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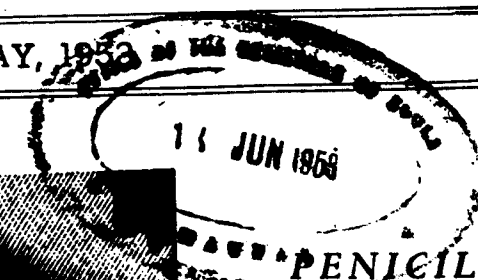
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THE
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PUBLISHED BI-MONTHLY 1482
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Vol. XXIX

MAY, 1953

No. 6



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Madras,
20th May 1953)

EDITOR.

Vol. XIX

May, 1953

No. 6

THE INDIAN VETERINARY JOURNAL

[THE JOURNAL OF THE ALL-INDIA VETERINARY ASSOCIATION]

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(ESTD. 1924)

EDITOR

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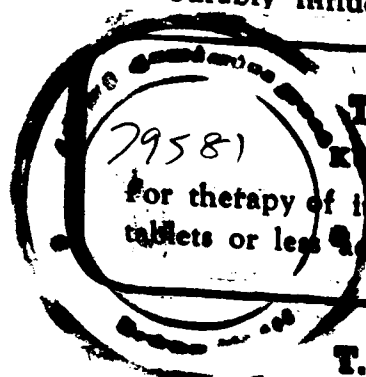
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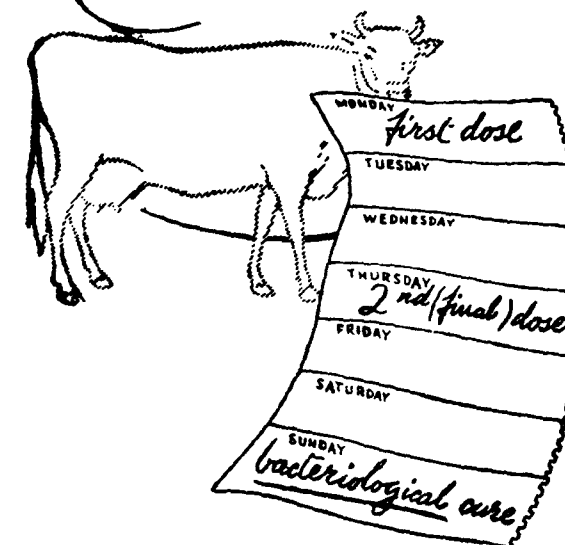
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MAY 1953

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Original Article

A STRIGEID, *DIPLOSTOMUM DUBOISI* N.Sp., FROM THE INDIAN KITE *MILVUS MIGRANS GOVINDA* SYKES

By

M. ANANTARAMAN

and

G. BALASUBRAMANIAM,

Helminthology Research Section, Madras Veterinary College.

The study of Strigeid trematodes from Indian hosts has received considerable attention in recent years. Bhalerao (1942) has reviewed the literature on these forms incorporating his criticisms about the identity of some of them. Of the fifty-nine species he has listed, a very large number are parasites of birds, and the genus *Diplostomum* is represented by three species, *D. ketupanensis* Vidyarthi 1937, *D. butei* Vidyarthi 1937 and *D. thapari* (*Neolalaria thaparia* Lal 1939) Bhalerao 1942, all of them from birds belonging to the group *Accipitres*. Nazmi Gohar (1932) has also recorded *D. tregenna* from a similar host, *Milvus migrans aegyptius*, outside India.

In the present paper, an account is given of a *Diplostomum* from the Indian Pariah kite *Milvus migrans govinda*. Fourteen specimens of this parasite were present in the intestines of a bird found dead in Madras and formed the basis of this description.

The body is in two distinct halves, the division between them being narrow and marked. The fore-body is shorter than the hind-body, flattened, oval in shape with rounded corners. There is a conspicuous curvature of the dorsal surface. The posterior margin occasionally shows an infolding ventrally to form a shallow cavity. The hind-body is flat and leaf-like, broadly rectangular in outline with a narrow posterior end.

On each side of and close to the oral sucker is a prominent muscular organ—the lateral accessory sucker or pseudo-sucker—epaulette-like in appearance. The acetabulum is transversely oval and situated on the ventral median line just in front of the hold-fast organ. The latter is a

large oval muscular organ with an irregular outline; the inner margin is slightly folded and encloses a longitudinal slit. Proteolytic glands are visible at its posterior region.

The mouth is triangular, surrounded by a sub-terminal oral sucker followed immediately by a sub-globular pharynx with its lumen slit-like in optical section. On either side of the pharynx are three rows of nuclei suggesting some accessory glands. The oesophagus is very short, often inconspicuous, bifurcating into the lateral caeca, each caecum extending posteriorly into the hind-body where it is often indistinct owing to the crowded genital components.

The male and the female genitalia are confined to the hind-body. The anterior testis is L-shaped and consists of a large arm on the right side of the worm with another extending across to the left side. The posterior testis is symmetrically bilobed and H-shaped, composed of two elongated lateral lobes connected anteriorly by a distinct isthmus and tapering gradually to the posterior end. Both testes show distinct lobulations on the surface.

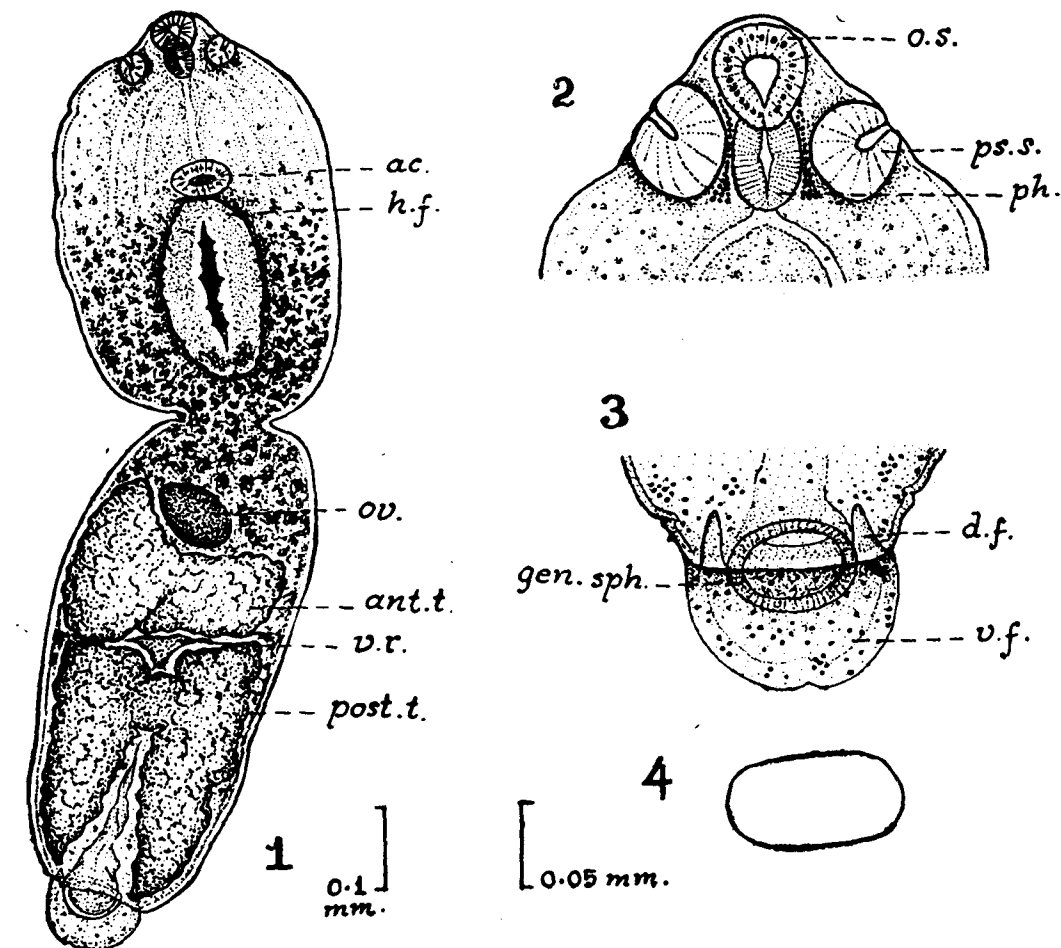
The ovary is located behind the 'hip' a little to the right of the median line. It is compact, globular in the lateral view and oval in the frontal view.

Vitellaria are distributed in both segments of the body in follicles but concentrated in the posterior half of the fore-body and the middle region of the hind-body. The vitelline reservoir is roughly triangular and lies on the median line between the anterior and the posterior testes.

The uterus, median in position and convoluted in its course, shows very few eggs. Eggs are oval and soft-shelled.

The posterior end of the worm is represented by the bursa copulatrix, made up of a large semicircular ventral flap and a smaller but broader dorsal flap. The genital opening is prominent, sub-terminal, directed dorsalwards and bounded by a strong sphincter which gives the appearance of a genital prominence or cone.

Apart from measurements, *D. tregenna* (Nazmi Gohar 1932) differs from this in having a hind-body triangular in outline and smaller than the fore-body and in the shape of the testes and hold-fast organ. *D. ketupanensis* (Vidyardhi 1937) differs in the shape and relative sizes of both segments of the body, in the ovary being median and sausage-shaped, and anterior testis being oval and confined to the side. *D. butei* (Vidyardhi 1937) also has an almost circular fore-body and a broadly oval hind-body; the hold-fast organ is circular and the anterior testis is dumb-bell shaped. In all these three, the posterior margin of the fore-body is turned in ventrally to a far greater extent than was observed in the present species. In *D. thapari*



ILLUSTRATIONS

Fig. 1. *Diplostomum duboisi* n.sp.—entire worm-ventral view.
2. do. anterior end magnified.
3. do. posterior end magnified.
4. do. egg.

ac—acetabulum.	ov.—ovary.
ant.t.—anterior testis.	ph.—pharynx.
d.f.—dorsal fold.	post.t.—posterior testis.
gen. sph.—genital sphincter.	ps.s.—pseudo-sucker.
h.f.—hold-fast organ.	v.f.—ventral fold.
o.s.—oral sucker.	v.r.—vitelline reservoir.

(*Neolalaria thaporia* Lal 1939) Bhalerao 1942, the division between the two halves of the body is indistinct, the hold-fast organ is trough-shaped, and the testes are dumb-bell shaped.

Actual measurements made on three largest worms are as follows:—

Total length	1,015	1,103	1,050
Forebody (Length X maximum Width) ...	438 X 350	455 X 368	438 X 368
Hind body (Length X maximum Width)...	578 X 333	648 X 298	613 X 280
Oral sucker ...	56 X 64	52 X 52	60 X 68
Pharynx ...	40 X 52	32 X 44	48 X 60
Pseudo-sucker ...	56 X 40	48 X 40	48 X 32
Ventral sucker ...	40 X 56	48 X 60	52 X 64
Hold-fast organ ...	152 X 100	164 X 96	128 X 124
Ovary ...	92 X 48	104 X 60	88 X 76
Ant. Testis ...	263 X 140	280 X 175	210 X 105
Post. Testis ...	315 X 123	350 X 105	350 X 88
Egg ...	80 X 52	76 X 40	64 X 48
Dist. between ovary and post. end ...	490	543	508
Dist. between Ant. end and Ant. end of ventral sucker ...	180	184	188

(All measurements in Microns)

The species under consideration is therefore regarded as a new one and will be named *Diplostomum duboisi* sp. nov. as a tribute to the contributions of Dr Georges Dubois of the University of Neuchatel, Switzerland, to this group of trematodes.

It is also to be noted that *D. duboisi* possesses a remarkable resemblance to *Bolbophorus orientalis* (Vidyarthi 1938) from *Buteo rufinus rufinus*. *B. orientalis*, however, differs from *D. duboisi* in having an oval fore-body pointed at the anterior end, a fusiform hind-body, the acetabulum located within the hold-fast organ and the anterior testis having an irregular V-shape. Dubois (1938) and Bhalerao (1942) consider *B. orientalis* to be a species of *Neodiplostomum*, doubting the presence of pseudo-suckers. It could as well be relegated to the genus *Diplostomum*, without having to question the presence of the pseudo-suckers, if it should be argued that the genital sphincter as observed in *D. duboisi* had been mistaken for a genital cone. Indeed, Vidyarthi (1937) himself describes only an *inconspicuous* genital cone.

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General Articles

THERAPY AND PROPHYLAXIS OF INDIAN EQUINE AND BOVINE TRYPANAOSOMIASIS BY 'ANTRYCIDE' FORMULATIONS

By

H. N. RAY, M.Sc., Ph.D., M.A.S., F.Z.S.I., F.N.I.,
Indian Veterinary Research Institute, Mukteswar,

G. V. SHORT, M.R.C.V.S.,
Imperial Chemical Industries Ltd.,

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Indian Veterinary Research Institute,
and

PHILIP A. HAWKINS, A.B., M.A., Ph.D., D.V.M.,*
Michigan State Veterinary College, U. S. A.

Introduction

Curd and Davey (1950)¹ reported the preparation of 4-amino-6 (2-amino-1:6-dimethylpyrimidin-4-amino) -1:2-dimethylquinolinium, of which four salts were first prepared, i.e. the dibromide, diiodide, dichloride and dimethylsulphate. As the first two behaved like the dichloride, only the last two were adopted. Dimethylsulphate is readily soluble up to 33% in water, but the dichloride is sparingly soluble to about 0.12%. It was claimed that the dimethylsulphate solution, injected subcutaneously, rapidly gave trypanocidal blood levels, while the dichloride, similarly given, formed a depot under the skin persisting for several weeks and affording protection for this time against artificial infection.

* Since deceased

These salts were subsequently prepared by Imperial Chemical (Pharmaceuticals) Ltd. as 'Antrycide' methylsulphate for therapy, and 'Antrycide' pro-salt for therapy and prophylaxis. The last contains the dimethylsulphate and dichloride salts in the proportion of 1.5:2.

Evaluation.—To assess the usefulness of these formulations in the treatment and control of trypanosomiasis in India due to *Trypanosoma evansi*, the following work was done.

A. Equine.—Three ponies were artificially infected with *T. evansi*. Their blood became positive after five days. Two ponies of 266 kg. and 300 kg. body weight were injected subcutaneously at 5 mg./kg. methylsulphate in 10% solution at seven days and four weeks after infection respectively. The number of parasites was 20-30 per microscopical field in fresh cover-glass preparations. Treatment of the second pony was delayed to allow extension of the disease and to test the efficacy of the drug in a heavily infected animal. The third pony was left as a control. Twenty-four hours after treatment the peripheral blood of both the treated ponies was microscopically negative, while the control remained positive until it died six weeks after infection.

Five c.c. blood from each of the treated ponies were injected into guinea-pigs after two and five weeks respectively. They remained negative for six weeks when the test was stopped. Blood sera of treated ponies were negative to complement fixation test after three months. The subcutaneous injection of the drug did not cause any toxicity either immediately or later.

Eighteen cases of natural equine trypanosomiasis in surrounding villages were successfully treated at the Indian Veterinary Research Institute (I.V.R.I.). In four cases the blood sera of the treated ponies were negative to complement fixation test after two, three, four and six weeks respectively. The remaining cases could not be followed critically, although the owners reported complete recovery.

B. Bovine.—(i) A preliminary test was done on three hill bulls (body weight 118-134 kg.) by injecting 1.75 Gm. 'Antrycide' pro-salt in 17.5 c.c. sterile distilled water in June, 1950, (=13 to 14.8 mg./kg.) with no toxic effects. Two of these animals were positive to complement fixation and stilbamidine tests (Ray, 1950) prior to treatment and were negative to both the tests one month later. A syringe challenge of one 10 weeks after treatment failed to yield positive serum when tested at 12 weeks, while the other, challenged at three weeks after treatment, gave a weak positive reaction to stilbamidine at seven weeks, though it was negative to all other tests.

All the three bulls were negative to all tests six months after treatment.

- (ii) Two well-grown cross-bred cows were each given 3.5 Gm. 'Antrycide' pro-salt in 15 c.c. sterile distilled water with no after-effects.
- (iii) To test the possibility of any additive toxic effect of 'Antrycide' in cattle undergoing rinderpest immunisation, six hill bulls were inoculated with attenuated rinderpest virus. A dose of 3.5 Gm. pro-salt was given to two simultaneously; to another two after three days, and to the two remaining after six days. No toxicity was seen during the following month.
- (iv) Six healthy Haryana cattle of the Izatnagar Dairy were given brisk running exercise in the sun during June, 1952, immediately before and after injection of 3.5 Gm. pro-salt subcutaneously. No ill-effects were seen, and the temperatures and general condition remained normal for the following month.
- (v) An assessment of latent surra was planned from February to May, 1950, with the co-operation of the Director of Animal Husbandry, U. P. Unfortunately the final evaluation of this work was prevented by a supervening infection, possibly of rinderpest, but figures are given of the latent trypanosomiasis found in Table I.

TABLE I

Species.	Total samples of serum.	Positive.	Percentage.	
Buffaloes.	400	160	40%	(By C. F. T. alone).
Cattle.	270	171	63%	(By all three tests).

Sera from 400 buffaloes were tested by complement fixation, and from 270 cattle by complement fixation, allergy, and stilbamidine tests. The technique of the allergic test is given below:

Surra antigen prepared from rats at Mukteswar at 1:4000 in normal saline dilution was injected 0.1 c.c. intradermally. Positive reactors showed a swelling, sometimes painful, at the site of inoculation. Negative cases showed no swelling. Skin measurements were taken with callipers before and after injection. 125 out of 270 were found to be positive to this test. (46%).

- (vi) Complement fixation and allergic tests were repeated on 450 cattle in May, 1951, with the co-operation of the Milk Commissioner-Cum-Administrator, W. Bengal, at the Government Cattle Breeding Farm, Haringhata.

99 (22%) positive reactors were divided into three groups of 33 each for treatment with (a) methylsulphate, (b) pro-salt and (c) as controls. The breeds were Haryana (92), Tharparkar (4), Sahiwal (2) and Buffalo (1).

Reference to the work of Curd and Davey (1950) on 9 strains of trypanosomes from Africa, Sudan and India showed that whereas *T. evansi* (Sudan) in mice broke through 2.5 mg./kg. methylsulphate (10 out of 34), *T. evansi* (Sind) was entirely susceptible to this concentration (13 cases) (see Table III). The I.C.I. workers estimated that 3 mg./kg. methylsulphate should give adequate control of all Indian strains, and this dosage rate was adopted. Groups (a) and (b) were treated in October, 1951.

Group (a) Methylsulphate.—10% sterile aqueous solution was given subcutaneously at the following rates: 200–350 kg. body weight 9 c.c.; over 350 kg. 12 c.c. Cattle in advanced pregnancy, on the border line between the first and second weight groups, were given 9 c.c. as being more suited to their true body-weight.

Group (b) Pro-salt.—35 Gm. of this formulation was shaken up in 120 c.c. sterile distilled water until suspended and the volume made up to 150 c.c. Of this final suspension the same dosage was given as for group (a).

Injection in both groups was given behind the shoulder below the hump, and the site was massaged for 15 seconds afterwards. No abortions occurred in treated pregnant cattle.

All three groups were retested by complement fixation in January, 1952. The results are shown in Table II.

TABLE II

Group.	Pre-treatment Tests.	Treatment.	Post-treatment C. F. Test.
(a) 33	+++	Methylsulphate.	—
(b) 33	+++	Pro-salt.	—
(c) 33	+++	Controls.	16 +++ 4 ±

13 controls showed a negative complement fixation, which may be presumed due to a drop in antibody titre or auto-sterilisation. (Mudaliar and Ray, 1947)².

Immediate and Delayed effects.—Any drug used for trypanosomiasis therapy or prophylaxis in cattle must be free of immediate or delayed toxicity or depression on (i) milk yield and (ii) body weight. To assess these factors records of (i) and (ii) were kept and analysed for a full year before treatment and a full year after treatment (up to October 1952) for groups (a), (b) and (c), making allowances for pregnancies and dry periods.

Immediate effects:—There were no local or systemic reactions in groups (a) and (b). Treated cattle continued to feed normally and there was no fluctuation in the milk yield.

Delayed effects:—(i) **Milk yield.**—A scrutiny of the year's records after treatment shows that whereas 7 control cows (6 negative on re-test in January, 1952) gave an increased average milk yield, 13 in group (a) and 13 in group (b) gave an increased average. 23 controls gave a decreased average milk yield (12 positive on retest), and 19 in each of group (a) and (b). In the treated groups, 31 intervening cases of mastitis were considered responsible for the drop in milk yield. In group (c), 15 cases of mastitis occurred.

(ii) **Body Weight:**—Analysis under this head is rather more difficult for milch cattle than for draft cattle, because of recurrent gestation. A significant fact, however, emerges from the figures, namely, that, of the 27 controls showing a decreased average body-weight, 16 were positive on re-test in January, 1952.

The resistance of various strains of trypanosomes to chemotherapeutic substances differs so widely that a close study of protocols is necessary to arrive at legitimate conclusions. The data given by Curd and Davey¹ are reproduced in Table III for ready reference.

TABLE III

Curative action of 'Antrycide' against various species of Trypanosomiasis in mice

Dose mg./kg. s/c.	T. rhodesiense (Tinde).	T. gambiense.	T. brucei (laboratory).	T. brucei (Mariani).	T. equiperdum.	T. equinum.	T. evansi (Sind).	T. evansi (Sudan).	T. congolense (Busimbi).
25	8 C	10 C	6 C	7 C					
12.5	8 C 14 R	21 C	3 C 4 R	16 C					
5	3 C 33 R	11 C 8 R	4 C 17 R	8 C 7 R	7 C	8 C	6 C	15 C	
2.5	30 R	4 R	22 R	2 C 6 R	18 C	16 C	13 C	24 C 10 R	15 C
1.25					22 C	15 C	16 C	2 C 16 R	40 C

Numbers indicate the number of mice cured (C) or relapsed (R)

Mice were infected by intraperitoneal or intravenous injection and were treated subcutaneously by a single dose of 'Antrycide' dichloride or methylsulphate within 48 hours after infection. Mice remaining free from clinical trypanosomiasis 30 days after treatment were regarded as cured.

From these data it will be seen that *T. evansi* (Sind) was killed by significantly lower concentrations than *T. evansi* (Sudan), so that the dosage of pro-salt recommended and used in this work (3 mg./kg.) should be quite adequate to ensure prolonged trypanocidal blood levels after the methylsulphate content of the dose has been excreted.

The excretion of 'Antrycide' was studied (Spinks 1950)* in four lots of rabbits, each given subcutaneous injections of either the methylsulphate (13.2 mg./kg) or the chloride (10 mg./kg) (equal doses of 'Antrycide' base). The results are given in terms of percentage of the dose recovered in urine per day in Table IV.

TABLE IV
Percentage of dose excreted on days 1-12

Salt	1	2	3	4	5	6	7	8	9	10	11	12 Days
Methyl sulphate ...	10	4	2	1	0.46	0.4	0.3	0.2	0.1	0.1	0.11	0
Chloride ...	0.9	1.1	1	0.8	0.7	0.36	0.26	0.26	0.2	0.18	0.18	0.16

Toxicity.—The high experimental dosage of pro-salt given successfully to three hill bulls (13 to 14.8 mg./kg. para. B(i) above), considered with the published reports, indicates a wide variation in toxic concentrations. Evans (1950)* reported the spectacular success in the Sudan of methylsulphate at 5 mg./kg. in more than 2,50,000 cattle exposed to infection by *T. congolense* and *T. vivax*; while six healthy cattle (370-500 kg. body weight) were given 12 mg./kg. in experimental toxicity tests in England. Salivation and occasional tremors were seen, but all were normal the following day (Davey 1950)*.

One factor that recurs in toxicity reports is the handling of cattle before and particularly after treatment, especially in tropical heat. Davey* showed that cattle mishandled by being chased in hot sun succumbed (3 out of 5) to 2.5 mg./kg. with symptoms akin to heatstroke; while Lewis (Priv. rep. quoted by Davey*) in Kenya reported similar deaths in two cattle chased for some time after receiving 2 gm. pro-salt.

On the other hand the experimental mishandling of healthy cattle without ill-effects, reported in para. B (iv) above, suggests that such reactions may be expected only in infected cattle.

Pellegrini and Bonelli (1951)* in Somaliland reported successful large-scale field trial and laboratory infection tests on cattle, camels, sheep, dogs, cats, horses and mules with methylsulphate at 5 mg./kg. against six strains of trypanosomes, including *T. evansi* (Sudan). The pro-salt, at recommended doses, protected cattle against re-infection for a maximum of six months.

Burdin and Plowright (1952)* conducted toxicity trials on E. African Zebu Cattle. They showed toxicity at 7.5 mg./kg. and death at 10 mg./kg. (presumably of methylsulphate), but no reaction to 5 mg./kg.; the animals receiving this last dose showed no signs of liver or other organic change on post-mortem examination.

Bukshi and others (Priv. communication 1952) report the use of both formulations in an outbreak of bovine surra in Uttar Pradesh in which 14 animals had died prior to treatment. Two clinical cases were given methylsulphate at 3 mg./kg. and about 400 exposed cattle and buffaloes were given pro-salt in appropriate doses. No local or systemic reactions were seen except in one animal given methylsulphate in which slight shivering occurred eight minutes after injection, but passed off within five minutes.

Some local swelling has been reported by various workers but, in our experience, these have been confined to the neck and were most probably aggravated by friction from tethering ropes, chains, and byre yokes. Injection behind the shoulder and below the hump, with massage is followed by good absorption, and no external evidence of the dichloride depot is seen after pro-salt use. Methylated spirit is preferable to Tincture of Iodine for skin sterilisation as being less irritant to the animals' skin, and also to the inoculator's fingers when a large number of injections have to be given.

Conclusions.—We know that Indian cattle infected with *T. evansi* remain carriers of the disease for approximately six months and are a continuous source of infection (Sen, 1936)*. We also know, from data available at the I. V. R. I. for 1948-50 inclusive, that the loss due to deaths from surra only in all species was Rs. 1,50,000 annually, in addition to a much greater loss from decreased working capacity in draft animals and milk yield in milch cattle.

In our study of trypanosomiasis aetiology we must bear in mind the ability of these protozoa to rapid adaptation to trypanocidal substances and of the consequent danger of drug-fast strains. The history of control measures is eloquent of such failures with arsenicals, antimonials, the flavines, trypan blue and, in a few instances, suramin and its analogues, especially in the virulent strain introduced by army animals from Burma during the last war. Immediate sterilisation of the peripheral circulation

is attended by some risk of cerebral embolism. A prolonged inhibitory action on trypanosome division is preferable, by which the whole reticulo-endothelial system and not just the peripheral circulation is sterilised. Ormerod (1951)¹⁰ studied the action of 'Antrycide' on the division of trypanosomes in mice and found that their infectivity in a treated mouse became markedly reduced as early as 45 minutes after dosing.

The control of surra in India is conditioned by (a) cost and (b) practicability. Cost must be assessed not only on the initial cost of the drug used but also on its therapeutic and prophylactic efficiency, since any relapses of infective strains occurring behind the inoculation "front" cancels all the work previously done. Retreatment, in such cases, is faced with the additional difficulty of a strain possibly rendered drug-fast to some degree by the previous ineffective treatment. The use of drugs with this intrinsic disability cannot be considered economical, however cheap they may be initially, and their use should be discouraged.

Practicability in the control of an endemic disease, such as surra in India, demands the quickest and simplest administration technique, when it is remembered that we have about one veterinarian to each 1,00,000 animals. Drugs requiring intravenous injection, therefore, have had to be accepted as a necessary evil in the past, with the risks of phlebitis attending accidental perivascular injection in intractable animals.

In the Antrycide formulations we have, for the first time, products of great promise for the cure and control of this disease by subcutaneous injection. We have shown that it can be used safely and effectively at the recommended dosage of 3 mg./kg. methylsulphate content with no relapse up to 3 months, and no toxicity at this concentration has been reported when animals have been properly handled. It is essential that infected cattle for inoculation shall be allowed to settle down if driven to a collecting point before treatment and shall have 12 to 24 hours' rest in shade afterwards, especially in hot weather. A plentiful supply of drinking water must be provided. No deleterious effect is produced on the milk yield and there is evidence to show that increased milk yields and body weights may be expected from cattle freed from trypanosomiasis by 'Antrycide' or auto-sterilisation.

'Antrycide', at the recommended dosage, therefore, has the following advantages over the arsenicals, antimonials and suramin and its analogues hitherto used:

- (a) It is of low toxicity, either local or systemic, immediate or delayed.
- (b) It is given by a single subcutaneous injection.
- (c) It has a wide latitude of dosage in properly handled animals.

- (d) The cost of treatment for the average animal (200-350 kg.) is approximately 25% (methylsulphate) and 50% (pro-salt) of the cost of a suramin course of treatment.
- (e) The methylsulphate gives a useful residual protection.
- (f) The pro-salt gives a solid protection for some months against Indian strains of *T. evansi*.

Summary

116 Cattle and 21 horses clinically or experimentally infected with *T. evansi*, were treated by Antrycide, methylsulphate and pro-salt in concentrations, varying from 3 mg./kg. to 14.8 mg./kg. without local or systemic reactions. In one trial, 99 cattle reactors were divided into equal groups for treatment by methylsulphate, pro-salt and controls. All treated cattle were negative to complement fixation test three months later while the controls showed 48 per cent positive and 12 per cent doubtful. Milk records for a year after treatment indicated an increased yield from negative cattle, either treated or auto-sterilised; while body-weight records showed a decrease in 81 per cent of the controls, of which 59 per cent were positive to complement fixation test three months after the start of the trial.

3 mg./kg. methylsulphate content was a safe and efficient dose for both formulations.

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STUDIES ON THE BLOOD OF MULES*

II Chemical

BY

GANTI. A. SASTRY, and M. R. DHANDA,

Department of Pathology, Indian Veterinary Research Institute
Mukteswar-Kumaon, India.

In these studies, the calcium, inorganic phosphorous, magnesium and protein contents of the sera of ten mules were estimated. The modern trend of appraisal of immunity being in terms of globulin, especially its gamma fraction, the different fractions of globulin were estimated in terms of alpha, beta and gamma.

Material and methods.—Blood was collected in the morning before feed at about 9 A.M. in wide test tubes, 6" x 1". After separation, the serum was centrifuged for 10' @ 3,000 r.p.m. and the clear serum was used for different estimations.

Calcium.—Clark-Collip modification of Tidsall method (1925) of estimation of calcium was adopted.

Inorganic phosphorous.—Fiske and Subbarow's (1925) method was used.

Magnesium.—Hawk, Osler and Summerson's (1947) modification of Dennis method was used.

Proteins.—As the procedure adopted is not found in any of the Indian journals, a detailed account of the same is rendered here.

This method is essentially that of Wolfson, Cohn, Calavary and Ichiba with slight modifications.

Reagents.

23% Sodium sulphate solution.—Dissolve exactly 23 grams of *anhydrous* sodium sulphate in distilled water at 37° C. Make up to 100 ml. with distilled water and store in an incubator at 37° C.

28% Sodium sulphite solution.—Dissolve exactly 28.0 grams of *anhydrous* sodium sulphite in distilled water at 28° C. The salt is difficultly soluble but can be dissolved with sufficient shaking. Make upto 100 ml. with distilled water and store at room temperature.

Saline ammonium sulphate solution.—In one liter volumetric flask dissolve 193 grams of ammonium sulphite in about 500 ml. of distilled water. Add 30 grams of sodium chloride, dissolve and make upto one liter with distilled water. Store at room temperature.

* Continued from page 405, previous issue.

Biuret reagent. Weishellbaum.—Prepare an accurately titrated 0.2 N. NaOH solution. Dissolve 90 grams of Rochelle salt in about 400 ml. of the 0.2 N. NaOH. Following solution, add 10 grams of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. When this copper sulphate has entirely dissolved, add 10 grams of potassium iodide and make up to 2 liters with 0.2 N. NaOH. Store in a rubber stoppered waxed bottle.

Standardisation.—A standard protein solution was made with pooled normal cattle serum, the protein content of which was accurately determined by the Kjeldahl method.

Using normal saline as a diluent, standards were prepared with the above mentioned serum with protein concentration of about 1.0, 2.0, 4.0, 6.0 and 8.0 gms. Total protein concentration of each dilute standard was determined by the total protein procedure given below. A standard curve from the data obtained was constructed. This standard curve was used in all the determinations detailed hereunder.

*Determinations.*1. *Serum total protein.*

- Pipette 0.4 ml. of serum into a test tube and add 9.6 ml. of distilled water from a burette. Mix well by inversion.
- Transfer 5 ml. to another test tube and add 5.0 ml. of biuret reagent and mix well by shaking.
- Prepare the blank with 5 ml. of distilled water and 5 ml. of biuret reagent. Save this blank for use in the determinations of albumin plus alpha globulin and gamma globulin.
- Let the solution stand at least 30 minutes. Since the colour is quite stable, reading may be deferred for as much as 24 hours. Read on the photoelectric colorimeter or spectrophotometer at a wave length of 540 millimicra.

2. *Serum Albumin plus alpha-globulin.*

- Place 4.6 ml. of 23% sodium sulphate solution in a test tube.
- Pipette 0.4 ml. of serum and mix thoroughly by inversion.
- Filter with a No. 42. whatman filter paper.
- Withdraw 2.5 ml. of the clear filtrate and transfer to a test tube. Add 2.5 ml. of distilled water and 5. ml. of biuret reagent and mix well by inversion. The blank is that required for total protein.
- After standing for at least 30 minutes read on the photoelectric colorimeter at a wave length of 540 millimicra.

3. Serum albumin.

- (a) Place 9.6 ml. of 28% sodium sulphite solution in a test tube.
- (b) Pipette 0.4 ml. of serum and mix well by inversion.
- (c) Filter with a No. 42 whatman filter paper.
- (d) Withdraw 5 ml. of the clear filtrate into a test tube and add 5 ml. of the biuret reagent. Mix well by inversion.
- (e) Prepare a blank by adding 5 ml. of the biuret reagent to 5 ml. of the sodium sulphite solution.
- (f) After standing for at least 30 min. read on the photoelectric colorimeter at 540 millimicra wave length.

4. Serum gamma-globulin.

- (a) Pipette 9.6 ml. of the saline ammonia sulphate solution into a sturdy 15 ml. centrifuge tube. Layer 0.4 ml. of the serum on top of this solution. Mix the two components by careful, slow, repeated inversions. Continue mixing until, within a minute or two, the gradually developing visible turbidity had reached an apparent maximum.
- (b) cork the tube securely and centrifuge at 2500 to 3000 r.p.m. for 30 minutes.
- (c) Taking extreme care not to disturb the precipitate, gently turn the uncorked tube to the horizontal and permit the supernatant to run off. No attempt should be made to ensure complete removal of the entire supernatant at this time.
- (d) Return the uncorked tubes to the centrifuge and spin at 3000 r.p.m. for 5 min.
- (e) Slowly invert the tubes and let them stand in this position on a layer of filter paper for a few minutes.
- (f) Add 5.0 ml. of the biuret reagent and 5 ml. of distilled water to the tube and shake briskly for 30 seconds. Let it stand for 15 minutes, centrifuge down any slight residual turbidity remaining and decant the supernatant into a test tube.
- (g) Use the blank as in the total protein.
- (h) After standing for at least 30 minutes, read on the photoelectric colorimeter at 540 millimicra. Divide the protein value by 2 to obtain the serum gamma-globulin concentration.

From the values obtained in 1, 2, 3 and 4 above, the Alpha, Beta and total globulins can be calculated.

The values obtained are embodied in Table 1.

TABLE I

Normal chemical constituents of the blood of Mules and Horses.

Animal.	Calcium in mgm. %	Inorganic Phosphorus in mgm. %	Magnesium in mgm. %	Total Protein gms. %	Albumin gms. %	Total Globulin gms. %	Alpha Globulin gms. %	Beta Globulin gms. %	Gamma Globulin gms. %	Albumin Globulin ratio.
Mules.										
17	14.4	2.10	1.94	7.7	3.6	4.1	0.55	1.2	2.05	0.88
19	12.76	2.0	2.20	7.3	3.6	3.7	1.0	1.25	1.55	0.973
21	14.58	2.57	2.0	7.0	3.6	3.4	1.2	1.0	1.2	1.06
16	13.86	2.93	2.17	7.0	3.9	3.1	1.2	0.7	1.2	1.26
20	13.68	2.82	1.98	7.3	3.6	3.7	0.85	1.3	1.55	0.973
25	12.61	2.81	1.98	7.0	3.4	3.6	0.95	1.2	1.45	0.944
8	13.68	2.75	1.82	6.3	3.6	3.7	1.25	1.5	0.95	0.973
24	14.22	2.31	1.77	7.6	3.4	4.2	0.95	1.575	1.675	0.809
11	15.12	2.13	2.30	7.0	3.15	3.85	0.90	1.4	1.55	0.813
15	13.5	2.01	2.1	7.0	3.4	3.6	0.75	1.3	1.55	0.944
Horses.										
464	13.86	2.81	2.03	7.0	3.4	3.6	0.75	1.55	1.3	0.944
555	13.5	2.97	2.1	6.5	3.4	3.1	0.7	0.85	1.55	1.09
584	13.32	2.72	2.25	7.5	3.15	3.85	0.95	1.225	1.675	0.813
579	15.57	2.45	2.13	7.7	3.4	4.3	0.95	1.675	1.675	0.79
575	13.86	2.83	1.61	7.2	3.7	3.5	0.82	1.13	1.55	1.06
416	13.50	3.03	1.93	7.8	3.15	3.85	1.35	1.25	1.3	0.813
573	13.50	2.5	2.1	7.5	3.6	3.9	1.2	1.4	1.3	0.926
574	13.69	3.09	1.93	6.4	3.15	3.25	0.95	0.95	1.35	0.969
488	13.86	2.54	1.81	7.5	3.4	4.1	0.95	1.475	1.675	0.83
471	13.32	2.4	2.13	7.4	3.6	3.8	0.95	1.175	1.675	0.95
Range.										
Mules.	12.76 to 15.12	2.0 to 2.93	1.77 to 2.3	6.3 to 7.7	3.15 to 3.9	3.1 to 4.1	0.75 to 1.25	0.7 to 1.575	0.95 to 2.05	0.809 to 1.26
Horses.	13.5 to 15.57	2.4 to 3.03	1.61 to 2.25	6.4 to 7.8	3.15 to 3.7	3.1 to 4.3	0.7 to 1.35	0.85 to 1.675	1.3 to 1.675	0.813 to 1.09

Discussion

From Table I it would be apparent that the calcium and phosphorus contents of the sera of mules are slightly lower than those of horses. The gamma globulin content of the sera of mules seem to be higher than those of horses.

This being probably the first time that the above constituents of sera of mules have been estimated, the values obtained by us could not be compared with the observations of other workers.

Summary

The calcium, inorganic phosphorus, magnesium and protein contents of the sera of ten mules were estimated and reported here. For comparison, these constituents of the sera of horses were estimated.

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MELANOMATA IN DOGS*

By

GANTI A. SASTRY, G.M.V.C., B.V.Sc.*

Indian Veterinary Research Institute, Mukteswar.

Melanomata, consisting of "definitely specialised cells known as melanoblasts, which produce pigment called melanin" (Feldman, 1932) are rarely observed in dogs, in contrast to their occurrence in the grey horses, which die of this tumour if they live long enough (Jaeger, 1909) and in human beings in whom these are "among the commonest of tumours" (Taylor and Tuttle, 1944)

The rarity of this tumour may be much better appreciated from the following account: Frohner (1895) could not find even a single melanoma out of 2289 canine neoplasms examined by him; Folger (1917) observed only three melanomas out of 2537 tumours he examined; of the

*Read at the 40th Indian Science Congress, Now at the Madras Veterinary College, as Assistant Lecturer in Pathology.

53 canine neoplasms examined by Crocker (1919) not a single one was melanoma; Feldman (1926, 27 & 28) examined 86 neoplasms of the dogs and found but three to be melanomata; seventy canine neoplasms of Cohrs (1927) did not contain even a single melanoma. A solitary tumour designated as "melanocytoblastoma" was encountered by Auler and Wernick (1931) among 585 neoplasms that had come to their notice. A subsequent collection by Feldman (1932) of 78 tumours of the dog, consisted of a single melanoma. For many years, Christiansen (1933) noted only one 'alveolar mixed melanosarcoma', though nearly 250 to 300 dogs were autopsied every year at the Royal Scandinavian Veterinary College. Of the 72 canine neoplasms in Jackson's collection (1936), but five were melanosarcomas. Mangrulkar (1944) failed to see even a single melanoma among 400 neoplasms, the collection of the Indian Veterinary Research Institute, Mukteswar. During the course of three years, Whitney (1945) found eleven melanocarcinomas among 145 canine tumours. Mulligan's (1949) collection of one thousand canine tumours included only forty-two melanomata. McFadyean's collection (1930) of two thousand neoplasms he bequeathed to the Royal College of Veterinary Surgeons, London, contained 78 melanotic tumours from "horses, cattle, sheep, dogs and cats", but details as to how many of these are canine could not be obtained. They cannot, in any event, be more than five.

Besides the above authors, Bunker (1882), Gray (1900), Roberts (1909), Ball & Cuny (1911), Petit and Germain (1912-13), Sammon (1913-14), Samson (1913-14), Taylor (1916), Seddon (1916-17), Vawter (1919), Petit (1919), Kansas Vety. College (1919), Pillai (1928-29), Chambers (1931), Mc Clelland (1941), Fedrigo (1942), Bloom (1942), Brock (1944), Hopper (1945), Ottosen (1947), Pires (1947-48), Rautmann, Leon and Brachmuller have reported the finding of this neoplasm in dogs. Of these, Mc Clelland had reported four and Ottosen fifteen and the rest one each. So, the total number of melanomata (benign and malignant combined) reported in available literature are but one hundred and twelve, including one of mine detailed below, (in computing this total, it is assumed that the collection of McFadyean might probably have included Gray's (1900) as it was McFadyean who had diagnosed it as such).

Report of a case.—The subject was a three year old male, non-descript terrier dog, admitted as an in-patient at the Madras Veterinary College Hospital on 12-1-1942, with a tumour on the external surface of the left thigh, subcutaneously, about four inches from the stifle joint. The dog had very extensive patches of melanosis on the skin, some discrete and others more or less confluent. The tumour on the thigh

was incised for biopsy and revealed a "melanotic sarcoma". As the condition of the animal did not improve, it was destroyed on 6-2-42.

Autopsy findings.—The carcase was of a well nourished male dog, with a fair amount of subcutaneous fat. There was an abscess on the left side of the upper jaw and the corresponding submaxillary lymphatic glands were swollen, somewhat, consequently. On section, these glands were darker, cellular and 'juicy'.

The spleen contained six nodular lesions, upto the size of a grape, in the pulp, causing a varying degree of projection of the splenic surface. The malpighian corpuscles were more prominent than normal. The organ itself appeared but only slightly enlarged and pale.

The nodules were firm to soft and were smoky grey. On incision they were more or less dark and homogeneous. There was no demonstrable capsule and the nodules had to be dissected out as they were too adherent to the parenchyma and would not 'shell out'.

All other organs appeared normal and metastases were not noticed elsewhere.

Microscopical appearance.—The primary tumour on the thigh presented no difficulty to diagnose as a malignant melanoma, as it was dark and most of the cells contained the pigment. The cells were, for most of the part, spindle shaped (Pl. 29, Fig. 1). Those of the metastases in the spleen varied widely in histological picture. Most of the cells in these nodules were polyhedral and some were oval. Those containing the pigment were spindle as in the primary tumour. Even in the same field one could see clearly the two types of cells polyhedral and spindle shaped the latter containing the pigment (Pl. 29, Fig. 2). The cells were packed in sheets without either a whorl, or alveolar arrangement, that is so characteristic of human neval melanomata. Inter-cellular substance was not noticeable, nor was there much of connective tissue stroma. In the more anaplastic type of cells, melanin was not demonstrable and the nuclei were large, eccentrically placed with much fragmented chromatin. Well-developed nucleoli (in most of the cells two) could be seen. The n : N ratio was about 1 : 10. Mitotic figures were rather frequent. In the less anaplastic type of cells, melanin was observable and these cells were elongated. The cytoplasm of these cells was much obscured by the melanin pigment present. Well-developed blood vessels were not present but primitive capillaries were present in sufficient numbers to ensure a good supply of blood. In some situations, the tumour cells themselves seemed to make the lining of the capillaries. Dendritic forms were not observed.

Incidence of Melanomata in dogs.—The age, sex and breed distribution of melanomata for which detailed information is available is given in Table I.

TABLE I

Serial No.	Author.	Breed.	Sex	Age.	Benign or Malignant.	Site of primary Tumour.	Site of metastases.
1.	Ball and Cuny (1911-12).	Pug.		7	M.	Internal toe of left paw.	Subcutis and internal organs.
2.	Petit and Germain (1912-13).	French Poodle.		—	M.	Undersurface of tail.	In all organs.
3.	Sammon (1913-14).	Gordon Setter.		5	M.	Toe of left fore leg.	On the skin and all organs except liver.
4.	Taylor (1916).	Spaniel.		3 or 4	B?	Interdigital space.	
5.	Seddon (1916-17).	Irish Terrier.		1	M.	—	All organs except liver.
6.	Vawter (1919.)	—		8	M.	Prepuce.	All organs.
7.	Kansas Vet. College 1919.	M		8	M.	Sheath.	All organs.
8.	Pillai (1928-29).	Airdale.		Middle aged.	M.	Hock and Foot subcutaneously.	All organs.
9.	Chambers (1931).	Aberdeen Terrier.	M	10	M.	Inguinal region.	?
10.	McClelland (1941).	Chow.	M	8	M.	Metacarpal region.	Popliteal, inguinal and mesenteric lymph nodes and lungs.
11.	do.	Chow.	M	10	M.	Dorsum of tongue.	Fraenum linguae, cervical lymph nodes and lungs.
12. } & 13. }	do.	Boston terriers.		—	B	Choroid.	No metastases.
14.	Bloom (1942).	Chow.	M	1-1/2	?	?	?
15.	Brock (1944).	Scotch terrier.	M	11	M.	Lip.	No necropsy conducted.
16.	Hopper (1945).	Scotch terrier.	F	6	M.	Fundus of eye.	Lungs, kidneys and brain.
17.	Mulligan 1948.	German shepherd.	M	14	B	Left metacarpus.	—
18.	do.	Boston terrier.	F	9	B	Right hock.	—
19.	do.	Airdale	M	14	M.	Right buccal and ventrum of tail regions.	—
20.	do.	Chihuanhau	F	9	B	Two cutaneous tumours.	—
21.	do.	Boston terrier.	M	12	M.	Right axilla.	—
22.	do.	Pekingese	F	12	B	Eyelid.	—
23.	do.	Scotch terrier.	M	2	B	Right metacarpus.	—
24.	Sastry (1953)	Terrier Non descripti	M	3	M.	Left thigh.	Spleen.

From Table I, it would appear that the incidence of this tumour is slightly higher in male dogs. The breeds commonly affected are the Boston terrier, Scotch Terrier and Airdales, though for a matter of that, any dog with a heavily pigmented skin may be greatly predisposed for this tumour, contrary to what is obtained in horses and man, in whom the grey horses and white man are more susceptible than the black or brown horses and the Negro respectively. Gray (1900) is of the opinion that Yorkshire Terrier is particularly susceptible for melanomata but none of the other authors have corroborated his view.

Finding that Airdale Terriers were particularly susceptible for neoplasms, Passey (1938) tried tar applications on the skin of these animals and was able to produce malignant melanomata in two dogs.

Again from Table I, the highest incidence is between the ages of 7 to 14, what Mulligan (1949) calls the 'canine cancer age'. The table also reveals that the majority occur in the head, thorax and the extremities. The eye and the soft tissues around the oral cavity are particularly prone to be affected by this tumour.

Metastasis and Malignancy.—Melanomata are the most malignant of all the neoplasms. The extensive distribution of secondary tumours in a case suggests that metastasis occurs by every available means. The organs most commonly affected secondarily are: the lungs, liver, kidneys and spleen, but no organ is immune from metastasis as it has been found even in the brain (Feldman, 1932). Though most of the internal tumours may be secondary in origin, still one should be alive, cautions Feldman (1932), to the possibility of primary tumour arising from aberrant collections of melanoblasts in such organs.

Melanotic deposits occur occasionally in such organs like the liver and lungs and so the mere presence of this pigment should not be construed as evidence of the presence of metastatic melanomata. The criterion is the demonstration of the presence of melanin producing tumour cells in such places.

Nature of the melanin pigment and histogenesis of melanomata.—Melanin pigment, produced by the melanoblast, (which all agree to give rise to melanomata), is a metabolite, that is the outcome of an intra-cellular enzymic action on certain melanogens, having chemical structure similar to Tyrosine, and Adrenaline. It is thought that the mother substance from which both adrenaline and melanine are formed is one and the same and that excess of this substance that is unused in Addison's disease is diverted to the skin where it is oxidised by the melanoblasts into melanin, bronzing ensuing thereby. (Boyd, 1947)

Ropeshaw (1933) suggests that the intra-cellular reaction of cystine on protamine results in the formation of melanin. As the

sulphydril compounds are present only in the epithelial layer, this process is limited to that layer of the skin. The cystine contained in the glutathione of the cytoplasm and the protamine of the chromatin are freed by enzymic cleavage. The reaction of cystine on protamine is aerobic and required the catalysation of iron, which is present in protamine.

The nature of melanin formation has been demonstrated by Bloch, Dihydroxyphenylalanine (DOPA for short) from the broad bean is converted into a black substance by certain cells, which he called Dopa positive. These cells are supposed to be true melanoblasts containing the oxidising enzyme. Dopa is supposed to be similar to the chromogen of the body. In amelanotic melanomas, the cells are Dopa positive, while the melanophores of the dermis are Dopa negative and so they are only mechanical carriers of the pigment but are not involved in the production of the same. The melanoblasts are present in the basal layer of the epidermis as branching cells, in the ciliary body of the choroid coat of the eye, in the pia-mater, in the retina and the medulla and zona reticularis of the adrenal (Jacobsen and Klinck, 1934).

The histogenesis of the melanoblast is still a vexed question and agreement has not been reached yet. There are two main schools of thought regarding its derivation: the mesodermal and the neuro-ectodermal. The adherents to the former are Post (1894), Ribbert (1887), Beattie and Dickson (1926) and Cowdry (1932, 1934). The ectodermal derivation was first proposed by Recklinghausen (1887) and later on by Unna (1893), supported by Soldan (1899), Dawson (1925), Mason (1926), Ewing (1930), Foot and Zeek (1931), Foot (1932), Laidlaw (1932, 1933) Jacobsen and Klinck (1934) and Taylor and Tuttle (1944). The latter school substantiate their theory by demonstrating exodermal elements in the melanomata arising from the naevi. Though the presence of naevi in animals (at least in horses) is denied (Jaeger) the cells of some varieties of tumours as found in animals were of such histology that the tumours had to be classified as melanoepitheliomas: McFadyean (1890), Caylor and Scholtthaeur (1926), Thomas (1929) and Jackson (1936).

Dawson, Innes and Harvey (1939) consider malignant cutaneous and ocular melanomata to be epithelial in origin while choroidal and meningeal melanotic cell aggregations to be of mesodermic pigment cell type. But Strong (1947) is of the view that primary melanomata of leptomeninges are of neuro-ectodermal in origin.

The observation of Stewart and Bonser (1948) that the epithelial cells can produce melanin when stimulated functionally, for eg. in the tumours of skin like benign calcified epitheliomas, basal cell

carcinomas and squamous cell carcinomas, confounds much an already confusing problem.

Jaeger (1909) from his extensive studies in equine melanomata concludes that the tumour cells are modified fibroblasts and hence the tumours genuine spindle cell sarcomas. Though this may be true for certain equine melanomata, it may not be tenable for tumours from all animals.

From the foregoing account it would be plain that no definite agreement has been reached regarding the genesis of the primary tumour cell and so it is advisable to designate all benign melanomata as melanomas and malignant varieties, malignant melanomas, as advocated by Feldman (1932) and Boyd (1947), until such time when the exact histogenesis will have been established. And so it is that Ewing (1934) gives a non-committal definition to these tumours: "melanoma is a pigmented tumour arising from a specific mesoblastic cell, the chromatophore, probably also from tactile cells lying in the epidermis and probably also from the nerve cells in the derma".

The opinion of Wright (1949) that trauma probably plays a part in the production of malignant melanomas is still to be confirmed though most of the workers believe that the benign tumours, sometime, sooner or later, may get transformed into the malignant variety and so radical excision of such are advocated.

It is known that soft rays with longer wave-lengths (ultraviolet, alpha and beta) and some times X-rays, stimulate the activity of melanoblasts. Increased pigmentation also results from exposure to actinic rays when the skin has been sensitised by substances such as impure naphthalene and creosote (Becker and Obermeyer, 1940). The question of therapeutic agents photosensitising the animals may have to be borne in mind as a possible cause of these tumours.

Diagnosis.—The diagnosis of this tumour is not difficult. A biopsy is always needed to diagnose if the tumour is benign or malignant. One may be confronted, some times, with the difficulty to decide whether a tumour in question is benign or not. The following Table II, compiled from Feldman's (1932) data, may be of use in this respect.

Green's method (1940, 1941) of transplating in the anterior chamber of guinea pig's eye is useful to diagnose amelanotic melanomas, when the tumour cells (though amelanotic in the original tumour) produce melanin. (Mohiyuddin, 1949)

Influence of heredity. Only two instances (not in dogs) of the influence of heredity in the transmission of this neoplasm are recorded.

Melanomata in Dogs by Ganti A. Sastry

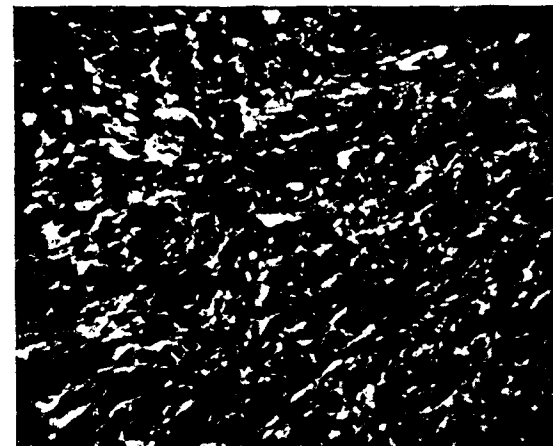


Fig. 1
Showing cells frankly spindle shaped with much pigment.
H & E (X 300)

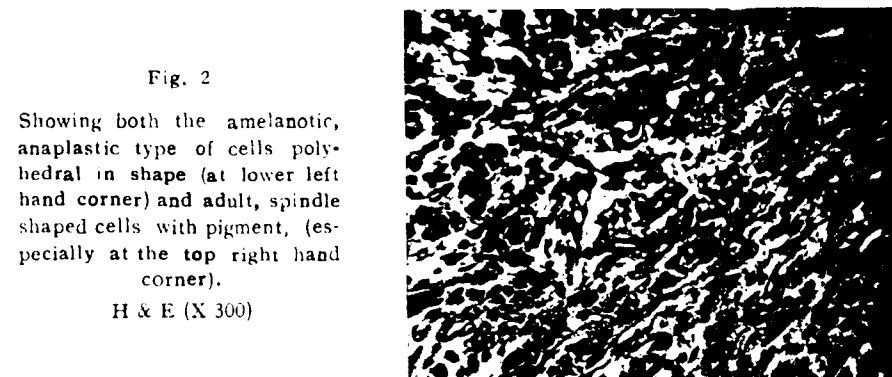


Fig. 2
Showing both the amelanotic, anaplastic type of cells polyhedral in shape (at lower left hand corner) and adult, spindle shaped cells with pigment, (especially at the top right hand corner).
H & E (X 300)

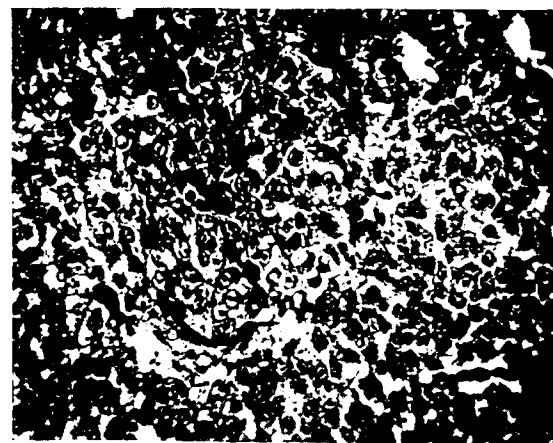


Fig. 3
Showing a highly anaplastic type of cells, distinctly polyhedral in shape, with little or no pigment. Mitotic figures can be seen.
H & E (X 300)

Cultivation of Berseem by Y. C. Gupta



Fig. 1.
General view of Berseem Crop.



Fig. 2.
Berseem Crop is being harvested with
bullock reaper.



Fig. 3.
Berseem Crop is being harvested with manual labour.



Fig. 4
Berseem Crop is hung on wires for hay
making.

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The first is Virchow's grey stallion, which was responsible for the transmission of melanomata to many of its descendants, specially the grey ones. The next is that of Caylor and Schlotthauer's Duroc hogs. Two out of a litter of 3, of a sow with a melanoma at the flank, developed similar melanomas at the identical site.

TABLE II

Distinguishing features of Benign and Malignant tumours.

BENIGN.	MALIGNANT.
1. Usually single.	1. Usually multiple.
2. Encapsulated mostly.	2. Not encapsulated usually.
3. Younger animals affected.	3. Animals in 'cancer age' affected mostly.
4. No recurrence after removal.	4. Recurrence after removal likely to occur and metastasis probable.
5. Most of the cells contain much melanin.	5. Many cells free of pigment.
6. Cells usually ovoid or spindle shaped. (not large)	6. Cells polyhedral and larger than normal.
7. Much stroma present.	7. Minimal amount of stroma present.
8. Mitotic figures not seen.	8. Mitosis common.

Comment. The material on hand was not sufficient enough to help to elucidate the vexed question of the genesis of the melanoblasts in this tumour. One point of interest in the case described here is the diffuse areas of melanosis on the skin of the dog, resembling the spots of a Dalmatian. Though there are extensive spots in the Dalmatian breed, it is very interesting to note that not one dog was reported to have had this tumour. The reason for this may be that this breed is not so favoured as others, for among 669 dogs examined by Mulligan (1949) only 5 were Dalmatians. It would be really interesting if all the veterinarians were to keep an 'eye' on the animals of this breed for the incidence of this neoplasm among them.

Summary

1. Melanomata as occurring in dogs is reviewed.
2. A case report of a malignant melanoma is detailed.
3. The theories of the histogenesis of the melanoblasts, without any attempts to elucidate that vexed question, are furnished.
4. Three photomicrographs are furnished.

Acknowledgments

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FAECES AS TEST-MATERIAL FOR PREGNANCY DIAGNOSIS
IN MAMMALIA

BY

Mrs. K. HARRIS, M.A.,

Department of Biology, Madras Veterinary College, Madras.

Introduction.—Following Bhaduri's work (1951) on faeces as test-material for diagnosing pregnancy in animals, this work was undertaken with a view to ascertain its practical application in Veterinary Field. Bhaduri prepared aqueous extracts of the dung of various farm animals and injected male toads and examined their cloacal fluids for sperms. He injected the extracts only into toads which did not show any sperm in their cloacal fluid. After the injections, the cloacal fluid was drawn at intervals and examined for sperms. Up to 20 c.c of faecal extract was injected within a period of about 2 hours. He waited for about 4 hours for the emission of sperms in the cloacal fluid. If there were no sperms, a negative reaction was recorded. Even if there were very few sperms, he claimed a positive reaction. He said that only faecal extract of pregnant animals produced a positive reaction in the male toad and the

faecal extracts of non-pregnant animals did not produce positive results. So he claimed that faeces can be taken as a test-material for pregnancy diagnosis in farm animals.

Materials and methods.—A measured quantity of faeces was mixed with a known quantity of distilled water and left in the frigidaire for nearly 36 hours. This time limit may be reduced up to 12 hours according to one's convenience, but at least twelve hours are essential for a definite result. The mixture was then decanted and allowed to filter through ordinary filter paper. The apparatus thus set for filtering was left in the frigidaire. This precaution was taken to prevent decomposition. Usually in about 12 hours enough filtrate was available for the injections.

Usually the faeces samples were collected from the early morning faeces. The quantity of faeces was measured by volume by filling up a glass dish marked to hold 250 c.c. This measured sample was mixed with 500 c.c. of distilled water. The same proportion was maintained even when the faeces sample had been of a semi-solid consistency. To obtain a concentrated extract the amount of distilled water added was reduced. Normally samples were collected from fresh dung. Sometimes the sample was taken directly from the rectum of the animal. The faeces samples were not always free from urine. Specially, this was the case with the faeces of guinea-pig.

Bufo melanostictus was the test animal used. The male toads were isolated and their cloacal fluids were examined microscopically. The toads which did not show any sperm were injected subcutaneously with the faecal extracts. The initial dose was 10 c.c. After an interval of half-an hour the second dose of 5 c.c. was given. For the third dose of 5 c.c. often it was necessary to wait for 45 minutes or even more. If the fluid already injected was not absorbed, the third dose was delayed to a longer interval. The site of injection did not matter much as long as the fluid was injected subcutaneously. The first dose of 10 c.c. was given in 5 c.c. amounts on each side, injected from the dorsal surface. The second and third doses were given ventrolaterally.

It was not possible to inject more than 20 c.c. successfully. Invariably the toad died immediately after receiving 25 c.c., whenever attempted. So there was not even a single occasion to record the reaction with 25 c.c. Similarly more concentrated faecal extracts also failed to produce any useful result. The concentrated extract proved so toxic that the toad died within a short period. Some times when the toad did not die, it became inactive and no cloacal fluid or urine could be collected from such toads. Even if collected the cloacal fluid was negative for sperms but contained numerous red blood corpuscles. During

post-mortem such toads had revealed conspicuously empty bladders with the result one might conclude that the toxicity had some inhibitory action on the kidneys of the toads. Those toads which showed sperms in the cloacal fluid or urine anytime within six hours after the first injection were recorded as having shown positive reactions.

Tests on various animals.—Dung samples from cows, she-buffaloes, bull-calf, breeding bulls, bullocks, ram, male and female guinea-pigs, horses, mares and asses were tested. The details of the tests are as follows:—

1. *Non-pregnant cows.*—Dung samples of three non-pregnant cows were tested at 7 different times. Strong positive results were obtained on 6 occasions. The calves of these cows were all about a month old at the time of testing, vide Table I.

TABLE I
Non-pregnant Cows.

Serial No. of Tests.	Date on which dung was collected.	Diet and other particulars about the animal.	No. of toads used.	Result.	Details of Results.
1	2-8-51	Belonging to Madras Veterinary College; fed on wheat bran, Bengal Gram Husk, Ground-nut oil-cake, Paddy Straw, Bone Meal, Shark Liver Oil, common Salt. Allowed to graze for sometime daily	1	+ve	With 20 c.c. after 3 hrs.
2	4-8-51		1	+ve	With 15 c.c. after 1½ hrs.
3	6-8-51		1	-ve	With 20 c.c.
4	9-8-51		1	+ve	With 20 c.c. after 3½ hrs.
5	17-7-51	Belonging to a local milkman.	1	+ve	With 20 c.c. after 3½ hrs.
6	20-7-51	Feeds mostly on grass besides other types of food.	2	+ve	With 10 c.c. after 1 hr.
7	10-8-51	Belonging to a local milkman Grass—a main item in its rations.	1	+ve	With 10 c.c. after 1 hr.

2. *Pregnant cow.*—One pregnant cow belonging to the Madras Veterinary College indicated a pregnancy of about four months on clinical examination. Its dung was tested nine times. Four times negative results and five times positive results were obtained. The first 8 tests were within a period of 40 days. The last test was a positive one 42 days afterwards. Once the positive result was obtained after a feed of a bundle of lawn grass (*Cynodon dactylon*). Nine days later it again gave a negative result, vide Table II.

TABLE II
Pregnant Cow.

Serial No. of Tests.	Date on which dung was collected.	Diet of the Cow.	Result.	No. of toads.	Details of results.
1	9-7-51	Usual College diet as in the case of of the non-pregnant cow.	+ve	1	With 20 c.c. after 3½ hrs.
2	14-7-51	Do.	+ve	2	With 10 c.c. after 1 hr.
3	18-7-51	Do.	-ve	1	With 20 c.c.
4	23-7-51	Do.	-ve	2	With 20 c.c.
5	2-8-51	Do.	-ve	1	With 20 c.c.
6	6-8-51	On 5th evening fed with a big bundle of grass in addition to its usual food. Dung collected on 6th morning.	+ve	2	With 15 c.c. after 1½ hrs.
7	8-8-51	Usual diet	+ve	2	With 10 c.c. after 1 hr.
8	15-8-51	Usual diet	-ve	1	With 20 c.c.
9	26-9-51	Usual diet	+ve	2	With 20 c.c. after 3 hrs.

3. *Bull calf*.—One bull calf about one and half years old gave positive results twice and negative result once, out of the three occasions tested within a period of eight days. It was fed on Bengal-gram husk, ground-nut oil-cake, rice bran, common salt, bone-meal, shark liver oil, and was allowed to graze on the fields for sometime daily—vide Table III.

TABLE III
Bull Calf.

Se. No. of Tests	Date on which dung was collected.	Results.	No. of toads used.	Details of results.
1	8-8-51	+ve	2	With 15 c.c. after 1½ hrs.
2	11-8-51	+ve	1	With 15 c.c. after 1½ hrs.
3	15-8-51	-ve	1	With 20 c.c.

4. *Breeding bulls*.—The dung of five breeding bulls produced strong positive results. They were fed with rice-bran, Bengal-gram husk, ground-nut oil-cake, paddy straw, bone-meal, shark-liver-oil and alfalfa. They were not allowed any grazing—vide Table IV.

TABLE IV
Breeding Bulls.

Serial No. of Tests.	Date on which dung was collected.	Bull No.	Results.	No. of toads.	Details.
1	9-8-51	Ongole Bull 289	+ve	1	With 10 c.c. after 1 hr.
2	15-8-51	Bull No. 448	+ve	2	With 15 c.c. after 1½ hrs.
3	18-8-51	Bull No. 447	+ve	2	With 15 c.c. in 1½ hrs. With 20 c.c. in 3 hrs.
	2-9-51	Bull No. 447	+ve	2	With 10 c.c. after ½ hr. With 10 c.c. examined after 3 hrs. only.
5	2-9-51	Conjeevaram Bull	+ve	2	With 10 c.c. after ½ hr. With 10 c.c. examined only after 3 hrs.

With a view to ascertain whether heat had any action on the agent causing the male toad reaction, the dung of Conjeevaram bull was mixed with distilled water in the usual proportion and allowed to boil for about three minutes. It was then filtered and the clear fluid was injected into toads. In one hour, after an injection of 10 c.c. a positive reaction was obtained. Similarly the dung of Sindhi Bull No. 14 was boiled and the extract was filtered and injected. Forty minutes after the injections of 6.5 c.c. of the extract, a positive reaction was obtained. It appears that the agent causing the Male-toad-reaction is not destroyed by heat up to boiling point.

5. *Buffaloes*.—The dung samples of one pregnant buffalo and one non-pregnant buffalo were tested. Both produced positive reactions. In addition to the usual fodder both were fed a big bundle of grass each evening. The dung was collected early in the morning—vide Table V.

TABLE V
Buffalo.

Serial No. of Tests.	Particulars about the animal.	Date of Dung Collection.	Result.	No. of toads	Details of results
1	Pregnant buffalo mated on 17-3-51	3-7-51	+ve	2	With 15 c.c. before 1½ hrs. With 10 c.c. after 1 hr.
2	Non-pregnant with a calf a month old.	10-8-51	+ve	1	

6. *Horses*.—The dung samples of three horses and two mares were tested. All produced strong positive reactions. From their owners, it was found that they were mostly fed on grass. Vide table VI.

TABLE VI
Horses.

Serial No. of Tests	Date on which faeces was collected.	Particulars about the animals.	Result.	No. of toads.	Details of results.
1	27-7-51	Mare	+ve	2	With 20 c.c. after 3 hrs.
2	1-8-51	Mare	+ve	1	With 15 c.c. after 1½ hrs.
3	7-8-51	Horse	+ve	1	With 15 c.c. after 1½ hrs.
4	7-8-51	Horse	+ve	1	With 20 c.c. after 3 hrs.
5	4-8-51	Horse	+ve	1	With 15 c.c. after 1½ hrs.

7. *Asses*.—Faeces of one pregnant ass, one non-pregnant ass and one jack ass were tested. Pregnancy and non-pregnancy were merely the versions of the owners. The animals were not examined clinically. They were all let on the fields during day. No food was given to them in their folds. All produced positive reactions in the male toad.—vide Table VII.

TABLE VII
Asses.

Serial No.	Date	Particulars about the animal.	Result.	No. of toads	Details of results.
1	12-8-51	Male	+ve	1	With 20 c.c. After 2 hrs.
2	12-8-51	Non-pregnant female	+ve	1	With 10 c.c. After 1 hr.
3	12-8-51	Pregnant female	+ve	1	With 10 c.c. After 1 hr.

8. *Ram*.—Dung of one ram belonging to the Madras Veterinary College gave positive reaction on all the three occasions tested. On the third occasion the dung was boiled with distilled water and then filtered. This also produced positive reaction, thereby indicating that heat upto boiling point does not destroy the agent causing male-toad-reaction.—vide Table VIII.

TABLE VIII
Ram.

Serial No.	Date on which dung was collected.	Food of the Ram	Result.	No. of toads.	Details of results.
1	8-8-51	Bengal-gram husk Rice bran Common Salt Groundnut oil cake Allowed to graze for sometime daily.	+ve	2	With 15 c.c. after 1½ hrs. With 20 c.c. after 2½ hrs.
2	11-8-51		+ve	1	With 10 c.c. after 1 hr.
3	14-8-51	Boiled extract.	+ve	1	With 10 c.c. after 1 hr.

9. *Guinea-pig*.—A female believed to be non-pregnant was isolated on 23rd July 1951 and kept separately. It was fed on different types of food and its faeces were tested 9 times within a period of thirty days. It gave positive results when fed exclusively on lawn grass (*Cynodon dactylon*), alfalfa, cabbage leaves and soaked skinned Bengal gram. On three occasions it was fed on a mixed diet of greens gathered from the wastage in the vegetable market. Then it gave a negative result once. On 24th August 1951 it was killed and postmortem confirmed its non pregnant condition.—vide table IX.

TABLE IX
Female Guinea-pig (non-pregnant)

Serial No. of Tests.	Date on which faeces was collected.	Diet.	Result.	No. of toads used.	Details of results.
1	24-7-51	Lawn grass	+ve	1	With 10 c.c. after 1 hr.
2	26-7-51	Lawn grass	+ve	2	With 10 c.c. Before 1 hr.
3	29-7-51	Soaked, skinned Bengal gram seeds.	+ve	2	With 15 c.c. after 3 hrs.
4	31-7-51	Cabbage leaves.	+ve	2	With 20 c.c. Before 3½ hrs.
5	4-8-51	Lawn grass	+ve	1	With 10 c.c. after 1 hr.
6	6-8-51	Mixed diet	-ve	1	With 20 c.c.
7	7-8-51	Mixed diet	+ve	1	With 10 c.c. after 1 hr.
8	11-8-51	Alfalfa	+ve	1	With 3.5 c.c. after 1 hr.
9	19-8-51	Mixed diet	+ve	1	With 10 c.c. after 1 hr.

One male guinea pig similarly gave positive results on all the five occasions tested within a period of 13 days. — vide Table X.

TABLE X
Male Guinea-pig.

Serial No.	Date on which faeces was collected.	Diet.	Result.	No. of Toads.	Details.
1	6-8-51	Mixed diet	+ve	1	With 10 c.c. after 1 hr.
2	7-8-51	Lawn Grass	+ve	1	With 15 c.c. after 1½ hrs.
3	18-8-51	Mixed diet	+ve	1	With 20 c.c. after 3 hrs.
4	8-8-51	Bengal Gram seeds, soaked & skinned.	+ve	1	With 20 c.c. after 2½ hrs.
5	12-8-51	Alfalfa	+ve	1	With 12 c.c. after 1 hr.

10. *Bullocks* :—The dung of two bullocks of the Madras Veterinary College produced positive results. They were fed on paddy straw. The dung was collected at 3 p.m. in the afternoon — vide Table XI.

TABLE XI
Bullock.

Serial No.	Date on which dung was collected.	Bullock	Result.	No. of toads.	Details.
1	29-8-51	No. 290	+ve	1	With 10 c.c. after 1 hr.
2	29-8-51	No. 359	+ve	1	With 10 c.c. after 1 hr.

Discussion :—The results did not show any variation between faeces collected from the ground and faeces collected from the rectum, as long as the faeces was in fresh condition. The results of the various tests clearly show that the positive reaction of the male toad produced by the faecal extracts of different animals cannot be taken as an indication of pregnancy. Since both male and female animals (pregnant and non-pregnant) have given positive results, it was surmised whether the food of the animals had any effect on the reaction. To elucidate this, various extracts of the usual food stuffs were injected and they also produced positive results.

Extracts of lawn grass (*Cynodon dactylon*), carrot-leaves, alfalfa (grown at Bangalore), skinned soaked Bengal gram seeds, cabbage leaves and paddy straw produced positive reaction in the male toad. The

extracts were obtained by grinding the vegetable product with distilled water and filtering through filter paper. 100 grams of vegetable product with 100 c.c. of distilled water was used. If the vegetable product was fresh and juicy, the extract was concentrated. If it was dry, the extract was diluted. By experience it was found that vegetable extracts should be used only in dilute conditions. Even then alfalfa and carrot-top were toxic in higher doses. Paddy-straw and cabbage leaves produced positive results only when injected fresh. — Vide Table XII.

TABLE XII
Vegetable products.

Serial No. of Tests.	Date.	Name of Material	Result.	No. of toads.	Details of Result.
1	18-7-51	Lawn grass, ground with distilled water and filtered	+ve	1	with 21.5 c.c. within 2 hrs.
2	19-7-51	" "	+ve	1	with 15 c.c. after 1½ hrs.
3	20-7-51	" "	+ve	1	with 20 c.c. after 3½ hrs.
4	10-8-51	" "	+ve	1	with 20 c.c. after 2½ hrs.
5	19-7-51	Carrot-top, ground with distilled water and filtered	+ve (More than 10 c.c. toxic)	1	with 10 c.c. after 2 hrs.
6	20-7-51	" "	+ve	1	with 10 c.c. after 3½ hrs.
7	27-7-51	Alfalfa leaves and stem ground with distilled water and filtered	+ve (More than 10 c.c. toxic)	1	with 10 c.c. after 4 hrs.
8	1-9-51	" " concentrated extract	+ve	1	with 5 c.c. after 3 hrs.
9	11-8-51	Bengal gram seeds, soaked and skinned, ground with distilled water and filtered	+ve	1	with 20 c.c. after 2½ hrs.
10	17-8-51	Cabbage leaves ground with distilled water and filtered	+ve (Effective only when extract is fresh)	1	with 10 c.c. after 1 hr.
11	24-8-51	Paddy straw ground with distilled water and filtered.	+ve (Effective only when extract is fresh)	1	with 10 c.c. after 1 hr.
12	24-8-51	" "	+ve	1	with 10 c.c. after 1 hr.

Distilled water extracts of wheat bran, rice bran, Bengal gram husk and bone-meal produced positive results. Similar extracts of ground-nut oil-cake and cluster-bean seeds gave negative results. Cow's urine, cow's milk and non-pregnant human urine gave negative results. Thus it appears that the male toad induced by the faecal extracts of various animals is similar to the reaction produced by the food the animal eats.

Conclusion.—In the light of the above tests, faeces cannot be taken as a reliable material for pregnancy diagnosis in mammalia. The reaction of the faeces is similar to the reactions produced by the food the animal eats.

Summary.—1. Faecal extracts of 3 non-pregnant cows, one pregnant cow, one bull calf, 5 breeding bulls, one pregnant buffalo, one non-pregnant buffalo, 3 horses, 2 mares, one pregnant ass, one non-pregnant ass, one jack-ass, one ram, one non-pregnant guinea-pig, one male guinea pig and 2 bullocks were tested on male toads. All produced positive reactions.

2. One male guinea-pig and one female guinea-gig were fed exclusively on lawn grass, alfalfa, cabbage leaves, and soaked, skinned Bengal gram. The faeces was collected every time and the faecal extracts were injected into male toads. All the tests produced positive reactions.

3. Distilled water extracts of lawn grass, alfalfa, carrot-top, cabbage leaves, paddy-straw, soaked skinned Bengal gram seeds, wheat bran, rice bran, Bengal gram husk and bone-meal produced positive reaction in the male toad.

4. The reaction of the faeces is similar to the reaction produced by the food the animal eats.

5. Faeces cannot be taken as a test-material for pregnancy diagnosis in mammalia.

Acknowledgment.

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REFERENCE

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CULTIVATION OF BERSEEM

BY

YASHPAL CHANDRA GUPTA, B.Sc. Agril. Assoc. I.A.R.I.,
Agricultural Officer,
Govt. of India, Cattle Cum Dairy Farm, Karnal.

Berseem, a leguminous fodder crop, appears to have been introduced in India during 1904 from Egypt and, from that time onwards, its cultivation is in progress in our country. Berseem is also known as Egyptian Clover. Its Botanical name is *Trifolium alexandrinum*. Cool to mild climate suits this crop, and it flourishes well in rich loamy soils. This forms an important winter fodder crop for cattle, especially Dairy animals.

Method of sowing.—The plot is given 2-3 ploughings followed with beaming. In the end a shallow operation is given before filling the plot with water for sowing. Berseem crop dies in patches, where the water stagnates in the field; hence the plots should be thoroughly levelled. Generally 20-25 pounds of berseem seed is sown per acre. The seed is sown from 1st October to 15th November and supplies green fodder from 15th November to end of May. Seeds of berseem are soaked in water overnight and then spread in the shade for a couple of hours in the morning. The seed is then mixed with culture prepared in gur solution and is broadcast in the standing water about 2-3 inches in depth in the plots. Sufficient care should be taken to drain out all surplus water from the field 24 hours after the seed is broadcasted. The crop needs irrigation every 10-15 days from October to February, and every week from March to May. Berseem crop thrives in all parts of our country.

Harvesting:—

(a) **For fodder.**—The first cutting or topping of Berseem is taken 40-45 days after sowing. Subsequent cuttings can be taken after every 30 days. Yield of first cutting is always less than the subsequent cuttings. Sarson is mixed at the rate of 1-2 chataks for every 10 seers of berseem seed. This would make up the deficiency in the first cutting. Generally 5-6 cuttings are taken. Average yield of berseem ranges between 500 to 700 maunds per acre. It can be harvested either with the help of bullock reaper or by manual labour.

(a) **For seed.**—Formerly it was thought that berseem seeds set in cooler zones only like N.W.F.P. and not elsewhere, hence for long time, seeds of berseem were imported from N.W.F.P. However, the experiments conducted at I.A.R.I., New Delhi, and its Sub-station at Karnal have proved that seed of

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berseem could be set elsewhere too. Later observations have indicated that the seed of berseem can be set almost at every place in India.

After taking 2-3 cuttings, the crop of Berseem is left for seed production. Roguing should be done thoroughly atleast twice in order to weed out chickorey etc. Chickorey is a damaging weed and unless thoroughly eliminated from the berseem seed, it will germinate with the berseem seed when sown. It will greatly effect the quality and yield of Berseem fodder.

The crop of Berseem left for seed does not require afterwards as much irrigation as for the crop harvested for fodder. When the seed matures, the crop is harvested and is thrashed either with bullocks or with power thrasher. We generally get 3-5 maunds of Berseem seed per acre.

Manuring.—Berseem is a crop which fixes nitrogen in the soil through the nodules present in the roots. It does not require any manure before sowing. The recent experiments conducted on the Berseem crop show that the application of phosphates to Berseem crop enhances the yield considerably. It is, therefore, advisable to apply Ammonium Phosphate to the standing crop of Berseem. Optimum dose recommended is 80 lbs of P_2O_5 per acre.

During 1951-52, a top dressing of fine powdered farm yard manure to the Berseem crop was given after every cutting, and higher yields of berseem fodder were obtained in comparison with other plots, which did not receive any similar top dressing.

Feed Value.—Green Berseem is an excellent succulent fodder for the Dairy cows. From 10-20 seers are easily consumed by each milch cow. However, some care should be taken in feeding green Berseem since it tends to cause tympanitis. To avoid tympanitis, mixing of dry fodder such as oats or wheat straw is recommended.

Berseem crop can also be converted into silage along with straw. Extra moisture present in the berseem crop is absorbed by oats straw, and the crop of Berseem is preserved as silage. This also affords valuable succulent fodder which could be conveniently preserved for any length of time and could be utilized whenever wanted.

Berseem crop can also be preserved as hay. There are two methods of hay making. The first is that the Berseem crop is spread evenly on the ground and is given a few turnings and, when the crop dries, is collected in the morning and stored in the dry bhusa sheds. This method is not useful since the hay will be poor in proteins.

The second method is to hang green Berseem on the wires and allowed to remain in this condition for a couple of days till the whole crop

is dried and is then carted to the dry bhusa sheds. The second method is excellent as the drying process goes on very slowly, decolouration of leaves does not occur, the leaves also do not shed on the floor, and much of the protein will remain in the hay.

Rotation.—Berseem crop in the low lying areas is rotated with paddy crop and in other areas it is taken with maize crop. After Berseem crop, we generally get good out-turn of the succeeding crop.

Conclusion.—Berseem crop forms an excellent fodder crop of both milch and dry herd and, therefore, it is called the 'KING OF FODDERS'. Milk yield is enhanced considerably when green Berseem or Berseem silage, or Berseem hay is fed to the milch herd. Surplus Berseem should be preserved as Silage or Hay, and should be supplemented as fodder requirement during summer months, and this will maintain high milk yield in the cattle throughout the year.

PATHOLOGICAL EFFECTS OF CHLORDON AND TOXAPHENE INGESTED IN TOXIC AMOUNTS

By

B. CHOUDHARY G.B.V.C. M.S. (U.S.A.)

Calcutta

(Continued from page No. 291 of *I. V. J.* Vol. XXIX No. 4 January 1953.)

II Toxaphene

Three other goats were administered lethal quantities of a 40 per cent wettable toxaphene powder orally in gelatin capsules and in varying doses (See Table II). The clinical symptoms were as follows:

At first there was impaired appetite, later to be followed by total inanition. There were dilatation of pupils, rapid and superficial respiration, groaning, bleating, grinding of teeth, restlessness and stiffness in the hind quarters. The flanks were tucked up and they moved with posterior limbs apart. There were wriggings of the tail, frequent attempts to micturate, high coloured urine and oliguria. Later, nervous irritability increased, startling was produced with slightest provocation and convulsions developed. During convulsive fits, the respective goat was on the ground, the head was backwards, and there was paddling of the limbs. Muscular tremors were consistently present. The convulsions were transitory and lasted for about 2 to 3 minutes. These were followed by a varying period of intermediate relaxation accompanied with marked depression. There was a marked loss of weight due to anorexia, dehydration and exhaustion. Prior to death, there was laboured respiration succeeded by dyspnoea, cyanosis, and asphyxia. Death supervened with or without convulsions.

TABLE II
Results of feeding goats with toxaphene (40% Wettable powder).

Serial No. of goat.	Day of experiment.	Age of animal.	Live weight in kg.	Active ingredient in mg. per kg. of body weight.	Total quantity of active ingredient in mg.	Total quantity of 40% preparation of the active ingredient given.	CLINICAL RESULTS.
2	1	1 yr.	22.27	25	556.75	1,391.625	No effect
	2	"	"	"	"	"	No effect
	3	"	21.82	"	545.5	1,363.75	No effect
	4	"	"	"	"	"	No effect
	5	"	21.6	37.5	806.8	2,017	No effect
	6	"	"	"	"	"	No effect
	7	"	21.5	50	1,075	2,687.5	No effect
	8	"	"	75	1,612.5	4,031.25	No effect
	9	"	20.14	100	2,014	5,035	Stiffness, staggering gait, restlessness, moving with hind legs apart, bleating, groaning, frequent attempts to micturate, high colored urine, quick and shallow respiration, dilatation of pupil, increased nervous irritability, convulsion, muscular tremor, paddling of the limbs during convulsion. The symptoms appeared on the 9th day and began to aggravate more and more until it died early in the morning of the 11th day.
	10	"	"	"	"	"	
	11	"	18.70	150	2,805	7,012.5	

TABLE II (Continued)
Results of feeding goats with toxaphene (40% Wettable powder).

Serial No. of goat.	Day of experiment.	Age of animal.	Live weight in kg.	Active ingredient in mg. per kg. of body weight.	Total quantity of active ingredient in mg.	Total quantity of 40% preparation of the active ingredient given.	CLINICAL RESULTS.
5	1	2 yrs.	25	100	2,500	6,250	No effect.
	2	"	"	"	"	"	Symptoms were similar to those of goat No. 2. The goat died towards the end of the 4th day of experiment. It was seriously sick and so the dose of toxaphene was not repeated after the 3rd day.
	3	"	24.5	150	3,675	9,187.5	
6	1	2 yrs.	150	150	4,200	10,500	Symptoms were the same as above but ushered in with such a gravity that the goat was extremely sick towards the end of the 1st day and died at the beginning of the 2nd day. The second dose of the compound was not given as that was thought unnecessary.

Macroscopic appearance

Lungs were edematous, hyperemic, heavy and firm to touch. The alveoli, bronchioles and bronchi contained frothy serous fluid slightly tinged with blood. The trachea was partially filled with frothy exudate and the mucous membrane was hyperemic.

The heart was enlarged and the epicardial surface petechiated along the course of coronary vessels and about the trigona fibrosa of the atrio-ventricular junction. There seemed to be an excessive quantity of pericardial fluid.

The mucosae of the abomasum were congested, thickened, and slightly eroded at the pylorus.

The small intestine was congested and ecchymotic. The mucosa was marked with spotted erosions and covered with thick mucus. Slight hemorrhage was evidenced by the faint, sanguinous nature of the contents. The lesions were more vivid from the posterior end of the jejunum to the ileum. The lesions in the large intestine were less severe. There were a few dead whip and nodular worms. The mesenteric lymph nodes were hyperemic, edematous, and enlarged.

The liver was enlarged, hyperemic and glossy. The gall bladder wall was ecchymotic. The kidneys exhibited irregular large and small areas of congestion on the surface continuous with the cortex and medulla on the cut surface.

The vessels of the meninges, brain, and spinal cord were markedly injected and distended with blood. There was some evidence of hemorrhage in the brain.

In goat No. 5 the petechiae in the sub-epicardium were very few in number, whereas, in goat No. 6, the petechial hemorrhage involved a larger area along coronary vessels.

Histopathology

Brain and Spinal Cord. Congestion, edema, infiltration of round cells, and a few neutrophils were found throughout but were especially severe in the medulla. There were spotted softening and degeneration of neurons in the brain and spinal cord. The meningo-cortical vessels were somewhat thickened and there was extensive perivascular edema in the brain and spinal cord. Obvious neuronophagia existed in all three cases, but, in goat No. 6, it was coupled with hemorrhage particularly in the medulla.

Heart. Congestion, edema, hemorrhage, round cell and neutrophilic infiltration, cloudy swelling and fatty metamorphosis of the cardiac muscles were a consistent finding. Circumscribed hemorrhages in the sub-epicardium

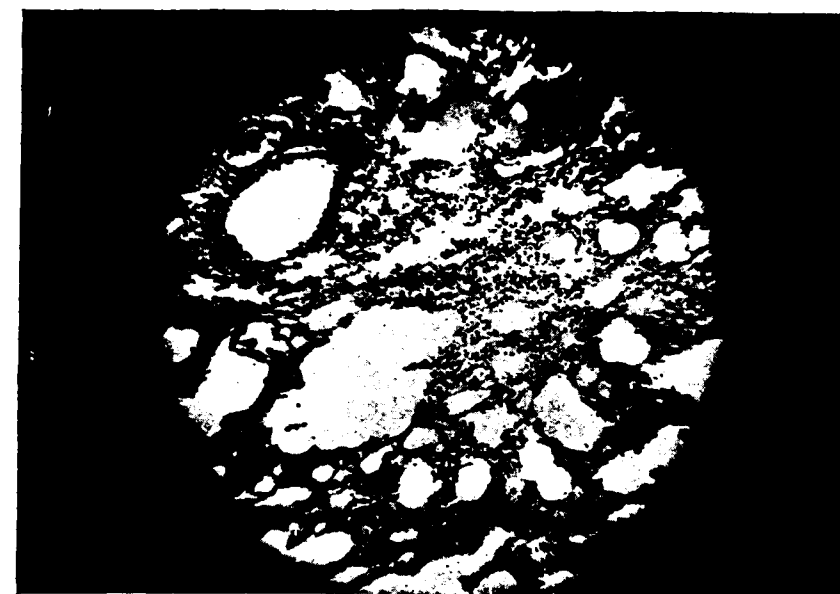
Effects of chlordon and toxaphene by B. Choudhury

Fig. 1. Lung of Goat No. 1 poisoned with Chlordon X 130 diam. (Size 2, 3)



Fig. 2. Small Intestine of Goat No. 4 poisoned with Chlordon X 130 diam. (Size 2, 3)

Effects of chlordon and toxaphene by B. Choudhury

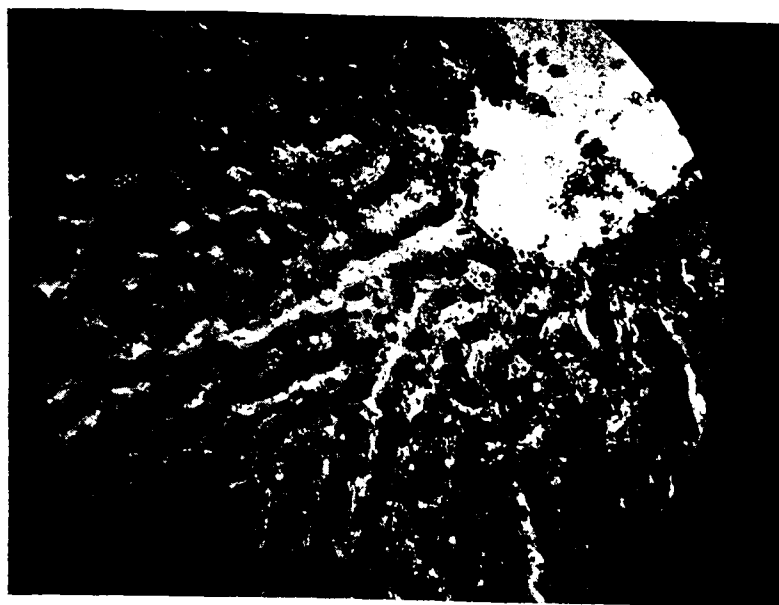


Fig. 3. Liver of Goat No. 3 poisoned with Chlordan X 439 diam.
(Size 2/3)



Fig. 4. Kidney of Goat No. 3 poisoned with Chlordan X 830 diam.
(Size 2/3)

along the course of coronary vessels was noted. The architecture of the muscle fibers was changed. There was rupture of the muscle cells, apparent escape of myohematin, and consequent pigmentation.

Lungs—Congestion, edema, hemorrhage, hemosiderosis and migration of round cells and neutrophils were observed. There was swelling of the alveolar walls, epithelial desquamation, emphysema and atelectasis. Rupture of the alveolar walls, interstitial hemorrhage and infiltration of round cells and neutrophils were observed. There was interlobular and alveolar edema with solidification of exudate in the alveoli. Some bronchioles were ruptured and others collapsed. They contained serous exudate and desquamated epithelium. The pulmonary vessels were highly injected with blood and many capillaries were plugged with emboli.

Lymph nodes—The bronchial and mesenteric lymph nodes were congested, swollen and edematous with lymph sinuses distended. Monocytes were found with phagocytized brownish pigment which appeared to be hemosiderin.

Spleen—There were congestion, hemorrhage, swelling, and necrosis of some reticular cells (karyorrhexis). There were also observed the perivascular edema and hemosiderosis. The sinuses were engorged with lymphocytes red blood cells, a few neutrophils, and protein fragments.

Adrenals—There were congestion, cloudy swelling, scattered focal necrosis, and slight round cell and neutrophilic infiltration. The intercellular spaces were distended with cell debris and coagulated protein material. Both the medulla and cortex were affected.

Kidneys—There were marked cortical and medullary congestion hemorrhage, hemosiderosis, and thrombosis of smaller vessels. Cloudy swelling, coagulation necrosis, fatty metamorphosis and cellular desquamation were observed. The intertubular edema, capillary congestion, and round cell infiltration had resulted in the compression of tubules and consequent distension of the proximal end of some tubules and Bowman's space. The tubular epithelium was undergoing cloudy swelling, fatty metamorphosis, coagulation necrosis, and desquamation.

Most of the glomeruli were enlarged and packed up with desquamated endothelium. There were epithelial desquamation in Bowman's capsule and formation of granular and cellular casts. Numerous vacuoles were formed in the glomerular tufts showing the rate of desquamation of cells. There were engorgement and distention of the tufts but in some there was partial hyalization. Some of the distended Bowman's capsules contained atrophied glomeruli due to pressure of the regurgitated urine from the occluded tubules. The capillary tufts were enlarged to the point of completely filling Bowman's space in some instances by proliferation of

endothelium, infiltration of neutrophils and edema. There were localized areas of coagulation necrosis under the renal capsule in the cortex due to anemic infarction. Both the cortical and medullary portions were equally involved.

Abomasum.—The mucous membrane was covered with exudate containing desquamated epithelium, round cells, plasma cells, neutrophils, and mucin. There was an increased activity of the mucus producing cells. Focal coagulation necrosis with complete sloughing of the mucous lining was the most striking feature. There was extensive edema under the mucosa muscularis. Exudation was evidenced in the muscular layer by the infiltration of round cells, but the changes in the mucous and submucous layer were more pronounced.

Small Intestines.—The intestinal mucosa was covered with catarrhal exudate containing round cells, plasma cells, neutrophils, goblet cells, desquamated epithelium, cell fragments and mucin. The tips of the villi were sloughed off in places and the crypts were filled up with inflammatory exudate and mucin. The mucous membrane was hemorrhagic throughout. There was edema under the mucosa muscularis and in the muscular layers. The edema contained numerous reactive cells.

The micropathology of the caecum was similar to that of the small intestine but beyond that it diminished abruptly.

Liver.—Congestion, hemosiderosis, cloudy swelling, fatty metamorphosis, and focal necrosis around the central veins were pronounced. The reticulo-endothelial cells were swollen and increased in number. There was a moderate infiltration of round cells and neutrophils. The sinusoids were distended with shreds of coagulated protein, chromatin, and cell debris. Some of the hepatic cells were shrunken, causing narrowing of the cord and concomitant enlargement of the adjacent sinusoids.

Discussion

It has been shown by experimental evidence and experience that all the insect toxicants developed so far are harmful to animal life. This remains true of the groups of compounds represented by chlordan and toxaphene. This experiment elucidates the fact that they are capable of producing death in warm-blooded animals such as goats, provided these animals are exposed to sufficient doses. Death usually supervenes as a result of serious pathology of many of the vital organs of the animal system e.g., gastro-enteritis, hepatitis, carditis, pulmonary edema (hemorrhage in the case of toxaphene), encephalomyelitis, etc. The impaired or total loss of function of these organs collectively or singly renders life of the animal impossible. Such detrimental effects of these chlorinated hydrocarbons are manifested clinically, macroscopically and microscopically.

Effects of chlordan and toxaphene by B. Choudhury

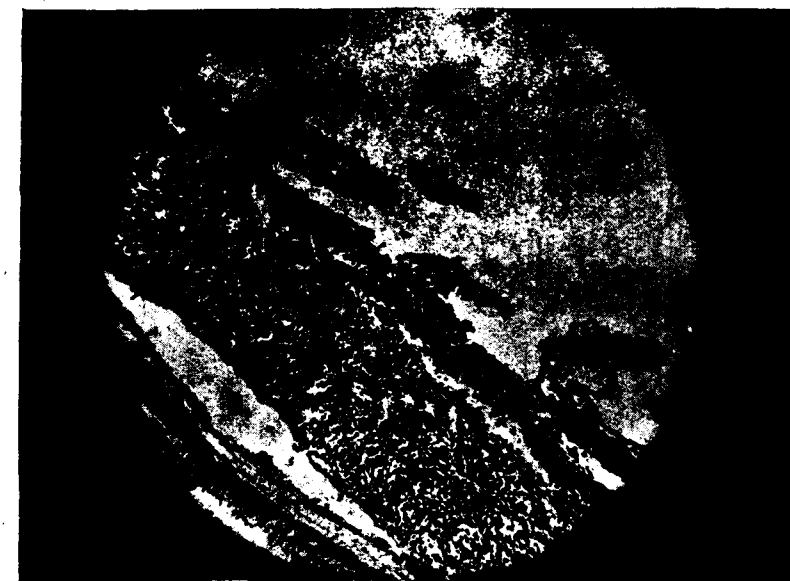


Fig. 5. Small Intestine of Goat No. 5 poisoned with Toxaphene X 170 diam.
(Size 2/3)

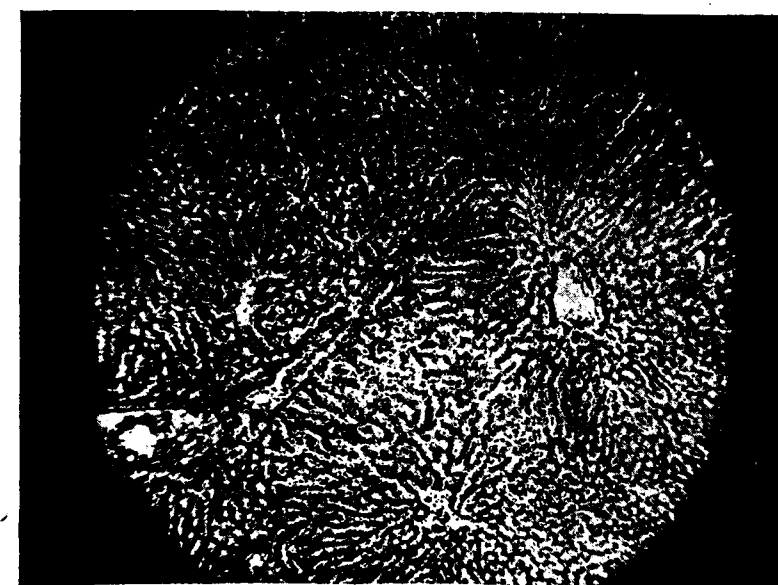


Fig. 6. Liver of Goat No. 5 poisoned with Toxaphene X 180 diam.
(Size 2/3)

Trans-thoracic partial oesophagectomy in a dog.



Radiograph showing the newly formed rib three months after resection.

[Vide article by M. N. Menon. Page No. 537.]

Both chlordan and toxaphene seem to behave in about the same way, but the difference that was observed in this experiment and those conducted by Batte and Turk (2) was in the gravity of the lesion produced by an individual compound. The pathology produced with the administration of the fairly large doses of chlordan (750 mg. per kg. daily) was sufficient to kill goat Nos. 1 and 3 on the third day and goat No. 4 on the second day of the experiment. More conclusively, goat Nos. 1 and 3 withstood three consecutive doses at the rate of 750 milligrams per kilogram of live weight whereas goat No. 4 died four hours after the administration of the second dose of the compound given at the same rate. This wide range of individual susceptibility corroborated the findings of Ingle (7).

The goats appeared to be slightly more susceptible to chlordan than the sheep as the latter could tolerate a daily dose of 770 mgs. per kg. for at least seven days (22). A daily dose of 750 mgs. per kg. in goats was able to produce an appreciable amount of toxic effects within 48 hours, ending in death within 72 hours. The pathological lesions produced by chlordan were gastro-enteritis (without diarrhoea), hepatitis with necrosis around the central veins, acute glomerulo-nephritis, and severe pulmonary congestion and edema. There was evidence of endomyocarditis microscopically and grossly there was evidence of cardiac enlargement. There was congestion of the meninges, brain and spinal cord. This was coupled with softening and degeneration of these organs. They showed a slight tendency to liquifaction necrosis microscopically. The immediate cause of death was judged to be asphyxia originating from the extreme pulmonary congestion and edema.

Toxaphene produced lesions which were many times more severe than those produced by approximately 5 to 7 times the quantity of chlordan. The lesions produced by toxaphene were similar in nature to those of chlordan but there was a difference in intensity of the affection of several organs. The pathology caused by toxaphene could better be explained as per-acute morbid conditions in comparison with those produced by chlordan being only acute. This was manifested by a tendency to hemorrhage from the gastro-intestinal tract, kidneys, lungs, heart and brain. The hemorrhagic condition of the heart was restricted to the sub-epicardium and only of petechial nature. The petechiae were noticed along the course of the coronary vessels and mostly on the trigona fibrosa. The number of petechiae varied considerably. For instance, goat No. 5 showed only a few petechiae whereas the other two of the lot showed numerous ones. This factor substantiated the findings of Ingle (7) and Welch (22) who mentioned the variable range of lesions from animal to animal.

Both chlordan and toxaphene produced gastro-enteritis and injured the endothelium of the capillaries and lymphatics. Moreover, the diseased condition of the intestinal epithelium facilitated their absorption. The

injured endothelium of the capillaries of the brain, lungs, heart, gastro-intestinal tract, kidneys, etc., became abnormally permeable resulting in the edematous condition of those organs. The edema went on increasing rapidly due to the partial suspension of the filtration process in the kidneys and increased accumulation of effete materials in the tissues. The vicious cycle thus established was no doubt responsible for the striking pathology.

The convulsive symptoms probably originated from congestion edema, emboli, hemorrhages (in the case of toxaphene), focal miliary softening, and chromatolysis of the neurons in the brain and spinal cord.

The petechiae in the sub-epicardium were due to the more acute diseases and degenerated condition of the capillary endothelium of the heart produced by toxaphene. The dilatation of the right side of the heart was due to the pulmonary congestion and the unusually increased efforts for oxygenation of blood.

The lesions in the lungs were so marked that they over-shadowed the pathology of all other organs. Death in every case was seemingly due to the rapid cut in the respiratory capacity as the pronounced edema prevented the blood from oxidation. The reduction of pulmonary oxidation of blood put a stop to the tissue respiration. Eventually the function of all organs of the body was hampered due to the dearth of oxygen and death supervened. This was the reason for the fatal termination irrespective of convulsion. The inflammatory and the mid-zonal necrotic condition of the hepatic lobules (16) aggravated the entire process furthermore in consequence of the suspension of intra-hepatic metabolism.

The cumulative effects of these chlorinated hydrocarbons may be partially a mechanical proposition. Their accumulation in the system was no doubt increased by the renal dysfunction whereas the rate of absorption of the compounds remained the same or became more rapid due to the pathological condition of the intestinal epithelium.

The pathological diagnosis of chlordan or toxaphene poisoning depends upon the history, clinical manifestation, macroscopic and histopathological changes in the different vital organs. They do not produce any characteristic lesion which could be considered as specific one, because some what similar conditions are produced by sodium chloride, chloroform, cocklebur, DDT, santonin and possibly others.

Summary

Chlordan and toxaphene, like all other chemicals used as insect toxicants, are toxic to warm-blooded animals and may produce grave symptoms or even death. The pathogenicity of chlordan and toxaphene includes the gastro-intestinal tracts, liver, kidneys, suprarenals, heart, lungs, lymph nodes of the affected organs, brain, and spinal cord. The immediate cause or

death seems to be retarded oxygenation of blood due to extensive pulmonary edema, emphysema and atelectasis. The pulmonary lesions are probably coupled with gastro-enteritis, hepatitis, nephritic change, endocarditis, myocarditis, adrenal pathologies and changes in the brain and spinal cord. In other words, these chemicals affect most of the vital organs of the body but pulmonary lesions are the most severe.

The cumulative effects of these chlorinated hydrocarbons is seemingly enhanced by the severe renal pathology produced and the suppression of urine production.

Toxaphene is approximately 5 to 7 times more toxic than chlordan in warm blooded animals such as goats. The susceptibility to both the compounds is variable from goat to goat. They affect the highly specialized endothelial cells of lymph and blood vascular system causing inflammation, edema, emboli, and hemorrhage (toxaphene) in the tissues of the vital organs. The petechial hemorrhages in the epicardial surface of the heart are not observed in goats poisoned with chlordan but this was a common factor associated with toxaphene poisoning.

Both chlordan and toxaphene seem to be especially toxic to endothelial and nervous tissue. Congestion of meninges, brain and spinal cord is usually produced and in the central nervous system focal miliary softening, necrosis of neurons, perivascular edema, infiltration of round cells, and neuronophagia are constant findings. These changes are clinically manifested by increased reflexes, convulsions and muscular tremors.

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BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT OF THE DOG WITH SPECIAL REFERENCE TO STREPTOCOCCI*

BY

DR. K. S. SHETTY, G.M.V.C., PH.D. (Edin),
Director, Veterinary Department, Hyderabad-Deccan.

Introduction

In 1941, Francis, while summarising the Public Health Report on the study of bacterial flora of naso-pharynx of individuals of the population in London and South East England, (Swaker, Bedford, Lovell and Rosher 1938) drew the attention of veterinary research workers to the fact that there had been no adequate investigation of the bacteria normally present in the naso-pharynx of any species of domestic animals or of the factors which affected the carrier rate: Bosworth (1947) quoted the need for an adequate survey of staphylococci in dogs in normal health and sickness, while Levi (1946) thought that a detailed study of the normal inhabitants of the naso-pharynx of the cat would be of value in interpreting bacteriological findings in cases of snuffles. Much has been said in recent years about the incidents of haemolytic streptococci in dogs and their pathogenicity (Pilot, Buck, Davis and Eastman, 1936; Hare and Fry, 1938; Stafseth, 1940; Hare, 1946; and Garside, 1947) but the frequencies of these organisms in relation to other possible pathogens have not been studied.

Taking these factors into consideration, it was decided to make a general survey of the various organisms present in the upper respiratory tract of dogs and cats under the guidance and supervision of Prof. T. J. Mackie of the Department of Bacteriology and acting Dean of the Faculty of Medicine, University of Edinburgh. An important factor which weighed in selecting these two species of animals was the Public Health aspect in which Prof. Mackie was genuinely interested. It was thought that the results of this investigation would provide information as to whether or not these animals were frequent carriers of human pathogens. In modern society the dog and cat have become such household pets that they are considered as members of the family having free access practically to everything and every place in their master's homes and, if these animals harbour any of the human pathogens, transmission to human beings may be easily accomplished. Several reports regarding the high frequency of haemolytic streptococci in the throats of dogs as stated above, repeated reports in the medical literature of Pasteurella infection in man following cat-bites and in one instance dog-bite (Kapel and

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Holm, 1930; Schenk, 1938; Allin, 1942; Allott *et al*, 1944; and Hansman Tully, 1945) and the cases recorded of Tularaemia following (Smiles, 1931; Collins 1933-34) also influenced this aspect of the study.

Source of material and method of collection

This part of the study comprised the examination of the upper respiratory tracts of one hundred dogs of different breeds and of different ages varying from two months up to fourteen years. Seventy-five of these animals were for all practical purposes considered normal or had at least no visible signs of any respiratory troubles, while the remaining twenty five were classified as sick ones with one of the following complaints:—nasal catarrh, bronchitis, convulsions chorea, anaemia and debility mostly associated with distemper. Care was taken not to select for study any dog which had received treatment in any form.

All the dogs were made available at the outpatient clinic of the Royal (Dick) Veterinary College. Mostly the dogs destroyed at the clinic either by the intravenous injection of saturated solutions of magnesium sulphate or by the intracardial injection of nembutol were used for study. Such destroyed dogs were preferred for examination for the obvious advantages that the parts could be well rubbed and a good quantity of mucus made available for examination. Further, it was found not practicable by any device to swab the naso-pharynx of a living dog satisfactorily. Even a swab improvised on the pattern of West's post-nasal swab used for swabbing the human naso-pharynx was not of much avail in dogs.

As soon as the dog was destroyed, both nostrils, and the surrounding skin were thoroughly wiped with cotton wool soaked in absolute alcohol. Then a sterile cotton wool swab was passed through one nostril into the nasal cavity as far as possible, withdrawn and passed into the other nasal cavity in the same way. Then the mouth of the dog was kept wide open by an assistant, the tongue was pressed down, exposing both the tonsils, which were rubbed with a second swab. Again a third swab, which was slightly bent at the tip was inserted behind the soft palate and passed through the naso-pharynx to a depth of about one inch, collecting a sufficient quantity of mucus from this site also.

In living dogs, when swabbing the nose, the same precautions were taken in wiping outside the nostrils with absolute alcohol. While an assistant was holding the dog with its head raised, the swab was gently passed through the nostril into the nasal cavity, removed, and again passed into the other one. It was not possible to pass the swab far inside the nasal cavity, nor was it advisable to rub hard as was done in killed dogs; nevertheless sufficient mucus was always available on the swab. Similarly, while an assistant opened the mouth of the dog, both tonsils were touched and a satisfactory swab obtained. The naso-pharynx was not swabbed in the living dog.

In a dozen dogs, swabs were taken from the tonsils before destroying and again after destroying, while further more, the tonsils of six of them were excised under strict aseptic precautions and triturated with sand and saline for the purpose of making separate cultures and comparing the results in order to check and confirm the efficiency of mere swabbing of the parts.

Technique and Procedure

Immediately after obtaining the swabs with the specimens of mucus, cultures were made on the following media and incubated at 37° C. aerobically:

1. Five percent horse blood agar plate.
2. Chocolate agar plate.
3. MacConkey agar plate.
4. One percent glucose broth.

The swab was rubbed over a small area at the edge of each plate and then successive stroke inoculations were made in the remainder of the plates by means of a platinum loop, tip being charged several times from the area inoculated directly with the swabs. After rubbing on the plate, the swab was dipped into a tube of glucose broth. Culture, from the nose, naso-pharynx and tonsils were marked as N, NP, and T respectively bearing also the date.

The plates were examined after 18 hours incubation and the presence of different types of colonies and their approximate numbers were noted. The blood agar plate was examined particularly for the presence of colonies of haemolytic streptococci and such colonies when present were subcultured to another blood agar plate. Other prominent colonies were also subcultured on the blood agar plates. The subcultivation of streptococci at this stage was necessary as it was found by experience that on longer incubation the whole medium became haemolysed owing to the activity of other organisms, such as staphylococci or coliform organisms, when it became difficult to pick up haemolytic streptococcal colonies with any degree of certainty.

Smears were made from the glucose broth cultures, stained by Gram's method and examined, and the morphology of different organisms and their frequency noted.

The original plates were again examined after twenty-four hours' and forty-eight hours' incubation, and if any new type of colony was noticed, it was subcultured on to blood agar. The chocolate agar plate was specially examined after 48 hours' incubation for colonies resembling those of haemophilus, and any such colonies were subcultured on fresh plates of the same medium.

Subcultivation was carried out until pure cultures of representatives of all the different colonies on the original plates were obtained. Films of these cultures were then prepared and the morphology and reaction to Gram's stain of the organism was examined.

Results. The bacterial flora of the nasal passages differed in several respects from those of the naso-pharynx and tonsils. Examination of the primary culture plates, especially the blood agar plates, showed some striking differences regarding the amount of growth observed on them. The growth was less copious and the different types of colonies were fewer in number on the culture plate from the nose than from that of the naso-pharynx, while it was

highest in the tonsils culture. The organisms isolated and their incidence in the normal and sick dogs are given in the following tables.

TABLE I.

Summary of the incidence of different organisms in the normal and sick dogs.

Organisms	Normal dogs (75)		Sick dogs (25)		Difference Percent.	
	No. Positive	Percent Positive	No. Positive	Percent Positive		
1. Staphylococci	53	70.7	21	84	↓	13.3
2. Beta hæmolytic Streptococci	48	64	17	68	↓	4
3. Non-hæmolytic streptococci.	58	77.3	24	96	↓	18.7
4. Gram negative cocci	45	60	40	56	—	4
5. H. bronchisepticus	14	18.7	30	52	↓	33.3
6. Coliform bacilli	16	21.3	12	48	↓	26.7
7. Diphtheroid bacilli	14	18.7	7	28	↓	9.3
8. B. subtilis group	12	16	5	20	↓	4
9. Bacterium alkaligenes	27	36	15	60	↓	24
10. Bacillus of Friedlander	—	—	4	16	↓	16
11. Hæmophilus cani	9	12	2	8	—	4
12. Proteus sp.	12	16	7	28	↓	12
13. Leptotrichia sp.	18	24	7	28	↓	4
14. Unidentified group (a)	45	60	16	64	↓	4
do (b)	36	48	11	44	—	4
do (c)	27	36	10	40	↓	4

Haemolytic Streptococci

Methods of identifications.—Single colonies showing beta-haemolysis on the primary blood agar plate were subcultured to fresh blood agar plates and after eighteen hours' incubation at 37°C the plates were examined for haemolysis and a film was made, stained and examined. If found to be small cocci in chains, it was recorded as haemolytic streptococci. A test for soluble haemolysin was not performed. In every case a colony picked up from the primary plate, presumed to be of haemolytic streptococci by its characteristic disc-like appearance with a clear zone of haemolysis, definitely proved to be so when subcultured and examined.

All strains were identified by Lancefields precipitation method to ascertain the distribution of various groups in the nose, naso-pharynx and tonsils of normal and sick dogs.

TABLE II
Summary of the incidence of different organisms in the Nose, Naso-Pharynx and Tonsils of Normal and Sick dogs.

Class	Situation	Normal			Sick		
		N	NP	T	N	NP	T
Staphylococci %		56.7	34	24	84	58.8	36
Beta hæmolytic streptococci %		13.3	48	62.7	32	47	68
Non-haemolytic streptococci %		28	46	65.3	48	52.9	76
Gram-negative cocci %		38.7	48	26.7	32	35.3	32
Hæmophilus bronchisepticus %		14.7	10	4	52	41.2	20
Coliform bacilli %		14.7	4	5.3	20	17.6	28
Diphtheroid bacilli %		14.7	6	2.7	16	11.6	4
B. subtilis group %		16	—	—	20	—	4
Bacterium alkaligenes %		21.3	6	20	40	35.3	32
Bacillus of Friedlander %		—	—	—	8	6	8
Hæmophilus canis %		5.3	6	4	8	5.9	4
Proteus sp.		6.7	4	6.7	20	11.8	16
Leptotrichia sp. %		—	18	24	—	23.5	24
Unidentified	Group (a)	8	58	60	8	5.9	64
	Group (b)	5.3	32	37.3	8	35.3	36
	Group (c)	1.3	30	28	8	29.4	36

The acid extraction method of Lancefield (1933) was employed for the purpose of grouping. The organism was grown in 100 ml. of glucose broth and, after centrifugation of the culture, the deposit was extracted with 3 ml. N/25 HCL in saline by boiling for fourteen minutes in a water bath. After cooling and adding two drops of phenol red indicator, the extract was neutralised with N/1 NaOH solution, centrifuged and the clear extract was tested against the precipitating sera. All the sera excepting that of group M, were obtained from Messrs. Burroughs Wellcome & Co. Serum of group M was not available from any of the commercial laboratories but was kindly supplied by Dr. R. M. Fry of Cambridge.

All the strains of haemolytic streptococci isolated in this investigation, without a single exception, fell into one or other of Lancefield's group, and the acid extraction method was found perfectly satisfactory for all of them. All the strains which showed group G reaction were tested for cross precipitation with group C serum. But in no case was cross-precipitation observed. It may, however, be pointed out that all the strains were grouped when freshly isolated. The various groups were tested for their fermentative ability as complementary tests for their identifications: group G in aesculin, lactose, raffinose, glycerol, litmus milk, and for the hydrolysis of sodium hippurate; group C in lactose, trehalose and sorbitol; group L and M in lactose, trehalose, sorbitol, mannitol, salicin and litmus milk; group A in lactose, sucrose, trehalose, sorbitol, salicin, raffinose, mannitol, insulin, litmus milk, and for the hydrolysis of sodium hippurate; and group F in aesculin, trehalose, sorbitol, lactose, salicin and litmus milk.

Result

The percentage of carriers of haemolytic streptococci observed in this investigation was not so high as that recorded by Pilot *et al* (loc. cit), which reached upto 92 per cent in young dogs, but it compared favourably with the result of one of the surveys of Garside (loc. cit) in which the positive carrier rate was recorded as 60 per cent among the general dog population of Edinburgh city.

The difference in the carrier rate of streptococci in the two groups of dogs, normal (64 per cent) and sick (68 per cent), is not very significant. For all practical purposes the combined result (65) per cent may be taken as the normal figure.

A striking difference in the incidence of this organism in the nose, naso-pharynx and tonsils was observed; while the positive carrier rate in the nose was 13.3 per cent, it increased in the naso-pharynx to 48 per cent., whereas it was as high as 62.7 per cent. in the tonsils. Here again the difference in two groups of dogs was not very marked, except in the nose where it increased from 13.3 per cent. in the normal to 32 per cent. in the sick. In all positive cases, this organism was present in the tonsils, excepting in one dog, where it was found only in the naso-pharynx. There was not another instance where it was present in the nose or naso-pharynx and absent in the tonsils.

One hundred and fourteen strains of this organism thus isolated were distributed amongst six serological groups of Lancefield, the most predominant of them being group G, to which 48.2 per cent of the strains belonged. The next in frequency was group M, which totalled 23.7 per cent. Groups C and L

were 13.2 per cent and 10.5 per cent respectively. Groups A, which formed 2.6 per cent. of the total strains, was found present in the nose and tonsils of only one normal dog and in the tonsils of one sick dog. Similarly, group F, which formed 1.8 per cent, of the strains, was present in the tonsils of one and in the nasopharynx of another normal dog.

TABLE III.

Percentage Carrier Rates for Haemolytic Streptococci

Group	Number of dogs swabbed	Number positive	Percent Positive
Normal	75	48	64
Sick	25	17	68
Combined	100	65	65

TABLE IV.

The frequency of Haemolytic Streptococci in the nose, naso-pharynx and tonsils.

Situation	Normal Dogs.			Sick Dogs.			Combined.		
	No. swabbed	No. positive	Percent positive	No. swabbed	No. positive	Percent positive	No. swabbed	No. positive	Percent positive
Nose	75	10	13.3	25	8	32	100	18	18
Naso-pharynx	50	24	48	17	8	47	67	32	47.7
Tonsils	75	47	62.7	25	17	68	100	64	64

In one dog, group A strain was isolated from the tonsils, whereas the nose of the same dog yielded group G strain; similarly, in another dog, group C was isolated from the nose, while the naso-pharynx and tonsils were positive for group G. Excepting these two instances, in all the rest of the dogs the strains belonging to the same group were recovered from more than one situation whenever it was present.

On the whole, 28 per cent. of the normal and 36 per cent. of the sick dogs harboured group G, while group M decreased from 20 per cent. in the sick. Group C was isolated from 8 per cent. of the normal and 12 per cent. of the sick.

TABLE V

Number of strains and groups of Haemolytic streptococci isolated from nose, naso-pharynx and tonsils of normal and sick dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total Strains
Nose	9	4	2	2	1	—	18
Naso-pharynx	17	3	7	4	—	1	32
Tonsils	29	8	18	6	2	1	64
Grand Total	55	15	27	12	3	2	114

48.2% Group G; 13.2% Group C; 23.7% Group M; 10.5% Group L; 2.6% Group A; 1.8% Group F.

TABLE VI

Number of strains and groups of Haemolytic Streptococci isolated from nose, naso-pharynx and tonsils of normal dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total Strains
Nose	5	2	2	—	1	—	10
Naso-pharynx	11	3	6	3	—	1	24
Tonsils	21	5	15	4	1	1	47
Total	37	10	23	7	2	2	81

45.7% Group G; 12.3% Group C; 28.4% Group M; 8.6% Group L; 2.5% Group A; 2.5% Group F.

Similarly 5.3 per cent. of the normal and 8 per cent. of the sick showed group L; group F was recovered from only 2 per cent. of the normal and none at all from the sick; group A too was found in 1.3 per cent. of the normal and 4 per cent. of the sick dogs.

The biochemical reactions of the 114 strains are set out in detail under their respective groups in Table IX, X, XI, XII, XIII, XIV. None of the group G strains hydrolysed sodium hippurate. All without exception fermented aesculin, lactose was fermented by all strains except five. Only three strains fermented raffinose, and five strains failed to clot milk.

These reactions are in agreement with the reactions of the three types described by Smith and Sherman (1938), but the fermentation of glycerol is an

TABLE VII

Incidence of different groups of Haemolytic Streptococci in normal and sick dogs.

Class	Group G		Group M		Group C		Group L		Group F		Group A	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Normal (75)	21	28	15	20	6	8	4	5.3	2	2.7	1	1.3
Sick (25)	9	36	3	12	3	12	2	8	—	—	1	4
Combined (100)	30	30	18	18	9	9	6	6	2	2	2	2

TABLE VIII

Number of strains and groups of Haemolytic Streptococci isolated from nose, naso-pharynx and tonsils of sick dogs.

Situation	Group G	Group C	Group M	Group L	Group A	Group F	Total Strains
Nose	4	2	—	2	—	—	8
Naso-pharynx	6	—	1	1	—	—	8
Tonsils	8	3	3	2	1	—	17
Total	18	5	4	5	1	—	33

54.5 % Group G; 15.2 % Group C; 12.1 % Group M; 15.2 % Group L; 3.0 % Group A; 0.0 % Group F.

additional factor. Glycerol is not fermented by anyone of the three types described by these authors, but the possible explanation is that their classification was based on the strains of human origin, whereas the same authors observed that group G of animal origin had the property of fermenting this sugar. Forty-three out of fifty-five strains fermented glycerol. Action on this sugar was rather slow and invariably it took three to five days to bring about the complete reaction. Five strains which did not clot milk and fermented only aesculin may be classified as "pyogenes-like" G's.

Group M strains which come next to G in number were found more frequently in normal dogs than in sick dogs. Growth was extremely poor in glucose broth, and often serum had to be added to the medium to obtain sufficient growth for the purpose of performing the precipitation test. On blood agar, the colonies were small with a wide zonal of beta haemolysis. The biochemical reactions were rather constant. All the strains with the exception of two fermented lactose but not trehalose, mannitol, sorbitol or salicin. This reaction is in agreement with the findings of Fry (1941). But the two exceptions fermented sorbitol in addition to lactose. All the strains produced slight acid in litmus milk, with the exception of two, which had no action. None clotted milk.

Group C formed the third group in numbers. From the reactions in lactose, sorbitol and trehalose, it was clear that the three types mentioned by Bazeley and Battle (1940) were encountered. Of the fifteen strains, five fermented lactose and sorbitol (Type 2), four fermented trehalose (type 4) six fermented trehalose and lactose (type 5). Types 1 and 3 were not met with. It should, however, be noted that the strains studied by Baxeley and Battle

were all from horses. Five strains fermenting lactose and sorbitol belonged to the "animal pyogenes" group, while the remaining ten fermenting lactose and trehalose or trehalose alone belonged to the "human C" group (Sherman 1937).

Colonies of group L, when first isolated, were very small with a wide zone of haemolysis. Unlike group M, these strains grew well in glucose broth. Lactose and trehalose were fermented by all the twelve strains, while mannitol and sorbitol were not acted upon by any of them.

Contrary to the findings of Fry (1941), the reaction in salicin was variable: eight strains fermented this sugar, while four did not. It should, however, be mentioned that the action on this sugar by some of the strains was rather slow (2-4 days).

The remaining two groups A and E were very sparse in number. Three strains of group A, isolated from one normal and one sick dog, showed the typical reactions of streptococcus.

Two strains of group F were isolated from two normal dogs—one from the tonsils and the other from the naso-pharynx. They grew very slowly on blood-agar plates forming transparent minute pin-point colonies surrounded by a narrow zone of beta-haemolysis. Both the strains fermented aesculin, lactose and salicin, but not trehalose and sorbitol; both curdled milk.

Non-Haemolytic Streptococci

Method of identification.

All strains of streptococci which produced alpha-haemolysis (partial haemolysis of the blood corpuscles immediately surrounding the colonies), or no change at all on horse blood agar plate, were recorded as non-haemolytic streptococci. The biochemical reactions of all the strains were studied in arabinose, maltose, sucrose, lactose, trehalose, raffinose, inulin, glycerol, mannitol, sorbitol, and salicin. The action on litmus milk, the production of ammonia in peptone water, and the ability to grow on media containing bile salt were studied. The majority of the inulin-fermenting strains were further tested for bile solubility and their pathogenicity was tested by inoculation into mice. These two tests completely eliminated any doubt that such strains were not pneumococci as all strains tested were non-bile-soluble and non-pathogenic for mice, even though inulin was fermented, which is an important biochemical characteristic of pneumococci.

Result

77.3 per cent. of the normal dogs and 96 per cent. of the sick dogs were found to be harbouring these organisms in one situation or another. The carrier rates in the nose, naso-pharynx and tonsils were 28 per cent. and 65.8 per cent respectively.

TABLE IX

Biochemical Reactions.

Group G.

Strain	Sodium Hippurate	Aesculin	Lactose	Raffinose	Glycerol	Litmus Milk
4 T1	—	+	—	—	—	A
4 NP1	—	+	—	—	—	A
5 T1	—	+	+	—	—	AC
5 NP1	—	+	+	—	—	AC
6 N2	—	+	—	—	—	A
6 T1	—	+	—	—	—	A
6 NP3	—	+	—	—	—	A
7 T11	—	+	+	—	+	ACR
7 NP3	—	+	+	—	+	ACR
11 NP1	—	+	+	—	+	AC
11 T1	—	+	+	—	+	AC
14 NP4	—	+	+	—	+	AC
14 T6	—	+	+	—	+	AC
16 T1	—	+	+	+	+	AC
21 T1	—	+	+	—	+	ACR
24 NP2	—	+	+	—	+	AC
24 T2	—	+	+	—	+	AC
27 T1	—	+	+	—	—	A
28 N1	—	+	+	—	+	AC
28 T1	—	+	+	—	+	AC
25 NP3	—	+	+	—	+	AC
35 T1	—	+	+	—	+	AC
41 NP3	—	+	+	—	+	ACR
41 T3	—	+	+	+	+	ACR
43 T1	—	+	+	+	+	AC
43 NP1	—	+	+	—	+	AC
52 N1	—	+	+	—	+	ACR
52 T1	—	+	+	—	+	ACR
54 T5	—	+	+	—	+	ACR
56 N4	—	+	+	—	+	ACR
56 T7	—	+	+	—	+	ACR
59 T2	—	+	+	—	+	ACR
60 T2	—	+	+	—	+	ACR
69 T3	—	+	+	—	+	ACR
73 N2	—	+	+	—	+	AC
73 T2	—	+	+	—	+	AC
1 NP4	—	+	—	—	+	AC
1 Tf	—	+	—	—	+	AC
4 Td	—	+	—	—	+	AC
4 NPd	—	+	+	—	+	ACR
6 Nb	—	+	+	—	+	ACR
6 Te	—	+	+	—	+	AC
6 NPe	—	+	+	—	—	AC
8 Na	—	+	+	—	—	AC
8 Tg	—	+	+	—	+	AC
12 Na	—	+	+	—	+	AC
17 Na	—	+	+	—	+	ACR
17 Ta	—	+	+	—	+	AC
20 Te	—	+	+	—	+	AC
20 NPe	—	+	+	+	+	ACR
24 NP4	—	+	+	+	+	ACR
24 Ta	—	+	+	—	+	ACR
25 NP4	—	+	+	—	+	ACR
25 Ta	—	+	+	—	+	AC

+ Presence of a reaction
 — Absence of a reaction
 ACR Acid, clot and reduction
 AC Acid and clot

TABLE X

Biochemical Reactions.

Group M.

Strain	Lactose	Trehalose	Mannitol	Salicin	Sorbitol	Litmus Milk
1 T1	+	—	—	—	—	A
1 NP1	+	—	—	—	—	A
9 NP2	+	—	—	—	—	—
9 T1	+	—	—	—	—	A
9 NP5	+	—	—	—	—	A
19 T1	+	—	—	—	—	A
22 T1	+	—	—	—	—	A
25 T1	+	—	—	—	—	A
37 T1	+	—	—	—	—	A
39 NP3	+	—	—	—	—	A
39 T1	+	—	—	—	—	A
40 T1	+	—	—	—	—	A
42 N1	+	—	—	—	—	A
42 NP1	+	—	—	—	—	A
42 T1	+	—	—	—	—	A
47 NP1	+	—	—	—	—	A
47 T1	+	—	—	—	—	A
48 NP1	+	—	—	—	—	A
48 T4	+	—	—	—	—	A
67 T1	+	—	—	—	—	A
71 T1	+	—	—	—	—	A
74 T1	+	—	—	—	—	A
75 T1	+	—	—	—	—	A
13 Ta	+	—	—	—	—	A
14 Ta	+	—	—	—	—	A
22 NP4	+	—	—	—	—	A
22 Ta	+	—	—	—	—	A

+ Presence of a reaction.

— Absence of a reaction.

A Acid production.

An attempt was made to classify one hundred and thirty three strains of this organism isolated from both normal and sick dogs into different groups by their physiological and more particularly their biochemical reactions as recommended by Sherman (1937) since no serological method is practicable for that purpose.

As shown in Table XXVI all the strains forming either acid and clot or acid alone in litmus milk, fermenting maltose, sucrose and lactose and not fermenting glycerol, mannitol, sorbitol and arabinose, were classified as *Streptococcus salivarius*. Eighty six (64.7 per cent) belonged to this group.

TABLE XI

Biochemical Reaction.

Group C.				
Strain	Lactose	Sorbitol	Trehalose	Remarks
2 NP3	+	+	—	Type 2
2 T1	+	+	—	Type 2
7 N1	+	—	+	Type 5
10 N1	+	—	+	Type 5
10 T1	+	—	+	Type 5
20 T1	+	+	—	Type 2
20 NP1	+	+	—	Type 2
23 T1	—	—	+	Type 4
23 NP1	—	—	+	Type 4
70 T5	+	+	—	Type 2
7 Nb	—	—	+	Type 4
7 Tb	—	—	+	Type 4
10 Na	+	—	+	Type 5
10 Te	+	—	+	Type 5
20 Td	+	—	+	Type 5

+, — Presence or absence of a reaction.

TABLE XII

Biochemical Reaction.

Group L.						
Strain	Lactose	Trehalose	Manitol	Salicin	Sorbitol	Litmus Milk
3 NP1	+	+	—	Slow	—	A
3 T5	+	+	—	Slow	—	A
13 T1	+	+	—	Slow	—	A
13 NP3	+	+	—	Slow	—	A
13 NP1	+	+	—	—	—	A
49 T1	+	+	—	—	—	A
49 T1	+	+	—	+	—	A
9 Nc	+	+	—	+	—	A
9 Tb	+	+	—	+	—	A
9 NP6	+	+	—	+	—	A
15 Na	+	+	—	—	—	A
15 Ta	+	+	—	—	—	A

+, — Presence or absence of a reaction.
 A Acid production.
 AC Acid and clot.

TABLE XIII

Biochemical Reaction.

Group A.			
	12 T _a	57 N ₅	57 T ₃
Sodium Hippurate	—	—	—
Glucose	+	+	+
Lactose	+	+	+
Sacrose	+	+	+
Trehalose	+	+	+
Sorbitol	—	—	—
Salicin	+	+	+
Raffinose	—	—	—
Manitol	—	+	+
Inulin	—	—	—
Litmus Milk	ACR	AC	AC

+, — Presence or absence of a reaction
 AC Acid and clot
 ACR Acid, clot and reduction

TABLE XIV

Biochemical Reaction.

Group F.		
	31 NP ₄	58 T ₄
Aesculin	+	+
Trehalose	—	—
Sorbitol	—	—
Lactose	+	+
Salicin	+	+
Litmus Milk	AC	AC

+, — Presence or absence of a reaction
 AC Acid and clot

Those strains which showed no haemolysis on blood agar, clotted milk, fermented mannitol sorbitol and salicin and were bile resistant were classified as *Streptococcus faecalis*. Twenty one (15.2 per cent) belonged to this group.

Seventeen strains (12.8 per cent) which did not curdle milk and did not ferment lactose, but in other respects showed same reactions as *Str. salivarius* were identified as *Streptococcus equinus*.

The strains which were bile-resistant invariably curdled milk, fermented arabinose, lactose, raffinose and salicin were recorded as *Streptococcus bovis*. Only nine strains (6.8 per cent) belonged to this group.

TABLE XXIV

Percentage carrier rates for non-haemolytic streptococci.

Class	Number of dogs swabbed	No. Positive	Per-cent positive
Normal	75	58	77.3
Sick	25	24	96

TABLE XXV

The frequency of non-haemolytic streptococci in the nose, naso-pharynx and tonsils.

Situation	Normal Dogs			Sick Dogs			Combined		
	No. swabbed	Positive No.	Per cent	No. swabbed	Positive No.	Per cent	No. swabbed	Positive No.	Per cent
Nose	75	21	28	25	12	48	100	33	33
Naso-pharynx	50	23	46	17	9	52.9	67	32	47.8
Tonsils	75	49	65.3	25	19	76	100	68	68

Discussion

Haemolytic Streptococci:—Although the isolation of B-Haemolytic Streptococci from the tonsils of apparently normal dogs and from cases of tonsillitis and pharyngitis have been reported by some workers, any information about their presence and frequency in the nose and naso-pharynx has been completely lacking upto the present, though their presence in these situations has been assumed. Pilot *et al* (1936) did not group their strains. Garside (1947) grouped only a few of the strains which he isolated from the tonsils of 200 normal dogs in his two surveys and most of them belonged to Group G. But it was recorded by Garside that 82% of the total strains isolated from different situations in dogs, mostly from infective processes, belonged to Group G. Similarly Hare and Fry (1938) observed that 61 per cent of the total strains isolated from various conditions in dogs belonged to Group G.

Though the percentage (48.2 per cent) of group G strains isolated in this investigation fell short of the above figures, it still remained the predominant group. It is interesting that such high percentages as 23.7 per cent. of

group M and 10.5 per cent of group L were isolated. Such figures have not so far been recorded.

The frequency of group M strains in sick dogs (12 per cent) was decidedly less than their frequency in normal dogs (20 per cent); while all the remaining groups except group F showed higher frequencies in sick dogs, as shown in Table XVII. And also, group M and group F appear to have no pathogenic significance in these animals. The investigation also revealed that there is possibility of existence of more than one serological group.

One of the important questions arising from the results of this investigation is whether the claims made by Hare (1946) can be upheld. Hare maintains that B-haemolytic streptococci are never found in normal dogs and that when they are isolated from supposedly normal dogs it indicates a cyclinical warning of a chronic infection. But the present investigation shows that forty-eight (64 per cent) of the seventy-five apparently normal dogs harboured haemolytic streptococci and it does not seem possible that such a high percentage of dogs which appeared perfectly healthy at the time of swabbing could have been in the waning phase of the infection. In any case, where there is no sign of even chronic infection, the animal has to be classed as a carrier until the indicative signs of the chronic infection are recorded.

Such a high percentage of carriers among the dog population does support the observations of the importance of haemolytic streptococci as pathogens of dogs, for transmission of these organisms to susceptible animals by licking through the agency of common feeding troughs or by droplet infection is easily accomplished. It is also an easy possibility that new born puppies contract infection from their own dam either from infected vaginal exudate during parturition or by post-natal licking as suggested by Hare.

Isolations of groups C, G and A from normal and sick dogs indicate the possibility of cross infection between dog and man, as groups C and G are not infrequently found in human infections and group A is a proved human pathogen.

Non-Haemolytic Streptococci:—Non-haemolytic streptococci of the throat of any species of animal have not so far been studied. Even in the medical field these organisms still remain ill-defined, and they are usually referred to as the streptococci of the salivary group, *Streptococcus viridans* and indifferent streptococci according to the degree of greening produced on blood agar, or simply as the mouth streptococci.

In their early classical work, Andrews and Horder (1906) described the predominant streptococci of the human throat as *Str. salivarius* and *Str. mitis*, but since they were not able to draw a distinction between these two species, they considered that *Str. mitis* was a variant type of *Str. salivarius*. Although, subsequently, Niven, Smiley and Sherman (1941 a, 1941 b) and Sherman,

Niven and Smiley (1943) differentiated *Str. salivarius* from *Str. mitis* by a few physiological characters, one may still consider that the difference is not so marked as to warrant separate names for them. For the purpose of this study, however, all the strains showing the biochemical characteristics of *salivarius* and *mitis* as described by Sherman *et al* (1943) were classified as *Str. salivarius*.

It is interesting that strains belonging to all groups were isolated from the upper respiratory tracts of dogs. *Str. salivarius*, which is the predominant organism of the human throat, seems to be a normal inhabitant of the throats of dogs also, since they were so frequently found in these animals. 64.7 per cent of non-haemolytic streptococci isolated from normal and sick dogs belonged to this group. Other groups were *Str. faecalis* (12.2 per cent), *Str. equinus* (12.8 per cent) and *Str. bovis* (6.8 per cent). These organisms which are considered to be the inhabitants of the intestinal tracts of man and animals are likely to gain access into the naso-pharynx of dogs on account of the constant habit of these animals of licking and sniffing faecal materials.

As with the haemolytic streptococci, the frequency of these organisms was the highest in the tonsils (68 per cent), less in the naso-pharynx (47.8 per cent) and lowest in the nose (33 per cent) in both normal and sick dogs.

What part these organisms play in health and disease in dogs is beyond the knowledge of any veterinary pathologist at present, and much more research will be necessary before arriving at any conclusion. However, the greater frequency of these organisms in all situations in sick dogs (96 per cent) than in normal dogs (77.3 per cent) possibly indicates that they play some part in disease of these animals. It may also be stressed that the biochemical characteristics of many of the strains isolated in dogs are similar to those isolated from human throat, indicating the possibility of cross infection between man and animal.

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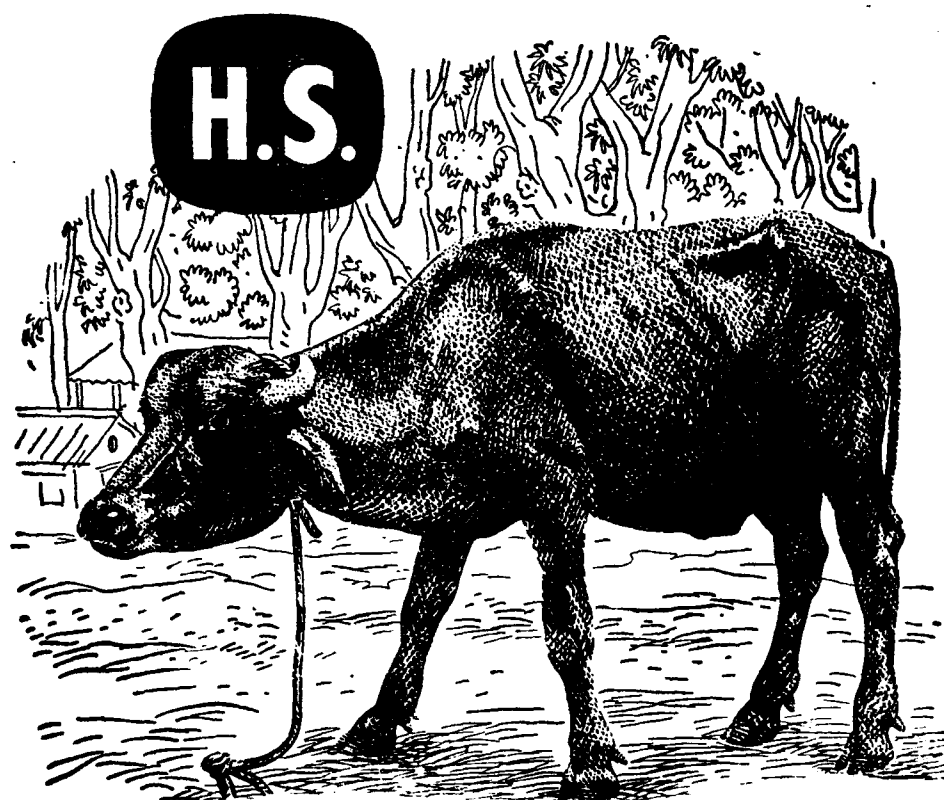
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Editorial

THE XVth INTERNATIONAL VETERINARY CONGRESS

From the periodical bulletins we have been publishing in these pages, our readers would be aware, we are sure, of the forthcoming XVth International Veterinary Congress which is to be held this year at Stockholm from August 9th to 15th. This is a great international event, very important for the Veterinarian and for the Veterinary profession all over the world. It is an occasion for pooling our knowledge about the advances and problems of our profession, for exchange of ideas, views and experiences and for creating social contacts. Ordinarily this Congress is held once in four years but, unfortunately, owing to World War II, its sessions were interrupted for nearly twelve years. And, although the XIVth International Veterinary Congress was held in the year 1949, after the cessation of hostilities, still it was not as representative as the previous gatherings mainly due to post-war conditions. Naturally therefore, this Congress has assumed great importance and it should be the endeavour of every country in the world to depute representatives to it and get benefitted from the variety and wealth of information that would be available there. We had taken it for granted that our country also would, in due course, arrange to send representatives to it, as it has done for many an international event. We are, therefore, very much surprised to learn that this is not going to be and that this country will not be represented at this Congress. The reasons for this decision are not available but, whatever the reasons may be, we are sure the

Veterinary profession in this country will feel that the Government have treated it in the proverbial cindrella way. This XVth International Congress is practically the first Congress to be held after the attainment of our Freedom and, therefore, our country stands to benefit by participating in it. It will give us and the Veterinary profession here an international status which it did not have all along. Further, with the elimination of distances by the modern quick and rapid means of communications, the world has shrunk a great deal. The barriers and boundaries between nations and countries have practically disappeared and the peoples of the world have been brought nearer to each other as never before. Transport of livestock and livestock products take place with surprising ease from one country to another. Diseases of animals and humans are likely to appear and spread with increasing rapidity and suddenness. Prophylactic measures become useless when a few nations fail to fall in line with accepted standards and regulations. At a time when the over-all shortage of food requirements for the people of different countries is causing great anxiety to the ruling powers and at a time when great efforts are being made to increase agricultural production and animal products, it is highly imperative that livestock problems are tackled with understanding and judgment on an international basis, because, as has often been reiterated, the success of the agricultural operations depends upon the efficiency of the livestock. We, in this country, have to make a lot of leeway before we could catch up with the advances made in other countries in livestock problems and we, also, suffer from the want of an adequate number of trained Veterinary personnel. Under these circumstances, it would be a great pity, we would even say a tragedy, if our country does not participate in International Veterinary gatherings, contribute to the discussions therein with our experiences and finally help in the formulation of measures for the rational approach to the problems of livestock industry. We, therefore, request the Government to reconsider its decision and arrange to send a delegation of Veterinary Officers to this important XVth International Veterinary Congress.

COMMUNICABLE DISEASES

Diseases communicable to human beings from animals have been the subject of close study at the hands of research workers in comparative veterinary medicine for a long time past. With increasing accumulation of knowledge, more and more diseases are added to the list and Public Health authorities are alerted frequently of the danger of such maladies. In countries where strict vigilance is maintained over these affections, investigations are regularly undertaken to study the normal conditions in animals in a more detailed way than what had been done before and, as a result, the people in those countries are much more aware of the nature of communicable diseases. One such study is of the bacterial flora of the upper respiratory tract of dogs as these animals have attained an importance in our modern civilisation owing to their intimate contact with human beings. In this issue of the *Journal* an investigation of this kind carried out by Dr. K. S. Shetty of Hyderabad-Deccan in Edinburgh is recorded fairly *in extenso* for the benefit of our readers. It shows there is lurking danger from these animals to humans if they were to be fondled far too intimately. We very much wish that some similar investigations are undertaken in this country also so that we may know exactly whether the findings in other countries apply here also.

While the Public Health aspect of communicable diseases has thus been under close study, the reverse aspect of it viz. the Animal Health aspect of it and the nature of the diseases communicable from human beings to animals have not received much attention as far as we could gather. It is, therefore, very interesting to read in the *Onderstepoort Journal of Veterinary Research* for March 1952 an article by P. J. J. Fourie postulating the proposition:—Tuberculosis in Man, an Animal Health problem. We extract elsewhere in this issue the summary and conclusions of that author and draw the special attention of our readers to the following observations:—This experience with the Onderstepoort herd makes it abundantly clear that man as a source of infection of the bovine with the human type of organism, with consequent sensitization, and man with open pulmonary lesions due to the

bovine type of organism as a dangerous source of infection for bovines, can no longer be regarded as a rare academic curiosity, but must be faced as an important practical issue, where a serious attempt is being made to eradicate tuberculosis from cattle and to maintain them free from sensitization. Again, while reviewing the literature on the subject, the author quotes instances where herds free from tuberculosis had become infected by humans with open pulmonary tuberculosis due to bovine type of organism. This is a clear warning to us in this country. According to the information so far collected and available, the incidence of tuberculosis in the cattle of this sub-continent is very low. On the other hand, its incidence in humans is fairly high. Many a suffering people go about their avocations owing to economic factors and also due to absence of adequate sanatorium facilities. Some of these people might even be engaged in dairies and in the tending of cattle. These diseased persons are likely to spread the disease to animals through their expectorations, creating thereby a great Animal Health problem. Here, therefore, is a great danger lurking amidst us and it is up to us, Veterinarians, to be wide awake and keep things under proper check and control before they assume big proportions difficult to tackle.

Notes and News

NON-VEGETARIAN FOODS

Dr. Horace Thornton, Consultant in Food Hygiene to WHO and FAO, with working experience in 22 different countries in Europe, the Middle East, and the Americas, has just completed a three-month assignment in India. At the request of the Government of India he advised on the introduction of a modern and scientific approach to the inspection of foods of animal origin, slaughter-house construction and operation, and food-market practices. His terms of reference attach special importance to the question of primary infection in animals and secondary contamination by man of food products liable to cause food-borne diseases.

"American Reporter"

Clinical Articles

TRANS-THORACIC PARTIAL OESOPHAGECTOMY IN DOGS

By

M. N. MENON, G.M.V.C., B.V.Sc., Assoc. I.D.R.I., M.R.C.V.S.,
Lecturer in Surgery, Madras Veterinary College.

(Continued from page 380 of I.V.J. for March '53 — Vol. XXIX, No. 5)

In continuation of my article on the surgical removal of *Spirocerca lupi* nests on the oesophageal wall in dogs published in the March 1953 issue of the Indian Veterinary Journal, a second operation on similar lines was performed on 8th March 1953 for another demonstration class with the same amount of success.

The subject for operation was procured from the Lethal Chamber of the Corporation of Madras. Examination of a sample of its faeces revealed ova of *Spirocerca* indicating the presence of mature parasitic nest or nests communicating with the digestive tract. No further attempts at diagnosis and location of the nodule was made as the operation was being performed purely for the demonstration of operative technique.

The anaesthetic used was Sagattal (M & B) being tried for the first time. The dosage recommended is 1 c.c. for every 5-lb body-weight and accordingly 5 c.c. of the solution was given I/V at the rate of 1 c.c. a minute. (The dog weighed 25-lbs as recorded prior to commencement of operation). The animal 'went under' quietly by the time the injection was completed.

The right side chest wall which was suitably prepared was incised on the same lines and at the same site as in the previous case after prior intubation and establishment of positive pressure ventilation of the lungs. On retraction of the adjacent ribs a parasitic nodule was found visible on the oesophageal wall at a level behind the base of the heart. What appeared to be a small surface nodule was found on dissection to be quite diffuse and irregular in extent. Consequently enucleation was a little more difficult. The pedunculated track communicating with the lumen of the oesophagus was clamped and stitched off below and the nodule severed close to its base along the jaws of the clamp. The mediastinal pleura could not be stitched into position over the oesophagus as it was found to tear off. The operation was completed in much the same manner as described before.

Before the respiration pump was disconnected, 2 c.c. of Picrotoxin were given i/v to help speedy revival of normal respiration.

The temperature of the patient remained normal till the third day after operation when a rise by 1 degree was recorded which rose to 1.5° the following day. Examination of blood smears revealed nothing specific.

Consequently the dog was put on a course of Penicillin which restored the temperature to normal. One point which drew attention was the accumulation of some sero-sanguineous fluid under the skin over the suture line noticed on the fourth day being possibly pleural effusion which had escaped through the wound on the chest wall. This fluid was drained off by removing the dependant stitch. Thereafter the recovery was uneventful. All sutures were removed on the ninth day. Incidentally it may be stated that Sagittal anaesthesia was found to be very satisfactory, confirming the results of experimental trials conducted by me.

Reproduced alongside is an X-ray graph of the chest of the dog operated previously to show the new growth of rib 3 months after operation and showing no trace of local lesion along the oesophagus. This animal is still under observation.

My thanks are due to Messrs. May & Baker (India) Ltd., Madras., for the supply of Sagittal, to the Principal, Madras Veterinary College for his continued interest and to Mr. Jambulingam the college artist for the photo.

A CASE OF HERMAPHRODISM IN A HORNED MILCH GOAT

By

K. V. DIVEKAR, G.B.V.C., P.G.,

*Asst. Artificial Insemination Officer,
Bombay State, Poona.*

Hermaphrodisism or intersexuality occurs more frequently in goats than in any other farm animals, but there seems to be no accurate date of its frequency. Most animals reported as hermaphrodites are not true ones. They exhibit a condition known as pseudo-hermaphrodisism i.e., the external genital organs are usually of one sex, either male or female. (Hermaphrodites do not breed, they are a total except for their value as meat). It has also been noted by many authors in other countries that horned hermaphrodites in dairy goats are very rare or perhaps do not occur at all.

Addington & Cunningham (1935) of the New Mexico station in a study of the heredity of horns in goats noted four hermaphrodites, all polled and they appeared in matings between animals heterozygous for the polled condition. Paget (1943) reported that horned hermaphrodites are a very rarity known only in occasional instances among British Goats. Asdell (1944) has observed in about 200 intersexual goats, both in England and in America, and found all to be hornless.

Eaton and Simmons (1939) have reported hermaphrodisism to be inherited as a simple recessive. If, however, as has been suggested by Asdell (1944), only females are affected, matings between heterozygous normal will give 7 normals; 1 hermaphrodite. Divided as to sex, this ratio

becomes 4 males, 3 females and 1 hermaphrodite. Matings of homozygous recessive bucks with heterozygous does should give 50% males, 25% females and 25% hermaphrodites. There was one such male in the Sannen breed. The sex ratio for goats is usually reported with a high excess of males.

There seems to be some disagreement as to the sexual nature of the hermaphrodites. Witschly (quoted by Allen) considers them generally females.

Coming now to the subject of this paper, a case of hermaphrodisism in a horned dairy goat was recorded at Nasik in 1949.

The goat, about 10 months old, was admitted in the hospital on 3-4-1949 with a history that it made frequent attempts at micturition which itself was noticed to be difficult and painful.

On examination, the genitalia appeared to be rather abnormal. The so-called vaginal passage could admit only a small finger. The clitoris could not be noticed. Two protrusions of the size of a betelnut were noticed posterior to the teats at a distance of about an inch. On closer examination, it was found that the clitoris was deeply embedded. It was therefore operated upon to make sure of the structures. After making a deep incision in the region, the clitoris proper was well exposed. The tissues round about were clipped so that the slit could remain open. The wound was treated in the usual way. After the wound had completely healed up, the owner was requested to bring the animal again after a fortnight to observe changes, if any; but, he brought it after four months with a history that it was exhibiting masculine characters and had started behaving in a peculiar way. On examination, it was found to be true that the animal had become predominantly masculine. The neck appeared to have become massive and the voice changed to that of a he-goat. When was shown to a she-goat, it had great inclination to mount and when allowed it did so on several occasions. The small two protrusions at the posterior part of the udder were now found to have further developed and assumed the size of a large elongated jambul fruit about half an inch in length. The clinical observations suggested that the two protrusions might be testicles. It was therefore decided to operate and remove them so as to ascertain their true nature. This was done and the Principal, Bombay Veterinary College, very kindly examined them and reported as follows:—

“The tissue is from testicles. One can see the vas deferens. The seminiferous tubules are destroyed and instead several abscesses full of polymorphonuclear leucocytes are seen. These are surrounded by an overgrowth of connective tissue proliferation which has a tendency for malignancy. The connective tissue also shows fatty degeneration, Stained with Grams. No organisms present.”

The owner turned up after two months with a history that the animal was now showing signs of heat and standing close to he-goats. On examination, it was found that the vagina could admit the index finger and that the os uterus was well dilated. There was change in the voice, the massiveness of the neck had become reduced and the teats enlarged. It was evident from the symptoms that the goat was in full heat and therefore the owner was advised to have it covered.

No further observations could be made since the owner disposed it off after about a month or so after it was mated.

Eaton (1945) has stated that since all hermaphrodites are hornless there is a possibility that the genes for hornless and hermaphroditism may be linked. This leads to considerations that a single gene may be responsible for both the polled and hermaphroditic characters. There is possibility that the genes for hornlessness and hermaphroditism probably exert their primary influences on the endocrine system during sex differentiation but are only secondarily responsible for the appearance of hermaphrodites. Hermaphrodites are supposed to be due to excessive or delayed hormone secretions during or immediately prior to sexual differentiation.

As the incidence of such a case is rare in horned goats this merits publication.

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CORYNEBACTERIUM PYOGENES MASTITIS FOLLOWING POX*

BY

RAM NARAIN MOHAN,
Veterinary Investigation Officer,
Livestock Research Station, Mathura, U. P.

In this paper are recorded some observations made in 1948-49 winter on several State farms in Uttar Pradesh in connection with the occurrence of outbreaks of cow-pox accompanied by suppurating mastitis, chiefly due to *Corynebacterium pyogenes*, in many lactating buffaloes and a few cows. Pox was also reported later from the localities in East Punjab from where these animals were purchased.

*Read at the Indian Science Congress, 40th Session, Lucknow, January 1953.

A varying percentage of animals, particularly buffaloes, on different farms, were found affected to a varying degree of severity with skin lesions which were clinically typical of pox. The eruptions appeared generally in large numbers on most parts of the body, especially the udder and teats and the sides and the under-surface of the abdomen, in some cases, also on the vulva and the perineum. The lesions on the teats were generally more pronounced than those on other parts of the body. Repeated handling of the teats during milking, and injuries occurring in other ways, soon converted these lesions into large wounds. They were often situated around the teat orifice.

Many lactating buffaloes affected with pox lesions, especially on the teats, developed suppurating mastitis in one or more quarters. A few calves which had suckled at these udders developed purulent gingivitis and peridental abscesses resulting in loosening of one of more incisor teeth. Calf mortality figures during the period were rather high. Treatment of the mastitis with various drugs (acriflavine, proflavine hemisulphate, sulphonamides, penicillin, etc.) proved of little avail and over 100 quarters became more or less completely blind. Propamide infusions through the teat canal helped chiefly in reducing the consistency of the purulent udder secretion thereby making evacuation a little easier than otherwise. Many apparent cures (cessation of flow of pus through the teat canal) were later followed by crops of abscesses in the mammary glands breaking out on different points. A few udders became gangrenous and the affected animals died. Mortality due to generalised pyaemia, etc., was low and suppuration of joints was rare.

Bacteriological Examination

In view of the typical clinical picture of rather severe pox, no attempt was made to conduct transmission tests.

The udder secretion of 25 affected quarters of 21 buffaloes on three different farms was examined culturally with the following results. *C. pyogenes*, generally in pure culture, was isolated from 24 quarters. From the remaining one quarter, as well as from another included under *C. pyogenes*, *Streptococcus agalactiae* was isolated as the predominant organism. A few cultures of *C. pyogenes* and the two cultures of *Str. agalactiae* were forwarded to Mukteswar and the diagnosis was confirmed in each case after the usual cultural and biochemical tests there.

Some time later, a few cases of suppurating mastitis associated with *C. pyogenes* were encountered in goats in another State farm during an outbreak of goat-pox accompanied by severe lesions on teats.

Discussion

The occurrence of suppurating mastitis in cows and buffaloes in India due to *C. pyogenes* has already been recorded (Mohan, 1945.)

Mastitis of this type, often called "summer mastitis" in British veterinary literature, is said to affect non-lactating udders much more frequently than lactating udders. The present occurrence of *C. pyogenes* mastitis in a large number of lactating udders was evidently due to cow-pox predisposing the udders to secondary infection.

That *C. pyogenes* is widely distributed among apparently healthy as well as diseased animals all over the world is now well recognised and the organism has been found associated with a wide variety of disease conditions, often as a secondary invader (Lovell, 1950). The present writer has had experience of the frequent existence of *C. pyogenes* in such disease conditions as suppurating mastitis in cows, buffaloes and goats, pneumonia in calves, sheep and goats (particularly following primary injury by lungworms and such viruses as pox and rinderpest), purulent peritonitis following suppurating worm nodules in the intestines, suppurating lymph nodes and abscesses occurring in different parts of body.

No satisfactory treatment for mastitis and other infections caused by *C. pyogenes* has yet become available and at least two such conditions, viz., mastitis and pneumonia, are a menace to livestock industry. There is also no satisfactory method of immunising animals against *C. pyogenes* infections, though some degree of success has been claimed for the toxoid in raising antitoxic titres and in treatment of mastitis in cows and sheep (Weitz and Langridge, 1947; Brown and Stuart, 1943). Further research for developing satisfactory methods of treatment and for producing a high level immunity is indicated.

Summary

Over 100 lactating buffaloes and cows on organised farms were found affected with suppurating mastitis during outbreaks of cow-pox. No attempt was made to recover the pox-virus, but the lesions were characteristic of that disease. The udder infection was usually associated with pox lesions on the teats. Many calves suckling at such udders became affected with peridental suppuration.

C. pyogenes was recovered in almost pure cultures from the purulent udder secretion of several animals. Mastitis due to this organism ("summer mastitis") is believed generally to be an affection of dry cows.

Similar cases of suppurating mastitis, generally due to *C. pyogenes*, have been encountered in lactating goats affected with pox lesions on the teats.

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A NOTE ON BOVINE FROTHY 'BLOAT' AND ITS TREATMENT

BY

G. B. SINGH, B.A., M.R.C.V.S.,

Director Animal Husbandry and Veterinary Services, Orissa
 and

M. M. RAO, G.B.V.C.,

Veterinary Assistant Surgeon, Cuttack.

Tympany in cattle is caused by interference with the normal gas elimination, the resulting gas pressure arresting diaphragmatic movement and probably the blood flow through the adjacent large vessels, until death from asphyxia occurs. Prolonged research into the nature of this interference has postulated the theories reviewed briefly hereunder.

1. *Excessive gas formation*.—This theory is acceptable only if it can be shown that gas formation ever overtakes the animal's capacity for elimination. The opposite has recently been shown to be true, i. e., cattle can release far more gas than the rumen can produce.

2. *Food compaction*.—Compact foods, such as legumes, rapidly eaten, were thought to collect into a dense mass mechanically preventing gas release. This theory is incompatible with the quick response to appropriate treatment.

3. *Toxic gas*.—Possible toxic gases produced by fermentation are carbon monoxide, hydrogen sulphide and hydrocyanic acid. No abnormal content of the first two has been proved in affected animals and, while some clovers contain enough of the last to kill cattle if the acid were in the free state, the rate of production and elimination renders it non-toxic. South African workers tried unsuccessfully to cause tympany in sheep by giving cyanides.

4. *Insufficient roughage*.—Thought to cause atony of the rumen, possibly true. There is no evidence that such atony is a significant factor in acute tympany.

5. *Allergy*.—There is some evidence that an allergen may be involved in certain types, particularly associated with oesophageal spasm, responding to adrenalin.

6. *Muscle paralysis*.—Not to be confused with rumenal atony, muscle paralysis has been shown by British workers to be due probably to a flavone, a yellow crystalline substance isolated from lucerne. Flavones are natural plant pigments so that all flavones are probably paralysants to some extent. Grass has less flavone than legumes, and possibly other counteracting muscle stimulants in grass limit its action. The position of flavones in rumenal physiology is the subject of current research.²

7. *Permanent foam*.—When the surface tension of a fluid or semi-fluid medium is lowered, gas is imprisoned in frothy bubbles having a proportionately higher bursting resistance, until a point is reached where it is practically impossible to burst the bubbles by any mechanical means. If such a surface tension reduction occurs in the rumen, we may expect a tympany resistant to relief by stomach-tube or rumenal puncture. These signs were seen in the 10 case histories now reported.

Treatment of Frothy 'Bloat'. Agents increasing surface tension are indicated to burst the foam and release the gas. South African, and American² works have drawn attention to the use of polymerised methyl silicone, and British research has produced an ethylene oxide derivative of ricinoleic acid, which has been marketed as 'Avlinox' (Imperial Chemical Pharmaceuticals Ltd.) and which is stated to be non-irritant and non-toxic.

Eight semi-acute tympanic cattle were stomach-tubed, with no escape of gas, and then treated with 'Avlinox' 8-10 c.c. in 2½-4 oz. of water given orally. Recovery was complete in 4-6 hours.

Two acute cases were treated as follows:

- (a) Rumenal puncture by trocar and canula failed to release any gas. 'Avlinox' 10 c.c. in 3 oz. water was injected through a catheter in the canula, into various parts of the rumen, and the canula left in for two days. Recovery was complete in 6 hours.
- (b) A similar case was not punctured because the owner objected. 'Avlinox' 8 c.c. in 2½ oz. water was injected into the rumen through the flank by syringe and a large bore 5" needle. Recovery was complete in 6 hours. In no case was re-treatment necessary.

Summary

'Avlinox', an ethylene oxide derivative of ricinoleic acid (I.C.P. Ltd.), is effective in the treatment of frothy tympany ('bloat') in cattle in single doses of 8 to 10 c.c. in 2½ to 4 oz. water, either orally or by direct injection into the rumen. No toxic or irritant signs were seen. 10 case treatments are reported.

Acknowledgments

We wish to thank Imperial Chemical Industries (India) Ltd., for supplies of 'Avlinox'.

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SUBCUTANEOUS EMPHYSEMA AFTER PUNCTURE OF RUMEN IN A COW

BY

K. CHOTAJAN, B.V.Sc.,

Veterinary Assistant Surgeon, Hosur, Madras.

On 26-5-52 at 6-30 A.M., I was called in to attend on a cross-bred cow with the complaint that she had developed tympanitis since early hours of the day. On detailed enquiry it was gathered that she was fed with badly-preserved horse-gram the previous evening. A drench with turpentine and oil was given and frequent kneading of abdomen was advised.

By about 9 A.M., the tympany had increased enormously and the animal had fallen down struggling hard for breath. To relieve the distress of the animal, the rumen was punctured with a trocar and cannula taking all the usual precautions. Antizymotics were poured in through the cannula and the cannula was retained in position by means of tapes.

On visiting the animal again at 11-30 A.M., I observed a diffuse crepitating swelling over the whole of the left side of the trunk and back as far as the shoulder giving the animal a ballooned appearance. The crepitant swelling was typical of a Black-quarter lesion. The temperature was only 102.2° F and the animal was brighter than what it was in the morning. Hence the complaint was recorded as that of subcutaneous emphysema as a sequel to puncture of the rumen.

The cannula was removed and massaging was advised towards the opening of the wound.

At 5-30 P.M., the animal was found in the same condition. The punctured wound was not patent. The opening was therefore enlarged and massaging was done with the help of 3 or 4 persons. About 50% of the air was thus expelled out. Advised owner to continue massaging at intervals.

27-5-52 6 A.M.—The swelling had almost disappeared and the animal was brighter and ruminating. Temperature was 103° F. Stimulants were given.

At 6 P.M., the animal was again dull and the temperature shot up to 105.4° F. Infection due to secondary organisms was suspected and the animal was put on Penicillin.

28-5-52. 6 A.M. Temperature had come down, the swelling completely reduced and the animal was inclined to feed. The wound had become septic but it healed with ordinary dressings in the next few days.

Conclusion

The case is recorded only to show that subcutaneous emphysema may sometimes occur as a sequel to puncture of rumen and that it should be differentiated from Black-quarter. The condition is, however, not serious and yields to treatment.

Acknowledgment

My thanks are due to Dr. A. Alagar, G.M.V.C. District Veterinary Officer, Salem, for his kind permission to publish this article.

MILK FEVER IN A SHE-BUFFALO

By

R. N. DESAI, G.B.V.C., M.S., Ph.D.,
Banaras Hindu University.

A she-buffalo of the University Dairy, in a fairly good condition, calved on 9th December 1952 and was quite normal till the next day, when she was reported to be off-feed from the morning. She milked in the morning as usual but did not yield anything in the afternoon.

When examined, she was lying and was not inclined to move or get up. The muzzle was dry. The eyes were dull with staring expression. The conjunctiva was congested. The ears were drooping and cold. The croup and the hind legs were also extremely cold. The rectal temperature was 99.8° F. After sometime she adopted a typical milk fever attitude, with the neck resting on the shoulder. Later on, she got up of her own accord and walked about 10 yards to the water-trough with difficulty. Her gait was unsteady and the hind legs wide apart. After drinking some water she could not come back to her place and lay down on her way. There too she adopted the same posture. During this period of observation she passed urine but in small quantity and without the characteristic odour of acetone. The case was highly suggestive of milk fever and she was given subcutaneously 300 c.c. of a 20% solution of Calcium-boro-gluconate. Within about 15 minutes after the injection, she got up and walked around. Immediately she was taken near the fire place to warm her up. About an hour after, she started rumination and remained in a standing position for quite a long time, which she could not do before the treatment.

The next morning, she did not give milk though she took her feed. She was put on green fodder. In the after-noon, she was given another 250 c.c. of the same solution but this time intravenously. By evening she allowed her calf to suckle but did not yield much.

On 13-12-52 morning she took her feed normally and gave about 2½ seers of colostrum. Within about three days time she came to her normal yield.

Discussion

Milk fever in buffaloes is rare and hence this case is recorded. This buffalo is one of the heavy yielders of the dairy. It is the practice on the farm that the colostrum is not sold out as it is tabooed by certain people for use. So it is distributed to the farm servants. To derive the full advantages of this, the gowala must have milked the buffalo completely. Is it possible that this total stripping precipitated the onset of the disease

A CASE OF SEVERED BACK TENDONS IN A COW

By

R. C. CHOUDRY, B.V.Sc.,
*Veterinary Assistant Surgeon,
Kotkhai, Himachal Pradesh.*

Subject:—A non-descript, dark-grey cow, aged about six years, in calf about six months.

History:—On the 22nd June 1952, the owner of the animal came over to the hospital and reported that his cow, described above, had fallen from a height three days previously and that, since then, it was not able to move about.

Symptoms and Diagnosis:—On examination of the animal in its stall, it was found to have sustained a deep-cut wound, about 2" long, in the left hind shank at the junction of the upper and middle third posteriorly. The animal was not placing much weight on that leg. The toes were turned upwards, the pastern was more or less parallel to the ground and the rudimentary digits were almost touching the ground. Further detailed examination after casting the animal and securing the leg revealed that the back tendons had become completely severed and that even the suspensory ligament was greatly damaged. The wound was septic and discharged a blood-stained foetid pus.

Treatment:—As the proximal cut ends of the tendons had receded very much and as the part was highly septic, no attempt was made to suture the tendons. The part was thoroughly washed with strong antiseptics and all the loose and dead tissues were completely removed. Packing the wound with a gauze of Proflavine (1-1000), a couple of stitches was put in to prevent gaping of the wound and also to retain the gauze safely in. The part was then completely covered with plenty of cotton wool and bandaged. Thereafter, splints were applied extending from the hock to the hoof, leaving a window for the dressing of the wound. The animal was released and tied short so as to restrict its movements.

Daily treatment to the wound was carried on in the usual way. It healed without much difficulty and by the 21st of July 1932, i.e., a month after the accident, the animal was able to bear weight on the leg without any descension of the fetlock. A thick nodular cicatrix had formed at the place of the wound which, too, disappeared a great deal in course of time.

When seen 7 months after, the animal was found moving about practically without any lameness.

This case is recorded only to illustrate that a case of severed back tendons need not necessarily terminate in gross defects and that it may heal without any bad after-effect.

SPIROCERCOSIS IN A DOG

By

D. S. PARAMANAND RAO, B.V.Sc.

Assistant Veterinary Surgeon, Seetarampet, Hyderabad-Dn.

Having read the article on Canine Filariasis by Mr. C. A. McGaughey, Faculty of Veterinary Science, University of Ceylon, published in the Veterinary Record No. 51, Vol. 62, I would like to publish the following case of Spirocercosis in a dog and its successful treatment with Hetrazan.

At the Veterinary Hospital, Seetarampet, an Alsatian dog was under treatment for skin disease. This was cured in a few days and the case discharged. Later on, the owner brought the dog with a history of frequent vomition, off feed and loose motions. The condition of the animal was poor and its temperature was normal. Suspecting worms, the faeces were examined and ova of *Spirocerca* were found. The animal was thereupon put on a course of Hetrazan and 500 grms (10 tablets) of it were given daily for ten days. Glucose injections were also given every day intravenously.

After ten days, there was a remarkable improvement in the condition of the dog and there was no recurrence of the vomition. The animal was also very lively. The motions were normal and were negative for *Spirocerca* ova on microscopical examination.

I acknowledge my thanks to Dr. Mahalingam for his valuable guidance and encouragement given in writing this article.

Dicephalous Monstrosity in a Murrah Buffalo



Photo showing the monster with two heads, two necks and four forelimbs.

[Vide article by M. V. Sapre, Page No. 549.]

A Freak Pup



Photo showing the pup with the head shaped like that of a calf elephant with the upper jaw having a tapering snout.

(Photo by the courtesy of Hindu)

[Vide article by V. Venkatarayan, Page No. 350.]

EARLY SEPARATION OF PLACENTAL MEMBRANES AS THE CAUSE OF DYSTOKIA IN A COW

By

S. VENKATA NARASU, G.V.Sc.

Veterinary Assistant Surgeon, Padampur, Orissa.

On 25th August 1952, a cow was brought to the dispensary with the history that it was in labour since the previous night and that no progress had been made. On clinical examination, it was found that the presentation was normal with the head and the two fore-limbs in the vaginal passage. The cause of the delay in calving was not quite apparent at once but, on closer examination, it was found that a portion of the placenta covering the entire fore-head and eyes of the foetus was obstructing its easy exit. I just raised the membranes with my fingers and immediately the foetus came out without any further interference and with it the placenta also. The operation took only two minutes.

The after-treatment was in the usual way and the cow was discharged the same day with the calf. Is it possible that the difficulty in calving was due to an unusually early separation of the placenta?

DICEPHALOUS MONSTROCITY IN A MURRAH BUFFALO

By

M. V. SAPRE, B.V.Sc.,

Jabalpur, Madhya Pradesh.

The enclosed photograph of a *Dicephalous* monstrosity in a Murrah buffalo will be found interesting by the members of the profession as such cases are rare.

The monster consisted of really two calves fused at the thorax. It had two heads, two necks, four fore-limbs, four hind limbs and two tails with a single heart and diaphragm. The presentation was anterior but, owing to the large size of the monster, parturition could not be effected easily. Embryotomy had to be done and the two calves were separated and extracted individually.

The mother was none the worse for the ordeal and is doing well, giving about 12 seers of milk per day.

A FREAK PUP

BY

V. VENKATANARAYANAN, G.M.V.C., B.V.Sc.,
Veterinary Assistant Surgeon, Corporation of Madras.

A mongrel bitch in her fourth pregnancy gave birth to a freak along with two ordinary pups on January 4, 1952. The freak lived only for a few minutes.

Description of the freak:—It was a well developed pup of a comparatively large size and with normal features except in the head which was shaped like that of a calf elephant. The upper jaw had a long tapering snout about one inch long while the lower jaw was shaped like that of an elephant's.

The photograph enclosed herewith has been obtained through the kind courtesy of *The Hindu*, Madras. The specimen of the freak is preserved in the Basin Bridge Veterinary Dispensary of the Corporation of Madras.

Correspondence

[The views and opinions expressed in correspondence are those of the writers themselves and are not always endorsed by the Editor. Ed. I. V. J.]

Wanted a textbook of anatomy on the ox

It was decided by common consent, that in India the anatomy of the ox should be taught in all the Veterinary Colleges as the type and that the anatomy of the other domestic animals should be studied in a comparative manner.

As no text book was in existence in the English language on the anatomy of the ox, the Indian Council of Agricultural Research requested certain Indian Veterinary Anatomists to prepare a text book on the anatomy of the ox. Rao Sahib H.N. Chelva Iyengar, Lecturer in Anatomy, Madras Veterinary college, was engaged in this work for about four years at the instance of the I.C.A.R. He was helped in this work by a technical assistant – a Veterinary Graduate – an artist photographer, a typist, peons and others. In addition to the pay an allowance of the staff, a great deal of material in the shape of animals, stationery, artists equipment, etc., must have been spent. I know for certain that the work while in progress was periodically and systematically supervised by the then Director, Veterinary Services, Madras, as well as by Officers of the I.C.A.R. Finally at the completion of the work by the end of 1944 an honorarium of Rs. 2000/- was also awarded to Rao Sahib Chelva Iyengar. I believe that about Rs. 20,000/- would have been spent on this venture. From the little I had seen of the work,

the book was taking a good shape. For some unaccountable reason the book has not yet seen the light of the day. Teachers and students on the subject are seriously handicapped for want of a proper textbook on bovine anatomy. In certain Universities bovine anatomy is not given the required prominence as the teachers are still continuing with the teaching of the anatomy of the horse.

Is it possible that this mystery would be cleared up by the authorities of I.C.A.R. and publish the book?

Hyderabad-Dn.. } K. S. NAIR,
 15-9-52 } *Dean & Professor of Animal Husbandry,
 Osmania University, Hyderabad – Dn.*

[Note by the Editor I.V.J : On referring the matter to the I.C.A.R., we are informed that the publication of the above monograph is under consideration.]

XVth International Veterinary Congress Organising Committee
 C/o Isaksson State Veterinary Medical Institute
 Stockholm 50, Sweden.

Dear Colleagues,

The Organizing Committee have the honour of inviting you to apply for Special Membership of the XVth International Veterinary Congress, which will take place in Stockholm, August 9th-15th, 1953.

Special Membership is intended for Authorities, Veterinary Medical Schools, Veterinary Faculties, Libraries, and Veterinary Associations, which will not be represented at the Congress and for colleagues who are unable to attend the Congress in person. Special Members will receive the Proceedings of the Congress, Part I (including nearly 300 sectional reports) and Part II (including 10 plenary lectures and the contributions to the discussions after the sectional reports).

The Proceedings of the Congress, Part I, will be published before the beginning of the Congress, and Part II as soon as possible after its conclusion.

The price of the proceedings of the Congress, Parts I and II, is 45 Swedish kronor including postage and expedition expenses.

Application for Special Membership should be made to the XVth International Veterinary Congress, *Stockholm 50*, Sweden, before August 1st 1953, using an application form of the tenor below. Please write distinctly!

With collegial regards.

Very truly yours,
 FOR THE ORGANIZING COMMITTEE
 AXEL ALEGREN AZEL ISALSSON
Chairman Secretary.

Application for Special Membership of the XVth International Veterinary Congress in Stockholm 1963.

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Abstracts

Infectious coryza of fowls in India*—By S. B. V. RAO, L.V.P., Division of Poultry Research, Indian Veterinary Research Institute, Izatnagar, U.P.

The prevalence of Infectious Coryza characterised by inflammatory lesions of the upper respiratory tract, in specialised poultry farms all over India, a serious menace of growing chickens, has been confirmed. An appraisal of the etiological factors was made. Coryzas caused by two independent disease-entities viz. (1) *Haemophilus gallinarum* and (2) a coccobacillary organism or a virus have been studied. Chemotherapy with Lugol's Iodine, sulphamezathine and dettol were found to be inefficient in the latter type of coryza described.

Inflammatory exudate from early cases of coryza was found to be highly infective to 10-12 weeks old chicks, when instilled intranasally, but innocuous by other routes, while penicillin-treated exudate proved non-pathogenic. Pooled suspension of infective exudate standardised to Brown's Opacity Tube 10, in N. S. S., when injected in 0.5 c.c. to 1 c.c. dose intramuscularly, immunized chicks against subsequent infection for an undetermined period.

* Paper presented at the Fourth Meeting of the Disease Investigation Officers' Conference, Poona, March 1953.

A note on day-old chick vaccination against fowl-pox using egg propagated fowl-pox virus vaccine*—By S. G. IYER, S. B. V. RAO & B. M. Thakral. Division of Poultry Research, Indian Veterinary Research Institute, Izatnagar, U.P.

Literature on baby chick vaccination against fowl pox has been briefly reviewed. The consensus of opinion of workers is that immunization of chicks below six weeks of age using fowl pox skin lesion virus proves unsatisfactory owing to severe systemic reactions resulting in a heightening of inter-current infections and/or retardation in growth. In the present study however day-old chick have been successfully vaccinated with a field strain of fowl pox virus which has been modified by passages in embryonated eggs. The vaccine was prepared from infected membranes in the fifteenth passage of the virus, in concentration of 4 and 8 per cent in 25 per cent glycerine-broth and pH 7.4, and produced uniformly good "takes", followed by solid immunity so far up to twelve months. Other interesting observations are that neither systemic reactions resulting in growth retardation nor lowering of resistance to inter-current infection were noticed as was reported by previous workers abroad who used fowl pox scab virus for immunizing purposes in early chick vaccination. Most of the incontact group of unvaccinated chicks developed immunity without showing visible symptoms of infection.

Extract

Tuberculosis in man, an animal health problem.—By P. J. J. FOURIE, Section Pathology, Onderstepoort. *Onderstepoort Journal of Veterinary Research*, Vol. 28, No. 3, March 1952.

In testing the Onderstepoort herd of experimental animals, totalling 224 animals, 10 positive reactors were found in 1949. Since tuberculosis was eradicated from this herd in 1940 by slaughtering all reactors and doubtful reactors and since with the exception of one animal, no further cases were encountered at the annual tests during the last 9 years, this result was not only surprising, but also alarming.

Seven of the reactors were from a group of gallsickness vaccine reservoir animals, which were kept in complete isolation in so far as other cattle were concerned. Their hay and bedding were sterilized. The only food which was not sterilized was the silage and the concentrate ration. Since the Onderstepoort

* Paper read at the 40th Indian Science Congress January, 1953.

silage was shown to contain 4 per cent. acid, it was believed that any tubercle bacilli which might be present, would be destroyed. The concentrate ration could carry viable tubercle bacilli, but even if that were the case, the source of such infection was much more likely to be human than bovine. As these animals become too old or are for some other reason no longer suitable as donors of blood for the vaccine, they are slaughtered and in every case a post mortem examination is made by a veterinarian. During the last 30 years not a single case of tuberculosis was found in this group of isolation stables. Lesions were present in six out of the seven animals. The 7th had actinomycosis, which was thought to have been responsible for the sensitization in this animal. An attempt was made to type the organism from all six animals. In four the bovine type was identified. In one the organisms were lost and in the other, owing to difficulties in obtaining good cultures for the biological examination, the results are not yet available.

It was concluded that a human being with open pulmonary lesions due to the bovine type of organism must have been responsible for the infection. All the available humans were tuberculin tested, medically examined and screened. A number of positive tuberculin reactors were found, but no clinical cases were found, on medical examination and on screening. Since the native personnel is constantly changing, it was unfortunately not possible to examine all those who were in close contact with these animals during the last two years.

In another group of three positive reactors no lesions were found and it is considered that these animals were sensitized by inspection with the human type of organism. The native attendant in close contact with these animals was shown to have pulmonary tuberculosis on screening, but up to now the organisms could not be identified in his sputum by direct smear examination, nor by biological examination.

This experience with the Onderstepoort herd, makes it abundantly clear that man as a source of infection of the bovine with the human type of organism, with consequent sensitization and man with open pulmonary lesions due to the bovine type of organism, as a dangerous source of infection for bovines, can no longer be regarded as a rare academic curiosity, but must be faced as an important practical issue, where a serious attempt is being made to eradicate tuberculosis from cattle and to maintain them free from sensitization. Where the bovine as the source of infection has been eliminated and tuberculin tests are not carried out annually, or at the most every two years, serious and disastrous setbacks may occur, when a human being with open lesions, due to the bovine type of organism, introduces the infection to a herd.

Queer Jobs.—R. P. JONES, M.R.C.V.S., Veterinary Adviser, Department of Livestock Development, Bangkok, Thailand.

One morning on arrival at the clinic of the Veterinary Faculty for my usual short visit I found the entrance blocked by several large packing cases. These, I was told, contained 14 large, sick pythons sent in by a wild-animal exporter, who reported that two showing similar symptoms had died at his establishment.

I have had to devise various methods of restraint for most species of indigenous wild animals submitted for treatment, but this looked like a major problem as there were two or three snakes in each packing case. I visualized, while attempting to secure one, its roommates would escape with myself enveloped in the exodus. The attendant who came with the snakes volunteered to grasp one by the neck if four other men would control the body. A packing case was cautiously opened, the attendant made a sudden lunge and gripped a snake with both hands behind the head, when out slithered yards of python. An interesting rough and tumble display followed while the coils were straightened out.

All the pythons had necrosis of the gums, and of the skin and underlying tissues of the upper and lower jaws. Dead tissue was removed and the wounds curetted which seemed to cause no pain though I felt I detected, at this stage, an increased malignancy in the stare the patients bestowed upon me. But when tincture of iodine was applied to the raw surfaces, there was a general upheaval. The pythons, under this initial and subsequent milder treatment, together with daily injections of procaine penicillin in oil, all duly recovered. The injuries were attributed to the snakes using their heads as so many battering rams to try to force an escape from their cages, but no explanation can be offered as to the source of the necrotic infection involving all pythons in the consignment, which was sufficiently extensive in the two untreated ones to cause death.

On the first day, the presence of the snakes was responsible for a considerable drop in the income of the clinic. Ladies arriving with their pet dogs and cats gave one horrified look at what was taking place, dug their chauffeurs in the back and cars turned around and disappeared. A more secluded spot was selected for subsequent treatments.

Pythons are quite common in local rice mills where the Chinese proprietors, in spite of appreciating their meat as a succulent delicacy, carefully cherish them as killers of rats, and prohibit their capture and destruction. Here, too, if when leaving the club after a rather late night, members of the stronger sex see a large snake gliding about, it is a matter only for quiet comment. It does not create panicky self-diagnosis among the departees, such as might happen in more temperate climates, resulting in a firm decision for the future to imbibe stimulants in strictly medicinal doses. Nor is the actual sight

of a python gliding along the floor of a cub bar-has occasionally happened-considered to be a fantasy; it calls for speedy organisation and good teamwork with the object of eviction, but such an intrusion is definitely regarded as unethical, bad sportsmanship and, of course, the later the hour the more heinous the offense.

Twenty-four elephants were recently assembled at Bangkok for shipment to Borneo for work there in the forests. The elephants had travelled down on foot from the North, a distance of about 650 kilometers. On arrival they were tethered, well separated one from another, and hobbled. Among them was one tusker in must. Secured by extra chains on his legs to a large tree.

I was awakened out of a peaceful sleep one night to be informed that two elephants, both tuskers, were fighting and I was requested to attend as one was already wounded. Although deeply sensible to the implied compliment to my capability of soothing enraged pachyderms, yet having no reputation as a referee of fights, I considered the suggestion that I become involved a somewhat tall order. However, when I arrived all was peaceful, with one elephant dead from tusk wounds through the chest and in the hindquarters.

During the night the tusker in must had quietly and persistently worked on the chains securing him to the tree and, on gaining freedom and probably because of some former disagreement possibly while en route down from the North, attacked the other tusker, still chained up, and quickly disposed of him. He then returned to his tree, draped a chain around his leg with his trunk, adopted a pose of perfect innocence and resumed his feeding.

The question arose, as to how to prevent a further furious mood, particularly during embarkation. Opium is frequently given in areas adjacent to which the poppy is grown and where it can be obtained without much red tape. Here, the difficulty of quickly obtaining that drug in sufficient quantity was too great, so chloral hydrate was offered in a large volume of molasses. But the elephant although appreciating the delicacy of the vehicle, at once became suspicious and rejected it. Recourse was then had to a drastic cut in his rations. The photograph shows the method of embarkation which took place without incident.

— N. A. V. October 1952.

Precocious Gestation.—A case wherein a calf of two and one-half months conceived is reported by Daily 1, who quotes French Veterinarians on the subject. In this instance the patient became prostrate and there was inappetence on March 12, 1947. The heifer was thin in flesh and the conclusion was that existing anemia was due to parasitism. The patient died three days later and autopsy showed that advanced pregnancy existed—parturition time was near. The heifer was eleven and one-half months of age at the time. This author concludes that while parturition in heifers at 13 to 18 months of age is relatively frequent this case was an exception and worthy of recording.

— N. A. V. October 1952.

Association News

The second meeting of Punjab Subordinate Veterinary Services Association of Ferozopore District was held at the Civil Veterinary Hospital, Moga, on 7-2-53 from 3 to 6 P. M. Nearly all the members attended the meeting.

Dr. Karnail Singh, Veterinary Assistant Surgeon, Anandpur Sahib, President of the Punjab Veterinary Service Association presided. The minutes of the last meeting were read by the Secretary, Dr. Mohan Lal Bhatia, Veterinary Assistant Surgeon, Moga, and were approved by the house. In the course of his report, Dr. Bhatia spoke as follows on the Role of Veterinarians in free India.

It was said that Veterinarians were not only more practitioners, but in true sense the veterinarians are the custodians of human health of the country. The Government is paying much attention towards the health of people by opening more medical clinics and hospitals. It is also spending crores of rupees on the cereals of which the deficiency is only 10%. But practically very little is being done on the improvement of Livestock breeds for more milk yield. There is 600% deficiency of milk in India. Human health is not merely to be had from wheat, grams and other cereals, nor is to be recouped from medicines in hospitals only. But it is to be had from perfect health making commodities viz. milk, butter, ghee, meat, eggs, etc., Milk, meat and eggs contains all that is required for forming developing and keeping in perfect condition of human bones, flesh, blood and brain. All these comes from good livestock etc. Until and unless we improve our livestock, our poultry, our goat and sheep flock and grow more fish we can never improve the health of our nation. Milk consumption in India is the lowest than in any other country on earth, inspite of the fact that India contains the largest number of cattle found any where on earth. But majority of these are of very little value. So a great deal remains to be done for the improvement of our livestock breeds. All these duties of paramount importance have been entrusted to the veterinarians in Punjab.

But what is our fate. The veterinarian spends more than six years at the college, two in passing F.Sc. and four in Veterinary college and then is able to get grade of Rs. 100/—to Rs. 300/—. It is the lowest grade which is being given to any Government servants who has undergone such a high training. The pay grades of nearly all the Government department were increased in 1946, some even by 40% but the grade of Veterinarians remained the same as in 1929. Due to high cost of living by 500% and due to no increase in our pay over the grade of the year 1920, we are very badly hit, and are unable to make both ends meet.

Dr. Mohan Lal Bhatia, further said that the work of the Veterinarian is purely a missionary work, and therefore it was the duty of the government to keep them above want.

It was also considered necessary to request the Government to enact legislation regarding prophylactic vaccination of cattle in Punjab against Rinderpest and Haemorrhagic septicaemia if these diseases were to be totally eradicated.

Then Dr. Mohan Lal Bhatia, Veterinary Assistant Surgeon, Moga, was unanimously re-elected as Secretary of the Ferozepore District Sub Veterinary Services Association, Moga.

The meeting ended with a vote of thanks to the President, Dr. Karnail Singh.

MOHAN LAL BHATIA,
Secretary Ferozepore District
Veterinary Services Association, Moga.

Dated : 9th Feb. 1953.

College News

BENGAL VETERINARY COLLEGE

List of students who passed the Diploma Examination
for the year 1952-53

(In order to merit).

- | | |
|-----------------------------------|---------------------------------|
| 1. Benudhar Mishra | 14. Sinnadurai Krishnanandan |
| 2. Samuel David Monoharam Paulraj | 15. Bertram Thilagaretnam Hoole |
| 3. Rajaghatta Nanjappa Rajanna | 16. Kulandaivelu Thamotheram |
| 4. Joseph Louis | 17. Kanapathi pillai Arulchothy |
| 5. Stanly David Joyavickrama | Sivapada Ghose |
| 6. Amalendu Roy | 18. Thillyamapalam Ramanathan |
| 7. Sovamoni Tripathi | 19. Ponniah Sanmugrajah |
| 8. Santosh Kumar Das | 20. Dharmendra Singh Gangwar |
| 9. Ariyapala Kuruneru | 21. Bhupendra Chandra Dandapat |
| 10. Velapody Sivalingham | 22. Lincoln Dassanayake |
| 11. Chinmoy Das | 23. Arabindu Biswas |
| 12. Aragula Srinivasa Rao | 24. Lalit Kumar Biswas. |
| 13. Pranab Kumar Biswas | |

Calcutta, }
10-4-53. }

K. C. MUKERJEE,
Vice-Principal.

Review

Manual of Infertility and Artificial Insemination in Cattle.—By P. G. MILLAR, M.R.C.V.S. and N. P. RAS. Publishers :—Messrs. Bailliere, Tindall and Cox, Ltd., London. Price 25s.

With the increasing attention to livestock breeding by the Veterinarian who has only latterly realised that it is a vital part of his assignment, the

complexities of the subject are coming up to the foreground for close and intensive study. Of these, infertility in cattle has become a much-discussed subject and a good bit of literature is slowly growing around it. The book under review is, therefore, of topical interest and will be found useful by all Veterinarians and by livestock owners also. It has been written in a simple and easy style with great understanding of the subject.

The authors have dealt with the subjects they have chosen under four sections. The first section contains a descriptive anatomy and physiology of the reproductive organs of the cow and the bull with chapters on pregnancy diagnosis and semen evaluation. The second section deals with the factors leading to infertility genetic, developmental, infective and environmental. It is good that genetic factors are given the importance due to them in the causation of infertility. Under the infective factors are considered the several diseases that cause infertility. The third section deals in greater detail with the infertility in cows and bulls. We, however, find no mention in this section or in the previous section any detailed study of granular vaginitis as a cause of infertility. Probably, it is not so prevalent or common in the experience of the authors. The last section outlines the principles of Artificial Insemination and also the latest methods of storage and dilution of semen.

The book is well illustrated and this enhances its value to the practising Veterinarians and also to the students.

Veterinary Anaesthesia.—By JOHN G. WRIGHT, M.V.Sc., F.R.C.V.S. Third Edition, 1952. Publishers :—Messrs. Bailliere, Tindall & Cox, Ltd., London. Price 21s.

This third edition of this useful book has as its main features a description of the use of ether intravenously in the horse, close circuit anaesthesia in sheep with cyclopropane, ethyl chloride as a general anaesthetic for cats, Pentothal as a short acting anaesthetic in horses, Anavenol and Anavenol 'K'—as a general anaesthetic and positive pressure ventilation of the lungs with the Knight Wood's apparatus.

Set to a difficult task, the author has done his best in the collection of information taken from every source available including his own extensive experience in the varied use of a variety of anaesthetics in a variety of animals under varying conditions and thereby facilitating the student preparing for his examination and the practitioner looking for a ready reference.

There are a few statements in the book with which the reviewer and many others may not agree. For example, the author appears to be sceptical about the value of paravertebral block for rumenotomy, when he says, "For rumenotomy the author has discarded the method in favour of the more simple local infiltration described on page 16. In fact, for rumenotomy there is no better and more easier method than paravertebral block for such anaesthetic effect without interference in the field of operation."

Again he says "Ethyl chloride and Nitrous Oxide have their champions but in the author's opinion they are inferior to ether", Ether alone as a general anaesthetic for cats could positively be dangerous whereas ethyl chloride alone has been found to be a very safe and handy anaesthetic in cat practice.

Referring to epidural anaesthesia, the author has stated that in the horse the epidural cavity is less easy to locate and that the technique is more difficult. While the "cavity" may be "less easy" to locate the technique is just as easy as in cattle if the horse would not resent the manipulations the cattle are put to.

Again, Picrotoxin in doses of 1-2 c.c. given intravenously is definitely the best and quite a cheap antidote for barbiturate excess and respiratory failure in small animals. The effect is almost immediate so long as the heart continues to beat. But, the author of this book says that he understands that it is sometimes employed in man, but that he has had no experience of its use in animals.

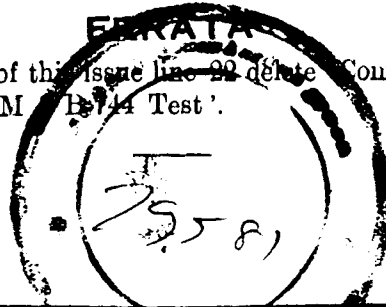
Lastly, a historical review of anaesthetics and anaesthesia and their mechanism of anaesthesia could have been included in the book and we are sure they would have enhanced the value of the book.

M. N.

KZ m2, N24

In page 472, of this issue line 22 delete 'Compliment Fixation' and substitute 'Ray's M. B. 744 Test'.

N53.29.6



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By

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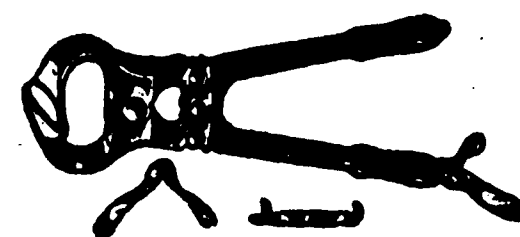
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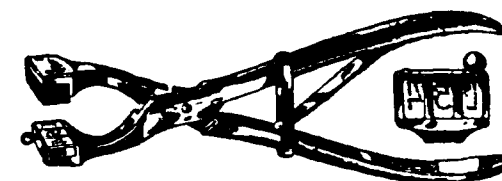
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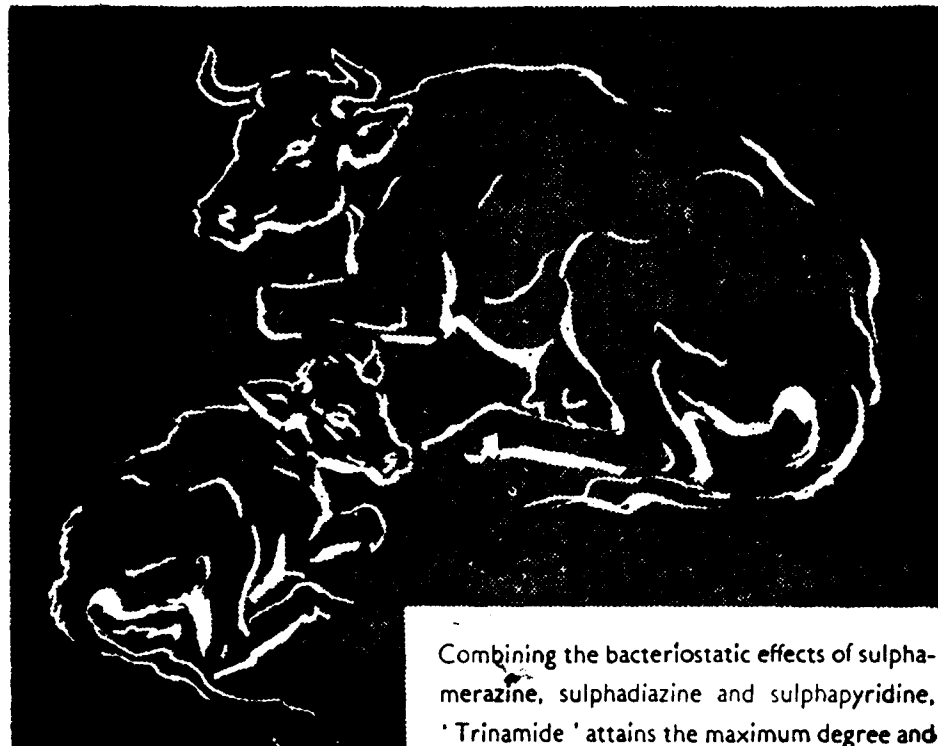
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