PROCEEDINGS

OF THE

INDIAN ACADEMY OF SCIENCES

VOL. XVII

SECTION B

BANGALORE CITY
PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
1943

CONTENTS

SECTION B—VOL. XVII

No. 1—January 1943

No. 2—February 1943

No. 3—March 1943

No. 4—April 1943

ON THE BREEDING HABITS AND DEVELOPMENT OF AN INDIAN CARP, *CIRRHINA MRIGALA* (HAMILTON)

By Hamid Khan, M.Sc., LL.B. (Punjab), Ph.D. (Cantab.), F.A.Sc.
Game Warden, Punjab

Received March 22, 1943

A reasonably complete knowledge of life-histories and habits of fishes is needed as a basis for the adoption of adequate measures for conservation of our fisheries and for the production of maximum quantity through proper propagation. It is, however, a matter of regret that the breeding habits and development of Indian Carps specially the larger species, such as *Cirrhina mrigala* (Hamilton), *Labeo rohita* (Hamilton), *Catla catla* (Hamilton), and others are very meagrely known. The author published in 1924, a general account of the breeding habits of *Cyprinidæ* and described the early stages in the development of *Labeo gonius* (Hamilton) (Hamid Khan, 1924, 1925). Jones (1938 *a*, *b*; 1941) studied the development of small-sized Cyprinoid species, namely *Danio malabaricus* (Jerdon) and *Garra ceylonensis ceylonensis* (Bleeker).

Material and Methods

Cirrhina mrigala is one of the commonest carps in India and greatly prized as a food fish. It is widely distributed in the rivers in the Punjab, Bengal, Deccan, Sind and Burma. It grows to about 3 feet in length and attains a weight of 18 pounds or so.

Observations on its breeding habits were carried out at the Departmental Fish Farm, Chhenawan, where the fish spawned on the 25th, 18th and 17th July, in 1928, 1929 and 1933 respectively. On these occasions ripe females were netted from spawning grounds actually in the act of spawning and were stripped. The eggs were obtained in a wash basin and fertilized by mixing them with milt from the male. The excess of milt was washed off after 15 minutes and the fertilized eggs were placed in hatching trays, which were left floating in the tank. The development stages were studied after every five minutes till the completion of segmentation, and then after every half an hour till hatching. After hatching, the study was continued every four hours. Stages were fixed in Bouin's fluid for histological study, while observations on blood circulation were recorded on

live specimens, as in the preserved specimens it becomes impracticable to trace the course of blood. Sketches were drawn with Baker's Drawing Eyepiece or Camera Lucida.

Spawning

Cirrhina mrigala, like most of the carps, breeds in July during monsoon rains. The fish become ripe in May and wait for the first heavy flood to lay their eggs. If the floods are insufficient to inundate their spawning grounds, the fish refuse to spawn and the eggs degenerate within the ovaries. If the floods occur in time the fish leave the main stream, run up the side streams and move on to the inundated fields where water is shallow. Female is often followed by two or three males, and very frequently reverse cases are also seen. On such occasions fish are not shy and can easily be caught with a net or killed with a stick or a stone. In the inundated fields, males and females play together with their bodies pressed and tails slightly bent round each other and splash the water. Many a time it is noticed that their heads are thrust out of water. They remain in this position for a short time with their caudal portion pressed together, and it is at such time that eggs from the female are expelled and immediately fertilized by the male. The eggs are not laid at one place as the fish remain on the move with tremendous splashing and churning of water and go on repeating the process of egg-laying. The actual expulsion of the eggs has not been observed due to turbidity of flood water where the eggs are laid, but the eggs from a female caught in the act of spawning simply flow out of its urino-genital aperture with a slight pressure on its belly. The spawning lasts for a few hours and generally begins during the night or in early hours of the morning and may continue till late in the afternoon. Fertilization is external and the male pours its milt or seminal fluid, which is milky white, non-sticky and non-granular, on the eggs immediately after they are expelled. It has been observed that the milt coagulates within two minutes of its expulsion in water and all the sperms die. Fertilization, in order to be effective, must, therefore, be a speedy process.

Stripping.—The stripping of a ripe female fish either before floods or as soon as it has entered the inundated fields is not practicable as it results in expulsion of hard opaque eggs, which are often mixed with blood and as such cannot be fertilized. But when the fish have indulged in sexual play and are actually spawning, the stripping is easy and the female yields its eggs with a slight pressure. These eggs are soft and translucent and can successfully be fertilized by mixing them with milt from the male. The male, however, yields its milt readily on stripping at all times during the breeding season.

Reproductive powers.—*Cirrhina mrigala*, like all other carps, possesses an extraordinary fecundity. A ripe female fish weighing 2 lb. has been found to contain 1,24,800 eggs in its ovaries, and another one, weighing 3½ lb. had 2,16,800 eggs (Hamid Khan, 1924).

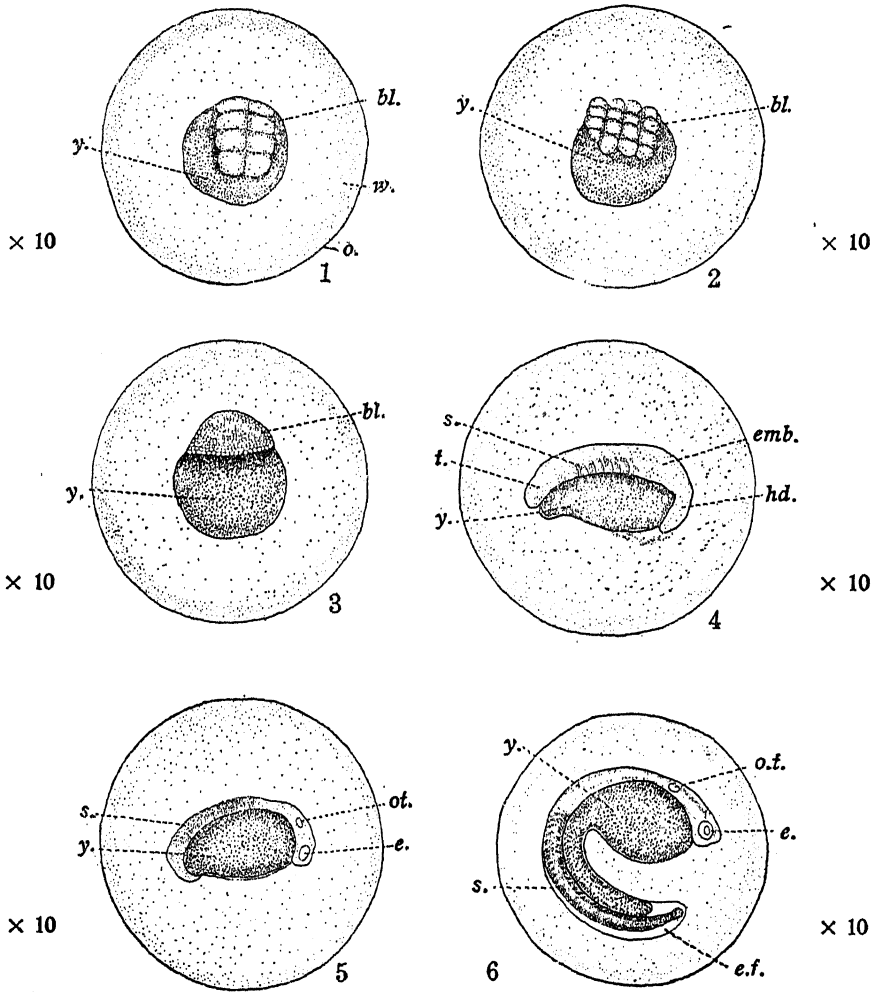
Development

Egg.—When fully ripe, the eggs are spherical in form, almost transparent, yellowish white in colour, non-adhesive, and non-floating. The yolk sphere contains no oil globule. Each measures 1·5 mm. in diameter and is heavily yolked. As soon as it falls into water it begins to swell and within 15 minutes attains a size of 3 to 4 mm. in diameter.

Segmentation.—As soon as fertilization takes place the protoplasm becomes concentrated at one pole of the yolk sphere to form blastodisc. Five minutes after fertilization the first act of cleavage occurs dividing the blastodisc into two cells. Segmentation is partial or discoidal and typically teleostean. After ten minutes four cells are seen, fifteen minute stage has eight cells. Eight blastomeres formed by the third cleavage lie in two symmetrical series of four cells each (Fig. 1). Sixteen blastomeres are seen after 40 minutes (Fig. 2). Blastoderm in advanced stage of cleavage is observed within two hours after fertilization (Fig. 3). During early stages of segmentation blastoderm is a lens-shaped mass of cells and as segmentation advances it becomes dome-shaped and spreads over the yolk. The peripheral margin of the blastoderm is thickened to form the germ ring. As blastoderm grows larger, the germ ring increases in width and advances around the yolk sphere. In three and half hours' stage the blastoderm has covered nearly half of the yolk sphere, and in four and half hours stage, it has spread almost over the whole of the yolk, with a yolk plug, forming the blastopore at one end. Rudiments of embryo appear in seven hours' stage in the form of a belt lying over the yolk sac, which has become elliptical in shape with well differentiated head and tail ends (Fig. 4, *emb.*). By the time the embryo is differentiated the blastoderm has covered the whole of the yolk sphere and the blastopore has closed.

Embryonic development.—Nine hours after fertilization the embryo becomes well defined with seven to eight somites. Segmentation of body advances rapidly and an hour later twelve somites are visible (Fig. 5, *s.*). Eyes (*e.*) appear as transparent objects on the head end. Some of the embryos of ten hours stage have sixteen to seventeen somites with well-developed head, eyes and possess a pair of otocysts with two otoliths (*ot.*) in each, and some of the embryos show movement in the egg shell.

In eleven to twelve hours' stage, there are twenty seven to thirty somites with definite movement of the tail. Yolk sac is prolonged posteriorly up to the tail end. In twelve to fourteen hours stage (Fig. 6), tail is much elongated and projects beyond the yolk sac, and its movements are quick. There are thirty-two somites (*s.*). Eyes (*e.*) are without pigment. Notochord is cellular. Embryonic fin-fold is visible (*e.f.*). In fifteen to sixteen hours'



TEXT-FIGS. 1–6. Development stages of *Cirrhina mrigala*.—Fig. 1. Eight-celled stage, fifteen minutes after fertilization. Fig. 2. Sixteen-celled stage, forty minutes after fertilization. Fig. 3. Two hours' stage. Fig. 4. Seven hours' stage with seven somites. Fig. 5. Ten hours' stage with 15 somites. Fig. 6. Fourteen hours' stage with 32 somites. *bl.*, blastoderm; *e.*, eye; *e.f.*, embryonic finfold; *emb.*, embryo; *hd.*, head; *o.*, outer egg membrane; *o.t.*, otocyst; *s.*, somite; *t.*, tail end of embryo; *w.*, space filled with water; *y.*, yolk.

stage heart has appeared as a simple tabular structure in all the embryos. Auditory and optic vesicles are visible.

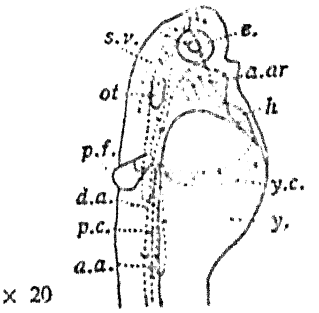
Hatching started at sixteen hours and continued up to nineteen hours. The hatching of a egg lying in a petri-dish was observed under the binocular. The tail ruptured the egg shell and the embryo wriggled out, tail first and came to the surface of water by swirling movement.

Newly hatched embryo (Fig. 7).—The length of newly hatched embryo ranges from 3·8 to 4 mm. The embryo is colourless without any pigment and almost transparent. It moves with a side to side or rotatory movement. Eyes (*e.*) are round and without pigment. Auditory and optic vesicles are visible. Otocysts (*o.t.*), with two otoliths in each, are present. Notochord is cellular. Heart (*h.*) pulsates with dorsal aorta (*d.a.*) which turns back at the caudal end to form the caudal vessel (*c.v.*) which in its turn runs forward to enter the yolk mass and breaks up into capillaries (*y.c.*). The yolk capillaries join together and enter the heart. Tail has become elongated. Yolk sac (*y.*) is prolonged posteriorly and the future anal aperture is marked by a slight depression near the posterior end of the yolk sac. The mouth is not open. Gills have not yet appeared. Embryonic fin-fold (*e.f.*) is present but fins are not yet formed.

Post-embryonic development.—Seven hours after the hatching, three aortic arches are visible and two hours later, the fourth makes its appearance (Fig. 8, *a.ar.*). Two of these arches send blood to the eye and the brain and two to the dorsal aorta. The blood vessel to the eye turns back over the otocyst, forms a loop and then enters the yolk sac. The caudal vessel (*c.v.*), breaks up into capillaries just behind the posterior end of the yolk sac and is continued anteriorly as a single vessel, which enters the yolk sac to form the yolk capillaries (*y.c.*). Segmental vessels have also appeared in the anterior part of the embryo. Heart pulsates at the rate of 140 beats per second and is tabular.

Eleven hours after hatching, pectoral fins appear as bud-like outgrowths. Five aortic arches and three to four branchial arches are also visible. Four hours later (Fig. 9), pectoral fins are prominently marked (*p.f.*), and the branchial region together with mandibular arch shows movement though mouth is not yet open. There are nine to eleven segmental vessels in the head region up to the otocyst, and two posterior to it (Fig. 9, *s.v.*). Segmental vessels are present in the caudal region as well. A vessel (Fig. 9, *a.a.*) branches from the dorsal aorta (*d.a.*), runs posteriorly, enters the yolk, and then runs anteriorly to join the vitelline vessels. There are five aortic arches (*a.ar.*), two anterior ones send the blood to the head and

the eye, the three posterior ones to the dorsal aorta. Blood circulation in the eye is complicated. Two blood vessels take the blood from the head, flow into the yolk and then pass on to the heart along with the yolk capillaries (Fig. 9, *y.c.*).

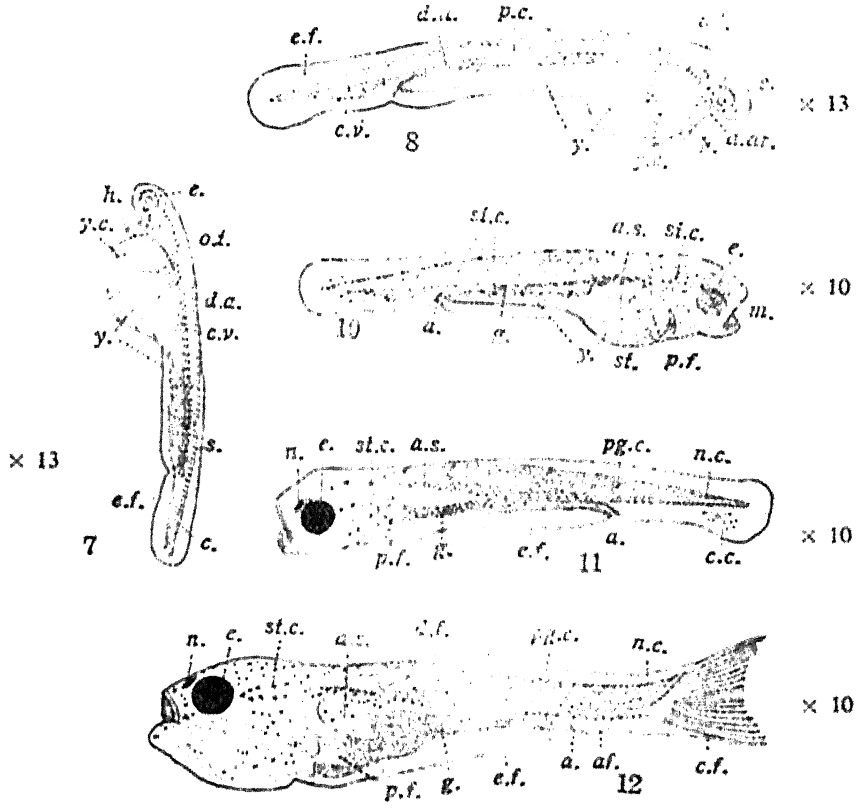


TEXT-FIG. 9. Embryo of *Cirrhina mrigala*, fifteen hours after hatching, showing circulation in the head. *a.a.*, blood vessel branching from dorsal aorta; *a.ar.*, aortic arches; *d.a.*, dorsal aorta; *e.*, eye; *h.*, heart; *ot.*, otocyst; *p.c.*, posterior cardinal vein; *p.f.*, pectoral fin; *s.v.*, segmental vessels; *y.*, yolk; *y.c.*, yolk capillaries.

By the end of the first day, *i.e.*, 24 hours after hatching, the pectoral fins are well developed. The embryo has increased in length, measures 5·6 mm. and shows typical teleostean circulation. Six aortic arches on each side function with full strength. Gills have appeared as lobular outgrowths attached to branchial arches and blood vessels pass through them. The blood in the first two aortic arches go direct to the head while in the last four arches it is pumped into the gills, and then to the dorsal aorta. The caudal lacunar circulation behind the future anus is now confined to two vessels with anastomosing branches. Operculum has just appeared but does not as yet extend over the gills. Depressions for the mouth and anus are well marked. Yolk sac is now reduced. There are four to five branchial arches. Air sac has appeared as a space over the yolk. Eyes are slightly pigmented.

Second day.—Embryo measures 5·8 mm. in length (Fig. 10). Mouth (*m.*) is open for respiration. Yolk sac (*y.*) is reduced and has, in addition to yolk capillaries, one vitelline vein at its dorsal region. Eyes (*e.*) have become pigmented, purple with yellow border dorsally. Yellow pigment over the head renders it opaque. Black stellate cells (*st.c.*) have appeared on the ventral and lateral surface of the notochord extending from posterior end of the head to the caudal portion. Air sac (*a.s.*) has extended posteriorly. Stomach (*st.*) is visible under the air sac in the yolk and has a lumen in it, but no lumen is seen in the intestine, which is a prolongation of the stomach and extends posteriorly to the future anus (*a.*). In the yolk there are only

small blood vessels taking blood to the heart. Caudal circulation has extended to the posterior end of the embryo. By the end of the second day,



TEXT-FIGS. 7, 8, 10, 11 and 12. Development of *Cirrhina mrigala*.—Fig. 7. Newly hatched out embryo. Fig. 8. Nine hours stage after hatching. Fig. 10. Second day embryo, 31 hours after hatching. Fig. 11. Four days' old embryo. Fig. 12. Twelve days' old embryo. *a.*, anus; *a.ar.*, aortic arches; *a.s.*, air sac; *c.c.*, caudal capillaries; *c.v.*, caudal vein; *d.a.*, dorsal aorta; *e.*, eye; *e.f.*, embryonic fin; *g.*, gut; *h.*, heart; *m.*, mouth; *n.*, nostril; *n.c.*, notochord; *o.l.*, otocyst; *p.c.*, posterior cardinal vein; *p.f.*, pectoral fin; *pg.c.* pigment cells; *s.*, somite; *st.c.*, stellate cells; *y.*, yolk; *y.c.*, yolk capillaries.

the gills are covered by the operculum. Air sac is filled with gas. Yolk sac is considerably reduced. Gullet is not yet open but lumen is appearing in the intestine. There are no anastomosing capillaries behind the anus. In some specimens two caudal capillaries are seen forming a loop ventral to notochord at the caudal region (Fig. 13, *c.c.*).

Third day.—Length is 6·3 mm. Mouth is open, gut is formed and in some specimens it has food in it, and opens to the exterior. Yolk sac is considerably reduced and lies ventral to the gut. Eyes are brilliantly reddish yellow and purple in colour with golden yellow border round the

pupil. There are two caudal capillary loops and stellate cells have appeared over them. By the end of the day stomach has enlarged. Outlines of vertebræ are visible. Pectoral fin has enlarged. Stellate and round pigment cells have appeared on the head and body. There are four caudal capillaries with radially arranged stellate cells. The fry are very active.

Fourth day.—(Fig. 11). Length of the fry has not increased. Yolk sac is completely absorbed. Caudal fin rays have appeared (Fig. 14, *c.r.*), and by the end of the day, six to seven rays are visible and the caudal capillary circulation has increased.

Fifth day.—Length 6·65 mm. Large stellate cells (Fig. 15, *st.c.*) are arranged laterally on the body along the ventral edge of notochord and dorsal border of body segments. There are six to seven caudal rays and four distinct and two indistinct basal cartilages (Fig. 15, *c.r.*, *b.c.*). Notochord is slightly bent at its posterior extremity. By the end of the day some of the specimens have twelve distinct caudal rays and six basal cartilages.

Sixth day.—Length has not increased. Notochord is well bent upwards and there are fourteen to sixteen caudal rays with six basal cartilages. Internally, liver is visible as a red spot near the air sac. Gut is full of food. Heart consists of three pulsating chambers and receives anterior and posterior vena cavæ, and one blood vessel from the stomach which breaks into capillaries in the liver region and then enters the heart.

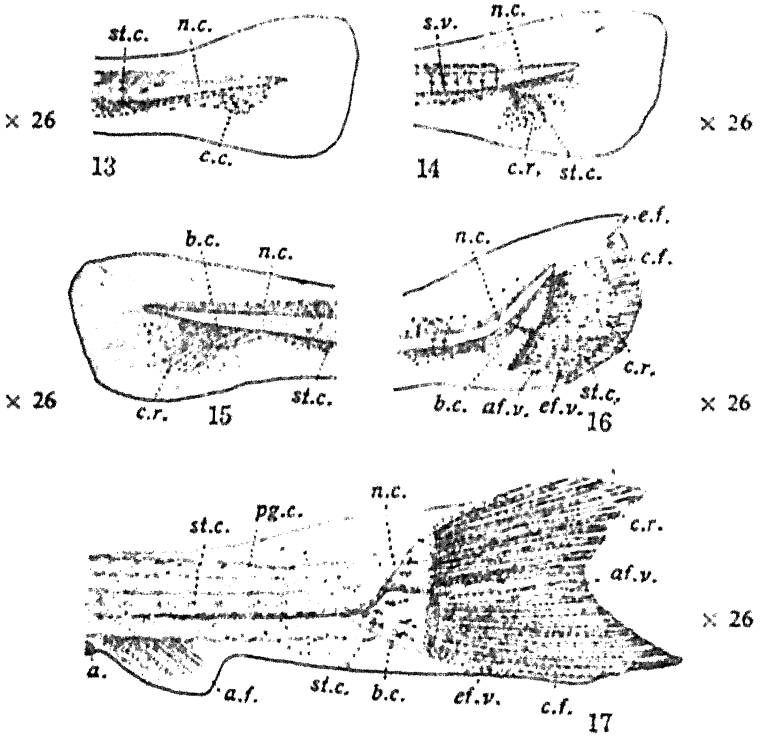
Seventh day.—Owing to curvature of body length has not increased. Caudal rays number seventeen to twenty. In specimens with seventeen to eighteen caudal rays, dorsal fin is appearing as an elevated ridge dorsally with one capillary vessel. In specimens with nineteen to twenty caudal rays dorsal fin has four fin rays and many capillaries.

Eighth day.—Length 7 mm. Structure practically the same as on the seventh day. Caudal fin has ten rays with three basal cartilages in its dorsal half and nine caudal rays and three basal cartilages on its ventral half. There are round pigment cells scattered over the body and stellate cells around the caudal rays and the notochord. Fry reared in small and large live cars showed considerable difference in their growth. Those reared in large live car, measuring 4′ × 3′ × 3′, had eighteen to twenty caudal rays, while those in small live car, measuring 2′ × 1′ × 1′, were under-developed and had six to ten caudal rays.

In 1928 fry were attacked by a protozoan ectoparasite belonging to Cychlochætæ, which infested the embryonic fin-fold, adhered to the gills

and the fry died in large number. Salt bath of 1% strength for 10 minutes cured many of the fry in early stage of the attack. On the 9th and 10th day, structure was almost the same as on the 8th day.

Eleventh day.—Length from 7 to 7·3 mm. Stellate cells round the caudal rays have become radially arranged (Fig. 16, *st.c.*). Caudal rays are 19 to 20 in number and are jointed. Notochord is bent dorsally. Dorsal fin has 4 to 6 rays. In the kacha pond, where weed had been planted to serve as food and also for protection, the fry showed extraordinarily rapid growth. These fry on the eleventh day measured 8 to 9·5 mm. in length and had fully developed dorsal fin with fifteen rays and well-formed caudal, and pectoral fins. It indicates the difference in rearing fry in confinement and under natural conditions.



TEXT-FIGS. 13–17. . Development of caudal fin of *Cirrhina mrigala*.—Fig. 13. Caudal fin of second day embryo. Fig. 14. Caudal fin of four days' old embryo. Fig. 15. Caudal fin of five days' old embryo. Fig. 16. Caudal fin of eleven days' old embryo. Fig. 17. Caudal fin of seventeen days' old embryo. *a.*, anus; *a.f.*, anal fin; *af.v.*, afferent vessel; *b.c.*, basal cartilage; *c.c.*, caudal capillaries; *c.f.*, caudal fin; *c.r.*, caudal fin ray; *d.f.*, dorsal fin; *e.f.*, embryonic fin; *ef.v.*, efferent vessel; *n.c.*, notochord; *pg.c.*, pigment cell; *st.*, stellate cells; *s.v.*, segmental vessels.

Twelfth day.—Length 8·05 mm. (Fig. 12). Caudal fin (*c.f.*) is forked. Anal fin (*a.f.*) has two capillaries and appears as a ridge of the embryonic fin-fold just behind the anus. Pelvic fins are also marked off. Dorsal fin (*d.f.*) has six to seven rays with a set of capillaries.

Fourteenth day.—There are fifteen jointed and five or six unbranched caudal fin rays. Dorsal fin has 9 to 11, and anal 2 to 3 rays.

During the third week, the fry measured 9 to 10·5 mm. in length. On the 17th day caudal fin is seen separating off from the embryonic fold with the development of the anal fin (Fig. 17, *a.f.*, *c.f.*) and is fully separated off from the embryonic fold when the anal fin is completely developed, and is forked. It has 15 three-jointed rays and 5 to 6 short unbranched ones. Dorsal fin has 13 to 15 rays and is distinctly separated off from the embryonic fin-fold. Anal has 4 to 6 rays. Pelvic fins are underdeveloped. On the 21st day embryonic fin-fold is present ventrally but anal is separated from caudal. Round black pigment cells are present over the body and head.

Scales appeared on the 24th day after hatching and the fry possessed all the characters of the adult. A month old fry of *Cirrhina mrigala*, netted from a pond, measured 45 mm. with fully developed fins, while those reared in boxes or live cars measured 20 to 25 mm. in length during the same period.

Discussion

The development of *Cirrhina mrigala* illustrates some interesting features which require further consideration:—

(1) *Swelling up of egg.*—It is a remarkable phenomenon, and has previously been recorded by the author (1924, p. 959 and 1925, Fig. 7, Plate I) in the case of other cyprinidæ and figured in the case of *Labeo gonius*. It has also been described by Jones (1938, *b*) in *Garra ceylonensis ceylonensis*. As the egg swells a space is created between the outer membrane and the developing embryo. This space is filled with water and gives the egg a glassy bead-like appearance. The water cushion between the developing embryo and the egg membrane apparently protects the egg from external shock which may otherwise injure the embryo, especially when the eggs, which are laid loose, are likely to be carried down the streams by heavy floods.

(2) *Rapidity of embryonic development.*—Like most of the other Indian Cyprinoids, whose life-history is known (Hamid Khan, 1924, 1925 and Jones, 1938 *a* and *b*), hatching in *Cirrhina mrigala* takes 16 to 20 hours. The development, evidently, is quickened by the warmth of the sun falling

on the eggs lying in shallow water and, unlike the Western species, which hatch out in 12 to 16 days, the Indian carps take 16 to 20 hours to hatch. Newly hatched embryo in *Cirrhina mrigala* is underdeveloped and resembles that of *Labeo gonius* (Hamid Khan, 1925) and of *Danio malabaricus* (Jones, 1938, *a*), but differs considerably from that of *Garra* (Jones, 1938, *b*). It has neither a mouth, nor gills, nor gill clefts.

(3) *Development of caudal fin.*—Formation of caudal fin rays is preceded by the appearance of caudal capillaries lying ventral to the notochord in the embryonic fin-fold. On the second day after hatching, the caudal efferent vessel gives a branch which forms a loop and then flows back into caudal vein. Soon after, two loops are formed (Fig. 13, *c.c.*). Fourth day marks the appearance of fin rays between the capillary vessels (Fig. 14, *c.r.*). There are six to seven rays with four distinct and two indistinct basal cartilages on the fifth day (Fig. 15), and the number of rays increases daily with fresh formation of caudal capillaries. On the ninth day there are fifteen jointed and three to four unjointed caudal rays with six basal cartilages. As notochord curves dorsally the rays are drawn towards dorsal surface and heterocercal condition of the fin becomes apparent (Fig. 16, *c.f.*). The embryonic fin forms a dorsal lobe and does not contain any rays (Fig. 16, *e.f.*), while the ventral lobe contains fin rays and forms the permanent caudal fin. A similar condition has been described by Agassiz (1878) in the case of Flounders and other bony fishes. The tail fin thus seems to resemble the usual Elasmobranch form or still more that of some Ganoids, *e.g.*, the Sturgeon (Balfour, 1885). With the development of fresh fin rays the dorsal lobe of embryonic fin finally disappears. Caudal fin rays on the dorsal and ventral border of the fin grow rapidly than those in the middle of the fin and thus give to the fin its forked and externally homocercal appearance (Fig. 17, *c.f.*). On the 17th day, with the increased growth of anal fin, caudal fin is seen separating off from the embryonic fin-fold (Fig. 17, *a.f.* and *e.f.*).

(4) *Disparity in rate of growth.* Fry reared in confinement and those reared under natural condition in a pond show remarkable difference in their growth. On the 10th and 11th day the fry bred in confinement were still underdeveloped, with 4 to 5 unjointed caudal rays, while those reared in a shallow pond had fully developed dorsal fin with 15 rays, and well formed caudal, and pectoral fins. A month old fry reared in ponds under natural environments measures 45 mm. in length with fully developed fins, while those in confinement during the same period measured 20 to 25 mm. in length.

Rearing and feeding of fry is a delicate problem. If proper care is not taken, the fry are often attacked by external parasites. Feeding on artificial diet, such as wheat and gram flour, produced unhealthy conditions, while feeding on natural diet of rotifers and young crustaceens produced healthy fry.

Conclusion

The development of *Cirrhina mrigala* illustrates the helpless nature of eggs and fry and stresses the need to devise means to protect them from many dangers to which they are exposed. The eggs are laid during floods which may carry them to places where their fate becomes uncertain. The male sheds its milt in water and there are very great chances of some eggs being left unfertilized. The eggs lie submerged on the grass or sink to the bottom and there is every likelihood of the spawning fields drying up before they are hatched. If, however, the eggs escape all these misfortunes and hatch out in time and run into a pond or stream, there they are likely to fall an easy victim to their enemies, namely, the predaceous fish, frogs and birds. Establishment of nurseries and hatcheries near the spawning grounds could only ensure the development of eggs and fry and lessen the chances of their loss and destruction.

Summary

1. *Cirrhina mrigala* breeds in July when streams are flooded by monsoon rains. If the floods are untimely or insufficient to inundate the spawning fields, the fish do not spawn and become egg bound.

2. The egg measures 1·5 mm. but swells to 3 to 4 mm. when it falls in water. Embryonic development is rapid. The eggs hatch out from 16 to 19 hours. Heart appears just an hour or so before hatching. Newly hatched out embryo measures 3·8 to 4 mm. with a yolk sac, which is prolonged posteriorly. It possesses a pair of otocysts with two otoliths in each, auditory and optic vesicles, a pair of eyes without any pigment and one dorsal vessel, which turns back at its posterior extremity to form the caudal vein.

3. Pectoral fins appear on the first day. Mouth opens on the second day for respiration. Gut is formed on the third day and opens to the exterior. Yolk sac is completely absorbed on the fourth day.

4. Formation of the caudal rays is preceded by the appearance of caudal capillaries on the second day after hatching. The capillaries increase in number and it is on the fourth day that the rays appear. Dorsal fin rays appear on the seventh day, and anal and pelvic on the 12th day

after hatching. Scales appear on the 24th day when the fry have fully developed fins, and possess all the characters of the adult.

5. A month old fry reared in ponds measures 45 mm. in length, while those reared in confinement measure 20 to 25 mm. in length.

REFERENCES

Agassiz, A. .. " On the young stages of some Osseous Fishes. 1. Development of the tail," *Proc. Amer. Acad. Arts and Sci.*, 1877, **13**, 117–25.

Balfour, F. M. .. " A Treatise on Comparative Embryology," 1885, **2**, 79.

Hamid Khan .. " Observations on the breeding habits of some fresh-water fishes in the Punjab," *Bomb. Nat. Hist. Soc. Journ.*, 1924, **29**, 958-62.

——— .. " Early stages in the development of some fresh-water fishes in the Punjab," *Bomb. Nat. Hist. Soc. Journ.*, 1925, **30**, 532-40.

Jones, S. .. " On the breeding habits and development of a Cyprinid, *Danio* (*Danio*) *malabaricus* (Jerdon) in Ceylon," *Ceylon Journ. Sci.* (C), 1938, *a*, *b*, 79-89.

——— .. " External features in the embryonic development of a Ceylon Mountain Carp, *Garra ceylonensis ceylonensis* (Bleeker)," *Ceylon Journ. Sci.* (C), 1938, *b*, **6**, 91-98.

——— .. " Larval development of *Garra ceylonensis ceylonensis* (Bleeker)," *Rec. Ind. Mus. Cal.*, 1941, **43** II, 117-120.

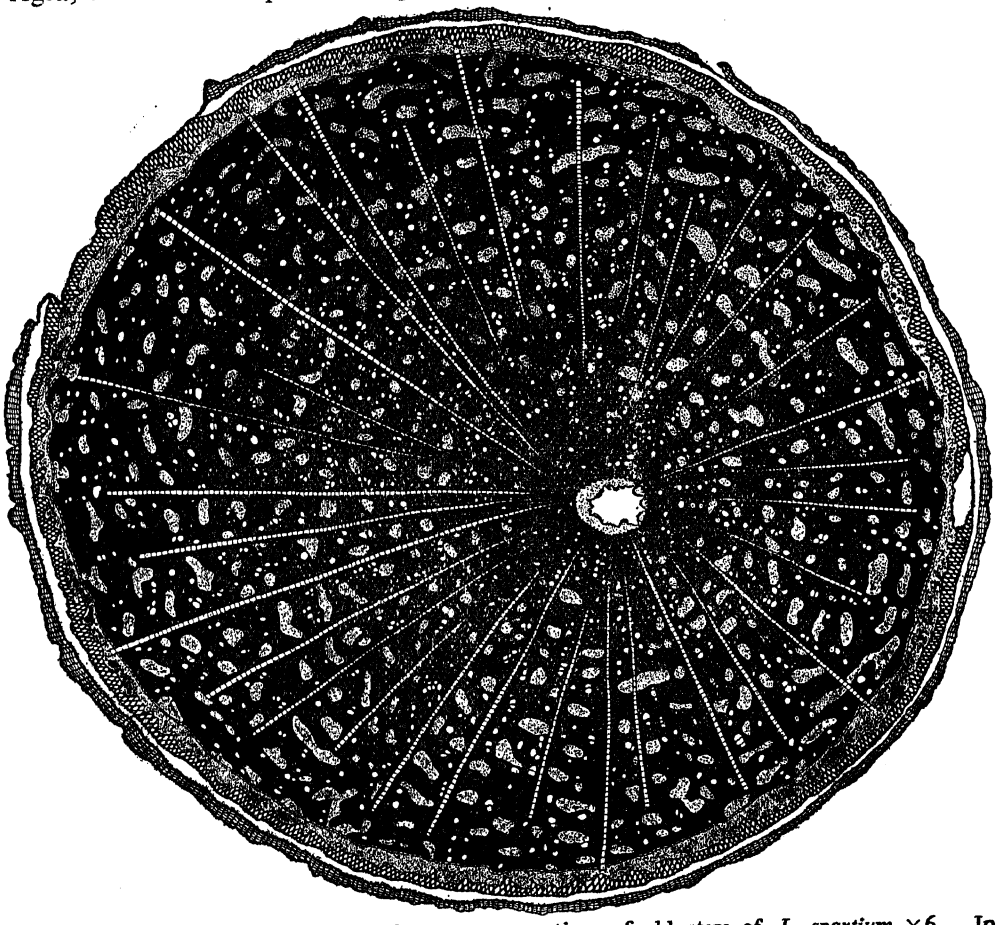
THE ORIGIN AND DISTRIBUTION OF INTER- AND INTRAXYLARY PHLOEM IN *LEPTADENIA*

BY BALWANT SINGH
(Department of Biology, Dacca University)

Received April 15, 1943
(Communicated by Dr. P. Maheshwari, D.SC., F.A.SC., F.N.A.SC., F.N.I.)

1. Introduction

ALTHOUGH intraxylary phloem is known to occur in a number of dicotyledonous families, the presence of interxylary phloem is comparatively rare. An examination of the stem of *Leptadenia spartium* Wight, collected from Agra, showed three phloem regions (Text-Fig. 1):—



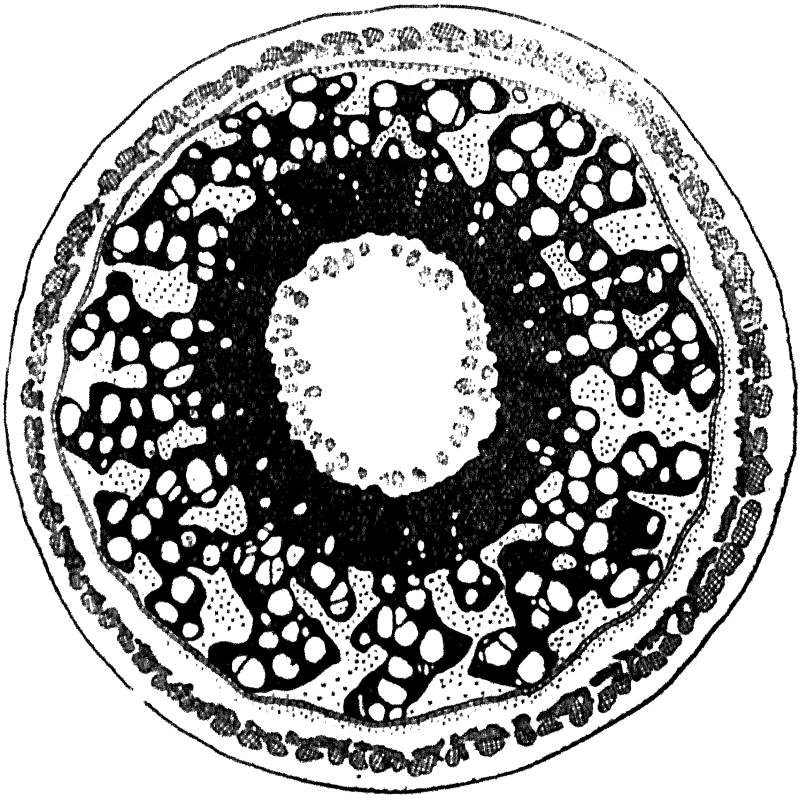
TEXT-FIG. 1. Outline drawing of transverse section of old stem of *L. spartium* ×6. In this and the next drawing the xylem parenchyma is represented by solid black, xylem vessels by empty spaces, cambium by a single line of cells, and the phloem islands by dotted portions within the wood.

(i) Outer normal phloem,

(ii) Intraxylary phloem adjacent to the pith, and

(iii) " Islands " of phloem scattered here and there in the secondary xylem.

Some material of *Leptadenia reticulata* W. and A., received from Prof. M. O. P. Iyengar of Madras, Prof. M. Sayeedud-Din of the Osmania University, and Mr. J. Venkateswaralu* of Coconada, was also examined and found to show the same abnormality (Text-Fig. 2), although other differences



TEXT-FIG. 2. Outline drawing of transverse section of stem of *L. reticulata* ×14.

were of course noticeable due to the shrubby habit of *L. spartium* and the climbing habit of *L. reticulata*. A reference to the available literature showed that no detailed account of the anatomy of *Leptadenia* is so far available. Of the previous workers only Maheshwari (1935) has called attention to the

* To all these gentlemen I offer my warmest thanks.

presence of the interxylary phloem; Sabnis (1921), Blatter, McCann, and Sabnis (1929), and Sayeedud-Din and Suxena (1940) make no mention of it. The only other genera of the Asclepiadaceæ in which interxylary phloem has been recorded are *Asclepias* L., *Morrenia* Lindl., and *Ceropegia* L. (Pfeiffer, 1926).

2. *Intraxylary Phloem*

In both the species the intraxylary phloem arises from the peripheral cells of the pith. New phloem cells continue to arise successively towards the outside even from the xylem parenchyma cells adjacent to the protoxylem elements, as in tobacco (Esau, 1938). The protoxylem elements are often crushed in the process and at places, in the thickest stems of *L. spartium* and *L. reticulata*, they could not be made out for that reason. In both the species there is also differentiated a cambial layer outside the internal phloem groups. This does not produce any secondary xylem, but the secondary phloem cut off by it towards the inside crushes some of the first formed phloem cells.

3. *Interxylary Phloem*

The phloem "islands" are not arranged in a regular order in either of the two species. They are smaller and fewer in the inner and older portions of the wood, but considerably larger and more numerous towards the periphery. In *L. spartium* (Plate I, Fig. 1) the islands are usually tangentially elongated but in *L. reticulata* (Plate I, Fig. 2) they have no definite shape. As many as 412 such islands were counted in a t.s. of the stem of *L. spartium* approximately 1·5 cm. in diameter. In *L. reticulata*, the number is appreciably smaller, for only 183 islands were seen in the cross-section of a stem 1·6 cm. thick.

A detailed study of the origin of the interxylary phloem revealed that at places the outer smooth boundary of the secondary xylem becomes indented (Plate I, Fig. 2) and the depressions are filled with bays of thin-walled tissue. This is because at these points the cambium begins to cut off, on the inside, groups of parenchymatous cells instead of secondary xylem. This is however only a temporary phase and it soon resumes its normal activity producing the usual secondary xylem elements (Plate I, Fig. 1). This process is repeated several times giving rise to a number of "islands" of thin-walled tissue embedded in the thick-walled cells of the wood. Some of the cells composing the islands, specially those in the centre of each island, are differentiated into sieve tubes and companion cells and the rest form phloem parenchyma.

As the islands become older and more deep-seated some of their central cells get crushed and degenerated. The obliterated tissue in *L. spartium* usually extends tangentially (Plate II, Fig. 3) or sometimes radially corresponding to the longer diameter of the island. In *L. reticulata* also the direction

of the crushed tissue varies with the shape of islands. Those cells of the medullary rays which happen to pass through the interxylary phloem groups, usually remain intact, however, even though the phloem cells on either side of them may be in an advanced state of degeneration. One reason for this peculiar crushing of the central phloem cells is that, in the outer parenchyma cells of the older islands, divisions accompanied by subsequent differentiation and enlargement of additional sieve tubes and companion cells continue to take place for a fairly long time. As the islands are surrounded by thick-walled xylem parenchyma, the expanding phloem elements cannot spread outwards and the whole force is therefore directed centripetally resulting in a crushing of the central cells.

A weak cambium-like layer† has occasionally been observed around some of the old phloem islands in the stems of *L. spartium* (Plate II, Fig. 3) as well as *L. reticulata* (Plate II, Fig. 4). It may begin to differentiate on any side or more than one side of the island. If it develops first on the inner side, the islands may partially resemble those of *Strychnos* although they originate quite differently in this case. As found by Hérail (1885) and subsequently confirmed by Scott and Brebner (1889), the phloem islands are here produced centrifugally by portions of the normal cambium which afterwards become slackened in activity. At the outer borders of these groups, however, new cambial segments arise from the pericycle or phloem parenchyma. These "complementary segments" bridge over the groups and join on to the main cambium, thus completing the general ring. Like the rest of the cambium they now cut off secondary xylem towards the inner and secondary phloem towards the outer sides. As a result, the groups of phloem lying below those segments get embedded in the xylem. The two processes continue to alternate so that an old stem shows quite a large number of phloem islands, each having a centripetally embedded cambium.

A. Comparison with the Phloem Islands of Strychnos

It is worthy of note that in a young stem of *Leptadenia* the phloem cells constituting the islands lie in the same radial rows as the cells of the wood both centripetally as well as centrifugally (Plate I, Figs. 1 & 2). In *Strychnos nux-vomica*, on the other hand, Scott and Brebner (1889) found that while the radial rows could readily be traced from the interxylary phloem into the wood on the inner side, no such continuity existed in the opposite direction. This difference is clearly due to the different modes of origin of the interxylary

† A secondary cambium has also been reported around the groups of phloem cells in the roots of certain Cruciferæ (Weiss, 1883); *Salvadora*, and some Cucurbitaceæ (Scott and Brebner, 1889); and *Ipomœa batatas* (Artschwager, 1924).

phloem in the two genera. In *Leptadenia* the islands as well as the xylem cells on its outer and inner sides are produced from the same cambium. In *Strychnos*, on the other hand, the island and the inner wood are products of the same cambium, but the cells of the outer wood are formed from the new complementary cambial segments, and do not therefore lie in the same radial rows as the cells of the interxylary phloem towards the inside.

Another difference is seen in the islands themselves. In *Strychnos*, in the older islands, the crushed phloem forms a sort of a cap on its outer side due to the activity of the embedded cambial segment which was once a part of the original cambium ring. This continues to add some new cells towards the outside causing pressure upon the older cells. In *Leptadenia*, on the contrary, the crushed phloem occupies the central place in the island, for reasons which have already been explained above.

5. Summary

1. In *Leptadenia spartium* and *L. reticulata* there are three phloem regions: (*a*) the outer normal phloem, (*b*) the intraxylary or inner phloem, and (*c*) the interxylary phloem which forms inclusions in the wood.

2. The patches of intraxylary phloem arise from the pith cells, but in later stages even the xylem parenchyma cells adjacent to the pith take part in their formation. In old stems a cambium is differentiated on the outer faces of these phloem groups and produces some secondary phloem centripetally.

3. The interxylary phloem, present in the stem, becomes differentiated from groups of thin-walled cells produced centripetally by the cambium. Later the cambium resumes its normal activity with the result that the phloem groups become embedded in the secondary xylem.

4. Owing to an enlargement of the cells in the island and the fact that it is surrounded on all sides by the woody cells of the xylem, there is often a compression and crushing of the phloem tissues in its centre.

5. A weak secondary cambium has occasionally been observed to differentiate on one or more than one side of some of the older phloem islands.

6. A comparison with *Strychnos nux-vomica* shows that in the latter the islands are always produced centrifugally from the cambium and later become embedded due to the formation of a complementary cambial segment on the outer side, whereas in *Leptadenia* it is the same cambium which produces both the secondary xylem as well as the phloem on its inner side.

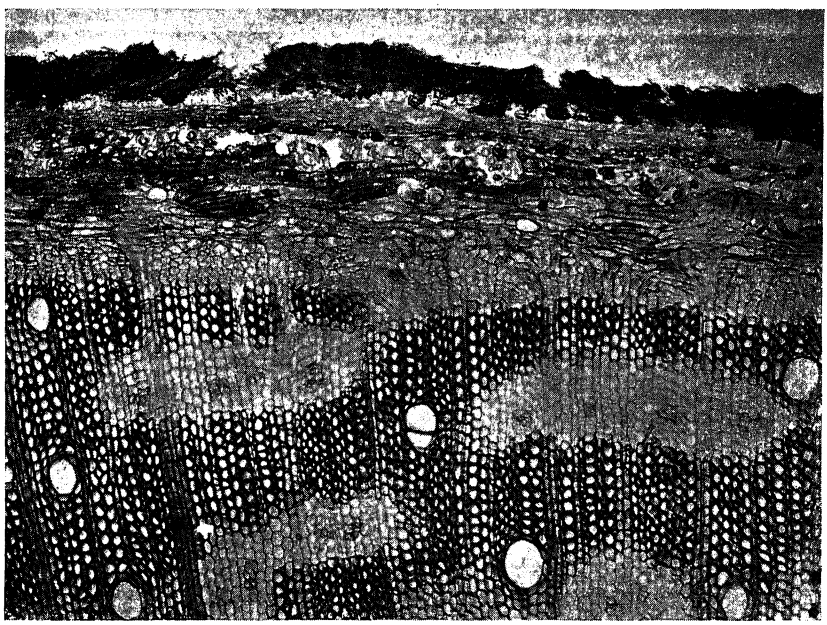


FIG. 1

L. spartium—outer portion of an old stem showing some newly formed islands of phloem. Note their tangential elongation. ×65.

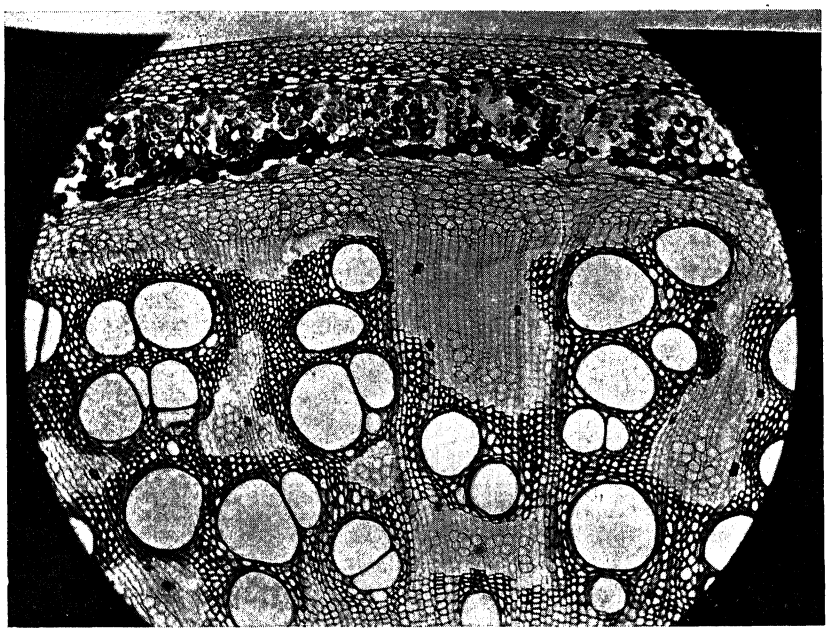


FIG. 2

Same in *L. reticulata*. Note the irregular shape of the islands. ×47.

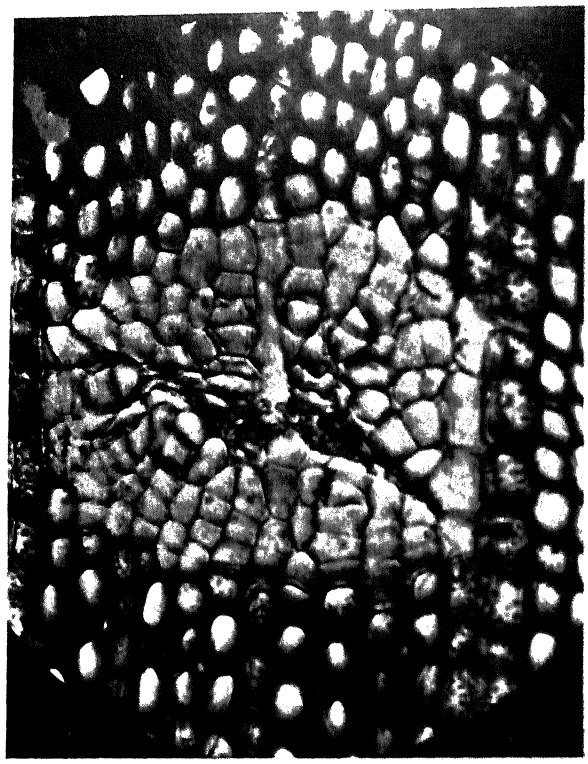


FIG. 3

L. spartium—An advanced stage showing the tangential crushing of the central cells of the island. × 290.

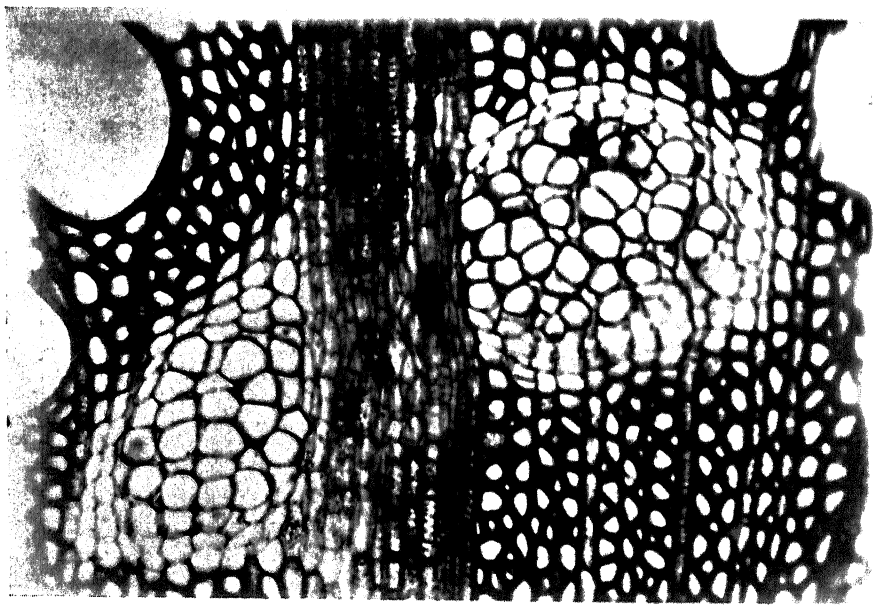


FIG. 4

Phloem islands in *L. reticulata*; note the cambium-like cells on the inner as well as outer sides of the island on the right. × 175.

6. Acknowledgements

I express my heartfelt thanks to my teacher, Dr. P. Maheshwari, for the guidance and help which he has so kindly rendered from time to time. I am also indebted to my friend, Mr. Sukumar Sen, for helping me in taking the micro-photographs, and to Prof. B. Sahni for his kindness in going through the manuscript.

LITERATURE CITED

1. Artschwager, E. .. "On the anatomy of the sweet potato root with notes on internal breakdown," *Jour. Agri. Res.*, 1924, **27**, 157–166.
2. Blatter, E., McCann, C., and Sabnis, T. S. "The flora of the Indus Delta," *Jour. Ind. Bot. Soc.*, 1929, **8**, 19–61.
3. De Bary, A. .. "Comparative Anatomy of the Vegetative Organs of the Phanerogams and Ferns," Engl. Trans., Oxford, 1884.
4. Esau, K. .. "Ontogeny and structure of the phloem of tobacco," *Hilgardia*, 1938, **11**, 343–424.
5. Hérail, J. .. "Recherches sur l'anatomie comparée de la tige des Dicotylédones," *Ann. Sci. Nat. Bot.*, 1885, 7, 2nd ser., 203–314 (Quoted from Scott, D. H., and Brebner, G., 1889).
6. Maheshwari, P. .. "The presence of interxylary phloem in the stem of *Leptadenia spartium* Wight," *Proc. Ind. Sci. Cong.*, 22nd Session, 1935, Calcutta.
7. Pfeiffer, H. .. "Das abnorme Dickenwachstum." In Linsbauer's *Handbuch der Pflanzenanatomie*, Borntrager, Berlin, 1926.
8. Sabnis, T. S. .. "The physiological anatomy of the plants of the Indian desert," *Jour. Ind. Bot. Soc.*, 1921, **2**, 1–19.
9. Sayeedud-Din, M., and Suxena, M. R. "On the anatomy of some of the Asclepiadaceæ," *Proc. Nat. Acad. Sci.*, India, 1940, **10**, 129–132.
10. Scott, D. H., and Brebner, G. "On the anatomy and histogeny of *Strychnos*," *Ann. Bot.*, 1889, **3**, 275–304.
11. Solereder, H. .. "Systematic anatomy of the Dictoyledons," Engl. Transl. Oxford, 1908.
12. Weiss, J. E. .. "Das markständige Gefässbündelsystem einiger Dikotyledonen in seiner Beziehung zu den Blattspuren," *Bot. Cbl.*, 1883, **15**, 280–295, 318–327, 358–367, 390–397, 401–415.

612·43 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore.

STUDIES ON MYXOSPORIDIA FROM THE COMMON FOOD FISHES OF BENGAL

By Mukundamurari Chakravarty
(Department of Zoology, Calcutta University)

Received June 16, 1943
(Communicated by Dr. G. D. Bhalerao)

CONTENTS

Introduction

The author has been studying, for the last few years the myxosporidian parasites of fishes, amphibians and reptilians obtainable in the vicinity of Calcutta and some of his previous observations were recorded before (1939, 1941). The present paper deals with a number of myxosporidians parasitic in the common food fishes of Bengal. The majority of the parasites described here are new, while a few call forth fresh attention. *Myxidium leiberkühni* Bütschli (1881) for instance, has been reported from various fishes inhabiting different parts of the world except India. Now it is reported for the first

time from India too but from a new host *Anabas testudineus*. Another species *Chloromyxum amphipnoui* though reported by Ray* (1933) from India but there is no account of it. A description of the latter, therefore, is also added here.

The fishes harbouring the parasites were either collected from the local tanks or purchased from Calcutta markets. Some of the fishes were kept in large aquaria in the laboratory.

The parasites, specially the spores, were observed in fresh conditions following the improved method given by Nemeczek (1926) and their measurements were taken. For extrusion of filaments, 1 to 10 per cent. KOH solution or Methyl Alcohol-giemsa method of the author (1939) proved satisfactory. Lugol's solution was used for detecting the iodinophilous vacuole and the nuclei. Smears of infected organs and tissues were fixed in Schaudinn's fluid as well as in Bouin-Duboscq. The latter was, however, mainly used for fixation of tissues meant for cutting sections. Delafield's and Iron-alum hæmatoxylin were used for staining both the smears and sections.

The number of fishes examined, the number of fishes parasitised, as well as a list of the parasites with their seat of infection and locality are given in Table I.

Description of Species

Leptotheca latesi n.sp.

(Figs. 1–7)

Host: *Lates calcarifer* (Bloch.). Of the five fishes examined, three were found infected.

Seat of Infection: Gall-bladder.

Locality: Bengal.†

Vegetative Form.—Both mature and young trophozoites are found in the permanent preparations of the gall-bladder of the host. The mature trophozoites (Figs. 1, 2) are circular in outline measuring 10–14 μ in diameter.

---

* I am indebted to Dr. H. N. Ray, Systematic Protozoologist, Imperial Institute of Veterinary Research, Mukteswar, for kindly placing his materials at my disposal for further investigation. Thanks are also due to Mr. D. Mukherji and Mr. G. K. Chakravarti who helped me in various ways.

† As it is difficult to ascertain the exact locality of the fishes purchased from markets owing to their being imported from different parts of the Province, 'Bengal' has been used as their locality.

No distinction could be made between the ectoplasm and the endoplasm of the parasites, the cytoplasm being uniformly granular. The youngest forms (Fig. 3) are also more or less circular in outline and contained two nuclei. Developing trophozoites (Fig. 4) with two to eight nuclei could also be observed in the smear preparation. Fully formed trophozoites contained two mature spores (Fig. 2), they are therefore disporous.

THE SPORE.—The mature spores (Figs. 5–7) are bean-shaped in lateral view with both the extremities rounded, while the developing young spores are more or less crescent-shaped. The valves of the spore are smooth and symmetrical. The sutural line is prominent both in fresh and stained specimens, but the sutural ridge could not be seen. The polar capsules are spherical in shape and equal in size; they are located one on each side of the sutural line and are surrounded by a delicate membrane. The sporoplasm which is granular, does not occupy the entire extracapsular cavity of the spore and is situated below the polar capsules. It has the form of a triangle, the apex of which is directed downwards and contains the two nuclei distinctly visible in stained preparations (Fig. 7). Dimensions: breadth of the spore 10·3 – 12·4 μ, sutural diameter of the spore 6·2 μ, polar capsules 3·1 μ in diameter, polar filament 50–80 μ long.

REMARKS.—The myxosporidian under report does not resemble any known species of *Leptotheca*. Its spores approach those of *L. inconstans* (Jameson, 1929), *L. agilis* Thélohan (1895) in size and to those of *L. informis* Auerbach (1910) and *L. longipes* Auerbach (1910) in shape.

Leptotheca macronesi n.sp.

(Figs. 8–11)

HOST: *Macrones gulio* (Ham.). Of five fishes examined, two were found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—Only a few mature trophozoites (Fig. 8) were found in living condition. They are circular in outline with an uniformly granular cytoplasm, containing several refractile granules. The trophozoites are monosporous and measure 10·3 μ in diameter.

THE SPORE.—The spores (Figs. 9–11) are elliptical in shape with the ventral side more or less flattened; their extremities are rounded but one of them is slightly narrower than the other. The valves are smooth, unequal and thin. The sutural line is fine. The equal and spherical polar capsules

are placed one on either side of the sutural line, and are surrounded by a delicate membrane. The granular sporoplasm occupies the entire extracapsular cavity of the spore and contains two nuclei. Capsulogenous nuclei are present at the bases of the capsules. Dimensions: breadth of the spore 10–14·4 μ, sutural diameter 6·18–7·2 μ, polar capsules 3·1 μ in diameter.

REMARKS.—The breadth of the majority of the spores is twice the sutural diameter, but in a few it is less than twice while in others it is slightly larger than sutural diameter. From the definition of the genera *Leptotheca* and *Ceratomyxa*, it seems that the myxosporidian under report stands in an intermediate position between the two genera.

L. macronesi n.sp. shows affinities to *L. constricta* Fujita (1923), *L. fisheri* (Jameson, 1929), and *L. inconstans* (Jameson, 1929).

Ceratomyxa scatophagi n.sp.

(Figs. 12–17)

HOST: *Scatophagus argus* (Bloch.). Among twenty-five fishes examined, ten were found infected.

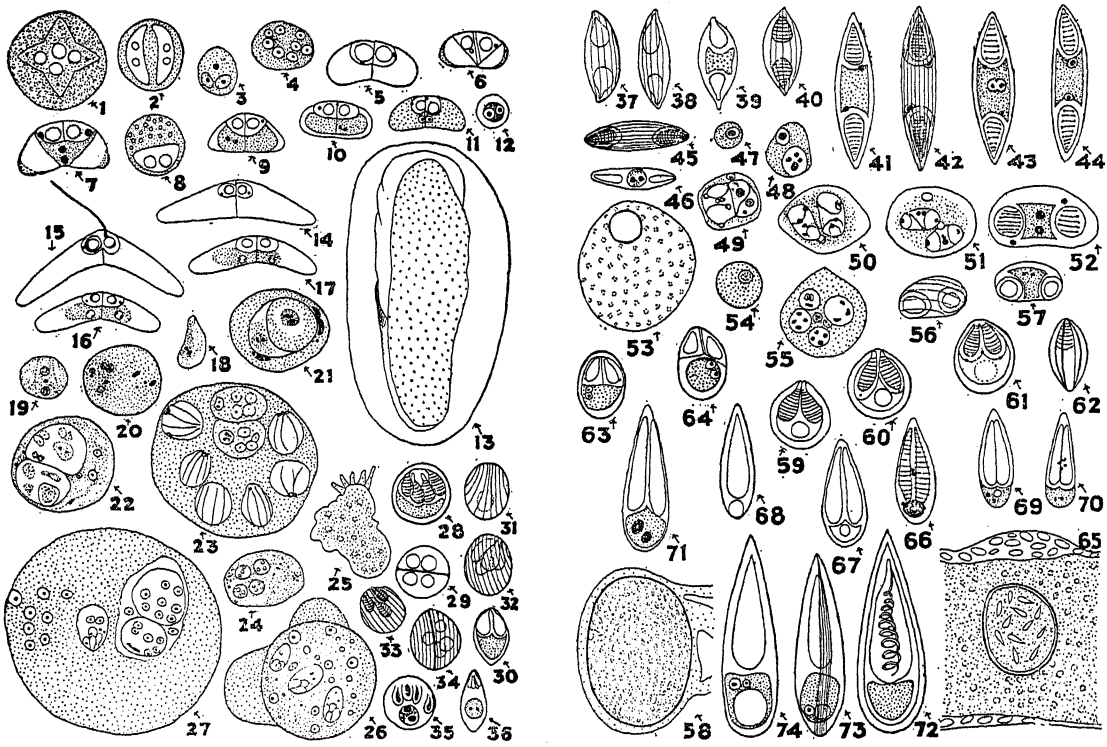
SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—The youngest forms (Fig. 12) observed in the smear preparations are circular in outline and are binucleate; the central granular endoplasm is surrounded by the hyaline ectoplasm. They measure 5 μ in diameter. Mature forms measuring 50–120 μ × 40–85 μ, are irregular in shape and the body can be distinguished into an outer hyaline ectoplasm and an inner granular endoplasm having nuclei irregularly scattered in it (Fig. 13). Unfortunately the sporulating trophozoites could not be found.

THE SPORE.—The spores (Figs. 14–17) are crescent-shaped in lateral view. The valves are alike being cylindrical in form with the terminal extremities rounded. The curvature of the valves varies to some extent from almost straight to concave. The sutural line is faintly marked. The polar capsules are equal and spherical and extrude the filament when treated with 10 per cent. KOH solution. The sporoplasm does not fill the entire extracapsular cavity of the spore, but is situated asymmetrically beneath the polar capsules and contains two nuclei (Figs. 16, 17). Dimensions: breadth of the spore 16–26 μ, sutural diameter of the spore 4·2–7·2 μ, polar capsules 2·5–3·1 μ in diameter, polar filament 30–50 μ in length.

REMARKS.—Awerinzew (1913) reported an unnamed *Ceratomyxa* from the gall-bladder of *Scatophagus argus* collected from Delagoa Bay, Africa. The



Figures were drawn under a camera lucida and magnified 833 times, unless otherwise stated.

Figs. 1–7. *Leptotheca latesi* n.sp.—Fig. 1. A fresh trophozoite. Fig. 2. A stained trophozoite containing two spores. Fig. 3. A young trophozoite from a stained smear. Fig. 4. A stained developing trophozoite. Figs. 5–6. Lateral view of fresh spores. Fig. 7. A stained spore showing sporoplasm and nuclei. × 1350. Figs. 8–11. *Leptotheca macronesi* n.sp. Fig. 8. A fresh trophozoite. Figs. 9–11. Lateral view of fresh spores. Figs. 12–17. *Ceratomyxa scatophagi* n.sp. Fig. 12. Youngest trophozoite from a stained smear. Fig. 13. A stained mature trophozoite. Figs. 14–15. Fresh spores, fig. 15 shows an extruded filament. Figs. 16–17. Stained spores. Figs. 18–36. *Chloromyxum amphipnoui* Ray.—Figs. 18–24. Trophozoites from *A. cuchia*; figs. 18–19, stained youngest trophozoites; figs. 20–23, developing trophozoites from stained smear; fig. 24, a fresh trophozoite. ×365. Figs. 25–27. Trophozoites from *H. fossilis*; fig. 24, a fresh trophozoite ×365; figs. 26–27, stained trophozoites. Figs. 28–34. Fresh spores; figs. 28 and 33, front view; fig. 30, side view; fig. 29, top view showing sutural line; figs. 31–34, showing striations. Figs. 35–36. Stained spores. Figs. 37–40. *Myxidium heteropneustesi* n.sp.—Figs. 37–39. Front view of fresh spores. Fig. 40. Side view of a fresh spore. Figs. 41–44.—*Myxidium procerum* var. *calcariferi* var. n. Figs. 41–44. Fresh spores; fig. 42. showing striations; note the position of nuclei in figs. 43 and 44. Figs. 45–46. *Myxidium leiberkühni* Bütschli.—Fig. 45. A fresh spore. Fig. 46. A stained spore. Figs. 47–52. *Zschokkella fossilæ* n.sp.—Fig. 47. A stained youngest trophozoite. Figs. 48–49. Stained trophozoites showing developing pansporoblast. Figs. 50–51. Stained mature trophozoites. Fig. 52. A fresh spore. ×1750. Figs. 53–57. *Zschokkella ilishæ* n.sp.—Fig. 53. A fresh trophozoite. Fig. 54. A stained youngest form. Fig. 55. A developing trophozoite from a stained smear. Figs. 56–57. Fresh spores. Figs. 58–64. *Myxobolus clarii* n.sp.—Fig. 58. A cyst from a section of testis. ×55. Figs. 59–61. Front view of fresh spores. Fig. 62. Side view of a fresh spores. Figs. 63–64. Stained spores. Figs. 65–71. *Myxobolus catlæ* n.sp.—Fig. 65. A cyst within the gill filament. From a section. ×360. Fig. 66. Front view of a fresh spore. Fig. 67. Iodine stained spore showing iodinophilous vacuole. Fig. 68. Side view of a fresh spore. Figs. 69–70. Stained spores. Fig. 71. Stained spore. ×1350. Figs. 72–74. *Thelohanellus rohitæ* (Southwell and Prasad).—Fig. 72. Front view of a fresh spore. Figs. 73–74. Stained spores; sutural ridge is shown in Fig. 73 (side view).

description and size of the vegetative forms given by him do not agree with the myxosporidian under consideration. Further, owing to the want of any description of the spores of the African form it is difficult for me to call these two myxosporidians synonymous. The spores of the parasite under report approach those of *C. menospora* Davis (1917) in size but they differ in shape. Moreover, the vegetative forms of these two species differ to a considerable extent. The spores of the present *Ceratomyxa* have some affinities with that of *C. urophysis* Fantham, Porter and Richardson (1940).

*Chloromyxum amphipnoui** Ray

(Figs. 18–36)

HOST.—*Amphipnous cuchia* (Ham. Buch.) and *Heteropneustes fossilis* (Bloch.). The hosts were kept alive for observation under laboratory conditions. Eight eels were examined and of these five were infected. Of the twelve cat fishes examined five were infected with *C. amphipnoui*, and two other hosts carried both *C. amphipnoui* as well as another species of *Myxidium* which is described elsewhere in this paper.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The trophozoites in the two hosts differ slightly in their structure, they are therefore described separately below.

In the eel *A. cuchia* the youngest forms (Figs. 18, 19) obtained from the permanent preparations of the gall-bladder are amœboid in shape. They are binucleate and in maximum width measure $4 \cdot 12–8 \cdot 24\,\mu$. As these forms increase in size, they become oval or spherical in shape with nuclei dividing. In forms measuring $12 \cdot 36\,\mu$ in diameter, 8–10 nuclei are generally found irregularly scattered in the cytoplasm which appears uniformly granular (Fig. 20). The nuclei later arrange themselves into groups and give rise to the pansporoblast (Figs. 21, 22).

The pansporoblast gives rise to two sporoblasts. The nuclei within the sporoblast divide and give rise to a single spore. The mature trophozoites vary from $14 \cdot 4\,\mu \times 16 \cdot 5\,\mu - 35\,\mu \times 40\,\mu$ in size and the smaller ones contain two mature spores while the bigger ones eight (Fig. 23).

Trophozoites of various stages and sizes containing developing and mature spores have been examined in living condition. They are oval or spherical in outline (Fig. 24) and do not produce any pseudopodia and

* The specific name as given by Bhatia (1938) is '*amphipnovi*' and I have retained for obvious reasons the name as given by the original author.

seemed non-motile. The cytoplasm of these parasites appear uniformly granula without any marked distinction between the ectoplasm and the endoplasm. There are, however, several refractile bodies scattered irregularly in it.

In the fresh smear preparations of the gall-bladder of *H. fossilis*, amœboid trophozoites were noted in the living condition and they exhibited a very sluggish streaming movement (Fig. 25). During the movement the parasites give out digitiform pseudopodia and curiously enough these are generally formed at the end opposite to the direction of movement. Though in locomotion they differ from the parasites of *A. cuchia* to which they closely resemble in the structure of the cytoplasm.

In permanent preparations only, fully formed trophozoites (Figs. 26, 27) are found in large numbers. Pansporoblasts are formed within the trophozoites and the nucleus of the former divides into two, four, eight and sixteen. The pansporoblast then divides into two sporoblasts each of which contains eight nuclei (Fig. 27). As in the other host a single spore is developed in each sporoblast.

In both the hosts therefore the pansporoblasts are disporoblastic while the sporoblast is monosporic.

THE SPORE.—The shape and size of the spores are exactly alike in both the hosts. In shape they are almost spherical in front view (Figs. 28, 33), and ovoidal in side view (Fig. 30), but when seen from the top at certain angles they appear rectangular. The valves of the shell are alike and are sculptured by striations (Figs. 31–34). These striations give various pictures when seen from different angles as shown in the figures. In top view they run parallel to the sutural line. The sutural line is very fine and is only visible when seen from the top (Fig. 29). The polar capsules are of equal size, oval in shape but anterior end attenuated. They are placed side by side within the spore; their attennuated end directed toward the anterior end of the spore. The capsules are provided with distinct coiled filament inside; the latter is visible in a fresh spore. The sporoplasm occupies the entire extracapsular space of the spore and when stained it shows two distinct spherical nuclei (Figs. 35, 36). Dimensions: length or breadth of the spore 8·24–10·3 μ, polar capsules 4·1–5·2 $\mu \times$ 3·1–4 μ, polar filament 35–50 μ long.

REMARKS.—The parasite described above was reported by Ray (1933) from the gall-bladder of *Amphipnous cuchia*. The same myxophoridian was also observed by me in the gall-bladder of the same host as well as in the gall-bladder of *Heteropneustes fossilis*. Although the vegetative forms from the two hosts differ in their structure in some respects, yet the spores of the parasites found in these two hosts are exactly alike both in shape and size.

C. amphipnoui shows close affinities with *C. chitosense* Fujita (1923) both in form and size of the vegetative forms as well as in the structure of the spores but the former differs in having striations on the valves of the spores, all the polar capsules being equal in size, and having spherical nuclei in the sporoplasm. It also resembles in some character *C. fluviatile* Thélohan (1892), *C. dubium* Auerbach (1908), *C. trijugum* Kudo (1920), *C. catostomi* Kudo (1920) and *C. sphæricum* Fujita (1927).

Myxidium heteropneustesi n.sp.

(Figs. 37–40)

HOST: *Heteropneustes fossilis* (Bloch.). Only two fishes were found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 37–40) are more or less spindle-shaped with bluntly pointed extremities. The valves are provided with fine striations, and the sutural line cannot be distinguished from them. The polar capsules are slightly ovoidal with their anterior ends pointed. They are equal in size and are provided with coiled filaments which are clearly seen in fresh spores. The granular sporoplasm in live spores is rectangular in shape. It is situated in the space between the polar capsules. Dimensions: length of the spore 14·42 μ, breadth of spore 6·18 μ, polar capsules 4·12–6·18 μ $\times$ 4·12 μ.

REMARKS.—The shape of the spores of the parasite under report does not resemble any known species of *Myxidium* so far described, but in size the spores approach to forms such as *M. oncorhynchi* Fujita (1923), and *M. kudoi* Meglitsch (1937). The spores show affinities to those of *M. giardi* Cépède, given by Schäferna and Jirovec (1934).

Myxidium procerum var. *calcariferi* var. n.

(Pl. II, Figs. 41–44)

HOST: *Lates calcarifer* (Bloch.). Five fishes were examined, only one was infected with this myxosporidian, and the three other with *L. latesi.*

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—Not found.

THE SPORE.—The shape of the spores is elongated and fusiform with terminal ends pointed (Figs. 41–44). The valves are equal and marked

with longitudinal striations, which run parallel to one another in the middle region of the spore, but converge at the pointed ends (Fig. 42). The sutural line could not be seen either in fresh or stained spores. The polar capsules are typically pyriform in shape and equal in size. The polar filaments are well marked in fresh condition and are extruded when treated with Methyl Alcohol-giemsa method. The sporoplasm occupies the entire space of the spore between the polar capsules, and contains two nuclei lying either side by side at the centre of the sporoplasm (Fig. 43), or placed at the base of the polar capsules widely separated from each other (Fig. 44). Capsulogenous nuclei at the base of the capsules are also distinctly marked in living condition. Dimensions: length of the spore 23–27 μ, breadth of the spore 6·18 μ, polar capsules 8·24 $\mu \times$ 4·12 μ, polar filament 25 to 30 μ in length.

REMARKS.—The shape and size of the spores of the myxosporidian under report are exactly like those of *Myxidium procerum* Auerbach (1910 *a*). The species under consideration, however, differs from the latter in having striations on the valves and also with regard to the position of the sporoplasm. Moreover it is found in a different host. As the structural difference is a minor one I propose to call it a new variety of *M. procerum*.

Myxidium leiberkühni Bütschli (1881)

(Figs. 45–46)

HOST: *Anabas testudineus* (Bloch.). Of the ten fishes examined only two were found infected with this parasite.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 45–46) are fusiform in shape with both the extremities pointed as described by previous workers. The valves of the spore are thin and marked with longitudinal striations. The shell though delicate is highly resistant, since 10 per cent. KOH solution could not penetrate the spore to bring about the extrusion of the polar filaments. The sutural line is very faint and can be seen with certain difficulty. The polar capsules are pyriform in shape and equal in size. The sporoplasm contains two nuclei and occupies the space in between the polar capsules. The size of the spores obtained by me approach to that given by Fujita (1924). Dimensions: length of the spore 12·4–15 μ, breadth of the spore 4·12–5 μ, polar capsules 4·12 $\mu \times$ 2·06 μ, polar filaments 15 μ in length.

Zschokkella fossilæ n.sp.

(Figs. 47–52)

HOST: *Heteropneustes fossilis* (Bloch.). Twelve fishes were examined but only one was found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The trophozoites are roughly circular in outline and in fresh conditions they do not exhibit any movement. The ectoplasm and the endoplasm are distinguishable both in live and stained specimens; the former appears non-granular while the latter is highly granular and contains nuclei of the different stages, as well as the developing spores. The youngest trophozoite (Fig. 47) when examined in smear preparation is $4 \cdot 12 \mu$ in diameter and is spherical in shape, having the nucleus placed excentrically and cytoplasm granular. The nucleus has a definite nuclear membrane with a spherical karyosome. As the trophozoite grows, it gives rise to two pansporoblasts, each of which is transformed into a sporoblast originating in a single spore (Figs. 48–49). The fully matured trophozoite (Figs. 50–51) contains two spores and measures $12 \cdot 36$–$16 \cdot 48 \mu$ in diameter.

THE SPORE.—The spores (Fig. 52) are more or less semi-circular in outline with one of the sides more or less straight or slightly concave and the angles rounded. The valves of the shell are thin and are not provided with any striations. The sutural line is in the form of an elongated 'S'. The polar capsules are placed at each end of the spore; they are spherical and equal in size having distinct coiled filaments. The sporoplasm is more or less rectangular in shape, granular in texture and contains two distinct nuclei. Dimensions: length of the spore $10 \cdot 3 \mu$, breadth of the spore: $4 \cdot 12$–$5 \cdot 18 \mu$, polar capsules $3 \cdot 1 \mu$ in diameter.

REMARKS.—*Zschokkella fossilæ* n.sp. shows close affinities to *Z. globulosa* Davis (1917), but differs from the latter in the following points. Trophozoites of *Z. fossilæ* have a distinct ectoplasm, are non-motile and do not produce any pseudopodia, while those of *Z. globulosa* have cytoplasm homogeneous and are slowly amœboid giving out short lobose pseudopodia. Moreover there is a difference between their spores.

Zschokkella ilishæ n.sp.

(Figs. 53–57)

HOST: *Hilsa ilisa* (Ham.). Six fishes were examined and of these only one was found infected.

SEAT OF INFECTION: Gall-bladder.

LOCALITY: Bengal.

VEGETATIVE FORM.—The live trophozoites (Fig. 53) are disc-shaped, non-motile and have uniformly granular cytoplasm. The stained specimens contain developing pansporoblasts (Fig. 55). The trophozoites in longest diameter measure 14·5–22·6 μ. The youngest forms obtained are uninucleate, and are circular in outline; they measure 4·12–6·18 μ in diameter (Fig. 54). Other stages could not be found.

THE SPORE.—The spores (Figs. 56, 57) are more or less semi-circular in shape, the basal line being slightly concave with the ends rounded. The valves are thin and are provided with longitudinal striations. The polar capsules are equal and spherical. The sporoplasm is situated in between the polar capsules but dorsally extends over the capsules. It contains two small nuclei. Dimensions: length of the spore 12·36 μ, breadth of the spore 6·18 μ, polar capsules 4·26 μ in diameter.

REMARKS.—The spores of *Z. ilishæ* n.sp. closely resemble in shape and size those of *Z. fossilæ* described above, but differ in having striations on the valves and in the structure of the sporoplasm. Further the vegetative forms of these two species are different to some extent.

Myxobolus clarii n.sp.

(Figs. 58–64)

HOST: *Clarius batrachus* (Linn.). Of twelve fishes examined, nine were found infected.

SEAT OF INFECTION: Gall-bladder, liver, testes, ovary and fat-bodies. All the infected fishes in their gall-bladder harboured mature spores while cysts were found distributed in the liver, fat-bodies and gonads.

LOCALITY: Calcutta.

VEGETATIVE FORM.—The only forms that could be obtained were the cysts and almost all of these contained mature spores. The cysts (Fig. 58) are broadly oval in shape and appear opaque white when seen under the microscope in living condition. They are surrounded by a cyst-membrane about 4–6 μ in thickness and they measure 780–975 μ × 604–877 μ.

THE SPORE.—The shape of the spores are subspherical in front view (Figs. 59–61) and lenticular in lateral view (Fig. 62). The shell of the spore is comparatively thick with equal valves. The sutural ridge is distinctly marked in fresh spores. The polar capsules are pyriform with their anterior

ends drawn out into a short narrow tube and exhibit distinctly the coiled filament. The sporoplasm which appears granular in fresh spores, is situated behind the polar capsules and occupies the entire extracapsular cavity of the spore. A spherical iodinophilous vacuole is present within it. Two nuclei are found in the sporoplasm of stained spores (Figs. 63, 64). Capsulogenous nuclei are also seen attached to the bases of the capsules. Dimensions: length of the spore 11·3–12·4 μ, breadth of the spore 10·3 μ, thickness of the spore 6·18 μ, sutural ridge 2·06 μ thick, polar capsules 6·18 μ × 3.09 μ, iodinophilous vacuole 3 μ in diameter, polar filament 50 μ long.

REMARKS.—Ray (1933 *a*) reported this myxosporidian from the liver and ovary of the same fish. The Myxosporidian under report differs from any known species of *Myxobolus* both in shape and structure of the vegetative forms and the spores. It, however, shows some affinities with *M. orbiculatus* Kudo (1920) and *M. intestinalis* Kudo (1920), but differs from both of them in the shape and size of the vegetative forms and in not having folds or markings on the shell of the spores.

Myxobolus catlæ n.sp.

(Figs. 65–71)

HOST: *Catla catla* (Ham.), *Labeo rohita* (Ham.) and *Cirrhina mrigala* (Ham.). Both adult and fingerlings of these three species of fishes were heavily infected with this myxosporidian. Of fifty specimens of each species examined forty-five in *C. catla*, thirty-eight in *L. rohita* and thirty-five in *C. mrigala* were found infected.

SEAT OF INFECTION: Branchiæ.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Cysts are found embeded in large numbers in the gill filaments. They are opaque white when alive and are either spherical or oval in shape. The cysts (Fig. 68) are embedded in the gill filaments. They measure 45–150 μ in largest diameter.

THE SPORE.—The shape of the spores is elongately pyriform in front view (Figs. 66, 67), with sharply pointed anterior and rounded posterior extremities. In lateral view they have the form of a spindle (Fig. 68). The shell of the spore is thin and the sutural ridge and the line could not be distinctly marked. The polar capsules are equal in size and elongated pyriform in shape. They occupy the major portion of the spore leaving for the sporoplasm a small space at their posterior end. The coiled filament is distinctly visible in fresh condition within the capsules. The sporoplasm

is generally spherical in outline containing two nuclei and a circular iodinophilous vacuole. Chromatin dots, about four in number, are seen between the polar capsules. Dimensions: length of the spore 14·5–16·5 μ, breadth of the spore 6·18 μ, thickness of the spore 5·15 μ, polar capsules 10·3–12·36 μ × 2·06–3·1 μ, polar filament 150 μ long.

REMARKS.—The parasite under report is new; it does not resemble any known species of *Myxobolus* so far described. Although it has some affinities with *M. capsulatus* Davis (1917), *M. koi* Kudo (1920) and *M. angustus* Kudo (1934) it sharply differs from them in essential features.

Some of the myxosporidian parasites are regarded as pathogenic by some workers and I have touched this aspect in a previous paper (1939). It will be interesting to mention here that infection in the fingerlings of the host fishes with *M. catlæ* proved fatal under laboratory conditions.

Thelohanellus rohitæ (Southwell and Prasad)

(Figs. 72–74)

This parasite was described by Southwell and Prasad (1919) from the gills of *Labeo rohita* which they collected from Turag river, Mirpur, in the District of Dacca, Bengal. I found the spores of this parasite infesting the gill of the same host. Southwell and Prasad's observations have been based on fixed material, and my observation given below made on fresh material are added as supplement.

HOST: *Labeo rohita.* Only one fish was found infected.

SEAT OF INFECTION: Branchiæ.

LOCALITY: Calcutta.

VEGETATIVE FORM.—Not found.

THE SPORE.—The spores (Figs. 72–74) are elongated pear-like or pyriform in shape with acutely pointed anterior and rounded posterior extremities as described by the previous authors. The size of the spores is slightly larger than that given by Southwell and Prasad (1918). The valves are thick and the sutural ridge is very prominent. The polar capsule has the same form as the spore. The former is provided with a highly coiled filament. The sporoplasm occupies the posterior portion of the spore and contains two nuclei and a spherical iodinophilous vacuole. Dimensions: length of the spore 30–33 μ, breadth of the spore 10–13 μ, polar capsule 16–20 μ × 7–8·24 μ, iodinophilous vacuole 4·5 μ in diameter, polar filament 206 μ in length.

TABLE I

Species of Fish	No. of fishes examined	No. of fishes infected	Seat of infection	Parasite	Locality
Amphipnous cuchia ..	8	5	Gall-bladder	*Chloromyxum amphipnoui* Ray	Calcutta
Anabas testudineus ..	10	2	,,	*Myxidium leiberkühni* Bütschli	,,
Catla catla ..	50	45	Branchiæ	*Myxobolus catlæ* n.sp.	,,
Cirrhina mrigala ..	50	35	,,	,,	,,
Clarius batrachus ..	12	9	Gall-bladder, liver, testes, ovary and fat bodies	*Myxobolus clarii* n.sp.	,,
Heteropneustes fossilis	12	7	Gall-bladder	*Chloromyxum amphipnoui* Ray	,,
		2	,,	*Myxidium heteropneustesi* n.sp.	
		1	,,	*Zschokkella fossilæ* n.sp.	,,
Hilsa ilisha ..	6	1	,,	*Zschokkella ilishæ* n.sp.	Bengal
Labeo rohita ..	50	38	Branchiæ	*Myxobolus catlæ* n.sp.	Calcutta
		1	,,	*Thelohanellus rohitæ* (Southwell and Prasad)	,,
Lates calcarifer ..	5	3	Gall-bladder	*Leptotheca latesi* n.sp.	Bengal
		1	,,	*Myxidium procerum* var. *calcariferi* var. n.	,,
Macrones gulio ..	5	2	,,	*Leptotheca macronesi* n.sp.	,,
Scatophagus argus ..	25	10	,,	*Certomyxa scatophagi* n.sp.	,,

Summary

1. Eight new species of myxosporidians belonging to the genera *Leptotheca*, *Ceratomyxa*, *Myxidium*, *Zschokkella* and *Myxobolus* have been described.

2. A new variety of *Myxidium procerum* is described.

3. A detailed description of *Chloromyxum amphipnoui* Ray is given and it is recorded from a new host.

4. *Myxidium leiberkühni* Bütschli is recorded from a new host in India.

5. *Thelohanellus rohitæ* (Southwell and Prasad) is recorded for the first time from Calcutta.

6. Infection with *Myxobolus catlæ* has been briefly discussed.

REFERENCES

Auerbach, M.* .. "Bemerkungen über Myxosporidien heimischer Süsswasserfische," *Zool. Anz.*, 1908, **32**, 456–65.

———* .. "Cnidosporidienstudien," *Ibid.*, 1910, **35**, 767–77.

———* .. "Biologische und morphologische Bemerkungen über Myxosporidien," *Zool. Anz.*, 1910 *a*, **35**, 57–63.

Awerinzew, S.* .. "Ergebnisse der Untersuchungen über parasitische Protozoen der tropischen Region Afrikas. III," *Ibid.*, 1913, **42**, 151–56.

Bhatia, B. L. .. *Sporozoa. Fauna Brit. India*, p. 336, Taylor & Francis, London, 1938.

Bütschli, O.* .. "Beiträge zur Kenntnis der Fischsporospermien," *Z. wiss. Zool.*, 1881, **35**, 629–51.

Chakravarty, M. .. "Studies on Myxosporidia from the fishes of Bengal, with a note on the myxosporidian infection in aquaria fishes," *Arch. Protistenkde*, 1939, **92**, 169–78.

——— .. "Observations on two myxosporidians *Zschokkella lissemysi* n.sp. from the gall-bladder of the tortoise, *Lissemys punctata* and *Zschokkella auerbachi* (Weill) from the gall-bladder of *Bufo melanostictus*, with a note on the genus *Zschokkella* Auerbach," *Journ. Roy. Asiat. Soc. Bengal*, 1941, **6**, 69–76.

Davis, H. S.* .. "The Myxosporidia of the Beaufort Region. A systematic and biologic study," *Bull. Bur. Fish.*, 1917, **35**, 201–43.

Fantham, H. B., Porter, A., and Richardson, L. R. "Some more Myxosporidia observed in Canadian fishes," *Parasit.*, 1940, **32**, 333–53.

Fujita, T. .. "Studies on Myxosporidia of Japan," *Journ. Coll. Agr., Hokkaido Imp. Univ.*, 1923, **10**, 191–248.

——— .. "Studies on myxosporidian infection of the crucian carp," *Japan Journ. Zool.*, 1924, **1**, 45–57.

——— .. "Studies on Myxosporidia of Japan," *Journ. Coll. Agr., Hokkaido Imp. Univ.*, 1927, **16**, 229–47.

Jameson, A. P. .. "Myxosporidia from Californian fishes," *Journ. Parasit.*, 1929, **16**, 59–68.

Kudo, R. .. "Studies on Myxosporidia. A synopsis of the genera and species of Myxosporidia," *Ill. Biol. Monogr.*, 1920, **5**, Nos. 3 and 4, 1–265.

——— .. "Histozoic Myxosporidia found in fresh-water fishes of Illinois, U.S.A." *Arch. Protistenkde.*, 1929, **65**, 364–78.

——— .. "Studies on some protozoan parasites of fishes of Illinois," *Ill. Biol. Mongor.*, 1934, **13**, 7–44.

Meglitsch, P. A. .. "On some new and known Myxosporidia of the fishes of Illinois," *Journ. Parasit.*, 1937, **23**, 467–77.

Nemeczek, A. .. "Beiträge zur Kenntnis der Myxosporidien-fauna Brasiliens," *Arch. Protistenkde.*, 1926, **54**, 137–49.

Ray, H. N. .. "Myxosporidia from India," *Proc. Indian Sci. Congr.*, 1933, Zoology, p. 259.

——— .. "Preliminary observations on Myxosporidia from India," *Curr. Sci.*, 1933 *a*, **1**, 349–50.

Schäferna, K., und Jírovec, O. .. "Zur Kenntnis des *Myxidium giardi* Cépède," *Zool. Anz.*, 1934, **105**, 266–70.

Southwell, T., and Prasad, B. .. "On some Indian Myxosporidia," *Rec. Ind. Mus.*, 1918, **15**, 344–48.

Thélohan, P.* .. "Observations sur les myxosporidies et éssai de classification de ces organismes," *Bull. soc. philom.*, 1892, **4**, 165–78.

———* .. "Recherches sur les Myxosporidies," *Bull. sc. France et Belg.*, 1895, **26**, 100–394.

* The author has not seen the original papers. The informations about the myxosporidians concerned are taken from Kudo (1920).

MASSEEELLA NARASIMHANII, A NEW SPECIES OF RUST ON *FLUEGGEA LEUCOPYRUS* WILLD.

By M. J. Thirumalachar

(Department of Botany, Central College, Bangalore)

Receied July 8, 1943

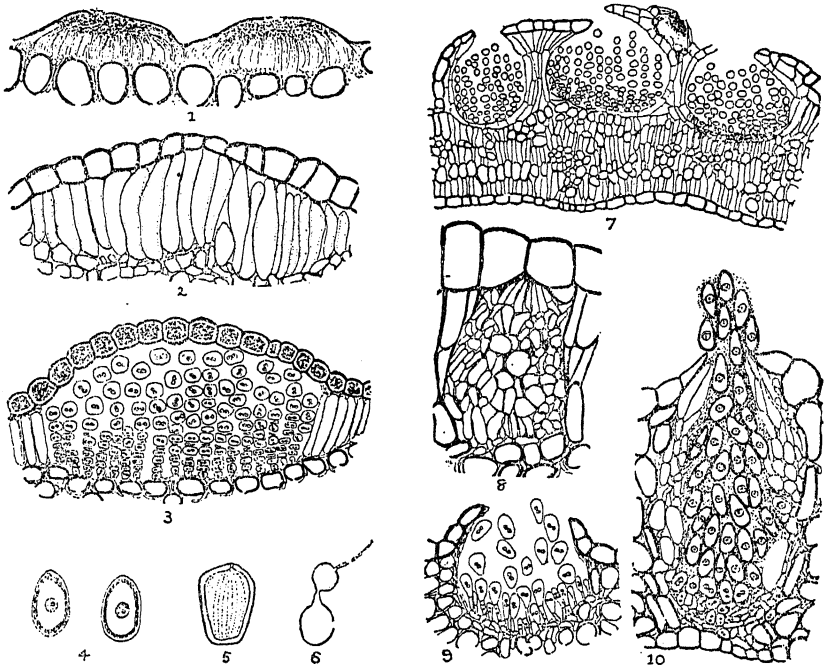
(Communicated by Prof. L. Rama Rao, F.A.Sc.)

Flueggea leucopyrus Willd. is a stiff thorny shrub with ovate-orbicular leaves and greenish white fruits. Some of these plants growing near Yashavantapur, Bangalore, were heavily rusted, and a detailed study undertaken by the writer proved it to be a new species of *Masseeella*. So far three species have been recorded for the genus, *Masseeella Capparidis* (Hobson) Diet., *M. Flueggeæ* Syd. on *Flueggea virosa* Baill. in the Philippines, and *M. Breyniæ* Thirumalachar. With the exception of *Masseeella Capparidis* for which only the telial stage is known, the other two species are autœcious eu-forms. Even so the *Masseeella* on *Flueggea leucopyrus* is also an autœcious eu-form, with pycnia and æcia developing in August-September, uredia and telia in the months of October to January.

Infection spots on which pycnia and aecia are distributed, are minute, and greatly hypertrophied. They are pale yellow and do not coalesce to form a patch. Pycnia are subcuticular (Fig. 1), minute, amphigenous, hyaline and applanate. They coalesce with one another if developed in close proximity. A pore formed by the rupture of the cuticle enables the pycniospores to escape. Subcuticular pycnia have also been noticed in *Masseeella Flueggeæ* Syd. and *M. Breyniæ*.

Aecia are amphigenous and erumpent, following the pycnia in development. They are applanate, spreading, opening out irregularly. They form large chambers due to the coalescence of more than one aecium on the same infection spot (Fig. 7). The aecial initial is formed by the formation of a plectenchyma beneath the epidermis. From the upper portion of the plectenchyma, long palisade-like cells are differentiated (Fig. 2), which swell at maturity and become transformed into loose parenchymatous cells. From the basal *fertile* portion of the initial aeciospores are abstricted off in chains. The pseudoparenchymatous cells disintegrate forming a space into which the aeciospores are abstricted. The sori spread horizontally by the differentiation of more basal cells from the hymenium followed by the disintegration of the pseudoparenchymatous cells. Aeciospores are white, ovate-ellipsoid,

minutely verrucose with an indistinct germ pore, measuring 20–24 × 17 μ. The aecia are without any peridia, which however is the characteristic feature of both *Masseeella Flueggeæ* and *M. Breyniæ*, in which the peridial cells are angularly globoid, abutting on the sides, with rugose walls. In some of the



Figs. 1-6.—Fig. 1. Subcuticular pycnia of *Masseeella Narasimhanii*. ×400. Fig. 2. Elongated palisade-like cells of the aecial initial. ×320. Fig. 3. Aecium. ×320. Fig. 4. Teliospores. ×320. Fig. 5. Mature teliospore showing longitudinally striate exospore. ×320. Fig. 6. Secondary sporidium. ×400.

Figs. 7–10.—Fig. 7. Section through the infected leaf showing aecia. ×100. Fig. 8. Telial initial. ×200. Fig. 9. Uredium. ×200. Fig. 10. Mature telium with teliospores embedded in gelatinous matrix. ×200.

sections of the *Masseeella* species on *Flueggea leucopyrus*, some of the aeciospores distributed at the margin of the sorus were found to be somewhat thick-walled, and without any cell contents. They might be degenerating aeciospores or may represent evanescent peridial cells. However in the majority of aecia peridia do not occur.

The applanate spreading type of aecium (Fig. 3), bordered by palisade-like pseudoparenchymatous cells, further marked out by the lack of any definite shape and peridia distinguishes the rust from the other species. In both *Masseeella Flueggeæ* and *M. Breyniæ*, the aecia are cupulate, with a definite shape, and possessing well-developed peridia. A *Masseeella* on *Flueggea*

species collected by Rhind in Burma was sent to Kew, England, tentatively identified as *M. Flueggeæ* (Butler and Bisby, 1931). It is manifest that the exact species can be determined only after observing the aecial stages.

Uredia are hypophyllous, white, minute, aparaphysate and pulverulent (Fig. 9). The infection spots do not get hypertrophied and are often associated with pycnial and aecial pustules. Urediospores are ovate-ellipsoid, white, minutely echinulate, and stipitate. The spores are binucleate, with indistinct germ pores. They closely resemble the aeciospores. The spores are dispersed by the rupture of the epidermis. In some cases they are developed in such large numbers on the surface of the leaves, that the entire leaf surface presents a white powdery appearance.

Telia are epiphyllous and very rarely amphigenous. The sori are deeply sunk within the host tissue, almost extending up to the lower epidermis (Fig. 10). They are flask-shaped opening out by an ostiole. The teliospores are abstricted off in chains and these emerge out embedded in a gelatinous matrix secreted by the hyphæ lining the sorus. The spore tendrils measure up to 7 mm. in length. The mucilage greatly swells in water and on drying up becomes a horny mass. The telial initials are formed beneath the epidermis by the concentraction of hyphæ (Fig. 8). The teliospores are one-celled, ovate-oblong, chestnut brown, slightly angular, with an apical germ pore. The wall layer is three partite, the exospore being longitudinally striate (Fig. 5), as in *Masseeella Flueggeæ* (Cummins, 1937) and *M. Breyniæ* (Thirumalachar, 1943). Young teliospores are thin-walled, hyaline and binucleate, which later fuse to form a syncaryon (Fig. 4). The spores were germinated and stained by the method suggested by the writer (1940). The basidium is four-celled, bearing globular basidiospores on short sterigmata. Secondary sporidia (Fig. 6) have been observed.

In respect to the structure and development of the teliospores all the species of *Masseeella* so far known, show remarkably close resemblance. The telia are invariably epiphyllous, the sorus being deep seated, lined with mucilage secreting hyphæ. The teliospores of *Masseeella Flueggeæ*, *M. Breyniæ*, and in the species under study, are of same shape and possesses three wall layers, the exospore being longitudinally striate. Further, the teliospores of *M. Flueggeæ* Syd. on *Flueggea virosa* and those of *Masseeella* sp. on *Flueggea leucopyrus* closely resemble each other as regards the size and shape. But the urediospores and æciospores differ in spore measurements. The urediospores of *M. Flueggeæ* measure 20–26 × 16–20 μ, whereas those of the present rust are smaller in size measuring 12·7–25 × 12·7–16 μ. On the other hand the aeciospores are larger in the latter form than in

M. Flueggeæ. They measure 13–16 × 16–23 μ in *M. Flueggeæ* as against 20–24 × 17 μ in the *Masseeella* species on *Flueggea leucopyrus*. In addition, the lack of peridium and absence of cupulate aecia in the *Masseeella* on *Flueggea leucopyrus* and other characters necessitate the erection of a separate species for its accommodation. The name *Masseeella Narasimhanii* is proposed in honour of Mr. M. J. Narasimhan, Director of Agriculture, Mysore State. The telial stages of this rust were also collected by Md. Taslim on 26–11–1936 at New Delhi on the same host, and the rust has evidently a wide distribution.

Masseeella Narasimhanii Spec. Nov.

Pycnia subcuticularia, complures, plus minus densissime aggregati, maculis contagium tumidulus. Aecia subepidermalia, irregularitier erumpentia, aliquanto applanata, flave albida, peridio evanidus vel absens; aeciosporæ alba, subglobosæ ovatæ vel ellipsoidæ, minutissime verrucosæ, poris germ. indistinctæ, magnitudinis 20–24 × 17 μ. Urediosori hypophylli, maculis obsoletis, subepidermicis, aparaphysatis; urediosporæ ovatæ vel ellipsoidæ, verrucosæ, membrana hyalina, solitarie ortæ, poris germ. obscuris. Telia epiphylla, rarissime singulæ, etiam hypophylla, profundi immersi, sporas in cirras filiformes, copiosæ aggregati, vel solitarie, nigro-brunneos, 3–7 mm. longos; teliosporæ oblong ellipsoidæ, vel fusiformæ, sessiles, leniter angulatæ, castanneo-brunnæ, spora membrana tripartitus, exosporæ subtilissime costatæ longitrosum, magnitudinis 22–31 × 14·5–17 μ., in massa mucosa sitæ, poris germ apicali, sporæ statim germinantes, promycelio externo, typice 4-cellulari; sporidis globosus, tenuiter membrana, secundario sporidiis formant.

Hab. In vivis foliis *Flueggea Leucopyrus* Willd., Yashavantapur, Bangalore, 15 9 1942, leg. M. J. Thirumalachar (Type), New Delhi, 26 11 1936. leg. Md. Taslim. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi.

Pycnia subcuticular, densely aggregated, hyaline, on swollen infection spots. Aecia subepidermal, applanate, yellowish white, opening irregularly, peridium evanescent or absent, aeciospores white, subglobose, ovate-ellipsoid, minutely verrucose, germ pores indistinct, measuring 20–24 × 17 μ. Uredial infections not causing hypertrophy, uredia white, hypophyllous, subepidermal and aparaphysate; urediospores ovate-ellipsoid, minutely verrucose, single indistinct germ pore, measuring 12·7–25 × 12·7–16 μ. Telia epiphyllous, rarely hypophyllous, in solitary curly hair-like columns, or densely aggregated, horny, teliosorus conical, deeply sunk, single-celled

spores abstricted in succession, which emerge out embedded in gelatinous matrix secreted by the hyphæ lining the sorus; teliospores spherical, fusifom or oblong-ellipsoid, slightly angular, chestnut brown, wall layer three partite, longitudinally striate, with apical germ pore, spores measuring 22–31 × 14·5–17 μ. Sporidia spherical, secondary sporidia also formed.

Hab.—On living leaves of *Flueggea leucopyrus* Willd. Yashavantapur, Bangalore, 15—9—1942, leg. M. J. Thirumalachar (Type), and New Delhi, 26—11—1936, leg. Md. Taslim. Type deposited in the Herb. Crypt. Ind. Orient., New Delhi.

In conclusion the wirter wishes to acknowledge his indebtedness to Dr. B. B. Mundkur, Inperial Agricultural Research Institute, New Delhi, for critically going through the manuscript and valuable suggestions, and to Dr. L. N. Rao, Professor of Botany, University of Mysore, for helpful suggestions and encouragement.

LITERATURE CITED

1. Butler, E. J., and Bisby, G. R. " Fungi of India," *Imp. Council Agri. Res. Scien. Monograph,* 1931, No. 1, 51.
2. Cummins, G. B. .. " Studies in the Uredinales of the Philippines," *Ann. Mycol. Berlin,* 1937, **35,** 130.
3. Sydow, H., and Petrak, F. .. " Micromycetes Philippinensis," *ibid.,* 1928, **26,** 424.
4. Thirumalachar, M. J. .. " A method for germinating and staining teleutospores," *Jour. Ind. Bot. Soc.,* 1940, **19,** 71–75.
5. ———— .. " *Masseeella Breyniæ,* A New species of Rust," 1943 (In Press.)

TENT CATERPILLAR (*MALOCOSOMA INDICA* WLK.) IN THE SIMLA HILLS

BY KHAN A. RAHMAN, B.SC. AGRI. (EDIN.), PH.D. (CANTAB.),
AND
ASA NAND KALRA, B.SC.AGRI. (PB.)
(Punjab Agricultural College and Research Institute, Lyallpur)

Received July 26, 1943
(Communicated by Dr. Hamid Khan, F.A.SC.)

Introduction.—During the course of an entomological survey of the orchards in the Kotgarh ilaqa (Simla Hills) in July-August, 1940 a large number of egg-masses, resembling those of the notorious Tent Caterpillar of America, were found deposited on branches and twigs of a number of apple and a few pear, plants. The twigs bearing these egg-masses were brought to the laboratory at Kotgarh and kept under observation. They hatched on the 20th March, 1941 and the adults which emerged from them in due course were identified as *Malocosoma indica* Wlk. by a Systematist of the Imperial Agricultural Research Institute, New Delhi.

This article embodies the results of observations made on *M. indica* Wlk. since its first discovery.

Food plants.—The pest has been recorded as occurring on apple pear, apricot, walnut, " shaigal " (*Pyrus* sp.), and " ban " (*Quercus incana*) and of these apple generally, and pear occasionally, suffer the most from its ravages.

Distribution in the Simla Hills.—The pest has so far been collected from Aina, Bhareri, Himtala, Kotgarh proper, Pomlai, Rhoga and Thanedhar in the Kotgarh ilaqa; Kudiali and Matiana on the Hindustan-Tibet Road (between Kotgarh and Simla); Cheriot Nal, Chharabra, Gahan, Mashobra and Sanjauli near about Simla; and from Kasauli.

Description of various stages : Egg.—About 1 mm. long, elongate, thimble-shaped and shining.

Full-grown larva.—Length 40 to 45 mm., breadth 4·5 to 5·5 mm. Head and dorsum black. Dorsum ornamented with (*a*) a broad greyish line (dotted with black) which extends antero-posteriorly medially and (*b*) four lines of crimson coloured dots, two on either side of (*a*). Legs black, prolegs light brown. Body cylindrical with a lateral tuft of white hairs on each segment. Dorsum sparingly clothed in fine black bristles arising from tubercles ; those on the last two abdominal segments being more numerous.

Last abdominal segment triangular and furnished with two lobe-like appendages at posterior end.

Pupa.—18 to 21 mm. long, 6 to 7 mm. broad, brown to dark brown, more or less smooth.

Adult: Male:—Wing expanse 29–32 mm. Light reddish in colour. Forewings traversed by two oblique, broad, whitish stripes enclosing whitish area in between. Antennæ thick, many jointed and bipectinate.

Female.—Wing expanse 35–37 mm. Light brown, stripes on the fore-wings rather less prominent. Antennæ similar to those of the male.

Life-history.—The pest hibernates in the egg-stage. Females lay eggs as broad bands round branches of plants. Each band, which consists of 200 to 400 eggs, is covered with a protective layer of dark brown gluey substance. When buds appear (about the middle of March) these eggs hatch out (Table I).

TABLE I. *Duration of egg-stage of* Malocosoma indica *Wlk. at Kotgarh*

Eggs laid on	Eggs hatched on	Duration of egg stage	
		Months	Days
22–5–1941	18–3–1942	9	27
30–5–1941	18–3–1942	9	19
4–6–1941	18–3–1942	9	14

Thus the egg-stage lasts for about 9 to 10 months.

The larvæ live gregariously. Soon after hatching they spin a silken nest at a convenient and sheltered place on the plant. The nest, or the tent as it is called, is at first small but it gradually increases in size as the caterpillars grow bigger until in some cases it may nearly be 1 to 1½ ft. in length. In cases of serious infestation there may be as many as 18 to 25 such ' tents ' on a single plant. These ' tents ' render a plant unsightly. The caterpillars spend the day in the ' tent'; they feed on the leaves of the plant at night. When full-fed the caterpillars seek out a protected place where they spin their cocoons. Duration of the larval stage varies from 39 to 68 days at Kotgarh (Table II).

TABLE II. *Duration of larval stage of* Malocosoma indica *Wlk. at Kotgarh*

No.	Eggs hatched on	Larvæ pupated on	Duration of larval stage (days)
1	20–3–1941	28–4–1941	39
2	20–3–1941	3–5–1941	44
3	20–3–1941	20–5–1941	61
4	20–3–1941	27–5–1941	68
5	18–3–1942	9–5–1942	52
6	18–3–1942	13–5–1942	56
7	18–3–1942	17–5–1942	60

The oval white cocoons, which were about 1" in length, were found to be made of compactly woven silk. Pupal stage was found to last for 8 to 22 days (Table III).

TABLE III. *Duration of pupal stage of* Malocosoma indica *Wlk. at Kotgarh*

No.	Larvæ pupated on	Moths emerged on	Duration of pupage stage (days)
1	30-4-1941	8-5-1941	14
2	8-5-1941	19-5-1941	11
3	16-5-1941	2-6-1941	17
4	8-5-1942	30-5-1942	22
5	1-5-1942	20-5-1942	19
6	8-5-1942	29-5-1942	21

Preoviposition period.—Preoviposition period was found to be 1 to 3 days.

TABLE IV. *Preoviposition period in* Malocosoma indica *Wlk. at Kotgarh*

No.	Pairs mated on	Eggs laid on	Preoviposition period (days)
1	20-5-1942	22-5-1942	2
2	30-5-1942	31-5-1942	1
3	1-6-1942	4-6-1942	3

In captivity a female moth lived for 3 to 5 days and a male 4 to 6 days.

Proportion of sexes.—Table IV below gives the proportion of sexes among the moths which emerged in the laboratory at Kotgarh during 1941 and 1942.

TABLE V. *Proportion of sexes*

Year	Percentage of males	Percentage of females
1941	56·2	43·8
1942	61·5	38·5

Seasonal history and duration of life-cycle.—There is only one generation of the pest in a year. Over-wintered eggs started hatching from the middle of March and continued till May-June. Emergence of moths began in the 3rd week of May and lasted till the beginning of June.

Table VI below gives the duration of life-cycle.

TABLE VI. *Duration of total life-cycle of* Malocosoma indica *Wlk. at Kotgarh*

No.	Eggs laid on	Eggs hatched on	Larvæ pupated on	Adults emerged on	Duration of total life-cycle
1	22–5–1941	18–3–1942	1–5–1942	20–5–1942	One year
2	4–6–1941	18–3–1942	16–5–1942	1–6–1942	One year

Nature and extent of damage.—The caterpillars feed on leaves gregariously. In cases of serious infestation the entire plant was found to be almost completely defoliated, mid rib and other harder veins of the leaves only were left behind. Such plants did not bear any fruit. In the absence of leaves young caterpillars were noticed to feed on tender bark. In years of serious infestation as many as 40 to 50% of the apple plants in an orchard were found to be infested.

Control.—1. As is evident from the life-history details, egg-stage lasts for about 9 to 10 months. Egg clusters which are laid round branches and twigs are the easiest to destroy, the most convenient time for the purpose being December-January. When pruning, branches and twigs with egg clusters, should be carefully searched out and collected and either burnt or buried about 1 foot deep in the soil.

2. The caterpillars should be killed by rubbing the 'tents' with rags dipped in kerosene oil and tied at the end of a pole. This control gave the best results when carried out from 12 noon to 3 p.m. on clear sunny days.

An open vessel containing water with a film of kerosene oil on surface was placed on the ground just underneath the 'tent' when applying the 'kerosenised rags' so that any larvæ which fell down at the touch of the 'rag' were killed in the treated water. The cost of operation was worked out at As. 4 per 10–15 tents.

NITROGEN REQUIREMENTS AND VITAMIN DEFICIENCIES OF *PHYTOPHTHORA PHASEOLI* THAXTER

BY R. K. SAKSENA AND K. S. BHARGAVA
(Botany Department, University of Allahabad)

Received July 15, 1943
(Communicated by Prof. Shri Ranjan, D.SC., F.A.SC.)

IT has been well established that the source of nitrogen, in the cultural nutrition of fungi, is an important limiting factor (Robbins, 1937, Leonian and Lilly, 1938), but for some even the proper source of nitrogen often may be ineffective without the presence of growth supplements. Robbins (1938), who studied the nutrition of some species of *Phytophthora*, found that they apparently required for their growth an external supply of thiamin. Leonian (1925, p. 448) has reported that *Phytophthora phaseoli* grows very slowly on the two media used by him. One contained malt extract (dry), KH_2PO_4, $MgSO_4$ and Bacto agar, while the other was made up of nucleinic acid, KH_2PO_4, $MgSO_4$, dextrose and Bacto agar. The latter medium was used without agar also. When he added a few c.c. of lima bean infusion to the media, the fungus showed rapid growth. His results indicate that *P. phaseoli* suffers from a deficiency of some growth supplements not amply represented in the original media used by him.

The present paper deals with the nitrogen requirements and vitamin deficiencies of *Phytophthora phaseoli* Thaxter.

For a comprehensive review on these subjects the reader is referred to Robbins (1937) and Robbins and Kavanagh (1942).

Material and Methods

The culture of *Phytophthora phaseoli* Thaxter was obtained from Centraal Bureau voor Shimmelcultures, Baarn, Holland.

The methods and technique employed in this investigation were essentially the same as described in previous papers (Saksena, 1941 *a* and *b*; Saksena and Bhargava, 1941).

Unless otherwise indicated the fungus was grown in 150 ml. Erlenmeyer Pyrex flasks containing 15 c.c. of a basal medium, which will afterwards be referred to as medium M, consisting of 0·5 gm. each of KH_2PO_4, $MgCl_2 \cdot 6H_2O$ and K_2SO_4, 5·0 gm. of pure dextrose (dextrosol of Corn Products Co.)

and 1 litre of distilled water. Magnesium chloride was prepared by the action of pure hydrochloric acid on the clean magnesium ribbon; the commercial guaranteed reagent ($MgCl_2$. $6H_2O$) was found to be of no use in these experiments, because it contained traces of ammonia. Asparagin and alanine were purified by repeated precipitations with alcohol.

Only guaranteed reagents (either from Merck or from British Drug House) were used. Thiamin used was Merck's vitamin B_1 and the yeast extract was a Difco product. Lima bean infusion was obtained by steaming 2 gm. of lima beans (*Phaseolus lunatus**) in 100 c.c. of water for one hour, while lentil extract was prepared according to the process used by Buston and Pramanik (1931). Casein was brought into solution by dissolving 1 gm. of casein in 8 c.c. of N/10 NaOH, then adjusting the reaction with HCl.

The stock cultures were maintained on oatmeal agar. All cultures were grown in triplicate. The range of temperature during the experiments was 20°–22°C. and the pH of the media was adjusted to 5. Generally the incubation period was of 15 days' duration. Throughout the experiments only Pyrex glass ware was used.

In order to decrease the effect of whatever vitamins might have been present in the original inoculum coming from cultures grown on oatmeal agar, subcultures from the growth in the first passage in liquid medium were made into corresponding medium for a second passage. A particular medium was thought suitable only when the fungus grew on it by subsequent transfers.

Experimental

Nitrogen requirements.

Series I. To the basal medium M, the following inorganic and organic nitrogen-containing substances were added singly in 0·1 per cent. concentration. The growth of the fungus in a medium indicated the utilisation of the particular mitrogen compound. Medium M served as a control.

Inorganic sources.—Ammonium nitrate, sodium nitrate and sodium nitrite.

Organic sources.—Amino acids: Glycine, *d*-alanine, purified *d*-alanine, *d*-valin, *l*-leucin, *d*-arginine, *d*-lysin, *l*-asparatic acid, *d*-glutamic acid, asparagin, purified asparagin, *l*-phenyl-alanine, tyrosin, histidin, tryptophane, proline, cystin and cystein hydrochloride. Amide: Acetamide. Amines: Urea and trihydroxy-triethylamine. Proteins: peptone, hydrolysed peptone, purified casein and butter milk.

* This is the host of *P. phaseoli.*

It was found that *P. phaseoli* grew only in media containing *d*-alanine, peptone, hydrolysed peptone and butter milk, while it did not respond positively to other media.

Series II.—Since the fungus grew in *d*-alanine and not in purified *d*-alanine, it was thought that in *d*-alanine was some unknown substance essential for its growth and that it got removed during the process of purification. The various washings obtained during purification were collected and evaporated to dryness. A little of this substance was added to medium M, containing purified *d*-alanine. Medium M with purified *d*-alanine served as a control. The two media were then inoculated with the fungus, which grew only in the former.

Relation to Vitamins.

The results obtained above indicated that the fungus required some growth substances for its growth and that they were present in *d*-alanine, peptone, hydrolysed peptone and butter milk. Since peptone has been found to have a small amount of thiamin or its intermediates (Robbins and Schmidt, 1938) the following procedure was adopted.

Series III.—The media used in the experiments described under Series I were inoculated with spore suspensions of *Phycomyces blakesleeanus* which requires thiamin or its intermediates for its growth (Schopfer, 1934). The results obtained were all negative except in the case of media containing *d*-alanine, asparagin, peptone, hydrolysed peptone and butter milk.

Series IV.—To the various media used in Series I was added thiamin (5 international units per 25 c.c. of the medium). They were then inoculated with *Phytophthora phaseoli.* Good growth was obtained in media containing *d*-alanine, purified *d*-alanine, peptone, hydrolysed peptone, purified casein and butter milk.

Series V.—The basal medium M and other media used in Series I were supplemented with the following products of natural origin, in the concentration noted against each:

(*a*) lentil extract	0·02%
(*b*) yeast extract	0·01%
(*c*) lima bean infusion	1 c.c. per 10 c.c. of the medium.

These were then inoculated with *P. phaseoli.* The fungus made good growth in all the cases except where the source of nitrogen was NH_4NO_3.

Toxic effect of ammonium salts.

Series VI (*a*).—From the experiments reported under Series V, it appeared that NH_4NO_3 had some toxic effect on the growth of the fungus. To ascertain, if it was so, the fungus was grown on a richer medium (Leonian, 1930, p. 673) containing proteose peptone 2 gm.; KH_2PO_4 0·5 gm., $MgSO_4$ 0·5 gm., succinic acid 0·2 gm., dextrose 5 gm. and water 1,000 c.c. supplemented with NH_4NO_3 and various growth substances. The results obtained are given in Table I, and shown in Fig. 1.

TABLE I

Dry weight of mycelium (in milligrams) of the fungus colony grown for 30 *days*

Medium	Dry wt.
1. Leonian's medium (under Series VI *a*)	7
2. Medium 1 + NH_4NO_3 0·2%	×
3. Medium 1 + thiamin (5 units per 25 c.c.)	15
4. Medium 3 + NH_4NO_3 0·2%	×
5. Medium 3 + ascorbic acid (1 unit per 25 c.c.)	15·5
6. Medium 5 + NH_4NO_3 0·2%	×
7. Medium 1 + lentil extract 0·02%	19
8. Medium 7 + NH_4NO_3 0·2%	×
9. Medium 1 + bean infusion (1 c.c. per 10 c.c.)	30
10. Medium 9 + NH_4NO_3 0·2%	19
11. Medium 1 + yeast extract 0·01%	18
12. Medium 11 + NH_4NO_3 0·2%	×

× Indicates absence of growth.

Series VI *b*.—To know exactly whether the ammonium ion or the nitrate ion is responsible for the toxicity Leonian's medium used in Series VI *a* was supplemented with thiamin (5 units per 25 c.c.), and NH_4Cl, $(NH_4)_2SO_4$ and $NaNO_3$ were added to it singly in 0·2% concentration. The fungus showed growth on Leonian's medium supplemented with thiamin and on the medium containing $NaNO_3$ while it did not grow on those to which NH_4Cl or $(NH_4)_2SO_4$ had been added.

Discussion and Conclusions

Earlier work of Leonian (1925, 1930) on the nutrition of the genus *Phytophthora* furnishes little information on the nutritional requirements of *Phytophthora phaseoli.* The inability of *P. phaseoli* to grow on nutrient media containing inorganic nitrogen (Series I) clearly indicates that probably it is one of those fungi which do not assimilate ammonia or nitrate but require some organic nitrogen. The positive reaction which the organism shows

with *d*-alanine and peptone justifies its being placed in the 'organic nitrogen organisms' classified and placed separately by Robbins (1937). Kincaid (Robbins 1937, p. 244) found that *Blepharospora cambivora, Diplodia Zeæ, Endomyces Magnusii, Phycomyces nitens,* thirteen species of *Phytophthora, Sphæronema fimbriatum* did not grow with either nitrate or ammonia as the source of nitrogen and dextrose as the source of carbon but grew with peptone. Similarly *Euglena deses* and *E. pisci-formis* do not assimilate nitrate or ammonia but require amino acids or peptone (Dusi, 1933).

But the relation of organisms to organic nitrogen is generally complicated by the possible contamination of organic nitrogen compounds with the presence of accessory growth factors, which are not generally found in pure inorganic salts. In the present case, *d*-alanine, peptone, hydrolysed peptone and butter milk are found to contain traces of thiamin or its intermediates (Series III). Since lentil extract (Hawker, 1936, p. 703) and yeast extract (Leonian and Lilly, 1938) in addition to some of the known vitamins contain amino acids and other organic nitrogen compounds, it is clear that they supply the necessary source of organic nitrogen in addition to thiamin (Series V).

That thiamin is also essential for the growth of *P. phaseoli* is shown by the fact that it fails to grow on medium M containing purified *d*-alanine but shows good growth in the presence of *d*-alanine (Series II), which has been demonstrated to contain thiamin or its intermediates (Series III). This conclusion is also supported by the experiment carried under Series IV where the addition of thiamin to medium M containing purified *d*-alanine or purified casein induces the growth of the organism. It may be noted here that the fungus grows well on medium M containing lime bean infusion (Series V). This shows that lima bean infusion is a source both of a suitable organic nitrogen and growth supplement for the organism.

Since peptone is a complex mixture containing minerals, amino acids, several vitamins as well as other nitrogen compounds, no important conclusion as regards nitrogen requirements of the fungus can be derived from its use. Similar is the case with lentil extract, yeast extract and lima bean infusion. The most important result is the presence of growth on medium M containing purified *d*-alanine supplemented with thiamin (Series IV). This shows that *P. phaseoli* requires a special amino acid as source of nitrogen for its growth and that it suffers from thiamin deficiency. Both amino acids and thiamin are considered necessary by Leonian and Lilly (1938) for *Coprinus lagopus, Chætocladium Brefeldii, Nyctalis asterophora, Pilaira moreaui* and *Pleurotus corticatus* also.

The other result to be interpreted is the failure of the organism to grow on a medium containing any ammonium salt. It is clear from the results of the experiments carried under Series VII *a* and *b* that ammonium salts have a toxic effect on its growth. Streets (1937) found ammonium compounds toxic to the root rot fungus when supplied in sufficient concentration. According to Blank (1941) and Blank and Talley (1941) poor growth with the ammonium salts can be attributed either to the rapid development of critical acidity or to a reduction in the availability of the trace elements. The media used by the authors do not contain high concentrations of ammonia, the salts of which are added in 0·2% concentration. There is no lack of trace elements in them since the fungus grows well in Leonian's medium with or without the addition of thiamin, yeast extract, lentil extract and bean infusion (Series VII *a*).

That a very acid condition of the media inhibits the fungal growth cannot be a fact in this case since the pH of the media containing ammonium salts, on which the fungus shows no growth, is the same, *i.e.*, pH 5 as that of the media without ammonium salts, on which the fungus grows. This conclusion is supported by the result of an experiment, not reported in the foregoing pages, that the fungus grows in 1% Difco bacto peptone solution, while it fails to do so when NH_4NO_3 is added to this solution, the pH in both the cases being 6·9.

It is evident that none of these factors are responsible for the absence of growth on the media supplemented with ammonium salts. Our results indicate that ammonium ion has a toxic effect on the growth of the fungus, but this toxicity may be due to the presence of some growth inhibitors in the purest ammonium salts obtained from the manufacturers (*i.e.*, *Proanalysi* of Merck's and Analar of British Drug House). It may be mentioned here that in a large number of fungi which have been or are being investigated in this laboratory, the same ammonium salts were used and none of them has so far been found toxic for any other fungus. The nature of toxicity will be dealt with in a subsequent note.

Summary

Phytophthora phaseoli Thaxter is unable to grow on a medium containing mineral salts, dextrose and inorganic nitrogen but requires for its growth a special amino acid (*d*-alanine) supplemented with thiamin. Other substances found to be suitable as nitrogen sources are peptone, hydrolysed peptone, casein, buttermilk, lentil extract, yeast extract and lima bean infusion, all of which, excepting casein, also supply the necessary growth substance.

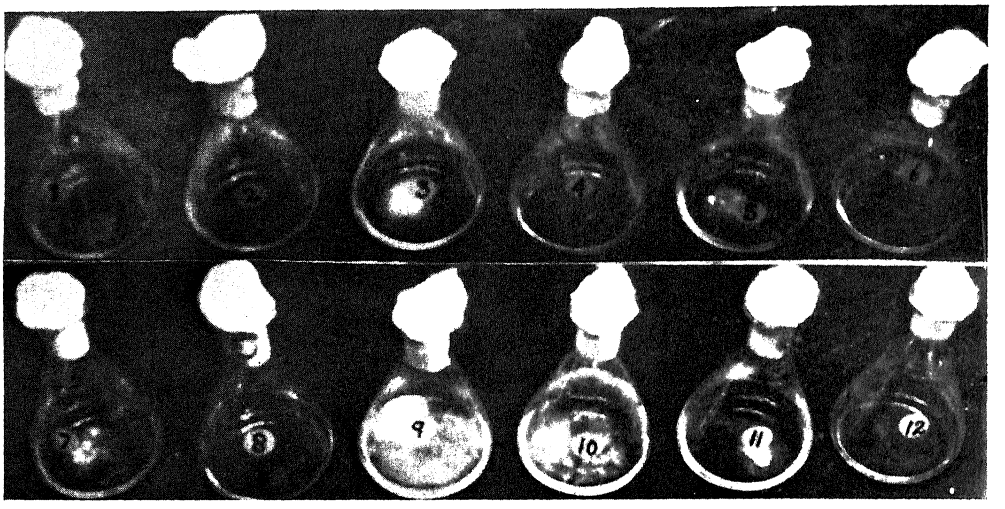


FIG. 1. Growth of *Phytophthora phaseoli* on various media used in Series VI *a*

(1) Leonian's medium. (2) Medium 1 + NH_4NO_3. (3) Medium 1 + Thiamin. (4) Medium 3 + NH_4NO_3. (5) Medium 3 + Ascorbic acid. (6) Medium 5 + NH_4NO_3. (7) Medium 1 + lentil extract. (8) Medium 7 + NH_4NO_3. (9) Medium 1 + bean infusion. (10) Medium 9 + NH_4NO_3. (11) Medium 1 + yeast extract. (12) Medium 11 + NH_4NO_3.

The fungus suffers from thiamin deficiency. Ammonium ion is found to be toxic for the growth of the fungus.

LITERATURE CITED

Blank, L. M. .. *Jour. Agr. Res.* (U.S.), 1941, **62**, 129–59.

——— and Talley, P. J. .. *Phytopath.*, 1941, **31**, 926–35.

Buston, H. W., and Pramanik, B. N. *Biochem. Jour.*, 1931, **25**, 1656–70.

Dusi, H. .. *Ann. Inst. Pasteur.*, 1933, **50**, 840–89.

Hawker, L. F. .. *Ann. Bot.*, 1936, **50**, 699–717.

Leonian, L. H. .. *Amer. Jour. Bot.*, 1925, **12**, 444–98.

——— .. *Ibid.*, 1930, **17**, 671–77.

——— and Lilly, V. G. .. *Phytopath.*, 1938, **28**, 531–48.

Robbins, W. J. .. *Amer. Jour. Bot.*, 1937, **24**, 243–50.

——— .. *Bull. Torrey Bot. Club*, 1938, **65**, 267.

——— and Kavanagh, V. .. *Bot. Rev.*, 1942, **8**, 411–71.

——— and Schmidt, M. B. .. *Bot. Gaz.*, 1938, **99**, 761–728.

Saksena, R. K. .. *Proc. Ind. Acad. Sci.*, 1941*a*, **14**, 141–48.

——— .. *Jour. Ind. Bot. Soc.*, 1941*b*, **20**, 183–89.

——— and Bhargava, K. S. .. *Proc. Nat. Acad. Sci. India*, 1941, **11**, 27–40.

Schopfer, W. H. .. *Arch. Mikrobiol.*, 1934, **5**, 511–49.

Streets, R. B. .. *Ariz. Agr. Exp. Stat. Tech. Bull.*, 1937, **71**, 299–410.

695-43 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore.

A FURTHER STUDY OF ATMOSPHERICS DURING THE MONSOON PERIOD

By Dr. N. S. Subba Rao, M.A., Ph.D.
(Annamalai University, Annamalainagar)

Received April 7, 1943
[Communicated by Prof. S. Ramachandra Rao, D.Sc. (Lond.), F.A.Sc.]

CONTENTS

Introduction

In a previous paper I explained that atmospheric activity curves show certain distinctive features during the period of the S.W. (or summer) Monsoon. It was also shown that with the aid of these curves, the formation of a meteorological depression in the Bay of Bengal, its movement inland, and finally its weakening and disappearance could be followed. The curves further show that with the withdrawal of the monsoon, heat thunderstorms which occur mainly in the afternoons become more pronounced.

During the year 1941, the S.W. Monsoon withdrew from the country by about the end of September and the N.E. Monsoon later established itself. The present paper discusses with the aid of data collected in 1941, the characteristics of atmospheric activity of the period (1) between the withdrawal of the S.W. Monsoon and the setting in of the N.E. Monsoon and (2) during the period of the N.E. (or winter) Monsoon.

(1) *Atmospherics during the period of transition*

As typical instances of the atmospheric activity of this period, the atmospheric activity curves from the 1st to the 5th October 1941, will be considered in relation to the weather charts of the same period.

The atmospheric activity curves are given in Fig. 1. The peaks of activity in the figure may be divided into two groups, (i) peaks K, L and M of early morning activity and (ii) peaks A, B and C of atmospheric activity in the evening and early night. These two groups will be discussed separately.

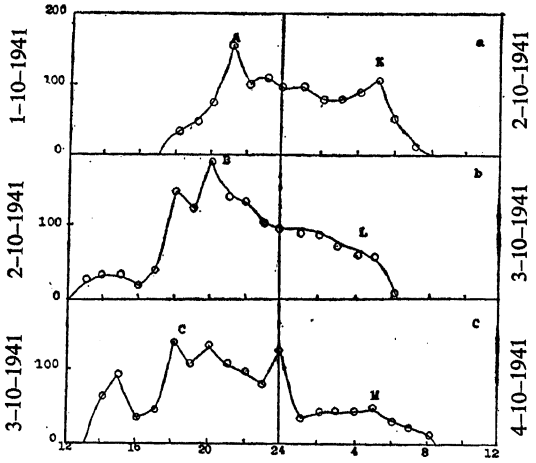
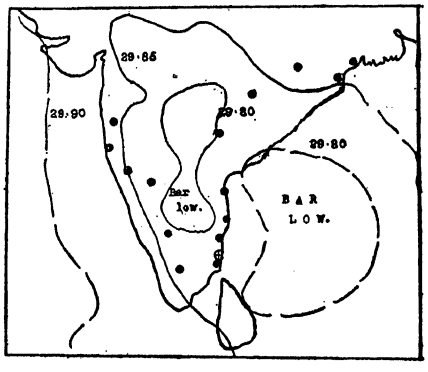
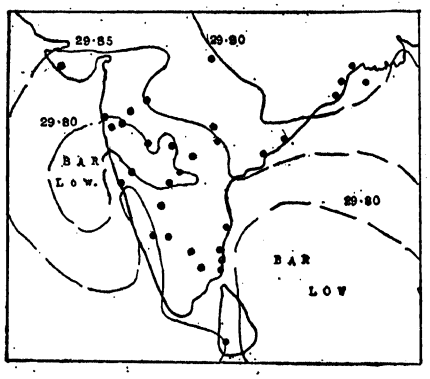


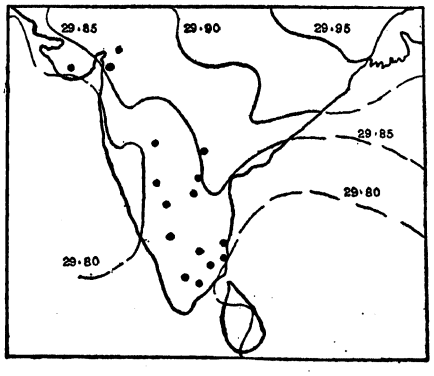
FIG. 1. Atmospheric Activity curves relating to the period of transition from the Summer to the Winter Monsoon



2 (*a*). 2–10–1941. At 8 hours.



2 (*b*). 3–10–1941. At 8 hours.



2 (*c*). 4–10–1941. At 8 hours.

FIGS. 2*a*, 2*b* and 2*c*. Weather charts relating to the period of transition from the Summer to the Winter Monsoon

● Indicates places from which thunderstorms have been reported during the previous 24 hours.

⊕ Indicates the position of the University Laboratories at Annamalainagar.

Peaks K, L and M.—The weather charts relating to this period are given in Fig. 2. Figs. 2*a*, 2*b* and 2*c* show the distribution of isobars at 8 hours on the 2nd, 3rd and 4th October respectively. On the 2nd a depression appeared in the Bay of Bengal close to the coast. By the morning of the 3rd this region of low pressure became diffuse. By the 4th this became more diffuse and unimportant.

It was pointed out (1943) in a previous paper that the formation of low pressure regions on the sea is associated with observed atmospheric activity in the early mornings. The peak K on the morning of the 2nd may therefore be associated with the low pressure region in the Bay of Bengal in Fig. 2*b*. We notice further that as the low pressure region in the Bay of Bengal becomes more and more diffuse the atmospheric activity represented by L and M decreases. Thus the occurrence and variation of early morning activity represented by peaks K, L and M can without doubt be ascribed to the low pressure region in the Bay of Bengal.

Peaks A, B and C.—Taking next the atmospheric activity in the early night, it must be remembered that during the period of the summer monsoon, large peaks of activity appeared in the early night only when a depression which had formed in the Bay of Bengal crossed the coast and moved inland. The peaks A, B and C resemble peaks of activity which accompanied the movement inland of the depression. In the present case, however, as is evident from the weather reports in Fig. 2, the depression that formed in the Bay of Bengal on the 2nd remained practically in its original position and became unimportant by the 4th. The peaks of atmospheric activity A, B and C cannot therefore be attributed to the effect of this depression.

Fig. 2 further shows that during the period between the 1st and 4th October, a diffuse low pressure area moved across the peninsula from east to west. It was over the peninsula on the 2nd and was off the west coast of India on the 4th. This 'low' over the peninsula became regularly intensified in the afternoon as can be seen from an examination of the weather charts giving the distribution of isobars at 17 hours on these days. (The charts showing this distribution are not reproduced.) This intensification of the 'Low' over the peninsula in the afternoons which is a regular feature of the pressure distribution in the non-monsoon months brings about conditions favourable for thunderstorm activity and for the generation of atmospherics associated with such activity. The peaks A, B and C are the effects of this intensification of the low over the peninsula.

The differences in the appearances of the peaks A, B and C may be explained by a detailed study of the thunderstorm activity of the period.

The regions from which thunderstorm activity is reported in the daily weather reports on these days are indicated by black dots on the weather charts. These reports of the thunderstorm activity refer to the previous twenty-four hours and do not give any indication of the time of the day at which these thunderstorms were active. But a study of a large number of atmospheric activity curves along with the related reports of thunderstorms has shown that there is a greater probability of thunderstorm activity along the coast line, occurring in the later part of the night, specially when a low pressure region exists on the sea and close to the coast. It would in this connection be extremely useful to analyse the actual times of occurrence of thunderstorms. This could not however be carried out for want of necessary data.

It was pointed out in an earlier paper (1943) that if thunderstorms are reported from a large number of areas, it is very unlikely that all these centres are active at the same time. The activity at one centre may be on the decline while that at another is reaching a maximum. Thus data on atmospherics collected from one observing station which cannot distinguish between atmospherics coming from various centres, is likely to show certain characteristic features. If only one narrow region is affected there will be a definite rate of increase of atmospheric activity, which will reach a maximum terminating in a peak, and after which there will be a regular decline resulting finally in zero activity. The atmospheric activity curve may therefore be expected to give a sharp well-defined peak of activity, the rise and decline of activity being very steep. If during a certain period, a large number of centres are active, assuming that the maximum activities at every centre do not necessarily coincide in time, the atmospheric activity curves will show large activity for a considerable time and well-defined peaks are not likely to be observed.

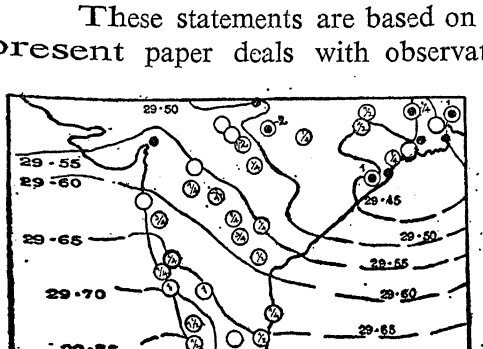
The low pressure areas on the evenings of the 1st and 2nd are concentrated into a narrow region while that on the 3rd is over a very large region. The areas from which thunderstorm activity is reported lie on the outskirts of the region of low pressure. It is no wonder therefore, that atmospheric activity in the evenings and early night shows sharp peaks like A and B as on the 1st and 2nd, while on the 3rd atmospheric activity at a large value continued from 18 to 24 hours and there are three peaks instead of one.

Thus during the period of transition large peaks of activity are observed in the evenings and the early night. This activity is governed mainly by the low pressure regions which develop in the centre of the peninsula and intensify by the evening each day during this period. Atmospheric activity in the later part of the night is on a small scale.

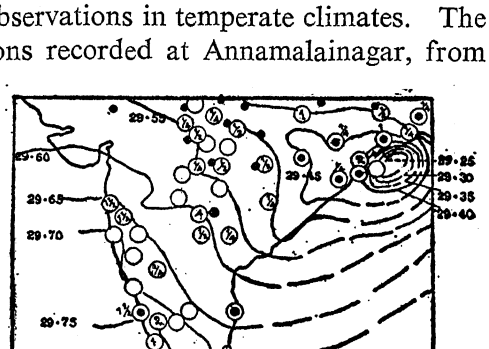
(2) *Atmospherics during the period of the Winter Monsoon*

Several observers have noticed that atmospheric activity during the winter months is on a much smaller scale than in the warmer months. One of the earliest of these is Jackson (1902) who noticed that Atmospherics are more frequent in summer and autumn than in winter and spring. Later, Wolf (1922) found that while during the warm half year atmospherics developed on the appearance of a new depression and disappeared on its filling up, no such relation could be noticed in winter. He says that even thunderstorms failed to give a notable increase in Atmospheric disturbance in winter. Watt (*Nature*, Vol. 127) found that the predominant source of the world's supply of Atmospherics lies in a region of summer afternoon.

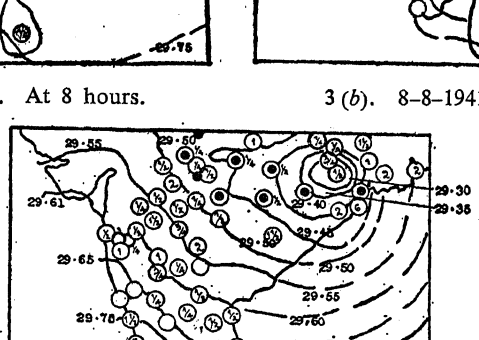
These statements are based on observations in temperate climates. The present paper deals with observations recorded at Annamalainagar, from



3 (*a*). 7–8–1941. At 8 hours.



3 (*b*). 8–8–1941. At 8 hours.



3 (*c*). 9–8–1941. At 8 hours.

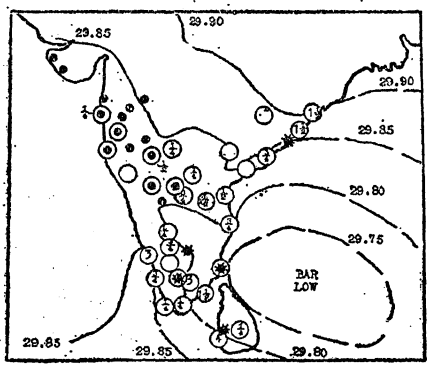
FIGS. 3*a*, 3*b* and 3*c*. Weather charts relating to the period 7th to 10th August 1941 during the Summer Monsoon

○ Indicates places from which rainfall is reported, the amount of rainfall during the previous 24 hours being indicated by the numbers inside the circles.

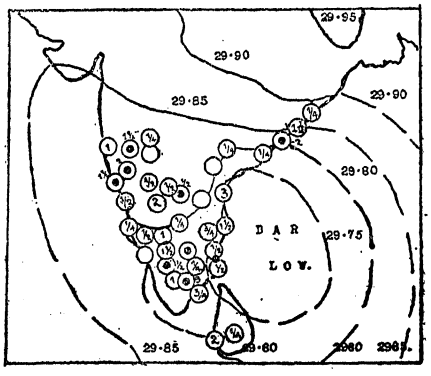
● Indicates places from which thunderstorms during the previous 24 hours have been reported.

October to December 1941 during the period of the winter monsoon. A study of atmospheric activity during this period shows no regular features of the type found in the period of the summer monsoon, and described by me in an earlier paper. I propose to discuss in this paper the probable causes for the striking difference noticed in the behaviour of Atmospherics during the two periods.

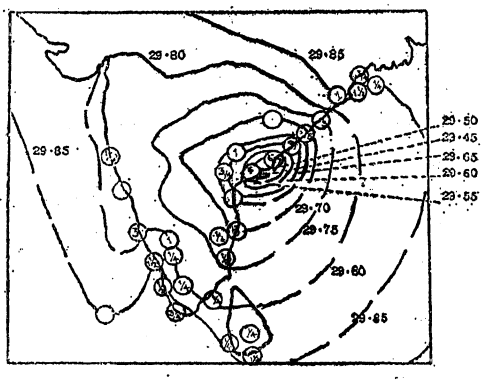
Two sets of weather charts, taken from the Indian Daily Weather Reports, are redrawn in Figs. 3 and 4. The first set refers to the period 7th–10th



4 (*a*). 5–10–1941. At 8 hours.



4 (*b*). 6–10–1941. At 8 hours.



4 (*c*). 7–10–1941. At 8 hours.

FIGS. 4*a*, 4*b* and 4*c*. The weather charts relating to the period 3rd to 8th October 1941 during the Winter Monsoon

- o Indicates places from which rainfall is reported, the amount of rainfall during the previous 24 hours being indicated by numbers inside the circles.
- ● Indicates places from which thunderstorms during the previous 24 hours have been reported.
- ✱ Indicates areas where thunderstorm activity attributable to the depression in the Bay of Bengal has occurred.

August 1941, during the summer monsoon and the other to the period 3rd–8th October 1941, the beginning of the North East Monsoon.

On these charts the circles indicate the regions from which rainfall is reported, during the previous 24 hours, the actual amount of rainfall being indicated by the numbers inside the circles. According to the convention followed in the mapping out of these charts rain less than ·09″ is neglected. A blank circle indicates rainfall between ·10″ — ·17″.

¼ — ·18″ to ·37″;
½ — ·38″ to ·87″;
¾ — ·68″ to ·87″;
1 — ·88″ to 1·24″;
1½ — 1·25″ to 1·74″;
2 — 1·75″ to 2·50″;
3 — 2·51″ to 3·49″, etc.

Weather charts in Figs. 3 *and* 4.—The charts refer to two similar cyclonic storms, one during the S.W. Monsoon and the other during the N.E. Monsoon. In their wake they brought considerable rain to the regions affected, *viz.*, Bengal and Orissa in the first case and S.E. Madras in the second case. In this respect therefore the two cyclonic storms are very similar.

Thunderstorm activity during these periods.—If we compare the thunderstorm activities during these periods we find a striking contrast. Areas from which thunderstorms are reported during the previous 24 hours are indicated by black dots in the weather charts. It is evident from Figs. 3*a* and 3*b*, that the deepening of the depression into a cyclonic storm is followed by increased thunderstorm activity. Thunderstorms are reported from a large number of areas and their positions give an indication of the probable direction of travel of the cyclone. In Fig. 3*c*, we find that as the depression slowly fills up thunderstorm activity is again on the decrease.

It will be clear from the above that thunderstorm activity increases with the deepening of the depression. The position of the areas of thunderstorm activity is mainly governed by the region of low pressure and the activity becomes feeble as the low pressure region fills up.

In Fig. 4*a*, we notice thunderstorm activity at certain places in the Peninsula, a large number of areas to the N.W., a few in the south of the Peninsula and a few along the east coast. From Fig. 4*b*, we find that the depression moved nearer the coast line. In striking contrast to what was noticed in the set of charts given in Fig. 3, we find that with the approach of the depression towards land thunderstorm activity is reported from fewer areas and is hence on the decline. In Fig. 4*c*, we find that the intensification of the depression into a cyclonic storm close to the coast between Masulipatam

and Cocanada caused widespread heavy rain along the Madras coast and the adjoining districts but no thunderstorms occurred in any of these areas.

Thus we find that thunderstorm activity which increases during the summer monsoon with the intensification of a depression, disappears under similar conditions during the winter monsoon. As most atmospherics orginate in areas of thunderstorm activity, it is no wonder that atmospheric activity during the period of the summar monsoon shows certain regularities, increasing activity being associated with the formation and accentuation of a depression and its decreasing activity with the movement and disappearance of the low pressure region. In the period of the winter monsoon these features are not noticed.

In this connection one other point may also be mentioned. In Fig. 4*a* the presence of a large number of thunderstorm areas might create an impression that thunderstorm activity is very much greater and further that all these might be due to the effect of the depression formed in the Bay of Bengal. A study of the weather charts for the previous two days, *i.e.*, 3rd and 4th, helps us to determine the true effect of the depression in the Bay of Bengal.

On the 2nd October a depression in the Bay was accompanied by a region of low pressure in the central region of the Peninsula. The chart for this day is given as Fig. 2*b* and thunderstorms were reported from areas lying on the coast line on either side of the land depression.

Judging from the way in which the areas of thunderstorm activity appear and disappear during the three days, 2nd, 3rd and 4th October, it appears as though the activity in the N.W. region of the Peninsula may be attributed to the depression on land noticed on the 4th and to the depression in the Arabian Sea (Fig. 2*c*). We are thus left with a few areas only, areas which showed no variation with the appearance or disappearance of the depression in the Arabian sea, or the depression on land. These areas are indicated in the figure thus✳. It will thus be evident that even when thunderstorm activity is reported and atmospherics are recorded at the observing station, they arise only from small isolated areas and that the total atmospheric activity itself is small.

One example may be given to show how small the atmospheric activity is when it exists. A deep depression formed in the Bay of Bengal on the 30th of November intensified into a cyclonic depression on the 1st December, became severe and passed inland by the night of the 2nd. Later this moved right across the Peninsula and entered into the Arabian Sea by the 4th and became unimportant by the 6th.

This particular example is of special interest as the cyclonic storm passed almost over the observing station. If there had been atmospheric activity of the intensity usually met with in the summer monsoon period, no record could have been obtained, as the recording equipment would have been upset on account of the powerful impulses given to the galvanometer. Although a few atmospherics were actually recorded during this period, the atmospheric activity must be judged as being of negligible amount, the little activity that was observed being attributable to very feeble isolated lightning flashes possibly occurring far above the monsoon clouds, invisible from the ground and unaccompanied by thunder. As the convention in the Indian Meteorological Department is that thunderstorm should be reported only when thunder is heard with or without precipitation, the Weather Reports do not mention any thunderstorm during this period.

Discussion

As stated already during the period of the Summer monsoon thunderstorm activity and the associated Atmospheric activity are on a large scale, while during the Winter monsoon they are of negligible amount. It was also pointed out that atmospheric activity is intimately connected with meteorological depressions, and that the study of the variation of Atmospheric activity from hour to hour and day to day enables us to follow the various stages in the formation, accentuation, movement inland and the subsequent filling up of the meteorological depressions that originate in the Bay of Bengal during this period.

It may be considered surprising that there is only a small amount of thunderstorm and Atmospheric activity during the period of the winter monsoon. Further, as has been already pointed out, the little Atmospheric activity that does exist, becomes feeble on the intensification of a depression.

Brunt (1934) considers the greater frequency and intensity of the meteorological depressions in Europe in winter as due to the larger differences of temperature between the pole and the equator in winter than in summer. The reason for the feeble thunderstorm activity during this period may be understood from a discussion of the various features involved in the formation of depressions and the generation of thunderstorm and the associated Atmospheric activity.

The most important of these factors is Solar Radiation. The effect of the sun in bringing about conditions favourable for the display of energy automatically in the atmosphere in the form of a thunderstorm is described by Napier Shaw and Captain Douglas. According to Napier Shaw (1930) the first condition to be satisfied is the existence of a state of liability in the atmosphere

caused by the establishment of a thick layer in convective equilibrium. Another condition is the provision at the right moment, of a sufficient quantity of air saturated with water vapour. Both these conditions are governed by the effect of Solar Radiation.

Convection on a large scale in air of a high degree of humidity is essential for the production of towering cumulus clouds whose base, according to Captain Douglas (1923) is usually between 5,000 to 6,000 feet, in severe thunderstorms, and whose tops attain 20,000 feet in summer and 15,000 feet in winter. Captain Douglas also states that the existence of at least one damp layer as low as 6,000 feet appears to be a necessary condition for the development of thunderstorms.

The electrification of thunderclouds is explained by the well-known theories of Wilson and Simpson. The mechanism suggested by Wilson as the cause of opposite charges on large and small drops inside a thundercloud depends upon the presence in the cloud of a number of slow ions. In the earlier stages of development of a thundercloud these may arise from natural sources, but later on they will be supplied in numerous numbers by brush discharges from water drops (Macky, 1933) drawn out by the field into pointed forms (Schonland, 1932). According to Simpson (1927) the generation of electricity is a consequence of the disruption of rain drops when caught in a vigorous convection current. A water drop with a radius of more than 0·25 cm. becomes flattened out and unstable when it falls through the air with the result that it breaks up into a number of smaller drops. Since the terminal velocity of a falling drop with a radius of 0·25 cm. is 8 metres per second, it follows that no drop of water can ever fall downward through an ascending current of air whose vertical velocity exceeds 8 metres per second.

Formation of a meteorological depression.—Convection again plays an important part in the formation of depressions. The formation of a depression involves the removal of large amounts of air for the fall of pressure. All theories put forward for explaining the formation of a depression are based on the existence of horizontal differences of temperature and the displacement of warm air by cold air. According to Brunt (1934) the most obvious method of removal of air is by means of a convection current, produced by inequalities of temperature, or water vapour content or by the effect of surface discontinuity. If warm damp air is set in motion by its buoyancy the continuance of the upward motion as an effect of the condensation of moisture is easily understood. Such an ascending current has a scouring effect on the environment, the process being aptly described as 'eviction of air'.

It is thus seen from the above that vigorous convection plays a most important role in the formation of cumulus clouds, in the generation of electrical charges in thunderclouds and also in the formation of depressions. Convection is dependent on Solar Radiation and therefore it would be instructive to consider how these effects are modified by the varying amounts of solar radiation received at various parts of the globe and at the various seasons.

It is well known that thunderstorms are more frequent and more violent in the tropics. Simpson (1927) attributes this in the first place, to the larger water vapour content of the air in the tropics which provides the greater energy for driving the currents upwards to much greater heights. Secondly, the height at which the freezing point is reached is much greater and finally the stratosphere is so much higher that there is more vertical room in which the thunderstorms can develop. By an extension of the same argument, the cause of the smaller thunderstorm activity during the period of the winter monsoon as compared with that of the summer monsoon may be traced to the lower temperatures during the former period.

There seems to be no doubt that convection is quite vigorous during the period of the winter monsoon. Napier Shaw (1930) points out that rainfall is the best index of convection and that the absence of rainfall is equally good evidence of the absence of any persistent ascending current. The large amount of rainfall that occurs on the outskirts of the depressions formed during the period of the monsoon, in the Bay of Bengal, is sufficient evidence of the existence of convection on a large scale. But there is no thunderstorm activity in spite of the large convection.

Brunt (1934) points out that in addition to the mechanism that gives rise to vertical convection in any region where a lowering of pressure is taking place, there must be present some mechanism for removing the air that has ascended. Otherwise this vertical motion cannot continue for any length of time. The simplest mechanism that may be thought of is an upper current whose direction differs from that of the current in the lower troposphere. Brunt takes the outward motion of cirrus clouds from the centre of a cyclonic system as evidence of the existence of such a current. In the absence of some means of removal of the evicted air a thunderstorm is a more likely occurrence than a cyclonic system. The absence of thunderstorm activity during the winter monsoon period even when vigorous convection is taking place may probably be due to the causes enumerated above.

Finally there is also the conductivity of the atmosphere. Considering the smaller space, in which thunderstorms during the winter may develop,

the intensity of the depressions formed during the period and the large amount of rainfall accompanying the formation of cyclonic systems, it may be expected that the conductivity of the atmosphere may rise to large amounts and give rise to leakage effects. Schonland (1932) from a study of the recovery curves of a thundercloud after a discharge showed that the electrical energy generated by the storm is only partially employed in the feeding of the flashes. At the moment just before the discharge most of the power of the machine is expended in overcoming various leakage effects. If the conductivity of the atmosphere becomes very large, it may happen that the potential of the thunderclouds may never reach the sparking value.

A review of recent work on Atmospherics in Nature (1936) states that it is now generally agreed that the majority of atmospherics encountered in Radio Communication originate in lightning flashes. When the storm is close to the receiver it is possible to identify the stronger atmospherics with the neighbouring flashes. It is therefore quite easy to understand that if the potential of the thunderclouds does not attain the sparking value no lightning flashes can occur and hence the amount of Atmospheric activity must be very small indeed.

Some observations on the variation of Atmospheric activity with precipitation and conductivity lend support to the above view. Wiedenhoff (1921) found that atmospheric activity was at a minimum with maximum conductivity at a moderate height with overcast sky, with maximum relative humidity and minimum temperature. Rothe (1921) found that atmospherics produced by storm clouds cease as soon as uniform rain starts.

Thus the striking difference in the variation of Atmospheric activity in the periods of the Summer and the Winter Monsoon may be traced to (i) the lower temperature, (ii) the smaller height available for the development of thunderstorm activity, (iii) the operation of a mechanism by which the ascending current of air is removed by means of an upper air current and (iv) the larger leakage effects that are likely to prevent the charges in the cloud building up to the sparking value.

Summary

The paper discusses the characteristics of Atmospheric Activity during (i) the period of transition from the South West (or the summer) monsoon to the North East (or the Winter) monsoon, and (ii) the North East (or the Winter) monsoon.

During the period of transition large peaks of activity are observed in the evenings and the early night. This activity appears to be governed mainly by the pressure regions which develop in the centre of the Peninsula by

the evening each day during this period. Activity in the later part of the night is on a small scale.

It was pointed out in a previous paper that during the summer monsoon, unsettled conditions in the Bay of Bengal give rise to continuous Atmospheric Activity throughout the night; the formation of a depression is accompanied by large atmospheric activity in the early mornings; the movement of the depression inland by an increase in early night activity and the disappearance of early morning activity. It was further shown that the various stages in the formation, movement inland and finally the filling up of the depression could be followed with the aid of the Atmospheric Activity curves.

During the period of the North East Monsoon, however, there is a striking contrast. With the setting in of the monsoon the atmospheric activity becomes very feeble. Further, the little activity that does occasionally exist disappears on the accentuation of depressions that are formed in the Bay of Bengal and the Arabian Sea during this period.

In the later part of the paper the probable causes for this difference in behaviour are discussed. It is suggested that this difference in behaviour may be due to (i) the lower temperatures, (ii) the smaller height available for the development of thunderstorm activity, (iii) the operation of a mechanism by which the ascending current of air is removed by an upper air current, and (iv) the larger leakage effects that are likely to prevent the charges in the cloud from building up to the sparking value.

In conclusion I have much pleasure in expressing my thanks to Dr. S. Ramachadra Rao, D.Sc. (London), Professor of Physics, for valuable guidance and helpful criticism.

REFERENCES

Brunt .. *Physical and Dynamical Meteorology*, Camb. Univ. Press, 1934.

Douglas, Captain.. *Dictionary of Applied Physics*, 1923, **111**, 41.

Jackson .. *Proc. Roy. Soc.*, 1902, **A 70**, 266.

Macky .. *Ibid.*, 1933, **A 133**, 565.

Marchand .. *Rev. Gen. Sc.*, Paris, 1921, **32**, 594.

Rothe .. *C. R. Acad. Sc.*, Paris, 1921, **175**, 840.

Schonland .. *Atmospheric Electricity*, Methuen's Monographs on Physical subjects, 1932.

Shaw, Napier .. *Manual of Meteorology*, Camb. Univ. Press, 1930, **111**; 280.

Simpson .. *Proc. Roy. Soc.*, 1927, **A 114**, 376.

Subba Rao .. *Proc. Ind. Acad. Sci.*, 1943, **17**, 83.

Watt, Watson .. *Nature*, 1925, 127.

Wiedenhoff .. *Jahrb Drahtl Telegraph*, Berlin, 1921, **18**, 242.

A STUDY OF *'CARPASEMINE'* ISOLATED FROM *CARICA PAPAYA* SEEDS

BY T. B. PANSE AND A. S. PARANJPE

(Pharmacology Department, Seth G. S. Medical College, Parel, Bombay)

Received July 21, 1943

(Communicated by Dr. K. Venkataraman, F.A.SC.)

THE seeds of *Carica papaya* are said to possess powerful anthelmintic and emmenagogue properties.[1] In order to verify these claims and other medicinal properties claimed for the seeds, a pharmacological study of the seeds was undertaken and as a part of the work the chemical analysis of seeds was first carried out.

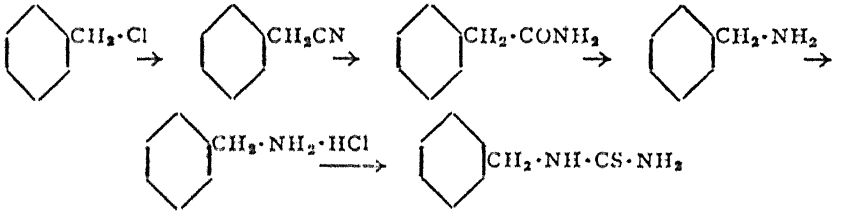
Reference to previous work on the subject showed that Greshoff[2] had isolated mainly from the leaves (and is also stated to have obtained from the fruit and seeds) of *Carica papaya* Linn., the alkaloid Carpaine, m.p. 121°. We have, however, isolated from the seeds a crystalline substance, melting at 165°. In a preliminary note[3] on the substance it was provisionally named 'Carpasemine' because of its alkaloidal character, such as the presence of nitrogen in the molecule and positive results for tests with reagents for alkaloids.

A careful examination of the constituent elements in the substance showed that it contained sulphur (instead of O_2 as was assumed in our previous note) together with carbon, hydrogen and nitrogen. The results of the ultimate analysis of the substance as well as those of its molecular weight determination by cryoscopic method, indicated the molecular formula to be $C_8H_{10}N_2S$, for the substance. The substance gave an acetyl derivative, m.p. 131° and also formed a methiodide compound, m.p. 103°. However, it did not form salts with the common acids. On boiling the substance, m.p. 165°, with sodium hydroxide, an " oil " (A) and a product (B), m.p. 148–9° (in low yield), were obtained. Evolution of ammonia was noticed during the reaction. The " oil " (A) formed a hydrochloride, m.p. 245–6°. While the product (B) formed an acetyl derivative, m.p. 130° and was not found to contain sulphur. On oxidation, the product (B) yielded a compound, m.p. 205–7°, which is under investigation. During the oxidation a distinct smell of benzaldehyde was noticed. Direct oxidation of the original substance, m.p. 165°, however yielded benzoic acid.

The oxidation results indicated that the substance, m.p. 165°, contained a benzene ring with a side chain. Evolution of ammonia on boiling with sodium hydroxide, suggested it to be a substituted ammonia derivative, which could also form acetyl and methiodide compounds. While its weak

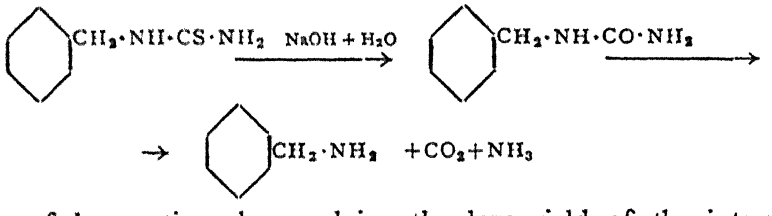
basic character together with the fact that it contained sulphur indicated it to be a phenyl derivative having probably as its side chain a heterocyclic ring containing S and N or a thiourea residue.

On referring to the literature, it was found that benzylthiourea (or benzylthiocarbamide) possessed properties similar to those enumerated for the substance, m.p. 165°. In order to confirm this assumption, a direct comparison of the natural product and synthetic benzylthiourea was considered nesessary. The synthetic benzylthiourea was prepared by the scheme indicated below :—



The mixed melting point of the product, m.p. 165° isolated from the papaya seeds with the synthetic benzylthiourea (Salkowski)[4] showed no depression and thus established the identity of the former with the latter. The identity was further confirmed by the mixed melting point of their acetyl derivatives.

It is known that substituted thioureas when boiled with aqueous alkali decompose into a substituted amine, carbon dioxide, hydrogen-sulphide and ammonia. In view of this fact, the " oil " (A) obtained on boiling the substance, m.p. 165° with alkali, was suspected to be benzylamine. The surmise proved to be correct as the mixed melting point determination of the hydrochloride of (A) and the hydrochloride of benzylamine showed no lowering. The other product (B) obtained on boiling the substance m.p. 165° with alkali, appeared from its properties to be benzylurea (or benzylcarbamide). It was subsequently identified to be benzylurea by a mixed melting point with synthetic benzylurea (Paterno and Spica).[5] The action of 20% aqueous sodium hydroxide solution on benzylthiourea may, on the basis of the results obtained above, be representated as follows :—



The course of the reaction also explains the low yield of the intermediate product, benzylurea, in view of the possibility of its further hydrolysis into benzylamine, carbon dioxide and ammonia.

The pharmacological study of benzylthiourea or 'carpasemine' is in progress and the results of the investigation will be published elsewhere in due course.

Experimental

Isolation of the substance, m.p. 165°, *from* Carica papaya *Seeds.*—Dried papaya seed powder (100 gm.) was digested in Prollius's fluid (approx. 550 c.c.) with occasional shaking during forty-eight hours. The liquor was then filtered off and the seed powder discarded. The filtrate was allowed to evaporate at the room temperature, when an oil together with some solid was obtained as a residue. The solid after separation from the oil was crystallised from boiling water. The repeated crystallisations of the solid resulted in the formation of beautiful bunches of colourless crystals, m.p. 165°, yield 0·35% of the seed powder. It may be mentioned here, that the yield of the substance was seriously hampered when the seeds were powdered long before the extraction. Analysis (Found: C, 57·95, H, 6·44, N, 16·3, S·19·3%, $C_8H_{10}N_2S$ requires C, 57·8, H, 6·0, N, 16·9, S, 19·3%). The molecular weight of the substance (determined by the cryoscopic method, using nitrobenzene as solvent) was found to be 163 which indicated the molecular formula $C_8H_{10}N_2S$ for the substance. The substance is bitter to taste. It is insoluble in cold water, petroleum ether and benzene but fairly soluble in alcohol and acetone. A mixed melting point of the substance with benzylthiourea (Salkowski,[4] m.p. 164°) showed no depression.

Acetyl derivative of the product, m.p. 165°—was prepared with sodium acetate and acetic anhydride as usual. The acetate crystallised from 40% alcohol into prisms, m.p. 131°. Analysis (Found: N, 13·2%; $C_{10}H_{12}N_2OS$ requires N, 13·46%). The mixed melting point of this acetate with the acetate of benzylthiourea (Werner,[6] m.p. 129–30°) showed no depression.

Methiodide of the product, m.p. 165°.—Equimolecular quantities of methyliodide and the product, m.p. 165°, were added to methyl alcohol and refluxed on a water-bath for four hours. Alcohol was then distilled off and a sticky residue was obtained. The residue, which solidified on keeping, was crystallised from benzene into fine hexagonal crystals, m.p. 103–5°. Analysis (Found: N, 9·1%, $C_9H_{13}N_2SI$ requires N, 9·0%). The product was fairly soluble in water.

Oxidation of the product, m.p. 165°.—0·5 Gm. of the product, m.p. 165°, was dissolved in warm water and (30 c.c) of potassium permanganate solution (2%) was added to it in dropwise manner. After the reaction was over the manganese dioxide precipitate was filtered off. The excess of potassium permanganate in the filtrate was then destroyed with a few drops of alcohol and manganese dioxide precipitate again filtered. The resulting clear filtrate,

on cooling was extracted with ether, which on dehydration with anhydrous sodium sulphate followed by evaporation yielded a solid, m.p. 120°. It was identified as benzoic acid.

The action of alkali on the product, m.p. 165°.—0·5 Gm. of the product, m.p. 165°, was refluxed with 20% aqueous sodium hydroxide solution (75 c.c.) for four hours. During the reaction a distinct smell of ammonia was perceptible. The alkaline reaction mixture on cooling, was extracted with ether. Dehydration of the ethereal layer with anhydrous calcium chloride followed by evaporation gave a crystalline solid and an oil. The solid was separated from the oil and crystallised from benzene into light lustrous needles, m.p. 148–9°. Yield 7%. Analysis (Found: C, 64·52; H, 6·75; N, 18·57%; $C_8H_{10}N_2O$ requires C, 64·00, H, 6·4, N, 18·66%). A mixed melting point of the product, m.p. 148–9°, with benzylcarbamide (Paterno and Spica,[5] m.p. 147°) prepared from benzylamine and potassium cyanate showed no depression.

Acetate of the product, m.p. 148–9°—was prepared with sodium acetate and acetic anhydride as usual. The acetate was crystallised from boiling water and was found to melt at 130°. Analysis (Found: C, 62·95, H, 6·42, N, 14·55%; $C_{10}H_{12}O_2N_2$ requires C, 63·0, H, 6·25, N, 14·6%).

Oxidation of the product, m.p. 148–9°.—0·225 Gm. of the product m.p. 148–9°, was dissolved in warm water and 15 c.c. of potassium permanganate solution (2%) was added to it in a dropwise manner. During oxidation bitter almond-oil-like smell was noted. After the reaction was complete, the manganese dioxide precipitate was filtered off. The excess of potassium permanganate in the filtrate was destroyed with a few drops of alcohol and the precipitate of the manganese dioxide again filtered off. The resulting clear filtrate was concentrated on a water bath and on cooling, deposited beautiful hexagonal crystals, m.p. 205–7°. The product is under further investigation.

Hydrochloride of the oil obtained in the alkali boiling of the product, m.p. 165°.—A few drops of the oil were treated with conc. hydrochloric acid when a solid was thrown down immediately. The solid crystallised from alcohol into thin rectangular plates melting at 245–6°. The hydrochloride did not show any lowering in the melting point when admixed with benzylamine hydrochloride (Curtius and Lederer,[7] m.p. 246°).

Synthesis of Benzylthiourea.—To a warm solution of sodium cyanide (52 gms.) in water (45 c.c.) was slowly added benzyl chloride (103 gm.) diluted with equal volume of alcohol. The reaction was completed by heating on a water-bath for three hours. Sodium chloride was removed by filtration and alcohol distilled off from the filtrate. Benzyl cyanide separated as an oil from the residual aqueous portion and purified by distillation under reduced pressure 10 mm. had a b.p. 115–20°. (Cannizzaro,[8] b.p. 115–20°.) Yield

80 gm. benzyl cyanide (10 gm.) prepared as above was mixed and shaken with sulphuric acid (15 gms. D. 1·82) care being taken that the temperature is not raised above 65–70°. After the heat of the reaction was over, the mass was poured in water. The resulting precipitate of phenylacetamide was purified by treatment with sodium bicarbonate solution, followed by washing with water and crystallisation from hot water, m.p. 155° (Purgotti,[9] m.p. 155–6°). To 135 gm. of phenyl acetamide (1/10 mol.) prepared as above was added bromine (8·0 gm. 1/10 mol.) dissolved in 4 molecular proportions of potassium hydroxide in 16 parts of water. The solution was heated rapidly and as soon as a clear solution was formed, it was distilled off to obtain benzylamine as an oil in the distilate, b.p. 185° (Hoogewerff and van Dorp,[10] b.p. 185°). The benzylamine by treatment with hydrochloric acid was converted into its hydrochloride, m.p. 245° (Curtius,[7] m.p. 246°). The hydrochloride on heating with equimolecular quantity of potassium sulphocyanide at a temperature of 120° for fourteen hours, yielded a brownish residue which after washing with cold water, was crystallised from boiling water into colourless needles. The benzylthiourea melted at 164° (Salkowski,[4] m.p. 164°). Yield 80%.

Summary

From the seeds of *Carica papaya* a substance, m.p. 165° ($C_8H_{10}N_2S$) has been isolated for the first time and named 'Carpasemine' to indicate its source. The chemical properties of 'Carpasemine' together with its degradation products have been studied and some new derivatives have been prepared from it. 'Carpasemine' has been identified to be benzylthiourea or benzylthiocarbamide by mixed melting point with the synthetically prepared benzylthiourea. The identity has also been confirmed through the mixed melting point of their derivatives.

The present investigation forms a part of the Indigenous Drugs Inquiry financed by the Indian Research Fund Association, to which our thanks are due.

REFERENCES

1 Kirtikar and Basu .. *Ind. Med. Plants*, Part I, 1918, 575.
2. Greshoff .. *Ber.*, 1890, **23**, 3537.
3. Panse and Paranjpe .. *Rasayanam*, 1941, **1**, No. 7, 215.
4. Salkowski .. *Ber.*, 1891, **24**, 2724.
5. Paterno and Spica .. *Ibid.*, 1876, **9**, 81.
6. Werner .. *Jl. Chem. Soc.*, 1891, **59**, 408.
7. Curtius and Lederer .. *Ber.*, 1886, **19**, 2463.
8. Cannizzaro .. *Ann.* 1855, **96**, 247.
9. Purgotti .. *Gaz. Chim. Ita.*, 1890, **20**, 173, 593, Anm. 2, quoted from Beilstein.
10. Hoogewerff and van Dorp *Res. des tra. chim.*, 1886, **5**, 253, quoted from Beilstein.

CHEMICAL COMPOSITION OF *CALOTROPIS GIGANTEA*

Part I. Wax and Resin Components of the Latex

BY P. BHASKARA RAMA MURTI* AND T. R. SESHADRI

(*From the Department of Chemistry, Andhra University, Waltair*)

Received August 25, 1943

Calotropis gigantea is a shrub common in the eastern and southern parts of India, Ceylon and Eastern Asia. The milky juice obtained from it is used for medicinal and insecticidal purposes and frequent cases of criminal poisoning by its means are known. The possibilities of obtaining a guttapercha-like solid from this latex have also been considered in the past. Its chemical composition was first investigated by Basu and Nath[1] who employed the dried latex. From the unsaponifiable portion of the ether-soluble matter they isolated, after repeated purification, a product which was considered by them to be a sterol of the formula $C_{28}H_{44}O$. It was named calosterol. They, however, noted that this substance did not form a precipitate with digitonin and in most of its reactions differed from ordinary sterols. As part of their investigations on African arrow poisons G. Hesse and co-workers[2] examined the mixed latex obtained from *Calotropis procera* and *Calotropis gigantea*. From the alcohol-soluble portion were obtained usharin, calotoxin and calactin which belong to the group of cardiac poisons.

Considerable quantities of the latex can be collected easily and the material is best preserved by adding a little chloroform. Some amount of preliminary investigation had to be done in order to work out the most suitable method of separating the components. The procedure adopted by Basu and Nath of drying the whole material so as to obtain a solid does not seem to be satisfactory, since it leads to undesirable decompositions and the separation of the various components from the resulting product is also difficult. Coagulation by simple heating or by treatment with acids with a view to separate fractions is better, but still not adequate. The most satisfactory procedure is to add enough alcohol to produce a filterable precipitate and a clear filtrate, and it renders the separation of the various components more easy. The soft coagulum (I) and the aqueous alcoholic solution (II) were separately examined.

* At present working in the Laboratories of the Board of Scientific and Industrial Research, Delhi.

The coagulum (I) was repeatedly extracted with boiling alcohol and subsequently with ether. It was finally separated into a sticky solid (I *a*), which was difficultly soluble in ordinary solvents and which is still under investigation and a portion easily soluble in ether (I *b*). The above separation rendered the study of the ether-soluble fraction more easy. This was a crystalline solid melting between 120° and 170°. With a view to understand the nature of the components present, colour reactions were carried out using several reagents. The most characteristics reactions were a deep pink colour with the Liebermann-Burchard reagent and a yellow solution with the Salkowski reagent and these indicated the presence of triterpene compounds. With a view to obtain some information about the exact condition in which these compounds occur and if possible to obtain a pure substance repeated crystallisation was carried out. A small amount of a fraction melting at 248–50° (solid A) could be isolated; it will be described later on. The remaining portion was, therefore, subjected to saponification and the unsaponifiable matter and the fatty acids analysed separately.

The unsaponifiable matter was a pale yellow solid and consisted mostly of resinols. It was divided into two fractions using solubility in alcohol: less soluble (B) and more soluble (C). Since no definite entity could be obtained from them by adopting methods of crystallisation, they were independently subjected to acetylation and benzoylation and these esters were subsequently subjected to fractional crystallisation. Such a procedure has yielded very good results in the hands of Heilbron and co-workers in similar cases. The existence of α- and β-amyrins and of lupeol in the waxy portion of *Decalepis Hamiltonii* and *Hemidesmus indicus* could be established by adopting this technique.[3] By the acetylation of (B) and crystallisation of the mixed acetates using ethyl acetate a definite compound melting at 250–51° could be obtained as the major product. Its sparing solubility was noteworthy; its crystal structure (elongated hexagonal plates) was very characteristic and was very sensitive to the presence of impurities; $[\alpha]_D^{32°}$, + 98·0° in benzene solution. With Liebermann-Burchard reagent it produced a pink solution which deepened slowly to purple. The colour faded very slowly and after several hours it appeared to be pale blue and eventually turned yellowish brown. With Salkowski's reagent it produced a yellow solution exhibiting powerful green fluorescence. These colour reactions seemed to be individual characteristics of the substance and also indicated that it belongs to the triterpene group. This surmise was supported by the results of combustion analysis which corresponded to the formula, $C_{32}H_{52}O_2$ and by a molecular weight determination. The latter value was obtained by employing the

saponification equivalent, since it could not be correctly obtained by the camphor method of Rast. Hydrolysis of the acetate gave rise to an alcohol melting at 204–5° $[\alpha]_D^{32°}$, + 102·0° in benzene solution and its composition corresponded to the formula, $C_{30}H_{50}O$. It formed no combination with digitonin. Since it did not appear to be identical with any known compound it has been given the name, α-calotropeol indicating its isolation from Calotropis. Its benzoate melted at 273–74°, $[\alpha]_D^{32°}$, + 74·3° in bezene solution. Thus the alcohol and its esters are all dextrorotatory. From a determination of the iodine value of the acetate the presence of one double bond in the compound could be established. From the mother liquors left after the separation of α-calotropeol-acetate, small quantities of the acetates of another new alcohol, β-calotropeol (see below) and of β-amyrin could be obtained with great difficulty. However, β-calotropeol was more easily obtained in good quantity as its benzoate from the crystallisation of the mixed benzoates produced by the benzoylation of the original resinol mixture (B).

By the repeated crystallisation of the mixed benzoates a substance melting sharp at 279–80° could be isolated. It was different from α-calotropeol benzoate, the mixed melting point being depressed and its specific rotation, $[\alpha]_D^{32°}$, + 69·0° in benzene solution was also different thus showing that they are different substances. However, from combustion analysis and molecular weight determination it was found to be isomeric. The free alcohol obtained from it melted at 216–17° and had the specific rotation, $[\alpha]_D^{32°}$, + 50·9° in benzene solution and was thus different from α-calotropeol. It was, therefore, named β-calotropeol. It resembled α-calotropeol in its composition and colour reactions. The individuality of β-calotropeol was further supported by the preparation of the acetate which was found to melt at 238°, $[\alpha]_D^{32°}$, + 43·9°, and to be quite different from α-calotropeol acetate, the mixed melting point being depressed. It should be mentioned here that the calotropeols were found to be stable and their acetates did not undergo any change on boiling with formic acid.

The more soluble fraction (C) of the unsaponifiable matter was similarly analysed. The acetate method seemed to be the most satisfactory in this case and by the crystallisation of the crude acetates, a pure substance melting sharp at 239–40° could be isolated. It had the formula, $C_{32}H_{52}O_2$ and gave colour reactions characteristic of pentacyclic triterpenes. On hydrolysis it yielded an alcohol melting at 196–97°. The acetate and the alcohol were found to be identical with β-amyrin acetate and β-amyrin respectively by comparison with authentic samples obtained from the roots of *Decalepis Hamiltonii*. The more soluble portion of the acetates yielded finally a fraction

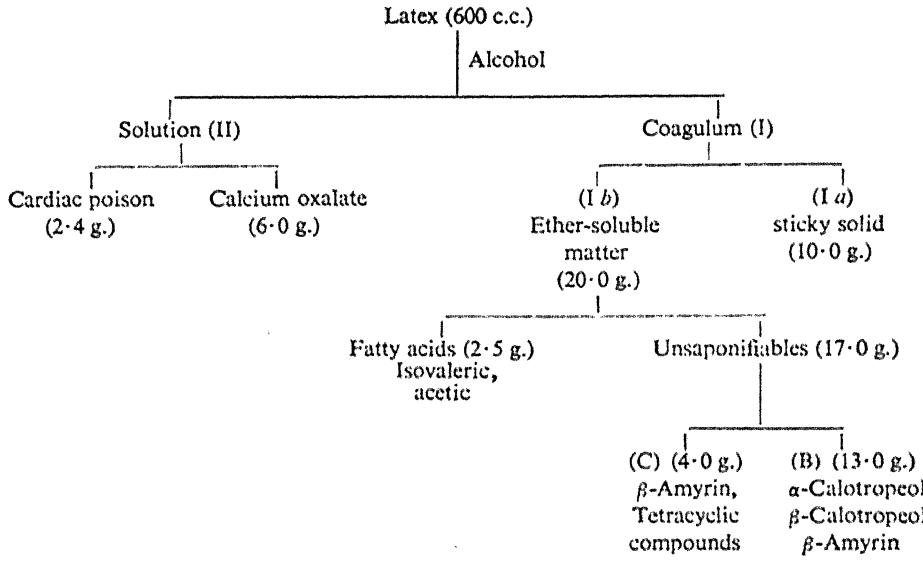
melting at 172–74° and its composition corresponded to the formula, $C_{32}H_{52}O_2$. It was dextro-rotatory. The colour reactions, however, were different from those of the above compounds; with both the Liebermann-Burchard and Salkowski reagents it produced yellow solutions with green fluorescence. This fraction seemed, therefore, to contain tetracyclic triterpenes and this surmise was confirmed by treatment with formic acid or with chloroform saturated with hydrogen chloride. The new product melted between 211 and 215° and gave the colour reactions of the pentacyclic triterpenes (similar to β-amyrin). Thus the presence of mixtures of β-amyrin and tetracyclic resinols was established.

By the decomposition of the soap obtained from the saponification of the ether-soluble portion of coagulum (I) were produced only steam-volatile fatty acids. The existence of acetic acid and of isovaleric acid in the mixture was indicated by the lanthanum nitrate test[6] for the former and the characteristic smell of the latter. An artificial mixture of the two acids could be prepared having practically the same smell as the steam-volatile acids obtained from the latex.

Summing up the results, the ether-soluble portion of the coagulum consists of the esters of triterpene alcohols (acetates and isovalerates). α- and β-calotropeols occur in equal amounts and they form the major portion; β-amyrin comes next and small amounts of tetracyclic compounds also seem to exist.

The aqueous alcoholic solution (II) left after the separation of the coagulum contained substances which could be extracted by means of ether and chloroform. These, after purification using chloroform and petroleum ether, yielded a colourless crystalline substance (mixture of compounds) melting at about 242°. Its colour reactions and solubility indicated that it was not a wax component but belonged to the group of cardiac poisons described by Hesse *et al.* This mixture was highly toxic to fish, gave tests for N and S indicating that compounds containing these elements were present. Further study of this material is in progress. The aqueous alcoholic solution from which all ether- and chloroform-soluble matter had been removed, deposited slowly a colourless solid, and this was identified as calcium oxalate by qualitative and quantitative analyses and examination of its crystalline structure. It is probable that the irritating effect of the latex when rubbed on the skin is due to the presence of this substance in a very fine crystalline condition. This phenomenon has been noticed in some other cases, one such being *Vitis quadrangularis*.[4]

The following chart embodies in brief the isolation of the various fractions and their compositions.



As already mentioned Basu and Nath investigated the ether extract of the dried latex and claimed to have obtained as the sole crystalline component of the unsaponifiable matter a new sterol called calosterol. Though its reactions were abnormal for a sterol they seem to have been led to this opinion by the formula, $C_{28}H_{44}O$ that they obtained for it. However, the carbon analysis of calosterol and of its acetate and benzoate agree closely with the values required for the monohydric triterpene alcohols, $C_{30}H_{50}O$ and their derivatives, though the recorded hydrogen values of the former are low all through. From the results of the present work and particularly from a comparison of the melting points it seems to be quite possible that the above authors were dealing with a mixture of resinols. It was probably contaminated with cardiac poisons since these are also soluble in ether and would get into the ether extract when the whole of the latex in a dry condition is extracted. The cardiac poisons give an immediate green colour with the Liebermann-Burchard reagent and their presence might have contributed to the colour changes observed with calosterol when treated with this reagent.

It may be mentioned in this connection that the latex of another species of Calotropis, *Calotropis procera* has been recently analysed by Hesse[5] *et al.* and has been found to contain a triterpene alcohol. From the abstract of the paper which alone is now available it could be seen that the sole alcoholic component of the latex is α-lactucerol. It has a melting point of 224–25°, $[\alpha]_D$, + 97·5° and its acetate and benzoate melt at 252° and 257° respectively. On boiling with formic acid it isomerises to iso-lactucerol melting at 201°; $[\alpha]_D^{20°}$, + 66·8°. The acetate and benzoate of iso-lactucerol melt

at 237° and 271° respectively. The alcohols are given the formula $C_{30}H_{50}O$ and they produce colour reactions similar to those noted for the calotropeols. They are described as pentacyclic triterpene alcohols. These observations are in accord with the results of the present investigation of the latex of *Calotropis gigantea*, that the components are resinols though the compounds present in the two species are different. They further support the conclusion that 'calosterol' should be considered to be a mixture of triterpene alcohols.

With regard to the composition of the latex of *Calotropis procera* Hesse *et al.* found it to contain (1) cardiac poisons and (2) esters of α-lactucerol with steam-volatile fatty acids. The present investigation shows that the latex of *Calotropis gigantea* contains (1) cardiac poisons, (2) esters of α- and β-calotropeols and β-amyrin with steam-volatile fatty acids and (3) calcium oxalate. Besides others the most characteristic difference between α-lactucerol and the resinols of *C. gigantea* is that the former undergoes isomeric change when treated with formic acid, whereas the latter are stable to such treatment.

Experimental

Fresh latex was collected in bottles containing a few drops of chloroform. In order to bring about coagulation of colloidal matter, it (600 c.c.) was treated with rectified spirits (200 c.c.), the mixture was well stirred and set aside for some hours. The coagulum (I) (35·0 g.) was separated from the aqueous alcoholic mother-liquor (II) by filtration under gentle suction and the two were examined separately.

The soft coagulum (I) was boiled with alcohol (500 c.c.) under reflux, filtered and the extraction repeated twice again using 250 c.c. of alcohol each time. The residue was then extracted repeatedly with boiling ether till no more went into solution. A small sticky portion (I *a*), pale yellow in colour was left behind; it was difficultly soluble in ordinary solvents, contained a little mineral matter and gave tests for phosphate. The combined alcoholic extract deposited on standing a bulky solid which was completely soluble in ether. It closely resembled the ether extract and was therefore added to it (I *b*). On evaporating the clear alcoholic solution a yellowish brown sticky solid was obtained. It resembled (I *a*) closely and the two were mixed together (10·0 g.). This is under further investigation.

Ether solution, (I *b*) (*Resinol esters,* 20 *g.*).—On distilling off the solvent completely the dry residue was obtained as a colourless, non-sticky solid

melting indefinitely between 120° and 170°. To effect, if possible, the isolation of pure compounds by direct crystallisation, it (25·0 g.) was dissolved in boiling ethyl acetate (300 c.c.) and the solution was left undisturbed for about 6 hours. A glistening mass of colourless crystals melting at 210–15° separated out. Two more crystallisations of this solid (5·0 g.) from the same solvent yielded a product melting at 230–35°. Further repeated crystallisation using ether and ethyl acetate alternately left a small amount of (0·5 g.) of a pure product melting at 248–50° (A). Description of this substance has been postponed for the present.

All the impure fractions were mixed together. The solid (20·0 g.) was dissolved in benzene (200 c.c.), N/2 alcoholic potash (500 c.c.) added and the contents were boiled under reflux for 15 hours. By this time a large amount of a crystalline solid (needles) was found to separate out. The solvents were removed by distillation as far as possible, water was added to the residual mixture and boiled. The unsaponifiable matter was isolated by ether extracting the aqueous suspension; during this operation no emulsions were formed and the soap solution remained clear all the time.

Fatty acids (*acetic and isovaleric acids*).—Before decomposing the soap solution with acids the last traces of alcohol were carefully removed by heating under reduced pressure. This precaution is necessary, since lower fatty acids readily form volatile esters with alcohols in the presence of mineral acids and thus give rise to complications. The acidified solution did not form any layer of insoluble fatty acids and hence the clear solution was subjected to steam distillation. The distillate (2 litres) was ether extracted, the extract dried over anhydrous sodium sulphate and finally distilled to recover the solvent. The liquid residue was strongly acidic in reaction and had an offensive smell. Treatment with lanthanum nitrate[6] produced a blue solution indicating the presence of acetic acid and the smell was characteristic of isovaleric acid. Careful comparison of the odour of a dilute solution of the mixed fatty acids with a similar solution containing authentic samples of acetic and isovaleric acids confirmed the presence of these two acids in the mixture.

The residue in the distilling flask was a clear solution. It was repeatedly extracted with ether and the ether solution evaporated. The absence of any residue showed that solid and liquid non-volatile fatty acids were absent.

Unsaponifiable matter, Resinols (17·0 *g.*).—The ether solution of the unsaponifiable matter was distilled in order to remove the solvent completely. The residue (17·0 g.) was a pale yellow solid containing some colourless crystals. It was readily soluble in benzene, ethyl acetate and

ether, but was much less soluble in both methyl and ethyl alcohols. When the whole solid was boiled under reflux with rectified spirits (400 c.c.) and the contents allowed to stand overnight a crystalline solid (B) separated out. It was filtered off and the alcoholic filtrate concentrated in stages. Since no more crystalline solid could be obtained by this process, the whole of the filtrate was evaporated to dryness and the residue (C) was separately examined.

Solid (B) (13·0 *g.*), *α- and β-calotropeols and β-amyrin.*—This fraction crystallised from alcohol in long colourless needles melting at 120–70°. Simple crystallisation from ordinary solvents a number of times did not yield sharp melting substances. Consequently esters (acetate and benzoate) were prepared and studied.

Acetate method (*α-calotropeol acetate*).—The resinol mixture (8·0 g.) was dissolved in acetic anhydride (20 c.c.), anhydrous sodium acetate (5·0 g.) was added and the mixture was kept boiling under reflux for 3½ hours in an oil-bath. It was cooled, diluted with water (300 c.c.) and kept in the ice-chest overnight. The separated solid was extracted first with excess of boiling ether and subsequently with boiling benzene. Only a little dark resinous matter was left behind and it was discarded. Both the ether and benzene solutions were decolourised by warming with 'norit' and concentrated independently. They yielded the same crystalline solid melting at about 230° which when recrystallised from ethyl acetate melted at 240-44°. The two fractions from the two solvents were mixed together and twice crystallised from ethyl acetate when a compound melting at 250–51° was obtained (0·5 g.).

The crystal structure was quite characteristic; the compound came out slowly in the form of glistening, elongated hexagonal prisms. It was very sparingly soluble in both ethyl and methyl alcohols, moderately soluble in ether and ethyl acetate and readily soluble in chloroform and benzene. With Liebermann-Burchard reagent it produced a pink solution which very slowly faded to a yellow brown. With the Salkowski reagent it formed a yellow solution exhibiting green fluorescence. Treatment with boiling formic acid did not produce any change. [Found: C, 82·5; H, 11·2; $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%]. $[\alpha]_D^{30°}$, + 98·0° in benzene solution. The molecular weight of the acetate was determined by finding the saponification equivalent. The solid was dissolved in benzene, excess of N/2 alcoholic potash was added to it and the contents were boiled under reflux for 3 hours. A blank experiment was conducted simultaneously. The unused alkali was estimated by titration with standard acid. The molecular

weight was found to be 462 and that required for the formula given above is 468. The iodine value was determined as follows: The substance (0·2180 g.) was dissolved in carbon tetrachloride (20 c.c.), an equal amount of iodine chloride solution was added and the contents were kept in the dark for 3 hours. The excess of iodine was then titrated in the usual way with sodium thiosulphate. The I. V. obtained was 57·4 and that required for one double bond in the above formula 54·2.

α-Calotropeol.—The acetate (2·0 g.) was dissolved in benzene (50 c.c.), an equal amount of 5% alcoholic potash was added to it and the mixture was boiled under reflux for 5 hours. The major bulk of the solvents was distilled off and the residue was diluted with water. The resulting solid was washed free of alkali and dried. It was dissolved in a mixture of boiling acetone (125 c.c.) and ether (20 c.c.) and allowed to crystallise slowly in the refrigerator. A good amount of crystalline solid was collected at the bottom of the flask and was recovered by filtration. It appeared as transparent rods and narrow plates under the microscope and melted at 204–5°. It resembled the acetate in its solubility in the ordinary organic solvents except benzene in which it was more sparingly soluble. It gave a bright pink solution immediately with the Liebermann-Burchard reagent ; the colour changed to purple in about an hour; after 2 hours it was pale blue and slowly faded to yellowish brown (5 hours). These changes could be brought about rapidly by the addition of a few drops of water. With the Salkowski reagent the sulphuric acid layer was coloured orange-yellow exhibiting deep green fluorescence. [Found: C, 84·6; H, 11·4; $C_{30}H_{50}O$ requires C, 84·5; H, 11·7%]. $[\alpha]_D^{32°}$, + 102·0° in benzene solution.

Benzoate of α-Calotropeol.—The crystalline alcohol (0·6 g.) was dissolved in benzene (40 c.c.) and pyridine (8 c.c.) and benzoyl chloride (7 c.c.) were added to it. The mixture was set aside for 12 hours and then heated under reflux for 3 hours on a water-bath. The solvents were removed under low pressure and the resulting residue was dissolved in ether-benzene mixture (150 c.c.). The solution was washed with 1% sodium hydroxide solution followed by aqueous sulphuric acid. Finally it was washed free of acid with water. It was dried over anhydrous sodium sulphate and gently warmed to remove the ether as far as possible. To the concentrate (20 c.c.) an equal amount of alcohol was added and the mixture was allowed to cool slowly. A crystalline solid melting at 273–74° was obtained and no change in the melting point was effected by crystallising it from ligroin (20 c.c.). It crystallised in the form of broad rectangular plates. [Found: C, 83·6; H, 9·8; $C_{37}H_{54}O_2$ requires C, 83·8; H, 10·2%]. $[\alpha]_D$, + 74·3° in benzene solution. It gave the same colour reactions as the acetate.

Isolation of acetate of β-calotropeol and β-amyrin.—The ethyl acetate mother-liquor left after removing the acetate of α-calotropeol was concentrated and the earlier fractions which were mixtures of this compound were removed. Later a small fraction melting at 232–35° was obtained. Repeated recrystallisation of this from ethyl acetate yielded a product melting at 238–39° which proved to be identical with β-calotropeol acetate (see below). Though the existence of β-calotropeol in the resinol mixture was thus indicated, its isolation in larger quantities was conveniently effected by the benzoate method.

The final ethyl acetate filtrates from which the acetates of the calotropeols had been separated and the original ether and benzene mother-liquors were all united, evaporated to dryness and the residue carefully studied. It (4·0 g.) was dissolved in ether (150 c.c.), an equal amount of alcohol was added and the contents were stirred well. Immediately a solid melting at 205–8° and containing acetates of calotropeols separated out ; it was removed by filtration. The mother liquor was concentrated in stages and five fractions were collected. The first of these melted between 173–85° and the last 90–135°. The last two fractions were coloured yellow. By fractionation of these several times from acetone and ethyl acetate, a small amount of a solid melting at 190–206° was collected. Further crystallisation from benzene-alcohol mixture and finally ethylacetate yielded a product melting at 239–40° and crystallising in the form of long prismatic rods. It produced a pink solution with the Liebermann-Burchard reagent and no change in the colour was effected by the addition of water; with the Salkowski reagent a yellow solution was formed. It was different from the acetates of the calotropeols and the mixed melting points with these compounds were considerably depressed. From its properties it appeared to be β-amyrin acetate and this surmise was proved to be correct by a mixed melting point determination using an authentic sample of β-amyrin acetate obtained from the roots of the Decalepis. On hydrolysis it yielded β-amyrin.

Benzoate method (β-calotropeol benzoate).—Solid (B) (5·0 g.) was dissolved in benzene (30 c.c.), benzoyl chloride (12 c.c.) and pyridine (10 c.c.) were added and the contents were set aside for 12 hours. Then the mixture was heated on a water-bath for 5 hours. The solvents were removed completely under low pressure and the residue was taken up with water. The aqueous suspension was extracted with a mixture of ether and benzene (2 : 1), (300 c.c.) in small lots and the ether-benzene layer was washed with 1% aqueous sodium hydroxide, dilute sulphuric acid and water in succession. The solution on concentration to about 100 c.c. deposited a solid (3·0 g.) melting at 200°. It was digested with ether (100 c.c.) and the ether-insoluble

portion was carefully separated by decanting off the solution. On crystallisation from a mixture of benzene and alcohol (50 c.c.) it yielded a fraction (0·8 g.) melting at about 260°. Further purification of the solid was effected by dissolution in petrol (50 c.c.) and cooling the solution in ice after concentrating to half the bulk. As a result a solid (0·4 g.) melting at 274–77° separated out and after one more crystallisation from the same solvent it was obtained as long rectangular plates melting at 279–80°. No more rise in the melting point could be effected by further purification. With the resinol colour reagents it reacted to produce coloured solutions similar to those given by α-calotropeol and its derivatives. [Found: C, 83·7; H, 10·4; $C_{37}H_{54}O_2$ requires C, 83·8; H, 10·2%]. $[\alpha]_D^{33°}$, + 69·0° in benzene solution. The molecular weight determined as already described by finding the saponification equivalent was 538, the value required for the above formula being 530. The mixed melting point with α-calotropeolbenzoate was considerably depressed.

β-Calotropeol.—The benzoate (0·8 g.) was dissolved in benzene (45 c.c.) and the solution was boiled with an equal amount of N/2 alcoholic potash for 5 hours. The major bulk of the solvents was distilled off and the residue was treated with water. The resulting solid (β-calotropeol) was filtered and washed free of alkali. It melted at 216–17°, after a crystallisation from benzene-alcohol mixture (2 : 1). On concentrating the mother-liquor in stages, only the above substance could be obtained. The alcohol as well as the benzoate were thus shown to be pure. The mixed melting point with α-calotropeol was found to be depressed (175–95°).

β-Calotropeol appeared as prismatic rods under the microscope. It was practically insoluble in alcohol and readily dissolved in ether, chloroform and benzene. In the last solvent its solubility was less than that of its acetate (see below). It produced the same colour reactions with the Salkowski and the Liebermann-Burchard reagents as α-calotropeol. [Found: C, 84·7; H, 11·2; and $C_{30}H_{50}O$ requires C, 84·5; H, 11·7%.] $[\alpha]_D^{32°}$, + 50·9° in benzene solution. [Found: iodine value, 57·8; $C_{30}H_{50}O$ requires for one double bond, 59·6].

β-Calotropeol Acetate.—β-Calotropeol (0·5 g.) was dissolved in acetic anhydride (10 c.c.), a few drops of pyridine were added and the solution boiled for 3 hours. On adding water and allowing the contents to stand overnight the acetate separated out as a colourless solid. It was crystallised from ethyl acetate (20 c.c.) and the resulting crystalline compound melted at 238°. Under the microscope it appeared as elongated hexagonal plates. When crystallised from benzene-alcohol mixture, it was obtained as a woolly

crystalline mass and it appeared as thin rods under the microscope. It was easily soluble in benzene and chloroform, moderately soluble in ether and ethyl acetate and practically insoluble in ethyl and methyl alcohols. It produced the same colour reactions as the benzoate. The substance was unaffected when boiled with formic acid. [Found: C, 81·7; H, 10·9; and $C_{32}H_{52}O_2$ requires C, 82·1; H, 11·1%.] $[\alpha]_D^{30°}$, + 43·9° in benzene solution. Mixed melting point with α-calotropeol acetate was found to be depressed (225–230°).

When the mother-liquors from the crystallisation of the benzoate mixture were worked up some more of the β-calotropeol benzoate could be obtained and it was not possible to isolate α-calotropeol or β-amyrin esters.

Solid (C) (4·0 *g.*) (*β-amyrin and tetracyclic compounds*).—It was yellow in colour and sticky to the touch and was found to be soluble in all organic solvents making purification by crystallisation difficult. From its colour reactions it was considered to consist mostly of resinols. To facilitate the isolation of crystalline products it was studied by the acetate method. Its acetylation was carried out in the usual way by boiling the solid (4·0 g.) with acetic anhydride (15 c.c.) in presence of sodium acetate for 3 hours. The mixture was diluted with water and after allowing to stand for some hours it was ether extracted. On concentrating the ether solution to about 30 c.c. a crystalline solid mixed up with an yellow amorphous substance separated out. Repeated washing with small quantities of ether removed the coloured portion leaving behind colourless crystals melting at 218–30°. Two more crystallisations of the above fraction (1·5 g.) from ethyl acetate resulted in the separation of a colourless crystalline compound melting sharp at 240°. It was identified as β-amyrin acetate by making a mixed melting point determination with an authentic sample.

The ether mother-liquor and washings, on careful concentration in stages, deposited some more of crude amyrin acetate. The final solution was evaporated almost to dryness when a residue was obtained in the form of a thick syrup. It was shaken well with acetone (30 c.c.) and on allowing the contents to stand for about 6 hours a colourless solid melting at 148–60° was deposited. The mother-liquor on careful manipulation yielded some more of the above solid. On crystallisation from acetone it was obtained in the form of broken cubes and it melted at 170–74°. Further purification was not attempted since the yield of the substance was only 0·5 g. [Found: C, 82·6; H, 11·2; $C_{32}H_{52}O_2$ requires C, 82·1 and H, 11·1%]; $[\alpha]_D^{30°}$, + 44·0° in benzene solution. It differed from β-amyrin acetate in producing a yellow solution exhibiting green fluorescence with Liebermann-Burchard

reagent in place of a pink coloured solution. This property was made use of in the above fractionation to detect and eliminate β-amyrin acetate which if present even in traces would invariably produce the pink solution. With the Salkowski reagent it produced a yellow solution with green fluorescence. The low melting point of the acetate coupled with the colour reactions indicated that it was probably a tetracyclic resinol mixture. This surmise was confirmed by effecting ring closure by the following two methods: Formic acid (20 c.c.) was added to the benzene solution (20 c.c.) of the substance and the mixture was boiled for 3 hours. The contents were largely diluted with water and the benzene layer was separated, washed free of acid and was concentrated to about 5 c.c. On the addition of an equal volume of alcohol and cooling the solution in ice, a crystalline solid melting at about 211–15° was obtained. This produced the characteristic colour reaction of the pentacyclic triterpenes, *i.e.*, pink solution with the Liebermann-Burchard reagent. However, the benzene-alcohol mother-liquor from the above compound yielded on evaporation a large amount of the unchanged original compound. This was dissolved in chloroform (15 c.c.), the solution was saturated with dry hydrogen chloride at 0° C. and kept at that temperature overnight. The residue obtained after removing the solvent was crystallised from benzene-alcohol and ethylacetate in succession resulting in the isolation of the transformation product in a better yield.

The final mother-liquors left after removing the crude tetracyclic compounds were examined in detail to see if any sterol-like substances could be detected. No fraction gave the proper sterol colour reactions when tested with the Liebermann-Burchard and Salkowski reagents. Solid (C) thus consisted mostly of β-amyrin and small quantities of tetracyclic compounds.

Filtrate (II) (Cardiac poisons and calcium oxalate).—The filtrate obtained from the coagulum (I) on cooling in the refrigerator deposited only a small amount of a slimy solid. Hence the whole solution was extracted repeatedly with ether employing 500 c.c. of it at a time. The ether solutions were united and extracted with 5% aqueous sodium carbonate twice. The ether layer was washed free of alkali and dried over anhydrous sodium sulphate; on distilling off the solvent it left behind a residue melting at about 235°. It was further purified by dissolving in chloroform (100 c.c.) and reprecipitating by the addition of petrol (500 c.c.). The purified solid (0·4 g.) was obtained in the form of colourless rods and melted at 241°–43°. It produced a green solution with the Liebermann-Burchard reagent and the colour of the solution changed to red after a day. It dissolved in strong hydrochloric acid producing a greenish blue solution. It tasted bitter leaving a tingling sensation on the tongue.

The carbonate solution was acidified with hydrochloric acid and was slightly warmed, when an offensive smell emanated. The acid solution was repeatedly ether extracted and all the ether solutions were united. The solvent was completely removed by distillation and the residue was dissolved in chloroform and reprecipitated by the addition of petroleum ether. The resulting product was very small and was not further studied.

The original filtrate (II) which had been extracted with ether, was extracted with chloroform in four lots employing 400 c.c. of chloroform each time. All the chloroform solutions were mixed and the solution was concentrated to about 25 c.c. No solid separated out even on prolonged cooling in the refrigerator. Then about 100 c.c. of petrol were added and the contents were well stirred when a solid slowly began to separate out. It was crisp and it melted at about 220° (yield 2·0 g.). It gave colour reactions very similar to those of the compound melting at 241°. They were both toxic to fish and in general resembled usharin and related compounds isolated by Hesse *et al.* from the mixed latex obtained from *Calotropis procera* and *gigantea.* No further detailed study was made of them.

After the filtrate (II) had been extracted with chloroform and the chloroform layer removed, a small quantity of an insoluble compound slowly began to separate out from the aqueous layer. It was found to be insoluble in alcohol also. Making use of this property, the substance could be completely separated from the aqueous solution by adding a liberal quantity of alcohol (600 c.c.) and leaving the contents undisturbed for a week. A sticky solid thus precipitated down at the bottom of the flask and was filtered at the pump. Further purification was effected by digestion with alcohol (500 c.c.) whereby it was obtained as a non-sticky solid (6·0 g). It neither melted nor burnt when introduced into a flame and hence was considered to be an inorganic salt. Further it was not soluble in any organic solvent or in water. In acetic acid medium it appeared as plates and bunches of needles under the microscope. Its resemblance in crystalline structure to an authentic sample of calcium oxalate was very marked. Further on ignition calcium oxide was obtained in quantitative yield. It readily dissolved in hot dilute sulphuric acid and the solution reduced potassium permanganata. From the above properties it was identified as calcium oxalate and its estimation was carried out by permanganate titration. Most of the solid (95%) was found to be calcium oxalate, the yield being 1% on the weight of the latex.

The aqueous alcoholic solution was concentrated to about 500 c.c. and was treated with hot neutral and basic lead acetate solutions in succession. The insoluble lead salts were collected and decomposed separately in the

usual way by passing hydrogen sulphide through the aqueous suspensions. After removing the lead sulphide the filtrates were extracted with ether and chloroform in succession. From the ether solution only a small quantity of a crystalline resin acid melting at 135° could be obtained. From the chloroform solution no compound could be obtained; all the chloroform-soluble substances had obviously been removed earlier. The aqueous filtrate on further concentration and hydrolysis with aqueous sulphuric acid yielded amorphous resins.

Summary

The latex of *Calotropis gigantea* contains (1) resinols as esters of steam-volatile fatty acids (acetic and isovaleric), (2) cardiac poisons similar to usharin and (3) calcium oxalate. The resinol portion consists mainly of two new alcohols, α-calotropeol and β-calotropeol in almost equal quantities and minor amounts of β-amyrin. The important properties of the calotropeols have been studied. It is suggested that 'Calosterol' of Basu and Nath should be a mixture of resinols contaminated with cardiac poisons. The recent report of Hesse *et al.* that the milky latex of *Calotropis procera* contains only α-lactucerol as its esters with steam-volatile fatty acids is discussed.

REFERENCES

1. Basu and Nath .. *Biochem. J.*, 1924, **28**, 1561.
2. Hesse *et al.* .. *Ann.*, 1939, **537**, 67–86.
3. Murti and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1941, **14**, 93.
4. ——————— .. *Ibid.*, 1939, **9**, 121.
5. Hesse *et al.* .. *Ann.*, 1941, **546**, 233 ; *B. C. A.*, 1942, **A 2**, 43.
6. Neelakantam and Row .. *Proc. Ind. Acad. Sci.* (A), 1941, **13**, 194.

FIXED OIL FROM *JATROPHA CURCAS* (LINN.)

BY A. R. SUKUMARAN KARTHA AND K. N. MENON
(*Maharaja's College, Ernakulam*)

Received July 24, 1943

Jatropha curcas or the angular-leaved physic nut flowers in May–June and the fruits ripen during the monsoon. The oil from the seeds has been analysed by many investigators and only two have published complete analysis of the component acids, the others only separating the soild and liquid acids and in some cases determining the proportion of the oleic and linoleic acids in the latter. Kajuka, Hata and Fujikawa[1] gave the component acids of the Formosan oil, of iodine value 130, as 14·4% palmitic, 9·6% stearic, 53·2% oleic and 22·8% linoleic acid. These results are not in agreement with the recorded iodine value and can be accepted only with caution. The same trouble arises in accepting the values recorded by Cruz and West.[2]

For the present investigation the seeds were collected locally and the oil obtained shows a higher density and lower iodine value than all other samples hitherto examined. The analytical constants of the Malabar oil together with the constants of other curcas oils are shown in Table I. The mixed acids (96·4%) gave 24·57% of solid acids and 75·43 of liquid acids. Complete analysis has shown that the constituent acids are myristic 1·37%, palmitic 15·61%, stearic 9·69%, arachidic 0·35%, oleic 40·9% and linoleic 32·08%. The Euphorbiaceæ oils in general contain only very small amounts of saturated acids and Jatropha oil is the first in the whole of the family which has been shown to contain more than 18% of saturated acids. The component acids of some typical Euphorbiaceæ fats are shown in Table II for comparison.

The component glycerides of Euphorbiaceæ have recieved very little attention. Hilditch and Priestman[3] records the glyceride composition of the fruit coat fat of *Stillingia sebifera*, and the present is the first report on the glyceride composition of the seed fat in this group. The analysis of the azelao glyceride mixture has already been published[4] and in this paper the data relating to the trisaturated glyceride content is reported. It is interesting to note that this oil does not obey the rule of even distribution.

The studies of Ivano and others[5] have resulted in the conclusion that a given plant species, capable of existence in different climates, produces, when

TABLE I

Authority		Density	Refr. Index	Acid Value	Sapon. Value	Iod. Value	Unsaponified %	Locality
Siam Govt. Laboratory	(1) ..	0·924	1·4640	7·5	192·6	101·1	..	Siam
	(2) ..	0·923	1·4623	12·84	202·9	98·2	..	Do.
Kinzo and Chinta	(3) ..	0·9152	1·4695	2·86	195·22	102·31	1·25	Formosa
Simon et Droit	(4) ..	..	1·4720	6·4–9·6	188–190	93·00	..	Barbadoes
	(5) ..	..	1·4730	4–8	176–180	97·98	..	Do.
Francis et Droit	(6) ..	0·9168	1·4720	4·5–5·5	176·00	93·00	..	Do.
	(7) ..	0·9205	1·4733	4·0–9·6	190·00	98·00	..	Do.
L. A. Drians	(8) ..	0·9106	1·4722	38·68	196·32	106·90	1·15	Belgian Congo
	(9) ..	0·9219	1·4675	2·91	193·20	96·20	0·50	Do.
Cruz and West	(10) ..	0·9820	1·4665	5·10	192·40	94·80	0·45	Philippine
Present work	(12) ..	0·9849	1·4669	26·27	196·10	90·84	0·20	Malabar

TABLE II. *Component Acids of the Euphorbiaccæ*

No.	Name	C_{16}	C_{18}	Ol	Linol.	Others	Authority
1	*Alewrites cordate*	6·5		19·4	..	74·1 Elæo-stearic	Mc. Kinney and Jameson[1]
2	*Croton tiglium*	1·3	0·5	55·8	28·8	11·3 C_{14} and 2·3 C_{20} acids	Flaschentragger and Wolffersdorff[2]
3	*Euphorbia lathyris*	5·8		91·9	2·3		Zoleol[3]
4	*Hevea brazilinsis*	8·3	8·6	23·6	43·3	14·7 Linolenic	Griffiths and Hilditch[4]
5	*Ricinodendron africanum* ..	10·4		17·0	12·0	44·0 X elæo-stearic* and 11% linolenic	Steger and Van Loon[5]
6	*Ricinus communis*		0·3	7·2	3·6	87·8 Ricinoleic	Panjutin and Rapport[6]
7	*Stillingia sebifera*	4·7	1·5	8·1	59·4	25·9 Linolenic	Jamieson and Mc. Kinney[7]
8	*Jatropha curcas*	15·61	9·69	40·9	32·08	0·35 C_{20} 1·37, C_{14}	Present work

1. Mc. Kinney and Jamieson, *Oil and Soap*, 1937, **14**, 2.
2. Flaschentragger and Wolffersdory, *Hebe. Chem. Acta*, 1934, **17**, 1444.
3. Sobol and others, *Maslob. Shir. Delo*, 1935, **11**, 132.
4. Griffiths and Hilditch, *J. Soc. Chem. Ind.*, 1934, **53**, 75 T.
5. Steger and Van Loon, *Rec. Trav. Chim.*, 1935, **54**, 988.
6. Panjutin and Rapport, *Chem. Unschan*, 1930, **37**, 130.
7. Jamieson and Mc. Kinney, *Oil and Soap*, 1938, **15**, 295.

grown in a cold climate, more unsaturated acids in its seed fats than when it is grown in a warmer climate. A perusal of the literature connected with Jatropha oils shows that probably more important than the general climatic condition, the minimum temperature to which the plant is subjected, influences the degree of unsaturation of the oil. The oil of curcas from Belgian Congo, practically on the equator and subject to extremes of heat and cold, has an iodine value of 97–102, Malabar (8° N.) oil has 90·84, Philippine (15° N.) has 95·00, Formosa (23° N.) has 103·0. The Belgian Congo seeds are subjected to much greater variations in respect of heat and cold so much so that the oil shows higher unsaturation than the Malabar oil or Philippine oil, and its unsaturated nature is on a par with Formosan oil from seeds grown in a much colder climate.

Experimental

450 Grams of clear dry oil were obtained by extraction of 972 grams of the crushed seeds, and gave the following constants :—

Density (30° C.)	0·9849	Refractive Index	1·4669
Acid value	26·27	Saponification value	196·1
Acetyl value	Nil	Iodine value	90·84
Water insoluble acids	96·4%	Non-saponifiable matter	0·2%

Constants of Mixed acids.—

Titre	26°	Refractive index	1·4583
Mean Molecular weight	277·2	Iodine value	94·85

The mixed acids were separated into solid and liquid acids showing the following constants :—

(*a*) *Solid acids.*—(24·57% of the mixed acids).

Titre	52·5°	Mean molecular weight	268
Iodine value	0·65	Refractive index	1·4496

(*b*) *Liquid acids.*—(75·43% of the mixed acids).

Mean molecular weight	282·1	Refractive index	1·4650
Iodine value	117·0		

The solid and liquid acids were converted into their esters, fractionated and worked up as usual. Careful fractionations followed by hydrolysis and analysis gave the following values :—

Solid acids :—

C_{14}—0·25; C_{16}—14·11; C_{18}—9·69; C_{20}—0·35;
Oleic—0·07; Linoleic—0·10

Liquid acids :—

C_{14}—1·12; C_{16}—1·50;
Oleic—40·83 Linoleic—31·98

Thus the composition of the total acids is found to be myristic 1·37%, palmitic 15·61%, stearic 9·69%, arachidic 0·35%, oleic 40·90% and linoleic 32·08%.

Permanganate Oxidation

120 Grams of the neutral oil gave 3·41 grams of neutral non-oxidisable portion corresponding to 2·84% of saturated glycerides.

Confirmation of the above value by an independent method could not be attempted due to lack of suitable methods. The oil is miscible in all proportions with dry acetone and could not be crystallised from it. The estimation of tristearin in the completely hydrogenated fat would be useless firstly because the oil contains nearly 10% of stearic acid and secondly because it contains nearly 16% of palmitic acid. The tristearin value will thus be about 55 and it has been proved[6] that this method is unreliable when the value lies between 40 and 75. The estimation of tri-C_{18}-glycerides by the partial hydrogenation method would not give any accurate results since the oil contains nearly 10% of stearic acid.

Summary

The constants, mixed acid composition and the trisaturated glyceride content of the oil from the seeds of *Jatropha curcas* (Linn.) are recorded.

REFERENCES

1. *J. Chem. Soc. Japan*, **53**, 1115.
2. *Philippine J. Sci.*, **61**, 437.
3. *J. Soc. Chem. Ind.*, 1930, **49**, 197.
4. *Proc. Ind. Acad. Sci.*, 1943, **17**, 114.
5. *Ber. D. Botan. Ges.*, 1926, **44**, 31 ; *Z. Angew. Chem.*, 1929, **42**, 292 ; *Chem. Unschan* 1931, **38**, 96 ; see also S. Jaschkevitsch, *Fat Chem. Unschan*, 1933, **40**, 197.
6. *The Chemical Constitution of Natural Fats*, p. 194.

THE CONDENSATION OF ALDEHYDES WITH MALONIC ACID

Part XV. Condensation of 5-Bromosalicylaldehyde and of 3: 5-Dibromosalicylaldehyde: Influence of Dissimilar Groups

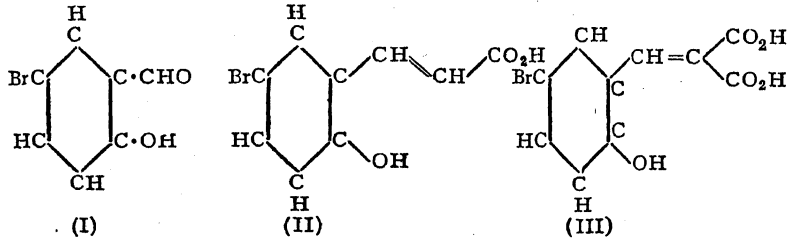
By Kantilal C. Pandya
and
Miss Rashmi Bala K. Pandya
(Chemistry Laboratory, St. John's College, Agra)

Received August 21, 1943

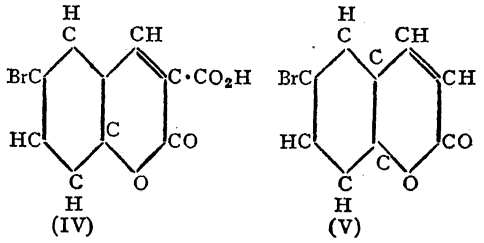
In Part XIV[1] it was shown that the presence of two nitro-groups on the aromatic ring, as in 2: 4-dinitribenzaldehyde, made the condensation with malonic acid extremely slack and it was only after many attempts that the yield of 50% of the theoretical was obtained. It is possible that two nitro-groups in different positions from the above might have behaved differently.

In Part XIII,[2] it has been shown that the influence of a chloro- or a bromo-group present on the aromatic ring of the aldehyde affected the reaction very favourably, as regards both the speed and the final yield, which includes its purity also. In Part I[3] it was shown that salicylaldehyde gave about 51% yield only with a small trace of pyridine : the question may be asked, if a chloro- or a bromo-group were placed on the aromatic ring in salicylaldehyde, would the yield be increased ? The investigations described in this paper give data for an answer in the affirmative.

Two bromo-salicylaldehydes have been condensed here with malonic acid, in the presence as well as in the absence of pyridine-traces. Three different products have been obtained from each of the aldehydes, while a fourth was obtained from each by Perkin's method. On the whole the yields in the aldehyde-malonic acid condensations have been much higher than those obtained by Perkin's method, and are also greater than the 51% obtained from the simple salicylaldehyde-malonic acid condensation, though in no case full theoretical yields are obtained as in the condensations with the chloro- and bromo-benzaldehydes.[2]



(I) (II) (III)

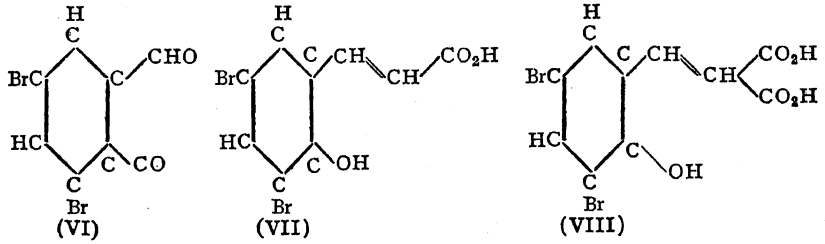


(IV) (V)

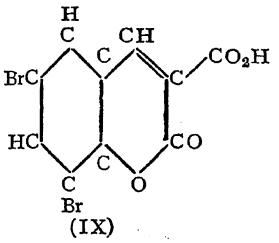
The 5-bromosalicylaldehyde (I) took some time to condense with malonic acid in the presence of pyridine, difficulties being caused by the sublimability of the aldehyde below the water-bath temperature. The sublimated amount was repeatedly scraped off and added to the reacting mass or was washed down with a drop or two more of pyridine. After prolonged heating 5-bromo-orthocoumaric acid (II) was obtained, but the yield was between 50 and 58%. 5-Bromosalicylidenemalonic acid (III) was also formed and was discovered in the filtrate of the bromo-orthocoumaric acid. It was only about 20–25% of the theoretical, but the total yield of the two would be about 78%, which is better than that from the ordinary salicylaldehyde.

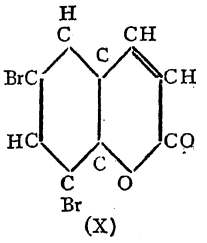
As the decarboxylating action of pyridine is well known it was expected that the condensation in the entire absence of pyridine might give more of the dibasic 5-bromosalicylidene-malonic acid (III). However, the main bulk of this condensation was 6-bromo-coumarincarboxylic acid (IV), which melted at 200° and which came out in yields of 92·5%. It was accompanied by another still higher melting product, m.p. 241°, which was too little to be properly investigated. It however melted with effervescence. This was not 6-bromocoumarin (V), which was obtained by following Perkin's method and which melted at 164°.

Condensations with the 3 : 5-dibromosalicylaldehyde (VI) were a little less difficult, as the sublimation was much less and could be controlled more easily. Perkin's method gave the expected 6 : 8-dibromocourmarin (X), m.p. 176°. When pyridine was present three products were obtained, two of which were the expected 3 : 5-dibromo-orthocoumaric acid (VII) and the 3 : 5-dibromosalicylidenemalonic acid (VIII), melting at 187–189° and 158°, or



(VI) (VII) (VIII)


(IX)


(X)

157–159°, respectively. These have been identified, but the third, melting at 327° or above, was all used up in purification and qualitative tests. It did not appear to have either a hydroxy or an aldehydo or a carboxylic group and had to be left over for the time. Condensation in the absence of pyridine gave as before 6: 8-dibromocoumarincarboxylic acid (IX), in yields of about 74%.

Experimental

Preparation of 5-Bromosalicylaldehyde.[4]—5 Grams salicylaldehyde were dissolved in 9·6 c.c. (10 g.) of glacial acetic acid. The flask was placed in freezing-mixture and a solution of bromine 1·8 c.c. (6·6 g.) in glacial acetic acid was added drop by drop with constant stirring. The 5-bromosalicylaldehyde was at once precipitated and washed and dried. The weight of the crude product on drying was 6·5 g., *i.e.*, 82·5% of theory. Recrystallised from dilute alcohol, it melted at 105° (the expected m.p. was 104–105°[4]).

Condensation by Perkin's method: the Preparation of 6-Bromocoumarin.[5]—2 G. 5-bromosalicylaldehyde, 4 g. freshly fused sodium acetate and 6 c.c. acetic anhydride were mixed and heated in a flask for five hours at 180–190°. It set to a solid mass at the end of 4·5 hours. The mass was repeatedly extracted with hot water until it gave no colour with ferric chloride. It was then repeatedly washed with ether until that also ceased giving colour with ferric chloride. The white mass left behind melted at 155-165°. It was dissolved in hot caustic soda and reprecipitated by dilute hydrochloric acid. Recrystallised from absolute ethyl alcohol it melted at 164°. (160°, Simonis[4]). The yield was 1 g. or 50%.

Condensation with Malonic Acid with a Trace of Pyridine.—5-Bromosalicylaldehyde 3 g., malonic acid 2·1 g. and 8 drops of pyridine were heated on an oil-bath at 95° for two hours and then at 100–105° for nearly 18 hours. About 8 drops more of pyridine had to be added during the course of this heating. There was much effervescence and the mixture liquefied in the first hour. The sublimed aldehyde was frequently scraped and thrown back into the flask or returned to the mixture by a drop or so of pyridine. After two hours the liquid became quite clear, effervescence had stopped and the

temperature was raised to 100–105°. Gradually the clear liquid became viscous and then set to a solid. The flask was cooled overnight and the contents were extracted with sodium bicarbonate (10%) solution. On acidifying the extract a white precipitate was obtained, which was filtered and washed with water. It melted at 145–148°. Crystallised from ethyl acetate and benzene, it melted at 150–152°. The yield was 50–55%.

It melted without effervescence, gave no colour with ferric chloride but decolorised Baeyer's reagent. Bromine found 32·5%, *bromo-orthocoumaric acid* $C_9H_7O_3Br$ requires 32·9%.

The filtrate was concentrated and on cooling deposited crystals which were removed and the mother-liquor on shaking up with ether and distilling off the ether gave more crystals. These melted at 170°, and on recrystallisation from ethyl acetate and benzene it melted at 175°. It melted with effervescence, gave no colour with ferric chloride and decolourised Baeyer's reagent immediately. A small portion of it was kept in an oil-bath at 180°: it melted and the effervescence continued for some time. After about 15 minutes it was taken out and, on cooling and reheating, melted at 148–150°. When mixed with the bromo-orthocoumaric acid, m.p. 150–152°, the melting-point did not change at all. Bromine found 27·57, 27·58%: *5-bromosalicylidenemalonic acid* $C_{10}H_7O_5Br$ requires 27·87%. Equivalent weight, found by titration with potassium hydroxide, 148·3; required = 143·5. The yield was about 24%, the total yield being about 78%.

Condensation without any Reagent: *6-Bromo-Coumarincarboxylic Acid* (IV)—3 G. 5-bromosalicylaldehyde and 2·1 g. malonic acid were mixed up and heated on the water-bath for 9 hours and then for 8 hours on an oil-bath at 100–105°. The sublimed aldehyde was scraped and added on from time to time. There was plenty of effervescence and after about two hours an opalescent liquid was formed. The mixture never became a clear transparent liquid. One hour after it had solidified again, the temperature was raised up by a change of the bath. The product at the end was extracted with ether to remove the unreacted aldehyde. A grey solid remained undissolved which was filtered off. In the crude condition it melted at 195–200° and weighed 3·7 g. Recrystallised from hot benzene, the melting-point rose only slightly to 200°. It was soluble in hot water and acetone as well, and insoluble in ether.

This experiment was repeated several times, and it was found that only six hours' heating on a water-bath was sufficient to give the same yield, *i.e.*, 92·5%, of the first solid, m.p. 200°, which was the *6-bromocoumarincarboxylic acid*: Bromine, found = 29·43, 29·44%, the acid $C_{10}H_5O_4Br$ requires 29·74%. It melted without effervescence and gave no colour with ferric chloride. It decolourised alkaline permanganate.

When the ethereal extract was treated with sodium dicarbonate solution, it gave much effervescence: excess was then added and the two layers separated. On acidfication, the lower layer gave a solid white substance, m.p. 237° (dec.) (y. = 0·3 g.).

This high-melting substance melted at 241° on further purification, but melted with effervescence. It was not in sufficient amount for a clear identification.

3-5-*Dibromosalicylaldehyde.*[6]—5 g. salicylaldehyde were taken in a flask and to this were added 17·5 g. of bromine (a little more than 2 mol. proportion) drop by drop and with vigorous shaking. The flask was kept at room temperature for two days with occasional shakes: on the third day the whole mass had set to a solid and was taken out with about 500 c.c. or more of water. This was washed free of the excess of bromine. Recrystallised from dilute alcohol it melted at 82–84° (m.p. 83°)[6]. Yield about 87% of the theoretical.

Condensation by Perkin's method: 6–8-*Dibromocoumarin.*[7]—1·4 G. dibromosalicylaldehyde, 2 g. fused sodium acetate and 3 g. acetic anhydride were mixed and heated in the usual way, 170–180°, when the whole mass solidified at the end of three hours. The cold mass was washed with plenty of water, the residue was dried and washed with ether repeatedly till the washing gave no colour with ferric chloride. The residue was dried and recrystallised from rectified spirit. It melted at 176°. Yield about 33%. (6:8-Dibromocoumarin melts at 174–176°).[7]

Condensation in the Presence of Pyridine: 3:5-Dibromo-salicylidenemalonic Acid and 3:5-Dibromo-orthocoumaric Acid.—Very many condensations had to be tried to get conditions of good yield, as three different substances were found to occur in the product of this single reaction. Higher temperatures than 100° gave better yields of a substance which seemed to melt at about 300°, but has not been identified. Two other products 3: 5-dibromosalicylidenemalonic acid (VIII) and 3:5-dibromo-orthocoumaric acid (VII) were also formed, but the amounts of their yields varied greatly with the temperature and the manner of heating. On the other hand oridinary water-bath heating for several hours even gave poor yields and much of the original aldehyde was found remaining unaffected but mixed up with resin.

2·8 G. dibromosalicylaldehyde, 1·4 g. malonic acid and 6 to 8 drops of pyridine were mixed in a flask and the whole was heated on an oil-bath at 110° for 4 hours. There was much effervescence and the mixture changed into a thick viscous mass, a little aldehyde that had sublimed was washed in with a drop or two of pyridine. The temperature was gradually raised to 125°,

and then the whole was left to itself overnight. The next day the temperature was raised up to 135°, and after one hour quickly to 160°. A solid mixed with much viscous matter appeared to have been formed and the heating was stopped. To the cold flask ether was added when much dissolved, leaving behind a solid which in the crude condition melted at 260°–270°. It was found insoluble in most of the usual organic solvents. Pyridine however dissolved it readily but gave crystals that were very impure and sticky. After a recrystallisation from pyridine-benzene, the substance was washed with dilute hydrochloric acid which removed some of its colour. The substance was then washed with alcohol, then with alcohol plus hydrochloric acid and finally with water to remove the acid. It was finally washed with alcohol and ether. It was now white in colour, and on heating began to get black at 290° and decomposed finally with effervescence at 295–300°. It was again treated with pyridine and benzene, and a little alcohol was added: after two days clear white crystals came out which on drying melted at 323–327°, becoming dark at 310°. It was insoluble in sodium bicarbonate solution and gave no effervescence with it. In fact it gave no indications of the presence of a hydroxy or an aldehyde or a carboxyl group, though it showed bromine.

From the ether-extract the two acids were separated. Addition of sodium bicarbonate solution to it gave effervescence, so excess was added and it was allowed to remain for a couple of hours when white crystals of a sodium salt came out. These were filtered off, treated with hydrochloric acid and with water. The solid obtained after recrystallisation melted at 185–187° without effervescence. This was *dibromo-orthocoumaric acid*, soluble in ether, alcohol, etc., but insoluble in benzene (hot or cold). Found Bromine = 49·38%, the acid $C_9H_6O_3Br_2$ requires 49·68%. The best yield = 31%.

The filtrate contained the soluble sodium salt of the dibromosalicylidenemalonic acid, which was decomposed by dilute hydrochloric acid. It melted with effervescence at 140–150°, but on recrystallisation from alcohol, it melted at 157–159°. Heated alone on a nacked flame for some time it gave off carbon dioxide and was changed into the monobasic acid, m.p. 187–189°. Heating with pyridine also brought about the same change. Found Bromine = 43·28%, the acid $C_{10}H_6O_5Br_2$ requires 43·71%. The best yield = 22%.

Condensation in the Absence of any Condensing Reagent: 3:5-4:6-*Dibromo-coumarincarboxylic Acid* (IX).—3:5—Dibromosalicylaldehyde 1·4 g. and malonic acid 0·7 g. were mixed and heated together for 5 hours on a water-bath and then for one hour in an oil-bath at 130°. In another experiment the heating was at 130° at the beginning but was then lowered to 110–120° after the whole mass had melted, the total amount being six hours. The

product in both cases was the same and the yield also was the same about 76%. The product was extracted with ether, when the dibromocoumarin-carboxylic acid was left undissolved. It was recrystallised from alcohol and melted at 224–226°. The ethereal extract was a mixture of the same acid with some of the unreacted aldehyde. Found Bromine = 45·72%, the acid $C_{10}H_4O_4Br_2$ requires 45·97%. It appeared to be dimorphous; crystallised from alcohol, it came out in white needles, and from benzene it came out in cubes.

Acknowledgements are due to Mr. Mahendra Rai Kikani for a few preliminary experiments on this subject.

Summary

5-Bromosalicylaldehyde and 3:5-dibromosalicylaldehyde have both been condensed with malonic acid in the presence of pyridine as well as in its entire absence, and the yields are on the whole greater than were obtained from the condensation of salicylaldehyde with malonic acid in the presence of pyridine, the highest being 92·5%. Perkin's reaction gave different products altogether and in much smaller yields.

REFERENCES

1. Pandya, Ittyerah, and Miss Pandya — *J. Uni. Bom.*, 1941, **10**, Part 3, 78.
2. Pandya and Miss Pandya .. *Proc. Ind. Acad. Sci.*, A, 1941, **13**, 112.
3. Kurien, Pandya and Surange *J. I. C. S.*, 1934, **11**, 823.
4. Piria .. *Annal.*, **30**, 171 ; Auwers and Bürger, *Ber.*, 1904, **37**, 3934.
5. Perkin .. *J. C. S.*, **24**, 41 ; Simonis and Wenzel, *Ber.*, **33**, 1962 and 2327.
6. Loewig .. *Berzilius' Jahresberichte*, **20**, 313.
7. Perkin .. *J. C. S.*, **24**, 42 ; Simonis and Wenzel, *Ber.*, **33**, 1964.

COLORIMETRIC ESTIMATION OF BORIC ACID WITH PENTAMETHYLQUERCETIN

By K. Neelakantam and S. Rangaswami

(From the Departments of Chemistry and Chemical Technology, Andhra University, Waltair, now at Guntur)

Received April 15, 1943

Several methods for the determination of boric acid are known and of these the alkalimetric method which is largely employed depends on the titration of boric acid with standard alkali in the presence of glycerol, mannitol or invert sugar. Gravimetric methods depending on the absorption of boric acid in a suitable reagent and determining the increase in weight are also known.[1] Both types of methods yield satisfactory results only with appreciable amounts of boric acid. Colorimetric methods which have been suggested in the literature for the estimation of small amounts of boron depend on the reaction of boric acid with (*a*) turmeric, (*b*) curcumin in presence of oxalic acid, and (*c*) quinalizarin dissolved in sulphuric acid. To these may be added the unusual colorimetric procedure of Stahl[2] which depends on the comparison of the intensities of the green flame colour given by methyl borate from the test and standard samples.

Hebebrand[3] showed that 0·1 mg. of boric acid gives a feeble brown tint whilst 10·0 mg. of the acid gives a bright rose-red colour with a 1% solution of turmeric and a colorimetric method was developed on this basis. The colouration is somewhat fugitive, and is destroyed by prolonged exposure to light and also by boiling. Bertrand and Agulhon[4] employing strips of turmeric paper carried out successfully the estimation of 0·1 to 0·0001 mg. of boric acid. This method depends on the comparison of the lengths of the colourations produced on the turmeric strips with the test and standard solutions of boric acid in presence of dilute hydrochloric acid. Schäfer[5] pointed out that concentrated hydrochloric acid itself gives a red colour with turmeric and this changes progressively to yellow on dilution with water. Hence the concentration of hydrochloric acid employed is of importance in this method.

The second colorimetric method depends on the red colour given by boric acid with curcumin in presence of oxalic acid. Using this method as much as 2·0 mg. of boron trioxide can be estimated (Snell[6]). Neither of the above two methods, however, is rapid.

The third colorimetric method depends on the quinalizarin-boric acid reaction in presence of concentrated sulphuric acid. The solution of the reagent in concentrated sulphuric acid is itself violet in colour and this changes to blue with increasing additions of boric acid, the actual colour produced being composed of varying amounts of red and blue. Scharrer and Gottschall[7] employed the method for the estimation of 120 to 12γ of boric acid. Smith[8] found that it was necessary to work with concentrations of boric acid less than 0·04 mg. per c.c. and that the lower limit was 0·002 mg. per c.c.

Using the photo-electric colorimeter Olson and De Turk[9] estimated amounts up to 0·03 mg. of boron. These authors found that maintenance of constant temperature during the estimation was important for obtaining good duplicates and that decrease in the concentration of the sulphuric acid decreased the sensitivity, so that a definite high concentration of acid had to be employed. Patricia W. Mansell[10] using the Lovibond tintometer found that about 2γ of boron was the most satisfactory quantity to measure. It is evident from the above that this method is available only for the estimation of very small quantities of boric acid.

Stahl (*loc. cit.*) found that quantities of the order of 0·3 mg. or more of boron trioxide in the upper limit and smaller quantities of the order of 0·0022 to 0·007 mg. could be determined with an accuracy of 0 to 15% by his method.

Several other colour reactions for boric acid of great sensitivity have been described in the literature, but no attempt seems to have been made to adapt them for colorimetric work. Rangaswami and Seshadri[11] have recently pointed out that the boric acid reaction characteristic of 5-hydroxy- and 5-methoxyflavones and -flavonols and 2-hydroxy- and 2-methoxy-chalkones first described by Wilson[12] and subsequently developed by them is capable of application for the detection of boric acid itself. These compounds in anhydrous acetone solution containing anhydrous citric acid undergo a change of colour on the addition of traces of boric acid. Compounds that are themselves coloured yellow become deeper yellow on the addition of boric acid while those that give only colourless solutions in acetone containing citric acid develop a yellow colour on the addition of boric acid. Using pentamethylquercetin, which gives a practically colourless solution in acetone containing citric acid only, as the reagent (Rangaswami and Seshadri, *loc. cit.*) showed that 1 part of boric acid in 30,000 parts of solution could be easily detected—a reaction of a fairly high degree of sensitivity. This reaction has now been investigated in detail with a view to develop a method for the colorimetric estimation of boric acid.

Experimental

Solutions:

(1) *Reagent.*—Dry pentamethylquercetin (225 mg.) was dissolved in anhydrous acetone and the solution made up to 100 c.c. with same solvent. For this purpose acetone (extra pure) was dehydrated by standing over fused calcium chloride for 72 hours and then distilled under anhydrous conditions.

(2) *Boric Acid.*—A pure sample of boric acid was finely powdered and dried in a steam oven for 6 hours. 200 mg. of the acid was accurately weighed out, dissolved in anhydrous acetone and the solution made up to 100 c.c. with the same solvent. 5 c.c. of this solution was diluted to 25 c.c. with the same solvent and used as the standard.

(3) *Citric Acid.*—Citric acid (pure) crystals were finely powdered and dehydrated by drying in a steam oven for 30 hours. 50 g. of the acid were dissolved in 500 c.c. of anhydrous acetone and the solution was filtered rapidly through a plug of cotton wool, to remove some finely divided insoluble material.

In preparing the above solutions carefully dried apparatus was used and the solutions were kept in well-stoppered dry bottles.

(4) *Potassium chromate.*—1·0 g. of potassium chromate (A.R) was weighed accurately, dissolved in water and the solution made up to 100 c.c. with water. 20 c.c. of the solution was pipetted out and diluted to 100 c.c. with water. The latter solution was employed in the experiments.

Preliminary Investigation

The colour (yellow) was developed by adding to a measured volume of the boric acid solution an excess of the reagent solution—more than one molecular proportion—and then diluting to volume with the 10% citric acid solution.

As the various aspects of this colour reaction had not been studied before, they were investigated now and the results are reported below :—

1. No colour was developed until the citric acid solution was added.

2. The yellow colour developed was progressively reduced in intensity and it finally disappeared altogether on dilution with anhydrous acetone free from citric acid. Experiments showed that this could not be a mere dilution effect.

3. Attempts to use the Lovibond tintometer for the measurement of colour and investigation of its variation, if any, with time were not successful on account of the volatility of the solvent at the temperatures obtaining in the

tintometer and also due to the rapid absorption of moisture of the citric acid solution.

4. Experiments carried out with the Duboscq type of colorimeter (balancing method) gave satisfactory results with identical solutions. With solutions which differed slightly in concentration of boric acid, however, erratic results were obtained showing thereby that Beer's Law does not hold good.

5. The intensity of colour in a given case depended on the actual amount of reagent solution added and possibly also on the amount of citric acid solution employed so that for good reproducibility definite conditions had to be prescribed.

6. The duplication method was more successful than the balancing method but here again the same factors as in (3) above precluded satisfactory results. Consequently it was necessary to work with closed vessels and with the minimum of exposure to air.

7. With quantities of boric acid less than 0·4 mg. the yellow colour developed was considerably reduced in intensity on dilution to 25 c.c. with the citric acid solution. Experiments showed that the magnitude of this reduction was quite out of proportion to what may be expected on the basis of dilution. With 0·4 mg. and above of boric acid apparently this did not occur. From this it appears that different final dilutions must be adopted depending on the amount of boric acid present in the test.

8. The yellow colours obtained resembled closely, though not perfectly, the pure yellow of potassium chromate solutions so that the former could be matched with the latter. In this manner permanent standards for use in routine work could be easily prepared.

Based on the above results the following empirical method has been worked out for the approximate estimation of small quantities of boric acid ranging from 0·4 to 1·8 mg.

Procedure

An aliquot part of the standard boric acid solution was pipetted out into a dry 50 c.c. Nessler cylinder having a graduation at 25 c.c., 5 c.c. of the reagent solution added and the volume made up to the 25 c.c. mark with the citric acid solution. After stirring, the cylinder was tightly corked. 15–20 c.c. of water were placed in another cylinder of the same dimensions and the matching was carried out by adding the standard chromate solution from the burette in the usual manner for the duplication method. Except during actual matching the cylinder containing the test solution was kept corked. The matching was repeated three or four times in each case. With quantities of

boric acid of the order of 1·2 mg. and above, the intensities of the yellow colour, when the whole of the solution (25 c.c.) was employed, were felt to be too deep for satisfactory matching. In these cases, therefore, after making up to 25 c.c. with the citric acid solution, the volume was halved using a measuring cylinder and the matching carried out. The total intensity (for 25 c.c.) was obtained in terms of the chromate solution by doubling the value obtained.

Results

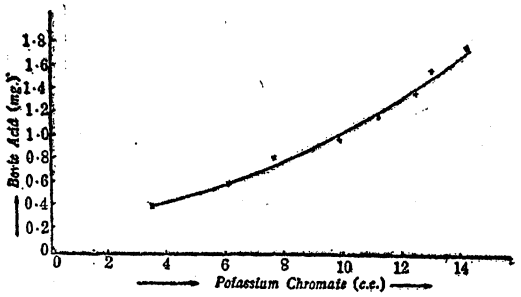
The results obtained are recorded in Table I. The amounts of boric acid were plotted against the mean volumes of the chromate solution and a mean curve was drawn (*cf.* Graph). To evaluate the magnitude of the

TABLE I

Boric acid mg.	Potassium chromate c.c.	Mean volume c.c.	Pot. chromate c.c. (interpolated)	Deviation %
1	2	3	4	5
0·4	3·2 3·3 3·7	3·4	3·4	nil
0·6	6·1 5·9 6·3	6·1	6·1	nil
0·8	7·9 7·4 7·6	7·6	8·0	−5·0
1·0	9·8 9·8 9·9	9·8	9·5	+3·2
1·2*	11·0 11·4 10·9	11·1	10·8	+2·8
1·4*	11·8 12·4 12·6	12·3	12·1	+1·7
1·6*	13·2 12·4 13·2	12·9	13·2	−2·3
1·8*	14·0 14·0 14·4	14·1	14·3	−1·4

* After developing colour only half the test solution was employed for matching.

errors in matching, the mean volumes of the chromate solution corresponding to the boric acid taken were obtained by interpolation on the graph



and are recorded in column 4. The percentage deviation of the mean volumes (experimental) from the interpolated figures was then calculated and the results are recorded in column 5.

In order to test the relationship thus established, the colour was developed with other known quantities of boric acid and then matched with the chromate solution as before. From the mean volume of the chromate solution required, the amount of boric acid was read out from the graph. The results obtained are given in Table II.

TABLE II

Boric acid taken mg.	Potassium chromate c.c.	Mean volume c.c.	Boric acid found mg.	Error %
1	2	3	4	5
0·48	5·5 5·6 5·7	5·6	0·55	+14·6
0·68	6·5 6·8 6·8	6·7	0·65	− 4·4
1·48*	13·9 13·6 13·9	13·8	1·7	+14·9

* After developing colour only half the test solution was employed for matching.

Discussion

The figures given in Table I, column 2, show a variation of 0·5 c.c. in matching except in two cases where it reaches 0·8 c.c. This increase obviously arises from the fact that the errors in matching are multiplied by two (*vide*

supra) according to the procedure adopted. The maximum deviation from the mean volumes (column 3) is only 0·5 c.c. Thus the figures in column 2 when compared with the mean volumes represent maximum errors in matching of about 15 and 3·5% on the lowest and highest limits of boric acid respectively. However, the deviations of the mean volumes from the mean curve range from 0 to 5·0% (*cf.* columns 4 and 5) so that if the mean volumes are employed for interpolation on the graph the deviation from the truth due to errors in matching does not exceed 5·0%.

The results given in Table II, however, show a maximum error of about 15% in the estimation of boric acid by interpolation of the mean volumes (column 3) on the graph. It appears probable that the higher error is partly due to the difficulty in measuring small volumes of boric acid solution in the highly volatile solvent, acetone. It may, however, be pointed out in this connection that errors as high as 10% are not unusual in visual colorimetric methods.

Regarding the mechanism of the reaction the following may be mentioned:—

In aqueous solutions boric acid reacts with polyhydroxy compounds such as glycerine, mannitol, etc., to form simple esters; with the *o*-hydroxy carbonyl compounds and in presence of the dehydrating agent, sulphuric acid, however, it yields inner complex (chelate) esters. The interaction of boric acid with the O-Hydroxy-carbonyl compounds, *viz.*, 5-hydroxy-flavones and -flavonols and the 2-hydroxy-chalkones, in presence of anhydrous citric acid may perhaps be explained on the assumption that the latter serves as a dehydrant, though the possibility of interaction between the hydroxy acid (citric acid) and boric acid as a preliminary to the former reaction could not be altogether ignored. The mechanism of the interaction of boric acid with the 5-methyl ethers of the above hydroxy-flavones and-flavonols and 2-methyl ethers of the above hydroxy-chalkones is obviously more complicated as no free hydroxyl, ortho to the carbonyl group, is available in any one of these compounds. The part played by the anhydrous citric acid is itself not quite clear and consequently the disappearance of colour on dilution with acetone, free from citric acid, could not be explained. The available evidence is meagre and hence the reaction must be regarded as empirical at present.

Regarding the method itself the following points may be considered:—

1. The use of the highly volatile solvent, acetone is apparently a serious disadvantage but it is of interest to note in this connection that even the more volatile ether is used for the extraction of nickel dimethyl glyoxime complex

in colorimetric work.[6a] The use of a less volatile solvent is an obvious solution of the difficulty but the choice is limited by the solubilities of the substances involved and also by the fact that hydroxylic solvents could not be employed.

The present method possesses the advantage that the blank is practically colourless whereas in the three colorimetric methods referred to already the blanks are coloured. Its chief advantage, however, is in its rapidity as compared with the turmeric and curcumin methods (*loc. cit.*), although the results obtained are only approximate.

3. The need for the rigorous exclusion of moisture is apparently a serious drawback of the method, but it may be pointed out that the drying of the reagents can be readily accomplished and the time taken in drying the few pieces of apparatus could not be regarded as of serious consequence. Further the need for stoppering the Nessler cylinder containing the test solution except during matching is not a great inconvenience in practice.

Summary

A new method for the approximate colorimetric estimation of boric acid depending on the yellow colouration yielded by boric acid with pentamethylquercetin in presence of citric acid in anhydrous acetone medium has been described. The method is empirical in character and gives results with a maximum error of about 15% with quantities of boric acid ranging from 0·4 to 1·8 mg.

The authors wish to express their thanks to Professor T. R. Seshadri for valuable help rendered in the course of this investigation.

REFERENCES

1. Mellor .. *A Treatise on Chemical Analysis*, 1938, 653.
2. Stahl .. *Z. anal. chem.*, 1931, **83**, 340 ; *cf. Chem. Abs.*, 1931, **25**, 2387.
——— .. *Z. anal. chem.*, 1935, **101**, 348 ; *cf. Chem. Abs.*, 1935, **29**, 5774.
3. Hebebrand .. *British Chem. Abs.*, 1902, **2**, 354.
4. Bertrand and Agulhon .. *Chem. Abs.*, 1914, **8**, 884, 1312.
5. Schäfer .. *Z. anal. chem.*, 1937, **110**, 11 ; *cf. Chem. Abs.*, 1937, **31**, 437.
6. Snell .. *Colorimetric Analysis*, 1936, **1**, 526.
6*a*. ——— .. *Ibid.*, p. 316.
7. Scharrer and Gottschall .. *Brit. Chem. Abs.*, 1935, B, 820.
8. Smith .. *Organic Reagents for Metals, cf.* Hopkin and Williams, Ltd., 1938, 109.
9. Olson and De Turk .. *Chem. Abs.*, 1941, **35**, 1723.
10. Patricia W. Mansell .. *Ibid.*, 1941, **35**, 6536.
11. Rangaswami and Seshadri *Proc. Ind. Acad. Sci.*, 1942, **16 A**, 129.
12. Wilson .. *J. A. C. S.*, 1939, 2303.

763-43 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

A NOTE ON POISSON DISTRIBUTION

By K. Sankara Pillai
(*Travancore University*)

Received March 23, 1943
(Communicated by Prof. K. B. Madhava, F.A.Sc.)

1. *Introduction*

Poisson distribution like the binomial, contains only one parameter and is of very wide application in statistical work. The usual estimate for the parameter is the sample arithmetic mean obtained by the method of maximum likelihood. The method of least squares yields in this case an estimate that cannot be expressed as a convenient function of the observed values. A slight modification in the method of approach to the problem of estimation allows a direct application of the least square principle and gives an estimate which is elegant though not as simple as the maximum likelihood estimate. A discussion on the estimate so obtained is given in this paper.

2. *Estimate for* m

Let the population frequencies be denoted by

$$Y_k = \frac{N \cdot m^k e^{-m}}{\Gamma(k+1)}, \qquad (k = 0, 1, 2, \cdots) \qquad (1)$$

and the observed frequencies by

$$y_k \qquad (k = 0, 1, 2, \cdots) \qquad (2)$$

Also

$$N = y_0 + y_1 + y_2 + \cdots + y_n.$$

We have in the population

$$Y_{k+1} = \frac{m}{k+1} \cdot Y_k \qquad (3)$$

Since the relation (3) exists in the population, we shall assume that in a sample also an approximately similar relationship holds good. Hence

$$y_{k+1} = \frac{m}{(k+1)} \cdot y_k + \epsilon_k \qquad (k = 0, 1, \cdots n-1) \qquad (4)$$

where the ϵ's are small. To estimate m we minimise

$$\sum_0^{n-1} \epsilon_k^2 = \sum_0^{n-1} \left[y_{k+1} - \frac{m}{k+1} \cdot y_k \right]^2.$$

By differentiation, we get the required estimate

$$m_2 = \frac{\sum_0^{n-1} [y_k \cdot y_{k+1}/(k+1)]}{\sum_0^{n-1} [y_k^2/(k+1)^2]} \quad * \qquad (5)$$

It may be interesting to note that in the case of the binomial distribution

$$Y_{k+1} = \frac{n-k}{k+1} \cdot \frac{p}{1-p} Y_k.$$

Using the method adopted above the estimate for p is

$$\Sigma \frac{n-k}{k+1} \cdot y_k \cdot y_{k+1} \Big/ \left[\Sigma \left(\frac{n-k}{k+1} y_k \right)^2 + \Sigma \frac{n-k}{k+1} \cdot y_k \cdot y_{k+1} \right].$$

The statistics m_2 satisfies the criterion of consistence, for the population value of m_2 is given by

$$\frac{N^2 \cdot e^{-2m} \cdot \sum_0^{n-1} \frac{m^{2k+1}}{\Gamma(k+2) \cdot \Gamma(k+2)}}{N^2 \cdot e^{-2m} \cdot \sum_0^{n-1} \frac{m^{2k}}{\Gamma(k+2) \cdot \Gamma(k+2)}} = m$$

Though the statistic is a consistent one, it is clear that it does not satisfy the criterion of sufficiency.

3. *Efficiency of* m_2

To determine the efficiency of the estimate m_2, we proceed to calculate the standard error of m_2 in large samples.

We have

$$m_2 = u/v \text{ where} \qquad (6)$$

$$u = \sum_0^\infty y_k \cdot y_{k+1}/(k+1); \qquad v = \sum_0^\infty y_k^2/(k+1)^2$$

Also let

$$U = \sum_0^\infty Y_k \cdot Y_{k+1}/(k+1); \quad V = \sum_0^\infty Y_k^2/(k+1)^2 \qquad (7)$$

In repeated samples, let us denote the small variation in the class frequency y_k by δy_k $(k = 0, 1, 2, \cdots)$ and the corresponding variations in u, v, m_2, by δu, δv and δm_2 respectively. Hence

$$y_k = Y_k + \delta y_k \ (k = 0, 1, \cdots \infty) \quad \dagger$$
$$u = U + \delta u, \ v = V + \delta v, \ m_2 = m + \delta m_2. \qquad (8)$$

---

* We shall assume that m_1 denotes the max. likelihood estimate, *viz.*,

$$m_1 = (y_0 + y_1 + \cdots + y_n)/n$$

† We assume $y_k = 0$ $(k = n+1, n+2, \cdots)$.

From (6) and (8) we have

$$V \cdot \delta m_2 = \delta u - m_2 \cdot \delta v. \tag{9}$$

Further,

$$\delta u = \sum_0^\infty [Y_k \cdot \delta y_{k+1} + Y_{k+1} \cdot \delta y_k] \div (k+1) \tag{10}$$

$$\delta v = \sum_0^\infty [2Y_k \cdot \delta y_k] \div (k+1)^2. \tag{11}$$

Using (10), (11) and (3) in (9), we have,

$$V \cdot \delta m_2 = \sum_1^\infty \left[\frac{Y_{k-1}}{k} - \frac{Y_{k+1}}{k+1}\right] \cdot \delta y_k - Y_1 \cdot \delta y_0 \tag{12}$$

Let

$$P_k = \frac{Y_{k-1}}{k} - \frac{Y_{k+1}}{k+1} \tag{13}$$

Equation (12) can now be written as

$$V \cdot \delta m_2 = \sum_1^\infty P_k \cdot \delta y_k - Y_1 \cdot \delta y_0. \tag{14}$$

Squaring (14) and taking expectations,

$$V^2 \cdot E(\delta m_2^2) = \sum_1^\infty P_k^2 \cdot E(\delta y_k^2) + Y_1^2 \cdot E(\delta y_0^2) - 2Y_1 \cdot \sum_1^\infty P_k \cdot E(\delta y_0 \cdot \delta y_k) + \sum_{k \neq j}^\infty \sum P_k \cdot P_j \cdot E(\delta y_k \cdot \delta y_j) \tag{15}$$

Since

$$E(\delta y_k^2) = Y_k \cdot (1 - Y_k/N) \text{ and}$$

$$E(\delta y_k \cdot \delta y_j) = -Y_k \cdot Y_j/N \qquad (k \neq j)$$

Equation (15) becomes,

$$V^2 \cdot \sigma_2^2 = \sum_1^\infty P_k^2 \cdot Y_k + Y_1^2 \cdot Y_0 - \frac{1}{N}\left[\sum_1^\infty P_k^2 \cdot Y_k^2 + \sum_{k \neq j}^\infty \sum P_k \cdot P_j \cdot Y_k Y_j - 2Y_0 \cdot Y_1 \sum_1^\infty P_k Y_k + Y_0^2 \cdot Y_1^2\right]$$

$$= \sum_1^\infty P_k^2 \cdot Y_k + Y_1^2 \cdot Y_0. \quad * \tag{16}$$

Substituting the value of P_k, we get after reduction,

$$\sigma_2^2 = \frac{e^m}{N} \frac{\sum_1^\infty \frac{m^{3k-1}}{k\,(k\,!)^3} - 2\sum_1^\infty \frac{m^{3k}}{k\,!\,(|k+1)^2} + \sum_1^\infty \frac{m^{3k-2}}{(k!)^3}}{\left[\Sigma \frac{m^{2k}}{(|k+1)^2}\right]} \tag{17}$$

* We assume that σ_1 and σ_2 are the standard errors of m_1 and m_2 respectively.

It has not been possible to obtain σ_2 in terms of elementary functions. The denominator can be expressed in terms of known series, it being equal to

$$[I_0(2m) - 1]^2 m^4$$

where $I_0(2m)$ is the Bessel Function of order zero with a purely imaginary argument. However the other series are rapidly converging and for particular values of m the sum may be calculated to any degree of accuracy.

The standard error of m_2, makes it possible to calculate the efficiency E of m_2, the standard error of the maximum likelihood estimate being equal to m/N

$$E = \sigma_1^2/\sigma_2^2. \tag{18}$$

For small values of m Table I below gives E. For large values of m the numerical calculation is heavy and hence we proceed to find an asymptotic formula for E.

The asymptotic expansions for the several series in equation (17) are as follows:—

$$I_0(2m) \sim \frac{e^{2m}\,[1 + 0\,(1/m)]}{2 \cdot \sqrt{m\pi}}\dagger \tag{19}$$

$$\sum_1^\infty \frac{m^{2k-1}}{k \cdot (k!)^3} \sim e^{2m}\left[1 + \frac{7}{9m} + 0\,(1/m^2)\right] \div (2\pi \cdot \sqrt{3} \cdot m^3) \tag{20}$$

$$\sum_1^\infty \frac{m^{3k}}{\underline{k} \cdot (\underline{k+1})^2} \sim \frac{e^{3m}\,[1 - 2/9m + 0\,(1/m^2)}{2\pi\sqrt{3}m^3} \tag{21}$$

$$\sum_1^\infty \frac{m^{2k-2}}{(k!)^2} \sim \frac{e^{3m}\,[1 + 1/9m + 0\,(1/m^2)}{2\pi \cdot m^3 \cdot \sqrt{3}} \tag{22}$$

Using these expansions, we get

$$E = 3 \cdot \sqrt{3}/8 = 0 \cdot 6495.$$

TABLE I

Efficiency of m_2

m ..	0	·5	1	1·5	2	3	4	5	10	∞
E ..	1	·4929	·3617	·3182	·3542	·4481	·5215	·5583	·5910	·6495

4. *Numerical Illustration*

In the example given below both m_1 and m_2 are calculated and the corresponding values of the frequencies tabled and compared.‡

† See *Asymptotic Developments of Functions defined by Series*, Walter B. Ford, p. 85. For (20), (21) and (22) see appendix.

‡ The example is taken from *The Theory of Probability*, Jeffries, p. 59.

TABLE II

The Frequency of Nuclei of Condensation in Aitken Dust Counter

Number	0	1	2	3	4	5	6	7	8
Observed Frequency	23	56	88	95	73	40	17	5	3
Calculated Frequency—									
1. m_1 (=2·93)	25	65	88	82	61	38	21	10	4
2. m_2 (=2·99)	20	60	90	90	67	40	20	9	3
Deviations—									
1. Based on m_1	−2	−9	..	13	12	2	−4	−5	−1
2. Based on m_2	3	−4	−2	5	6	0	−3	−4	0

Root mean square when m_1 is the estimate = 7·0

do. m_2 do. = 3·6.

5. *Conclusion*

The object of this paper has been to get an estimate for the parameter in the Poisson distribution, based on the method of least squares. The statistic obtained is found to satisfy the condition of consistence; it is, however, not a sufficient statistic. Its standard error has been found and its efficiency determined, and an asymptotic value for the latter obtained.

In conclusion, the author wishes to thank Dr. U. Sivaraman Nair for his kind help in the preparation of this paper. The author is also grateful to the University of Travancore for allowing him to carry on this work.

6. *Mathematical Appendix*

In what follows we shall prove a theorem pertaining to the asymptotic development of the function F (z) defined by the series,

$$\mathrm{F}(z) = \sum_{0}^{\infty} \frac{h(n)\cdot z^{3n}}{\Gamma(n+a_1)\cdot\Gamma(n+a_2)\cdot\Gamma(n+a_3)}.$$

The asymptotic expansion is based on the three Theorems given below§:

Theorem I. If the co-efficient $g(n)$ of the power series

$$f(z) = \sum_{0}^{\infty} g(n)\cdot z^n; \text{ radius of convergence} > 0 \qquad (1)$$

may be considered as a function $g(w)$ of the complex variable $w = x + iy$ and as such satisfies the two following conditions when considered throughout any arbitrary right half plane $x > x_0$:

§ See *Asymptotic Developments*, Ford, pp. 4, 30.

(*a*) is single valued and analytic,

(*b*) is such that for all $|y|$ sufficiently large one may write

$$|g(x+iy)| < K \cdot e^{\epsilon|y|} \tag{2}$$

where ϵ is an arbitrarily small positive quantity given in advance and where K depends only upon x_0 and ϵ, then the function $f(z)$ defined by (1) is analytic throughout any sector S (vertex at the origin) of the z-plane which does not include the positive half of the real axis and $f(z)$ within S is developable asymptotically as follows:—

$$f(z) \sim -\sum_{1}^{\infty} g(-n)/z^n \tag{3}$$

Theorem II. If condition (*b*) stated in Theorem I is changed to (*c*) $g(w)$ is such that for all $|y|$ sufficiently large one may write

$$|g(x+iy)| < K \cdot e^{(\pi+\epsilon)|y|}$$

other condition remaining the same, then the function $f(z)$ defined in (1), when considered throughout any sector S (vertex at the origin) of the z-plane which does not contain the negative half of the real axis, may be expressed in the form

$$f(z) = \int_{-l-\frac{1}{2}}^{\infty} g(x) \cdot z^x \, dx - \sum_{1}^{l} \frac{g(-n)}{z^n} + \xi_l(z) \tag{5}$$

in which 1 is any positive integer ≥ 1 and

$$\lim z \to \infty \; z^1 \xi_l(z) = 0.$$

Theorem III. Let $P(w)$ be a function of the complex variable $w = x+iy$, which is single valued and analytic throughout the half plane $x > x_0$, where x_0 has some negative value previously assigned; also suppose that as $w \to \infty$ along any ray emanating from the origin upon which $-\pi/2 < \arg w < \pi/2$, the same function satisfies the following condition, in which c is a constant whose value is independent of the ray selected,

$$\lim w \to \infty \; P(w) = c$$

then, as $z \to \infty$ along any ray emanating from the origin in the z-plane upon which $-\pi/2 < \arg z < \pi/2$, we shall have,

$$\lim_{s \to \infty} e^{-s} \cdot \int_0^{\infty} \frac{P(x) \cdot z^{x-1}}{\Gamma(x)} \, dx = c \tag{6}$$

where the integration is understood to be along the real axis.

Using these three theorems we proceed to prove:—

Theorem IV. Let F (z) be a function of the complex variable z defined by the series

$$F(z)=\sum_{0}^{\infty}\frac{h(n)}{\Gamma(n+a_1)\cdot\Gamma(n+a_2)\cdot\Gamma(n+a_3)}\cdot z^{3n} \tag{7}$$

in which a_1, a_2, a_3 are constants and in which $h(n)$ may be regarded as a function $h(w)$ of the complex variable $w=x+iy$ and as such satisfies the two following conditions:—

(a) $h(w)/\Gamma(w+a_1)\cdot\Gamma(w+a_2)\cdot\Gamma(w+a_3)$ is a single valued, analytic function of w throughout the finite w-plane,

(b). $h(w)$ is such that, when considered for values of w of large modulus lying in the right half plane $R(w)=x>x_0$ where x_0 is some assignable number, it may be expressed in the form

$$h(w)=\sum_{i=0}^{s+1}\frac{b_i\cdot\Gamma(w+k)}{\Gamma(w+k+i)}+\frac{\delta(w,s)\cdot\Gamma(w+k)}{\Gamma(w+k+s+1)} \tag{8}$$

in which the b_i's are constants and $\lim_{w\to\infty}\delta(w,s)=0 \qquad (s=0,1)$.

Then F (z) has the following asymptotic development

$$F(z)\sim\frac{e^{3z}}{2\pi\cdot\sqrt{3}\cdot z^{p}}\left[\sum_{0}^{\infty}\frac{C_m}{(3z)^m}\right],\quad -\pi/6<\arg z<\pi/6$$

where
$$p=a_1+a_2+a_3-2. \tag{9}$$

*Proof.** Let $f(z)$ be the function defined by the series

$$f(z)=\sum_{0}^{\infty}g(n)\cdot z^n, \tag{10}$$

where
$$g(n)=\frac{h(n/3)}{\Gamma(n/3)+a_1)\cdot\Gamma(n/3+a_2)\cdot\Gamma(n/3+a_3)}. \tag{11}$$

We have

$$F(z)=\tfrac{1}{3}\left[f(z)+f(z\cdot e^{2\pi i/3})+f(z\cdot e^{-2\pi i/3})\right. \tag{12}$$

so that our problem reduces to that of obtaining asymptotic expansions for the series occurring in (12).

$g(n)$ satisfies the conditions given in Theorem I and hence for all z of large modulus lying upon any ray for which $\pi/2<\arg z<3\pi/2$,

$$f(z)\sim-\sum_{1}^{\infty}\frac{h(-n/3)}{\Gamma(-n/3+a_1)\cdot\Gamma(-n/3+a_2)\cdot\Gamma(-n/3+a_3)\cdot z^n} \tag{13}$$

* The method of proof closely follows that adopted by Walter B. Ford in *Asymptotic Developments*, pp. 73.

$g(n)$ also satisfies the condition given in Theorem II and hence for all z of large modulus lying on any ray for which $-\pi < \arg z < \pi$,

$$f(z) = Q(z, l) - \sum_{1}^{l} \frac{h(-n/3)}{\Gamma(-n/3+a_1)\cdot\Gamma(-n/3+a_2)\cdot\Gamma(-n/3+a_3)\cdot z^n} + \epsilon_1(z) \quad (14)$$

where

$$Q(z, l) = \int_{-l-\frac{1}{2}}^{\infty} \frac{h(x/3)\cdot z^x}{\Gamma(x/3+a_1)\cdot\Gamma(x/3+a_2)\cdot\Gamma(x/3+a_3)}\, dx \quad (15)$$

and $\lim z \to \infty\ z^l \cdot \epsilon_1(z) = 0$

l being any arbitrarily large positive integer.

In order to study the behaviour of $Q(z, l)$ when z is large let us put

$$p = a_1 + a_2 + a_3 - 2 \quad (16)$$

and then write (15) in the form

$$Q(z, l) = \int_{-l-\frac{1}{2}}^{\infty} \frac{\Gamma(x+p+1)\cdot h(x/3)}{\Gamma(x/3+a_1)\cdot\Gamma(x/3+a_2)\cdot\Gamma(x/3+a_3)} \cdot \frac{z^x\, dx}{\Gamma(x+p+1)}. \quad (17)$$

Let us change the variable from x to t by the transformation $x+p=t$. Then we have

$$Q(z, l) = \int_{p-l-\frac{1}{2}}^{\infty} \frac{\Gamma(t+1)\cdot h\left(\frac{t-p}{3}\right)}{\Gamma\left(\frac{t-p}{3}+a_1\right)\cdot\Gamma\left(\frac{t-p}{3}+a_2\right)\cdot\Gamma\left(\frac{t-p}{3}+a_3\right)} \cdot \frac{z^{t-p}\, dt}{\Gamma(t+1)} \quad (18)$$

in which the path of integration extends from the point $t = p - l - 1/2$ to infinity in the direction of the positive real axis of t.

Now,

$$\Gamma(t+1) = \frac{3^{t+1/2}}{2\pi}\,\Gamma(t/3+1/3)\cdot\Gamma(t/3+2/3)\cdot\Gamma(t/3+1).$$

Using this result in (18),

$$Q(z, l) = \frac{\sqrt{3}}{2\pi z^p} \int_{p-l-\frac{1}{2}}^{\infty} \frac{H(t)\cdot h\left(\frac{t-p}{3}\right)}{\Gamma(t+1)} (3z)^t\, dt$$

in which

$$H(t) = \frac{\Gamma(t/3+1/3)\cdot\Gamma(t/3+2/3)\cdot\Gamma(t/3+1)}{\Gamma\left(\frac{t-p}{3}+a_1\right)\cdot\Gamma\left(\frac{t-p}{3}+a_2\right)\cdot\Gamma\left(\frac{t-p}{3}+a_3\right)}.$$

Using Stirling's formula and recalling (16), H (t) can be expanded as a factorial series. Hence, using (8) we write

$$\mathrm{H}\,(t)\cdot h\left(\frac{t-p}{3}\right)=\sum_{0}^{s}\mathrm{C}_n\,\frac{\Gamma\,(t+1)}{\Gamma\,(t+n+1)}+\frac{\sigma\,(t,\,s)\cdot\Gamma\,(t+1)}{\Gamma\,(t+s+1)}$$

where the C_n's are constants and $\sigma\,(t,\,s)=0\cdot(s=0,\,1,\,2\cdots)$ as $t\to\infty$ along any ray from $t=0$ in the positive half of the t axis.

Hence we write

$$\mathrm{Q}\,(z,\,l)=\frac{\sqrt{3}}{2\pi\,z^{p}}\left[\sum_{0}^{s}\mathrm{C}_n\int_{p-l-\frac{1}{2}}^{\infty}\frac{(3z)^t}{\Gamma\,(t+n+1)}\,dt+\int_{p-l-\frac{1}{2}}^{\infty}\frac{\sigma\,(t,\,s)\,(3z)^t}{\Gamma\,(t+s+1)}\,dt\cdot\right.\qquad(19)$$

Making use of Theorem II we get,

$$e^{3z}=\int_{p+n-l-\frac{1}{2}}^{\infty}\frac{(3z)^w}{\Gamma\,(w+1)}\,dw-\sum_{1}^{\mathrm{L}}\frac{1}{\Gamma\,(1-n)\;z^n}+\delta\,(z,\,\mathrm{L})$$

where

$$\lim z\to\infty\;z^{\mathrm{L}}\cdot\delta\,(z,\,\mathrm{L})=0\text{ and }-\pi<\arg z<\pi,$$

and L denotes the greatest integer in $l-n-\mathrm{R}\,(p)-1/2$, the path of integration extending to infinity in the direction of the positive real axis of w. By proper choice of l, L may be made independent of n and we then get

$$\lim z_{z\to\infty}\;z^n\cdot\delta\,(z,\,\mathrm{L})=0\text{ for }n=0,\,1,\,2,\cdots$$

Hence we get

$$\int_{p-l-\frac{1}{2}}^{\infty}\frac{(3z)^t}{\Gamma\,(t+n+1)}\,dt=\frac{e^{3z}}{(3z)^n}\,[1+\theta\,(z,\,\mathrm{L})]$$

where $\lim_{z\to\infty}z^n\cdot\theta\,(z,\,\mathrm{L})=0$ for $n=0,\,1,\,2,\cdots$

Thus the sum in (19) reduces to

$$e^{3z}\left[\sum_{1}^{s}\frac{\mathrm{C}_n}{(3z)^n}+\frac{\mathrm{C}_s\;\theta\,(z,\,\mathrm{L})}{(3z)^s}\right].$$

Also applying Theorem III the integral in (19) may be shown to be zero. Thus

$$\mathrm{Q}\,(z,\,\mathrm{L})=\frac{\sqrt{3}\;e^{3z}}{2\pi\,z^{p}}\left[\sum_{0}^{s}\frac{\mathrm{C}_n}{(3z)^n}+\frac{\eta\,(z,\,s)}{(3z)^s}\right]$$

where $\lim_{z\to\infty}\eta\;(z,\,s)=0$.

Using this result in (14) we finally get

$$f(z)\sim\frac{\sqrt{3}\;e^{3z}}{2\pi\,z^{p}}\,\Sigma\,\frac{\mathrm{C}_n}{(3z)^n}-\Sigma\,\frac{h\,(-n/3)\quad z^{-n}}{\Gamma\,(a_1-n/3)\cdot\Gamma\,(a_2-n/3)\cdot\Gamma\,(a_3-n/3)}$$

when $-\pi/2 < \arg z < \pi/2$. Further we note that

$$z^{-p}\, e^{-3z} \cdot \Sigma \frac{h(-n/3)}{\Gamma(a_1 - n/3)\cdot \Gamma(a_2 - n/3)\cdot \Gamma(a_3 - n/3)\cdot z^n}$$

can be developed asymptotically in the form

$$0 + \frac{0}{z} + \frac{0}{z^2} + \cdots$$

when $-\pi/2 < \arg z < \pi/2$. Thus we finally get

$$f(z) \sim \frac{\sqrt{3}\, e^{3z}}{2\pi\, z^p} \sum_0^\infty \frac{C_n}{(3z)^n}.$$

Substituting $z \cdot e^{2\pi i/3}$ and $z \cdot e^{-2\pi i/3}$ for z in (20) and summing the three expressions so obtained we get

$$F(z) \sim \frac{e^{3z}}{\sqrt{3}\, 2\pi\, z^p} \sum_0^\infty \frac{C_n}{(3z)^n}.$$

It will be clear that the region for which the expansion remains valid is $-\pi/6 < \arg z < \pi/6$.

The series (20), (21) and (22) required in the body of the paper are only particular cases of $F(z)$. Thus to get the asymptotic expansion of series in (20), we have

$$F(z) = \sum_p^\infty \frac{z^{3n}}{n\cdot \Gamma(n+1)\cdot \Gamma(n+1)\,\Gamma(n+1)}$$

Here

$$h(n) = 1/n,\ a_1 \quad a_2 \quad a_3 \quad 1, \text{ and hence } p \quad 1.$$

$$H(t) = \frac{\Gamma(t/3+1/3)\cdot \Gamma(t/3+2/3)\cdot \Gamma(t/3+1)}{\Gamma(t/3+2/3)\cdot \Gamma(t/3+2/3)\cdot \Gamma(t/3+2/3)}.$$

Now

$$\log \Gamma(x+h) = \tfrac{1}{2}\log 2\pi + x\log x - x + B_1(h)\cdot \log x - \Sigma(-1)^n \frac{B_{n+1}(h)}{n(n+1)\,x^n}$$

where B's are Bernoulli's polynomials of order n. Hence we get

$$\log H(t) = \frac{B_2(1/3) + B_2(1) - 2B_2(2/3)}{1\cdot 2\cdot t/3} + 0(1/t^2).$$

Using

$$B_2(h) = h^2 - h + 1/6$$

we get

$$\log H(t) = 1/3t + 0(1/t^2).$$

Hence

$$H(t) = e^{1/3t + 0(1/t^2)}$$
$$= 1 + 1/3t + 0(1/t^2).$$

Therefore

$$H(t)\, h\left(\frac{t-1}{3}\right) = \frac{3}{(t-1)} + \frac{1}{t(t-1)} + 0\,(1/t^3).$$

Converting the above into factorial series we get

$$H(t)\, h\left(\frac{t-1}{3}\right) = \frac{3}{t+1} + \frac{7}{(t+1)(t+2)} + 0\,(1/t^3).$$

Thus

$$C_0 = 0,\ C_1 = 3,\ C_2 = 7, \text{ etc.}$$

Hence

$$F(z) \sim \frac{e^{3z}}{\sqrt{3}\, 2\pi z}\left[\frac{1}{z} + \frac{7}{9z^2} + 0\,(1/z^3)\right]$$

Similarly the asymptotic expansions for (21) and (22) are obtained.

REFERENCES

1. Fisher, R. A. .. *On the Mathematical Foundations of Theoretical Statistics.*
2. Ford, Walter B. .. *Asymptotic Developments of Functions Defined by Maclaurin Series.*
3. Jeffries .. *Theory of Probability.*
4. Milne-Thompson, L. M. .. *The Calculus of Finite Differences.*

FLUCTUATIONS OF LIGHT INTENSITY IN CORONÆ FORMED BY DIFFRACTION

By G. N. Ramachandran

(From the Department of Physics, Indian Institute of Science, Bangalore)

Received September 22, 1943

(Communicated by Sir C. V. Raman, Kt., F.R.S., N.L.)

1. *Introduction*

The problem of determining the resultant of the secondary radiations emitted by a cloud of similar particles under the action of a primary wave is an important one in the field of optics. It might be supposed that when there are a large number, n, of such particles, distributed at random, the resultant intensity is just n times that due to a single particle. The late Lord Rayleigh (1871), however, showed that this is far from being the case, and that the resultant in any particular trial may be anywhere between o and n^2, and does not show any tendency to close upon the value n. He has also investigated (1880) the probability that the resultant intensity may lie between assigned limits of magnitude, and has derived an expression for the distribution of the resultant intensity.

In a general way, the existence of such fluctuations of intensity is illustrated by the well-known experiment of using a glass plate on which lycopodium powder is dusted as the diffraction screen, and viewing a source of light through it. The effects observed depend greatly on the size of the source and its spectral nature. Even as early as 1877, Exner observed the existence of radial streaks such as those shown in Fig. 3 (*a*), Plate I, when the corona is seen in white light. More recently, Laue (1916, 1917) and de Haas (1918) have observed the fluctuations in monochromatic light. In this case, the radial fibres reduce to a "mottled structure" of the type shown in Fig. 3 (*b*).* Laue's studies led him to suppose that the phenomena could not be completely explained on the basis of classical wave-optics. This contention was disputed by de Haas, who performed further experiments supporting the view that the phenomena are explicable on wave-principles.

In this paper, some theoretical considerations are presented regarding the nature of the fluctuations, and experiments performed with a view to

* This will hereafter be referred to also as the "fine-structure" or "structure" of the corona.

substantiate the theory are also described. It is found, as is shown below, that de Haas's explanation of the phenomena is inadequate, and does not go to the root of the problem. In particular, the Rayleigh statistical law of distribution of intensity has been quantitatively verified for the first time, making use of the fluctuations of intensity in the corona as the basis for measurement.

2. *The Nature of the Fluctuations in Monochromatic Light*

de Haas's explanation of the mottled structure consists in considering every pair of particles to give rise to a set of interference fringes crossing the field. These are superposed, and produce fluctuations of intensity. This explanation, however, is wholly inadequate. In fact, when the number of particles is very large, one must take the aggregate effect of *all* the particles into account and not merely the effect due to pairs of particles. Each particle gives rise to a corona of its own, and the resultant intensity in any direction is due to the collective action of the wave-fronts diffracted by all the particles in that direction.

The failure of the de Haas point of view may be brought out strikingly by the following analogy. Suppose we are considering the opposite case of a regular square diffraction grating, formed for example by a square mesh of wires. In this case, one may take each pair of meshes in the grating, and imagine it as giving rise to a set of interference fringes of the type imagined by de Haas. But, such a method gives us no idea at all of what the nature of diffraction pattern due to the complete grating would be. The latter could be determined only by taking the waves diffracted by all the meshes simultaneously into account and finding their total effect. In those directions in which all these waves reinforce, we get bright spots, while elsewhere we have dark areas.

In the same way, with the lycopodium powder also, one has to consider the waves diffracted by all the particles together. Each particle becomes the centre of secondary radiation, and in those directions in which the phase relations between the waves diffracted by the particles happen to be such that there is a large co-operative effect, there will be bright spots. Since the positions of the particles on the screen are unknown, one cannot determine the positions of these maxima of intensity; but it is easily seen that there must be a large number of such spots irregularly arranged in the field of view.

We now proceed to show that these spots are in fact as sharply defined as the original image of the source formed by a lens whose aperture is the same as that of the diffracting screen. Consider a point P in

the focal plane of the camera where due to the co-operative action of the waves from the various particles, we have a large intensity, say I_P. The amplitude at this point will be $u_P = \sum_1^n e^{ip_m}$ where p_m is the phase of the mth particle on the screen, and the summation is done for all the n particles present, it being assumed that the amplitude due to each particle is unity. Therefore,

$$I_P = (\sum_1^n \cos p_m)^2 + (\sum_1^n \sin p_m)^2 = n + \sum_{r=1}^n \sum_{s=1}^n \cos (p_r - p_s). \quad (1)$$

Consider now a neighbouring point P′. At this point, the phases of the waves from the various particles would be altered. Let the alteration of phase for the mth particle be δ_m. Then, the amplitude at P′ is

$u_{P'} = \sum_1^n e^{ip_m} e^{i\delta_m}$, and the intensity is

$$I_{P'} = \left[\sum_1^n \cos (p_m + \delta_m)\right]^2 + \left[\sum_1^n \sin (p_m + \delta_m)\right]^2. \quad (2)$$

This can be expressed in the form

$$I_{P'} = n + \sum_r \sum_s \cos (\delta_r - \delta_s) \cos (p_r - p_s) + \sum_r \sum_s \delta_s \sin (p_r - p_s).$$

If the δ's are small, this can be put in the form

$$I_{P'} = \left[n + \sum_r \sum_s \cos (p_r - p_s)\right] + \sum_r \sum_s \delta_r \delta_s \cos (p_r - p_s) + \sum_r \sum_s \delta_s \sin (p_r - p_s). \quad (3)$$

The second term is negligible, since it involves second order terms, so that

$$I_{P'} - I_P = \sum_{r=1}^n \sum_{s=1}^n \delta_s \sin (p_r - p_s). \quad (4)$$

Now, $\sin (p_r - p_s)$ is of the same order of magnitude as $\cos (p_r - p_s)$, so that $\Sigma\Sigma \delta_s \sin (p_r - p_s)$ is of a lower order compared with $\Sigma\Sigma \cos (p_r - p_s)$ which is the order of I_P. Thus, if δ is small, $(I_{P'} - I_P)$ is small compared with I_P.

Now, if we denote by $\delta\phi$ the angular separation of P and P′, then the largest value of δ_r or δ_s is given by $\pi b\, \delta\phi/\lambda$, where b is the width of the diffraction screen, and λ the wave-length of light. It is evident from (4) that $I_{P'}$ does not sensibly differ from I_P over a range of values of $\delta\phi$ within which the largest of the δ's does not exceed a fraction of 2π, and over this range of angles, the large intensity at P will persist. Putting the maximum value of the δ's for this to happen as, say, $\pi/4$ one gets the width of the bright spot as

$$\delta\phi = \frac{1}{4}\frac{\lambda}{b}. \quad (5)$$

Thus, the angular width of the bright spot is of the order of λ/b. Now, it is well known that the diffraction image due to a lens of aperture b also extends over an angle of the order of λ/b, so that the bright spot at P will be of the same sharpness as an image of the source formed by a lens of aperture b.

The argument used in the above discussion is, however, not restricted to the case when the intensity at P is a maximum. Actually, it is true whatever may be the intensity at P, so that one sees that the corona must actually consist of spots whose extension is of the same size as that of the image of the source. We have proved above that these images would not be spread out by an extent more than the aperture of the lens, so that we must expect the fine-structure to consist of sharp and well-defined images of the original source distributed at random. They are in fact the spectra formed by the irregular arrangement of the sources of secondary radiation produced by the particles.

Another interesting result, regarding the number of such spots in the field of view, comes out of the above discussion. As already remarked, the extent of the individual spots is given by the width of the aperture. Thus, we may divide the whole field of view into a number of areas, each of which is occupied by one spot. Now, on increasing the aperture, the area occupied by a spot decreases, so that more spots must appear in the field of view. To observe this phenomenon, the source of light must be small enough, so that the spots do not sensibly overlap. Otherwise, the fluctuations of intensity would all be wiped out by the overlapping, and the individual spots could not be discriminated. Under favourable conditions, however, the argument shows that the density or the number of spots per unit area must depend on the aperture employed, increasing with increase of its dimensions.

3. *Experimental Confirmation*

The verification of the above two deductions from the theory was done by the following experimental arrangement. The source of light, S (Fig. 1) was a pinhole, or an aperture of any size and shape as desired, which was illuminated by the filtered mercury radiation of 5461 A. U. At a certain distance from the source was placed a glass plate G, on which lycopodium powder was dusted. The resulting diffraction pattern could be brought to a focus on a plate C by a lens L. An aperture AB was placed in front of the lens, and its size and position could be adjusted. When necessary, the camera could be removed and the pattern directiy viewed with the eye.

Initial visual observations using a small circular hole as the source showed that the spots in the corona are also all circular in shape and of the

same size as the original source. On increasing or decreasing the size of the pinhole, the spots in the field of view also correspondingly became larger or smaller showing that they are images of the source. But the most convincing proof of this fact was obtained by using a triangular slit as the source, when every one of the spots assumed a triangular shape, and besides had the same size and orientation as the source. Photographs were then taken to illustrate this fact. They are reproduced in Fig. 4 (*a*) and (*b*), Plate I. Fig. 4 (*a*) was taken with a circular pinhole as the source, and Fig. 4 (*b*) with a triangular slit. They show in an unmistakeable manner that the spots are in reality images of the source.

The second conclusion from the theory was also verified by observation. On increasing the area of the aperture, it was found that the number of spots in the field of view increased, and *vice versa* as demanded by the theory.

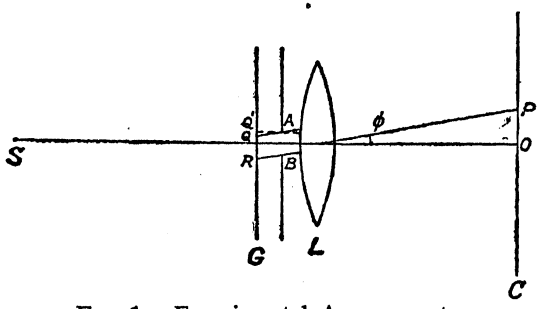


FIG. 1. Experimental Arrangement

During these experiments, a few other interesting phenomena were observed which also find a ready explanation on the idea that the spots are the spectra produced by the collective diffraction by all the particles.

(*a*) If one moves the screen containing the lycopodium powder keeping the eye fixed on the source, then the ring system is not found to undergo any change, but the fine-structure appears to move relative to the pattern of rings in the same direction as the motion of the screen. *Vice versa*, keeping the screen fixed, if one moves the eye, all the while looking at the source, then the fine-structure appears to move in a direction opposite to the motion of the eye. This effect is only the converse of the previous one. Also, if the screen is rotated, the fine-structure appears to rotate in the same direction.

These effects can be explained as being purely due to the geometry of the arrangement. Since the pupil of the eye is small, the whole of the screen does not contribute to the diffraction pattern nor is every portion of the pattern produced by the same portion of the screen. If we consider the corona in a direction ϕ (Fig.1), the intensity in this direction is due only to the waves diffracted by the region QR of the screen. Hence, if the screen is moved up, the

same portion QR is now responsible for the corona in a direction ϕ' parallel to Q'A. This happens for every portion of the screen, so that the fine-structure as a whole appears to move in the same direction as the screen relative to the rings. The effect of rotation is too obvious to need an explanation.

(*b*) During these experiments, it was found that the fine-structure not only moved bodily in the same direction as the motion of the screen, but that there were some internal changes as well. To follow these, the bodily motion was avoided in the following way. The aperture AB was placed in contact with the screen, and the lens L of the camera was large enough so that the whole diffraction pattern was caught by it. In this way, the difficulty of different portions of the screen giving rise to different portions of the corona was overcome. The whole of the screen was always operative. Now, on moving the screen in its own plane, the fine-structure pattern at the focal plane of the lens did not show any bodily movement; but there was an internal rearrangement of the spots.

This phenomenon could be explained as due to the fact that spherical waves are incident on the screen so that, on moving the screen in its own plane, the phase of the wave falling on each particle is altered. Thus, the phase relations between the waves emitted by different particles are changed, which leads to a change in the pattern of the spectra given by the particles and hence to a random rearrangement of the spots.

It is obvious that these internal changes must vanish if the incident wave is plane, for then a movement of the plate in its own plane should produce no change in the phase relationship between the different particles. This was tested out experimentally and it was found that with parallel light incident on the screen, the fine-structure pattern at the focal plane of the lens was entirely unaffected by moving the screen in its own plane. This shows that the explanation given above for the internal motion of the spots is correct.

(*c*) The phenomenon of the internal rearrangement of the spots can be observed with the eye also, if the aperture AB is made sufficiently small. In this case, on moving the aperture over the screen, different portions of the screen are operative, and since the arrangement of the particles in these are different, the spots in the field of view exhibit a beautiful "dance". The appearance is somewhat similar to that of the field of a spinthariscope, with bright spots appearing and disappearing at random positions.

4. *Fluctuations in White Light*

We have already shown how, in monochromatic light, the fine-structure consists of a series of images of the source. We now proceed to investigate

what happens if white light is used. Suppose that, with monochromatic light of wavelength λ, there is a bright spot at P, in the direction ϕ (Fig. 1). In the diffracting screen, draw the X-axis parallel to OP, and the Y-axis perpendicular to it, and let (x_m, y_m) be the co-ordinates of the mth particle on the screen with respect to these axes of co-ordinates. Then, (supposing the incident wave to be plane) the intensity in the direction ϕ is equal to

$$\left| \sum_{m=1}^{n} e^{ikx_m \sin \phi} \right|^2 \tag{6}$$

where k stands for $2\pi/\lambda$. Now, supposing the wavelength of the light to be changed to λ', and that $k' = 2\pi/\lambda'$, then it is readily seen from the above expression that the value of the intensity is unaltered provided we change ϕ to ϕ', such that

$$k \sin \phi = k' \sin \phi', \text{ or } \sin \phi/\lambda = \sin \phi'/\lambda'. \tag{7}$$

Hence, the bright spot, which was formerly at P, would now appear at another point P′ in the direction ϕ' given by expression (7) such that P′ lies along OP. This argument also is not restricted to the case when P is a bright spot, but is quite general. Hence, with an increase in the wavelength of the light, the whole pattern expands radially so that to every point P in the first case there is one, P′, in the second, such that

$$\frac{\text{OP}}{\lambda} = \frac{\text{OP}'}{\lambda'}. \tag{8}$$

If white light is used, there will be a range of wavelengths, so that there will be no discrete spots, but each spot will be spread out into a radial streak, whose colour is violet at the inner end and red at the outer end. The length of every such streak will be directly proportional to its distance from the centre, the streaks further away from the centre being longer.

As already remarked, such radial fibres were actually observed in white light by Exner. The author has observed these carefully and has found that one can actually follow the spectral colours in the order violet to red in any single fibre, as one goes away from the centre. Very close to the centre of the field, however, one can observe a few white spots, since the dispersion in them is very small on account of their proximity to the centre. The length of the fibres (the distance from the violet to the red end) is found to be proportional to their distance from the centre, and the length of any fibre at a certain distance is found to be a constant, within the limits of the experimental error.

In order to further demonstrate the correlation between the spots in monochromatic light, and the fibres in white light, the following experiment

was performed. A pointolite lamp was used to illuminate a fine pinhole, and a mercury green filter supplied by Adam Hilger & Co., was interposed. This transmitted only two bands, one in the green and the other in the red. The fine-structure now presented a beautiful appearance. The pattern was filled with red and green spots; but every green spot was accompanied by a red one, the two being along a radius, and the red spot being the outer one. It was also verified that the ratio of the distances of the red and the green spots from the centre was a constant, and was equal to the ratio of the mean wavelengths of the red and green transmission bands.

5. *Verification of the Rayleigh Law of Fluctuations*

Lord Rayleigh (1880) derived an expression for the distribution of intensity in the resultant due to n vibrations whose phases are at random. If the amplitude of a vibration is taken as unity, and as a special case, if the phases are assumed to have only either of values 0 or π, then it follows directly from Bernoulli's theorem that the chance of a positive amplitude between x and $x+\delta x$ is $\frac{1}{\sqrt{2\pi n}}\, e^{-x^2/2n}\, \delta x$. In the more general case of arbitrary phase, the probability that the resultant amplitude may lie between r and $r+\delta r$ comes out as

$$\frac{2}{n}\, e^{-r^2/n}\, r\, \delta r. \tag{9}$$

From this, it follows that the probability that the intensity may be between I and $\mathrm{I}+\delta \mathrm{I}$ is

$$p\,(\mathrm{I})\,\delta \mathrm{I} = \frac{1}{n}\, e^{-\mathrm{I}/n}\, \delta \mathrm{I}. \tag{10}$$

We can put this in a more convenient form. From (10), it can be shown that the average value of the intensity is n. Expressing I as a fraction f of the average value, *i.e.*, putting $\mathrm{I}/n = f$, (10) reduces to

$$p\,(f)\,\delta f = e^{-f}\, ef. \tag{11}$$

This gives the probability that the resultant intensity may lie between the fractions f and $f+\delta f$ of the average, and is the same function for all values of n. It is a maximum for $f=0$, and decreases as f increases.

This law can be verified by making use of the fine-structure in the corona. As already said, the fine-structure is produced as a result of the interference of the light diffracted by all the powder grains. The relative phases of these diffracted waves are not known but are distributed at random. Also, for different angles, ϕ, the phase relations are different, so that the whole pattern actually presents the effect of different combinations of these phase relations.

Hence, the resultant intensity in the pattern must show the same statistical law of fluctuations as demanded by Rayleigh's theory.

This was verified in the following way. A very fine pinhole illuminated by the mercury green radiation of 5461 Å.U. was used as a source of light, and a picture of the central portion of the corona was taken with an exposure of two hours on a Selochrome plate. To obtain a standard of intensities, a square grating was used instead of the lycopodium screen, and the same exposure was given for the grating also. The grating gave a spectrum, the intensities of whose spots are calculable. The plates for the two photographs were obtained by cutting a single quarter plate into two. Both were developed simultaneously in the same developing bath for the same time. Thus, a strictly comparable scale of intensities was obtained for determining the intensity of the spots in the corona. The photographs of the corona and the standard grating spectra are reproduced in Fig. 5 (*a*) and 5 (*b*), Plate I, respectively.

Now, in the corona, the average intensity falls away from the centre in the proportion $J_1^2(x)/x^2$ where $x = 2\pi a \sin(\phi)/\lambda$, a being the radius of the particle, ϕ the angle of diffraction, and λ the wavelength of the light. The corona taken in the present case extended upto a value of $x = 1{\cdot}5$, for which the average intensity was about half that at the entre. It was therefore divided into annular rings, the widths of which were all equal to $x = 0{\cdot}25$, by drawing circles of radii corresponding to $x = 0{\cdot}25$, $0{\cdot}5$ etc. Within each ring, the value of $J_1^2(x)/x^2$ did not vary much, and the average value for a ring was taken to be correct for that ring.

Using the grating spectrum, the intensity of whose spots could be calculated, an arbitrary scale of intensities was obtained. Then, the spots in each ring were classified into this scale, comparing them visually with the standard. The spots having an intensity less than 1 in the arbitrary scale, *i.e.*, those that could not be differentiated with the eye were found by calculating the total number of spots (dividing the area of the ring by the area of a spot) and subtracting from this the number of counted spots. Using this tabulated list, the average intensity (on the arbitrary scale) for that ring could be determined. Thus, the fraction of the total number of spots having an intensity between the fraction f_1 and f_2 of the average could be found. Dividing this by $(f_1 - f_2)$ we get the value of $p(f)$ at $(f_1 + f_2)/2$. Thus, $p(f)$ was evaluated for certain values of f. This was done for four rings, *viz.*, $x = 0{\cdot}25$ to $0{\cdot}50$, $0{\cdot}50$ to $0{\cdot}75$, $0{\cdot}75$ to $1{\cdot}00$, and $1{\cdot}00$ to $1{\cdot}25$. These were plotted in a graph and are reproduced in Fig. 2. The continuous curve represents the theoretical curve calculated from theory, and the experimental values are plotted by points.

A final average of all these four rings was obtained by dividing the intensities of the spots in each by the value of $J_1^2(x)/x^2$ for it, and then determining $p(f)$ and f taking them all into account. These are tabulated in the table below, and are represented by black dots in Fig. 2. It is clearly seen that the experimental points fit the theoretical curve very well, considering

f	$p(f)$	Theoretical	Difference
0·44	0·60	0·62	−0·02
1·29	0·33	0·28	+0·05
2·13	0·14	0·12	+0·02
3·45	0·02	0·03	−0·01
6·25	0·002	0·002	..

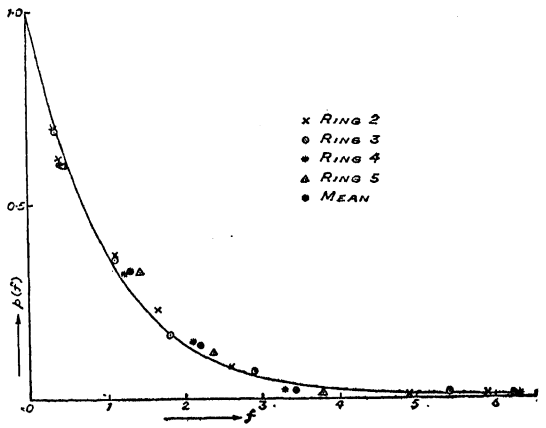


Fig. 2. Verification of Rayleigh's Statistical Law

that the comparison of intensities was done visually. Rayleigh's formula is therefore verified to be true for the distribution of intensity in the corona.

6. *Conclusion*

The above considerations regarding the fluctuations of intensity are true only for a static diffraction screen, *i.e.*, one in which the phase relations between the various particles is fixed in time. If the particles themselves are in motion, as in a gas or a cloud, then a continuous redistribution of phase takes place, and what one sees is the integrated effect of all these over a definite period. As Lord Rayleigh (1918) has shown, such a redistribution tends to make the resultant intensity (perceived as an average over a certain period) approach the average value n, this tendency being greater the larger the number of redistributions. Hence, for the light scattered by a gas, or a

cloud of particles, one is quite justified in regarding the scattered intensity in inclined directions as equal to n times that due to a single particle.

If the motion of the particles is slow, then the alteration in the position of the spots in the fine-structure will also be slow, and can be observed. Such a slow random motion takes place in Brownian movement, so that it must be capable of detection by this technique. This possibility, which was suggested by Prof. Sir C. V. Raman, is now under investigation.

I wish to express my sincere thanks to Prof. Sir C. V. Raman for suggesting the problem, and for the many helpful hints he gave during the investigation.

7. *Summary*

The fluctuations of light intensity in the diffraction corona produced by a large number of randomly distributed particles are investigated both theoretically and experimentally. The theoretical considerations show that the view put forward by de Haas is inadequate and that really the fluctuations arise owing to the interference of the waves diffracted by *all* the particles, which gives rise to a large number of sharp images of the source in monochromatic light. In white light, these spots must spread out, and produce radial streaks. These deductions from theory are all borne out by experiment.

Making use of the fluctuations of intensity in monochromatic light, verification has been made for the first time of Rayleigh's statistical law of distribution of intensity in the resultant of n vibrations of arbitrary phase. It is shown that the resultant intensity shows no tendency to close upon the average value, n, but that it is distributed over a wide range of values.

REFERENCES

1. de Haas, W. J. .. *Proc. K. Akad., Amsterdam*, 1918, **20**, 1278.
2. Exner .. *Sitzungsber. Akaa. Wien*, 1877, **76**, 522.
3. Laue, von .. *Phys. Gesselsch., Zürich*, 1916, 90.
4. ——— .. *Berichte deutsch. Phys. Gesselsch*, 1917, **19**, 19.
5. Rayleigh, Lord .. *Proc. Lond. Math. Soc.*, 1871, **3**, 267.
6. ——— .. *Phil. Mag.*, 1880, **10**, 73.
7. ——— .. *Ibid.*, 1918, **36**, 429.

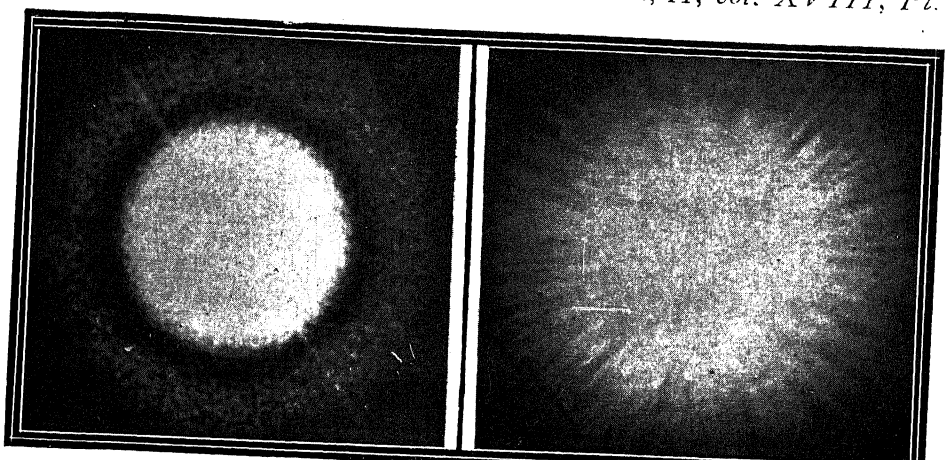


FIG. 3(a) FIG. 3(b)

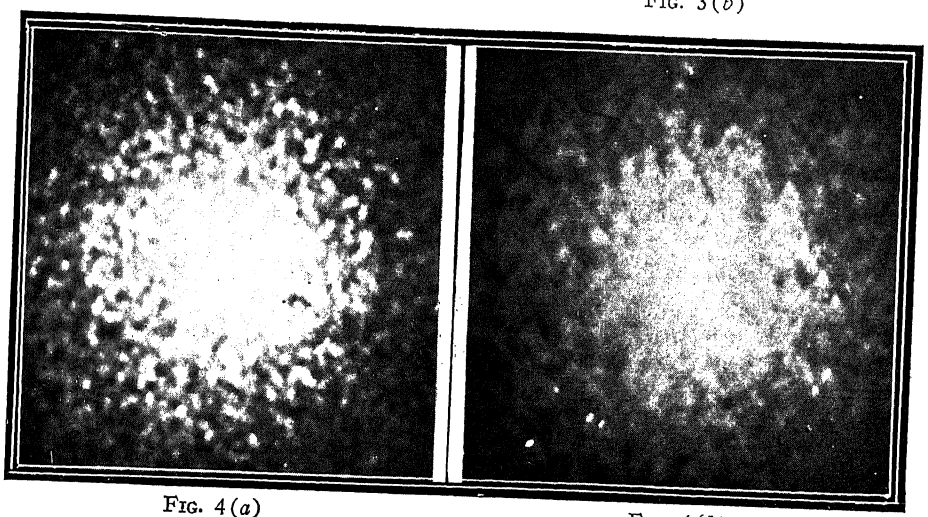


FIG. 4(a) FIG. 4(b)

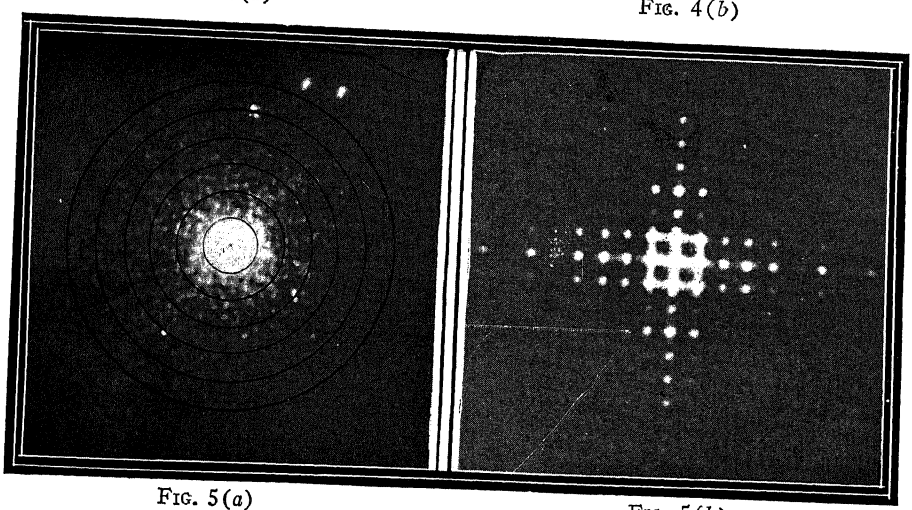


FIG. 5(a) FIG. 5(b)

CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part IV. A Note on the Bitter Principle of a Variety of *Citrus limetta*

BY T. R. SESHADRI

(From the Department of Chemistry, Andhra University, Waltair, now at Madras)

Received August 25, 1943

A VARIETY of *Citrus limetta* available in the northern and north-western parts of India during the hot weather has certain peculiar characteristics. It is quite juicy and the juice when carefully sucked out is almost insipid having only a faint sweet taste. But if the fruit should be crushed leading to pressure on the rags, the juice becomes exceedingly bitter. The bitter principle is present mainly in the rags. The thin peel which is rather difficult to remove is not so bitter. The seeds are very small. The external appearance of the fruits is quite similar to that of the sweet variety of *Cirtus limetta* (mussambi) common in Central and Western India and both go by the same common name, Mitta. The rind is yellow to orange in colour and smooth.

A fruit weighs on an average 200 grams and the juice yield is 100 to 120 grams (50 to 60%). The peels constitute 12–15%, the fresh rags 25–30% and the seeds about 0·7% of the fruit. The results of analysis of juice (average of several experiments) is as below: Brix 8·3°, reducing sugars 6·3%, total sugars 6·3%, sucrose nil, acidity 0·01% as citric acid. Thus there is very little acid and though the sugar content is not very low, sucrose is absent and consequently sweetness is poor.

The bitter principle was extracted from the rags by repeated treatment with cold alcohol and the extract concentrated to small bulk. With a view to detect the existence of more than one crystalline entity, the solid matter was isolated in fractions and then purified. By the addition of an equal volume of water to the alcoholic concentrate fraction (I) was obtained. Concentration of the mother-liquor during which most of the residual alcohol was evaporated, yielded fraction (II). The final mother-liquor (M) was separately examined. When fraction (I) was extracted with excess of boiling acetone, most of it dissolved leaving behind a very small quantity of resinous matter. On allowing the solution to concentrate a crystalline solid was obtained (fraction I A). Addition of water to the mother-liquor yielded an amorphous solid (fraction I B). Fraction (II) was also soluble in acetone and from it fractions (II A) and (II B) were obtained.

Fraction (I A) was insoluble in water, sparingly soluble in alcohol and readily soluble in acetone. It was best crystallised from acetic acid from which it came out as colourless rectangular prisms and tablets. The substance charred and burnt when introduced into a flame and left no residue. It melted at 292–94° with decomposition. When an alcoholic solution of it was treated with magnesium and hydrochloric acid no red or orange colour was developed. A dilute aqueous alcoholic solution was quite bitter; $[\alpha]_D^{30°}$, $-115{\cdot}1°$ in acetone solution. From these properties it seemed to be limonin and this surmise was confirmed by careful comparison with an authentic sample of limonin obtained from the seeds of Indian shaddock[1] and determination of the mixed melting point. Fractions (I B), (II A) and (II B), when subjected to crystallisation, yielded the same product thereby showing that there was only one bitter principle in all of them.

The aqueous mother-liquor (M) was made 7% acid with sulphuric acid and boiled for 2 hours. To start with there was a clear solution and at the end a dark brown resinous solid was found to have separated out. It was almost insoluble in acetone and an alcoholic extract (sparingly soluble in alcohol) gave no colour with magnesium and hydrochloric acid. No crystalline substance could be isolated from it. Thus the absence of naringenin in the resinous matter and of naringin in the aqueous mother-liquor was estabilished.

Consequently the only crystalline bitter principle of the above variety of *Citrus limetta* is limonin. This bitter substance has been formerly found to occur mainly in the seeds of the lemon, oranges and shaddock and it is invariably accompanied by more or less quantities of the closely related compound, isolimonin.[2,1]

It seems to be now clear that these complex bitter compounds of unknown constitution contribute also to the bitterness of the rags and peels of certain types of oranges. Isolimonin has been found by Higby[3] to be the main bitter component of the peels and rags of American Navel oranges and limonin to be present in the pulp of Valencia oranges. The present note records a further case of this kind. The constitution of limonin is quite indefinite. But its formula seems to be definite as $C_{26}H_{30}O_8$. Since the number of carbon atoms is near those of sterols and resinols, its reactions with the special reagents of these groups of compounds has now been studied. With the Liebermann-Burchard reagent it yields a very pale yellow solution. On the other hand, with the Salkowski reagent, the chloroform layer is colourless with a weak bluish violet fluorescence and the sulphuric acid layer first turns

yellowish brown and in the course of a few minutes becomes deep brown red. These characteristics are different from those of sterols and of resinols.

Summary

A variety of *Citrus limetta* has been found to contain limonin as the only bitter principle of the rags. The characteristics of the fruit and its juice are described.

REFERENCES

1. Seshadri and Veeraraghaviah .. *Proc. Ind. Acad. Sci.*, 1940, **11 A**, 505.
2. Köller and Czarney .. .. *Monatsh*, 1936, **67**, 248 ; 1937, **70**, 26.
3. Higby .. .. .. *J.A.C.S.*, 1938, 3014.

PIGMENTS OF COTTON FLOWERS

Part IX. A Note on the Occurrence of Populnetin in Indian Cotton Flowers

BY P. SURYAPRAKASA RAO AND T. R. SESHADRI

(From the Department of Chemistry, Andhra University, Waltair, now at Madras)

Received September 8, 1943

IN the course of their study of the colouring matter of the flowers of *Gossypium indicum*, Neelakantam and Seshadri reported the isolation of a new non-glycosidic compound.[1] The total pigment of the flowers was separated into five fractions according to the general scheme and studied. The first alcohol fraction contained mainly gossypin, a complex glycoside of gossypetin and the second (aqueous) fraction gave a small amount of the new substance which was not given any name at that time since it could not be studied in detail and its individuality was not quite definite. In the course of our examination of various samples of *G. herbaceum* flowers this substance has been again met with in small quantities and hence it occurs in this source also. Its main characteristics are (1) stability to aerial oxidation in alkaline solution thus indicating that it is a flavone, (2) lack of any colour changes in buffer solutions of pH on the alkaline side, (3) dissolution in concentrated sulphuric acid forming a yellow solution with a bright green fluorescence, (4) absence of any precipitate when an alcoholic solution is treated with neutral lead acetate, (5) formation of a yellow precipitate with basic lead acetate and (6) absence of methoxyl in its composition. These seemed to indicate that it was probably populnetin[2] and the idea was supported by the fact that populnetin occurs along with herbacetin in the flowers of *Thespasia populnea*[3] and hence could be expected to accompany it in the Indian cotton flowers also. But the melting point of the substance was rather low and the results of analysis were not definite due probably to the presence of inseparable impurities and the existence of hydration which seemed to vary with different conditions. The preparation and study of the acetyl derivative were not more helpful. The quantity available after these experiments was too small for further repeated purification and a detailed study independently. Consequently it was subjected to complete methylation using an acetone solution and excess of dimethyl sulphate and dilute sodium hydroxide. The product could be crystallised from alcohol from which it came out in the form of rectangular plates which were almost colourless. In its melting point, reactions and analysis, it was found to be identical with a sample of tetramethyl populnetin (described below) obtained by methylating an

authentic sample of populnetin isolated from *T. populnea*. It appears that definite purification is effected during methylation leading to the isolation of a pure methyl ether. The non-glycosidic substance should therefore consist mostly of populnetin.

Methylation of Populnetin

The flavone (1 g.) was dissolved in acetone (40 c.c.) and treated alternately in small quantities with 10% aqueous sodium hydroxide (50 c.c.) and dimethyl sulphate (10 c.c.). Finally the medium was made alkaline by the further addition of the alkali (50 c.c.) with vigorous shaking. In an hour an almost colourless crystalline compound separated out. It was filtered and recrystallised from alcohol using a little animal charcoal. Under the microscope it appeared as rectangular plates which were almost colourless. On heating it shrank at 95-100° (dehydration) and melted at 164-66°. It was insoluble in dilute alkali and did not yield any colour with ferric chloride. Its solution in concentrated sulphuric acid had a weak green fluorescence. [Found in the air-dried sample: C, 60·6; H, 6·2, and loss (H_2O) on heating at 110° for 2 hours *in vacuo*, 9·2%. $C_{15}H_6O_2$ $(OCH_3)_4$, $2H_2O$ requires : C, 60·3; H, 5·8 and H_2O loss, 9·5%. Found in the dehydrated sample: C, 66·2; H, 4·9%; $C_{15}H_6O_2$ $(OCH_3)_4$ requires C, 66·6; H, 5·2%.] The mixed melting point with the methyl ether obtained from the sample derived from the cotton flowers was undepressed.

From the experiments described above it is clear that populnetin occurs free in the Indian cotton flowers along with gossypetin, herbacetin and quercetin which are present mostly as glycosides. Thus there is a further case of the association which was first found in the flowers of the *Thespasia populnea*. It has already been shown that the substance is a tetrahydroxy flavone having a hydroxyl in the 4′-position. The disposition of the other groups in the benzopyrone part is still a matter of investigation. Its occurrence along with compounds having the 5:7:8-orientation of hydroxyls seems to suggest a similar disposition of the hydroxyl groups in it also.

Summary

The new non-glycosidic substance obtained from the Indian cotton flowers has been shown to consist mostly of populnetin from a comparison of the methyl ethers. The characteristics of the methyl ether of populnetin are described.

REFERENCES

1. Neelakantam and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1936, **4**, 54.
2. Neelakantam, Rao and Seshadri *Ibid.*, 1943, **17**, 26.
3. Rao and Reddy .. *Ibid.*, 1940, **12**, 372.

CHEMICAL EXAMINATION OF INDIAN ERGOT OF THE NILGIRIS

BY S. RANGASWAMI AND T. R. SESHADRI

(From the Department of Chemistry, Andhra University, Waltair, now at Madras)

Received August 25, 1943

SOME time back Thomas and Ramakrishnan[1] described their very successful experiments on the production of ergot of rye in the Nilgiris. Mr. Thomas sent us a sample as early as October 1942. We intended to make a detailed study of it as the first sample grown under Indian conditions and obtain information regarding the influence, if any, of habitat on the chemical composition. Owing to serious difficulties which we then had of laboratory and library we could not undertake the complete examination immediately; but after a proximate analysis the material was defatted and preserved. Further examination was carried out in March 1943 and the results were not without interest. Meanwhile Mukherji and Dey[2] published their note on the assay of Indian ergot and expressed their opinion that the Nilgiris ergot was at least of the British Pharmacopœial quality, if not better. Our results indicate that our sample is really of very high quality comparing favourably with the richest ever produced in different parts of the world and hence they are presented here.

Proximate analysis of the entire drug by standard methods gave the following values; the values reported by previous workers and taken from Barger's book on 'Ergot and Ergotism'[3a] are given within brackets for comparison.

Moisture	7·9% (4·4 – 10·0)
Ash	3·0% (2·2 – 7·0 ; average 4·0)
Total Nitrogen	3·57%
Fat (Petroleum ether extract)	27·3% (21·0%)

The fat which was a liquid had the following characteristics ; the figures within brackets have the same significance as above.

Refractive index at 32° C. ..	–1·466 (1·4685–1·4739 at 20° C.)
Saponification value ..	196·2 (178·4 – 196·9)
Iodine value (1 –hour Wij's)	–72·9 (69·55–73·8)
Unsaponifiable matter ..	1·7% (0·35–1·04; average 1·0)
Hehner number	96·5 (96·0–96·3)

The rest of the sample of ergot was powdered, defatted thoroughly with petroleum ether (boiling range 40–50°) by percolation, dried in the air and preserved in an air-tight container. The alkaloidal assay was carried out by the method of Hampshire and Page[4] as described in Garratt's book *Drugs and Galenicals*.[5] 7·0 gm. of the defatted sample were taken for the assay. The rest of the procedure was in general according to the description given in the above-mentioned reference except that a slightly larger volume of 1% tartaric acid was used for extracting the alkaloids from ether solution and the final tartaric acid solution had to be rediluted to give an easily measurable intensity of colour with the special alkaloidal reagent prepared according to the 1936 Addendum to the 1932 B.P. The concentration of alkaloids was obtained by using the Lovibond Tintometer of the B.D.H. pattern for measuring the intensity of colour. When the colour was developed using 1 ml. of the above solution and 2 ml. of the reagent, 8 blue units on the Lovibond Tintometer were taken to represent a concentration of 0·0001 gm. of anhydrous ergotoxine per ml. (*vide* Garratt, p. 112). The following values were obtained: Total alkaloids as anhydrous ergotoxine 0·585%; water-insoluble alkaloids as anhydrous ergotoxine 0·417%; water-soluble alkaloids as ergometrine 0·090%. The last figure was calculated by using the relation obtained by Hampshire and Page (*loc. cit.*) between ergotoxine and ergometrine, *viz.*, that the colour equivalent of ergometrine is 1·86 times that of ergotoxine. All the above figures are with reference to the defatted ergot sample. The corresponding values calculated on the original ergot sample will be 0·425, 0·303 and 0·0654% respectively.

In the meanwhile the note of Mukherji and Dey (*loc. cit.*) appeared in *Current Science* reporting values as low as 0·1213% for total alkaloids and 0·0237% for water-soluble alkaloids, based on the results of analysis by the same chemical method of Hampshire and Page and confirmed by pharmacological assays. With a view to see if our high value was due to any errors in the technique or in the assumed relation between ergotoxine content and blue value the sample was reassayed according to the standard method of 1932 B.P. modified by the 1936 Addendum. However, in view of our previous high results only 2·0 gm. of defatted ergot powder was taken. The rest of the procedure was according to the B.P. The total volume of the tartaric acid solution used in the extractions of the alkaloid from ether solution finally amounted to 52 ml. and this solution was further diluted with an equal volume of 1% tartaric acid for convenient colour matching. The comparison was done against a freshly prepared solution of ergotoxine ethanesulphonate taken from a freshly opened sealed tube of the compound procured from Messrs. B.D.H. and a Duboscq type of colorimeter was employed. The

value for total alkaloids obtained by this method was 0·465% as anhydrous ergotoxine calculated with reference to the defatted ergot and 0·338% on the entire ergot. It is well known that the B.P. method of assay includes only a portion of the water-soluble alkaloids.

The standard solution of ergotoxine ethanesulphonate was also utilised for checking the accuracy of the tintometric colour relation with the alkaloid content. Careful readings showed that the tintometer used in the previous assay entirely satisfied the colorimetric relation. 0·0001 gm. of anhydrous ergotoxine contained in 1 ml. of solution gave with the standard reagent under standard conditions a colour whose blue component was 8 units; so that the previous assay using only the tintometer for the evaluation of the total and water-soluble alkaloids was entirely correct.

Thus it is obvious that the sample of ergot examined by us is very rich in alkaloid content. There is nothing improbable in this high value. In his well-known book Barger[3b] gives numerous data for the alkaloidal content of various samples as obtained by different workers. Therein values as high as 0·38 and 0·414% are found for certain samples. More recently Bekes[6] has reported a value as high as 0·74% obtained in the course of large-scale experiments on the intensive production of rye-ergot. The same author has also studied the alkaloidal content of individual sclerotia[7] and reported values as high as over 1% of total alkaloids with several samples. It should however be noted that variations are possible in the qualities of the samples depending on differences in the conditions of collection and preservation and this may account for the differences between our results and those of others.

In any project for the production of ergot adequate precaution has to be taken to prevent infected rye grains finding their way into those collected for food, since ergot is a poison. A reliable chemical test for the presence of ergot makes use of the colour reaction for sclererythrin which is a crimson violet colouring matter present in the walls of the cortical hyphæ, probably as the calcium salt. The test was carried out as below with the present sample of ergot: About 0·1 gm. of the powdered ergot was skaken with 5 ml. of ether and a few drops of dilute sulphuric acid for 5 minutes. The ether solution was then decanted, diluted to 10 ml. and half the volume treated with 2 ml. of a saturated solution of sodium bicarbonate. A deep violet colour was formed in the aqueous layer. This colour test shows that the Nilgiris ergot easily conforms to the ordinary requirements of ergot for purposes of testing food grains.

Summary

A sample of Indian ergot of the Nilgiris has been studied in detail. It contains remarkably high percentage of total and water-soluble alkaloids. With respect to other components it is normal.

REFERENCES

1. Thomas and Ramakrishnan .. *Mad. Agr. J.*, 1942, **30**, 411.
2. Mukherji and Dey .. .. *Current Science*, 1943, **12**, 87.
3. Barger .. .. .. *Ergot and Ergotism*, Gurney and Jackson, 1931 ; *a.* pp. 141–5 ; *b.* pp. 204–5.
4. Hampshire and Page .. *Quart. J. Pharm.*, 1936, **9**, 60.
5. Garratt .. .. .. *Drugs and Galenicals*, Chapman and Hall, 1937, pp. 112–4.
6. Bekesy .. .. .. *Biochem. Z.*, 1940, **103**, 5–6, 368–383.
7. ——— .. .. *Ibid.*, 1940, **102**, 3–4, 187–197.

INTERFEROMETRIC STUDIES OF LIGHT SCATTERING IN BINARY LIQUID MIXTURES: PART I

BY K. SUNANDA BAI

(From the Department of Physics, Indian Institute of Science, Bangalore)

Received October 12, 1943

(Communicated by Sir C. V. Raman, KT., F.R.S., N.L.)

1. *Introduction*

THE blue opalescence of binary liquid mixtures in the vicinity of their critical solution temperature has been the subject of numerous investigations in the past. Smoluchowski (1908) was the first to put forward a thermodynamic explanation of this phenomenon as due to optical inhomogeneity caused by spontaneous local fluctuations in concentration in the mixture. Einstein (1910) extended this theory with certain simplifying assumptions, namely (1) that the liquids are incompressible; (2) that their specific volumes are negligibly small in comparison with those of the saturated vapours emitted by them; and (3) that the latter can be treated as ideal gases and on this basis derived an expression for the intensity of the opalescent light. This theory predicts an infinite intensity of scattering and also complete polarisation of the scattered light at the critical solution temperature. Experimental investigations of the opalescent light by several authors however showed that as the critical solution temperature is approached neither does the intensity of scattering tend to infinity nor is the polarisation of the scattered light perfect. In order to explain these difficulties Ornstein and Zernike (1914, 16, 18, 26) put forward a modified theory which considered the mutual influence of fluctuations in composition in neighbouring volume elements and according to which the intensity assumes a finite value at the critical temperature and is proportional to λ^{-2} and not to λ^{-4} as given by the formula of Smoluchowski-Einstein.

In order to explain the persistence of the opalescent phenomenon over a wide range of temperatures accompanied by remarkable changes in intensity and state of polarisation Raman and Ramanathan (1923) revised Einstein's theory taking into account the compressibility of the mixture and the varying orientations of the molecules. According to them the total intensity of light scattered by liquid mixtures is the aggregate of the separate effects of (1) fluctuations in concentration of the mixture, (2) fluctuations of its density and (3) the varying orientations of the anisotropic molecules.

Einstein's theory ignores the latter two effects which become of increasingly greater importance relative to the first at temperatures above or below the critical solution temperature.

The earlier investigators in this field suggested as an explanation of the opalescence before the thermodynamic explanation was put forward by Smoluchowski that the liquid mixture in the vicinity of the critical solution temperature behaves as an emulsoid. That this suggestion is not altogether baseless has been shown by Krishnamurti (1929) who found in X-ray diffraction studies with liquid mixtures that there are in general two types of liquid mixtures, one giving two separate rings for the two components and the other giving mainly one ring. The partially miscible liquids below their critical temperatures are found to belong to the first type and completely miscible liquids to the second type. The tendency for the formation of two distinct rings is explained by him by postulating the formation of large molecular clusters of the individual components. Recently Krishnan (1935) by means of a delicate optical method has shown that the molecules of critical composition mixtures in the neighbourhood of their critical solution temperatures have got a tendency to group themselves into clusters and that the finite value of the depolarisation of the opalescent light arises from the fact that the size of the clusters become comparable with the wavelength of light. Other experimental evidence such as the abnormal increase in the coefficient of viscosity and the thermal coefficient of magnetic and electric birefringence at the critical point support this point of view.

When a dust-free liquid is irradiated by a beam of monochromatic radiation and the light scattered by it is analysed by means of an ordinary spectrograph, a large part of the scattering which owes its origin to the fluctuations of optical density to have unaltered frequency. Examination of this "unmodified" scattering with the aid of a high-resolving power interferometer such as the Fabry-Perot Etalon however reveals it as split into three components one in the position of the original line and the other two appear displaced from it to the positions predicted by Brillouin's theory of light scattering (1922). The outer or Brillouin components owe their origin to pulsations of optical density taking place over an extended region in the fluid and travelling through the medium with the velocity of sound waves and thus giving rise to a Doppler effect. The appearance of a central component is attributed partly to the 'Q' branch of the orientation scattering and partly to the fluctuations of optical density of a quasi-static or static character. It is clear from the facts that an interferometric study of light scattering enables us to make a frequency analysis of the fluctuations of

optical density and to ascertain whether they occur with or without phase relationship within the fluid.

Such an investigation has not been carried out so far in the case of liquid mixtures and accordingly an interferometric study of the light scattered by liquid mixtures of both miscible and immiscible types was undertaken and the results are described in the present communication.

2. *Experimental Details*

The critical mixture chosen for the investigation consisted of (1) a polar and a non-polar liquid, *viz.*, methyl alcohol and normal hexane and (2) two polar liquids, *viz.*, *iso*-butyric acid and water. Two ordinary mixtures were also chosen. One was of two non-polar liquids namely benzene and *n*-heptane and the other of two polar liquids, namely ethyl alcohol and water which are miscible at all temperatures. Great care was taken to prepare all these mixtures perfectly dust-free and in the case of the critical mixtures with the correct critical composition, since the critical opalescence is most pronounced in such mixtures. The procedure adopted was as follows:—30% by weight of freshly distilled methyl alcohol was mixed with 70% by weight of distilled *n*-hexane and the mixture transferred into a flask having a capacity of about 400 c.c. This flask was connected to a Wood's tube of nearly 300 c.c. capacity and having a diameter of 3 cm. and length 20 cm. The flask containing the mixture was immersed in ice and the system evacuated and sealed off. The mixture was then distilled two or three times into the Wood's tube, each time washing the distillate back into the flask, the final distillation being carried out slowly till the whole of the mixture was almost completely distilled into the experimental tube. The same procedure was adopted for the *iso*-butyric acid-water mixture, the critical composition in this case being 50% by weight of each of the components. The two ordinary mixtures were also prepared in the same manner, their composition being 50% by volume of each of the components.

3. *General Statement of Results*

1. *Methyl Alcohol* n-*Hexane Mixture.*—The interference pattern obtained with a Fabry-Perot Etalon having 5 mm. separation for the 4810 A.U. radiation of the zinc-mercury amalgam arc, for this mixture at 30° C. is reproduced in Fig. (2) (*d*) [(the critical temperature being 29° C.). along with those of methyl alcohol and *n*-hexane (see Fig. (2) (*b*) and (*c*)]. Fig. 1 (*a*, *b* and *c*) gives the microphotometer curves for the same radiation. Both methyl alcohol and *n*-hexane have very intense and well-defined

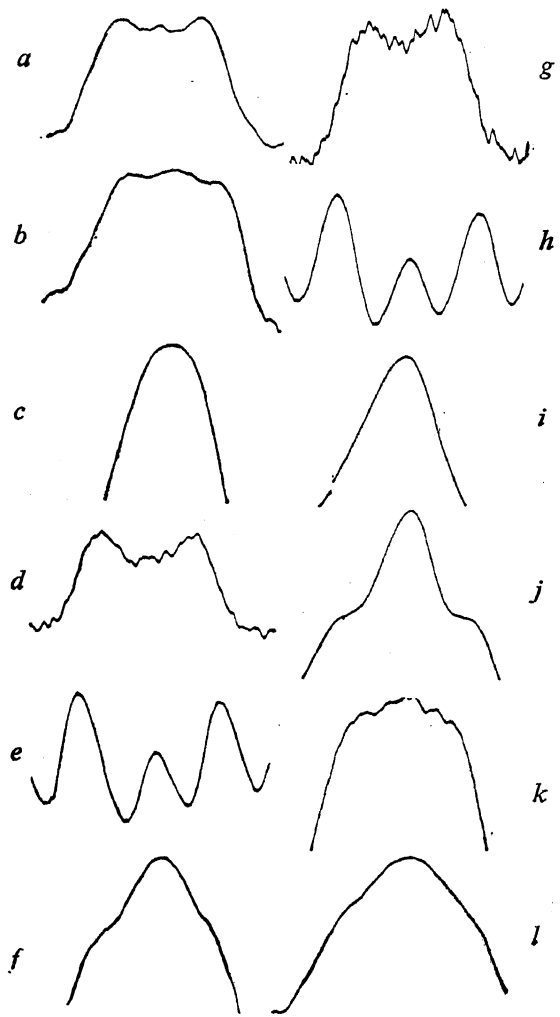


FIG. 1. Microphotometric Curves for Liquids

(*a*) Methyl alcohol
(*b*) *n*-Hexane
(*c*) Methyl alcohol–*n*-Hexane mixture
(*d*) Ethyl alcohol
(*e*) Water
(*f*) Ethyl alcohol-Water mixture
(*g*) *iso*-Butyric acid
(*h*) Water
(*i*) *iso*-Butyric acid–Water mixture
(*j*) Benzene
(*k*) *n*-Heptane
(*l*) Benzene–*n*-Heptane mixture

Brillouin components, their wave number shift $\delta\nu$ respectively being $0\cdot197$ cm.$^{-1}$ and $0\cdot21$ cm.$^{-1}$. The velocity of sound waves in these liquids is 1068 m./sec. for methyl alcohol and 1096 m/sec. for *n*-hexane. The

central component for methyl alcohol is very weak compared to the Brillouin components, while that for *n*-hexane has nearly the same intensity. In the mixture, however, we find the central component appearing with enormous intensity, but no trace of the Brillouin components is detected even in highly exposed plates.

2. *Iso-Butyric Acid-Water Mixture.*—The Fabry-Perot patterns for *iso*-butyric acid and water have already been investigated by Venkateswaran (1942) and his pictures and microphotometric curves for the same are reproduced respectively in Fig. 2 (*k*) and (*l*) and Fig. 1 (*g*) and (*h*). As can be easily seen from these photographs *iso*-butyric acid shows only an extremely weak central component and a strong continuum between the two intense Brillouin components. The spectral shift of the Brillouin components is 0·22 cm.$^{-1}$, the sound velocity being 1125 m./sec. Water on the other hand shows clearly all the three components the central component being slightly weak in comparison to the outer ones. The Brillouin shift for water is 0·28 cm.$^{-1}$, the velocity of sound being 1509 m./sec. The mixture at 30° C. [see Fig. 2 (*m*) and Fig. 1 (*i*)] however, shows no trace of the Brillouin components, as in the case of the mixture discussed above, in spite of the fact that the interference pattern is very intense.

3. *Benzene-n-Heptane Mixture.*—The interferometric patterns of benzene *n*-heptane and their mixture at 30° C. are respectively given in Fig. 2 (*p*), (*q*) and (*r*). Fig. 1 (*j*), (*k*) and (*l*) give the microphotometric curves for this mixture and its components. Benzene gives a very strong central component accompanied by two Brillouin components on either side of it, and of half its intensity; the spectral shift being 0·27 cm.$^{-1}$ and the velocity of sound propagation 1275 m./sec.; *n*-heptane on the other hand, gives two strong and diffuse Brillouin components with a fairly sharp central component of slightly higher intensity. The spectral shift of these components is 0·23 cm.$^{-1}$ and the velocity of sound propagation is 1161 m./sec. The mixture also unlike the above two critical mixtures shows all the three components. The mixture pattern has roughly the appearance of being a superposition of the patterns of the two component liquids. The velocity of sound propagation in this mixture is 1240 m./sec.

4. *Ethyl Alcohol-Water Mixture.*—The Fabry-Perot patterns obtained for the component liquids, *viz.*, ethyl alcohol and water by Venkateswaran (1942) are reproduced in Fig. 2 (*g*) and (*h*) along with that of the mixture Fig. 2 (*i*) obtained in the present investigation. Fig. 1 (*d*), (*e*) and (*f*) gives the microphotometric curves for the same. Here, as in the case of *iso*-butyric acid, ethyl alcohol gives slightly diffuse and very intense Brillouin components

accompanied by a sharp weak central component. As has been already mentioned in an earlier paragraph, water shows all the three components, the central component being slightly weaker than the outer ones. The mixture, on the other hand, shows a striking change. The central component exhibits a marked increase in intensity, the Brillouin components becoming relatively weaker than those of the component liquids. The velocity of sound propagation in water and ethyl alcohol is respectively 1509 m./sec. and 1130 m./sec. while that in the mixture is 1480 m./sec.

4. *The Effect of Temperature*

The two critical mixtures methyl alcohol-*n*-hexane and *iso*-butyric acid-water were also investigated at temperatures higher than the critical solution temperature. The interference patterns for methyl alcohol-*n*-hexane mixture at 40° C. and 50° C. are reproduced in Fig. 2 (*e*) and (*f*) and that of *iso*-butyric acid-water at 90° C. and 120° C. in Fig. 2 (*n*) and (*o*). The former shows no trace of the Brillouin components in spite of the fact that the mixture has been raised 21° C. above the critical temperature. The central component, however, becomes relatively sharper and weaker at higher temperatures. In *iso*-butyric acid-water mixture, on the other hand, the Brillouin components are clearly, though weakly seen at 90° C. The central component continues to be intense at all temperatures. The relative intensity of the shifted components to the central component becomes greater at 120° C.

Another fact of observation worthy to be recorded here is the relative intensity of the 5461 and 4358 A.U. radiations in the scattered spectrum. At the critical temperature, the green radiations are nearly as intense as the 4358 A.U. radiations; but as the temperature is raised by 20° C. or more, they become extremely weak, suggesting that the intensity of the opalescent light at the critical condition is proportional to λ^{-n} where n has a value much less than four [Andant (1924) and Rousset (1934)] and that at higher temperatures it tends to become proportional to λ^{-4}.

5. *Discussion of the Results*

The appearance of the Brillouin components in ordinary mixtures and the absence of the same in critical composition mixtures is very significant. One factor which might hinder the appearance of the Brillouin components is the abnormal increase in the coefficient of viscosity at the critical solution temperature reported by several authors such as Ostwald and Malss (1933) and Zofia Szafranska (1935). The effect of viscosity on the fine-structure components exhibited by pure liquids has been clearly brought out by

the studies of Venkateswaran (1942). Two important facts emerge out of his study on highly viscous liquids, *viz.*, (1) that the Brillouin components are present though feebly in the case of liquids like glycerine and castor oil, even at room temperature, (2) that the ratio of the intensity of the Brillouin components to the central component increases rapidly with rise of temperature. In the case of glycerine at 110° C. the intensity of the Brillouin components is comparable to that of the central component.

Similar temperature investigations on methyl alcohol-*n*-hexane and *iso*-butyric acid-water mixture ought to reveal marked changes in the relative intensity of the Brillouin components to the central component. On the contrary, the present investigations show no trace of the Brillouin components in the case of methyl alcohol-*n*-hexane mixture even when its temperature is raised by 20° C. above its critical temperature and for *iso*-butyric acid by 45° C. In the latter, the Brillouin components appear weakly at 90° C. and when the temperature is raised to 120° C. they brighten up, but not to the extent expected for a range of 30° C. These results therefore suggest that changes of viscosity as such cannot account for all the observed facts.

We are therefore obliged to interpret the absence of Brillouin components as indicating that the critical mixtures are composed of molecular clusters of size comparable to the wave-length of the sound waves and which are randomly distributed thus preventing the free propagation of sound waves in these mixtures. The results of the study of the effect of temperature on the light-scattering by these two mixtures indicate that they remain as emulsoids for a wide range of temperature above their critical points about 20° C. for methyl alcohol-*n*-hexane mixture and 45° C. for *iso*-butyric acid-water mixture. This conclusion is in agreement with the observations of Krishnan (1935) who finds that the range over which the clusters are detectable in the case of critical composition mixtures is more than 30° C.

On the other hand the appearance of the Brillouin components with intensity comparable to that of the pure liquids in ordinary mixtures indicates that there is no likelihood of the existence of large molecular clusters in them. X-ray and other optical evidences lend support to this view. The enhanced intensity of the central component however suggests that the composition scattering forms an important part of the total scattering in these mixtures as well.

In conclusion, the author desires to express her gretaful thanks to Prof. Sir C. V. Raman for his continued interest and encouragement in the course of the work. My thanks are also due to Dr. C. S. Venkateswaran for helpful suggestions.

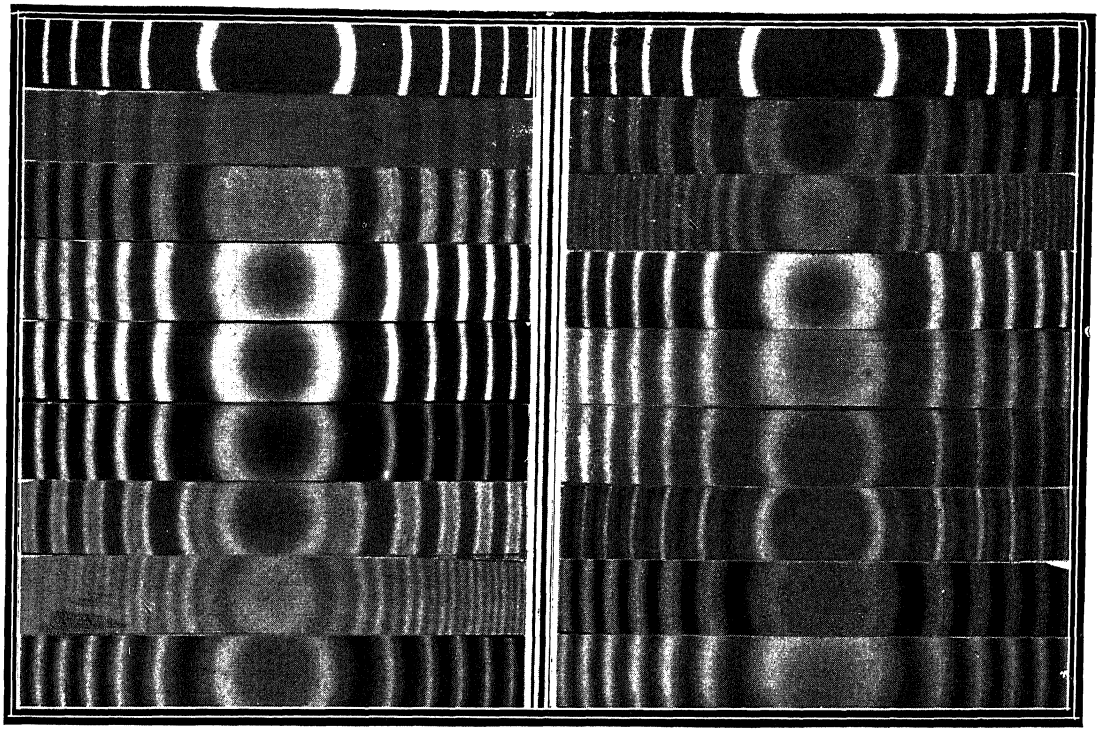


FIG. 2. Interferometric Patterns of Light Scattering in Liquids

(*a*) Direct Zn line (4810)
(*b*) Methyl alcohol (30° C.)
(*c*) *n*-Hexane (30° C.)
(*d*) Methyl alcohol–*n*-Hexane mixture (30° C.)
(*e*) Do. (40° C.)
(*f*) Do. (50° C.)
(*g*) Ethyl alcohol (30° C.)
(*h*) Water (30° C.) (4078 Hg)
(*i*) Ethyl alcohol–Water mixture (30° C.)
(*j*) Direct Zn line (4810)
(*k*) *iso*-Butyric acid (30° C.)
(*l*) Water (30° C.) (4078 Hg)
(*m*) *iso*-Butyric acid–Water mixture (30° C.)
(*n*) Do. (90° C.)
(*o*) Do. (120° C.)
(*p*) Benzene (30° C.)
(*q*) *n*-Heptane (30° C.)
(*r*) Benzene–*n*-Heptane mixture (30° C.)

6. *Summary*

The fine structure of the opalescent light of two critical composition mixtures, *viz.*, methyl alcohol-*n*-hexane and *iso*-butyric acid-water, has been studied at the critical solution temperature and higher temperatures. At the critical solution temperatures the Brillouin components are absent in either cases, while the central component is very intense. The former do not appear in the alcohol-hexane mixture even when its temperature was raised by 20° C. In the acid-water mixture, the shifted components are weakly present at 90° C. and found to increase in intensity at 120° C. In mixtures of miscible liquids namely benzene-*n*-heptane and ethyl-alcohol water also included in the present study, the Brillouin components are present prominently. The central component, however, shows enhanced intensity. These results indicate that a critical mixture at or above the critical solution temperature consists of molecular aggregates whose size is comparable to the wave-length of light and hence does not permit of the propagation of ordered sound wave trains of thermal origin. The significance of these results with reference to the Krishnan effect exhibited by binary liquid mixtures and the X-ray and other evidence is discussed.

REFERENCES

1. Andant, A. .. *Thesis*, Paris, 1924.
2. Brillouin, L. .. *Ann. de. Physique*, 1922, **12**, 88.
3. Einstein, A. .. *Ann. d. Phys.*, 1910, **33**, 1295.
4. Krishnan, R. S. .. *Proc. Ind. Acad. Sci.* A, 1935, **1**, 915.
5. Krishnamurti, P. .. *Ind. Jour. Phys.*, 1929, **3**, 331.
6. Ostwald, W., and Malss, A. *Koll. zeits.*, 1933, **63**, 61.
7. Ornstein, L. S., and Zernike *Proc. Amsterdam*, 1914, **17**, 79 ; 1916, **18**, 1520.
8. ———— .. *Phys. Zeits.*, 1918, **19**, 134 ; 1926, **27**, 761.
9. Raman, C. V., and Ramanathan, K. R. *Phil. Mag.*, 1923, **45**, 213.
10. Rousset, A. .. *Thesis*, Paris, 1934.
11. Smoluchowski, M. .. *Ann. d. Phys.*, 1908, **25**, 219.
12. Venkateswaran, C. S. .. *Proc. Ind. Acad. Sci.*, A, 1942, **15**, 363.
13. Zofia Szafranksa .. *Bull. Inter. Acad. Polonica. Class. Sci. Math. Nat.*, 1935 (A), **19**, 110.

ACTIVATION OF NITROGEN IN THE PRESENCE OF MERCURY

BY S. S. JOSHI AND A. PURUSHOTHAM

(From the Department of Chemistry, Benares Hindu University)

Received October 20, 1943

§1. APPRECIABLE divergence of opinion exists in regard to the effect of mercury on the life of active nitrogen. Strutt,[1] now Lord Rayleigh, observed: "Mercury gave an explosive compound, when its vapour was allowed to mix with active nitrogen." This was traced to the formation of mercury nitride. In a later paper[2] Rayleigh remarked: "No apparent effect is produced when the active gas is passed over clear cold metals or over a film of mercury held on copper; ....if a small quantity of mercury is placed in the bottom of a fairly wide tube, and shaken while the active gas passes over it, all luminosity is extinguished and the mercury becomes foul.....If the shaking is discontinued, the glow again passes." It must be emphasised that this role of mercury in the above-mentioned and similar experiments of Rayleigh and others is fundamentally different from the finding that on extreme purification nitrogen fails to give the afterglow and that the introduction of but a small proportion of a foreign substance, *e.g.*, mercury vapour, restores it.[2] To quote Rayleigh[2]: "A drop of mercury was placed inside a tube so that by warming, it could be made to give off vapour, which mingled with the nitrogen stream, a marked restoration of the glow was observed, though it was less brilliant than that obtained with the best catalysts." On the other hand, Kichelu and Basu[3] found: "For the measurement of pressure up to a certain stage, say 0·05 mm. Hg, a McLeod gauge was conveniently employed; but as the pressure of nitrogen became smaller any traces of mercury vapour had very destructive effect upon the life of active nitrogen, which quickly spent itself by acting upon mercury..... In the absence of liquid air it was found impossible to stop a slight diffusion of mercury vapour from the pump..... Even after these precautions, we did not find it possible to work with nitrogen below 0·02 mm." J. K. Robertson[4] also observed that "the addition of mercury vapour at once destroys the glow." As a manometric liquid and for working the evacuating pumps, mercury is almost indispensable in any work on gas reactions. In view of this and especially the above marked differences of observation in the literature, it was of interest to investigate the action of active nitrogen on mercury over a wide range of conditions.

§2. Fig. 1 shows the essential parts of the apparatus employed. The cylindernitrogen containing 0·78% oxygen was passed through a train of bulbs filled with alkaline pyrogallol to remove oxygen; it was then stored for about 3 days before use in an aspirator in which was hung a muslin bag containing some considerable quantity of freshly cut yellow phosphorus.

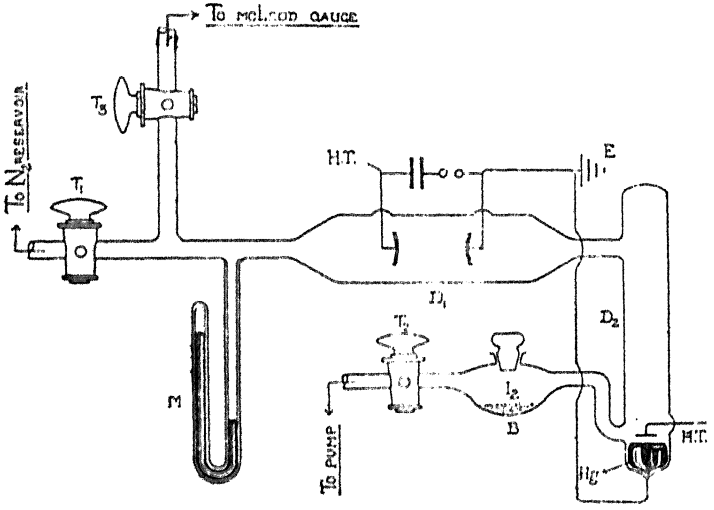


FIG. 1

No material differences were, however, observed so far as the nature of the results now to be reported are concerned, whether the gas was used after this purification or directly from the cylinder. By operating the suction pump and control of the stop-cocks the requisite pressure of nitrogen shown by the mercury manometer M was obtained; very low pressures were observed with a McLeod (not shown in the Fig.) connected at T_3. The gas was activated by a condensed, spark-in-series discharge in the Crookes' type tube D_1 fitted with well-cleaned aluminium electrodes about 1 cm. in diameter and 1·5 cm. apart; the electrode on the suction side was earthed. One more discharge tube D_2 disposed vertically and in series with D_1 contained some pure mercury at the bottom, which was earthed; the other electrode was connected to the H.T. as in D_1 when required. D_2 was immersed in an oil-bath whose temperature could be varied in the range 30–300° C. A small amount of powdered iodine in B served as a sensitive detector of active nitrogen on account of the very characteristic luminescence which it excites when brought into contact with iodine. This served to detect active nitrogen which was too feeble for visual observation. The various parts of the apparatus

between T_1 and T_2 were connected by fused glass-joints and the system was repeatedly tested for vacuum on a Töpler, before final observations.

§3. In the first series of experiments nitrogen streamed at a pressure of 2–3 cm. Hg was activated in D_1. The yellowish after-glow was well noticeable in D_2 on switching off the exciting voltage; with this on, and a well regulated suction, the glow was observable from D_2 upto T_2. It was just tinged with the characteristic green luminescence of mercury when the gas was flowing through D_2, which was kept at the ordinary temperature; was predominantly bluish-white due to iodine, in B; from T_2 to the exhaust end, the appearance was once more that of the normal after-glow of nitrogen. As the temperature of the bath was raised, the luminescence due to mercury predominated over the nitrogen glow in D_2. Above 140° C., mercury in D_2 boiled; this caused such an intensification of its luminescence that the after-glow of nitrogen was not perceptible in D_2. It is considered that this marked increase in the intensity of the mercury luminescence is appreciably due to transfer of energy by collisions of the second type, between part of active nitrogen and mercury vapour in D_2.[6] That the glow of active nitrogen was but masked or rather overshadowed by the mercury luminescence was shown by the fact that the streaming gas produced the characteristic emission of iodine in B and that the familiar after-glow of nitrogen was distinctly perceptible beyond B, *i.e.*, between T_2 and the exhaust.

§4. Two series of experiments were next made in which nitrogen was activated by exciting the discharge tube D_2 containing mercury as the earthed electrode: with D_2 at 28–30° C., the iodine detector B and the apparatus beyond it showed a well-marked after-glow over a wide pressure range of the streaming gas. The same results were obtained in which instead of streaming, the apparatus between T_1 and T_2 was filled with nitrogen at a pressure varied in the range 1–2 cm. Hg, and D_2 was excited intermittently. After each cessation of the discharge, an after-glow marked enough to be identified with a direct vision hand-spectroscope, was seen in D_2 and extending appreciably on either side of it, for a time which increased on decreasing the gas pressure. With the above arrangement *i.e.*, with static nitrogen, on reducing the gas pressure the duration of the after-glow increased progressively; it was 5–6 minutes at about 0·0018 cm. Hg.

In the second series of experiments, the bath surrounding D_2 was heated to 160° C., which produced a copious vaporisation of mercury. Nitrogen was then streamed at pressures varied from a few mm. to 2 cm. Hg. An *intense* after-glow was observed beyond D_2 upto T_2, which was in marked contrast when the activated gas was allowed to flow into neutral mercury

vapour.[2,3] It may be added that mercury in D_2 was fouled and showed the formation of mercury nitride when tested in the usual way.

There is considerable evidence to show the formation, under electrical discharge in mercury vapour, of the excited and meta-stable states; their proportion would increase with temperature. It is to be anticipated therefore, that subjecting nitrogen to an electrical discharge under such conditions would favour, as is actually observed, the intensification of the after-glow by (i) the diminution of the probability of collisional de-activation and (ii) the direct activation of the gas by energy transfer from the excited and metastable atoms of mercury,[6] and in collisions with the mercury ions moving under the action of the applied field.[7]

Summary

That active nitrogen is destroyed by contact with mercury is untenable as a general proposition. Their interaction produces the nitride and a variety of consequences depending upon the nature of conditions. When streamed into neutral mercury vapour, partial de-activation of active nitrogen and an excitation of the luminescence of mercury are the chief changes; the intensity of the latter increases with temperature and tends to overshadow that of nitrogen. When, however, the gas is excited with heated mercury as electrodes, the after-glow is markedly intense, which has been attributed to collisions with the excited and the metastable atoms of mercury, and its ions in the discharge space.

REFERENCES

1. Strutt .. *Proc. Roy. Soc.*, 1913, A **88**, 539–49.
2. ———— .. *Ibid.*, 1915, A **91**, 303–18.
3. Kichelu and Basu .. *Ind. Jour. Phys.*, 1929, **11**, 161–73.
4. Robertson .. *Canadian Journ. Research*, 1932, **7**, 444.
5. ———— .. *British Chem. Abst.*, 1933, p. 5.
6. Snaverly and Turner .. *Phys. Rev.*, 1931, **37**, 1684; *British Chem. Abst.*, 1933, 200.
   Kaplan .. *Ibid.*, 1931, **37**, 226.
   Okubo and Hamada .. *Phil. Mag.*, 1928, **5**, 372.
   Ruark .. *Ibid.*, 1928, **6**, 335.
   McLennan, Rudy and Anderson *Nature*, 1928, **121**, 537.
   Ruark, Foote, Rudnick and Chenault *J. Opt. Soc. Amer.*, 1927, **14**, 17.
7. Joshi and Purushotham. *Nature*, 1942, **149**, 250.

SOME ASPECTS OF THE BIOGENESIS OF ANTHOXANTHINS

By P. Suryaprakasa Rao and T. R. Seshadri

(From the Department of Chemistry, Andhra University, Waltair, now at Madras)

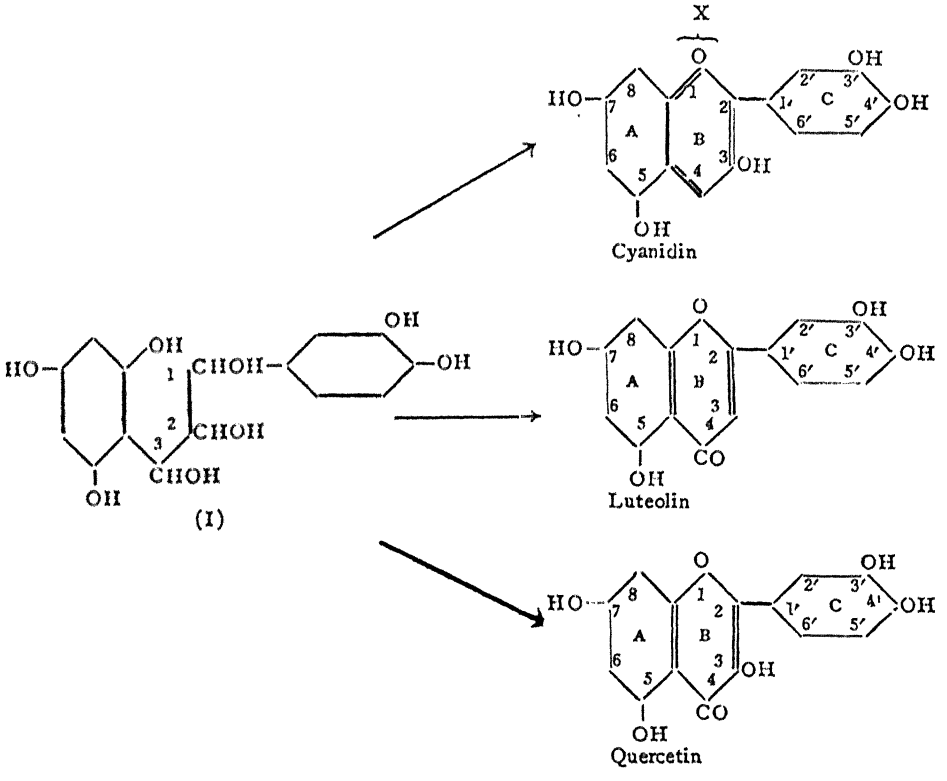
Received September 8, 1943

In a recent publication was given a brief account of our knowledge about naturally occurring flavones and flavonols whose constitutions have so far been definitely known.[1] They were arranged into different groups depending upon the number and the orientation of the hydroxyl groups in the benzopyrone part of the molecules. Their occurrence and their characteristic properties were also recorded. From the data thus presented it seemed to be possible to obtain information regarding the manner of their evolution in the plant kingdom. Possibility of success in this venture appeared to be greater particularly in view of the valuable conclusions which Robinson and his collaborators have recently arrived at in regard to the biogenesis of the anthocyanins.[2]

Anthocyanins and anthoxanthins are two groups of closely related sap soluble pigments. They are built upon the same pattern having 15 carbon atoms and 3 rings. Even before their chemical constitutions were definitely known, biologists were impressed by their association and they considered that anthocyanins were generated from the anthoxanthins by a process of oxidation taking place in the plant.[3] In support of this idea they could mention the comparatively later appearance of the bright red and blue colours due to anthocyanins and under conditions favourable to oxidation. When, as a result of the important work of Kostanecki, Perkin and others on the anthoxanthins and of Willstätter and his co-workers on anthocyanins, the chemistry of these two groups was made clear, the close chemical relation between them was established, but the anthocyanins represented a lower state of oxidation. Consequently the suggestion was made that anthocyanins were really products of reduction of flavones and flavonols, and no oxidation was involved.[3] On either of these two ideas which mean sequential evolution of the pigments, a correlation could be expected between the individuals of the different groups occurring in the same parts of the plants. For example, pairs like pelargonidin and kæmpferol, cyanidin and quercetin, delphinidin and myricetin, should occur. In the cases examined in this connection this correlation could not be found. Further, the presence

of pelargonidin with myricetin, delphinidin with kæmpferol was not uncommon. In the few cases where correlation was found, cyanidin and quercetin were involved and this could be considered as incidental to their prolific occurrence in the plant kingdom.

The difficulties were satisfactorily explained by Robinson who developed the idea of parallel origin of these groups of compounds from a common source[2]; different degrees of oxidation are involved in the production of anthocyanins, flavones and flavonols. According to his scheme no correlation is required between the members of the different groups occurring together. On the supposition that anthocyanins and other related substances are built up from simple carbohydrates through a series of aldol condensations, the hypothetical intermediate (I) was suggested as the basis from which all these compounds are derived. The intermediate can be constructed from two hexose units and one triose, and is in the same state of oxidation as a carbohydrate. The central three-carbon fragment of (I) may be modified in several ways to give different end products. For example, oxidation at C_1 leads to the formation of cyanidin, at C_3 to the flavone, luteolin and at both C_2 and C_3 or at C_1 and C_3 to the formation of the flavonol quercetin.



During the past several years Robinson and his collaborators have been engaged in a survey of anthocyanins occurring in nature. Making use of a quick method of analysis requiring small quantities of plant material they have been able to study a very large number of samples and thus provide sufficient material for statistical analysis. It has been found by them that the basic structures concerned are remarkably small in number being pelargonidin, cyanidin and delphinidin. Only a few rare cases correspond to flavones, *i.e.*, they lack in a hydroxyl group in the 3 position; gesnerin and carajurin are the rare examples. Further from their data it could be concluded that cyanidin is the primary member of the anthocyanidins and the production of delphinidin (oxidation) and pelargonidin (reduction) involve one stage more in evolution. These are remarkable results and give great support in favour of structure (I) as the common precursor.

The study of the biogenesis of anthoxanthins is more difficult for several reasons. In contrast to the few basic types found in the anthocyanins the variations in flavones and flavonols are quite numerous. There are at least 18 flavonols and 12 flavones of established constitutions. Quick methods of analysis of plant materials for anthoxanthins have not yet been developed and hence it has not been possible to analyse such large number of samples as for anthocyanins. Further, whereas anthocyanins usually occur single and in some cases with one more as minor component, anthoxanthin mixtures are more frequently complex. Consequently the number of cases of complete analysis are limited and deductions cannot therefore be comprehensive. However, as stated at the beginning it seems to be possible to arrive at some conclusions based on the following considerations :—(1) since anthocyanins and anthoxanthins are closely related, the results obtained from the more comprehensive study of the former could be accepted as applicable for the latter also, (2) the association of compounds in the same part of a plant, in different parts of the same plant and in closely related plants could be utilised as evidence of biogenetic relationship. In regard to alkaloids and other well-defined groups of naturally occurring compounds it is quite well known that plants of the same family contain the same compounds or closely related compounds. The differences amongst them are only in finer details and not in fundamentals. The minor changes mainly due to oxidation, reduction and dehydration could be brought about differently or to different stages in different members of a group of plants, in different parts of the same plant or even in the subdivisions of the parts. But the main framework may be said to be evolved in the same way.

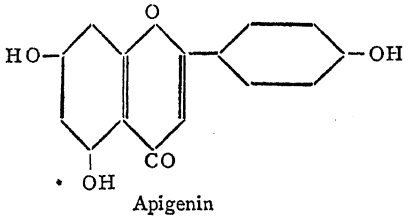
From consideration (1) given above, it follows that quercetin should be considered to be the simplest of the flavonols and luteolin the simplest of the

flavones and that the others require more stages for their evolution. This is also supported by the abundance of these two representatives in nature. Quercetin is the most frequently encountered anthoxanthin. Gisvold and Rogers[4] give figures showing that out of 268 cases where anthoxanthins have been identified, 220 have the 3′: 4′-dihydroxy orientation in the substituted phenyl group. It could further be stated in general terms that as in the case of the anthocyanins, here also variations in the number of hydroxyl groups in the side phenyl nucleus is possible by the introduction of one stage more of oxidation or reduction, the positions 3′ and 5′ being involved. Support for the above statement is available from the association of compounds as they occur in nature and it is given later in this paper. The state of complete reduction leaving no hydroxyl in the side phenyl nucleus is common in the anthoxanthins and is a distinctive feature. But a more prominent characteristic of these compounds which does not find a parallel in the anthocyanins is the large variation in the state of oxidation of the benzene ring (A). In the latter group of pigments, two hydroxyls are invariably present in the 5: 7-positions. Resoanthocyanins have not been found in nature. Carajurin represents an exceptionally rare case; but its occurrence indicates that the possibility of modification of ring (A) even in the anthocyanins cannot be excluded, though it may be uncommon. But in the anthoxanthins the variation in the number of hydroxyls is from 0 to 4. An explanation of this could probably be found in the marked difference in reactivity of the nuclear positions of anthoxanthins and anthocyanins after the structures have been formed from the common precursor, the modifications not taking place earlier. If quercetin and luteolin should be the simplest of the flavonols and flavones (containing hydroxyls in 5: 7 positions) the biogenesis of the others should involve further stages of oxidation and reduction. How far this conclusion is justified and in what manner the variations arise can be indicated in a general way by a study of the pigment associations made below. Though in most of the cases considered the pigments occur as glycosides, only the aglycones are mentioned and used in the discussion. The nature of the sugars involved and the positions they occupy do not seem to be relevant for the present purpose. Similar considerations are applicable to the methyl ethers also. Further the list is not claimed to be comprehensive; typical cases alone are taken up and attention is particularly focussed on the evolution of special structures found only in the anthoxanthins and not in the anthocyanins.

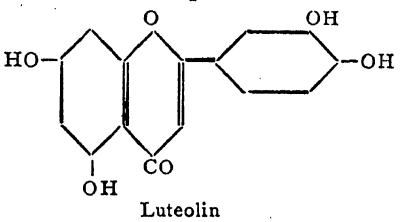
I. Some associations of flavones and flavonols in which the side phenyl nucleus has different number of hydroxyl groups are given below. It could be noticed that compounds with one hydroxyl (4′-position) or three

hydroxyls (3′, 4′ and 5′) are accompanied by those with two hydroxyls (3′ and 4′), indicating the evolution of the first two from the third. There seems to exist no definite cases where types with one and three hydroxyls in the side phenyl nucleus occur together, particularly to the exclusion of the 3′: 4′ dihydroxy compounds.

(*i*) The combination of apigenin and luteolin is frequently met with in different parts of the same plant or in plants of allied species and varieties.



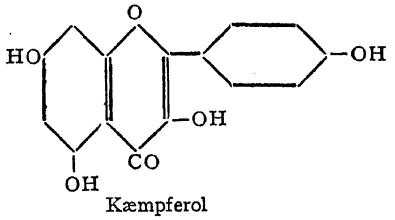
Apigenin



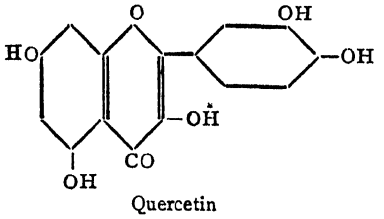
Luteolin

Apigenin occurs in the seeds of parsley, while in the stem and the leaves luteolin (as its monomethyl ether) is also present along with it.[5] Both of them are found in the yellow variety of *Antirrhinum majus*[6] and also in *Reseda luteola* (Weld.).[7] The two varieties of *Chrysanthemum* yield the two related pigments; *C. indicum* contains luteolin and *C. leucanthemum* apigenin.[8]

(*ii*) Kæmpferol is known to be found along with quercetin or its methyl ethers in several sources.



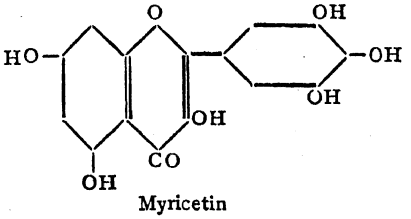
Kæmpferol



Quercetin

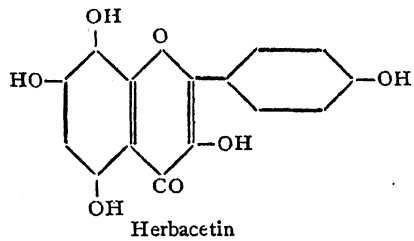
The stems of *Equisetum arvense* contain both these pigments, besides luteolin.[10] In senna leaves kæmpferol and isorhamnetin (3′-methyl quercetin) are present.[9] The same combination occurs in the flowers of *Delphinium Zalil* (Asbarg).[11] Kæmpferol and isorhamnetin along with kæmpferol monomethyl ether are found in *Rhamnus catharticus*.[9]

(*iii*) Quercetin and myricetin are both present in *Myrica nagi* and *Coriaria myrtifolia*.[9]

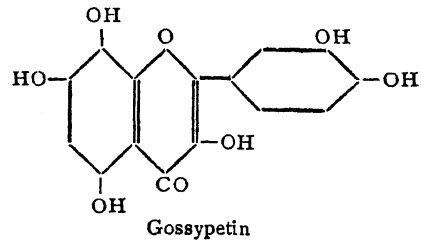


Myricetin

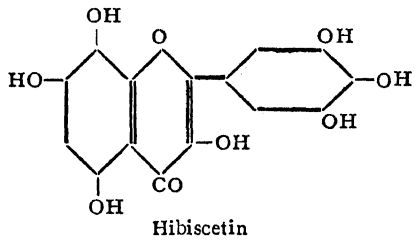
(*iv*) Indian cotton flowers (*Gossypium herbaceum* and *indicum*) contain gossypetin and herbacetin,[12] and in *Hibiscus sabdariffa* gossypetin and hibiscetin occur, the latter being the major component.[13]



Herbacetin

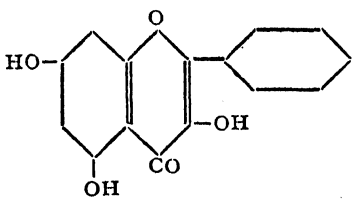


Gossypetin

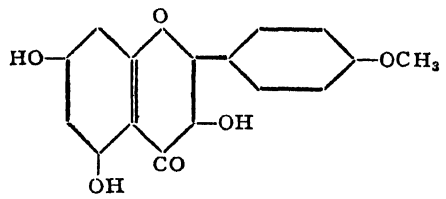


Hibiscetin

(*v*) Galangin, its monomethyl ether and kæmpferide occur together in the galanga root.[9] This example could be taken as indicating the sequence from the di- and monohydroxy side-phenyl nucleus to one devoid of hydroxyl groups.



Galangin



Kæmpferide

II. In some plants quercetin occurs along with other compounds which contain one more hydroxyl group in ring (A).

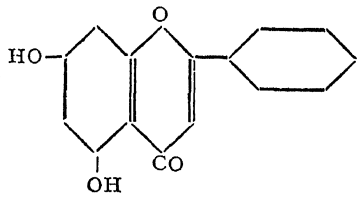
(*i*) The American cotton flowers, *G. hirsutum* contain as the sole component quercetin,[14] whereas the Indian varieties *G. herbaceum* and *G. indicum* contain gossypetin and herbacetin besides quercetin, gossypetin being the major component.[12]

(*ii*) The flowers of two species of *Thespasia* have so far been examined. *T. lampas* was studied by Perkin and was found to contain quercetin.[15] *T. populnea* has been shown by Rao and Reddy[16] to contain herbacetin besides populnetin (a flavone whose constitution is not yet settled[16a]).

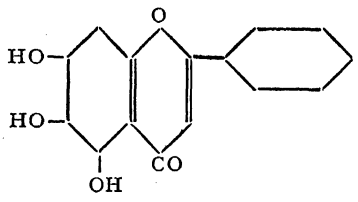
From the above data, it seems to be reasonable to infer that quercetin is the primary stage, and gossypetin indicates a higher stage of evolution involving oxidation which leads to the formation of the hydroxyl group in position 8. Herbacetin is a subsequent stage of reduction resulting in the disappearance of the hydroxyl group in 3′-position in ring (C).

III. 5:6:7 Combination of three hydroxyl groups in ring (A) occurs fairly frequently. This also seems to be an evolution from the more fundamental 5:7-dihydroxy types involving oxidation.

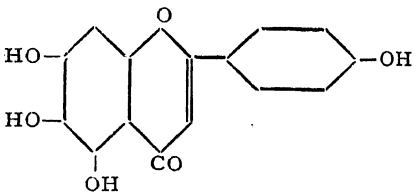
(*i*) The stem and root barks of *Oroxylum indicum* contain baicalein and its 6-methyl ether, oroxylin-A. Besides these two, the stem bark contains chrysin.[17] Thus the relation between the 5:7-dihydroxy and the 5:6:7-trihydroxy compounds is indicated.



Chrysin



Baicalein

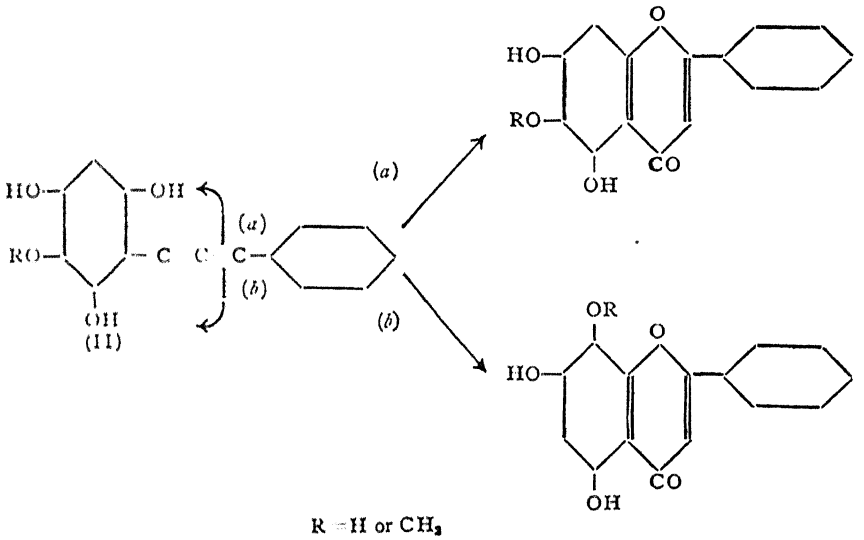


Scutellarien

(*ii*) Regarding the existence of stages in the reduction of ring (C) the composition of the parts of different species of *Scutellaria* is useful. The leaves and flowers of *S. baicalensis* and *S. altissima* contain scutellarein[18] whereas the roots of *S. baicalensis* contain baicalein.[19]

(*iii*) Another interesting point that should be noted here is that in the roots of *S. baicalensis*, wogonin, the 5:7:8 analogue of oroxylin-A is also found to be present[19] and the ether group is in the 8-position. There are two ways of explaining this phenomenon:

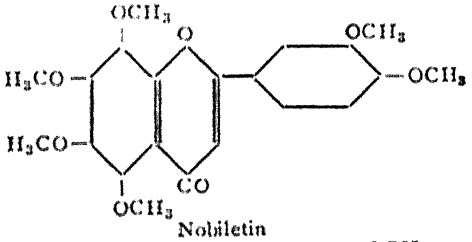
(*a*) the 5:6:7 and the 5:7:8 combinations arise independently from the 5:7-dihydroxy compound by oxidation affecting position 6 or position 8 independently; (*b*) the oxidation takes place at some stage prior to the closure of the pyrone ring and the ring closure takes place in two different ways, one leading to the 5:6:7 and the other to the 5:7:8-configuration:



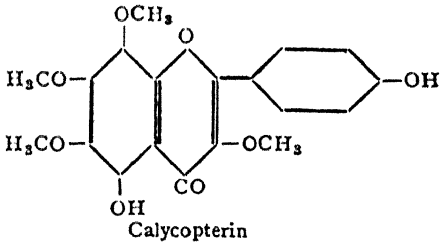
R = H or CH_3

The existence of the second possibility is indicated by some laboratory experiments. But they are not numerous enough to be conclusive. The association of 5:6:7 and 5:7:8 types is found so far in one case only. It may not be, therefore, possible to say definitely which of the above two alternatives is more plausible. The first is probably more simple and also avoids certain other difficulties. It is, however, more definite that they are both derived from the 5:7-dihydroxy precursor.

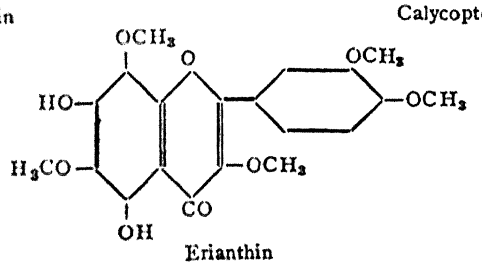
IV. So far cases of one stage oxidation of the benzene ring (A) have been discussed. The idea could be extended to complete oxidation resulting in tetrahydroxy benzopyrone systems. Several of this type have been recently discovered. It is noteworthy that all these, nobiletin, calycopterin and erianthin are partially or completely methylated presumably in order to escape oxidation.



Nobiletin



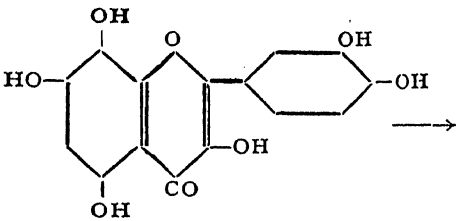
Calycopterin



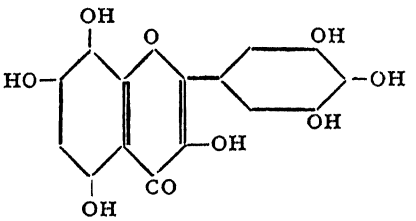
Erianthin

V. The following associations in closely related species indicate the existence of a stage of reduction of the hydroxyl group in position 7.

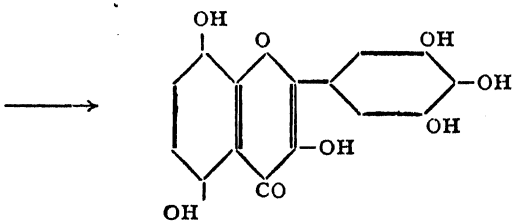
(*i*) *Hibiscus sabdariffa* contains hibiscetin as the main component and gossypetin and sabdaretin as the minor portion,[13] whereas the closely related species, *H. cannabinus* contains cannabiscetin as the sole component.[20] The constitution of sabdaretin is not known yet and hence it is omitted from consideration. The relationship between the others can be expressed by the following formulæ and it seems to be reasonable to conclude that cannabiscetin is derived from hibiscetin by a stage involving reduction affecting position 7.



Gossypetin



Hibiscetin

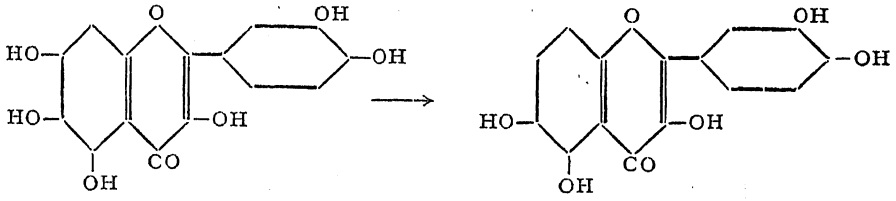


Cannabiscetin

It may be mentioned here that in one experiment a small amount of cannabiscetin was found in *Hibiscus sabdariffa*; but it could not be isolated from other samples.

(*ii*) In this connection may be considered the components of the species of *Primula*. Primetin, 5:8-dihydroxy flavone, is obtained from the leaves of *P. modesta*.[21] It is known that the flowers of *P. sinensis* contain pelargonidin or delphinidin glycosides depending on the variety.[22] Obviously the compounds containing the 5:7 combination of hydroxyls are common in these plants and the evolution of the 5:8 type through the intermediate 5:7:8 is possible.

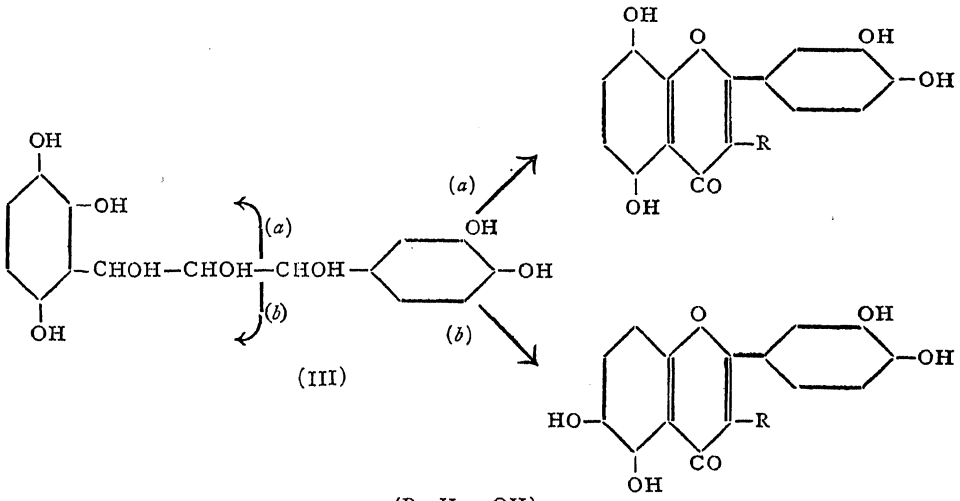
(*iii*) A very similar case arises in the two closely related flowers of the compositæ, *Tagetes erecta* and *T. patula*. The former contains quercetagetin and the latter patuletin in which the hydroxyl in the 7-position is missing.[23]



Quercetagetin Patuletin

The occurrence of the above-mentioned 5:8 and 5:6 combinations of hydroxyl groups which are not so common seems to be due to a stage involving reduction of 5 : 7 : 8 or 5 : 6 : 7 combinations whereby the 7-hydroxyl is lost. This process is obviously very rare but seems to exist definitely.

It could be suggested that the 5:6- and the 5:8-dihydroxy compounds are evolved from hydroxy quinol as shown below (III) instead of phloroglucinol present as part of the precursor (I).



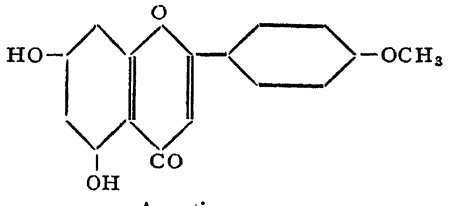
(III)

(R=H or OH)

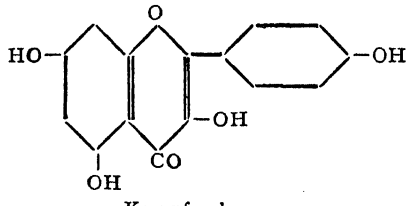
But this idea is neither supported by the considerations of associations and plant relations discussed above nor by the occurrence of hydroxyquinol nucleus in other groups of compounds found in nature nor by the reactivity of this nucleus.

VI. There are two cases of compounds, fisetin and robinetin in which there is no hydroxyl group in the 5-position, only one being left in the 7th position. Are they evolved quite independently of the fundamental 5:7 compounds or are they derived from them? The second alternative is supported by the following considerations:—

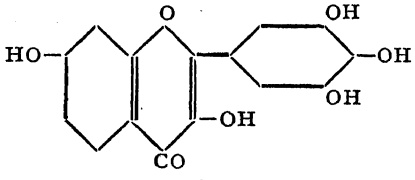
(*i*) The leaves and bark of *Robinia pseudacacia* contain acacetin,[24] the flowers contain kæmpferol,[25] whereas the wood (dye wood) contains robinetin.[26]



Acacetin



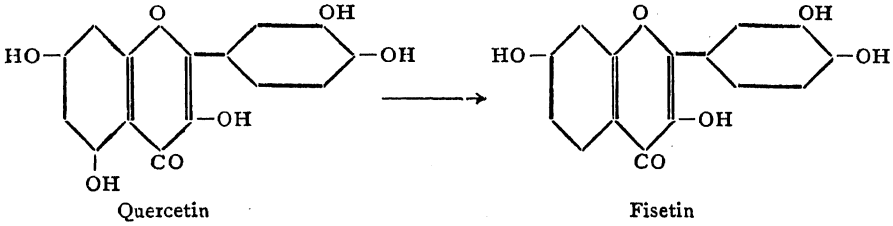
Kæmpferol



Robinetin

The association of 5:7 combinations with a compound containing only 7-hydroxyl group leads to the impression that it is derived from the fundamental dihydroxy compounds by a stage of reduction involving the hydroxyl in the 5-position. The possibility of oxidation or reduction in the side phenyl nucleus should be taken independently.

(*ii*) Other examples which support the above idea are obtained from "young fustic" and yellow cedar both of which are dye woods. The stem and branches of *Rhus cotinus* constituting "young fustic" contain fisetin, whereas the leaves of the plant (Venetian sumach) yield myricetin. In yellow cedar, fisetin is present in the stem and quercetin in the leaves.



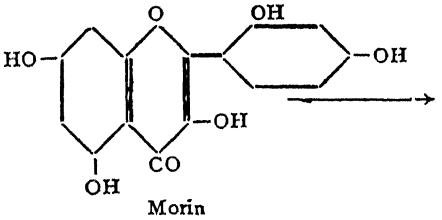
Quercetin Fisetin

It is not unreasonable to consider fisetin as representing a later stage involving reduction resulting in the disappearance of the 5-hydroxyl found in quercetin. Myricetin, of course, is related to quercetin in a different way.

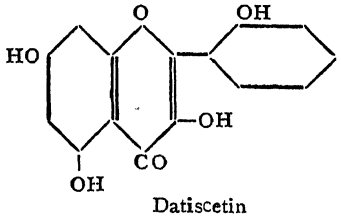
(*iii*) As a further example of this type may be mentioned pratol (7-hydroxy-4'-methoxy flavone) isolated by Power and Salway from the flowers of *Trifolium pratense*[27] and later by Rogerson from other species[28]; it occurs in these along with quercetin and isorhamnetin.

From the data presented above the following conclusions could be arrived at. (1) The theory of Robinson regarding the origin of anthocyanins and anthoxanthins from a common precursor is supported. (2) The conclusion of Robinson and his collaborators regarding the stage of oxidation of ring (C) in anthocyanins could be applied to anthoxanthins also, *i.e.*, 3′ : 4′ combination of hydroxyls is the simplest and 3′ and 3′ : 4′ : 5′ combinations involve more stages. In the anthoxanthins complete reduction of the hydroxyls in ring (C) is represented by a large number of examples. (3) Superimposed on the changes brought about in ring (C), ring (A) is quite easily susceptible to changes involving oxidation and reduction of nuclear positions. 5 : 7 Combination of hydroxyl groups seems to be the primary state; 5 : 6 : 7- and 5 : 7 : 8-trihydroxybenzene structures involve a stage of oxidation and 5 : 6 : 7 : 8-tetrahydroxybenzene nucleus results from a further stage of the same nature. 5 : 8-and 5 : 6-dihydroxy types seem to result from trihydroxy types involving a stage of reduction leading to the disappearance of the 7-hydroxyl. Similar reduction seems to produce fisetin and robinetin in which the hydroxyl in the 5-position is lacking.

There is one more point that requires consideration. It relates to the occurrence of the two rare cases of flavonols morin and datiscetin. The former is found in " old fustic " (wood of *Chlorophora tinctoria*) and in jak wood whereas the latter is present in the leaves and roots of the bastard hemp. In the roots datiscetin is accompanied by galangin.[29]

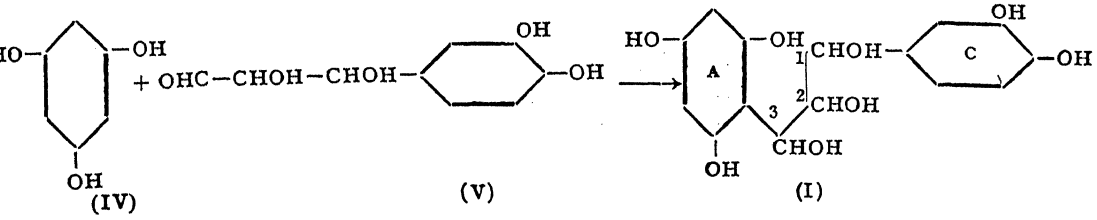


Morin



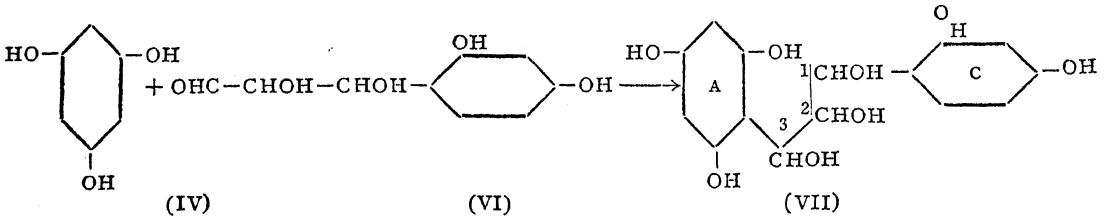
Datiscetin

They have one hydroxyl group in the 2′-position. Obviously they belong to a rare type, but their occurrence may be taken as indicating the existence of a small variation from the fundamental precursor proposed by Robinson. This precursor is considered to be obtained from the union of two parts (IV) and (V), (IV) being phloroglucinol representing a hexose unit and (V) a nine carbon system derived from a hexose and a triose.



(IV) (V) (I)

If the possibility of a variation in part (V) could be conceded as shown in (VI), then an alternative precursor (VII) having a resorcinol structure in the side phenyl nucleus instead of the catechol structure becomes available.



(IV) (VI) (VII)

By the subsequent oxidation at C_1 and C_3 or at C_2 and C_3 morin could be obtained and from it involving a stage of reduction datiscetin could be evolved. But the occurrence of these types does not seem to be more common. The association of galangin with datiscetin is noteworthy. It could probably be derived from both catechol and resorcinol types of the side phenyl nucleus by complete reduction of the hydroxyl groups present in this part.

Summary

Based on the theory of Robinson *et al.* regarding the biogenesis of anthocyanins and on a detailed consideration of the occurrence of anthoxanthins (flavones and flavonols) in plants, a scheme of evolution of the various types of anthoxanthins is developed and discussed. Besides the existence of stages of oxidation and reduction of the side phenyl nucleus, similar modifications by oxidation and reduction of the benzopyrone part is involved. The possibility of a variation in ring (C) of the common precursor (I) as shown in structure (VII) leading to certain rare cases having hydroxyl groups in 2′- and 2′: 4′-positions is suggested.

REFERENCES

1. Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1943, **17**, 119.
2. Robinson .. *Nature*, 1936, **137**, 172.
Robinson and collaborators .. *Phil. Trans. of Roy. Soc. of London*, 1939, **230 B**, 149.
3. Onslow .. *The Anthocyanin Pigments of Plants*, Cambridge University Press, 1925.
4. Gisvold and Rogers .. *The Chemistry of Plant Constituents*, Mineapolis, 1938.
5. Von Gerichten .. *Ber.*, 1876, **9**, 1124.
6. Wheldale and Bassett .. *Biochem. J.*, 1913, **7**, 441.
Scott-Moncrieff .. *Ibid.*, 1930, **24**, 753.
7. Perkin and Horsefall .. *J.C.S.*, 1900, **77**, 1314.
Chevreul .. *Ann.*, **82**, 53.
8. Rao .. *Proc. Ind. Acad. Sci.* (A), 1942, **15**, 123.

9. Perkin and Everest .. *The Natural Organic Colouring Matters*, 1918.
10. Nakamura and Hukuti *Br. Chem. Abs.*, 1941, **A ii**, 302.
11. Perkin and Pilgrim .. *J.C.S.*, 1898, 268.
12. Neelakantam, Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1935, **2**, 490.
    Neelakantam and Seshadri .. *Ibid.*, 1936, **4**, 54.
13. Rao and Seshadri .. *Ibid.*, 1942, **15**, 148.
14. Neelakantam, Rao and Seshadri .. *Ibid.*, 1935, **1**, 887.
15. Perkin .. *J.C.S.*, 1909, **95**, 1859.
16. Rao and Reddy .. *Proc. Ind. Acad. Sci.* (A), 1940, **12**, 372.
16*a*. Neelakantam, Rao and Seshadri .. *Ibid.*, 1943, **17**, 26.
17. Bose and Bhattacharya *J.I.C.S.*, 1938, **15**, 311.
18. Goldschmiedt and Molisch .. *Monatsh*, 1901, **22**, 679.
    Goldschmiedt and Zerner .. *Ibid*, 1910, **31**, 439.
19. Shibata, Iwata and Nakamura .. *J.C.S., Abs.*, 1923, **A i**, 592.
20. Neelakantam, Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1941, **14**, 105.
21. Nagai and Hattori .. *Br. Chem. Abs.*, 1930, **A**, 784.
22. Scott-Moncrieff .. *J. Genet.*, 1936, **32**, 117.
23. Rao and Seshadri .. *Proc. Ind. Acad. Sci.* (A), 1941, **14**, 289 ; *Ibid.*, 1941, **14**, 643.
24. Perkin .. *J.C.S.*, 1900, 423.
    Hattori .. *Ibid., Abs.*, 1925, **Ai**, 1443.
25. Sando .. *J. Biol. Chem.*, 1932, **94**, 675.
26. Schmid and Pietsch .. *Monatsh*, 1931, **57**, 305.
27. Power and Salway .. *J.C.S.*, 1910, **97**, 231.
28. Rogerson .. *J.C.S.*, 1910, **97**, 1006.
29. Kalff and Robinson .. *Ibid.*, 1925, 1968.

920-43 Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore.

CONTRIBUTIONS TO OUR KNOWLEDGE OF THE PYLORIC CÆCA IN THREE FAMILIES OF FRESH-WATER INDIAN FISHES (OPHICEPHALIDÆ, NOTOPTERIDÆ AND MASTACEMBELIDÆ), TOGETHER WITH SOME REMARKS ON THEIR PROBABLE FUNCTIONS

BY M. RAHIMULLAH, M.Sc., F.Z.S.
(Department of Fisheries, Hyderabad, Deccan)

With 1 Plate and 4 Text-Figures

Received September 9, 1943
(Communicated by Prof. R. Gopala Aiyar, F.A.SC.)

CONTENTS PAGE

1. Introductory

REFERENCES to up-to-date literature revealed that so far no regular work has been attempted on the pyloric cæca in the fresh-water fishes of India, and it is for this reason that my former teacher and colleague, Professor

The term pyloric cæca is retained throughout, though actually these structures arise from the duodenum.

B. K. Das of the Osmania University, suggested to me that it would be worthwhile to work out fully and to make a thorough comparative and systematic study and survey of these organs (and later on their physiology) in fishes of our own waters. From certain stray accounts it has been gathered that these structures have just been casually mentioned to be present in some 31 families of Indian fishes, including nearly 76 genera and about three times as many species, most of which are marine, some estuarine and a few fresh-water forms.

After consulting the relevant literature one would find that the structure (and to some extent the physiology) of the pyloric cæca has been briefly described by a handful of workers only, amongst which the most notable ones are Rosenthal (1824), Hyrtl (1864), Blanchard (1882), Stirling (1884), Fr. Day (1887), Bondouy (1897–99), Johnson (1907), Kostanecki (1913) and Dharmarajan (1936), whose works have been reviewed in this paper. Acting on Professor Das's suggestion I have first of all worked out the structures of these cæca in four species of the family Ophicephalidæ, and then one of Notopteridæ and three of Mastacembelidæ, thus making a total of eight species of fresh-water fishes commonly met with in Hyderabad, and the results of nearly a year's work of mine are embodied in the following pages.

2. *Historical Summary*

There are but a few small papers dealing with the account of the pyloric cæca in teleostean fishes:

In 1824 Rosenthal has just touched upon the condition of the pyloric cæca in the Sword-fish without giving any figure. Hyrtl (1864) has shown a very curious disposition and the mode of opening of the bile duct actually into the "appendices pyloricæ" in *Fistularia*, *Aulostoma* and *Acanthurus*—there being a single cæcum in *Fistularia*, two in *Aulostoma* and six in *Acanthurus*. The account given by Johnson (1907) is, however, an interesting one in which he has referred to "The individuality and variation of the pyloric cæca of the Centrarchidæ". The main object of his contribution is just to show that the pyloric cæca of certain members of this particular family, *viz.*, the Sun-fish, *Lepomis*, Black Bass, *Micropterus*, etc., are not similar, as generally assumed, but that they differ considerably in number and form: in other words, they show a lot of individual variations in a single family, that is to say, the pyloric cæca have an individuality of their own in every species of fish. Here the cæca vary in number, usually from 6 to 19, but in *Micropterus* they may be considerably branched, and these branches may be as many as 28.

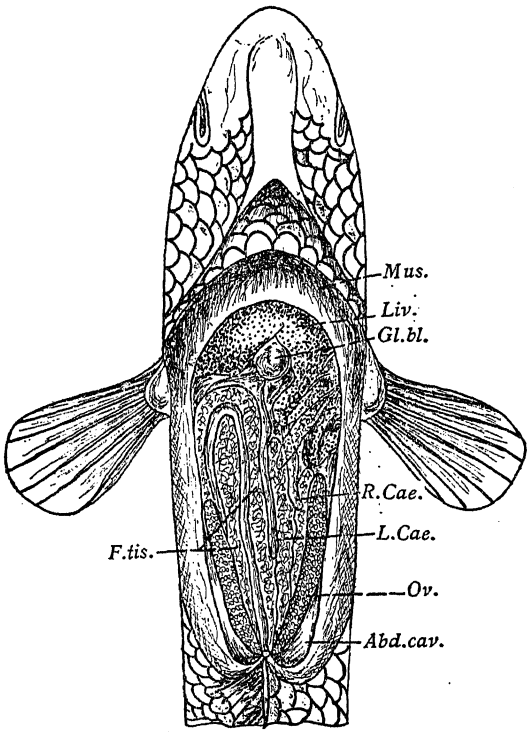
3. *Material and Technique*

In every case fresh material for histological study was obtained after pithing the live fish. In order to have a perfect fixation and preservation, both internally and externally, the two pyloric cæca were first of all injected with Bouin's fluid until they were quite turgid from the œsophageal side with the help of a small syringe after cutting off the œsophagus and inserting the nozzle at its distal end, and then finishing off the operation by giving two ligatures after fluid had run inside the whole length of the alimentary canal quite satisfactorily—one knot was tied at the remote end of the œsophagus and the other at the proximal end of the ileum slightly behind the cæca. The cæca and the associated parts of the gut were then removed and preserved in the same fluid for 8–24 hours. After having carefully dissected out the cæca from the surrounding tissues, each, as a rule, was cut into three portions, *viz.*, the *proximal*, the *middle* and the *distal* segments (except in the case of the Mastacembelidæ, in which the two cæca, being very small and very closely situated together, have been treated as a whole). Each piece of the cæca as also other parts of the alimentary canal were imbedded separately in paraffin blocks, and serial sections (both transverse and longitudinal), from 6 to 8 μ thick, were cut. They were variously stained, such as, for instance, in picro-indigo-carmine, Mallory's triple, Heidenhain's iron-hæmatoxylin, and Delafield's hæmatoxylin counterstained with eosin. Several freehand and camera lucida sketches were made, and many photomicrographs have also been taken and compared.

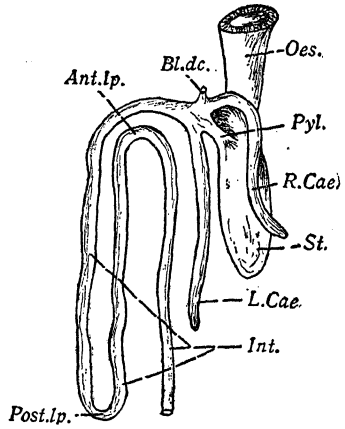
It is my most pleasant duty to record my sincere thanks here to Professor Dr. A. Subba Rau, B.A., D.Sc. (London), F.R.M.S., Principal and Head of the Zoology Department of the Central College, Bangalore, for his kindly going through the MS. and making some very useful suggestions as well as for accepting the paper for publication in this journal. I am very grateful to Professor R. Gopala Aiyar, Director of the Madras University Zoological Research Laboratory, for his kind help and friendly criticisms. I am also grateful to Professor B. K. Das for his help and guidance. I am very thankful to Professor A. B. Misra of the Benares Hindu University for certain valuable advice.

4. *The Pyloric Cæca of* Ophicephalus striatus *Bl. as a Type*

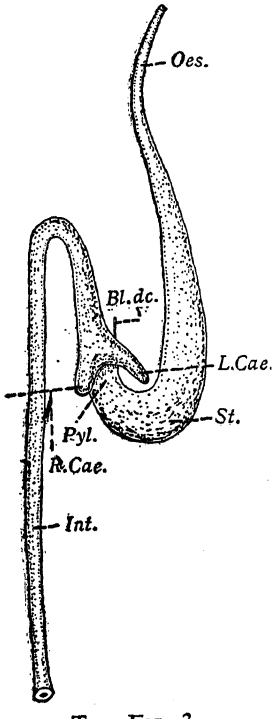
(*a*) *Topography* and *Morphology*.—As an example of the typical condition of the pyloric cæca, mention may first of all be made of *Ophicephalus striatus* Bl., the "Murrel" (the second largest member of the Fam. Ophicephalidæ), a fish which is very commonly found in Hyderabad. In this fish, there are two fairly large cæca (right and left, Text-Fig. 1, *a* and *b*,



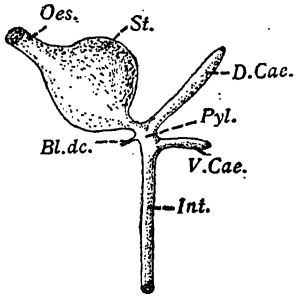
TEXT-FIG. 1 (*a*)



TEXT-FIG. 1. (*b*)



TEXT-FIG. 3



TEXT-FIG. 2

TEXT-FIG. 4

Text-Fig. 1 (*a*). Dissection of *Ophicephatus striatus* from the ventral aspect, showing the pyloric cæca *in situ* (× 1½). 1 (*b*). Alimentary canal of the same fish unravelled, showing the disposition of the cæca (× 1½). Text-Fig. 2. Anterior portion of the alimentary canal of *Notopterus notopterus* unravelled, showing the disposition of the cæca (× 2). Text-Fig. 3. Alimentary canal of *Mastacembelus armatus* unravelled, showing the disposition of the cæca (× 2). Text-Fig. 4. Ditto of *Fistularia villosa*, showing the disposition of the *single* cæcum (× 3).

R. cæ. and *L. cæ.*) which are tubular, digitiform structures arising just from the commencement of the small intestine; that is to say, immediately behind the pylorus (*Pyl.*) which is quite short. The bile-duct (*Bl. dc.*) often opens immediately behind the origin of the right cæcum, or sometimes in the very narrow interspace between the right and the left cæca. The right cæcum is usually somewhat smaller in size than the left one. The intestinal orifice of each cæcum is quite distinct though very small, but, unlike that of the rectal gland of Selachian fishes where there is a valve, it is unguarded by any valvular structure.

The cæca are invariably filled with some semi-digested food-material mixed up with a small quantity of mucous, and in certain rare cases some bile also. There is a large amount of fat (*F.tis.*) that conceals the greater portion of these cæca.

The intestine of this carnivorous fish, as one would naturally expect, is of a simple type and bears two loops—one at the anterior end (*Ant. lp.*) and the other at the posterior end (*Post. lp.*). Further, it is interesting to compare the relative lengths of the various parts of the alimentary canal with those of the cæca, and the ratios may be stated as follows:—

Cæca : Intestine, and Cæca : whole length of the alimentary canal $= \frac{1:3{\cdot}9\ \mathrm{R}}{1:3{\cdot}2\ \mathrm{L}}$ and $\frac{1:5{\cdot}2\ \mathrm{R}}{1:4{\cdot}2\ \mathrm{L}}$ respectively, where R denotes the right cæcum and L the left cæcum, and the length of the cæcum (either of the right side, or of the left side) has, in each case, been taken to represent as a unit.

(*b*) *Blood- and Nerve-Supplies.*—The blood-supply is quite interesting in a way that the cœliaco-mesenteric artery arises from the right side of the dorsal aorta and divides into three main branches :—

(1) Gastric artery going to the stomach.

(2) Cæcal artery bifurcating into two smaller twigs, and supplying the right and the left cæca.

(3) Anterior mesenteric artery supplying the proximal part of the intestine.

The blood is returned from these cæca by means of two factors, *viz.*,

(1) The cæcal vein draining blood from the right and left cæca and emptying itself into the Hepatic Portal vein. Into each of these cæcal factors veins from the stomach also open.

(2) A small vein arising independently from the right cæcum and dipping into one of the tributaries of the Hepatic Portal vein.

As regards the nerve-supply it is worthy of note that the visceral branch of the *right* Vagus divides into four small branches, *viz.*, the cardiac, gastric, intestinal and cæcal, supplying the heart, stomach, intestine and the two cæca respectively, whereas the *left* visceral has no cæcal branch, but in other respects it is exactly like its right counterpart.

(*c*) *Histology.*—After a careful examination and study of a large series of transverse and longitudinal sections of the pyloric cæca the following histological details add a great interest to our knowledge, and may be briefly mentioned thus—here the figures of *Ophicephalus marulius* Ham. have been given merely for the sake of convenience :—

(1) Roughly speaking, the internal structure of the proximal region of the pyloric cæca (Pl. IV, Fig. 2) is pretty similar to and built upon the same general plan as that of the small intestine (Pl. IV, Fig. 1).

(2) The cæcal villi (Pl. IV, *Cæ. vil.*, *i.e.*, the folds of the mucosa of the cæcum) are, as a rule, very prominent and extend for a considerable distance inside the cæcal lumen.

(3) The various layers which compose the wall of the cæcum, from without inwards, are (Pl. IV, Fig. 2) :—

(i) Serous coat = visceral peritoneum (*Ser.*).

(ii) Layer of longitudinal muscle-fibres (*Long. musc.*) = thin envelope.

(iii) Layer of circular muscle-fibres (*Circ. musc.*) often twice or three times as thick as No. (ii).

(iv) Sub-mucosa (*Sub. muco.*) consisting of connective-tissue, some blood-capillaries (*Bl. cap.*) and nerve fibres.

(v) Mucosa usually consisting of a two- or three-cell deep layer, made up mostly of columnar or stratified epithelial cells amongst which a large number of goblet cells are also to be found. It is thrown into a very large number of deep folds which form finger-like structures (or the " cæcal villi ", *Cæ. vil.*) penetrating into the cæcal lumen—the central core of each " cæcal villus " is highly vascular, having many fine blood-capillaries which traverse and extend up to its distal end, and probably thus increase the absorptive surface of the cæcum.

Curiously enough, in the *distal* region of each cæcum (Pl. IV, Figs. 3 & 5) the " cæcal villi " grow in size and multiply tremendously, interdigitate and fuse with one another thus presenting the appearance of a sort of spongy structure (or what has been designated here as " spongy-tissue ", *Int. dig.*

muc. fl.) filling up and practically obliterating the whole of the cæcal lumen (*Cæ. lum.*) in other words, this sort of excessive branching and highly folded arrangement of the "cæcal villi" evidently provides greater area for the absorption of the digested soluble food in this region of the cæcum.

5. Condition of the Cæca in Some other Fresh-Water Indian Fishes

(A) *Other Species of the Family Ophicephalidæ* :

In the other three species of the family Ophicephalidæ which have been investigated, *viz.*, *Ophicephalus marulius* Ham. (the largest species), *O. punctatus* Bl., *O. gachua* Ham. (the smallest species), the general structure of the cæca (together with their blood- and nerve-supplies) is practically just the same as that described for *O. striatus* Bl., but there are a few minor variations which may be very vriefly pointed out as follows :—

(1) The pyloric cæca are club-shaped in *O. punctatus*, whereas they are of a tapering nature in *O. gachua*.

(2) The various ratios of the cæca for the other three species, as those described above for *O. striatus*, are :—

(i) *O. marulius* $= \frac{1:6{\cdot}2\ \text{R}}{1:5{\cdot}2\ \text{L}}$ and $\frac{1:8{\cdot}7\ \text{R}}{1:7{\cdot}3\ \text{L}}$ respectively,

(ii) *O. punctatus* $= \frac{1:6{\cdot}6\ \text{R}}{1:4{\cdot}8\ \text{L}}$ and $\frac{1:8{\cdot}6\ \text{R}}{1:6{\cdot}2\ \text{L}}$ respectively, and

(iii) *O. gachua* $= \frac{1:7{\cdot}1\ \text{R}}{1:5{\cdot}7\ \text{L}}$ and $\frac{1:8{\cdot}5\ \text{R}}{1:6{\cdot}8\ \text{L}}$ respectively.

(R and L, wherever being used, always signify right and left cæca as in the case of *O. striatus*.)

(3) The "cæcal villi" of *O. punctatus* are relatively very large—they are nearly twice the size of the "intestinal villi".

(4) The simplest type of "cæcal villi" are to be found in the smallest species of this family, *viz.*, *O. gachua*, in which they do not multiply and fuse together in the distal region of the cæcum (Pl. IV, Fig. 4) as they do in all other species of the Ophicephalidæ as well as in most other fishes that I have studied so far.

(B) *Notopterus notopterus* (Pallas):

(*a*) *Topography and Morphology.*—In this fish the whole of the alimentary canal is relatively very short (Text-Fig. 2). The intestine (*Int.*) lies below the large gas-bladder and is thrown into a semi-circular loop with the

convex surface directed upwards. There are two curved conical cæca,* one of which is dorsal in position (*D. cæ.*), and the other ventral (*V. cæ.*), the former being bigger than the latter, and both lying hidden between the globular stomach (*St.*) and the intestine (*Int.*), and running closely parallel to the latter. The pylorus (*Pyl.*) is very small.

The ratio of the lengths of the *dorsal* and the *ventral* cæca : intestine = 1 : 2·5 and 1 : 4 respectively (the length of either of the cæca being taken as a unit). Ditto dorsal and ventral cæca : whole length of the alimentary tract = 1 : 3 and 1 : 5 respectively.

(*b*) *Blood- and Nerve-Supplies.*—(1) The cœliaco-mesenteric artery gives off three branches:—

(i) Intestinal—supplying the whole of the intestine.

(ii) Gastric—supplying the stomach and also sending a small branch to the posterior part of the dorsal cæcum.

(iii) Cæcal artery divides into two branches : one going to the dorsal cæcum and the other to the ventral.

(2) The blood is drained from the cæca by two ways:

(i) Two cæcal veins bring back blood independently from the dorsal and the ventral cæca and ultimately join the Hepatic Portal vein.

(ii) Small veins draining blood from the posterior ends of both the cæca and falling into the intestinal factor of the Hepatic Portal.

As regards the nerve-supply it may be mentioned that the visceral branch of the *left* Vagus, unlike the previous case, innervates the stomach and the two cæca, whereas its *right* counterpart sends off twigs to the intestine and the dorsal cæcum only.

(*c*) *Histology.*—The salient features in the histology of the cæcum of *Notopterus* as distinguished from those of the ophicephalids are as follows:

(1) Elongated digitiform "cæcal villi" and several large goblet cells are present in the cæcal epithelium towards the *Proximal* and the *Mid*-regions of the cæcum.

(2) In the *distal* region of the cæcum, however, the "villi" do not proliferate so copiously as they do in the Ophicephalidæ, but most of them penetrate inwards towards the centre of the cæcal lumen, and some of them also unite with one another (*Int. dig. muc. fl.*—Pl. IV, Fig. 5), forming many narrow inter-communicating passages or channels (*Ch.*) inside the cæcal

* In this figure the cæca are displayed after being straightened out.

lumen—all these modifications are evidently meant to increase the absorptive surface of the cæcum.

(C) *Various Species of the Family Mastacembelidæ:*

Three members of this family, *viz.*, *Rhynchobdella aculeata* (Bloch), *Mastacembelus armatus* (Lácep.) and *M. pancalus* (Ham.) have been studied, but here I shall confine myself in describing the condition in *Mastacembelus armatus* (Text-Fig. 3) as a typical case, which agrees with the other two species practically in all essential respects.

(*a*) *Topography and Morphology.*—In *M. armatus* (as also in the other two species of this family) there are two cæca (right and left, Text-Fig. 3, *R. cæ.* and *L. cæ.* respectively) which are relatively very small as compared with those of *Notopterus notopterus* (Pall.) and the several species of the Fam. Ophicephalidæ. They are short, stumpy, finger-like structures originating from the junction of the duodenum with the pylorus (*Pyl.*) and to some extent adhering to the sides of the latter. They are practically equal in size, but sometimes the left cæcum may be just slightly bigger than the right one.

The ratio of the length of any one of the two cæca (right or left—both being regarded to be of equal size): intestine = 1: 14·6, and the cæcum: whole length of the alimentary tract = 1: 25·0 (length of either cæcum representing a unit as in previous cases).

(*b*) *Blood- and Nerve-Supplies.*—(1) The cœliaco-mesenteric artery gives off 4 branches:

(i) Gastric—supplying the stomach.

(ii) Intestinal—supplying the intestine.

(iii & iv) Two independent cæcal arteries, and supplying the right and the left cæca.

(2) The blood is returned from both the right and the left cæca by a single cæcal vein which joins the intestinal factor of the Hepatic Portal vein.

Regarding the nerve-supply it may be mentioned that the *right* visceral branch of the Vagus, as in the Ophicephalidæ, sends off small branches to both the cæca, the stomach and the intestine, whereas its *left* counterpart mainly innervates the stomach and the mesentery.

(*c*) *Histology.*—In the *distal* region of the pyloric cæca of the Fam. Mastacembelidæ the "cæcal villi" (Pl. IV, Fig. 6) are comparatively more developed than in the Notopteridæ: here they form a lot of infoldings and interdigitation and are all compacted together, having the " villi " massed up side by side in a slightly oblique manner, and roughly presenting the appearance

of a small gland. Not infrequently, however, the mucous folds also unite with one another, chiefly towards the posteriormost part of the cæcum, as observed in other fishes, thus increasing its absorptive surface. Another noteworthy fact is that only a few goblet cells are present along the lumen epithelium of this group.

As far as histological structures are concerned in the other two species, *viz.*, *M. pancalus* and *R. aculeata*, it is worthwhile to remember that there is no deviation from the typical condition as just described above in the case of *M. armatus*.

6. *Discussion*

Here, I will just deal very briefly with the nature and significance of the pyloric cæca in a summarised form as they exist in various groups of fishes in general :

(1) The pyloric cæca are absent in Cyclostomata, Dipnoi, and practically in all Elasmobranchs, but there is a considerable variation as to the number, form and structure of these cæca in various members of the Teleostomi, some of which are already described in most of the text-books on fishes. A very good summarised comparative account of these cæca in fishes, found outside India, is given in *Handbuch der Vergleichenden Anatomic der Wirbeltiere*, Vol. III (1937), by Pernkopf, Lehner and Jacobshagen.

(2) For instance, amongst the Ganoids the pyloric cæca are absent in the Bow-fin (*Amia*). In the " Bichir " (*Polypterus*) there is a single cæcum whereas in other members of this group, *viz.*, Sturgeon (*Acipenser*), Spoonbill (*Polyodon*), and Gar-pike (*Lepidosteus*) the cæca are very well developed.

(3) Again, in certain groups of Teleosts, the cæca are entirely absent as, for example, in the Cat-fishes (Siluridæ), Pikes (Esocidæ), Toothed-carps (Cyprinodontidæ), Wrasses (Labridæ), Plectognathi including the Globe-fishes, the Porcupine-fishes and lastly in the Pipe-fishes (Syngnathidæ).

(4) Whilst in some others, including both the European as well as the Indian types, the cæca may be very numerous (*i.e.*, at least more than 50) as, for example, in Salmon (*Salmo*), whiting (*Gadus merlangus*), Mackerel (*Scomber scombrus*), and certain Clupeidæ. They may be many in number as in the " White Pomfret " (*Stromateus sinensis*), *Sphyræna*, the " Hair-tail " (*Trichurus*), the " Pompano " (*Caranx*), etc. ; moderate number (*e.g.*, 16) in the so-called " Bombay Duck " (*Harpodon*); 5–7 in *Acanthurus* ; 3–5 in certain Pleuronectids ; only a few (3, for example) in *Premnas* and *Tetradrachmum* (Fam. Glyphidodontidæ) ; two in *Notopterus*, Ophicephalids, Sand-eels (*Mastacembelidæ*), the " Gourami "; (*Osphronemus*), etc.;

and only one in *Fistularia villosa* (Fam. Fisturlariidæ, Text-Fig. 4), and besides these, there are, of course, several other genera and species that are not mentioned here—it would be too cumbersome a list to deal with all of them within the limited space at present.

(5) It is also very interesting to note that in certain extreme cases, *e.g.*, Sturgeon, Whiting and Tunny (*Thunnus thynnus*) where the cæca are not only numerous, but most of them (or in some cases, all of them) are also united together by means of connective tissue to form a compact, gland-like mass communicating with the intestine, either by a *single* wide duct (as in Sturgeon) or by *several* small orifices as in other examples. Such a condition of the pyloric cæca would naturally lead to the assumption that probably they have some sort of secretory function, supplementing the actions of the digestive glands, such as the liver and the pancreas. At any rate one might say that such cæca must be of some important use in connection with the digestive functions of the fish in which they occur and have assumed such a compact, gland-like character. In other words, in such cases they may be said to represent acessory digestive glands.

7. *Summary and General Conclusions*

(1) The pyloric cæca are true outgrowths of the *proximal* portion of the small instestine (*i.e.*, the duodenum), as has been corroborated by their histological structure, and hence the name pyloric cæca is really a misnomer: the correct name for them should be " intestinal cæca ".

(2) They should not be mixed up with the " cæcal " or " rectal gland " of Selachian fishes, which has been so thoroughly worked out by Miss D. R. Crofts in recent years (*cf. P.Z.S.*, 1925). *That is to say they are not homologous with the " cæcal " (or " rectal gland ") of Selachian fishes, nor with any other cæcal outgrowth of other vertebrates, because all such latter structures take their origin between the large and the small intestine, whereas the pyloric cæca are given off immediately behind the pylorus as true outgrowths of the first part of the ileum.*

(3) From the presence and nature of the semi-digested liqud food-contents inside the lumen of the pyloric cæcum and the opening of the latter into the ileum as well as in due consideration of a fairly large amount of vascular supply (particularly the drainage of the blood into the portal system) and also in due recognition of the significance of the very structure of those copiously distributed digitiform " cæcal villi ", comparable to the true intestinal villi, the following physiological functions may possibly be attributed to them:—

(*a*) Might serve as accessory food-reservoirs in these fishes—the intestine in most of these fishes being of shorter lengths (so far investigated), but this fact could not be generalised yet until a very large number of fishes has been thoroughly examined.

(*b*) Probably a part of digestion might take place.

(*c*) Some absorption of the digested food may probably also take place (*cf.* from the nature of the highly vascular "cæcal villi").

(*d*) According to some authors (Mordacai, 1882; Blanchard, 1882; Stirling, 1884; and Bondouy, 1897 and 1899), who have worked on the physiology of pyloric cæca in certain other fishes, the following probable functions have been assigned to them:

That they are said to produce diastatic and trypsin-like enzymes which effect some digestion of the carbohydrates and the proteids, and thus help and supplement the digestive processes of other juices poured into the alimentary canal. (I have, however, no sufficient physiological data at my disposal just at present to fully test and justify the validity of this statement and, at any rate, I am presently engaged in carrying on a series of physiological experiments and biochemical tests on the contents of the pyloric cæca, and my results will shortly be communicated in later papers.

(*e*) It is not yet quite certain if diet has really any marked effect or influence on the relative size and structure of these cæca, and this point can only be definitely settled when a very large number of fishes, belonging to various families and living in different environment (and having different diet) has been thoroughly investigated, especially from this point of view—at this stage, in absence of any further data, it would rather be too hazardous to speculate anything.

(*f*) It is also very doubtful whether the number and nature of the pyloric cæca are of any taxonomic value in the study of fishes—this point could also be elucidated after a thorough systematic examination of a very large number of different species and families of fishes with which I am engaged at present.

REFERENCES TO LITERATURE

Anile, A. .. "Contrabatio alla conceaza della piloriche nei Teleostei," *Publicazione Staz. Zool. Napoli*, 1918, **2**.

Blanchard, R. .. "Sur les fontions des appendices pyloriques," *C. R. Acad. Sci.*, Paris, 1882.

Bondouy, Th. .. "Sur les phénomènes digestifs dans les tubes pyloriques chez quelques téléostéens," *Bull. Soc. Scient. Med. Quest.*, 1897.

——— .. "Du rôle des tubes pyloriques dans la digestion chez la téléostéens," *Arch. Zool. Expre.*, 1899.

Bondouy, Th. .. "Recherches sur la valeur physiologique des tubes pyloriques de quelques téléostéens," *C. R. Acad. Sci.*, Paris, 1899.

Bridge, T. W. .. *Fishes*, C. N. H. Series, 1922.

Crofts, D. R. .. "The Comparative Morphology of the Cæcal Gland (Rectal Gland) of Selachian Fishes, with some references to the Morphology and Physiology of the similar Intestinal Appendages throughout Ichthyopsida and Sauropsida," *P.Z.S.*, 1925, Part I, 101–88.

Dawes, Ben .. "The Histology of the Alimentary Tract of the Plaice (*Pleuronectes platessa*)," *Q.J.M.S.*, 1930, **73**, No. 290.

Day, F. .. "Notes on Cæcal appendages of fresh-water Trout," *Journ. Nat. Fish. Cult. Assoc.*, 1887.

———— .. "Notes on the Cæcal appendages of Cardigenshire Trout," *Ibid.*, 1887.

———— .. *Fishes, Fauna Brit. Ind.*, 1889, **1** and **2**.

Del vecchio, C. .. "Richerche sulla struttura dello stomaco dei pesci con speciale righuardo alla parte pilorica," *Atti. Soc. Ital. Sci. Nat.*, 1917, **56**.

Dharmarajan, M. .. "The Anatomy and Histology of the Alimentary System of *Otolithus ruber* (Bl. Schn.)," *Proc. Ind. Sc. Cong.*, 1936.

Goodrich, E. S. .. "Cyclostomes and Fishes", *A Treatise on Zoology* (Lancester Series), 1909.

Hyrtl, C. J. .. "Über die Einmundung des Ductus choledochus in eine Appendix pyloricæ," *Sitzber. Akad. Wiss.*, Wien, 1864.

Jacobshagen, E., Pernkopf, E., and Lehner, J. .. *Handbuch der Vergleichenden Anatomie der Wirbeltière*, 1937, **3**.

Johnson, R. H. .. "The individuality and variation of the pyloric cæca of the Centrarchidæ," *Trans. Wiscon. Acad. Sci.*, 1907.

Kostanecki, K. .. "Zur Vergleichenden Morphologie des Blinddärmes unter Berücksichtigung seines Verhältnisses zum Bauchfell," *Anat. Hefte*, 1913.

Kyle, H. M. .. *The Biology of Fishes*, 1926.

Norman, J. R. .. *A History of Fishes*, 1931.

Oppel, A. .. *Lehrbuch der vergleichenden mikoskopischen Anatomie der Wirbeltiere*, 1896–1900, **1–3**.

Owen, R. .. *Comparative Anatomy and Physiology of Vertebrates*, London, 1886, **1–3**.

Panicker, M. K. N. .. "Histology of the Alimentary tract of *Therapon quadrilineatus*," in MS.

Rahimulla, M. .. "A contribution to the Structure and Probable Functions of the Pyloric Cæca in the Fam. Ophicephalidæ," *Anat. Anz.*, **Bd.** 1935, **80**, Nr. 1/4.

———— .. "On the Structure of the so-called Pyloric Cæca in a Marine Genus *Platycephalus*," *Proc. Nat. Acad. Sci., Ind.*, 1941, **2**.

———— .. "On the Structure and Functions of the so-called Pyloric Ceaca in the two genera of fishes, *Lactarius* and *Osphronemus*," *Journ. Osm. Univ.*, 1941.

Riggio, G. .. "Sopra un caso di notevole ramificazione dei ciechi pilorici di *Centrolophus pompilus* Cuv. et Val.," *Nat. Sicil*, 1894.

Rosenthal, F. C. .. "Über die Blindärme im Schwertfische," *Ztschr. Physiol.*, 1824.

Stirling, W. .. "On the chemistry and histology of the digestive organs of fishes," *Rept. Scot. Fisheries*, 1884.

EXPLANATION OF PLATE

Fig. 1. Camera lucida sketch of a part of the transverse section of the intestine of *Ophicephalus marulius*, showing the very prominent "intestinal villi".

Fig. 2. Ditto of the *proximal* region of the pyloric cæcum of the same fish, showing the very prominent "cæcal villi".

Fig. 3. Photomicrograph of the transverse section of the *distal* region of the pyloric cæcum of the same fish, showing the tremendous interdigitation and fusion of the "cæcal villi" to form a sort of "spongy tissue".

Fig. 4. Camera lucida sketch of the transverse section of the *distal* region of the pyloric cæcum of *Ophicephalus gachua*, showing very simple arrangement of the "cæcal villi".

Fig. 5. Photomicrograph of the transverse section of the *distal* region of the pyloric cæcum of *Notopterus notopterus*, showing the fusion of the "cæcal villi" and the formation of the intercommunicating channels.

Fig. 6. Ditto of *Mastacembelus armatus*, showing the compact gland-like arrangement of the "cæcal villi".

EXPLANATION OF THE ABBREVIATIONS USED IN THE TEXT-FIGURES AND THE PLATE

Abd. cav., Abdominal cavity; *Ant. lp.*, Anterior loop; *Bl. cap.*, Blood capillaries; *Bl. dc.*, Bile duct; *Cæ.*, Cæcum; *Cæ. lum.*, Cæcal lumen; *Cæ. pr.*, Proliferating "villi" of the cæcum; *Cæ. v.*, The so-called cæcal villi; *Ch.*, Inter-communicating channels inside the cæcal lumen; *Circ. musc.*, Layer of circular muscle fibres; *D. Cæ.*, Dorsal cæcum; *F. tis.*, Adipose tissue; *Gl. bl.*, Gall-bladder; *Int.*, Intestine; *Int. dig. muc. fl.*, Interdigitation and fusion of the mucous folds of the cæcum; *Int. vil.*, the so-called intestinal villi; *L. Cæ.*, Left cæcum; *Liv.*, Liver; *Long. musc.*, Layer of longitudinal muscle fibres; *Mus.*, Muscles; *Oes.*, Oesophagus; *Ov.*, Ovary; *Post. lp.*, Posterior loop; *Pyl.*, Pylorus; *R. Cæ.*, Right cæcum; *Ser.*, Serosa; *St.*, Stomach; *Sub. muco.*, Sub-mucosa; *V. Cæ.*, Ventral cæcum.

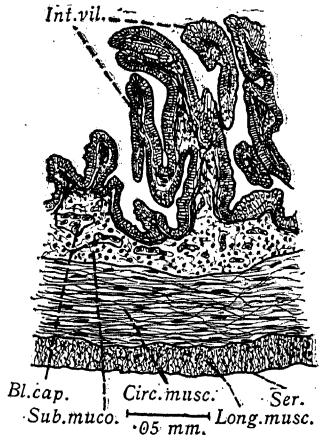


FIG. 1

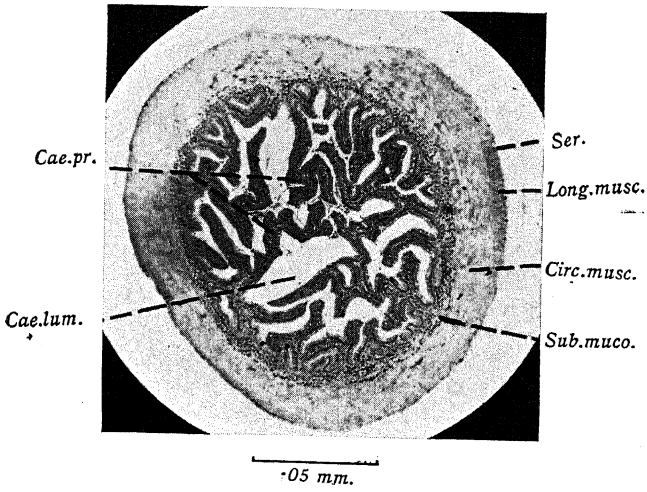


FIG. 3

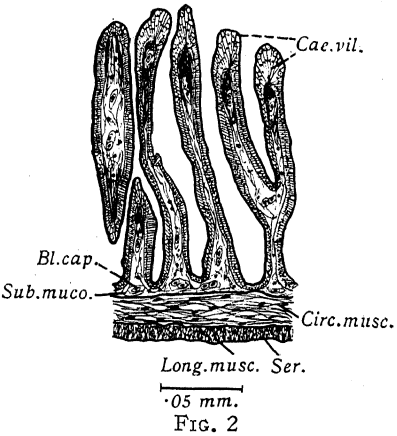


FIG. 2

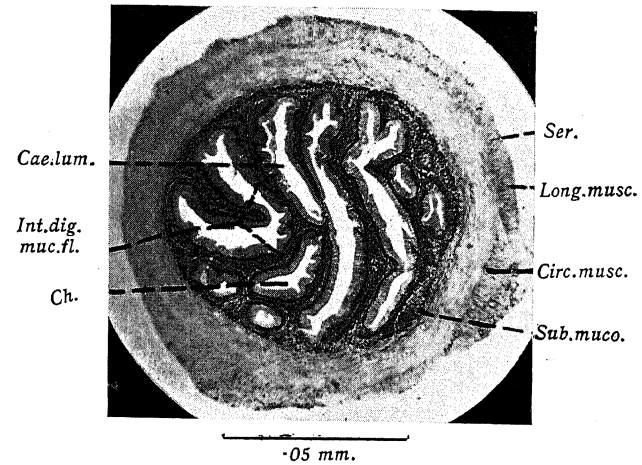


FIG. 5

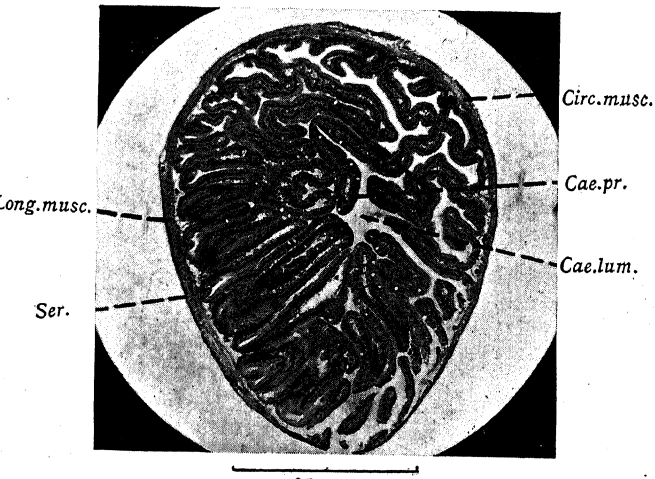


FIG. 6

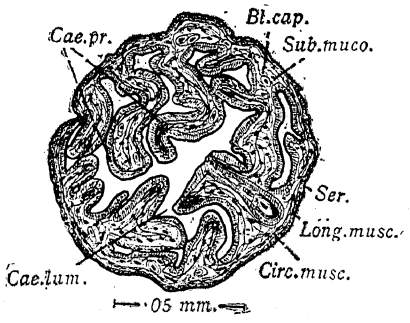


FIG. 4

STUDIES ON THE HELMINTH PARASITES OF KASHMIR*

Part II. On Two New Trematodes of the Subfamily *Pleurogenetinae* Looss (1899) with a Review of the Genus *Pleurogenes* Looss (1896)

BY B. L. KAW, M.SC.

(Lecturer in Biology, S.P. College, Srinagar, Kashmir)

(From the Department of Zoology, The University, Lucknow)

Received August 25, 1943

[Communicated by Dr. G. S. Thapar, M.SC., PH.D. (London), F.A.SC.]

THE trematode parasites, described in the present communication, form a part of the author's collection, from 1938–1941, from Kashmir. The entire work on these forms was done in the Zoological Laboratories at Lucknow under the supervision and guidance of Dr. G. S. Thapar to whom the author is indebted for his valuable suggestions and helpful criticism during the course of the work, and for keeping at the disposal of the author his personal library which is full of valuable literature. The author also wishes to thank Dr. S. L. Hora, Director of Fisheries, Bengal, for the identification of the hosts and Dr. M. B. Lal for advice and revision of the manuscript.

Prosotocus kashabia (n.sp.)

Earlier reference to Indian species of this genus is that by Mehra and Negi (1928), who described *P. indicus* from the intestine of *Rana tigrina*. Srivastava (1933) gave an account of *P. infrequentum* and Pande (1937) of *P. himalayai*, both from *Rana cyanophlyctis*. The present communication adds another species to the genus obtained from the intestine of *Rana cyanophlyctis* at Srinagar, Kashmir.

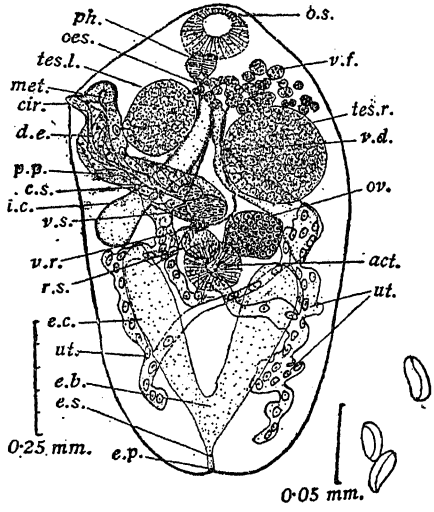
Description.—Body is more or less oval and spinose bearing exceedingly small spines arranged in regular rows.† Length of the body varies from 0·75 to 1·44 mm. and the maximum breadth is 0·38–0·66 mm. Oral sucker is subterminal, more or less oval, broader than long and 0·075–0·15 × 0·1–0·17 mm. in size, with mouth facing ventrally. Ventral sucker is equatorial or slightly post-equatorial, circular, 0·1–0.13 mm. in diameter and lies at a distance of 0·35–0. 67 mm. from the anterior end.

---

* For Part I of the "Studies on the Helminth Parasites of Kashmir," *vide Proc. Ind. Acad. Sci.*, 1941, **13**, 369–78.

† Spines are not shown in the figure.

Pharynx is globular, 0·03–0·06 × 0·05–0·07 mm. in size and œsophagus is longer than pharynx and measures 0·09–0·23 × 0·02–0·04 mm. Intestinal bifurcation is situated in the anterior quarter of the body. Intestinal cæca do not extend beyond the posterior limits of acetabulum, and its blind ends are slightly post-equatorial.



A (1) A (2)

Fig. A (1) *Prosotocus kashabia* (n.sp.) from *Rana cyanophlyctis* (dorsal view).
Fig. A (2) *Prosotocus kashabia*, eggs.
(*N.B.*—In all the figures spines have not been shown.)

Testes are pre-acetabular, and lie at the level of intestinal fork, extracæcal or overlapping the intestinal cæca in front of ovary. They are spherical, slightly oblique and unequal, the left one 0·13 × 0·12—0·17 × 0·15 mm. and the right one 0·15 × 0·12—0·22 × 0·22 mm. Cirrus sac is pre-acetabular, 0·3—0·43 × 0·08—0·11 mm. in size, and lies at a distance of 0·02—0·06 mm. from it. Its proximal basal portion is swollen and oblique and the distal tubular portion is more or less straight and lies near left margin. Vesicula seminalis lies inside the basal swollen portion of cirrus sac and consists of two parts, *i.e.*, the proximal broad sac-like portion and distal coiled tubular portion. Pars prostatica is flask-shaped measuring 0·06 × 0·03 mm. and is enclosed by prostate gland cells. Ductus ejaculatorius and cirrus are present in the distal half of cirrus sac, near lateral margin.

Ovary is somewhat pear-shaped and is post-testicular but pre-acetabular in position, being slightly on the right side. It lies dorsal to cæcum and is smaller than both the testes, measuring 0·06 × 0·09—0·11 × 0·14 mm. in

size. Receptaculum seminis varies in size and position. It may be pre-acetabular or acetabular and is spherical or oval 0·02 × 0·05 —0·05 × 0·09 mm. in size. Vitellaria bear vitelline follicles, all aggregated towards the right side of œsophagus between it and the right margin. The follicles overlap œsophagus and also anterior part of right testis. Two vitelline ducts are present which fuse to form a small triangular vitelline-vesicle near the receptaculum seminis. Owing to the asymmetry in the position of vitellaria, both the vitelline ducts arise from the same side. Uterus presents a few loops and is both pre- and post-acetabular, but never extends in front of intestinal fork. Metraterm is present but not very well differentiated. Genital atrium is indistinct and genital pore is sinistral, pre-acetabular and nearly marginal, lying on a level in front of intestinal bifurcation. Eggs are oval and measure 25—30 × 12 μ.

Excretory bladder Y-shaped with a small median stem, 0·06 mm. long and the lateral cornua are slightly inflated (0·1—0·11 mm. broad) and extend in the acetabular region, upto the blind ends of intestinal cæca. Excretory pore is terminal.

Discussion.—The present form differs from *P. fuelleborni* in the position of genital pore, which in the latter case is post-testicular and lies at a level posterior to the intestinal fork. It also differs from *P. confusum* and *P. fuelleborni* in the position of ovary and cirrus sac, for in the latter two species, ovary lies median and cirrus sac extends not only in acetabular and pre-acetabular regions but also in the post-acetabular region. It resembles *P. indicus* and *P. infrequentum* in the shape and position of gonads but can readily be distinguished from them by the peculiar one-sided position of vitellaria. All the vitelline follicles are confined in the present species towards the right side of œsophagus and in this character it resembles *P. himalayai.* These two species differ from each other in the size of body, the length of œsophagus, the posterior extension of intestinal cæca, the position of receptaculum seminis and shell gland mass, the position, shape and size of gonads and the extent of uterine loops. *P. himalayai* has body, and œsophagus proportionately long, intestinal, cæca extend a little beyond acetabulum, receptaculum seminis and shell gland mass is post-acetabular and ovary is extracæcal and larger than the testes. In the present form, on the other hand, the length of body is about twice that of its breadth, œsophagus lies in the anterior fourth of the body, intestinal cæca do not extend beyond the level of acetabulum, receptaculum seminis and shell gland mass is acetabular or pre-acetabular, ovary is smaller than the testes and never entirely extracæcal and uterine loops are fewer than that of *P. himalayai.* It is therefore described as a new species *P. kashabia.*

Pande (1937) reports the presence of only one vitelline duct in his species *P. himalayai* and on the basis of this observation presumes the existence of only one vitelline gland. *P. kashabia* is similar to *P. himalayi* in the distribution of vitelline follicles and the author has observed two well-marked vitelline ducts in it. Moreover the one-sided position of vitellaria is also present in certain species of *Pleurogenoides*, for example in *Pleurogenoides sphericus* Klein (1905) and *Pleurogenoides taylori* Tubangui (1928). In the former, two vitelline ducts have been traced to arise from the right side where all the vitelline follicles appear to have aggregated. The author believes that both the vitelline glands are present in those species of *Prosotocus* and *Pleurogenoides* which show this peculiar one-sided distribution of vitelline follicles. It appears that owing to the enlargement and anterior extension of cirrus sac and possibly in order to facilitate its movements and that of metraterm during the act of copulation the vitelline follicles of the left side have shifted towards the right side of œsophagus. In the light of above observations, regarding the number of vitelline ducts and vitelline glands, the species *P. himalayai* needs re-examination.

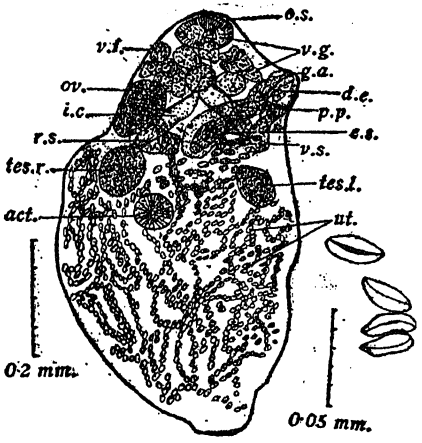
Key.—

1.	Genital pore post-testicular	*P. fuelleborni.*
	Genital pore testicular or pre-testicular ..	2
2.	Ovary median; cirrus sac post-acetabular to pre-acetabular	*P. confusum.*
	Ovary lateral; cirrus sac pre-acetabular ..	3
3.	Vitellaria on both sides	4
	Vitellaria confined on one side only ..	5
4.	Genital pore in the level of testes; ovary and testes more or less equal in size ..	*P. indicus.*
	Genital pore pre-testicular; ovary much smaller than testes	*P. infrequentum.*
5.	Ovary larger than testes; intestinal cæca extend a little beyond acetabulum ..	*P. himalayai.*
	Ovary smaller than both testes; intestinal cæca do not extend beyond acetabulum ..	*P. kashabia* (n.sp.)

Pleurogenoides Bufonis (n.sp.)

Travassos (1921) created the genus *Pleurogenoides* to include such species of *Pleurogenes* as have short intestinal cæca, never extending beyond acetabulum. *Pleurogenoides* comprises thirteen species and are chiefly parasites of Amphibia. *P. tener, P. minus* and *P. pabdai* are exceptional, the former is a reptilian parasite reported from *Chamæleo basiliscus* and the latter two

are piscine trematodes found in the intestine of *Esox lucius* and *Callichrus pabda* respectively. The genus has a wide distribution being reported from all continents. There are four Indian species:—*P. gastroporus* Lühe (1901) and *P. sitapurii* Srivastava (1934) are reported from *Rana cyanophlyctis*,



B (1) B (2)

Fig. B (1) *Pleurogenoides bufonis* (n.sp.) from *Bufo viridis* (ventral view).

Fig. B (2) *Pleurogenoides bufonis*, eggs.

P. sphericus Klein (1905) from *Rana hexadactyla* and *P. pabdai* Pande (1937) from the fish *Callichrus pabdai*. Mehra and Negi (1928) added *P. gastroporus* var. *equalis*, a new variety, from *Rana tigrina*, but Bhalerao (1936) fused the variety with *P. gastroporus* Lühe (1901). The present communication adds another species collected from the intestine of the toad, *Bufo viridis*, in August 1940, at Srinagar, Kashmir. The species appears rare, for only one out of thirty-five toads examined, was infected with this parasite.

Description.—Body is delicate, thin and transparent. It is oval in form, measuring 0·73—0·82 mm. in length and 0·42—0·45 mm. in maximum breadth and bears large number of eggs in the posterior two-third of the body. The anterior part is studded with rows of minute spines which alternate in the adjacent rows and disappear beyond the level of ovary. Suckers are feebly muscular and owing to the presence of large vitelline follicles and brown ova, their outline can be traced with difficulty. Oral sucker is subterminal and oval, measuring 0·08—0·1 × 0·05—0·06 mm. Acetabulum is equatorial or slightly pre-equatorial, smaller than oral sucker and circular in outline, with 0·06—0·07 mm. in diameter. Excretory pore is terminal.

Pharynx is present, measuring 0·022 × 0·014 mm. and œsophagus is moderately long, 0·044 mm. in length. Intestinal cæca are short, subequal, divergent, slightly dilated, pre-equatorial, pre-acetabular and pre-testicular.

Testes are round to oval, symmetrically placed, and pre-acetabular in position. They are subequal; the right testis is 0·09—0·12 × 0·06—0·07 mm. and the left testis 0·08—0·1 × 0·06—0·08 mm. Cirrus sac is well developed, sac-like and 0·23 × 0·06 mm. in size. It is sinistral, lies on the ventral side of the left intestinal cæcum and extends obliquely from the level immediately in front of testes to the genital pore. Vesicula seminalis is coiled and is divided into basal sac-like portion, 0·11—0·12 × 0·035—0·04 mm. in size and the distal tubular region. Pars prostatica is globular to flask shaped, measuring 0·035—0·055 × 0·025 and is followed by ductus ejaculatorius and cirrus.

Ovary is oval, 0·12 × 0·07—0·08 mm. in size. It is extracæcal and pre-testicular and lies between the vitelline follicles and the right testis. Receptaculum seminis is 0·052 × 0·048 mm. in size and lies dorsal to the right intestinal cæcum between ovary and right testis. A diffused shell gland mass is present just near the receptaculum seminis on its inner side. Vitellaria extend dorsally in the entire anterior region of the body upto the level of ovary, intestinal fork and the distal portion of cirrus sac. There are 9–18 vitelline follicles of large size circular to oval, measuring 0·02 × 0·02—0·05 × 0·04 mm. They are so conspicuous that they mask the outlines of oral sucker, pharynx and œsophagus. Uterus is well developed, and occupies about the posterior two-third of the body. As the parasites are all mature, the uterine coils are indistinguishable. Metraterm lies dorsal to cirrus sac and opens into the genital atrium which lies in the slight protuberance, present on the left body margin at the level of the intestinal fork and contains both male and female openings. It communicates outside through the genital pore which lies on the left body margin at the level of intestinal bifurcation or a little in front of it. Eggs are oval and slightly elongated, 28—30 × 12—14μ in size and yellowish to dark brown in colour.

Discussion.—As will be discussed later in this paper the author agrees with Travassos (1921, 1930 and 1931) in retaining *Pleurogenoides* as a separate genus comprising thirteen species. A new distome, *Pleurogenoides bufonis* has been added in the present communication. *P. bufonis* has acetabulum equatorial or slightly pre-equatorial and thus the species differs from *P. sphericus*, *P. solus*, *P. tener* and *P. sitapurii* in all of which acetabulum is distinctly post-equatorial. In the extra-cæcal position of ovary

the new distome resembles *P. pabdai*, *P. madians*, *P. minus* and *P. japanicus* but differs from the species *P. freycineti*, *P. arcanum*, *P. taylori*, *P. stromi* and *P. gastroporus*. It can be separated from *P. pabdai* by the shape of body and intestinal cæca and relative position of acetabulum and testes. It differs from *P. minus* in the arrangement of vitelline follicles, the form of cirrus sac, the position of genital pore and the posterior extension of intestinal cæca. It is different from *P. medians* in the position of testes in relation to body and acetabulum and in the number and size of vitelline follicles. It differs from *P. japonicus* in the relative position of the matraterm to the cirrus pouch, arrangement of follicles in vitellaria and size of ovary in relation to testes. It is therefore considered as a new species under the genus.

Key.—The following key is extended and slightly modified from Srivastava (1934) :—

1.	Acetabulum distinctly post-equatorial ..	2
	Acetabulum equatorial or pre-equatorial ..	5
2.	Oesophagus present	3
	Oesophagus absent	*P. sphericus.*
3.	Intestinal cæca extend upto the acetabulam ..	*P. solus.*
	Intestinal cæca do not extend upto the acetabulum	4
4.	Excretory pore terminal	*P. tener.*
	Excretory pore subterminal	*P. sitapurii.*
5.	Ovary extracæcal	6
	Ovary not extracæcal	10
6.	Acetabulum pre-equatorial; posterior end of the body bifid	*P. pabdai.*
	Acetabulum equatorial or slightly pre-equatorial; posterior end of the body not bifid ..	7
7.	Intestinal cæca extend upto the level of acetabulum	*P. minus.*
	Intestinal cæca do not extend upto the level of acetabulum	8
8.	Testes pre-equatorial; vitelline follicles few and of large size	*P. bufonis* (n.sp.).
	Testes equatorial; vitelline follicles numerous and of small size	9
9.	Intestinal cæca reach to the level of the testes	*P. japanicus.*
	Intestinal cæca do not reach to the level of the testes	*P. medians.*

10.	Oesophagus present	11
	Oesophagus absent	12
11.	Genital atrium opens on the left body margin	*P. freycineti.*
	Genital atrium opens subterminally halfway between the left intestinal cæcum and body margin	*P. arcanum.*
12.	Testes situated anterior to the ends of the intestinal cæca	*P. taylori.*
	Testes situated behind the ends of the intestinal cæca	13
13.	Vitellaria consists of a few large follicles, precæcal	*P. stromi.*
	Vitellaria consists of a large number of small follicles scattered all over the cæca and meeting in the median line	*P. gastroporus.*

Review of the Genus Pleurogenes *Looss* (1896)

Pleurogenes Looss (1896) forms a composite group of heterogeneous species and Travassos (1921 and 1928) divided it into two genera—*Pleurogenes* and *Pleurogenoides*—the former with elongated intestinal cæca extending posteriorly beyond acetabulum and the latter with short cæca which do not extend beyond acetabulum. Travassos restricted the genus *Pleurogenes* to such species as *P. claviger*, *P. loossi* and *P. lobatus* and all the remaining species were placed by him under the new genus *Pleurogenoides*. Mehra and Negi (1928) gave them only sub-generic status and called the two genera *Pleurogenoides* and *Pleurogenes* as *Pleurogenes* (*Pleurogenes*) and *Pleurogenes* (*Telogonella*). Srivastava (1934) supported Mehra and Negi but Macy (1936) retained *Pleurogenes* and *Pleurogenoides* as separate genera in his key on Pleurogenetinæ. The author believes that the view expressed by Mehra and Negi and later supported by Srivastava is not correct. *Pleurogenoides* and *Pleurogenes* differ not only in the length of intestinal cæca but also in the position of testes which is always acetabular or pre-acetabular in the former and distinctly post-acetabular in the latter. *Pleurogenes orientalis* definitely resembles in both these characters to the species of *Pleurogenes* Travassos (1921) and can be safely placed under it. These differences between *Pleurogenes* and *Pleurogenoides* are no less important and significant than what we notice between *Mehraorchis* (an unfortunate nomenclature) and *Prosotocus* and hence they must be given separate generic status.

Stafford (1904) created the genus *Loxogenes*, for *Pleurogenes arcanum* (syn. *Distomum arcanum* Nickerson, 1900), on account of the genital pore being present on the ventral surface, midway between the left intestinal cæcum and the body margin. Klein (1907), Mehra and Negi (1928) and Srivastava (1934) do not subscribe to this view and have placed *Loxogenes arcanum* under *Pleurogenes*, but Tubangui (1928), Fuhrmann (1928) have retained *Loxogenes* as a separate genus. Krull (1933) added one more species to the genus from *Rana clamitans* and called it *Loxogenes bicolor*.

Loxogenes arcanum Stafford (1904) and *Loxogenes bicolor* Krull (1933) possess more points of difference than resemblance. Apart from the difference in the length of cæca, the distribution of vitelline follicles and the form of excretory bladder, they differ in the topography of gonads as well as in the position of cirrus sac. *L. arcanum* has (*a*) short cæca, (*b*) vitelline follicles present across entire body from pharynx to acetabulum, (*c*) excretory bladder with short stem and inflated cornua, (*d*) ovary pre-acetabular between two testes and (*e*) cirrus sac sinistral. *L. bicolor* has (*a*) elongated inflated cæca, (*b*) vitellaria mainly confined laterally, (*c*) excretory bladder with long stem and small cornua, (*d*) ovary acetabular and testes post-acetabular and (*e*) cirrus sac dextral. Thus the two species cannot be retained under one and the same genus. As the position of genital pore which is the most important generic character is disputed in *L. arcanum* (on ventral surface according to Nickerson, 1900 and Stafford, 1904; on dorsal surface behind pharynx and near intestinal fork according to Osborn, 1912), the exact systematic position of the species becomes difficult. In view of its resemblance with *Pleurogenoides* in the topography of gonads, form of excretory bladder, sinistral position of cirrus sac and extent of intestinal cæca it may be tentatively named *Pleurogenoides arcanum*.

The description of the adult worm of *Loxogenes liberum* Senso (1907) being in Japanese script is not available and its metacercariæ described by Yamaguti (1937) shows difference from *L. bicolor* in the shape of excretory bladder and sinistral arrangement of cirrus sac. As the form of excretory bladder remains constant during development and difference in this character is of generic importance the two species, *L. bicolor* and *L. liberum*, cannot be placed in one and the same genus.

Loxogenes bicolor differs from *Pleurogenes* and *Pleurogenoides* in (*a*) long inflated intestinal cæca, (*b*) shape of excretory bladder, (*c*) dextral position of cirrus sac and genital opening, (*d*) median and acetabular position of ovary and (*e*) folded condition of testes. Thus the author agrees with Krull (1933) and Macy (1936) in retaining *Loxogenes* as a separate genus and further thinks that *Loxogenes bicolor* is its sole representative.

Loxogenes *Krull* (1933) *Emended*

Generic diagnoses.—Medium sized distomes with broad flattened and thick bodies; cuticle spinous; oral sucker subterminal and well developed; acetabulum small, weakly muscular and slightly pre-equatorial; prepharynx absent; pharynx present; œsophagus short and slender; intestinal cæca long, reaching near the posterior end of body and inflated; testes large, elongated dorsoventrally and folded, post-equatorial and opposite each other; cirrus sac long and slender, pre-equatorial and dextral; ovary rounded, lobed, or irregular in shape and pre-testicular, lying dorsal to acetabulum; receptaculum seminis and Laurer's canal present; shell glands well developed; vitellaria follicular, preacetabular with greatest concentration laterally; uterus voluminous both pre- and post-acetabular; metraterm short and well differentiated; genital pore ventral and pre-acetabular lying slighty right of median line; excretory vesicle Y-shaped with a long stem and short cornua; excretory pore posterior and terminal; parasites of Amphibia.

Type Species Loxogenes bicolor

Ozaki (1926) described a fluke from the bile ducts of the Japanese frog, *Polypedates buergeri* and placed it under *Pleurogenes* as *P. lobatus.* This worm shows the following differences from *Pleurogenes* :—(1) genital pore is situated at a level posterior to intestinal bifurcation, (2) Ovary is median and acetabular in position, (3) gonads (both ovary and testes) are greatly lobed and (4) vitelline follicles are cæcal and arranged in a number of small bunches.

Owing to its difference from *Pleurogenes,* in the position of genital pore and ovary and arrangement of vitellaria, this worm cannot be included in the genus. The author therefore creates a new genus *Pleurolobatus* for the reception of *Pleurogenes lobatus.*

Pleurolobatus (*n.g.*)

Generic diagnosis.—Distomes of moderate size; body oval and spinose ; oral sucker subterminal; acetabulum smaller than oral sucker and pre-equatorial ; pharynx and prepharynx present; œsophagus short; intestinal cæca long extending to the last quarter of body length; testes large, irregularly lobed, equatorial and post-acetabular lying ventral to cæca; cirrus sac long and conical, pre-acetabular and more or less transversely placed; ovary large, irregularly lobed, acetabular and slightly pre-acetabular and median; receptaculum seminis and Laurer's canal present; shell glands median and in front of ovary; vitellaria follicular in five to ten groups on each side, lying ventral to cæca between intestinal fork and

testes; uterus mostly post-testicular with more or less transverse loops; metraterm long and well differentiated; genital pore dorsal, pre-acetabular, close to left margin, behind the level of intestinal fork; excretory vesicle V-shaped; excretory pore terminal; parasites of Amphibia.

Type Species Pleurolobatus **Lobatus**

Thus the old complex genus *Pleurogenes* Looss (1896) is split into four genera—*Pleurogenes* as emended by Travassos (1921), *Pleurogenoides* Travassos (1921), *Loxogenes* Krull (1933) emended and *Pleurolobatus* (n.g.) and the distribution of the various species is as follows :—

1. *Pleurogenes* Looss (1896) emended by Travassos (1921) includes *P. claviger* Looss (1899), *P. intermedius* Issaitschikow (1926), *P. loossi* Travassos (1930) and *P. orientalis* Srivastava (1934).
2. *Pleurogenoides* Travassos (1921) includes *P. medians* Olsson (1876), Looss (1899), *P. tener* Looss (1899), *P. gastroporus* Lühe (1901), Mehra and Negi (1928), *P. sphericus* Klein (1905), *P. arcanum* Klein (1905), *P. freycineti* Johnson (1912), *P. solus* Johnson (1912), *P. taylori* Tubangui (1928), *P. stromi* Travassos (1930), *P. minus* Pigulewsky (1931), *P. sitapurii* Srivastava (1934), *P. japanicus* Yamaguti (1936), *P. pabdai* Pande (1937) and *P. bufei* (n.sp.).
3. *Loxogenes* Krull (1933) emended, includes *L. bicolor* Krull (1933) syn. *Pleurogenes bicolor* Srivastava (1934).
4. *Pleurolobatus* (n.g.) includes *Pleurolobatus lobatus* (syn. *Pleurogenes lobatus* Ozaki, 1926).

LIST OF REFERENCES

1. Bhalerao, G. D. .. "Studies on the Helminths of India; Trematoda III," *Jour. Helminth.*, 1936, **14**, No. 4.
2. Fuhrmann, V. O. .. "Trematoda" in Kukenthal and Krumbach's *Handbuch der Zoologie*, 1928.
3. Krull, W. H. .. "*Loxogenes bicolor*, a new pigmented fluke from the frog *Rana clamitans*," *Trans. Amer. Micr s. Soc.*, 1933, **52.**
4. Looss, A. .. "Weitere Beitrage Zur Kenntnis der Trematodenfauna Aegyptens," *Zool. Jaharb. Syst.*, 1899, 12.
5. Macy, R. W. .. "A new genus and species of Trematodes from the little brown bat and a key to the genera of Pleurogenetinæ," *Proceed. U. S. Nat. Mus.*, 1936, **83.**
6. Mehra, H. R., and Negi, P. S. .. "Trematode parasites of the Pleurogenatinæ from *Rana tigrina* with a revision and synopsis of the subfamily," *Allahd. Univ. Stud.*, 1928, **4.**
7. Modlinger .. "*Prosotocus* spp.," *Stud. Zool. Budapest*, 1930, **1.** (Not available.)
8. Modlinger .. "Trematode Ungarischer Chiropterin," *Ibid.*, 1930, **1.** (Not available).

9. Pande, B. P. .. "Two new fish Trematodes from Allahabad," *Proc. Nat. Acad. Sci., Ind.*, 1937, **7**, Part 2.

10. ——— .. "*Prosotocus himalayai* (n.sp.); a frog Trematode (Lecithodendriidæ)," *Ibid.*, 1937, **7**, Part 3.

11. Pigulewsky, S. W. .. "Neue Arten von Trematoden aus Fischen des Dnjeprbassins," *Zool. Anz.*, 1931, **96**.

12. Srivastava, H. D. .. "On new Trematodes of frog and fishes of the United Provinces, India; Part II. Three new trematodes of the subfamily Pleurogenetineæ (Fam. lecithodendriidæ) from *Rana cyanophlyctis* of Oudh," *Bull. Acad. Sc. U.P., Allahabad*, 1939, **4**, No. 4.

13. ——— .. "On new Trematodes of frog and fishes of the United Provinces, India; Part III. On a new genus *Mehraorchis* and species of *Pleurogenes* (Pleurogenetinæ) with a systematic discussion, and revision of the family Lecithodendrüdæ," *Ibid.*, 1934, **4**, No. 4.

14. Travassos, L. .. "Contribuicoes Para O Conhecimento Da Fauna *Helminthologica brasileira*, XV, Sobre as especies Brasileiras da Fauna Lecitho dendriidæ Assisteule do instituto Oswald Cruz," *Arch. de. Esc. Sup. de Agric. e. Med. Veter., Nictheroy*, 1921, **5**, Nos.1, e.2.

15. ——— .. "Pesquizas Helminthologicus realisadas em Hamburgo, V, Genero *Prosotocus* Looss, 1899 (Trematoda; Lecithodendriidæ)," *Mem. Inst. Osw. Cruz.*, 1930, Tomo. 24, Fasc. 2.

16. ——— .. "*Ibid.*, VI, Genero *Pleurogenoides* Travassos 1921 (Trematoda; Lecithodendriidæ)," *Ibid.*, 1930, Tomo. 24, Fasc. 2.

17. ——— .. "*Ibid.*, VIII, Informacœ Sobre O genero *Pleurogenes* Looss 1896, (Trematoda; Lecithodendriidæ)," *Ibid.*, 1930, Tomo. 24, Fasc. 4.

18. Tubangui, M. A. .. "Trematode parasites of Philippine Vertebrates," *Phil. Journ. Sci.*, 1928, **36**, No. 3.

19. Ward, H. B., and Whipple, G. C. .. '*Fish-water Biology*,' 1918.

20. Yamaguti, S. .. "Studies on the Helminth Fauna of Japan; Part 14, amphibian Trematodes," *Jap. Jour. Zool.*, 1934–36, **6**.

21. ——— .. "Second intermediate host of *Loxogenes liberum*," *Jour. Parasit.*, 1937, **23**.

LETTERING

act., acetabulum; *cir.*, cirrus; *c.s.*, cirrus sac; *d.e.*, ductus ejaculatorius; *eg.*, egg, *e.b.*, excretory bladder; *e.c.*, excretory cornua; *e.p.*, excretory pore; *e.s.*, excretory bladder, median stem; *g.a.*, genital atrium; *g.p.*, genital pore; *i.b.*, intestinal bifurcation; *i.c.*, intestinal cæcum; *mo.*, mouth; *met.*, metraterm; *æs.*, œsophagus; *o.s.*, oral sucker; *ov.*, ovary; *ovd.*, oviduct; *ph.*, pharynx; *prp.*, prepharynx; *p.p.*, pars prostatica; *r.s.*, receptaculum seminis; *s.g.*, shell gland; *tes.*, testis; *tes.r.*, right testis; *tes.l.*, left testis; *ut.*, uterus; *v.d.*, vitelline ducts; *v.f.* vitelline follicles; *v.g.*, vitelline gland; *v.r.*, vitelline reservoir; *v.s.*, vescicula seminalis.

(Type specimens and cotypes are deposited in Dr. Thapar's Helminthological Collections, Lucknow University).

ROOT INITIATION IN THE ADULT AXES OF A FEW DICOTYLEDONOUS SPECIES

(With 14 Text-Figures)

BY AMIYA DATTA AND GIRIJA P. MAJUMDAR
(Botanical Laboratory, Presidency College, Calcutta)

Received August 9, 1943
(Communicated by Prof. H. Chaudhuri, F.A.SC., Lahore)

Introduction

ORIGIN of shoot borne adventive roots is described in text-books as *endogenous*, exceptions being recorded in the family of Cruciferæ, *e.g.*, in *Cardamine pratensis* (Hansen, 1881) and *Nasturtium austriacum* (= *Roripa austriaca*, Wilson, 1927). In both these cases the origin is described as *exogenous* and is reported to be associated with axillary buds (Priestley and Swingle, 1929).

In assigning the position of the endogenous root initials in an organ De Bary (1884) makes the general statement that it must be "in or close to vascular bundles or masses of wood or bast" (p. 315). Lemaire (1886) made extensive studies of the "origin of naturally occurring adventive roots in the hypocotyls, stolons and rhizomes" of herbaceous dicotyledons and observed that the origin of these roots might be (1) exclusively in the pericycle, (2) partly in the pericycle and partly in the endodermis and inner cortex, (3) exclusively in the "subphloem meristem", *i.e.*, cambium, and (4) partly in the cambium and partly in the pericycle. He, however, concluded that the pericyclic origin is by far the commonest. Van Tieghem and Douliot (1888) fix the place of origin entirely in the pericycle so far as the young hypocotyls, epicotyls, stolons and rhizomes are concerned; if, however, they state, the pericycle loses its "root-forming property", as in old stems, the adventive roots arise in the phloem parenchyma, both primary and secondary; and still later in cambium itself. Eames and MacDaniels (1925), on the other hand, give the position as the pericycle in the cases of young stems and in older axes, where the pericycle is no longer active, in the secondary phloem (p. 238).

Priestley and Swingle (1929) distinguished the shoot borne adventive roots into two categories on the basis of their origin, namely, (1) those formed behind the apical meristem and (2) those arising upon old stems which have

ceased to extend longitudinally, but in which radial growth alone is proceeding (p. 62). They then pointed out that in young stems these initials differentiate practically always near but to the side of a vascular group, *i.e.*, on a primary ray. In old stems on the other hand the site of initiation of a lateral root moves inwards from the region of the pericycle to the living cells of the ray that lie close to the newly differentiated xylem and phloem. The root initials are formed in association with a group of cells bordering upon the vascular cambium (pp. 63, 64).

We have undertaken this investigation in order to determine in a general way the origin of naturally occurring adventive roots on adult axes of herbaceous plants, and at the same time to re-examine the validity of the general statement of Priestley and Swingle with regard to the root initiation in old stems.

By "adult" or "old" axes we would mean the hypocotyl and internodes which have ceased to elongate longitudinally, but in which the radial extension is still going on, as contemplated by Priestley and Swingle in their second category of shoots bearing adventive roots.

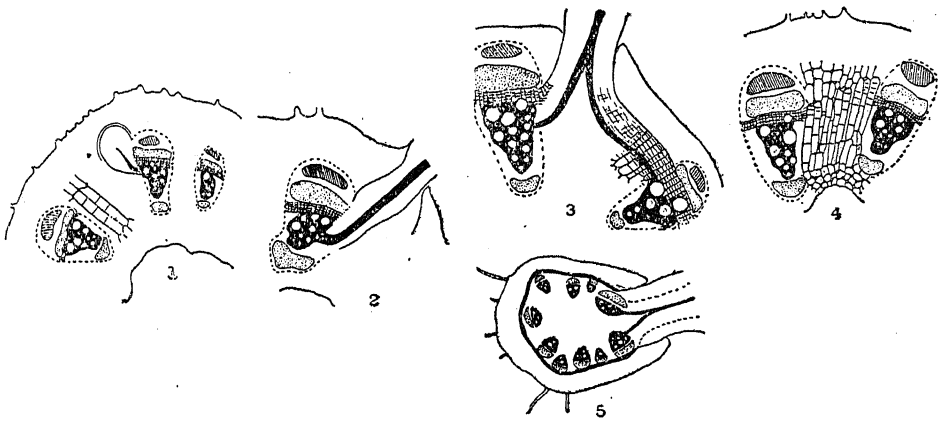
There is much controversy with regard to the use of the term 'pericycle' (Solereder, 1908; Strasburger, 1930). In this and subsequent papers we accept the definition of Van Tieghem and Douliot which runs as follows: The pericycle is "the layer or layers between the endodermis and the external phloem of a fibrovascular bundle continuous across the medullary rays but not distinguished on the inside from the medullary ray" (Carlson, p. 119, 1929).

We propose to report on the origin of naturally occurring lateral roots in the adult internodes of herbaceous dicotyledons, monocotyledons and of pteridophytes, and the present paper forms the first of the series that are expected to follow with the progress of our work. The results embodied in this paper are based on a study of free-hand sections of the hypocotyls or internodes of the species reported, and the text-figures have all been drawn under a microprojector.

Observation

Cucurbita maxima (Text-Figs. 1–4) develops a fairly elongated hypocotyl during its seedling stage of growth. The vascular bundles, ten in number, are typically bicollateral and are arranged in a ring with sclerenchymatous bundle caps characteristically associated with each of these ten bundles. These caps are very prominent in bundles near the base of the hypocotyl, but as one proceeds towards the top the cells of the cap lose their sclerenchymatous nature and do no longer give lignin reaction with aniline sulphate

or aniline chloride solution. The outer vascular cambium is much wider than the inner one, and extends laterally into a layer or two of the ray cells on the sides of each bicollateral bundle.



TEXT-FIGS. 1–5. Origin of shoot-borne lateral roots in *Cucurbita maxima* and *Oxalis corniculata*.—Figs. 1–4. *Cucurbita maxima*. T.S. of hypocotyl showing the origin of adventive roots. Fig. 4 shows the nature of the radial growth of the hypocotyl. Fig. 5. T.S. of the adult internode of *Oxalis corniculata*. ×15.

The vascular bundles in the hypocotyl thus differ from those in the stem in their arrangement round the pith, and in having isolated sclerenchymatous bundle-caps of procambial origin instead of a continuous sclerenchymatous cylinder of the adult internodes described as pericyclic in position.

Lateral roots begin to appear near the basal region of the hypocotyl which has already ceased to elongate, and proceeds to a certain distance up the organ. The root initials take their origin in the flanks of the outer cambium in the formation of which the adjoining ray cells also take part (Figs. 1 and 2). There may be two adventitious roots originating from the two sides of a vascular bundle or rarely two groups of initials from the flanks of the adjoining bundles join together, sometimes in the middle, sometimes to one side, of the intervening ray to form a single lateral root (Fig. 3). The root initials after their organization into the primordium bend through about 90° in their passage through the cortex towards the periphery of the hypocotyl without causing any or causing a very slight disturbance to the bundle-cap which retains its original position with reference to the bundle.

When the lateral roots begin to appear the hypocotyl in this region has already ceased to elongate, but the radial growth is still maintained. This is accomplished in two ways, namely, (1) by the radial growth of individual

bundles from the two fascicular cambia, particularly from the outer one, and (2) by the enormous radial elongation of ray cells which divide tangentially with a view, it appears, more to resist lateral pressure than for any other purpose (Fig. 4). Sometimes regular interfascicular cambia are organised, but they do not unite with the outer fascicular cambia to form a continuous cambial ring as in the normal axes of dicotyledons. These interfasicular cambia help the radial extension of the ray cells and also very frequently form isolated phloem patches in the rays.

Oxalis corniculata (Text-Fig. 5) is a creeping plant. Adventitious roots from adult internodes are rare in this plant. The few that have been found were given off from the lower side of the trailing shoots growing in moist shady places. There are ten bundles arranged in a ring and at no time a regular cambial ring has been seen to form though the growth and extension of the bundles, particularly of the bigger ones, is maintained by the fascicular cambia. The endodermis with casparian strips is found to persist, and a complete cylinder of thick-walled pericycle of varying depth is a special feature of the internodal structure of this plant.

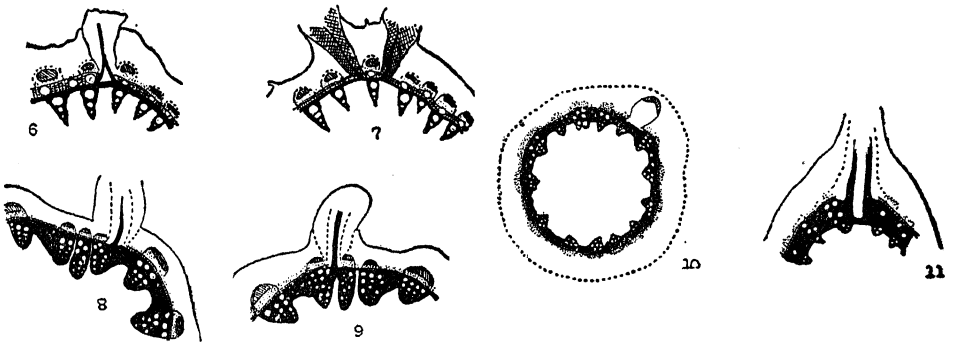
In the formation of the shoot-borne roots two groups of initials from the flanks of the adjoining vascular bundles join together in the middle of the intervening ray to form a single lateral root (Fig. 5). Vascular connection is later made with both the bundles. The root initials originate, as in *Cucurbita,* in the cambial and ray cells adjoining it.

Mikania scandens (Text-Figs. 6, 7) a twining and spreading herb which delight in moist places, particularly near the edges of a pool. Lateral roots from internodes of adult stems are very rare in this plant. The detailed developmental and adult anatomy of this plant is being worked out by Mr. I. Banerji of the Department of Botany, Calcutta University, and we propose to record here only the barest outline of the adult stem structure.

The large number of vascular bundles are surmounted each by a sclerenchymatous bundle-cap. Interfascicular cambium rarely produce any xylem vessels. In the formation of the root initials both the vascular and interfascicular cambia take part. In Fig. 6 the lateral root is median in position with reference to the medullary ray and vascular connection is made with both the bundles. In Fig. 7 two lateral roots are formed one on each side of a vascular bundle.

Tagetes patula (Text-Figs. 8, 9), an annual garden herb, but may be made to continue through many seasons if proper care is taken. It is towards the end of the first season that lateral roots, sometimes in profuse numbers, are given out from adult internodes. The origin of these roots are definitely

related to the trace bundles as their vertical arrangement can be followed along the course of these bundles in the internodes. Good varieties of *Tagetes* is propagated mainly by cuttings.



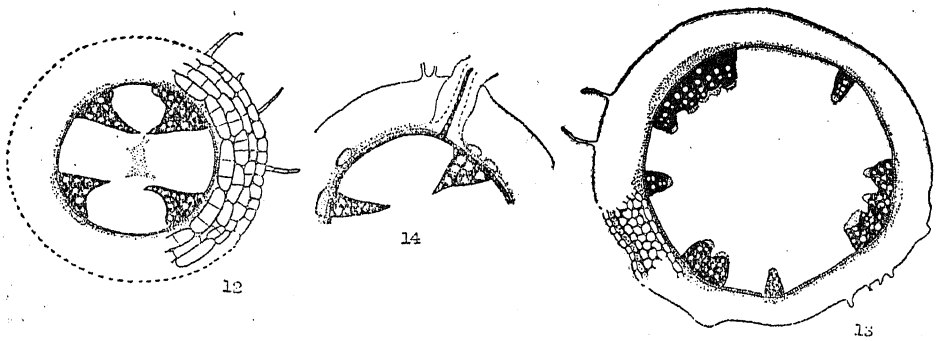
TEXT-FIGS. 6–11. Origin of shoot-borne lateral roots.—Figs. 6–7. T.S. of adult internodes of *Mikania scandens*. Figs. 8–9, that of *Tagetes patula* and Figs. 10–11, that of *Ageratum conyzoides*. ×15.

The arrangement and structure of the vascular bundles in adult internodes are typically of the *Helianthus* type. The lateral roots are given out from the flanks of the fascicular cambium of a vascular bundle. Fig. 8 shows the origin of a lateral root from the flank of a synthetic bundle in the making. The bundle-cap is not disturbed. Fig. 9 shows a root developed between two vascular bundles which has made vascular connections with both of them.

Ageratum conyzoides (Text-Figs. 10, 11).—This is also an annual herb but erect in habit. In all the specimens collected the production of lateral roots were found to be confined to the first three or four internodes growing prostrate on the ground. The anatomical structure of the axis is typically that of the woody vine type, *i.e.*, an unbroken cylinder of primary and secondary wood with no medullary rays. The lateral root arises from the flank of a vascular canbium (Fig. 10) and when it makes vascular connection with two bundles it is seen to occupy the whole of the intervening medullary ray outside the interfascicular cambium (Fig. 11).

Solanum nigrum (Text-Figs. 12–14).—This is an annual herb. Bhaduri (1933) points out that " plants identified as *S. nigrum* by Prain (1903), Hooker (1875) and others differ considerably in both morphological and cytological characters " (p. 58), and he could easily distinguish three types of *S. nigrum* from his critical studies of cytology and morphology of these plants ($n = 12$, $n = 24$, $n = 36$; pp. 60–61). We have not been able to collect, examine and compare the anatomical structure of all the three types

of Bhaduri, but Figs. 12 and 13 show that the internode and hypocotyl of this plant differ widely in their anatomical structures.



TEXT-FIGS. 12–14. Origin of lateral roots and the structure of internodes and hypocotyl in T.S.—Figs. 12 and 13. T.S. of the hypocotyl and internode respectively of *Solanum nigrum*. Fig. 14. T.S. of hypocotyl showing origin of lateral root. Figs. 12 and 14, ×15 ; Fig. 13, ×25.

Fig. 14 shows the origin of a lateral root in the hypocotyl of *S. nigrum*. It will be seen that there are only 4 primary bundles and a cambium ring is very early differentiated. The root initials originate in the interfascicular and fascicular cambia close to a vascular bundle, and the vascular connection is made with the secondary xylem and phloem.

Discussion

Adventive roots may arise on any part of a stem, but the normal position is the node. The present study as has been mentioned in the Introduction is confined to the origin of naturally occurring roots in the nodeless segments of adult shoots of a few dicotyledonous herbs ; and in this respect our observations differ from those of others dealing mostly in the regeneration of roots on stem and leaf cuttings (Swingle, 1940).

From the two reported cases it may be assumed that exogenous adventitious roots are developed only in association with axillary buds. In *Cotoneaster Dammeri* development of lateral roots in association with axillary buds is a normal feature. Miss Wolfe (1934) noted their origin in the parenchyma at the flank of the cambium of the branch (bud) trace. It is doubtful if one can call this an exogenous origin.

Lemaire and Van Tieghem and Douliot studied the origin of naturally occurring roots on hypocotyl, rhizomes and internodes, but they do not, so far as we are aware, appear to have noted any difference in the origin of lateral roots in young and adult organs. Priestley and Swingle are perhaps the first to note this difference. They formulated their second type of origin from a study of the hypocotyl in *Helianthus* and *Ricinus*, and of the

epicotyl of *Vicia* and *Solanum*. The six cases reported here are additional examples.

Trécul (1846) suggested the possibility of the existence of preformed root initials in stems of certain species. 32 years later Vöchting (1878) reported regular occurrence of preformed root premordia in the cuttings of Willow. Goebel (Vol. II, p. 275) reports the presence of pre-existing sub-cortical "root germs" in *Salix*, and these primordia are found to develop on the cuttings used for vegetative propagation in these plants. Van der Lek (1924) noticed occurrence of such preformed root germs in young branches of *Salix*, *Populus* and *Ribes nigrum* closely associated with cambium and the end of medullary rays at the outer edge of xylem. Pre-existing "root germs" have also been noticed by Swingle (1925) in apple stems and by Sandison (1934) in *Lonicera japonica*. Though *Tagetes* is often propagated by cuttings in this country we have so far failed to discover the presence of preformed root germs in this plant or in other plants examined by us. Preformed root-primordia have not also been found in *Salix caprea*, *S. aurita*, *Populus alba* and *Vitis vinifera* (Carlson, 1938).

In all the cases studied by us the site of origin is shifted to the flanks of a vascular bundle close to *cambium*, *interfascicular cambium* or *ray cells* abutting these meristematic tissues. *Cambial origin* has been reported by Corbett (1895–96) in herbaceous stem cuttings of *Geranium*, *Coleus* and allied plants; by Smith (1925, 1928) in *Coleus* and *Clematis*, by Taylor (1926) in *Acanthus montanus*, by Sandison (1934) as a response to wounding in *Lonicera japonica*, and by Arlot Smith (1936) occasionally in *Begonia maculata* and *B. semperflorens*.

Origin in *interfascicular cambium* has previously been recorded by Regal (1876) in *Begonia maculata* (= *Begonia argyrostigma*); by Connard and Zimmerman (1931) in cuttings of *Portulaca oleracea* and by A. Smith in *Begonia maculata* and *B. semperflorens*.

Pericyclic origin has been observed in *Coleus Blumei* by Carlson (1929), in *Veronica Beccabunga* by Priestley and Swingle (1929); origin in the *secondary phloem* either within or between the bundles by Carlson (1933) in the cuttings of Dorothy Perkins rose; and in the *ray parenchyma* between a poorly defined pericyclic region and the interfascicular cambium by A. Smith (1942) in the cuttings of *Tropaeolum majus*. The case reported by Crooks (1934) is very interesting in so far that in cuttings of the upper part of the hypocotyl of Flax seedlings he noticed initiation of a root by the activity of the ray cells in the regions respectively of the pericycle, phloem and pith.

According to Priestley (1931) and Priestley and Swingle (1929) three conditions must be satisfied for the initiation of a root in an organ, namely, (1) presence of a meristem or potentially meristematic cells, (2) the mother tissue must be free from air spaces and (3) placed very near the xylem and phloem. The first is necessary because by the division of its elements new growing centres of roots are organised; close proximity to vascular supply ensures adequate supply of nutritive materials and absence of air space will permit a steady diffusion of solutes (pp. 65, 66). The presence of a meristematic tissue, *i.e.*, cambium, renders grafting a practical process.

In roots the pericyclic origin of lateral roots is obviously an advantage, but in adult hypocotyl and internodes the bundles are collateral with phloem and in many cases with additional sclerenchymatous bundle-caps on the outside. Therefore to effect direct xylem connection the origin must be close to xylem in the flanks of a fascicular cambium. Intercellular spaces are already formed throughout the radial course of ray cells, the only region free from such intercellular spaces being the cambium, interfascicular cambium and the ray cells recently formed in the neighbourhood of cambial ends. The ray cells are relatively smaller and are filled with active protoplasm. When all these facts are taken into consideration the ends of the cambium, the outer region of the interfascicular cambium and the living cells just cut off from the meristematic tissue and the proximity of the newly differentiated xylem and phloem are the ideal places for the origin of a lateral root upon a radially expanding nodeless axis.

The radial growth of the hypocotyl of *Cucurbita maxima* is very interesting, but this kind of growth in thickness has already been reported in the stems of *Coccinia* (= Cephalandra), *Trichosanthes*, *Wilbrandia*, *Anisosperma* and *Alsomitra* (Potter, Schenk, Herail, in Solerder I, p. 395). In all these cases, as in the hypocotyl of *C. maxima*, the interfascicular cambia help primary rays in keeping pace with the radial expansion of the bundles.

Summary

Six additional cases of naturally occurring shoot-borne adventive roots on adult axes of herbaceous dicotyledons have been described in this paper.

These support the view of Priestley and Swingle that the site of initiation of a lateral root in adult hypocotyls and internodes moves inwards from the pericycle to the flanks of the vascular cambium close to the newly differentiated xylem and phloem.

The radial growth of the hypocotyl of *Cucurbita maxima* is maintained by the independent growth of the vascular bundles, the interfascicular

cambium helping the primary rays in keeping pace with the growth in thickness of the former.

LITERATURE

1. Bhaduri, P. N. .. "Chromose numbers of some Solanaceous plants of Bengal," *Jour. Ind. Bot. Soc.*, 1933, **12**, 56–64.
2. Borthwick, A. W. .. "The production of adventitious roots and their relation to Birds-eye formation in the wood of various trees," *Notes Roy. Bot. Gard.*, 1905, Edin. **4**, 15–36.
3. Carlson, M. C. .. "Origin of adventitious roots in *Coleus* cuttings," *Bot. Gaz.*, 1929, **87**, 119–27.
4. ———— .. "Origin and development of roots in cuttings of Rose and Willow," *Contrib. Boyce Thompson Inst.*, 1933, **5**, 313–30.
5. ———— .. "The formation of nodal adventitious roots in *Salix cordata*," *Amer. Jour. Bot.*, 1938, **25**, 721–25.
6. Clark, W. A. .. "Vegetative propagation in Cotoneaster," *Trans. & Proc. Bot. Soc.*, Edin. 1932, **31** (2), 256–61.
7. Connard, Mary H., and P. W. Zimmerman "The origin of adventitious roots in cuttings of *Portuloca oleracea* L.," *Contrib., Boyce Thompson Inst.* 1931, **3**, 337–46.
8. Corbett, L. C. .. "The development of roots from cuttings," *W. Va. Agric. Expt. Stn., Ann. Rep.*, 1895–96, **9**, 196–99 (A. I. Smith, 1936).
9. Crooks, D. M. .. "Histological and Regeneration studies on the Flax seedlings," *Bot. Gaz.*, 1934, **95**, 209–39.
10. Eames and MacDaniels .. *Introduction to Plant Anatomy*, 1925.
11. Goebel, K. .. *Organography of Plants*, Part II, 1905, p. 274, Eng. Ed. (quotes Van Tieghem and Douliot).
12. Jimenez, P. G. .. "Callus and root-formation in stem cuttings of Kapok, Achuete and Santol," *Philippine Agr.*, 1937, **26**, 585–635.
13. Van der Lek, H. A. A. .. *Over die wortelvorming van houtige stekken*, H. Veeman and Zonen, Wageningen, 1924–1925 (Carlson, 1929).
14. Lemaire, A. .. "Recherches sur l'origine et le development des racines laterales Chez les Dicotyledones," *Ann. Sci. Nat. Bot.*, Ser. VII, 1886, **3**, 163–274 (Carlson, 1929).
15. Prain, D. .. *Bengal Plants*, 2 vols., 1903.
16. Priestley, J. H. .. "Vegetative propagation from the standpoint of Plant Anatomy," Reprint, *Proc. 9th Int. Hort. Congress*, 1931.
17. ———— .. "Problems of Vegetative Propagation," *Jour. Roy. Hort. Soc.*, 1926, **51**, 1–16.
18. ——— and C. F. Swingle "Vegetative propagation from the standpoint of Plant Anatomy," *Technical Bulletin U.S. Dept. of Agriculture*, 1929, No. 151.
19. Regel, F. .. "Die vermehrung der Bigoniaceen aus ihren Blättern," *Jena, Zeitschr. Med. Naturwiss* (N.F. 3), 1876, **10**, 447–92 (A. I. Smith, 1936).
20. Sandison, S. .. "Rooting of cuttings of *Lonicera japonica*, a preliminary account," *New Phyt.*, 1934, **33**, 211–17.

21. Smith, A. I. .. "Adventitious roots in stem cuttings of *Begonia maculata* and *B. semperflorens*," *Amer. Jour. Bot.*, 1936, **23**, 511–15.

22. ———— .. "Adventitious roots in stem cuttings of *Tropæolum majus* L.," *ibid.*, 1942, **29**, 193–94.

23. Smith, E. P. .. "A comparative study of the stem structure of the genus *Clematis* with special reference to anatomical changes induced by vegetative propagation," *Trans. Roy. Soc.*, Edin., 1928, **55**, 643–44.

24. ———— .. "The origin of adventitious growth in *Cotcus*," *Trans. & Proc. Bot. Soc.*, Edin., 1925 (1936), **29**, 145–50.

25. Swingle, C. F. .. "Regeneration and Vegetative Propagation," *Bot. Rev.*, 1940, **6**, 301–55.

26. ———— .. "Burrknot formations in relation to the vascular system of the apple stem," *Jour. Agri. Res.*, 1925, **34**, 533–44.

27. Solereder .. *Systematic Anatomy of the Dicotyledons*, Eng. Ed., 2 vols., 1908.

28. Strasburger, E. .. *Text-Book of Botany*, Eng. Ed., 1930.

29. Taylor, G. .. "The origin of adventitious growth in *Acanthus montanus*," *Trans. Proc. Bot. Soc.* Edn., 1926, **29**, 291–96.

30. Van Tieghem, P., and H. Douliot "Recherches comparatives sur l'origine des membres endogenes dans les plantes vasculaires," *Ann. Sci. Nat. Bot.*, 1888, Sér. VII, **8**, 1–660 (Carlson, 1929).

31. Trécul, A. .. "Recherches sur l'origine des racenes," *ibid.*, 1846, Sér. III, **5**, 340–44 (Carlson, 1938).

32. Vöchting, H. .. *Über Organbildung im Pflanzenreich*, Theil 1, Bonn. (Carlson, 1938).

33. Wilson, C. L. .. "Adventitious roots and shoots in an introduced weed," *Bull. Torr. Bot. Club*, 1927, **54**, 35–38.

34. Wolfe, F. H. .. "Origin of adventitious roots in *Cotoneaster Dammeri*," *Bot. Gaz.*, 1934, **95**, 686–94.

ON TWO TREMATODES FROM FISHES IN INDIA

By G. D. Bhalerao, D.Sc., Ph.D., F.A.Sc.
(Helminthologist, Imperial Veterinary Research Institute, Izatnagar, U.P., India)

Received October 5, 1943

For the material dealt with in this paper, the writer is indebted to Professor B. K. Das of the Osmania University, Hyderabad (Deccan), and to Professor J. N. Karve, of Poona.

Stomachicola muræsocis Yamaguti, 1934

Twelve specimens collected by Mr. M. Rahimullah at Ennur from the stomach of the marine eel, *Muræsox cinereus* were forwarded to the writer by Professor B. K. Das. On examination, these proved to be *Stomachicola muræsocis* Yamaguti, 1934. The specimens, in most respects, conform to the description of this species given by Yamaguti (1934), but differ from it in some minor anatomical features.

The worms measure 14·3–56·5 mm. in length and their maximum breadth which is attained in the region of the receptaculum seminis or that of the ventral sucker, is 1·3–3·3 mm. Even the smallest specimen was sexually mature. The following measurements refer to a specimen measuring 49 mm. in length and 2·7 mm. in maximum breadth. The oral sucker measures 0·47 × 0·47 mm. The pharynx measures 0·36 × 0·3 mm., and the œsophagus is 0·35 mm. long. The sinuous intestinal cæca, filled with dark amorphous contents, terminate normally at the same level, at the posterior end of the body, but in some cases the two cæca terminate at slightly varying levels. The ventral sucker is large and measures 1·8 mm. in diameter. The testes measure 0·53–0·8 × 0·33–0·42 mm. and are normally symmetrical, but in some cases the right testis may be slightly anterior to the left. The oval vesicula seminalis lies immediately posterior to the ventral sucker and measures 0·72 × 0·54 mm. The pars prostatica is long and sinuous. It is surrounded by unicellular prostatic cells and unites with the terminal portion of the uterus to form the ductus hermaphroditicus. This structure is enclosed in a small hermaphroditic pouch, which measures 0·4 × 0·23 mm. The genital pore is situated slightly behind the oral sucker.

The ovary, as described by Yamaguti (1934), is approximately kidney-shaped with its concavity directed posteriorly, and lies medially behind

the level of the testes, measuring 1·22×0·4 mm. The receptaculum seminis is very large, measures 0·5×0·4 mm., and lies behind the ovary. The vitellaria are tubular, not follicular. As described by Yamaguti (1934) the right vitelline gland normally consists of three tubes, of which the hindermost is subdivided into two similar tubes. The left vitelline gland, however, consists of two tubes of which the posterior bifurcates into two similar tubes. It may thus be seen that the vitellaria consists of seven tubes in all. Certain deviations from the normal were, however, noticed. Thus in one specimen there were five tubes on the right side, and only two on the left. In another specimen there were, on the right side, five tubes passing posteriorly and two very short but stout tubes passing anteriorly and the normal number of three tubes on the left side, thus making a total of ten tubes. Yamaguti states that the uterine coils extend into the tail region for about one-fifth of its length, but this statement could not be confirmed. In the specimens under discussion, the uterine coils were found extending into the tail region from about two-ninths to one-third of its length. The eggs are numerous and small, and measure 0·0115–0·0165 × 0·008–0·009 mm. They are much smaller in size than those in the specimens described by Yamaguti.

It will be seen, from the foregoing account, that the material at the writer's disposal differs from that described by Yamaguti in respect to the œsophagus, the vitelline tubes, the position of the genital pore, the extension of the uterus into the tail region and the size of the eggs; but the degree of differentiation is not sufficiently marked to justify the creation of a new species. It may be mentioned that Yamaguti also pointed out a few variations occurring in this species.

Clinostomum indicum Bhalerao, 1940

Through the courtesy of Professor J. N. Karve an opportunity was obtained to examine a small collection of flukes obtained from the subcutaneous tissue of the fish *Notopterus notopterus*, from Poona. All the flukes were immature, but on closer examination and comparison with the allied forms, they proved to be a new species. The short description which follows is based on three entire mounts and three sectionized series.

The worms are thick, fleshy, cream coloured, and elliptical in shape, with both the extremities rounded. The anterior border and the sides are inverted, so that the worm is canoe-shaped. In preserved specimens, the cuticle is transversely striated, and beset with spines which are noticeable only in sectionized specimens. Beneath the cuticle, there is a well-developed layer of longitudinal muscle and below this a layer of circular muscle.

There are numerous dorso-ventral muscles and many large sub-cutaneous glands. The worms measure 9·5–11 mm. in length and their maximum breadth, which is attained in the region of the genital glands, is 3·5–4·7 mm. The mouth is situated subterminally at the anterior end and is surrounded by an

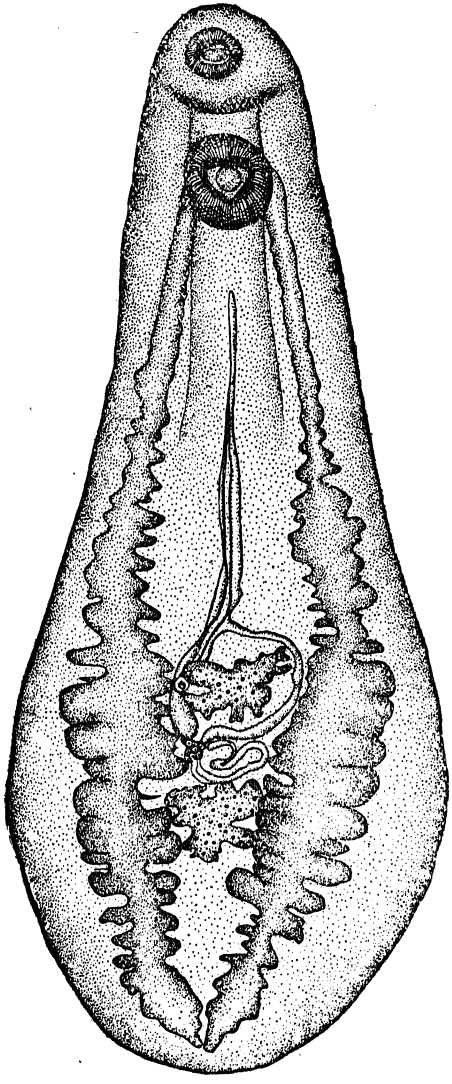


Fig. 1. Ventral view of *Clinostomum indicum* n.sp.

oral sucker measuring 0·475–0·67 × 0·375–0·57 mm. There is no pharynx. The œsophagus was observed to be short, though its actual length could not be determined on account of the peculiar bend at the anterior end of the worm. The intestinal cæca, filled with the blood of the host, pass along

the sides of the body, and terminate slightly in front of the posterior end. They are deeply diverticulated both internally and externally and some of the diverticula are subdivided into two or four digitations. The ventral sucker is very large, measures 0·86–1·18 × 0·82–0·96 mm., and is situated close to the anterior end of the body.

The excretory pore is situated subterminally at the posterior end in a small depression on the dorsal side, and leads into a V-shaped bladder with short arms. The terminations of the intestinal cæca come into close contact with the cornua of the bladder and in some cases cause an actual depression on its proximal face. In some species of this genus, it has been claimed that the intestinal cæca open posteriorly into the excretory bladder. Price (1938) while redescribing the species *Clinostomum intermedialis* Lamont (1920), remarks "intestinal cæca sinuous, apparently opening into excretory vesicle", showing that he is sceptical about the relation existing between the intestinal cæca and the excretory bladder. Special attempts were therefore made to obtain precise information concerning this relationship in this species, these two structures being studied in two series of horizontal and in another series of dorso-ventral sections. As a result, it was ascertained that the intestinal cæca do not actually open into the excretory bladder. The two structures are intimately juxtaposed, thus giving the false impression, in entire mounts, that they directly communicate with one another. From the side of the bladder emerges a fairly prominent branch on either side, which passes anteriorly almost as far as the oral sucker.

The genital pore is situated submedianally on the right side of the anterior testis. The testes are branched, and lie centrally, one behind the other, at the middle of the posterior half of the body. The anterior testis is slightly larger than the posterior, the measurements being 1·23–1·32 × 0·67–0·8 mm. and 1·1–1·14 × 0·062–0·925 mm. The vas efferens of the anterior testis emerges from its centre and passes posterolaterally on the right side. The vas efferens of the posterior testis emerges from its centre and goes first to the right side and then turns towards the left. The two vasa efferentia unite with each other slightly behind the cirrus sac and form a very small vas deferens. Special efforts were made to determine whether there is, in this species, a vesicula seminalis externa as is described in some species, *e.g.*, in *C. piscidium* Southwell and Prashad (1918). For this purpose and for obtaining the details of the contents of the serous sac, presently to be described, a detailed study of the serial sections was made. It was ascertained that the vesicula seminalis externa is absent in this species. The cirrus sac is more or less pear-shaped, having a slight bend near its proximal end. It lies on the right side, between the anterior testis and the right

intestinal cæcum. It measures 0·8–0·84 × 0·28–0·35 mm. and extends from the genital pore to the anterior border of the ovary. The cirrus sac contains the vesciula seminalis, which is very coiled and occupies slightly more than half the space inside the cirrus sac. The vesicula seminalis is followed by the ductus ejaculatorius, which is 0·24 mm. long and 0·08 mm. thick. The terminal portion of the male genital duct is the cirrus which is about 0·17 mm. long and is slightly muscular. In entire mounts it appears almost globular on account of its thick musculature. Baer (1933) remarks that Osborne (1912), in his description of the species *C. marginatum*, claims that the cirrus is the pars prostatica, since it is surrounded by numerous cells. The writer agrees with Baer (1933) in that the cells surrounding the cirrus are simply myoblasts and that a real pars prostatica is lacking in Clinostomatidæ.

The ovary is somewhat irregular body, measuring 0·185–0·28 × 0·23–0·25 mm., and lies on the right side, internal to the right intestinal cæcum, almost midway between the two testes. The oviduct is a long thin duct which winds about considerably in the inter-testicular area. At a short distance from its origin, it gives out a fairly long Laurer's canal. The shell gland is small and situated immediately behind the ovary. The vitellaria consist of very small follicles extending in the inter-cæcal area behind the ventral sucker. A thick utero-duct is seen in the inter-testicular area and curves round the anterior testis on its right side. The utero-duct meets the uterus centrally, slightly in front of the anterior testis. The uterus is 2·88–3·3 mm. long and passes anteriorly in the middle line to a distance of 0·61–0·65 mm. behind the ventral sucker. The terminal portion of the uterus, the metraterm, is a stout duct, lined internally with a thick cuticle, and opens into the genital atrium situated on the right side of the anterior testis.

It is apparent from the foregoing description that the form in question is the metacercarial stage of *Clinostomum* sp. According to Price (1938) there are only five recognized species of the metacercaria of *Clinostomum*, *viz.*, *C. pseudoheterostomum*, *C. dictyotum*, *C. chrysichthys*, *C. delagi* and *C. piscidium*. Referring to the key, it is seen that the form from *Notopterus notopterus* approximates to *C. delagi* Tubangui (1933). It can, however, be distinguished from it by the larger size and shape of the body, the relative size of the two suckers, the deeper indentations of the intestinal cæca, the branched nature of the testes, the position of the genital glands, the size and position of the cirrus sac, the situation of the shell gland and the length of the uterus. These points of difference being sufficient, it is proposed to regard the species described here as a new one, for which the name *Clinostomum indicum* is suggested.

Specific diagnosis of *Clinostomum indicum* Bhalerao, 1940

Metacercarial form. Length 9·5–11 mm. Maximum breadth 3·5–4·7 mm. Cuticle—spiny. Oral sucker 0·475–0·67 × 0·375–0·57 mm. Pharynx absent. Intestine with well-developed diverticula. Ventral sucker 0·86–0·118 × 0·82–0·96 mm. Genital pore on the right side of the middle of anterior testis. Genital glands in the middle of posterior half of body. Testes large and branched. Cirrus sac 0·8–0·84 × 0·28–0·35 mm. Ovary 0·185–0·28 × 0·23–0·25 mm. Vitellaria extending behind ventral sucker. Uterus 2·88–3·3 mm. long.

Host.—Notopterus notopterus.

Location.—Subcutaneous tissue.

Locality.—Poona.

REFERENCES

Baer, J. G. .. "Note sur un nouveau Trematode, *Clinostomum lophophallum* sp. nov. avec quelques considerations generales sur la famille des Clinostomidæ," *Rev. suisse Zool.*, 1933, **40**, 317–42.

Osborne, H. L. .. "On the structure of *Clinostomum marginatum*, a Trematode parasite of the frog, Bass and Heron," *J. Morph.*, 1912, **23**, 189–229.

Price, E. W. .. "A redescription of *Clinostomum intermedialis* Lamont (Trematoda: Clinostomidæ), with a key to the species of the genus," *Proc. Helminth. Soc. Wash.*, 1938, **5**, 11–13.

Yamaguti, S. .. "Studies on the Helminth Fauna of Japan, Part 2. Trematodes of Fishes I," *Jap. J. Zool.*, 1934, **5**, 249–541.

THE PANJAL TRAPS: ACID AND BASIC VOLCANIC ROCKS

By P. N. Ganju, M.Sc.
(*Lecturer in Geology, University of Lucknow*)

Received September 17, 1943
(Communicated by Prof. L. Rama Rao, F.A.Sc.)

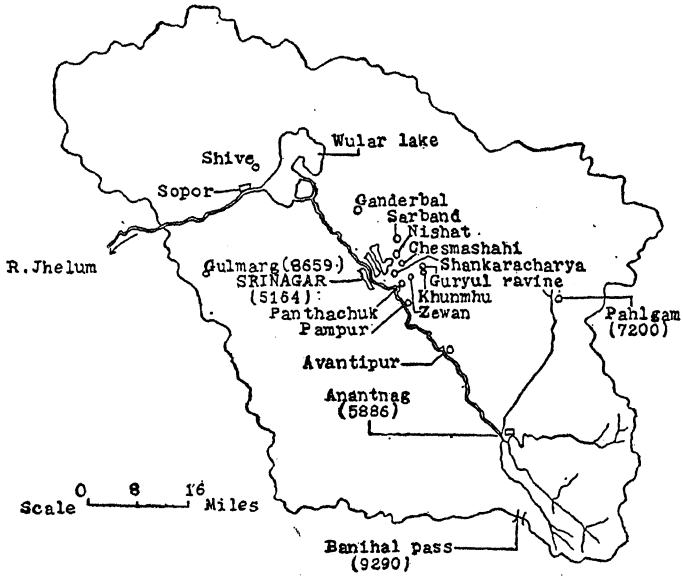
The material on which this paper is based was collected by the author, mainly during the field work done in the summer of 1937 under the very valuable guidance of Prof. Raj Nath and partly alone, again in the summer of 1941. The material was collected from various localities in the vicinity of Srinagar and at such far off places as Gulmarg, Banihal pass, Pahlgam and Shive (near Sopore). The work was carried out in the Geology Department of the Benares Hindu University where the material is preserved.

Towards the close of the last century Lydekker and McMahon (1883, p. 218) established the volcanic origin of the Panjal traps. Middlemiss (1910, p. 235) described these rocks as "Genuine old basic lava flows". The late Professor K. K. Mathur (1933, p. 126) in a letter to *Current Science* described a specimen of rhyolite occurring in the Panjal traps at a locality named Panthachuk near Srinagar. About this discovery West (1935, p. 492) remarked "Although these rhyolites may be quantitatively unimportant, neverth.less their discovery is of much interest." Mr. D. N. Wadia (1939, pp. 412–13) describes these traps as "A basic variety of augite andesite or basalt of acidity varying 49% to 60% of a prevailing dark or greenish colour.....Acid and intermediate differentiation products also occur locally and in small masses, *e.g.*, trachyte, ceratophyre, rhyolite, acid tuffs, etc."

The Panjal traps generally overlie the Agglomeratic slate series of Upper Carboniferous age but at most places these lie unconformably over much older rocks. The upper limit is also different at different places. Sometimes these are found overlain by Lower Permian beds, as in the Vihi district where the traps are overlain by Gondwana plant bearing beds, while at other places the volcanic activity continued till Upper Triassic.

The presence of the acid rocks in the Panjal traps made their study very interesting. The author, therefore, studied this problem in greater detail. The results obtained clearly prove the true nature of acid volcanic rocks and their occurrence on a much larger scale than that previously thought of.

The trap rocks were studied at Cheshmashahi, Sarband to Nishat along the canal road behind the Moghul Gardens, Panthachuk, Khunmhu, Zewan, Avantipur, Pahlgam, Banihal pass, Gulmarg, Ganderbal and Shive. The relative position of these places is shown in the outline map of Kashmir (Text-Fig. 1).



TEXT-FIG. 1. Outline sketch map of Kashmir showing the localities where the Panjal trap rocks were studied

The acid volcanic rocks have been found occurring in great amount at the localities of Panthachuk, Avantipur and Cheshmashahi. At other places the basic volcanic rocks predominate.

At Panthachuk, the Panjal trap formation appears to show a sort of bedded structure and each bed may have been an individual outflow of lava. These beds are of different thickness and the bedding planes merge one into another after a short distance which makes their individual study rather difficult. With the idea of finding variation in constituents, specimens were collected at intervals of different heights from the base of the formation to its top and from what appeared to be different beds of lava flows. Near the top there is a vein formed of calcite and quartz and about 20 ft. below this there is an another vein having similar constituents. The specimen K/P6 lies immediately below the lower vein. The specimens K/P3–K/P6 were taken at intervals of different heights (Text-Fig. 2) one over the other.

The Acid Volcanic Rocks

In hand specimen the acid volcanic rocks are compact and greyish in colour. Quartz can be easily seen with the help of a lens. The specific gravity varies from 2·69 to 2·79 at the two extremes.

Under the microscope these rocks (Figs. 1, 2 & 3) are porphyritic. Among the phenocrysts euhedral to subhedral crystals of quartz are quite abundant. Crystals of alkali felspars are present in fairly large amount. Some of these are turbid. A few crystals of plagioclase felspars showing polysynthetic twinning are also present in some specimens (P/4 and K/C1 from Panthachuk and Cheshmashahi respectively). A general characterstic, however, is the presence of green chloritic matter produced as a result of alteration. The chlorite usually occurs in association with felspars. The ground mass is usually turbid and is crypto- to micro-crystalline and shows crystals of quartz and felspar at places (Fig. 3). In a specimen from Cheshmashahi (K/C1) the ground mass is glassy and shows a sort of flow structure. Magnetite and ilmenite are present in fairly large amount. In a specimen from Panthachuk (K/P5) veins filled with secondary quartz are present.

Seven rocks were analysed, five from Panthachuk and one each from Cheshmashahi and Avantipur. The values of analyses and norm are given in Tables I and II.

The Basic Volcanic Rocks

These rocks are compact and dark green in appearance. Small pieces of ferromagnesian minerals are present in sufficient quantity and can be seen in hand specimen. The specific gravity of these varies from 2·8 to 3·0 at the two extremes.

Under the microscope the basic volcanic rocks (Fig. 4) are porphyritic. Phenocrysts of plagioclase felspars are present in fairly large amount. As a rule these rocks are very much altered and have undergone secondary silicification. Epidotisation is also a common phenomenon in these. Green chloritic product produced as a result of altertion is present in sufficient quantity. Big cavities filled with secondary quartz, secondary mica and epidote are fairly common. The ground mass is usually crypto-crystalline and at places consists of minute needle-shaped prisms of felspar most of which show polysynthetic twinning (*e.g.*, K/G4 from Guryul ravine). Magnetite and ilmenite is present in fairly large amount. Specimens from near Ganderbal are very much metamorphosed and show schistose structure.

TABLE I

Analyses of Acid and Basic Volcanic Rocks

	Acid							Basic
	Panthachuk					Cheshmashahi	Avantipur	Sarband-Nishat Canal Road
	K/P3	K/P4	K/P5	K/P6	P/4	K/C1	K/A3	K/SN2
SiO_2 ..	66·87	64·96	66·22	64·89	68·43	63·59	64·99	57·37
Al_2O_3 ..	15·04	16·61	15·57	16·78	15·39	16·36	17·06	18·70
TiO_2 ..	0·90	0·94	1·00	1·08	0·93	1·09	0·93	1·16
MnO ..	0·04	0·09	0·05	0·06	0·03	0·02	0·06	0·10
Fe_2O_3 ..	1·47	1·97	2·77	1·54	1·41	1·58	1·94	3·24
FeO ..	2·46	2·99	2·61	2·62	2·84	2·86	1·76	3·88
CaO ..	3·36	2·87	2·72	3·28	1·99	4·45	3·44	5·86
MgO ..	1·03	1·10	1·21	0·98	0·66	1·44	1·32	2·64
Na_2O ..	4·22	3·85	2·36	4·68	3·68	5·32	3·52	3·75
K_2O ..	3·87	4·71	5·62	3·28	4·82	2·52	4·36	2·27
H_2O (-) ..	0·28	0·32	0·19	0·21	0·43	0·22	0·23	0·28
Total ..	99·54	100·41	100·32	99·40	100·61	99·45	99·61	99·25
Sp. gr. ..	2·77	2·74	2·70	2·73	2·69	2·76	2·75	2·77
Microscopic characters	Porphyritic. Fairly large phenocrysts of quartz. Ground mass glassy.	Porphyritic. Phenocrysts of quartz and alkali felspar. Ground mass more or less glassy.	Porphyritic. Phenocrysts of quartz and orthoclase felspar. Ground mass contains crystals of quartz and felspar. Secondary quartz present in cavities.	Porphyritic. Phenocrysts of quartz and orthoclase felspar. Ground mass more or less glassy.	Porphyritic. Phenocrysts of quartz, alkali and a few plagioclase felspar. Ground mass mostly glassy with a few crystals of quartz and felspar.	Porphyritic. Much decomposed. Phenocrysts of alkali and a few plagioclase felspar. Ground mass glassy showing flow structure. Cavities filled with secondary minerals are common.	Porphyritic. Phenocrysts of quartz only. Ground mass mostly glassy with a few quartz and felspar crystals.	Porphyritic. Phenocrysts of plagioclase felspar only. Ground mass contains needle-shaped crystals of felspar. Cavities filled with secondary minerals very common.
Analyst	P. N. Ganju							

TABLE II

Norm of Acid and Basic Volcanic Rocks

	Acid					Basic		
	Panthachuk					Cheshma-shahi	Avantipur	Sarband-Nishat Canal Road
	K/P3	K/P4	K/P5	K/P6	P/4	K/C1	K/A3	K/SN2
Quartz ..	19·32	15·84	22·92	16·20	22·32	12·24	17·46	9·66
Orthoclase ..	22·79	27·80	33·36	19·46	28·36	15·01	27·24	13·34
Albite ..	35·63	32·48	19·91	39·30	31·44	45·06	29·86	31·44
Anorthite ..	10·56	14·17	13·34	15·00	10·01	13·06	16·95	27·52
Corundum ..	..	..	0·70	..	0·41	..	..	..
Diopside ..	5·00	..	..	1·14	..	7·23	..	1·35
Hypersthene ..	1·99	5·07	4·05	3·65	3·61	2·19	3·43	8·70
Magnetite ..	2·08	2·78	3·94	2·32	2·09	2·32	2·78	4·64
Ilmenite ..	1·67	1·82	1·82	2·12	1·67	2·12	1·82	2·12
Water ..	0·28	0·32	0·19	0·21	0·43	0·22	0·23	0·28
Total ..	99·32	100·28	100·23	99·41	100·34	99·45	99·77	99·05

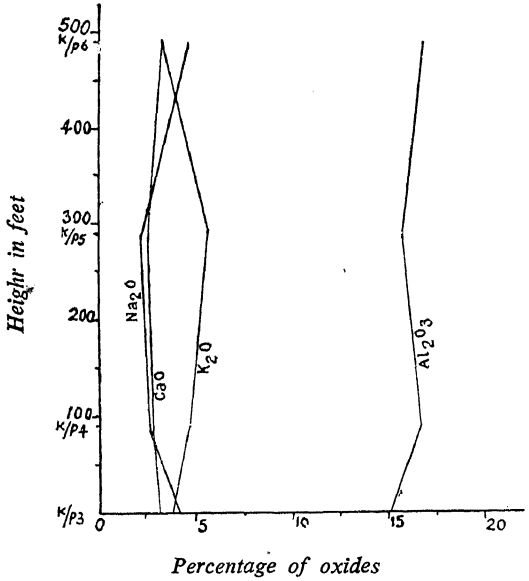
Only one rock sample K/SN2 from Sarband-Nishat Canal road was analysed. The values of analyses and norm are given side by side with the acid volcanic rocks in Tables I and II, for comparison.

Discussion

From the microscopic description, chemical analysis and other characters of Panjal traps occurring at Panthachuk, Avantipur and Cheshma-shahi, it is quite clear that acid volcanic rocks of the nature of rhyolites, trachytes and dacites do occur in the Panjal traps. That these acid rocks have not been formed by secondary silicification of andesites is also quite clear from the photomicrographs given (Figs. 1–3) where crystals of original quartz as well as those of the alkali felspars can be very clearly seen. A comparative glance at the analysis values of acid and basic rocks also proves beyond doubt the true nature of these acid rocks. It is true that most of the basic rocks have undergone secondary silicification, the secondary quartz being present in veins and amygdales but it is clearly different from the primary quartz seen in true acid volcanic rocks.

At Panthachuk, as already stated, specimens were collected at different heights from the base of the formation. Some of these were analysed chemically (see Table I) and the variation of various oxides with height has

been plotted (Text-Fig. 2). As is clear no definite change can be observed and the variations seem to be more or less irregular. The calcite and quartz veins at Panthachuk may have been formed by the steam and carbon dioxide evolved during the eruptions. These together may have decomposed the felspars producing calcium carbonate and silica deposited in the form of veins.



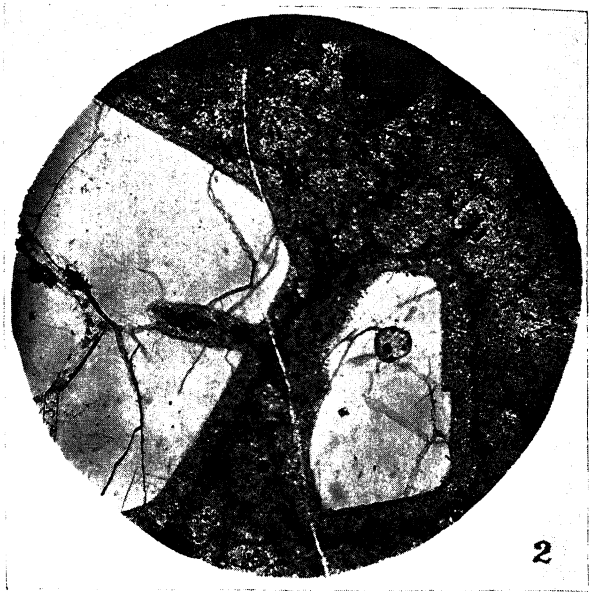
TEXT-FIG. 2. Graph showing the variation of different oxides with 'height' at Panthachuk

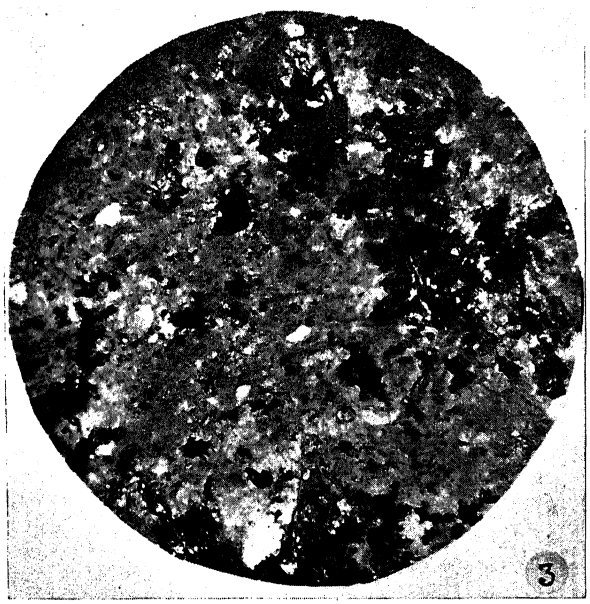
The acid rocks, however, show higher specific gravity than is usual. This may be explained as due to the high quantity of magnetite and ilmenite that these have been observed to contain.

As regards the quantitative importance of these, the author has traced the outcrop of acid rocks at three places within the distance of about 24 miles from Cheshmashahi to Avantipur. This shows that quantitatively these occur on a fairly large scale and it is likely that these occur on a much greater scale in Pir Panjal and other hills. It will be very interesting to get an idea of the total amount of the acid rocks as compared to basic rocks, their age, the time interval between the two outflows and their origin. The author hopes to continue the study of these points in detail.

Conclusion

The presence of rhyolites in the Panjal traps at Panthachuk was first brought to notice by late Prof. K. K. Mathur. The author studied the





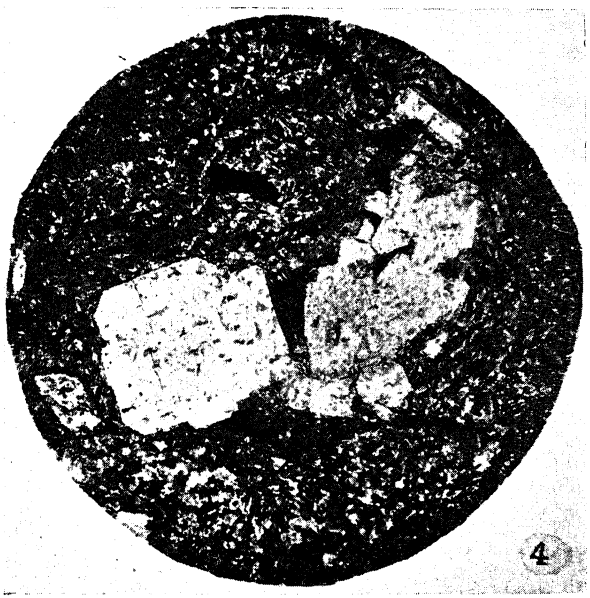


Fig. 1. Acid volcanic rock from the base of the quarry at Panthachuk, Srinagar, Kashmir. No. K/P 3.

Fig. 2. Acid volcanic rock from a height of about 300 feet at Panthachuk quarry. No. K/P 5.

Fig. 3. Acid volcanic rock from the Panjal trap formation at Avantipur, Kashmir. No. K/A 16.

Fig. 4. Basic volcanic rock from the Panjal trap formation along the Sarband-Nishat Canal road, Srinagar, Kashmir, No. K/SN 2.

Panjal traps in some detail and as a result the acid rocks have been found to occur at two other places also, *viz.*, at Cheshmashahi and Avantipur. These acid volcanic rocks are of the nature of rhyolites, trachytes and dacites and are very clearly different from basic volcanic rocks like andesites and basalts. These rocks are fairly abundant and must have played an important part in the Panjal volcanic activity.

Acknowledgment

I wish to express my great indebtedness to Prof. Raj Nath, M.Sc., Ph.D., D.I.C., for kindly suggesting this problem and the very valuable guidance he gave me throughout the work.

REFERENCES

Lydekker, R. .. "The geology of the Kashmir and Chamba territories and the districts of Khagan," *Mem. Geol. Sur. Ind.*, 1883, **22**, 211 24.

Mathur, K. K. .. "The Panjal Traps," *Curr. Sci.*, 1933, **2**, No. 4, 126.

.. "Panthachuk (Srinagar, Kashmir) Rhyolite," *ibid.*, 1935, **3**, No. 10, 492.

Middlemiss, C. S. .. "A revision of the Silurian-Trias Sequence in Kashmir," *Rec. Geol. Sur. Ind.*, 1910, **40**, 232–36.

Wadia, D. N. .. *Geology of India*, 1939, 410–14.

West, W. D. .. "Panthachuk (Srinagar Kashmir), Rhyolite," *Curr. Sci.*, 1935, **3**, No. 10, 492.

1083-43 Printed at The Bangalore Press, Mysore Road, Bangalore City, by G. Srinivasa Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

STUDIES ON THE CORPUS LUTEUM IN *RHINOBATUS GRANULATUS* CUV.

By Miss Mary Samuel, M.Sc.
Research Assistant
(From the University Zoological Research Laboratory, Madras)

Received October 21, 1943
(Communicated by Prof. A. Subba Rau, F.A.SC.)

CONTENTS

Introduction

Although considerable work has been done on the histology and development of the corpus luteum, a great deal of controversy still exists regarding its formation, and the origin of the luteal cells. Hitherto, the subject has been mainly studied in relation to mammals and our knowledge of the organ in the lower vertebrates, especially in fishes is very poor. It was therefore felt that a study of the corpus luteum in the Elasmobranchs would be well worth an investigation.

Previous History

Various views have been held in regard to the mode of formation of the corpus luteum and the origin of the luteal cells in mammals. Von Baer (1827), the first author to discuss the formation of the corpus luteum, believed the organ to have an exclusive connective tissue origin, the follicular epithelium not having any share in its formation. The hypothesis of Paterson (1840), that the structure was formed from the blood coagulum left in the lumen

of the Graafian follicle, after escape of the egg, gained practically no support. Bischoff (1842) came to the conclusion that the luteal cells are entirely derived from the follicular epithelium, which undergoes remarkable hypertrophy and histological changes. He adduced further evidence in support of his theory by his subsequent study of the ovaries of the dog, guinea-pig and the roe. His work is of great importance for he is one of the few early workers who studied all the stages in the formation of the corpus luteum. The theories of Von Baer and Bischoff received considerable support from subsequent investigators.

Extensive work has been done on the corpus luteum of various mammals such as the mouse, rabbit, guinea-pig, *Tarsius*, *Tupaia*, *Sorex*, Sow, rat, marmot, sheep, ferret, bat, monkeys, marsupials and monotremes and certain reptiles by various authors. It is not proposed to review in detail the vast literature relating to the formatin of the corpus luteum in all cases, as complete references have been given by many authors like Van der Stricht (1912), and Marshall (1906 and 1922) although important papers must be referred to. Sobotta (1896) was the first author to make a systematic study of the formation of the corpus luteum by applying experimental methods which went far to confirm the view held by Bischoff. Among the supporters of Bischoff's theory we find Heape (1897), Stratz (1898), Kreis (1899), Belloy (1899), Honore (1900), Sandes (1903), Cohn (1903), Völker (1904), Marshall (1904), O'Donoghue (1911, 1914 and 1916), Robinson (1918), Hill and Gatenby (1926) and Deanesly (1930) all of whom agree that the follicular epithelial cells hypertrophy and give rise to luteal cells.

After the publication of Sobotta's important work, several investigators have strongly supported the theory originally put forward by Von Baer and as adherents to his theory may be mentioned Clark (1898), Doering (1899), His (1899), Paladino (1900), Nagel (1899), Jankowski (1904) and Williams (1904).

Apart from these three hypotheses, a fourth was put forward by Schrön (1863), and was supported by Rabl (1898), Leo-loeb (1906), Meyer (1911), Van der Stricht (1901 and 1912), and more recently by Corner (1915 and 1919). These were of opinion that the luteal cells of the mature corpus luteum are derived from both the follicular epithelium and the cells of the theca interna. In this connection mention may be made of some of the more important workers like Miller (1914), Novak (1921), Gatenby (1924) who have done work on human corpus luteum.

To Giacomini (1896) belongs the credit of being the first to study the gland in Elasmobranch fishes. He found a well-marked glandular organ

very similar to the corpus luteum of mammals in *Myliobatus bovina* which he described as a more or less solid body in which the enlarged epithelium is penetrated by an extensive ingrowth of connective tissue and blood vessels so that the corpus luteum appears as a " glandular organ full of epithelial tubes ". Buhler (1902) was unable to discover any great hypertrophy of the follicular epithelium in the spent follicles in cyclostomes and certain teleosteans. Cunningham's (1898) observation on the ruptured follicles of teleosteans led him to similar conclusions. Wallace (1904) drew our attention to the peculiar form assumed by this body in *Spinax niger*, which shows certain resemblances to the mammalian corpus luteum. Regarding the ruptured follicles of the ovary of *Zoarces*, Wallace states that the follicular epithelium undergoes a slight hypertrophy by simple enlargement of its cells but the follicle does not show any further advancement.

Material and Methods

Rhinobatus granulatus is a typical ovoviviparous Elasmobranch like most Batoids. The material for the study was taken from a female specimen collected in August 1940 from the Madras Coast. The ova were large and the two ovaries contained eggs in all stages of development. In each ovary, over twenty corpora lutea of varying stages from the freshly ruptured follicle to the fully developed solid, glandular corpus luteum were present. The ruptured openings of these follicles through which the egg had escaped to the exterior had closed up. The advanced corpora lutea were yellowish in colour while the freshly ruptured follicles were coloured pinkish white.

The corpora lutea were preserved by several methods. The fixatives successfully employed were Bouin, Carnoy, Regaud, Champy, Flemming, 5% formalin and corrosive sublimate. The material was dehydrated and cleared in cedar wood oil in the usual manner and paraffin method of embedding was employed in all cases. Sections were cut 4 to 7 microns thick. The stains employed were iron hæmatoxylin and Mallory's triple stain. Of these Heidenhain's iron hæmatoxylin was the stain most frequently used and was found to give the best results.

The Follicular Wall of the Ripe Ovum

The ripe ovum is enveloped by the egg membranes and a clearly delimited outer follicular wall. The former, as in all Elasmobranchs, consists of the inner zona radiata and the outer vitelline membrane which is closely attached to the zona radiata and lies immediately inside the follicular epithelium.

In the fairly ripe condition of the egg the follicular wall consists of three layers—the follicular epithelium, the membrana propria and the theca folliculi (Ph. M.1). The most remarkable feature in *Rhinobatus granulatus* is the presence of two kinds of cells in the follicular epithelium. This layer is quite distinct and delimited from the theca folliculi. It consists of a large number of small cylindrical cells intercalated among which are a small number of large vesicular cells. Of these two sets of cells, the smaller cells are long and columnar with oval nuclei densely filled with deeply staining fine chromatin granules and a well marked nucleolus. The bigger cells have clear cytoplasm and more or less central vesicular nuclei. These nuclei are lightly staining and possess a distinct nucleolus but a peripheral chromatin reticulum is indistinct. The resemblance of these large cells to small oocytes is striking. Semper (1875) went to the extent of suggesting that these peculiar large cells were primitive ova destined to become permanent ova. Giacomini (1896) in describing the follicle of *Chimæra* mentions that the epithelium consists of large cells scattered among which are small cylindrical cells. *Rhinobatus granulatus* resembles *Raia, Scyllium* (Balfour, 1878), *Torpedo* (Schultze, 1875, as stated by Wallace, 1904), *Myliobates, Trygon* (Giacomini, 1896) and *Chimæra* (Wallace, 1904) in this feature of possessing two kinds of cells in the follicular epithelium. But it differs from the condition in *Chimæra* in the larger cells being comparatively fewer. The membrana propria is a very thin layer of elongated cells closely investing the follicular epithelium.

Surrounding the follicular epithelium is a layer of connective tissue—the theca folliculi. This layer is readily distinguishable from the follicular epithelium and consists of a fairly compact internal portion formed of connective tissue cells and fibres and an external portion of more protoplasmic and elongated cells loosely scattered and almost parenchymatic in nature. These are the theca interna and theca externa. In the higher vertebrates the connective tissue theca also shows a differentiation into the theca interna and theca externa. This distinction becomes very much more marked after the rupture of the follicle. The theca interna is 5 to 10 cells in thickness and at its maximum is about twice as thick as the follicular epithelium. The cells are smaller than the small cells of the follicular epithelium and their cell boundaries generally indistinct. Their nuclei are oval or rarely spherical and possess a distinct reticulum and a small nucleolus. The cytoplasm is faintly alveolar in texture and stains lightly. Fine connective tissue fibres run amongst them. These layers of cells and fibres are disposed parallel to one another (Ph.M.1).

The theca externa is thinner and consists of loosely arranged elongated cells and fibres. Thin-walled blood vessels occur in this outer region. The endothelium, surrounding the theca is not well defined. Wedged in amongst the concentrically arranged elements of the theca externa are groups of small cells with deeply staining spherical nuclei and with indistinct cell boundaries. The cells have probably originated from the stroma cells and have remained unaltered.

The follicular wall of the fully mature egg shows that the columnar cells of the follicular epithelium have increased in number. The nucleus has assumed a more vesicular form and the cytoplasm has become more granular and deeply staining. There is a reduction in the number of the large cells. In the theca interna the connective tissue cells show a tendency to group themselves round small spaces giving the appearance of tubules in section (Ph. M. 2 and Text-Fig. 1). The cells constituting the tubules have slightly increased in size and the nucleoli have become more distinct. The connective tissue fibres run in between these tubules but they still retain their parallel arrangement.

The theca externa is a thin concentrically disposed layer surrounding the theca interna. The cells constituting this layer are highly protoplasmic. Cell boundaries cannot be made out. A slight increase in the number of blood vessels as well as in the free thecal cells in this layer has been observed.

Corpus Luteum

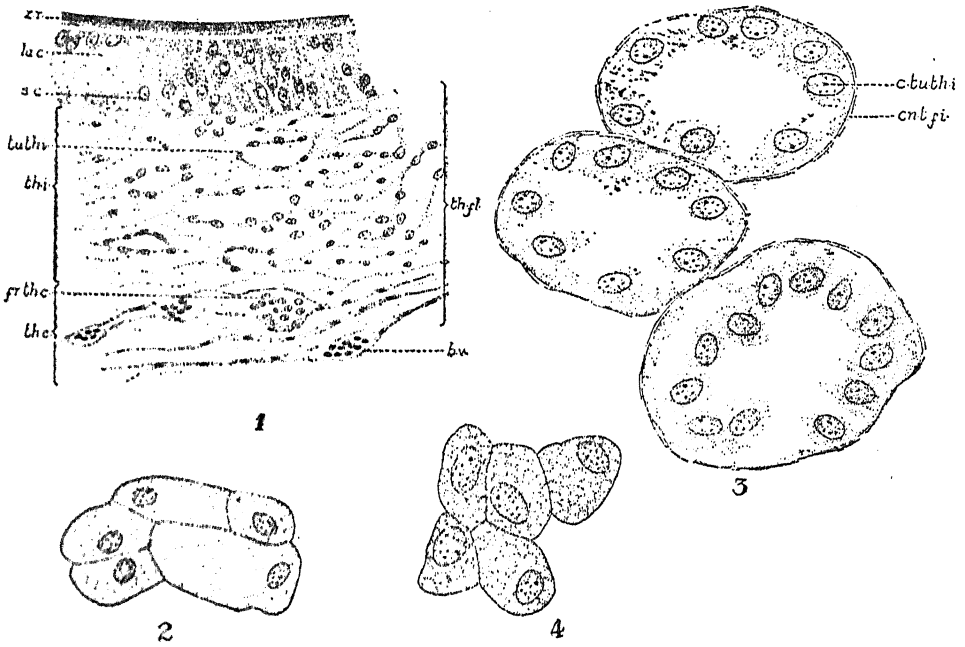
Stage I.—The corpora lutea are elongated flask-shaped bodies the mouth closed early in development with the ingrowth of the surrounding connective tissue. No stage showing the actual opening or rupture was available. The corpora lutea are very much smaller than the ripe unruptured follicle and it is evident that considerable contraction has taken place by the collapse of the wall as a result of the rupture and subsequent escape of the ova. The changes undergone by the follicular wall of the corpus luteum resulting in the final formation of the solid gland is described from within outwards in the following account.

Photomicrograph 3, shows a low power view of portion of a transverse section of the corpus luteum. There is a central lumen of considerable size containing a few globules of a darkly staining substance, probably yolk granules left behind after the escape of the egg. The detailed structure of the follicular wall is illustrated in Photomicrograph 4. The three layers of the follicular wall have undergone marked histological changes. The follicular epithelium is thrown into folds due to the escape of the ovum and the

consequent contraction of the follicular wall. It has also undergone considerable change and exhibits a multilayered appearance in contrast to its previous single-layered condition. Soon after the rupture, the follicular cells undergo enormous hypertrophy. Mitotic divisions have been described in the follicular epithelial cells in the early stages of the formation of the corpus luteum in the case of certain marsupials (O'Donoghue, 1914, 1916), in sheep (Marshall, 1904) and in certain lizards (Weekes, 1934). In the present form, however, such divisions have not been observed in the epithelial cells and the multilayered condition must have been brought about mainly by the hypertrophy of the follicle cells together with the contraction the follicle undergoes due to the escape of the egg. Instead of there being two sets of cells, small and large, the follicular epithelium is now composed of a more or less continuous layer formed by the hypertrophy of the small cells now arranged in varying thickness of 1–4 cells. The big cells have now completely disappeared. The disappearance of the large cells has been noticed in reptiles and is regarded by Hett (1924) as being absorbed by the egg, being of the nature of nutritive cells. Wallace (1904) is of opinion that the large specialised cells of the follicular epithelium of *Chimæra* are "nutritive" cells and they degenerate and disappear before the maturation of the egg.

The follicular epithelium is separated from the connective tissue theca probably due to the pressure caused by the contraction of the wall after ovulation. The cells forming it are deeply staining varying in shape from a polyhedral to a spherical condition with distinct cell-boundaries (Ph. M. 5 and Text-Fig. 2). They have rather large spherical or oval deeply staining nuclei which typically contain one or two nucleoli and a well marked reticulum of chromatin granules. The cytoplasm is moderately stained.

The connective tissue sheath is very different from that of the unruptured follicle and the distinction between the theca interna and theca externa is well marked (Ph.M. 4). The theca interna follows the wavy contour of the follicular epithelium and is seen to send long finger-shaped projections of connective tissue fibres and cells in between the folds of the epithelium. There is great increase in size and numbers of the tube like structures of the theca interna (Text-Fig. 3 and Ph.M. 6). The cells constituting the tubules have greatly increased in size but at this stage they are smaller than the hypertrophied follicular epithelial cells. They have definite oval nuclei with distinct nucleoli and a well-marked reticulum with peripheral chromatin granules. In a few cases the boundaries of these cells can be made out. On a careful examination the connective tissue fibres are now seen to have wedged themselves all round the tubules closely



TEXT-FIGS. 1–4. *Rhinobatus granulatus* Cuv.—Fig. 1. T. S. of a fully mature egg showing the structure of the follicular wall. ×400. Fig. 2. A portion of the follicular epithelium (Stage I) magnified to show the hypertrophied follicular epithelial cells. ×900. Fig. 3. A few tubules of the theca interna (Stage I) showing the theca interna cells and the fibres investing the tubule. ×900. Fig. 4. A portion of the follicular epithelium (Stage II) showing the follicular epithelial luteal cells. ×900.

investing them and giving the appearance of definite walls to the tubules. The formation of these tubule-like structures in the theca interna is of considerable interest from the point of view of comparative histology as I am not aware of any record of such structures in the theca interna in other groups of animals.

The theca externa has but slightly increased in thickness. There is however an increase in the number of blood vessels. A few blood capillaries invade the theca interna and run in between the above-mentioned tubules. The groups of small spherical free thecal cells with darkly staining nuclei and clear cytoplasm have definitely increased in number, and some of them have penetrated in between the tubules of the theca interna.

Stage II.—A transverse section of the corpus luteum at this stage of development shows considerable resemblance to the previous stage and so the more important differences alone need be enumerated. The central lumen is further reduced. The folded appearance of the follicular

epithelium is still retained. No marked increase in number of the epithelial luteal cells is noticed and their histological character has altered but slightly. The majority of the cells are oval or spherical in shape and are now slightly smaller than in the preceding stage (Text-Fig. 4). The cytoplasm stains lightly and appears reticulate. The nuclei, mostly eccentric in position, contain a large irregular mass of chromatin or karyosome and a few smaller nucleoli with all of which a well-marked reticulum studded with small chromatin granules is associated.

The connective tissue theca takes an important part in the formation of the early corpus luteum. It has further thickened and the tubular structures in it have now become very definite and slightly more numerous. No great increase in the size of the cells of the thecal tubules is noticed but in the majority of them the cell-boundaries have become clearly marked (Text-Fig. 5). They continue to be definitely smaller than the epithelial luteal cells. The nuclei and nucleoli are perfectly distinct and moderately stained.

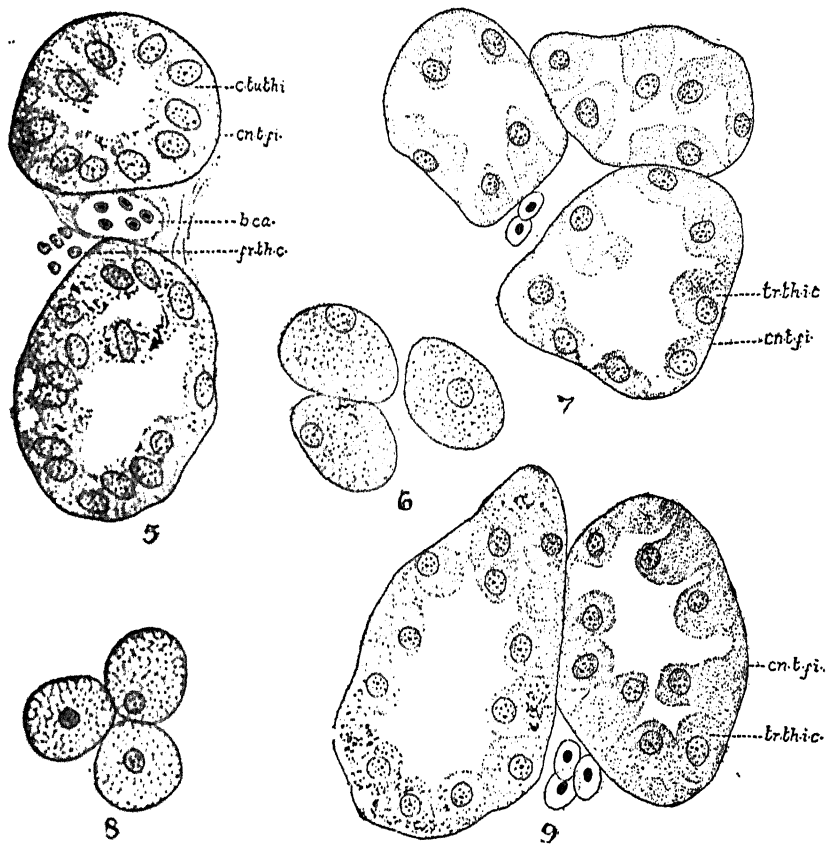
An advance is also shown in the commencement of ingrowths of strands of cells from the theca externa. There is a noticeable increase in the number of blood vessels in the theca externa and the projecting ingrowths above mentioned carry the blood vessels and groups of free thecal cells among the tubules of the theca interna.

Stage III.—A comparison with the previous stages shows a marked advance (Ph.M. 7). The central lumen is a little more reduced in size. Appreciable changes have taken place in the follicular epithelium. The folded condition has disappeared on account of a breaking up in the continuity of the layer and there is only a faint suggestion of the original folds. The epithelial luteal cells show a tendency to aggregate themselves into small masses or into more or less diffuse groups and most of them come to lie near the theca interna. They have assumed a more or less spherical shape in contrast to their previous columnar and elongated condition (Text-Fig. 6 and Ph.M. 8).

Accompanying these changes fine deeply staining chromatic granules have made their appearance in cytoplasm. Cytoplasmic vacuoles now begin to appear which owing to the large number of deeply staining granules are at first not readily distinguishable.

The theca interna is distinctly delimited from the follicular layer and still retains its folded appearance, though the interspaces between the folds become filled up by the approximation of the epithelial luteal cells (Ph. M. 7).

The more internally placed tubules have become larger in size than the superficial ones. The cells of the larger tubules have undergone further histological changes (Text-Fig. 7 and Ph.M. 9). The shape of these cells constituting the tubules is variable, but a number of them are spherical. The majority of the nuclei have become distinctly spherical and contain in addition to the nucleolus, a network of numerous chromatin granules, and



TEXT-FIGS. 5–9. *Rhinobatus granulatus* Cuv.—Fig. 5. A portion of the theca interna (Stage II) magnified to show the tubules of the theca interna, the blood capillaries and the free thecal cells. ×900. Fig. 6. Follicular epithelial luteal cells (Stage III). ×900. Fig. 7. The tubules of the theca interna (Stage III) showing the transforming theca interna cells. ×900. Fig. 8. A few follicular epithelial luteal cells (Stage IV). ×900. Fig. 9. The tubules of theca interna (Stage IV) magnified to show the transforming theca interna cells. ×900.

are of the same size but less deeply staining than those of the luteal cells of the follicular epithelium. The cytoplasm is rather dense and contains fine granules of very minute size. The fibres of connective tissue investing the tubules are quite distinct. There is a slight increase in the blood vessels

passing in between the tubules. An advance on the earlier stages is observed in the invasion of strands of connective tissue fibres along with blood capillaries and free thecal cells in between the groups of epithelial luteal cells. No appreciable change is observed in the theca externa.

Stage IV.—Photomicrograph 10 represents a portion of a transverse section of the corpus luteum at this stage. It is now slightly more advanced and is interesting in that the epithelial luteal cells show further cytological changes. The structure of the follicular wall is illustrated in Photomicrograph 11. The epithelial luteal cells form the conspicuous elements. They are distinguished both by the cytoplasmic vacuoles and by their large size and spherical shape (Text-Fig. 8 and Ph.M. 12). Though there is no noticeable increase in size of the epithelial luteal cells, an increase in number as compared with the previous stages is observed. The lumen is further reduced as a result of the luteal cells becoming more evenly spread out. The cytoplasm of the luteal cells is occupied by the spherical vacuoles which are much more marked than in the previous stage and consequently these cells are rendered more conspicuous than in the earlier stages examined. As a result of the great development of the vacuoles the chromatic granules are wedged in the intervacuolar cytoplasm. The nucleus does not show any change in its cytological details.

Another point of interest is that the theca interna cells constituting the inner tubules exhibit the first evident signs of transformation into luteal cells similar to those shown by the follicular epithelium (Text-Fig. 9 and Ph. M. 13). The first stage in the transformation of these cells into luteal cells is the appearance of chromatic granules together with an enlargement of the cytoplasm and a greater definition of the cell membrane. The majority of nuclei become spherical with distinct chromatin reticulum and deeply staining nucleolus. Cytoplasmic vacuoles begin to appear which owing to the large number of fine granules, are not readily distinguishable. The connective tissue fibres surrounding the tubules are well marked. The distinction between the cells of the theca interna and the epithelical luteal cells is maintained. There is an increase in the invasion of the blood capillaries and free thecal cells in between the tubules. The ingrowth of connective tissue fibres from the theca interna in between the groups of epithelial luteal cells initiated in the last stage has become more prominent.

The theca externa is thicker than in the earlier stage. This increase in the thickness of the connective tissue is accompanied by a corresponding increase of blood vessels. The invading strands of cells of the theca externa carrying blood vessels and free thecal cells into the theca interna are very prominent (Ph.M. 10).

Stage V.—This stage is interesting in that the follicular part and the theca interna has become indistinguishable (Text-Fig. 10 and Ph.M. 15). The luteal cells now almost fill the internal lumen, except for a narrow slit in the centre of the gland (Ph.M. 14).

The epithelial luteal cells have undergone further histological changes (Text-Fig. 11 *a* and Ph.M. 16). They are now polygonal or spherical with distinct large vesicular nuclei containing one or two nucleoli and a peripheral reticulum rich in chromatin granules. The vacuoles with which the cytoplasm is honey-combed have become smaller and spherical. Another change connected with the mature corpus luteum is the development of larger chromatic granules which take up an inter-vacuolar position. They are very much marked at the angles of the honey-combed vacuoles. Hence the inter-vacuolar regions take a deep stain which therefore emphasises the boundaries of the vacuoles so as to produce the conspicuous honey-combed appearance characteristic of the cytoplasm of the fully developed luteal cells. They have reached the height of their histological development and the main feature of this stage is that all the cells are in the same stage of activity. The vacuoles are very prominent and occur in every luteal cell and are so numerous that there is only little cytoplasm in the cell. In abundance and sharpness of outline and uniformity of structure, these vacuoles and their exquisitely honey-combed appearance are very striking. The ring-like vacuoles have been described in the luteal cells of higher mammals like Ungulates, Carnivora, Rodents and Primates. To my knowledge, they are now described for the first time in the luteal cells of an Elasmobranch. Simultaneous with the merging of the sheath portion and the follicular part there is an invasion of the connective tissue fibres a few of which surround the groups of inner epithelial luteal cells.

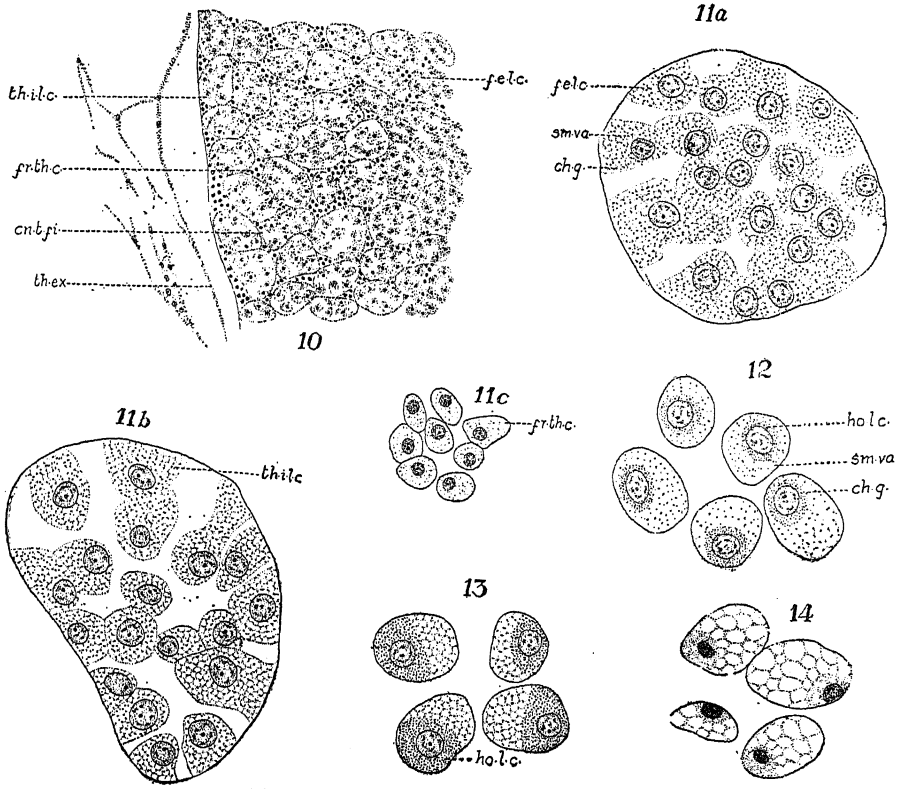
Towards the superficial region of the original theca interna the cells constituting the tubules are slightly smaller than the cells towards the inner region. The fibres investing these cells still persist round them retaining the tubular appearance. The smaller cells of these tubules pass imperceptibly into larger and more glandular luteal cells of the inner tubules. As a result of the enlargement of the cells composing the tubules some of them have been pushed into the lumen of the tubules giving them a solid appearance. A careful study of the cells of tubules of the theca interna reveals that they have undergone remarkable cytological changes. Text-Fig. 11 *b* and Ph.M. 17 illustrate the changes undergone by the cells during the metamorphosis of these thecal into luteal cells. Comparison with the earlier stages shows an increase in size of the cells of the theca

interna. With the transformation of the theca interna cells into luteal cells changes similar to those occurring in the follicular epithelial cells take place. The nucleus which hitherto has remained oval and small gradually enlarges, the charomatin granules getting concentrated in a uniform manner on the inner surface of the nuclear wall. When the nucleus has reached its maximum growth it presents a characteristic vesicular appearance (Text-Fig. 11 *b* and Ph.M. 17). With the assumption of luteal condition the nucleolus has by its special prominence become a distinct feature of the nucleus. The chromatin material as well as the nucleolus are distinct and deeply stained. The spherical vacuoles become very clear and the whole cytoplasm is honey-combed with ring-like vacuoles as in the epithelial luteal cells. The honey-combed appearance is due to mutual compression. Consequently there is no difference now in size or cytological structure between the luteal cells developed from the theca interna and the luteal cells formed from the original follicular epithelium.

The groups of free thecal cells may be easily noted in the corpus luteum. These are much more abundant and more conspicuous than in any of the earlier stages. The free cells of the theca can be clearly distinguished from the luteal cells by their smaller size, round, vesicular, deeply staining nuclei containing large amount of chromatin and by the uniformly staining cytoplasm (Text-Fig. 11*c* and Ph.M. 16). They are interspersed in between the groups of luteal cells. A careful study reveals the changes they have undergone. The cytoplasm is densely packed with minute vacuoles. These cells vary from round to oval shape and the nucleus generally has an eccentric position. The origin of these cells can be easily traced from the outer region of the theca folliculi. In the earlier stages they occur as clumps of theca cells situated in the periphery of the corpus luteum wedged in between the concentric layers of the theca externa. Later on they extend in between the tubules of the theca interna. Later still a small number grow along with the connective tissue fibres in between the diffuse groups of epithelial luteal cells. In the fully developed corpus luteum, these cells are found about the periphery and also along the connective tissue fibre ingrowths which penetrate and surround the groups of luteal cells. They also fit into the interstices between the groups of luteal cells. They form a special group different in histological character, location, and origin, from the luteal cells. The presence of a regular system of vacuoles very much like that found in the luteal cells suggests that these cells have a function similar to that of luteal cells.

The theca externa is very much reduced and consists of only a thin layer, about three cells deep, investing the corpus luteum. The luteal tissue

is held together by a framework of reticular connective tissue fibres which forms a dense network round the groups of luteal cells. There is an increase in the vasicular tissue in the theca externa and an invasion of blood vessels and free thecal cells into the luteal tissue and their extension along the connective tissue framework is clearly seen.



TEXT-FGIS. 10–14. *Rhinobatus granulatus* Cuv.—Fig. 10. T. S. of portion of corpus luteum (Stage V) showing the luteal tissue formed from the theca interna and the follicular epithelium and the penetration of connective tissue fibres and free thecal cells. ×80. Fig. 11*a*. A group of follicular epithelial luteal cells (Stage V) showing the elaborate system of cytoplasmic vacuoles and chromatic granules in the inter-vacuolar cytoplasm. ×900. Fig. 11*b*. A group of theca interna luteal cells showing similar cytoplasmic structures as the follicular epithelial luteal cells. ×900. Fig. 11*c*. A group of free thecal cells. ×900. Fig. 12. Luteal cells (Stage VI) magnified to show the changes in the cytoplasm. ×900. Fig. 13. Luteal cells (Stage VI) showing the homogeneous staining area of the cytoplasm. ×900. Fig. 14. Luteal cells (Stage VII) showing commencing degeneration by the appearance of large irregular vacuoles. ×900.

From a study of this stage it is clear that the follicular epithelial cells and the cells of the theca interna forming tubules enter into the formation of the luteal elements.

Stage VI.—In a still more advanced stage the corpus luteum has become solid throughout. It is a glandular organ composed of closely packed cells arranged in anastomosing columns and masses, between which run the blood vessels and groups of free thecal cells (Ph.M. 18). It is invested by a thin connective tissue envelope composed of protoplasmic cells and fibres of the theca externa. There is a general rupture of the tubules with the result that the luteal cells are somewhat spread apart, but are still held together by means of protoplasmic connections. The previous stage passes imperceptibly into this stage though a microscopic study shows that the cells constituting the corpus luteum exhibit a diversity in their cytoplasmic structure. Most of the luteal cells have an immense number of vacuoles of very constant size and shape as in the preceding stage. The intervacuolar spaces are broader and the chromatic granules are larger and stain deeply. A number of luteal cells show changes both in their shape and cytoplasmic structure as represented in Text-Fig. 12. The shape of the cells is variable. The cytological structure of these fully formed luteal cells is as follows. The nucleus is usually spherical and large but not so chromatic as in the previous stage though one or more nucleolar bodies and numerous small chromatin granules are present. It is noted that the cytoplasm as previously, is honey-combed with spherical vacuoles which are not so numerous as in the preceding stage. There is a homogeneous staining area round the nucleus while the vacuoles are confined to the periphery (Text-Fig. 12). It appears to consist of a group of very fine chromatic granules concentrated round the nucleus. These granules show up fairly with iron-alum and hæmatoxylin. In Mallory's triple stain they appear as light orange granules, diffusely scattered round the nucleus. It is also observed that the nucleus too is stained orange. In some of the cells the homogeneously staining granular area extends to the periphery (Text-Fig. 13). Regarding the nature of the chromatic granules of the luteal cells they may be of an albuminous nature since they are best preserved by mercury salts and are not dissolved by alcohol or water from fixed tissue. The presence of these granules recalls the condition of luteal cells as described by Corner (1915) in Swine.

The free thecal cells described in the previous stage show considerable increase in number but the cytological character of the cells remains unchanged. They become uniformly scattered about the luteal cells. In addition strands of protoplasmic cells could be seen growing from the theca externa more or less in the form of fine root-like processes in between the folds of the wall of the corpus luteum. In some places they can even be seen to penetrate into the central region. There is a great reduction in the theca externa.

Stage VII.—The histological character of the luteal cells of this stage has altered and careful examination shows that there is a marked increase in the homogeneous staining area of the cytoplasm with a corresponding reduction of vacuoles. Luteal cells in various stages, from a condition of full preservation having vesicular nuclei and honey-combed cytoplasm to stages of degeneration, could be noticed. Degenerating cells are now few and are found near the periphery of the corpus luteum. The vacuoles in the cytoplasm of the degenerating cells show variation in size and in some of the cells they become enlarged (Text-Fig. 14) accompanied by a reduction in size of the nuclei. In a number of cells the nuclei stain intensely. The boundaries of these cells can no longer be made out. Free deeply staining nuclei without the enveloping cytoplasm are occasionally found. A clumping of chromatin of the nucleus in the degenerating cells is observed as a result of which neither the nucleolus nor the chromatin granules are visible. In this connection it is interesting to note that the degenerating changes set in only late. The theca externa shows marked reduction and is seen as a very thin envelope of connective tissue cells and fibres surrouding the corpus luteum.

Discussion

We shall take the structures which finally form the corpus luteum in the order of their importance.

Luteal Cells.—The origin of the luteal cells in the different groups of vertebrates has been the subject of considerable amount of controversy. The general consensus of opinion at present seems to be that luteal cells are derived either exclusively from the follicular epithelium or from both the follicular epithelium and the theca interna. From the study of the formation of the corpus luteum in *R. granulatus* it is clear that the follicular epithelial cells and the theca interna cells which form tube-like structures develop and transform themselves into luteal cells of the fully developed corpus luteum. The theca interna cells which remain distinct from the follicular epithelial cells in the early stages, undergo interesting histological changes and metamorphose into luteal cells very much like those formed from the follicular epithelium so that in the fully developed corps luteum there is no difference between the luteal cells constituting the organ.

It has been shown that the small cells of the follicular epithelium of the unruptured follicle, after ovulation develop and form large spherical or polygonal glandular cells which exhibit similar cytoplasmic changes as in certain mammals. The large cells undergo reduction and finally, in the ruptured

follicle, disappear completely. The same phenomenon of decrease in numbers and final disappearance of the large cells has been noticed in the reptiles.

Corner (1915) recognises an endoplasm and exoplasm in the cytoplasm of the luteal cells in the Sow. With regard to the cytoplasmic changes undergone by the luteal cells this author distinguishes seven periods in the history of the corpus luteum of which the most important are the Preparatory, Exoplasmic, Transitional, Endoplasmic and beginning of retrogression. According to him the corpus luteum being an organic body each stage passes imperceptibly into the next stage. In the exoplasmic stage he describes the highest development of the exoplasmic region when the exoplasm is " occupied by a most curious and elaborate system of vacuoles". In the later stages a homogeneous staining area, the endoplasm, develops round the nucleus which gradually increases. As the endoplasmic zone increases there is a corresponding decrease of the exoplasmic area. Finally the whole cell is practically occupied by the homogeneous cytoplasm. He also observes that in the later periods the lipoid globules which form the regular vacuoles " have practically disappeared from the cell, being found if present, as a few globules at the periphery ". Still later " many cells contain one or two globules twice as large as the nucleus, in the cytoplasm ". The luteal cells of stages V, VI and VII of the present study correspond with the exoplasmic, endoplasmic and retrograssive periods respectively, of the Sow's corpus luteum. The fully formed luteal cells in *R. granulatus* are large, each cell having a large characteristic vesicular nucleus containing one or two large nucleoli and peripheral reticulum rich in chromatin granules. The cytoplasm becomes honey-combed with a regular system of spherical ring-like vacuoles in non-osmic fixatives.

It is interesting that the structure of the luteal cells and the cytological changes undergone by them in *Rhinobatus granulatus* agree in important respects with those of Sow, described by Corner (1915) in spite of their widely separated position in the vertebrate series.

Cohn (1903) was the first author to describe these remarkable vacuoles of the luteal cells in the corpus luteum of the rabbit. These ring-like bodies in the luteal cells have been described in such higher mammals as Ungulates, Carnivores, Rodents and Primates. Although Van der Stricht (1912) describes in detail the secretory appearance in the corpus luteum of bats he does not mention the ring-like structure in the luteal cells. Clark (1898) who studied the luteal cells of swine says that they become full of vacuoles and their cytoplasm shrunken. Regarding this statement of

Clark, Corner (1915) says "he was probably actually describing the exoplasmic vacuoles, which we shall see to be evidences of cellular activity, not of senescence".

Practically all workers agree on the point that "the deposition of fat in the luteal cells is not a sign of degeneration but represents a normal function of an endocrine nature". Most of them distinguish two periods in the deposition of fat in the luteal cells. A detailed study of the fatty inclusions in the luteal cells of bats is made by Van der Stricht (1912). The author recognises two periods in the deposition of fat, the first being 'epithelial in nature representing a secretion of the corpus luteum, but that the later reappearance of presumably fatty material is a sign of senescence in the tissue'. Corner's (1915) observations on the luteal cells regarding the nature of deposition of fat is in close agreement with that of the above author. Corner (1915) says 'the later deposition of fat I take to be a sign of senility of the tissue whereas the large amount of lipoid in the early lutein cell would seem to be correlated with physiological activity'.

Gatenby (1924) working on the human corpus luteum of ovulation is of opinion that the lutein granules are lipin and not true fat since "they do not occur in the same irregular manner in which true fat appears in cell; they do not vary much in individual size; they do not always reduce the osmic acid vigorously and finally they correspond to position with the expected arrangement of the mitochondria". This statement of Gatenby's is in agreement with the views of Cesa-Bianchi (Gatenby, 1924). According to Gatenby (1924) the mitochondria swell in size and become loaded with lipochrome which gives the corpus luteum its characteristic appearance. He concludes that since the lutein granules are disposed in the same manner as the mitochondria and all the granules are of equal size they are formed from the mitochondria. As further evidence in support of this view he states that in no case "the neutral fat appears within any cell in the form of chains of minute granules all of the same size".

Owing to the lack of specimens it has not been possible to study the cytoplasmic structures, *viz.*, mitochondria and golgi and the secretory products of the luteal cells. However, the fact that all the vacuoles of the cytoplasm are regular in arrangement and equal in size, point to the conclusion that the globules formed in the early active stages are lipoid in nature. It may be suggested that the lipoid globules formed become surrounded by chromatic granules which are also present in the cytoplasm and the former are readily removed by the reagents employed in fixing, and dehydration. Hence the position of the lipoid globules are indicated by rows of ring-like

vacuoles with chromatic granules in the inter-vacuolar cytoplasm. The appearance of large vacuoles at the periphery of the cell in the last stage without the uniformity of size observed in the earlier stages may be due to the formation of fat. This may be a sign of commencement of fatty degeneration of the cell as suggested by Corner (1915) and others. It may also be stated that the appearance of these large vacuoles in the cell is accompanied by degenerative changes in the nucleus of such cells.

Another element of histological interest in the luteal cells of *Rhinobatus granulatus* is the chromatic granules. These are best shown in corrosive sublimate fixed material stained with iron hæmatoxylin. They are fine in the earlier stages but appear to grow larger and more apparent. Cesa-Bianchi (1908) describes similar granules densely crowded about the nucleus. The presence of such chromatic granules, has also been demonstrated by Corner (1915), scattered diffusely through the endoplasm. Regarding the nature of these granules Cesa-Bianchi (1908) states that since they are not dissolved by ether, alcohol or water from the fixed material and are preserved by mercury salts they are of an albuminous nature. Corner (1915) also supports this view. The chromatic granules in the cytoplasm of luteal cells of *Rhinobatus* are very probably of the same nature.

Theca interna.—The discussion of the origin of the luteal cells is inseparable from the question regarding the role the theca interna plays which is still a matter of controversy. The histological changes undergone by the theca interna cells in this case are very interesting. As has been described the cells of the theca interna undergo a unique process of development arranging themselves round a clear space forming tubule-like structures. Both in size and cytological structure the cells constituting these tubules are different from the follicular epithelial cells in the early stages of the formation of the corpus luteum. The nuclei are oval and the cell limits are ill defined. During the transformation of these cells into luteal elements they swell up and the cell-membrane becomes distinctly marked. The nucleus gradually assumes a vesicular form, the chromatin granules concentrating in a uniform manner at the inner surface of the nuclear wall. With the assumption of luteal condition the nucleolus becomes a prominent feature of the nucleus. When the cell reaches its maximum growth the whole cytoplasm is honey-combed with regular ring-like vacuoles as in the epithelial luteal cells. They also show the presence of chromatic granules. Consequently in the fully developed corpus luteum there is no difference in size or cytological structure between the epithelial luteal cells and the theca luteal cells.

With regard to the fate of the theca interna cells Van der Stricht (1912) considers that the theca interna of the follicle incorporates interstitial cells which become indistinguishable from the true lutein cells developed from the follicular epithelium and function as lutein cells in the fully developed corpus luteum. Corner (1915) has shown in the swine, that the theca interna cells take an important part in the constitution of the fully developed corpus luteum and some of the cells of the theca interna " come to resemble the true lutein cells very closely; indeed, at times it is almost impossible to distinguish them ". Novak's (1921) figures of the corpus luteum in his book *Human Menstruation and Its Disorders*, are in agreement with those of Corner's in the Sow. In a more recent paper Corner (1919) states that the theca interna cells may approximate very closely to the luteal cells and there is a confusing resemblance between some of the theca cells and follicular luteal cells. However, Corner distinguishes the theca interna cells from the luteal cells by the presence of vacuoles and rings in the luteal cells in his latest results (1921). It may be noted that there is no such difference between the two luteal elements of *Rhinobatus*. It may be summarised that according to the above authors the theca interna cells give origin to glandular cells showing striking resemblance to the epithelial luteal cell. This is in close agreement with the results of the present study.

Gatenby (1924) working on the human corpus luteum found clumps of small luteal cells situated near the theca externa which pass imperceptibly into larger luteal cells. Gatenby puts forward two views regarding the question of the origin of the luteal cells and the fate of the theca interna cells, one, " that the luteal cells are of mixed origin; that most of the theca interna cells so closely approximate in cytological arrangement to the membrane cells that it is impossible to distinguish the two; and finally that the stellate cells are fibroid elements derived from free cells of the theca externa", and the second which the author seems to favour "to look upon the stellate cells as theca interna elements, and thus to deny the theory of mixed origin of lutein cells ". The author, however, concludes that before more material has been studied it is not possible to draw a definite conclusion regarding the stellate elements. He further observes that it is difficult to deny the mixed origin of luteal cells because of the possibility that the follicular epithelial cells and thecal cells which are both derived originally from the mesoderm could undergo the same kind of specialisation and differentiation.

Apart from the theca interna cells which transform into luteal cells, another category of thecal cells has been described interspersed between the luteal cells in the fully developed corpus luteum. In the early stages these occur as groups of free thecal cells situated in the periphery of the ruptured

follicles wedged in between the concentric layers of the theca externa. Later on, these cells invade the theca interna along with the ingrowths of protoplasmic cells of the theca externa and lie in between the tubules of the theca interna. Later still a small number grow along with connective tissue fibres in among the diffuse groups of follicular luteal cells. In the fully developed corpus luteum these cells are found along the connective tissue fibres which penetrate in between the groups of luteal cells and also fit into the interspaces between the groups of luteal cells. In the fully developed condition they are spherical or oval with deeply staining vesicular nuclei. The cytoplasm is packed with minute regular vacuoles as in the luteal cells but the chromatic granules are not noticed. They at no time approximate to the luteal cells in size. These cells form a special group of cells different in histological character, location and origin from the luteal cells. In the corpus luteum of Sow, Corner (1915) describes two kinds of cells apart from the luteal cells, and denotes them as additional cells of the corpus luteum, type 1 and type 2. These free thecal cells seem to resemble in certain respects additional cell type 1 of Corner's description but there are no elements corresponding to type 2 of Corner.

Theca externa.—There is considerable variation in the behaviour of the theca externa in different animals. According to Sobotta (1896 and 1897) in the mouse and rabbit the theca externa does not take any part in the formation of the corpus luteum and the connective tissue ingrowth takes place exclusively from the theca interna. Marshall (1904) states that the connective tissue element of the corpus luteum in sheep is contributed both by the theca interna and theca externa. In the human corpus luteum Gatenby (1924) describes that the connective tissue ingrowths in the form of columns and lamellæ carrying blood vessels take place from the theca externa while small cells probably derived from the theca interna form the general supporting tissue. In *R. granulatus* the theca externa early gives rise to connective tissue ingrowths which carry blood capillaries and free thecal cells. In the later stages there is an extensive invasion of connective tissue elements of the theca externa in among the luteal tissue carrying blood vessels in the same manner as is observed in mammals and certain reptiles. The theca externa forms the enveloping sheath and also provides a delicate investment around the groups of luteal cells in the fully mature corpus luteum.

The present investigation shows that the corpus luteum in *R. granulatus* is a well-developed glandular organ exhibiting a close resemblance to the mammalian corpus luteum but showing noteworthy peculiarities of its own. As in the mammals the cavity of the discharged follicle is eventually filled

in by the luteal cells and ingrowth of connective tissue cells and fibres. It has also been possible to confirm the mixed origin of the luteal cells as it is shown that they are derived not only from the follicular epithelium, but a fair proportion of them is developed out of the cells of the theca interna.

Summary

1. A detailed description of the histology and development of the corpus luteum in *R. granulatus* from a very early stage upto the formation of a solid glandular body is given.

2. All the three elements of the follicular wall of the ruptured Graffian follicle, *viz.*, follicular epithelium, theca interna and theca externa take part in the final formation of the corpus luteum.

3. The luteal cells are shown to be formed by the hypertrophy of the cells of the follicular epithelium and the theca interna. The mixed origin of the luteal cells is described in a lower vertebrate for the first time.

4. The histological differentiation which takes place in the cells of the theca interna during their transformation into luteal cells is described in detail.

5. The theca externa forms both the enveloping sheath and the supporting framework of the fully developed corpus luteum. There is an extensive invasion of connective tissue elements of the theca externa carrying blood vessels and free thecal cells into the luteal tissue.

6. It has also been shown that degenerative changes set in very late. The close analogy between the formation of the corpus luteum in the Elasmobranchs and the mammals is pointed out.

Acknowledgements

I wish to express my grateful thanks to Professor R. Gopala Aiyar, Director, University Zoological Research Laboratory, Madras, for his valuable help and suggestive criticisms during the course of these investigations and to the University of Madras for awarding me a Research Studentship.

REFERENCES

Belloy* .. "Recherches sur l'origine des corps Jaune de l'ovaire chez le rat et le cochon d' Inde," *C. R. de l' Assoc. des Anatomistes* Premierè Session, Paris, 1899.

Bischoff, T. L. W.* .. "Entwickelungsgeschichte des Kaninchenes," *Braunschweig*, 1842.

Buhler, A.* .. "Entwickelungsstudien menschlicher corpora lutea," *Verhand d. Anat. Gesellschaft. in Pavia*, 1900.

———— .. "Ruckbildung der Eifollikel bei wirbelthieren," *Morph Jahrb.*, 1902, **30.**

Cesa-Bianchi, D.* .. "Di alcune particolarita di strutturi edeifenomeni di secrezione del corpo luteo," *Internat. Monatschr. f. Anat. u. Physiol., Leipzig*, 1908, **25.**

Clark, J. G.* .. "The origin, growth, and fate of the corpus luteum as observed in the ovary of the pig and man," *Johns Hopkins Hosp. Rep., Baltimore*, 1898, **7.**

Cohn, F. .. "Zur Histologie und Histogenesis des Corpus Luteum und des Interstitiellen Ovarialgewebes," *Arch. f. Mikr. Anat.*, 1903, **62.**

Corner, G. W. .. "The corpus luteum of pregnancy, as it is in swine," *Contributions to Embryology*, 1915, **2,** Carnegie Institution of Washington.

————* .. "On the origin of the corpus luteum of the Sow from both granulossa and theca interna," *Amer. Jour. Anat.*, 1919, **26.**

———— .. "Cyclic changes in the ovaries and uterus of the Sow, and their relation to the mechanism of implantation," *Contributions to Embryology*, 1921, **13,** Carnegie Institution of Washington.

Cunningham, J. T. .. "On the histology of the ovary and of the ovarian ova in certain Marine fishes," *Q. J. M. S.*, 1898, **40.**

Deanesly, R. .. "The corpora lutea of the mouse with special reference to Fat accumulation during the Oestrous cycle," *Proc. Roy. Soc. Lond.*, Ser. B, 1930 *a*, **106.**

———— .. "The development and vascularisation of the corpus luteum in the mouse and rabbit," *ibid.*, Ser. B, 1930 *b*, **107.**

Doering .. "Beitrag zur streitfrage über die Bildung des Corpus Luteum," *Anat. Anz.*, 1899, **16.**

Gatenby, J. B. .. "Notes on the Human ovary, with special reference to a corpus luteum of ovulation," *Proc. of the Roy. Irish Academy*, 1921–24, **36.**

Giacomini* .. "Contributo all, 'Istologia' dell' Ovario dei Selaci", *Ricerca laboratoria di, Anatomia normale della Roy. Universita di Roma*," 1896, **5.**

Heape, W. .. "The menstruation and ovulation of *Macacus rhesus*, with observations on the changes undergone by the discharged follicle, *Phil. Trans. Roy. Soc. Lond.*, Ser. B, 1897, **188.**

Hill, J. P., and Gatenby, J. B. .. "The corpus luteum of the Monotremata," *Proc. Zool. Soc., Lond.*, 1926, 715–63.

His* .. "Discussion," *Verhand. d. Anat. Gesell., in Tübingen*, 1899.

Honore, C. H. .. "Recherches sur l'ovaire du lapin. I. Note sur les corps de call et exner et la formation du liquor folliculi. II. Recherches sur la formation des corpus jaunes," *Archives de Biologie, Tome*, 1900, **16.**

.. "Recherches sur l'ovaire du Lapin. III. Notes sur des follicules de. Graaf a'plusieurs ovules," *ibid.*, 1900, **17.**

Jankowski .. "Beitrag zur Entstehung des corpus luteum der säugethiere," *Arch. f. mikr. Anat.*, 1904, **44.**

Loeb, L.* .. "Über die Entwicklung des corpus luteum beim Meer schweinchen," *Anat. Anz.*, 1906, **28.**

Marshall, F. H. A. .. "The œstrous cycle and the formation of the corpus luteum in sheep," *Phil. Trans. Roy. Soc. Lond.*, Ser. B, 1904, **196.**

.. "The development of the corpus luteum"—A Review, *Q. J. M. S.*, 1906, **49.**

.. *The Physiology of Reproduction*, 1922.

Meyer, R.* .. "Über corpus luteum Bildung bein Menschen," *Arch. f. Gynæk, Berlin*, 1911, **93.**

Miller* .. "Corpus luteum, Menstruation und Graviditat," *ibid.*, 1914, **101.**

Nagel* .. "Uber neure Arbeiten auf dem Gebiete der Anatomie der weiblichen Geschlechtsorgane," Merkel and Bonnet's *Ergebnisse d. Anat. u. Entwickelungs*, 1899, **8.**

Novak, E.* .. *Menstruation and its Disorders*, Appleton, 1921.

Paterson* .. "Observations on corpora lutea," *Edinburgh Med. and Surg. Jour.*, 1840.

O'Donoghue, C. H. .. "The growth-changes in the Mammary apparatus of Dasyurus and the relation of the corpora lutea thereto," *Q. J. M. S.*, 1911, **57.**

.. "The corpus luteum in the non-pregnant Dasyurus and polyovular follicles in Dasyurus," *Anat. Anzeig.*, 1912, **B 41.**

.. "Über die corpora lutea bei einigen Beuteltieren," *Archiv. f. mikros. Anat.*, 1914, **Bd. 84.**

.. "On the corpora lutea and Interstitial tissue of the ovary in the Marsupialia," *Q. J. M. S.*, 1916, **61.**

Paladino .. "Per la Dibuttata Questione sulla Esenza del corpo luteo," *Anat. Anz.*, 1900, **18.**

Rabl, H.* .. "Beitrag zur Histologie des Eierstocks des Menschen und der säugetiere, nebst Bemerkungen über die Bildung von Hyalin und pigment," *Anat. Heft, I. Abt., Wiesbaden* 1898, **11.**

Robinson .. "The formation, rupture and closure of ovarian follicles in Ferrets," *Trans. Roy. Soc. Edin.*, 1918, **52.**

Sandes, F. P. .. "The corpus luteum of *Dasyurus viverrinus*, with observations on the growth and atrophy of the graafian follicle," *Proc. Linn. Soc. N.S.W.*, 1903.

Schrön, O.* .. "Beitrag zur kenntniss der Anatomie und physiologie des Eierstocks der Säugethiere," *Zeitschr. f. wiss. Zool. Leipzig*, 1863, **12.**

Sobotta, J. .. "Über die Bildung des corpus luteum bei der Maus," *Archiv. f. mikros. Anat.*, 1896, **Bd. 47.**

Stratz* .. *Der Geschlechtsreife Säugethiereierstock*, Haag, 1898.

Van der Stricht, O.* .. "La Rupture du Follicule ovarique et l'Histogénèse du corps jaune," *C. R. De l'Assoc. des Anatomistes*, 1901.

——— .. "Sur le processus de l'excretion des glandes endocrines," *Arch. de Biol.*, 1912, **27.**

Von Baer, C. E.* .. "De ovi mammalium et Hominis Genesi, Epistola" 4°, *Lipsiæ, L. voss.*, 1827.

Wallace, W. .. "Observations on ovarian ova and follicles in certain Teleostean and Elasmobranch Fishes," *Q. J. M. S.*, 1904, **47.**

Williams* .. *Obstetrics*, New York and London, 1904.

* Have not been referred to in original.

EXPLANATION OF PHOTOMICROGRAPHS

Rhinobatus granulatus Cuv.

PLATE VI

PHOTOMICROGRAPH 1. T.S. of a fairly ripe egg showing the structure of the follicular wall ×200.

„ 2. T.S. of the follicular wall of the fully matured egg showing the tubule-like structures of the theca interna. ×200.

„ 3. T.S. of the corpus luteum (Stage I). ×48.

„ 4. T.S. of the corpus luteum (Stage I); a portion magnified to show the follicular epithelial folds, the tubules of theca interna and the theca externa. ×80.

„ 5. Follicular epithelium (Stage I), magnified to show the highly hypertrophied follicular epithelial luteal cells. ×400.

„ 6. A portion of the theca interna (Stage I) magnified to show the cells of the tubules of theca interna, the connective tissue fibres and the free-thecal cells. × 400.

PLATE VII

PHOTOMICROGRAPH 7. T.S. of the corpus luteum (Stage III). ×48.

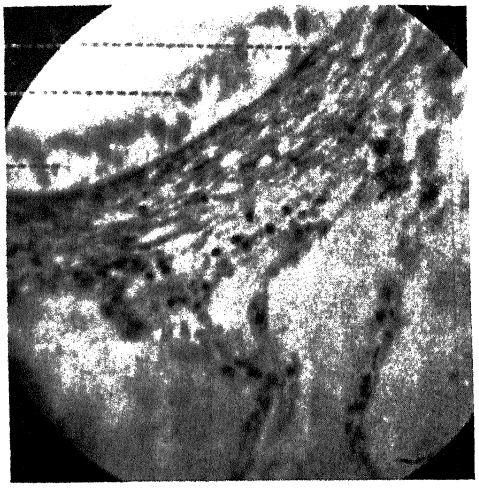
„ 8. A magnified view of the follicular epithelial luteal cells (Stage III). ×400.

„ 9. A portion of the theca interna (Stage III) magnified to show the transforming cells of the theca interna. ×400.

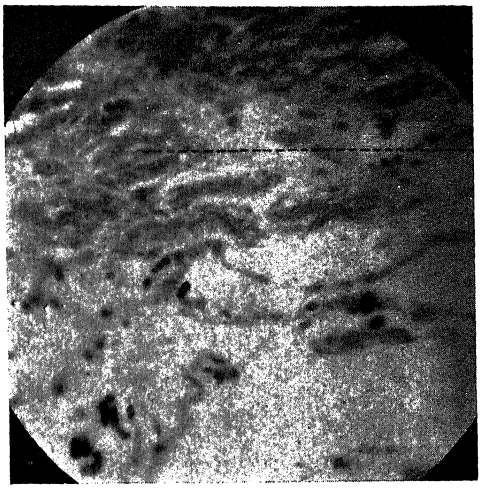
„ 10. T.S. of the corpus luteum (Stage IV) showing the ingrowth of the protoplasmic cells of the theca externa. ×48.

„ 11. T.S. of the corpus luteum (Stage IV); a portion of the wall of the corpus luteum magnified to show the tubules of theca interna and the follicular epithelial luteal cells. ×80.

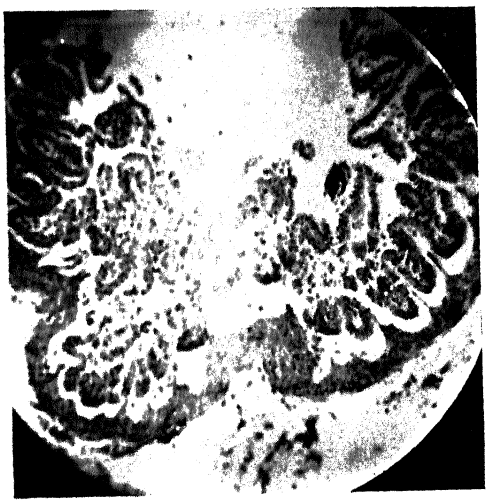
„ 12. Magnified view of the follicular epithelial luteal cells (Stage IV). ×400.



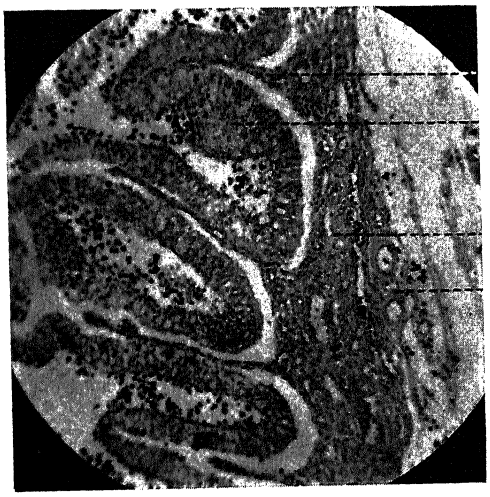
1



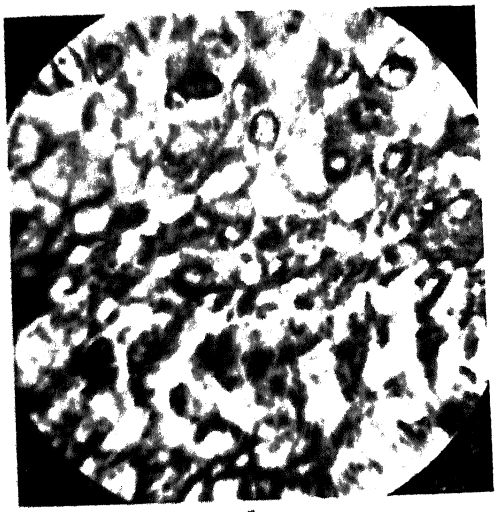
2



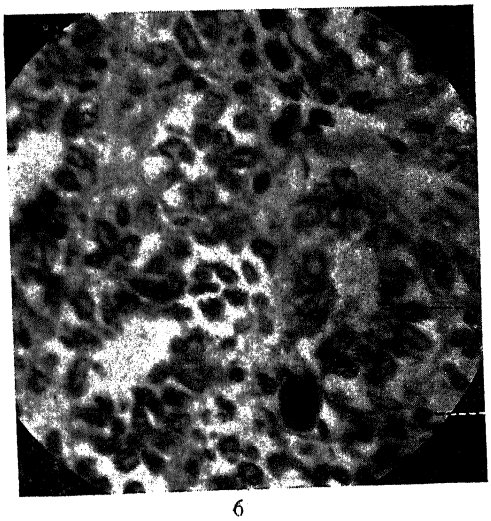
3



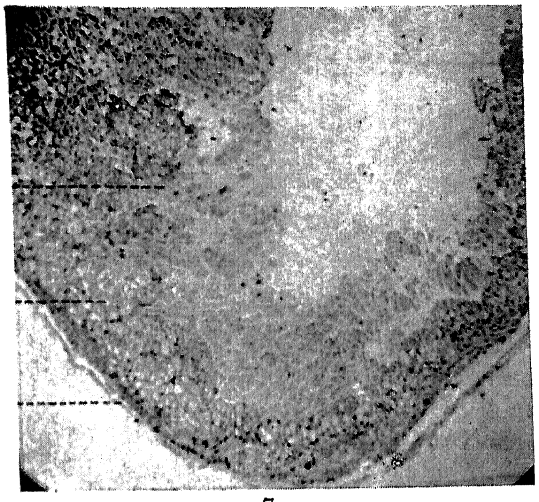
4



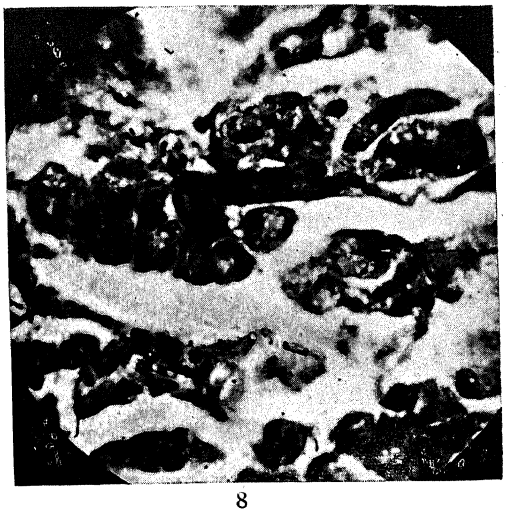
5



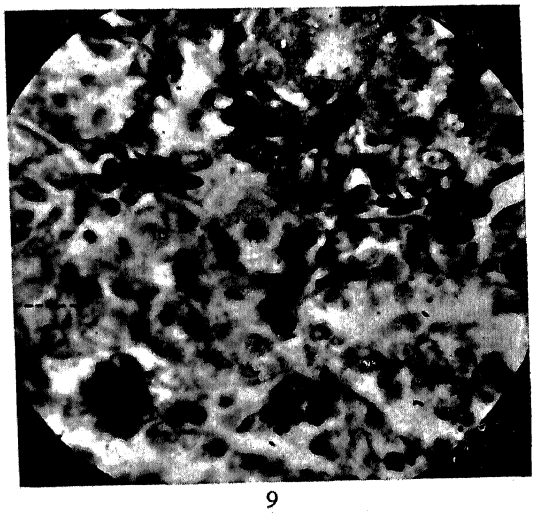
6



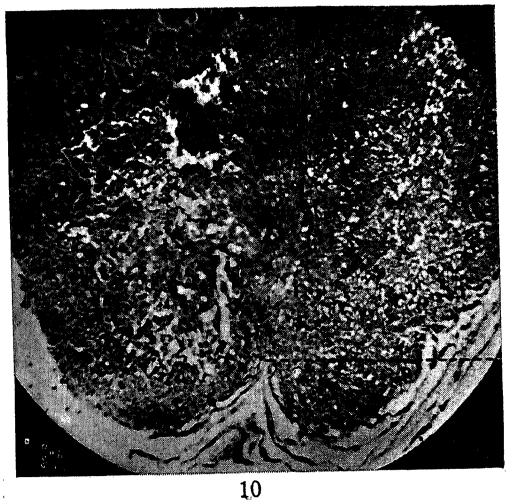
7



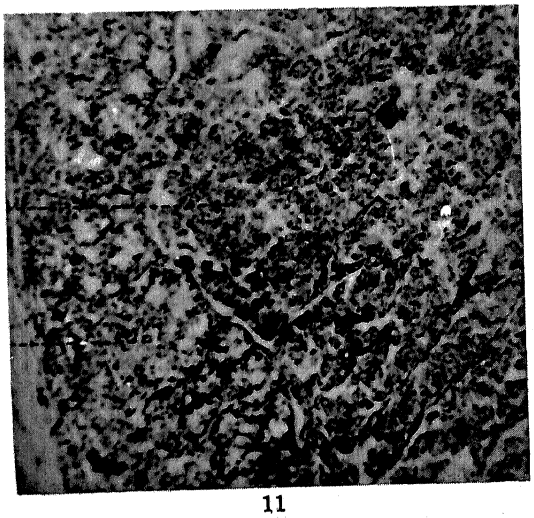
8



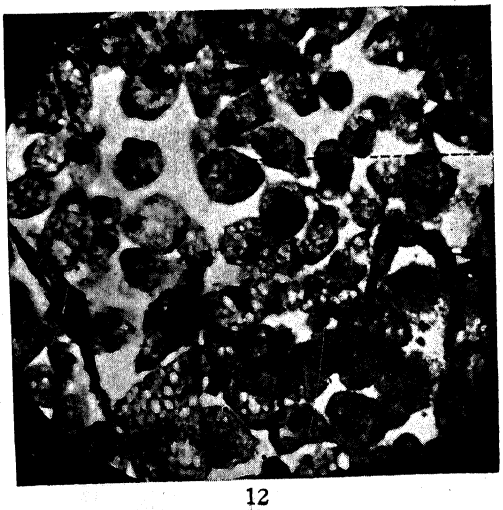
9



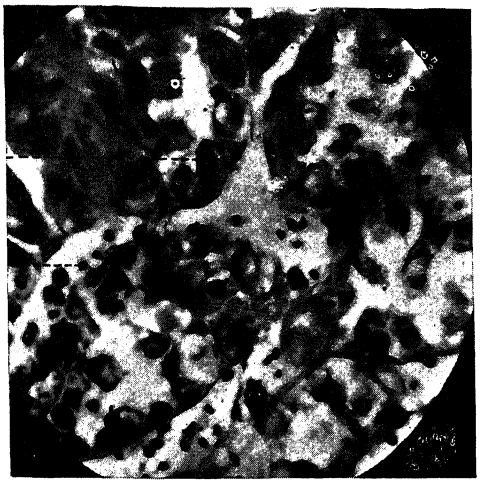
10



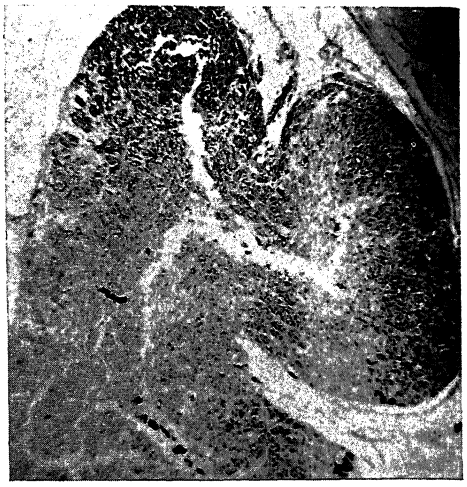
11



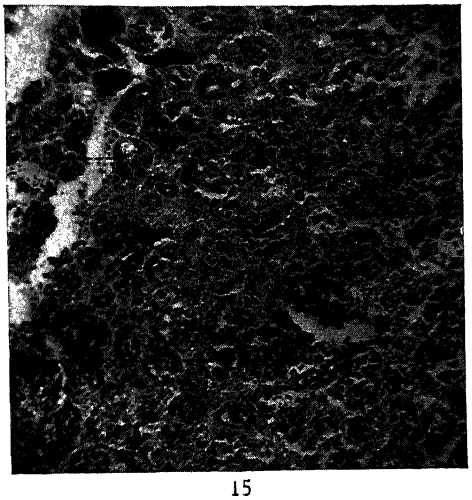
12



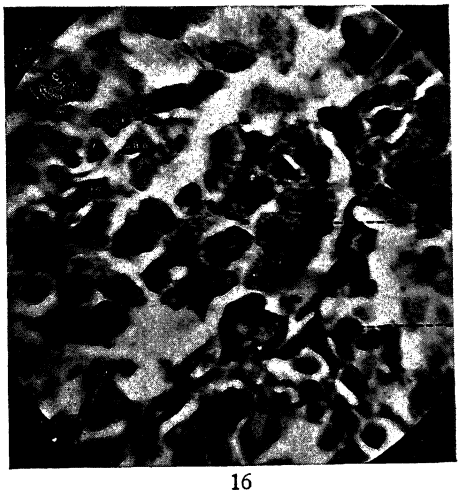
13



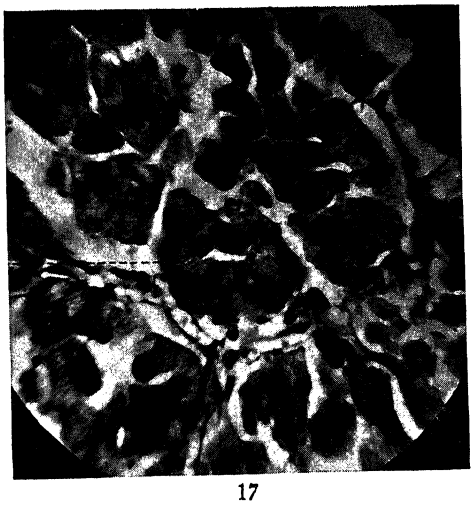
14



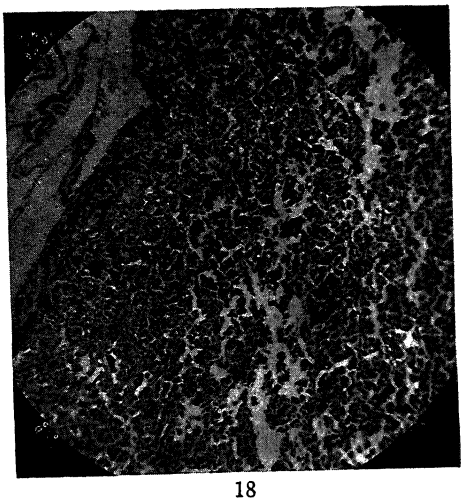
15



16



17



18

PLATE VIII

PHOTOMICROGRAPH 13. A portion of the theca interna (Stage IV) magnified to show the transforming theca interna cells and free thecal cells. ×400.

„ 14. T. S. of the fully developed corpus luteum (Stage V). ×48.

„ 15. T. S. of the corpus luteum (Stage V); a portion magnified to show the tubules of theca interna luteal cells and follicular epithelial luteal cells. ×80.

„ 16. A magnified view of a group of fully developed follicular epithelial luteal cells, the free thecal cells and the connective tissue fibres in between the groups of luteal cells (Stage V). ×400.

„ 17. Theca interna luteal cells (Stage V) magnified to show the similarity between the theca interna luteal cells and follicular epithelial luteal cells. ×400.

„ 18. T. S. of the solid corpus luteum (Stage VI) showing the general rupture of the tubules. ×80.

N.B.—All figures have been drawn with the Camera lucida. Text-Figs. have been reduced to half and Photomicrographs by one-eighth the magnifications given.

KEY TO LETTERING

b.ca. .. Blood capillaries.
b.v. .. Blood vessel.
c.tu.th.i. .. Cells of the tubules of theca interna.
ch.g. .. Chromatic granules of luteal cells.
cn.t.fi. .. Connective tissue fibres.
cn.t.ig. .. Connective tissue ingrowths.
d.l.c. .. Degenerating luteal cell.
en. .. Endothelium.
f.e. .. Follicular epithelium.
f.e.c. .. Follicular epithelial cell.
f.e.fo. .. Follicular epithelial fold.
f.e.l.c. .. Follicular epithelial luteal cell.
f.e.fo. .. Follicular epithelial fold.
fg.th.i. .. Finger-like ingrowths of the theca interna.
fr.th.c. .. Free thecal cell.
ho.l.c. .. Homogeneous staining area of the cytoplasm of the luteal cells.
ig.th.ex. .. Ingrowths of protoplasmic cells of theca externa.
l.c. .. Luteal cells.
la.c. .. Large cell of follicular epithelium.
m.p. .. Membrana propria.
s.c. .. Small cell of the follicular epithelium.
sm.va. .. Small regular vacuoles of luteal cells.
th.ex. .. Theca externa.
th.fl. .. Theca folliculi.
th.i. .. Theca interna.
th.i.l.c. .. Theca interna luteal cell.
tr.th.i.c. .. Transforming theca interna cell.
tu.th.i. .. Tubules of theca interna.
z.r. .. Zona radiata.

1084.43—Printed at The Bangalore Press, Bangalore City, by G. Srinivasa Rao, Superintendent,
and Published by The Indian Academy of Sciences, Bangalore

INDEX TO VOL. XVII (B)

AUTHORS' INDEX

TITLE INDEX