bile pigments have been circulating through the tissues for some time, while progressive absorption of pigment takes place. In tissues richly supplied by blood even a fairly severe degree of jaundice may be masked by the haemoglobin. In order therefore to detect a jaundice in the early stages, or alternatively to ascertain the efficacy with which the liver is excreting bile, it is essential to examine the blood serum for the presence of these pigments. This procedure has been adopted throughout. Experimental animals were regularly bled from the jugular vein and 10 c.c. citrated blood centrifuged at high speed. The supernatant plasma was drawn off into separate test tubes of uniform bore. Thereafter the colour was described as also its depth, and the v. d. Bergh reaction carried out where necessary. In all cases where the plasma was water clear or practically so, and the v. d. Bergh reaction negative, this was regarded as indicating a free and efficient bile excretion from the liver.

In order to test out the above point, 126 clinically healthy Merino sheep (ranging from 2-4 tooth in age) were bled and the plasma examined. The following results were obtained:—

- 20 water clear and v. d. Bergh negative.
- 100 very slight yellowish trace, v. d. Bergh negative.
- 6 definitely yellow—direct v. d. Bergh positive.

This indicates that by far the greatest majority of these animals show a clear or practically clear blood serum free from bile pigments while a small minority may be affected by a sluggish bile excretion as shown by the colour of the plasma and the presence of pigments. The healthy Merino sheep may thus be looked upon as an animal with a very efficient bile excretion and in which a physiological bilirubinaemia is not the rule. Regular examination of the blood serum therefore serves as an index of the bile flow.

2. Bile Flow as recorded from Biliary Fistulae.

In article 6 of this series the results of experimental ligation of the extra-hepatic bile tracts in sheep were recorded. In order to gain a more precise insight into the amounts of bile excreted over given periods, a further series of experiments were undertaken. For this purpose young Merino sheep (2-4 tooth old) and in good condition were selected. After a preliminary starvation of 24-36 hours a laparotomy was performed in the right flank under general anaesthesia using chloral hydrate intravenously. The common bile duct was then double ligated with thin silk and severed between the ligatures. An 8-inch length of fairly stiff rubber tubing (bore 4 m.m.) into one end of which a short funnel shaped glass tube had been inserted, was used as a cannula for the gall bladder. A small incision was made through the free end of the bladder and the flanged end of the tube tied into the bladder by means of a purse string suture, the other end of the tube being withdrawn to the exterior through a stab wound close to the laparotomy. Due to the fairly long gall bladder in the sheep it could as a rule be drawn up close to the stab wound. The laparotomy wound was subsequently closed with the usual three layers of sutures. The only dressing used was a thin layer of colloïd-iodoform over the injured skin. The bile was collected in a flat thick-walled glass bottle strapped on to the side of the animal, the fistula tube penetrating a tight fitting cork stopper on the bottle. Sheep were usually operated on in pairs, and in some instances where liver bile was studied, the cannula was inserted into the neck of the gall bladder close up to the cystic duct while the gall bladder itself was removed. All animals were kept on a ration of veld hay, green lucerne and crushed yellow maize with water ad lib. Apart from the daily recording of the bile flow, the animals were bled each morning and the serum examined for pigments, as also the conjunctiva for clinical jaundice. All the faeces was collected in linen bags strapped on to the animals and weighed at 12 hourly intervals i.e. at 6 a.m. and 6 p.m. at the same time that the bile volume was recorded. Furthermore the animals were weighed twice weekly. Rectal temperatures were taken in the morning and in the evening.

The following records were obtained from the different sheep placed in experiments:—

Two Merino lambs (No. 1 and No. 2) were kept under observation for 6 days prior to operation. During this period both animals were feeding very well. In both sheep samples of blood withdrawn on several occasions showed the serum to be water clear and free from

bile pigments. The animals were fed during the day only, all food being withdrawn from them at night. The following table indicates the weight of faeces collected over this period.

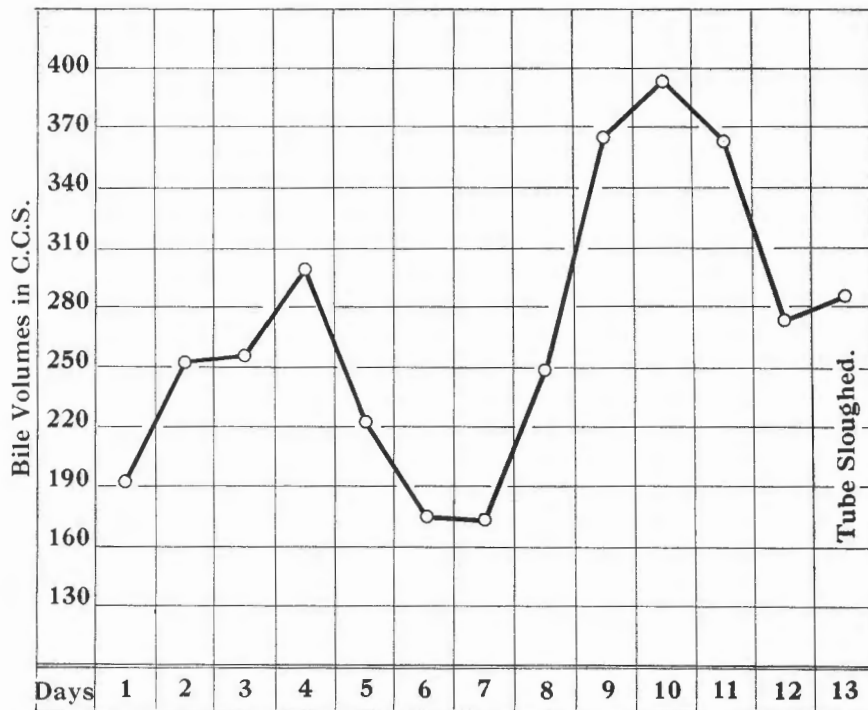
Day.	Sheep No. 1 (2 tooth hamel). Weight 57.5 lbs.	Sheep No. 2 (2 tooth hamel). Weight 50.5 lbs.
1. Day.....	204 grams	171 grams
Night.....	107 „	10 „
2. Day.....	113 grams	99 grams
Night.....	77 „	55 „
3. Day.....	116 grams	92 grams
Night.....	24 „	96 „
4. Day.....	250 grams	178 grams
Night.....	132 „	155 „
5. Day.....	240 grams	168 grams
Night.....	154 „	169 „
6. Day.....	129 grams	130 grams
Night.....	106 „	117 „

It is thus seen that on an average sheep No. 1 excreted 275 grams faeces daily of which 175 grams was passed out during the day and 100 grams during the night. For sheep No. 2 the corresponding figures were 240 grams daily, i.e. 140 grams by day and 100 grams by night. Regular examination of the faeces showed that in both sheep it consisted of well-formed glistening dark green pellets.

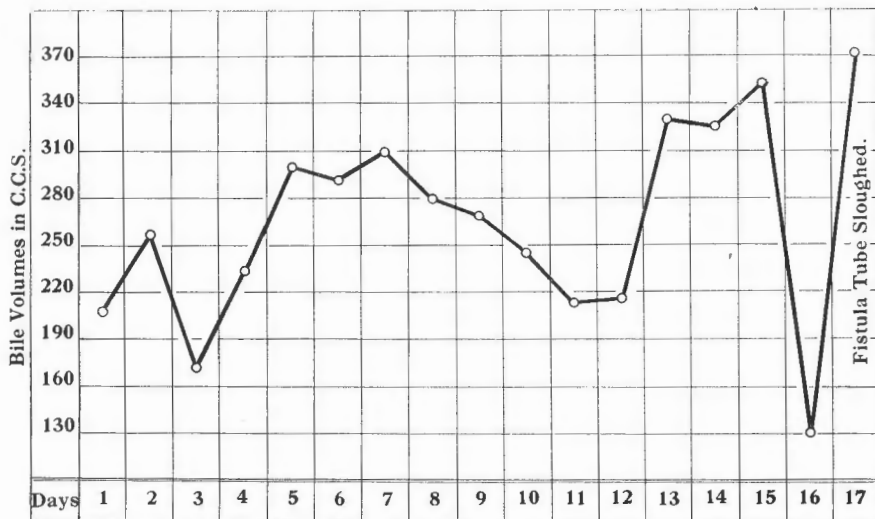
On the seventh day both sheep were operated and a rubber cannula inserted into the gall bladder after ligation of the common bile duct. Again the faeces were collected as well as all the bile excreted. For the rest the treatment remained as before. As will be noticed from the accompanying graphs (No. 1 and No. 2) the volume of bile eliminated daily showed fairly wide fluctuations, although it was flowing freely from the tube. Furthermore there was no constant relationship between the "day" yield and the "night" yield. Thus on some days the former was slightly greater and vice versa, although on the whole it was found that approximately equal quantities were eliminated by day and by night. Consequently there was no suggestion of a constant diurnal rhythm in the bile excreting activity of the liver. The average 24 hourly excretion in the two sheep was however remarkably close. Thus for sheep No. 1 this was 270 c.c. as compared with 265 c.c. for sheep No. 2.

The Bile.—Daily examination of the bile showed it to be of a very dark green colour, although on some days the shade was lighter. Usually it was clear and transparent, although again at times varying degrees of turbidity were noticeable. The viscosity throughout was very low due in all probability to little secretion of mucus from the bile tracts. Moreover as the bile was being rapidly drained from the gall bladder little opportunity was given for water absorption and hence for a concentration of the bile.

GRAPH No. 1.
Bile Volumes of Sheep No. 1.



GRAPH No. 2.
Bile Volumes of Sheep No. 2.



The Serum.—On the whole the blood serum remained water clear, although on some days a very slight yellowish coloration was noticeable and giving a doubtful direct van den Bergh reaction. From this one is led to conclude that the bile excretion from the liver, except for an occasional slight sluggishness, remains very efficient in spite of the fact that no bile enters the intestines and thus rendered incapable of exerting any cholagogue action. In sheep No. 1 the bile cannula sloughed away on the 15th day after operation leaving a very small constricted skin wound through which practically no bile was passing. This was followed by a rapid increase in bile pigments in the serum, which in 48 hours was of a deep yellow colour. At this moment too the animal developed acute photosensitisation with swellings of the lips and ears due to phylloerythrin present in the bile and regurgitating with it into the general circulation. After 4 days the photosensitisation passed off while the serum gradually lost the yellow coloration and again became water clear after 8 days, in spite of the fact that the fistula wound was completely closed by this time. When the animal was killed for post-mortem examination 4 months afterwards, restitution of the common bile duct was found to have taken place with bile entering the duodenum in the usual way.

In sheep No. 2 the tube sloughed on the 19th day after operation with bile continuing to flow from the skin fistula. On the 26th day the animal was killed for post-mortem on account of its poor condition.

Faeces.—Up to the time of the sloughing of the tubes the appetite of both animals remained good. The average daily yield of faeces for Sheep No. 1 amounted to 170 grams and for sheep No. 2 it was 95 grams. In both cases the consistence of the faeces showed little change from the normally well formed pellets. The colour however changed from the usual greenish black to a lighter greyish brown following the prevention of bile from entering the intestines.

Once it was established that by inserting a rubber cannula into the gall bladder of a sheep, its total daily yield of bile could be caught up and accurately measured, further experiments were undertaken with the object of ascertaining how the bile flow could be influenced by various factors, i.e. either inhibited or stimulated

FACTORS CAUSING INHIBITION OF BILE FLOW.

As indicated previously (article 5) sheep dosed with the plant *Lippia Rehmanni* (Pears) develop an acute photosensitisation accompanied by jaundice, both symptoms closely resembling those seen in true geeldikkop. In order therefore to gain further insight into the genesis of icterus especially of the obstructive or regurgitative type, i.e. of the factors which inhibit the prompt excretion of bile from the liver, an extensive series of experiments were undertaken with the plant *Lippia Rehmanni*. As will be indicated later, various other substances including some well-known liver poisons were also tested out on sheep although in no case was the typical jaundice of geeldikkop or *Lippia* poisoning produced.

The Lippia material consisted of large quantities of mature plants collected during the summer months. This was dried in the shade, then very finely pulverised in a mill and stored in stoppered jars. Experimental animals were drenched by stomach tube either with watery suspensions of the plants or 96 per cent. alcoholic extracts after evaporation of the alcohol.

1. *Drenching of Alcoholic Extracts of Lippia to Normal Sheep.*

Seven young Merino lambs (2 tooth) were each dosed with alcoholic extract from 500 grams Lippia and killed at regular intervals for post-mortem examination and collection of materials. In each case the serum was definitely yellow within 24 hours while after 48 hours it had changed to a deep brownish yellow and giving a strong direct van den Bergh reaction. On the third day all these animals showed a light but definite clinical icterus on the sclera and conjunctiva. Furthermore at this stage they all became acutely photosensitive with swelling of the head and accompanied by intense skin irritation. No haemolytic processes could be detected as daily determinations of the percentage volume of red cells remained constant and the serum free from haemoglobin.

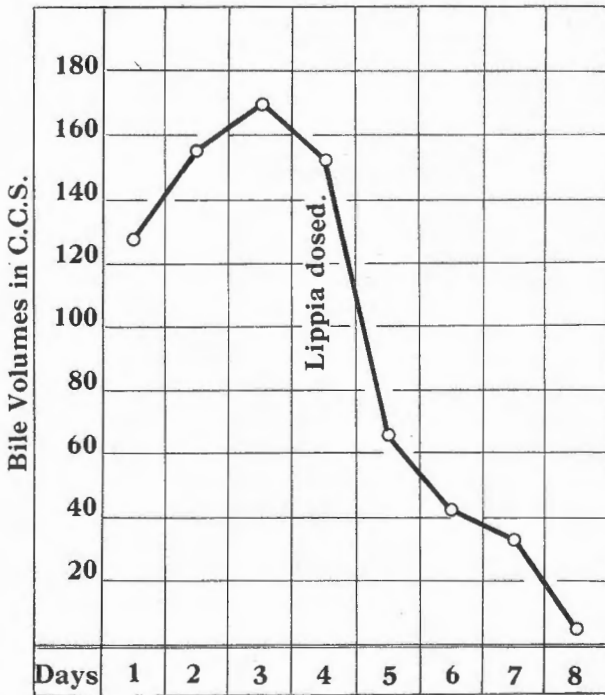
2. *Drenching of Lippia to Sheep with Biliary Fistula.*

As Lippia causes such a prompt bilirubinaemia, it was hoped that its quantitative effect on the bile flow would be more clearly shown after dosing it to animals with a biliary fistula. The following graph illustrates the effect of 500 grams Lippia dosed to a fistula sheep, a similar reaction being noted in several sheep treated in this way (Graph 3). Thus it is seen that an average daily bile flow of 150 c.c. was reduced to 66 c.c. within 24 hours after dosing Lippia, while by the 4th day, the flow was practically completely stopped, whereas the jaundice rapidly became more pronounced. Furthermore the colour of the bile undergoes a marked change after dosing the animal with Lippia. Thus in an animal showing a continuous flow of dark greenish bile from the fistula, when dosed with the alcoholic extract from 400 grams Lippia, the colour changes from the dark green to a light yellowish green within 5 hours of dosing. After 24 hours it changes to a clear pale sea green, whereas 3 hours later the bile flow is practically colourless and water clear resembling the so-called "white bile" (see coloured plate). It is only after a period of 4-5 days when the liver is regaining its power of bile excretion that the colour of the bile also deepens, i.e. a renewed elimination of the pigments takes place. At the same time the serum clears up and a general improvement in the health of the animal becomes noticeable, since during the period of severe jaundice all animals appear markedly depressed with loss of appetite, decreased ruminal movements and a stasis of the large intestines resulting in constipation. In this respect all the findings appear to be identical with those described for true geeldikkop or those resulting from artificial obstruction to the bile flow through ligation of the bile tracts (Quin 1933).

FACTORS INTENDED TO CAUSE STIMULATION OF BILE EXCRETION.

Seeing that the plant *Lippia Rehmanni* possesses such a strong paralysing effect on the bile excretion, various experiments were undertaken in an attempt to overcome the jaundice and to re-establish the bile flow. It should be mentioned that in no single case of this type of jaundice are the extrahepatic bile tracts occluded, e.g. there is no undue distension of any part of the tract. Furthermore on post-mortem, bile flows through easily into the duodenum even after gentle pressure on the gall bladder.

GRAPH No. 3.
Effect of Lippia on bile flow of sheep.



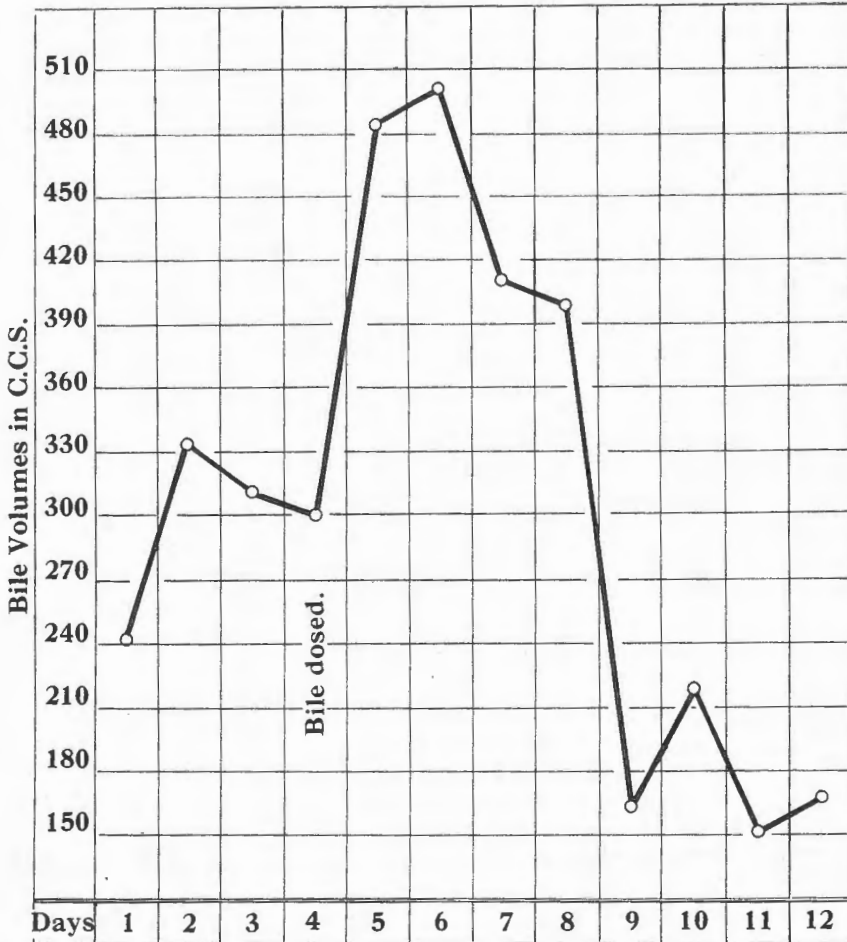
One is thus forced to conclude that some intrahepatic disturbance of the normal bile excretion is the primary effect caused by the Lippia. That being the case, it was reasonable to expect that the administration of certain materials might through their cholagogue action, stimulate the liver to renewed excretion. With this object in view, various materials were tested out, starting with the more commonly known cholagogues.

1. *Dosing of Bile to Sheep with a Bile Fistula.*

Seeing that bile through the action of its contained bile salts, is generally regarded as a particularly efficient cholagogue, large quantities were collected from sheep and also from other animals for dosing to experimental animals whose bile flow in turn, was closely followed over definite periods. Four sheep with a biliary fistula were dosed with sheeps bile 1,000 c.c. daily for 2 consecutive days. As will be

seen from the accompanying graphs of the bile flow of two of these sheep (graphs 4 and 5) the average daily yield increased from 300 to 450 c.c. in the one case and in the other from an average of 220 c.c. to 366 c.c. per day over a period of 4 days. From these results it is clear that the dosing of large quantities of bile causes a transitory though definite increase in the bile flow of sheep with a biliary fistula.

GRAPH No. 4.
Effect of dosing bile on the flow of bile in sheep with fistula.



2. *The Cholagogue Action of Various Substances administered to Fistula Sheep after being Poisoned with Lippia.*

In these experiments the object was to ascertain the strength of various substances, some of which are reputed to possess definite bile driving powers, in overcoming the jaundice caused by the administration of Lippia. For this purpose 28 experimental sheep were used. Some of these carried bile fistulae and in which the daily bile flow could be recorded, while in other sheep daily comparisons of the serum colour served the purpose of ascertaining the degree of

osteomalacia. Sonntag (1916) in comparing the fluorine content of normal bones with that of dogs fed on sodium fluoride finds that the values were increased from 0.03 per cent. to 1.73 per cent. fluorine calculated on the dry bases. Bethke (1929) *et al*, working with pigs, found that the ash content of femurs of normal pigs fed bone meal and limestone contained 0.0409 and 0.0231 per cent. fluorine, whereas the percentage of this element in the femurs of pigs fed NaF and rock phosphate was .528 per cent., .793 per cent., 1.107 per cent., 1.0934 per cent. and 1.0369 per cent. respectively. Christiani (1927) maintains, that the fluorine in bones is an index of fluorine toxicity. Brandl and Tappeiner (1891) found abnormal deposition of fluorine in various tissues after continuous administration of fluorine. The same workers examined the bones of a dog on fluorine and noticed glistening crystals deposited in the bones. They regarded these crystals as crystalline compounds similar to fluorite. Bethke, Kick, Edgington and Wilder (1929) found that the fluorine content of the bones of pigs fed rock phosphate or NaF was directly proportional to the fluorine content of the ration. According to Phillips and Hart (1935) the feeding of fluorine to hens resulted in a distinct and measurable increase in the F content of the eggs. It appears that at least a larger proportion of the fluorine remained with the acetone insoluble portion of the fat-like substance of the egg-yolk. This suggests that F was deposited with the egg in combination with the complex lipoids of the yolk. While it is fairly definite that fluorine is deposited in the egg under conditions of fluorine feeding, it appears on the other hand that the composition of milk is influenced with the greatest difficulty from extraneous sources. In a study on the effect of fluorine ingestion on the nutritional qualities of milk Phillips, Hart and Bohstedt (1934) detect no increase of fluorine in the milk of fluorine fed cows. They conclude that it is not easy to influence the fluorine content of milk. From a study by Klement (1935) on the analysis of the inorganic substance of bones and teeth of land and sea mammals and land and sea birds, it appears, that mammals and birds of the sea have a distinctly higher fluorine content in their ash than animals living on land. Thus land mammals have an average fluorine content of 0.05 per cent. while sea mammals have a fluorine content of 0.55 per cent. The fluorine content of land birds is 0.11 per cent., sea birds 0.32 per cent., fresh water fish 0.03 per cent. and sea water fish 0.43 per cent. This difference is ascribed to the higher fluorine content of the sea water in which these animals are continually living. It seems therefore fairly definite that while there is thus far no indications of a biological significance of fluorine, that the tissues nevertheless in the presence of a continual supply of fluorine may store appreciable amounts of this element. Marine animals living in an environment of higher fluorine concentration consequently stores greater amounts in their ash than fresh water animals.

Although there exists definite evidence of the retention of fluorine by the animal body, the form in which fluorine is deposited is still a matter of conjecture. McClure and Mitchell (1931) conclude that if fluorine is consumed in the more soluble form of the sodium salt, it may cause the deposition of an abnormal constituent

in the bones or an abnormal deposition of a non-calcium constituent, possibly a fluoride or a mineral other than calcium as is evidenced by an increase in the percentage of ash in the bones above normal. Trebitch (1927) claims that fluorapatite $3[\text{Ca}_3(\text{PO}_4)_2]\text{CaF}_2$ occurs in dental enamel and is responsible for the hardness of the teeth. Klement (1935) found that fossil bones are high in fluorapatite, which is formed by a continual process of absorption of fluorine from the soil water. From the extensive studies on the form of the calcium phosphate compound in bones, it appears that it does not exist as a tertiary calcium phosphate but rather as a phosphate of the apatite form $3[\text{Ca}_3(\text{PO}_4)_2]\text{CaX}$ in which X can be F, Cl_2 , $(\text{OH})_2$ or CO_3 . Robinson and Soames (1925) results on bones analysis support the general conception that the chief bone constituent is an apatite. Basset (1917) is of the opinion that an hydroxy-apatite is the chief inorganic compound in bones. It can readily be conceived how theoretically in the presence of fluorine the hydroxy or carbonate groups can be replaced, to form a fluorapatite. According to Klement, this is what actually happens in fossil bones, where a continual absorption of fluorine from the soil water takes place over a long period of time. However the possibility of fluorapatite deposition in the bony tissue is largely discredited by the fact that the results on the fluorine content of bones thus far reported on have not nearly approached the theoretical value of 3.77 per cent. of fluorine in fluorapatite. Consequently to attempt a definition of the fluorine complex in tissues at present would be merely speculation, and must await the results of further studies.

That the retention of fluorine has a direct bearing on the calcium and phosphorus metabolism has been shown by several workers. Channels (1930) found that the incisors of rats on a diet of white bread and milk plus fluorine contained slightly less calcium than the normals without fluorine. McClure and Mitchell (1931) found a decrease in the calcium content of the ash of rats receiving fluorine. Forbes and associates (1921) obtained weaker bones in pigs when rock phosphate was fed. The weaker bones were characterized by a high phosphorus content and a low percentage of calcium and carbon dioxide. Lantz and Smith (1934) compared the calcium and phosphorus metabolism of albino rats of various ages fed diets to which 0.05 and 1 per cent. of fluorine had been added, with matched rats as controls. Rats receiving the fluorine ration retained much less calcium and somewhat less phosphorus than the controls, whether the values were expressed as total quantities, percentage of intake or mgms. per gram weight. Older animals showed these differences less markedly. Fluorine fed rats excrete far more calcium in the faeces than the controls.

Of the actual amount of fluorine retained in the body little is known. Gautier and Clausmann (1913) estimate the daily excretion by man of 0.23 mgm. F in the urine and 0.80 mgm. F in the faeces. Brandl and Tappeiner (1891), feeding a dog for 21 months on a fluorine ration containing 402.9 grams fluorine, found 330.5 gms. excreted. It was only when the dosage reached 0.5 gm. fluorine that an excretion of this element was noticed. At lower doses no excretion was noticed. It appears that the system can cope with a continuous

a light yellow colour which in spite of continued dosing, did not reach the stage of a definite clinical icterus. Neither was photosensitisation noticeable in any of these sheep. In one case the dosing was continued for 43 days starting with daily doses of 0.5 c.c. and increasing up to 8 c.c. daily until a total amount of 110 c.c. had been administered. After the second dose of 8 c.c. the animal died suddenly with symptoms of acute respiratory distress. The only lesions that could be found were those of pulmonary oedema and fatty changes of the liver. Although bilirubinaemia was repeatedly observed during the period of dosing, no clinical jaundice could be established, neither was the carcass icteric. Given subcutaneously chloroform is far more toxic as amounts of 2 c.c. have been found to cause death within 24 hours with the same lesions as described above.

Phosphorus.—This severe liver poison was administered to experimental sheep either subcutaneously as phosphorated cod liver oil in 1 c.c. doses or as ordinary yellow phosphorus .01 gram dissolved in olive oil and dosed by stomach tube. One sheep dosed with a total of 14 c.c. phosphorated oil over a period of 8 days showed a definitely yellow serum on the seventh day with a direct van den Bergh reaction (35 mgs. per litre). Clinical jaundice however was slight. The animal died on the ninth day with severe fatty changes of the liver and catarrhal gastro-enteritis. Jaundice although present was very slight. One sheep injected with daily doses of 1 c.c. phosphorated oil for 5 days died on the tenth day with a pale yellow serum giving a faint direct van den Bergh reaction.

Altogether 5 sheep were poisoned with phosphorus. In every case very severe fatty changes of the liver were noted, the consistence being very soft and friable and the organ engorged with blood. General icterus if present was slight.

Carbon Tetrachloride.—Two sheep were dosed daily over long periods as follows:—

- (a) One sheep was dosed with a total amount of 524 c.c. carbon tetrachloride over a period of 42 days, starting with a daily dose of 4 c.c. This was gradually increased to 20 c.c. of which 13 consecutive doses were given before the animal died on the 43rd day. Every dose of carbon tetrachloride was mixed with an equal volume of alcohol (96 per cent) before dosing. Daily examination of the serum was carried out. After the third dose it turned a light yellow with a faintly positive direct van den Bergh reaction. The serum colour gradually increased to a deeper yellow up to a maximum of 29 mgs. bilirubin per litre.
- (b) One sheep was dosed 304 c.c. carbon tetrachloride over a period of 35 days in doses as described above, the only difference being that the carbon tetrachloride in this case was dissolved in equal volumes of olive oil. The serum in this case also turned a deep yellow with a maximum reading of 44 mgs. bilirubin per litre. The animal died on the 36th day.

Both animals were kept on a ration of hay, green lucern, crushed maize and meat meal. The only clinical symptoms noticeable in these two animals during the experimental period consisted in a progressive loss in condition, frequent drowsiness, a slight and often indefinite jaundice with increasing pallor of the mucous membranes and a gradual drop in the red cell count.

On post-mortem examination, both carcasses showed considerable emaciation and a well marked anaemia, also pulmonary oedema and an extensive fatty degeneration of the liver and kidneys and atrophy of the ruminal wall. There was no sign of icterus in the carcasses.

Manganese Chloride.—Two sheep dosed with manganese chloride in doses of 5 gram daily and gradually increased to 15 grams died on the 12th day after a total amount of 112 grams had been dosed. In both cases the serum turned a pale yellow without however clinical jaundice becoming visible. On post-mortem examination severe liver degeneration was the main lesion noticeable.

DISCUSSION.

In the various experiments outlined above, attempts have been made to elucidate some of the important factors influencing the normal bile excretion in Merino sheep. This work was undertaken primarily with the view of explaining the severe generalised icterus seen in the disease geeldikkop caused by excessive ingestion of wilted *Tribulus* plants. In this disease the icterus is of an exceptionally intense paralytic nature in which the liver appears to lose all power of bile excretion, with the result that the bile is regurgitated into the blood and lymph stream. Except for the bile pigmentation there is, however, little morphological change of the liver parenchyme to indicate the type of damage caused. The effect, therefore, seems to consist very largely of a functional derangement of the liver. In previous articles of this series, it was pointed out that administration of the plant *Lippia* although in no way related to *Tribulus*, provoked a closely similar or identical symptom complex in experimental sheep. Moreover the symptoms following surgical obstruction to the bile flow appeared to be the same. The only difference between the latter condition and that caused either by *Lippia* or *Tribulus* is the marked cavernous like dilatations of the extra and intrahepatic bile tracts above the point of obstruction following ligation of the common bile duct. In *Lippia* and *Tribulus* poisoning it would appear that the liver makes no such attempt at accommodating the bile in its own channels.

In the sheep there is no physiological bilirubinaemia comparable to that found in some other species of domesticated animals. Judging from the clear appearance of the serum and the absence of pigments from it one can conclude that bile elimination is very efficient and that the liver treats the bile pigments as non-threshold bodies. In spite of this efficiency, however, the bile flow can be very severely depressed or totally inhibited in cases of *Tribulosis* or *Lippia* poisoning thus leading to a pronounced degree of icterus. From the results obtained from sheep with a biliary fistula it is seen that the daily bile flow amounts to volumes of over 200 c.c. When, therefore,

elimination is interfered with, the severity of the jaundice can be well understood. The kidneys under these conditions compensate to some degree for the loss of the liver function as shown by the intensely yellow brown urine voided. The compensation however is not complete since the jaundice persists as long as liver action remains disturbed.

Investigations carried out on the effect of cholagogues on the bile flow of these experimental animals show that the dosing of bile definitely causes an increase in the bile flow from the liver. Bile elimination may however proceed at a steady rate in the absence of any bile constituents returning to the liver, i.e. the entero-hepatic bile salt circulation is not essential for the continued excretion of bile in the sheep. Moreover the appetite and digestion of such fistula animals are fairly well maintained although a slow and progressive decrease in the body weight is frequently noticeable, this being more evident in some animals than in others.

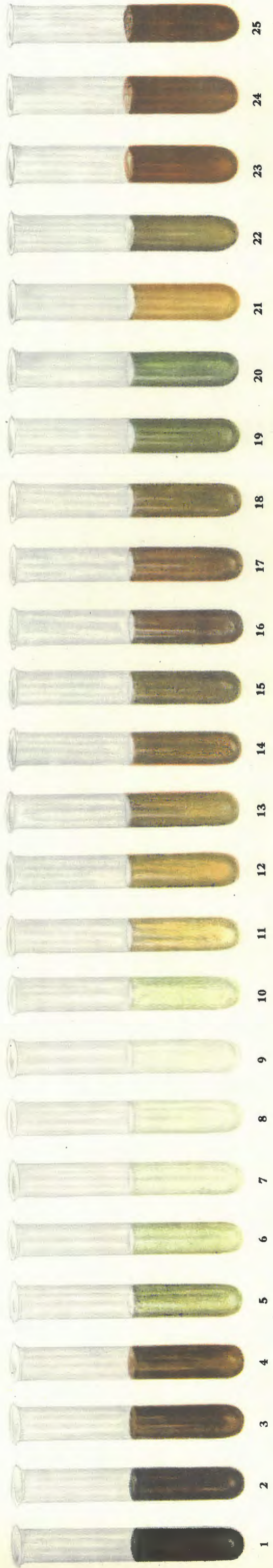
Under the influence of Lippia poisoning, the bile flow of experimental sheep can be very promptly depressed and ultimately even completely inhibited. The main effect of the Lippia toxin on the liver is as yet not clearly understood. It appears to have a paralysing effect on the normal bile excretion for although the liver cells themselves are still capable of allowing the bile to pass through as indicated by the direct van den Bergh reaction, the bile tracts including the smallest bile capillaries seem to be incapable of moving the bile in the normal manner and at the normal rate towards the large bile tracts. This sluggishness in the bile flow results in some of the biliary constituents, e.g. the pigments to escape into the small blood and lymph vessels and so carried back into the general circulation where an extensive and severe jaundice may be caused. The difficulty in explaining the genesis of the icterus arises from the fact that morphological changes in the liver may be slight even in very severe cases of jaundice. This point has also been stressed by other workers. Thus Cantarow and Stewart, studying the morphological changes in the liver and bile passages of cats with total biliary obstruction, state that "these observations seem to indicate that there is no demonstrable correlation in individual instances, between the changes in the liver and bile ducts and the serum bilirubin concentration at any given time during the period of total bile stasis".

The various experiments undertaken with the object of re-establishing the normal bile flow after the onset of Lippia jaundice, have been disappointing in that none of the cholagogues or purgatives used had any definite beneficial effect on the icterus and on the bile flow. Improvement, when it did come about, appeared as a slow process and governed by the powers of the body itself.

Other liver poisons, such as chloroform and phosphorus which were also studied, caused acute and severe fatty changes of the liver and frequently accompanied by bilirubinaemia. In no case however was the same intense and persistent jaundice of "geeldikkop" or Lippia poisoning observed.

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COLOUR CHANGES IN FISTULA BILE OF SHEEP SUFFERING FROM LIPPIC POISONING.

- 1-4.—Different shades of ordinary fistula bile.
- 5.—Bile colour 5 hours after dosing Lippia.
- 6.—Bile 24 hours after Lippia.
- 7.—Bile 25 " "
- 8.—Bile 26 " "
- 9.—Bile 27 " " (colourless, "white bile").
- 10-11.—Bile colour 2 days after Lippia.
- 12-13 " " 3 " "
- 14-15 " " 4 " "
- 16-17 " " 5 " "
- 18-19 " " 6 " "
- 20-21 " " 7 " "
- 22-23 " " 8 " "
- 24-25 " " 9 " "