THREE NEW RECORDS OF NEMATODE WORMS FROM PUNJAB AND THE UNITED PROVINCES

In the paper are recorded *Schwartziella nodulosa* (Schwartz, 1928) and *Trichostrongylus colubriformis* from buffaloes and *Capillaria bovis* from buffaloes and goats in Punjab and U.P. Specimens were collected from U.P. in February 1944 from buffaloes slaughtered at Bareilly. *Capillaria bovis* was recovered in August 1944 from two goats received from the General Disease Investigation Section of the Imperial Veterinary Research Institute, Izatnagar. Examination of the intestines of buffalo calves in Punjab was undertaken in October 1944 and in Bareilly it was continued to nodules only.

Schwartziella nodulosa

As the parasite is economically important its incidence was investigated and in the table are given the numbers found in the nodules as well as those lying free in the intestinal lumen respectively.

Number of Parasites

(*a*) Embedded in nodules—
31, 5, 11, 88, 44, 35, 19, 31, 10, 25.

(*b*) Free in the lumen—
69, 48, 18, 51, 90, 52, 50, 89, 22, 65.

At Sialkot all the twenty calves ranging in age from one to two years harboured the parasite. A very large number of adult buffaloes examined at Izatnagar were found to be free from worms.

The parasite was originally described by Schwartz (1928) as *Cooperia nodulosa* from a carabao. Le Roux (1936) and Travassos (1937) transferred the species to their newly created genera Schwartziella and Paracooperia respectively. Matoff (1938) records the worm from Bulgarian buffaloes and states that it occurs in the nodules in the cæcum also. The author has examined a large number of nodules from cæcum but has never met the parasite. Instead œsophagostome larvæ have always been found.

Capillaria bovis

The parasites were collected from buffalo calves and goats in small numbers, five being the largest met with in a calf. The parasite has not previously been collected from these animals in this country.

Host—*Capra hircus* and *Bos bubalus*.
Location—Small intestine.
Locality—Izatnagar, U.P.

Trichostrongylus colubriformis

The parasite was twice collected from calves at Izatnagar. It has not been previously recorded from this animal.

Host—*Bos bubalus*.
Location—Small intestine.
Locality—Izatnagar, U.P.

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Le Roux, P. L., *Jl. Helm.*, 1938, **14** (2), 113–18. Matoff, K., *Helm. Abstracts*, 1938. **7**, No. 260. Schwartz, B., *Proc. U.S. Nat. Mus.*, 1928 **74**, Art 20. Travassos, L., *Monograph Do Inst. Oswaldo Cruz.*, 1937, **1**, 1–511.

ACETYLCHOLINE TRANSMISSION AT NERVE ENDINGS

During experiments on frogs' stomach in December 1944 and January 1945, it was noticed that stomach muscle of certain frogs were absolutely refractory to the action of acetylcholine in 10⁻⁶ to 10⁻⁵ dilutions, even after treatment with eserine (1 in 10⁶ to 1 in 10⁵), though they responded normally to alternating current, direct current and potassium. As this seemed anomalous, it was decided to investigate the action of acetylcholine on other tissues, such as the heart and rectus abdominis of the same frog, the stomach muscles of which were refractory to the action of acetylcholine. The following effects were observed:

1. *A Natural Atropine Effect.* The stomach was inexcitative to acetylcholine (1 in 10⁶ to 1 in 10⁵), even after treatment with eserine. The heart was also found refractory even after eserinization. In the former contraction was produced by electric current, and in the latter complete standstill was produced by vagus stimulation. The rectus abdominis was sensitive; so also dog stomach and rabbit gut, thus eliminating any doubt regarding the potency of acetylcholine (BDH) employed.

2. *"Acetylcholine Escape".* In these hearts acetylcholine may have a temporary mild inhibitory effect; the muscle, however, soon adapts, and normal beats are restored after four to five contractions in the continued presence of acetylcholine. Before return to the normal the beats are augmented. The recovery is not due to destruction of acetylcholine as shown by the fact, that change of solution has no effect. Further if the muscle has adapted to low concentrations of ethylcholine (1 in 10⁶) higher concentrations (1 in 10⁵ to 1 in 10⁴) are rendered inert. Inhibition is produced if acetylcholine is alternated with Ringer solution. These results are identical with those found in plain muscle (Singh, 1942). After adaptation to acetylcholine, the vagus stimulation still brings the heart to a standstill.

3. *Augmentation of the Beats.* Far from producing inhibition, the beats were augmented. The heart may become hyperirritable in high concentrations of acetylcholine (1 in 10⁴), as shown by numerous extra systoles. There may be even contracture as found in plain muscle.

4. *Effect of Initial Length.* The inhibitory effect of acetylcholine is augmented by increase in initial length of the heart fibres. Acetylcholine which may produce no effect on the heart may produce inhibition, if the latter is distended by increasing the perfusion pressure. These effects are identical with those found in plain muscle, wherein it was found that with increasing length of fibres, not only contraction but also inhibition was enhanced.

In the above experiments, purely mechanical effects may produce identical results, an increase in perfusion pressure may prevent the heart from contracting, and so simulate inhibition. The effects of acetylcholine, therefore, should be controlled by saline only and by the action of atropine. Moderate distension of the heart thus favours excitation as well as inhibition. This is necessary as otherwise when

the excitability is increased, the heart may not relax properly.

5. Acetylcholine in small concentrations potentiates the response to electric current in frog stomach (Singh, 1939). It is possible that chemical transmission potentiates electrical transmission.

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Singh, I., *J. Physiol.*, 1939, **96**, 367; *Ind. Journ. Med. Res.*, 1942, **30**, 629.

A NEW BACTERIAL LEAF-SPOT ON *PIPER BETLE*

A NEW type of bacterial leaf-spot disease was observed on *bangla* variety of pan (*Piper betle*) at Ramtek during December 1943. So far, under field conditions, the disease has not been observed to occur on *kapuri*, *kakher* and *gangari* varieties. The mode of infection and symptoms of this leaf-spot disease are entirely different from those described by Raghunathan (1926, 1928), Park (1934), Nirula (1931) and Asthana and Mahmud (1944). As far as known to the authors this disease has not so far been reported from anywhere else.

The earliest symptom of the disease is the presence of extremely minute pale-yellow spots either on the lower or upper sides of the leaves. Within a couple of days the initial spots turn dark-purple in colour and are slightly raised. There are no corresponding spots or discolouration on the other side of the leaf. Yellow coloured zones with water-soaked areas are not formed round these spots as in the leaf-spot disease described by Raghunathan (1926). The spots may appear inbetween the veins or on or along the veins. In the former cases they are more or less round or roughly angular while in the latter they are irregularly elongated or branched like fern leaves. They vary considerably in size, measuring from 1 mm. to 1 cm. across, and are generally apparent only on one side of the leaves. In cases where rotting has advanced considerably these spots are visible on both the sides. The infected leaves gradually turn yellow and fall off. The disease has not been observed to cause any damage to roots, stems or petioles.

Healthy leaves of *kapuri* and *bangla* varieties were artificially inoculated by pure cultures of the pathogen. Inoculations were either carried out by spraying the leaves with a suspension of the bacterium in sterile distilled water or simply by smearing the leaves with the pure cultures of the organism. Under both the methods positive results were obtained on either of the varieties. *Kapuri* variety proved as susceptible to the disease as *bangla*. Under moist conditions the symptoms of the disease appeared within 12 to 18 hours. Infection appeared with equal readiness on both the sides of the leaves of all ages. On re-isolation the same pathogen was isolated from all the infected leaves. Inoculations of roots, stems and petioles gave negative results.

Parenchymatous cells are chiefly infected, the pathogen being intra-cellular. In the earlier stages of infection the organism is found only in the epidermal cells but later on it invades spongy and palisade cells. In highly advanced stages of rotting the pathogen is occasionally seen in the phloem and xylem vessels as well, though mostly in the former. The invaded cells slightly enlarge in size, turn dark-lemon and disintegrate. The leaves turn yellow and gradually drop off. Two to three spots are enough to kill a leaf. In some cases the parasite enters the host through the stomata but in others the entry appears to be directly through the epidermal wall. There are no stomata on the upper side of pan leaves of *bangla* and *kapuri* varieties yet the entry of the organism is easily effected by spraying or keeping a small bit of inoculum there. In some cases the organism has been found to enter through the stomata on the underside of the leaves while in others the epidermal cells are clogged but the neighbouring stomata and the cells beneath them are absolutely free of it. All these show that the presence of stomata or wound are not at all essential for the entry of this bacterium into the host cells.

On bouillon-agar plates the organism produces sky-white colonies within 12 to 18 hours which later on turn maize-yellow. The colonies are round, thin and flat, glistening and marked with ridges. In texture they are dry and brittle. The colonies have a distinct central area surrounded by an outer ring and a lobed margin; the lobes occasionally branching out fern-like and appear fan-shaped. On bouillon-agar streak the growth is echinulate in formation but in stab cultures it is filiform.

The pathogens are rod-shaped bacterium, measuring $1{\cdot}2 \times 2{\cdot}5\,\mu$ and occur in pairs or in chains of 3 to 12 or even more cells at a time. They are fairly motile both in solid and liquid media and in young and old cultures. Twenty-four to 48 hours-old cultures show very brisk motility of the sinuous swimming type, rarely straight and frequently with spells of quick and sudden tumbling on the short axis. At 30° to 38° C., the sporulation, even in young cultures, is in abundance. The spores remained viable even when they were heated to 90° C. for ten minutes.

The characteristics of the pathogen as described above are quite different from those of *Bacterium betle* Reg. (1926, 1928). It is presumed to be a new species and is provisionally named *Bacillus betle*.

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November 17, 1944. K. A. MAHMUD.

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