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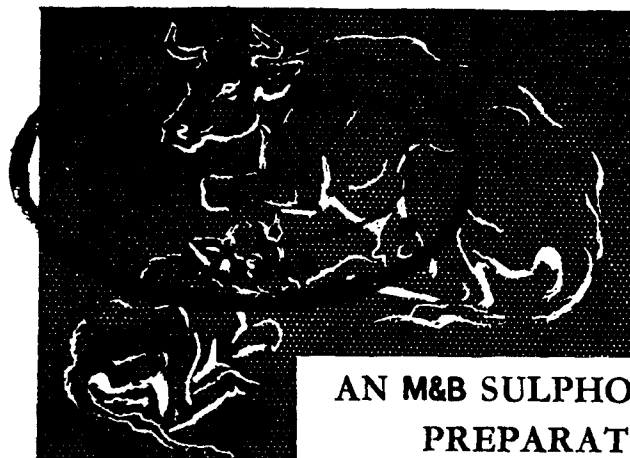
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Vol. XXX

MARCH, 1954

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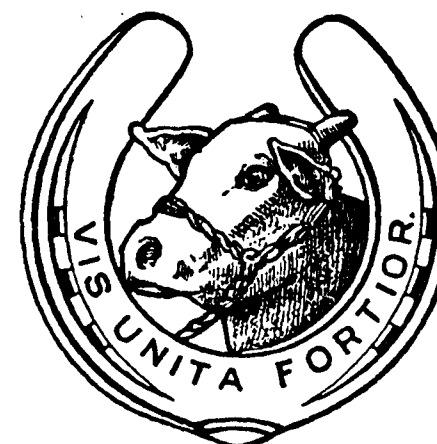
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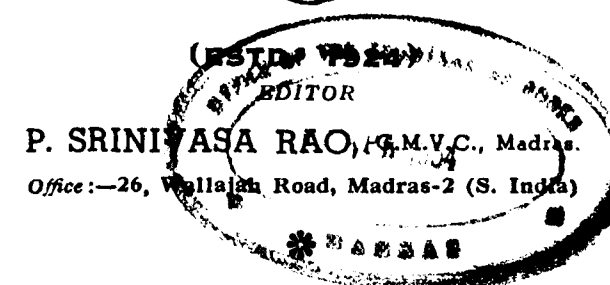
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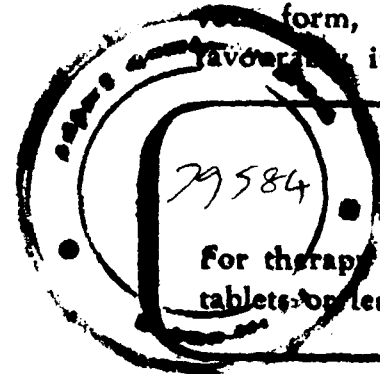
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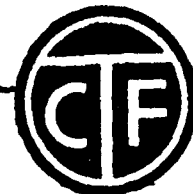
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Vol. XXX

MARCH, 1954

No. 8

CONTENTS

	PAGE
I. General Articles	
The role of "Bulk" or "Roughage" in the ration of dairy cows and buffaloes.—By V. Mahadevan	359
Localisation of vaccinia virus in the brains of sheep, calves and rabbits.—By R. D. Katiyar	363
Seasonal variation of serum calcium, magnesium and inorganic phosphorus in sheep, goats and buffaloes.—By H. S. Lakke Gowda	366
Preliminary investigations on the bacterial fermentation of paddy straw.—By M. Saunvasi Raju and S. Varadarajan.	371
Studies on the bacteriology and pathology of Bovine Endometritis.—By I. M. Azizuddin.	376
Chronic Bovine Haematuria.—By S. Datta	388
II. Editorial	
Shortage of Veterinary Personnel	407
III. Notes and News	
British Veterinary Codex—Corresponding Members.	410
PERSONAL.—M. Habib Khan	410
G. A. Shivnani	410
Veterinary and Animal Husbandry Department, Hyderabad-Dn.	411
OBITUARY.—A. Moberly	411
C. Madhavan Nambiyar	411
IV. Clinical Article	
Treatment of <i>Ascaridia Gallii</i> in Poultry with (A) Carbon Tetrachloride and (B) oil of Chenopodium.—By Ram Narain Mohan	412
A short note on Amoebic Dysentery in a lioness at Bombay.—By S. R. Rao and J. N. Barua	417
Lumbar Paralysis in a she-goat.—By J. Seetharaman, and H. Narasimha Shetty	420
Paraplegia in a dog.—By J. Seetharaman and K. S. Prabaker Rao	422
V. College News	
Madras Veterinary College—Golden Jubilee Celebration—Notice of	424
Punjab Veterinary College.—B.V.Sc., Examination Results, 1952 and 1953	424
VI. Association News	
All-India Veterinary Association—Hyderabad Branch, Proceedings of the 1st General Body Meeting	426
VII. Correspondence	
Use of "Lorexane" cream in the treatment and prevention of Myiasis in Livestock.—By G. V. Short	427
Appointment of Dr. J. D. Sampath Kumaran.—By M. V. Sapre	428
Hundredth anniversaries of the birthdays of Paul Ehrlich and Emil V. Behring by A. G. Brocke	429
VIII. Abstracts	
Organisation and execution of anti-epizootic measures in the U.S.S.R.—By N. Leonov	430
Studies on Fowl Spirochaetosis with special reference to Penicillin therapy and the development of an egg-adapted vaccine for its control.—By S. B. V. Rao, B. M. Thakral and M. R. Dhanda	430
IX. Reviews	
Animal Nutrition Research in India.—By K. C. Sen	431
Administration Report of the Veterinary Department, Hyderabad-Dn, for the year 1952-1953	432

THE Indian Veterinary Journal

(The Journal of the All-India Veterinary Association)

Vol. XXX

MARCH, 1954

No. 5

General Articles

THE ROLE OF "BULK" OR "ROUGHAGE" IN THE RATION OF DAIRY COWS AND BUFFALOES

BY

V. MAHADEVAN, M.A., D.Sc., F.R.I.C.,

Lecturer in Animal Nutrition,
Madras Veterinary College.

1. The term "Bulk".—Bulk of a feed means the total dry matter content of it in the ration of an animal. The most part of it consists of crude fibre or the indigestible portion of the feed by the digestive juices secreted by the animal. Though bulk may also be interpreted to signify the water absorbing nature of the feed, it is not understood in this way, when we speak usually of bulk in the ration of dairy cattle.

2. The importance of 'Bulk' in dairy rations.—A satisfactory ration must provide the proper amount of bulk, needed for the cow or buffalo, which has a digestive tract adapted to utilise a large quantity of bulky feed without which she is neither normal nor contented. The bulky feeds such as hay, straw, silage and grass invariably provide nutrients needed by the animal at a lower cost than the concentrates. They are also the chief source of Calcium and almost the sole source of vitamins A and D in the feeds of dairy cattle. Furthermore, bulky feeds help to give the desired 'lightness' to the ration, enabling the digestive juices to penetrate the food mass more completely and thereby facilitate digestion. The ration should include as much roughage as the animal will eat. When its quality is better, the animal will eat more and the amount of concentrates required will be less.

Therefore, the effects of bulk and fibre in the ration are :—

- To avoid the formation of dough like mass in the stomach to escape digestion,
- To maintain the normal rumen activity and other allied physiological processes of the animal,
- To supply an abundance of protective nutrients necessary for the health of the animal,

- (d) To supply heat and energy to the animal,
- (e) To maintain the normal production of milk, and its butter fat content,
- (f) To produce certain distention of the digestive tract to eliminate dung or faeces easily,
- (g) To produce a laxative effect,
- (h) To avoid a discontented and restless feeling in the animal and
- (i) To maintain the normal health of the animal.

3. "*Bulk*" and *Rumen activity*.—The bulk consists mostly of crude fibre of variable composition containing cellulose, hemicellulose, pectins, lignin and other polysaccharides. The digestibility of crude fibre in ruminants is, on an average, about 55 to 60 per cent, ranging from 20 to 80 per cent, depending upon the nature and maturity of fodder crop. Cellulose and hemicellulose are utilised by symbiotic digestion due to the activity of bacteria and micro-organisms, which are responsible for the fermentation in the rumen. Lignin is not affected.

The chief products of this symbiotic digestion are the lower fatty acids, among which Acetic acid accounts for about 69 per cent, besides large amounts of gases like Carbon dioxide, Methane, Nitrogen, Hydrogen, Hydrogen sulphide and Oxygen, the latter three being in traces, and heat. The high production of acids in the rumen fermentation is very essential for the normal health of the animal, when it is known that as much as 300 grams of Sodium bicarbonate are produced in the salivary secretion of a cow per day and the whole of it has to be neutralised by acids rapidly in the rumen to maintain the favourable environment for the activity of the micro-organisms at a pH 7 or a little less. A greater portion of the acids is absorbed rapidly too. It is also important to know that the Carbon dioxide produced is about 67 per cent of the total gases and amounts to as much as 280 litres per day from the rumen of a lactating cow, which must be expelled quickly as otherwise its accumulation will lead to bloat or to uncomfortable feeling in the animal. To expel this large volume of gas, eructation is a normal and absolutely necessary process in the ruminants. The normal rumination and eructation, or belching out in their turn, depend upon the stimulation of nerve fibres in the rumen by coarse roughage. Thus the eructation is a reflex rather than a voluntary act caused by the action of coarse roughage or 'bulk'.

The total amount of lower fatty acids resulting from the bulk in the rumen fermentation of a cow, expressed as Acetic acid equivalent, ranges from 1310 to 1637 grams per day and supplies available nutrient

energy of 4572 to 5713 Calories. The fasting metabolic rate or basal metabolic energy rate of a cow varies from 4770 to 9840 Calories per 24 hours. Therefore, the energy available from the lower fatty acids produced by the bulk in the rumen could account for 46 to 120 per cent of the fasting requirements of the cow. Thus the bulk or roughage, by undergoing fermentation in the rumen, is a major source of energy to the animal.

Even the types of micro-organisms in the gastro-intestinal tract and the products of digestion, even within the limits of normal diet of a cow, are specifically influenced by the nature and composition of the ration. The protozoa content of the rumen liquor increases from 200,000 to 2,000,000 per milli-litre when starch and protein are added to a bulky diet of hay. The micro-flora is also subject to seasonal variation.

Besides acids and gases, bacterial polysaccharides are also produced. Both dietary protein and non-protein nitrogen are utilised by the micro-organisms for the synthesis of their own structural proteins. Thirty per cent of the dry matter content of 40 to 60 gallons of the fermenting material in the rumen of a cow constitutes the micro-organisms viz., bacteria, protozoa and yeast, which are responsible for the rumen activity. They synthesise besides proteins, valuable nutrients like all the B group vitamins, which, in turn, become available to the cow. The thirty per cent of the dry matter content of the rumen inješta, constituting the micro-flora, becomes an easily digestible animal protein of high biological value, to be used by the cow, thus serving as a supplement to the injected food. Hence the food supply to the animal is indirectly increased by the bulk responsible for the rumen activity, in addition to the energy derived directly from the fermentation.

4. *Balance of Bulk in the dairy ration*.—The optimum balance of bulk in the ration is also important in the economy of animal's health and production. The amount of bulk in the ration of an animal depends on the species, anatomy of the digestive tract and the production sought. Too much of bulk lessens the consumption of digestible nutrients and makes the animal discontented and grunting. Too low an amount in bulk causes indigestion and the animal may even go off the feed and symptoms of bloat may appear. Roughage in the finely ground state does not promote normal rumen function and increases the tendency to bloat. Rapid consumption of large quantities of lush immature legume produces bloat. But eating of large amounts of grasses does not produce this effect, because rapid consumption of grass is more difficult and most grasses have leaves with rough surfaces which are effective in eliciting the eructive response to discharge the gases. The absence or reduction of bulk reduces markedly the milk and butter fat yield.

5. *The amount of Bulk in the dairy rations.*—The quantity of bulk in the feed is calculated on a dry matter basis, expressed as percentage of body weight of the animal and is to fall within a certain limit to give satisfactory results. The exact percentage is open to dispute, for the digestive capacity is influenced by the breed of the animal and the nature of the feed which it had during the development of its rumen.

It is not advisable to lower the amount of bulk or roughage used below that required to supply the nutrients for the maintenance of the animal. This would be about 1½ pounds of good hay or 4½ pounds of silage (1 pound of hay is equal to 3 pounds of silage.) per 100 pounds body weight of the animal. The amount of feed required above the maintenance requirements is figured on the basis of the amount of milk being produced and the percentage of butter fat in the milk. Similarly, growth and pregnancy conditions require additional amounts of feed. On the basis of energy value of the feeds, good hay is worth about 60 per cent as much as the concentrate mixture and silage is worth about one-third as much as good hay.

For a dry cow or a poor milking cow, 1.5 to 2.0 per cent of its body weight should be dry matter or bulk. For good milking cows, 3 to 3.5 per cent of their weights as dry matter is given in England. The American practice is to allow free use of bulky foods *ad libitum*, without the use of concentrates and it works out at 2.5 per cent average for the stock. Indian cattle appear to be conditioned to a somewhat lower level of dry matter or bulk requirements. For Indian cows, an allowance calculated at 2.5 to 3 per cent of their weights is suitable according to age, body weight, the number of its lactation, breed, milk yield and the nature of the feeds. A cow, weighing 800 pounds and giving 20 pounds of milk, requires about 20 pounds of dry matter. For buffaloes, 3 to 3.5 per cent of dry matter is found satisfactory according to the size, breed and the number of lactations they had. A buffalo, weighing 1200 to 1400 pounds, in lactation, is given 37 to 45 pounds of dry matter in the ration for normal health and optimum production. As the lactation advances, it will require the higher rate in the scale.

The scientific way of calculating the bulk or dry matter content of a mixed ration for a dairy cow in its various stages of growth, lactation, pregnancy, etc., can be done from the requirements of "Total Digestible Nutrients" as advised by the National Research Council for dairy cattle in America. To convert the 'T.D.N.' into pounds of feed on an air-dry basis, the percentage of digestible nutrients in the feed as a whole should be known. If we assume good roughage or bulky food as green grass, or silage, or good hay available and 75 per cent of the

total ration on dry basis consists of digestible nutrients, the "T.D.N." in pounds required for the particular weight of the cow and its physiological condition, multiplied by $\frac{100}{75}$ or $\frac{4}{3}$ gives the pounds of nutrients or feed on an air-dry basis. The table given below describes the allowances for different classes of dairy cattle in pounds of 'T.D.N.' and the corresponding pounds of air-dry feed or bulk in a mixed ration satisfying all the other nutrient requirements.

TABLE 1
Recommended 'T.D.N.' and Feed on an air-dry basis for dairy cattle.

Weight of the animal in pounds.	Daily allowance per animal.	
	'T.D.N.' in pounds.	Total feed (air-dry) in pounds.
FOR GROWTH.		
400	6.5	8.7
600	8.5	11.3
800	10.0	13.3
1000	11.0	14.7
1200	12.0	16.0
FOR MAINTENANCE (*).		
700	6.0	8.0
1000	8.0	10.7
1200	9.5	12.7
1400	11.0	14.7
FOR PREGNANCY.		
1000	14.0	18.7
FOR LACTATION PER POUND OF MILK.		
Percentage of Butter Fat.		
3	0.04	0.37
4	0.045	0.49
5	0.05	0.42
6	0.055	0.56

(*). When calculating the allowances for lactating heifers that are still growing, the figures for growth rather than those for maintenance should be used.

LOCALISATION OF VACCINIA VIRUS IN THE BRAINS OF SHEEP, CALVES AND RABBITS

By

R. D. KATIYAR, G.B.V.C., P.G. (Muk.)
Veterinary Officer, Government Vaccine Depot,
Patwa Dangar, Nainital, U.P.

Dermal strain of vaccinia virus is maintained by passage through calves, sheep or rabbits by scarification of skin. It has dominantly dermal properties and proliferates profusely in skin. Dermal inocula-

tion of skin by scarification is the method usually adopted for the manufacture of the vaccine lymph in the Vaccine Institutes.

A number of workers have failed to produce any evidence of encephalitis after dermal inoculation with the vaccinia virus. Berger (1929) showed that injury to the skin, did not cause the virus to produce encephalitis after dermal inoculation.

Other workers have, however, been successful in their attempts to cause localization of virus in the brain by peripheral injection. Evidence of cerebral involvement after peripheral inoculation has been furnished by Blanc and Caminepetres (1924 b, 1926).

During 1947, the author met with calves with the following symptoms:—On the 4th day after vaccination with the vaccinia virus the calves went down with the sub-normal temperature of 97°F. The calves were dull and off feed. Bending of the neck towards one side was observed. They succumbed within 24 hours. On post-mortem nothing unusual in any organ except congestion of brains and meninges were noticed. The spinal cords and brains were removed and on titration for presence of the vaccinia virus on rabbits gave confluent and semi-confluent reactions in 1:1000 and 1:4000 dilutions respectively. This observation prompted the author to find out whether localisation of vaccinia virus occurs in the brain after dermal scarification.

Experiments.—Rabbits were destroyed on different dates from the date of successful vaccination. Their brains were removed aseptically and grinded in 50% glycerine in the ration of 1:5.

Sheep and calves which died during post-vaccinal period were post-mortemed. Their brains were removed and grinded similarly in 50% glycerine.

These brain materials were titrated on rabbits to detect the presence of the vaccinia virus. The rabbits vaccinated with the brain tissues were re-vaccinated after 15-22 days with vaccine lymph of known potency to test whether these rabbits developed immunity due to vaccination with the brain tissues.

The results obtained are given below:—

(1) Localisation of vaccinia virus occurs in the brains of rabbits, sheep and calves after dermal vaccination with it.

(2) Vaccinia virus, after localisation in the brains, disappears after 14-15 days in the rabbits, 16-18 days in the sheep and 23-25 days in the buffalo calves from the date of vaccination.

(3) After localisation, it causes vaccinal encephalitis and death in about 1% cases. In such cases concentration of the virus is higher than in the rest.

Acknowledgment.—I am exceedingly grateful to Major P. K. Topa, M.B.B.S., D.P.H. Superintendent, Government Vaccine Depot, U.P. Patwa Danger, Nainital for his guidance, kind permission to carry out this work and editing this article. My thanks are also due to Sarveshi H.D. Thapliyal, M.D. Tewari and T.C. Tripathi for their help.

Rabbit No.	No. of days after vaccination when brain was collected.	Titration result of the vaccination with the brain tissues on the abdomen of the rabbits.	Revaccination of the rabbit on the back to test immunity with calf lymph in 1:5.		Whether vaccinia virus was present in the brain or not.
			No. of days after primary vaccination.	Result.	
1	4	D. 6 vesicles	15	Failed to react	Vaccinia virus present
2	4	D. 8 "			
3	4	D. 10 "			
5	11	D. 10 "	15	Failed to react	do.
0		D. 12 "			
12	12	D. 12 "			
13	14	D. 8 "	20	do.	do.
16	15	No reaction	15	Confluent reaction	Vaccinia virus absent
18	16	" "	20	" "	do.
19	23	" "	15	" "	do.
<i>Sheep No.</i>					
6	5	Semi-confluent	20	Failed to react	Vaccinia virus present
7	10	D. 10 vesicles	20	do.	do.
25	12	D. 6	15	do.	do.
40	14	D. 8	15	do.	do.
56	16	D. 6	20	do.	do.
58	16	D. 4	20	Confluent reaction	Vaccinia virus absent
72	18	No reaction			
80	19	" "	20	do.	do.
94	22	" "	20	do.	do.
<i>B. c. lf No.</i>					
20	5	D. 12 vesicles	20	Failed to react	Vaccinia virus present
86	7	D. 12 "	20	do.	do.
80	12	D. 10 "	22	do.	do.
116	13	D. 12 "	20	do.	do.
128	15	D. 15 "	18	do.	do.
130	19	D. 6 "	20	do.	do.
142	23	D. 8 "	20	do.	do.
156	25	No reaction	20	Confluent reaction	Vaccinia virus absent
166	26	No reaction	20	do.	do.
202	28	No reaction	20	do.	do.
208	29	No reaction	20	do.	do.

Note:— D = discrete reaction.

SEASONAL VARIATION OF SERUM CALCIUM, MAGNESIUM AND INORGANIC PHOSPHORUS IN SHEEP, GOATS AND BUFFALOES

By

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The importance of establishing the normal constituents of blood of different species of farm animals, at different stages of growth, at different seasons of the year and for different breeds of the species, was found to be of an immense significance in diagnosing various pathological and metabolic disorders. Malnutrition, or any other disease conditions, often influence the change in the composition of this body-fluid and hence the importance of blood studies in relation to health and diseases of farm animals. Experimental observations carried out in India on the blood composition of animals either in health or in diseases, or under different conditions of living, are not many.

Pal, Momin and Mullick (1945) found that, in cows, serum calcium and magnesium showed significant differences in their monthly variations, the summer values being higher than the winter values. Hammersma (1937) noticed similar variations in cattle. Craige and Godd (1941) found the calcium content of horses' serum to be at its maximum in summer. Duncun, Lightfoot and Huffman (1940) noticed in cattle that magnesium values of winter were greater than those of summer. Allcroft and Green (1938) found, from a group of cows of Hereford breed kept under natural conditions, that magnesium showed minimum in December and maximum in August. Winter values were less than half of the summer values. Rusoff, Frye and Scott (1951) found no evident effect of season on the blood composition of red Sindhi-Jersey cross breeds, but found significant differences between monthly values. Long *et al.* (1952) found that no correlation with season, age or breed could be established with calcium and phosphorus content of the blood of mature cows and heifers of Angus, Hereford and Short-horn beef breeds.

In view of these varying observations made on this subject by different workers, the present investigation on the seasonal variation of calcium, magnesium and phosphorus in the serum of sheep, goats and buffaloes is only an additional information to these results.

Experimental Methods

Five buffalo-bulls, five ewes and five he-goats, all of local breeds, aged about two and half to three years, were selected for the

purpose. Feeding and management were under supervision. The animals remained in sound health throughout the experimental period of one year. Blood was drawn from the jugular vein in the mornings before any feed was offered to the animals. Practically the same period was observed for bleeding every month and usually the animals were bled during the third week of every month. The first bleeding was undertaken during March 1952 and the last during February 1953. Whole blood was drawn into a clean well-dried one ounce bottle and was allowed to clot by keeping the bottle in a slanting position. The serum was then collected and analysed for calcium (Ca), Magnesium (Mg) and Inorganic Phosphorus (P). Calcium was determined by the method of Kramer and Tisdall (1921), as modified by Clark and Collip (1925). Magnesium was determined by the method of Fiske and Subbarow (1925), as modified by Holzapfel (1934). Inorganic phosphorus was determined by the method of Fiske and Subbarow (1925).

Results and Discussions

The average values of all the five animals for each month are given in table I, and the average values of all the twelve months for each animal, are given in table II, both with statistical analysis. The results are expressed in mgms. per 100 c.c. serum. From the tables I & II, the following observations can be made :—

Calcium (Ca):—Serum calcium levels of the three species studied showed that high variations occurred from month to month. The minimum average values were recorded during the month of May and June, the hottest period of the year, in all the three species. The average minimum values so recorded were 9.16, 9.86 and 10.14 in sheep, goats and buffaloes respectively. The maximum average values of 14.06 in the case of sheep and 14.48 in the case of goats were recorded during January and the maximum average value of 13.28 for buffaloes was recorded during November. Both these months could be considered as winter period. These results are not in agreement with the findings of Pal, Momin and Mullick (1945) in cows, Hammersma (1937) in cattle, and Craige and Godd (1941) in horses, who found that the calcium values of summer were higher than the winter values. In our findings the winter values were higher than summer values. This may probably be due to species differences or due to the growing nature of these animals, who were still young or it may be due to the peculiarity of the local breeds. When these results were statistically analysed it was found that variations due to season was found to be significant (Table I) while the variations due to animals was found to be non-significant (Table II).

Magnesium (Mg.):—While the variations in the values of calcium in all the three species were statistically found to be significant, the

TABLE I
Monthly variations of Serum Calcium, Magnesium and Inorganic Phosphorus of Sheep, Goats and Buffaloes (mg/100 c.c. Serum) and the statistical analysis (average of five animals).

Species and Elements	1952,												Significance (S) or Non-Significance (N.S.)	Critical Difference	
	1953														
	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	F		
Sheep															
Calcium (Ca)	11.56	11.90	11.24	9.16	10.56	10.76	13.44	13.76	12.08	13.68	14.06	11.18	14.15	S	1.54
Magnesium (Mg)	3.33	2.97	2.14	3.12	1.92	2.06	2.67	2.16	2.39	3.04	4.17	2.49	1.52	N.S.	—
Inorganic Phosphorus (P)	8.15	9.84	11.43	8.98	9.87	9.82	8.90	8.43	5.92	10.72	11.84	7.77	16.42	S	1.55
Goats															
Calcium (Ca)	10.06	11.48	9.86	9.88	11.08	10.32	12.98	12.36	12.24	11.56	14.48	11.85	7.03	S	2.01
Magnesium (Mg)	2.81	2.60	2.50	3.29	2.16	2.26	2.73	2.31	3.46	3.59	3.90	2.66	15.11	S	0.56
Inorganic Phosphorus (P)	10.12	13.23	11.51	9.51	9.10	10.70	9.87	9.55	9.01	9.90	11.17	9.54	3.69	S	2.40
Buffaloes															
Calcium (Ca)	10.46	11.60	12.80	10.14	11.54	10.76	11.30	11.76	13.28	11.92	12.80	11.38	3.56	S	1.93
Magnesium (Mg)	3.07	2.96	2.15	3.42	2.08	2.18	2.71	2.57	2.76	3.65	4.22	2.76	0.85	N.S.	—
Inorganic Phosphorus (P)	6.40	10.37	8.47	7.12	8.34	7.95	8.15	9.34	7.07	12.48	9.51	6.75	14.50	S	1.73

Note:—Theoretical value of F for 11.d.f. against 44 at 1 p.c. is 2.68

TABLE II
Seasonal variations of Serum Calcium, Magnesium and Inorganic Phosphorus of Sheep, Goats and Buffaloes (Mg/100 c.c. Serum) and the statistical analysis (average of twelve months).

	Animal numbers (for each species)					F	Significance (S) or Non-significance (N.S.)	Critical difference
	1	2	3	4	5			
Sheep								
Calcium (Ca)	11.4	12.2	12.2	11.6	12.3	.27	N.S.	—
Magnesium (Mg)	2.41	2.61	2.64	2.64	3.23	.67	N.S.	—
Inorganic Phosphorus (P)	9.47	9.24	8.84	9.49	9.66	1.34	N.S.	—
Goats								
Calcium (Ca)	11.8	11.3	10.9	11.8	11.8	1.33	N.S.	—
Magnesium (Mg)	3.06	2.73	2.93	2.71	2.87	2.08	N.S.	—
Inorganic Phosphorus (P)	10.19	10.46	10.29	10.16	10.26	.08	N.S.	—
Buffaloes								
Calcium (Ca)	11.9	11.8	11.9	11.1	11.5	1.32	N.S.	—
Magnesium (Mg)	2.90	2.83	2.94	2.83	2.90	0.12	N.S.	—
Inorganic Phosphorus (P)	8.43	8.38	8.09	8.87	8.72	1.09	N.S.	—

Note:—Theoretical values of F for 11.d.f. against 44 at 1 p.c. is 2.68

variations due to season in the magnesium values were not found to be statistically significant in the case of sheep and buffaloes but they were found to be significant in the case of goats. There seems to be not much difference in the actual values of magnesium in each month from species to species. The average maximum values were recorded during January and the average minimum values were recorded during July in each species. It may be stated that the maximum and minimum values of serum magnesium do not depend upon the species thus studied. A perusal of the Table I will indicate that the period between May and October may be treated as a period of low-Mg. level and the period between November and April as the period of high-Mg. level, in all the three species. The minimum average values thus recorded were 1.92, 2.16 and 2.08 in sheep, goats, and buffaloes respectively, and the

maximum average values recorded were 4.17, 3.90 and 4.22 in sheep, goats and buffaloes respectively. These results at least in the case of sheep and buffaloes agree more or less with the findings of Rusoff, Frye and Scott (1951) and Long *et al.* (1952) who could not establish any correlation between Mg. content of serum of cattle with the season, age or breed. The high values of summer and the low values of winter as obtained by Allcroft and Green (1938), Pal, Momin and Mullick (1945) and Hammersma (1937) could not be found with these species.

Inorganic Phosphorus (P):—Highly significant differences in the inorganic phosphorus of serum of sheep, goats and buffaloes could be seen among the monthly variations as well as in individual animals. Statistical examinations of these variations had shown that the variations due to animal factor has no significance (Table II), whereas the variations due to season was found to be highly significant (Table I) in all the three species. Two periods of high phosphorus levels could be observed, one in April-May and the other in December-January. In between these two periods, i.e. between June and November, no appreciable variations could be seen except in the case of sheep where a gradually decreasing values from May to November could be found. Thus the period between June and November could be considered as a dormant period. A general fall in inorganic phosphorus occurred in all the three species during February-March.

The twelve months' average values for all the five animals of Calcium were 11.9, 11.5 and 11.6 for sheep, goats and buffaloes respectively, and that of Magnesium were 2.71, 2.86 and 2.88 for sheep, goats and buffaloes respectively, and there seemed to be very little difference, from species to species, as far as Calcium and Magnesium values were concerned. But the values of inorganic phosphorus differed from species to species as could be seen from the values for sheep, goats and buffaloes which were 9.31, 10.27 and 8.50 respectively. The average values for goats were higher than the values for sheep and buffaloes, and the average values for buffaloes were lesser than sheep and goats. However, the values for inorganic phosphorus of serum of these three species were found to be higher than the values obtained by Kehar & Murty (1951) in adult buffaloes, Kehar and Murty (1945) in Haryana bulls, and Murty and Kehar (1950) in Kumaoni bullocks.

Summary

The monthly determinations of serum calcium, magnesium and inorganic phosphorus of sheep, goats and buffaloes, were made for one complete year and the results were statistically examined. The monthly variations of calcium and inorganic phosphorus of sheep, goats and buffaloes were all found to be statistically significant, but the magnesium

values of sheep and buffaloes were found to be non-significant while those of goats were found to be significant. None of these elements had any significant variations with the species.

Acknowledgment

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PRELIMINARY INVESTIGATIONS ON THE BACTERIAL FERMENTATION OF PADDY STRAW

By

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and

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Introductory

Any method of processing of paddy straw with a view to enhancing its nutritive value is of great benefit, since the digestibility of the nutrients of the straw is low owing to the presence of ligno-cellulose coating. In recent times the scarcity of fodder has become very acute and all the available straws of cereals and millets are not adequate. It

is estimated by Kulandai (1948) that the annual shortage of fodder in Madras State alone works out to 67.640 million pounds. Of the several methods that suggested themselves, it occurred to us that increasing the digestibility of the straw by processing would help a great deal to reduce the shortage. The processing aims at improving the nutritive value and the digestible co-efficient of the straw. Since the alkali treatment of straw was not economical, bacterial fermentation method to enhance the nutritive quality of the straw was tried. Literature on this subject being scarce, an experimental plan was devised by us as under.

Experimental

Lots of two hundred pounds of finely chopped paddy straw were taken for the experiment. Two lots of straw were soaked in water for 12 hours and, after removal from water, were spread over a cement floor to form a flat heap 12 inches high. To each lot, 20 lbs. of molasses, 7 lbs. of superphosphate and 7 lbs. of ammonium sulphate were added and thoroughly mixed. To one lot, an inoculum of bacteria built up in the laboratory from well-fermented cattle manure was added while, the other lot received an inoculum prepared from fresh horse dung. Both the lots were stirred thoroughly.

The temperature of the heap was noted at different places every day for a fortnight. The heaps were disturbed and re-made twice during the first week to permit proper aeration (vide table I). This was

TABLE I

Showing the temperature of the straw during the process of fermentation.

Day	Straw treated with cattle manure inoculum. °F.	Straw treated with horse dung inoculum. °F.
1	98.0	101.5
2	114.0	120.0
3	121.0	129.0
4	138.0	135.0
5	136.0	134.0
6	134.0	133.0
7	139.0	130.0
8	127.0	126.0
9	122.0	120.0
10	120.0	116.0
11	125.0	123.0
12	119.0	119.0
13	106.0	105.0
14	92.0	91.0
	90.0	89.0

repeated on alternate days for four days and everyday for another four days by which time the temperature had reached the peak and began to drop down.

At the end of the first week of fermentation, the material was analysed for ammoniacal nitrogen. As this was present in appreciable quantity, ten pounds of molasses were further added to hasten bacterial activity and convert the mineral form of nitrogen to microbial protein.

At the end of the second week of fermentation, the material was dried in the sun. The final product was rather dull in colour but did not have any bad taint. The finished product was 135 lbs. in weight. Thus there was a loss of 65 lbs. on the basis of 200 lbs. of original straw. This worked out to a loss of 32.5 per cent.

Analytical

The samples of fermented straw were analysed for their feeding value as well as for cellulose, lignin etc. Nitrogen partition was made and the different fractions of nitrogen estimated. Data are presented in tables II and III.

TABLE II

Showing the feeding value of fermented straw compared with the original straw as well as straw merely soaked and dried, with the values of cellulose and lignin (Results on dry basis).

Heads of analysis.	Original straw. %	Soaked in water for 12 hrs. and dried. %	Cattle manure inoculum.			Fresh horse dung inoculum.		
			After inocu- lation. %	After 7 days. %	After 14 days. %	After inocu- lation. %	After 7 days. %	After 14 days. %
Protein	2.78	2.68	5.34	8.56	9.25	6.86	10.00	9.32
Ash	21.52	20.67	23.74	34.41	34.41	24.60	32.47	35.02
Crude fibre	35.75	37.71	33.59	15.57	18.71	23.10	17.27	18.01
Ether extractives	1.04	1.13	1.12	1.49	0.78	1.17	1.57	0.97
Carbohydrates (by difference)	38.91	37.81	36.51	39.97	36.85	39.27	38.69	36.68
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Insolubles	16.19	18.24	17.33	24.22	23.69	16.37	24.25	24.21
Lime (CaO)	0.73	0.83	1.96	2.58	2.67	1.86	2.64	2.59
Phosphoric acid (P ₂ O ₅)	0.13	0.15	1.11	1.85	1.25	1.03	1.33	1.56
Total hydrolysable sugars	24.73	25.85	23.19	17.59	15.45	22.04	17.07	15.87
Cellulose	3.50	3.87	3.75	...	2.15	3.60	...	2.20
Lignin	46.37	53.58	39.77	...	34.65	40.72	...	32.43
Ammonia	nil	nil	0.21	...	0.199	0.299	...	0.153

TABLE III
Showing the changes in the different fractions of nitrogen due to fermentation.

Heads of analysis.	Original straw, %	Horse-dung inoculum, %	Percentage increase on the original straw.	Cattle manure inoculum, %	Percentage increase on the original straw.
Total nitrogen	0.5676	1.7650	210.9	1.7460	261.3
Humin nitrogen	0.1124	0.2486	121.2	0.2037	81.3
Ammonical nitrogen	nil	0.0920	...	0.1100	...
Albuminoid nitrogen	0.3710	1.3820	272.5	1.3400	261.1
Undetermined nitrogen	0.0842	0.1424	69.11	0.0903	7.2
Acid insoluble humin	0.1124	0.2486	121.2	0.2040	81.5
Amide nitrogen	0.0270	0.0414	53.3	0.0619	77.4
Nitrate nitrogen	0.0054	0.0054	nil	0.0094	74.1
Albuminoid to total nitrogen	65.32	78.07	19.52	76.63	17.32

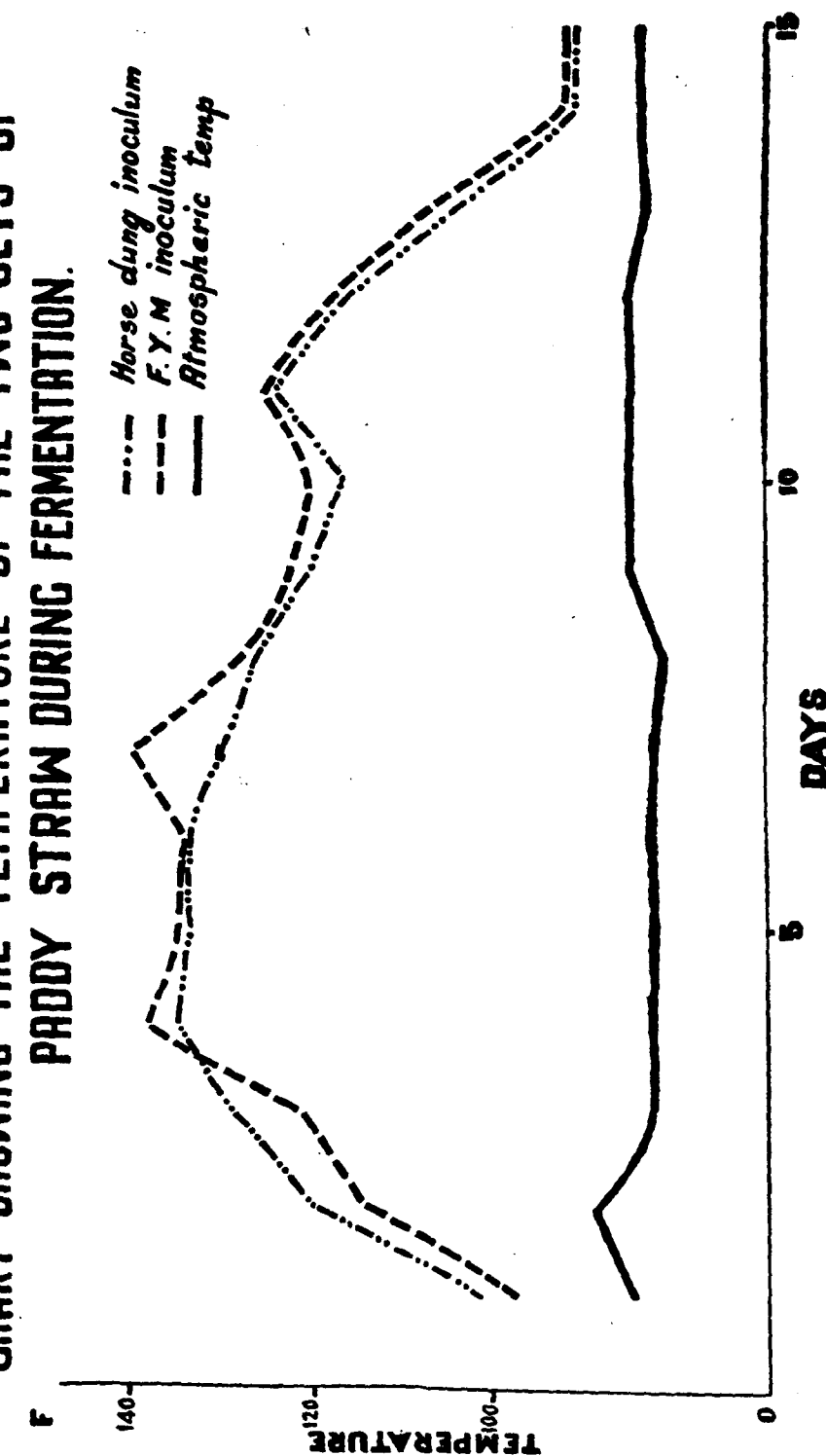
Discussion

Bacterial fermentation of paddy straw had resulted in a considerable loss of acid hydrolysable sugars, crude fibre and cellulose. Consequent on the loss of decomposable sugars and the addition of superphosphate and ammonium sulphate as starters the ash (including insolubles, lime and phosphoric acid) and crude protein contents had increased to a marked extent. All the ammoniacal nitrogen added in the form of ammonium sulphate was converted completely into microbial protein. Albuminoid nitrogen constituted 78.4 per cent in the straw treated with horse-dung as an inoculum, and 76.6 per cent in the case of farm-yard manure treated straw as against 68.4 per cent in the original unfermented straw. As a result of fermentation there was generally an increase of 8 to 10 per cent of albuminoid nitrogen in both the cases. There was only a slight increase in the acid soluble humin nitrogen. There was no appreciable change in lignin, ether extract, or moisture content due to fermentation (vide table IV).

TABLE IV
Increase in the nutritive value after fermentation.

Heads of analysis.	In 200 lb. of original straw, lb.	In 135 lb. of fermented straw, cattle manure inoculum, lb.	In 135 lb. of fermented straw horse dung inoculum, lb.
Protein	5.56	12.48	12.58
Mineral matter	43.04	46.45	47.27
Crude fibre	91.50	25.25	24.32
Ether extractives	2.08	1.53	1.31
Carbohydrate (by diff.)	77.82	49.74	49.52
Lime (CaO)	1.46	3.48	3.50
Phosphoric acid (P ₂ O ₅)	0.26	3.15	2.11

CHART SHOWING THE TEMPERATURE OF THE TWO SETS OF PADDY STRAW DURING FERMENTATION.



Casual feeding trials made revealed that the animals did not relish the straw.

Summary

1. Paddy straw was fermented with inocula prepared from both horse dung and well-fermented cattle manure.
2. The fermented straw was higher in nutritive value with low fibre content.
3. Casual feeding trials revealed that cattle do not readily take to it.

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STUDIES ON THE BACTERIOLOGY AND PATHOLOGY OF BOVINE ENDOMETRITIS*

Endometritis—its classification and relationship to sterility †

By

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Introduction

It is generally agreed that the greatest economic loss suffered by the dairy industry is that due to sterility, either temporary or permanent. Efficient dairying and breeding demands that a cow shall give birth to a healthy calf every twelve months and be in milk for at least 300 days in the year. If, on the other hand, the dairyman is obliged to keep a dry cow or one in low lactation he suffers an economic loss in direct proportion to the cost of maintenance for any time beyond three months before she again becomes pregnant. In addition, he loses the potential profits of dairying over the same period of time. An account concerning the incidence of infertility in Great Britain contained in the report of the Economic Advisory Council's Committee on cattle diseases (1934) states that of the annual 58.2 per cent wastage in dairy cows due to diseases, 26.8 per cent resulted from diseases of the reproductive system.

* The research reported in this paper was carried out at the Royal (Dick) Veterinary College, Edinburgh and the Hannah Dairy Research Institute, Ayr.

† Paper read to the Section of Medical and Veterinary Sciences, 38th Session, Indian Science Congress, Bangalore, 1951.

The Survey Committee of the National Veterinary Medical Association of Great Britain and Ireland (1941) considered that the chief cause of infertility was endometritis of various types, usually secondary to *Brucella abortus* infection. It also stated that not more than 10 per cent of all causes of sterility in average herds was due to "Physiological derangement" and that 80 per cent of the loss from infertility was preventable. The same report estimated the monetary loss from milk and calves due to the presence of sterility, to the tune of £ 7,600,000 annually.

Albrechtsen (1920) was the first to emphasise the fact that the chief cause of infertility in cows was endometritis which was the result of bacterial infection. His belief has since been supported by well known obstetricians including Williams (1921), Oppermann (1924), Richter (1926), Nielsen (1926) and others. Quinlan (1929) who made an extensive study of sterility in cattle, came to the conclusion that chronic endometritis was responsible for 70 to 80 per cent of sterility in cows in South Africa.

Though it is thus generally recognised that endometritis caused by bacteria is the most important cause of sterility, very little information was available on the bacteria responsible for such a condition. Contributions to the literature in this field have been largely clinical, although some notable work has been done in bacteriology and pathology. Much of the work that has been done seems to have been directed mainly towards the study of the pathogenicity of *Brucella abortus* and *Trichomonas foetus* for the reproductive system. Very few workers have taken the trouble to ascertain the nature of other bacteria which might be present in the genital tract either alone or in association with the *Brucella* group of organisms. It is frequently recorded that cultures of *Brucella abortus* were obtained but generally no mention is made of the presence or absence of other organisms. Whenever organisms other than *Brucella* have been encountered the prevailing view has been that they were contaminants. It is apparent that this view is rather a broad assumption unsupported by any scientific evidence. Contagious abortion, trichomoniasis and tuberculosis no doubt play a great role in the production of pathological conditions resulting in sterility or impaired fertility, but it is not unlikely that the so-called contaminants or saprophytes may be responsible for the inflammatory condition either primarily or secondarily. It is but rational to expect that any organism given the right conditions can invade the uterus.

Unfortunately most of the workers in this field have occupied themselves with a study of the pathological conditions of the genital tract without any previous knowledge of the bacteria in normal as compared with abnormal genital tracts. The question which naturally

arises at this stage is—what is the bacterial flora of the normal genital tract of the cow. It is obvious that an answer to this question will help a great deal in the proper understanding of the bacterial aspect of endometritis. Though a fair amount of work has been done recently on the bacterial flora of the vagina of cows, the question of the bacterial content of the normal uteri of the large domestic animals is still open to doubt. There are two schools of thought: some research workers (Lutje, 1924; Klimmer and Haupt, 1929) hold that the uteri of healthy animals are normally sterile; a conclusion supported by similar views in human medicine, where in healthy women the Fallopian tubes, the uterine cavity and the upper third of the cervical canal are free of micro-organisms (Shaw, 1945). The other group of authors (Sohnle, 1901; Day, 1917; Beller, 1925, and others) have almost without exception been able to demonstrate bacteria in non-pregnant as well as pregnant uteri of cows.

In the light of these observations it seemed to the author that a contribution to the problem of sterility in cattle could be made by a study of the bacteriology and pathology of endometritis. The investigation recorded herein was therefore pursued in an endeavour to obtain accurate information along the following lines, viz., to determine the bacterial flora in a series of normal pregnant and non-pregnant uteri and their associated Fallopian tubes and cervixes of cows; to determine the bacterial flora in a series of abnormal uteri, their Fallopian tubes and cervixes, and to correlate them with the pathological features of these structures and to test the influence of some of the predominant organisms isolated upon the genital tract of experimental animals.

Materials

The observations herein reported are based on a study of 231 genital tracts of cows of which 131 were apparently normal and 100 abnormal. All these genital tracts were obtained from cows slaughtered in the abattoirs of Ayr and Edinburgh. They were all from the Ayrshire breed of cows. Following the collection of the genital tracts at slaughter, an effort was made to obtain the breeding history of each animal by forwarding a questionnaire to the original owner of the animal, in which the following information was requested.

Identification number.

1. Age?
2. Date of last calving?
3. Number of calvings?
4. Whether the calving had been normal?
5. Whether the placenta had been retained?

6. Whether the animal had shown "whites" after calving?
7. Had the cow ever aborted?
8. Had the cow been blood tested and, if so, with what result?
9. Had the cow been vaccinated against *Brucella abortus* infection? If so, when and with what vaccine?
10. Had the cow been served since last calving? If so, how often, and on what date was she last put to the bull?
11. Had there been any evidence of discharge during heat or after service? If so, what was the character of discharge?

In most cases it was possible to ascertain the age of the cow and the number of calves it had had with details of all its previous calvings, i. e. whether normal or abnormal and whether the placenta had been retained or any discharge had occurred.

Procedure and Technique.

When collecting the specimens for examination the genital tract was taken immediately after the slaughter of cow and tied with a sterile ligature, a hand's breadth from the external genital orifice, wrapped in a sterile towel and transported to the laboratory in a clean container. At the time of the slaughter of the cow a sample of blood was obtained for carrying out agglutination test against *Brucella abortus* antigen. Whenever there was evidence of acute or suppurative mastitis the udder was also collected for bacteriological examination.

The bacteriological examination of the genital tracts so obtained was made within one to three hours of slaughter of the cow. In a small number of cases where it was not possible to do the cultural examination immediately, the uteri were kept in the refrigerator overnight.

Material for cultural examination was taken from each of the following sites - cervical canal, corpus uteri, right and left uterine horns and right and left uterine tubes. When peri-uterine abscesses, cystic ovaries and other abnormal structures were present, material from these was also obtained.

The following technique was observed in obtaining inocula from the various sites.

Cervical canal:— The genital tract was spread dorso-ventrally on a clean enamel tray. An area on the dorsum of the cervix mid-way between the external and internal os uteri was sterilised by searing with a heated spatula. Then an incision was made with a sterile scalpel into the canal, a sterile platinum loop introduced into the canal and rubbed against the mucosa. In this manner it was generally possible to obtain sufficient material on the loop for purposes of inoculation.

Body, Horns and Tubes:—The corpus uteri were seared just below the bifurcation of the horns, the horns and the Fallopian tubes midway along their lengths. These sterilised sites were opened with sterile precautions and a long platinum (4 inches) loop introduced into the respective cavities and rubbed against the mucosa, taking care not to touch the edges of the incision. In some cases, especially the normal ones where sufficient material could not be obtained on the loop, about 5 cc's of warm glucose broth was introduced into the lumen of the uterus and pipetted in and out several times by means of a Pasteur pipette, or a piece of mucosa was removed with sterile forceps and rubbed directly on the surface of media. If there was fluid or discharge in the uterus it was withdrawn into sterile pipettes. When pregnant uteri were cultured the same procedure was followed.

In addition a piece of the cervical seal of mucus was removed and cultured. The amniotic fluid was withdrawn into sterile pipettes and spread profusely on the surface of solid media and about five c.cs were inoculated into serum-glucose-broth. Material from the utero-chorionic space and the foetal and maternal cotyledons, if they are large enough to yield pieces of tissue, was also obtained.

Media:—Material thus obtained from each of the sites was seeded directly on to the following media, with a view to satisfying the optimum growth requirements of different types of bacteria.

1. Three blood agar plates (containing 5-10 per cent normal horse blood); one each for aerobic, anaerobic and micro-aerophilic incubation.

2. Three glucose-serum-broth tubes (bouillon containing one per cent glucose and ten per cent normal horse serum.)

3. One crystal-violet-liver-agar plate for micro-aerophilic incubation.

4. One bacto-tryptose-agar plate containing 2.5 per cent horse blood for micro-aerophilic incubation.

For anaerobic incubation McIntosh and Fildes' anaerobic jar was used while for micro-aerophilic incubation, 5-10 per cent CO₂ was introduced into the jar containing the cultures.

All cultures were incubated at 37°C. The aerobic cultures were examined after twenty-four and forty-eight hours' incubation, while the anaerobic and micro-aerophilic cultures after three days, five days and seven days. The various colonies were picked and sub-cultured into serum broth, and after testing for purity, the strains were stored in cooked meat medium, at room temperature for further study.

5. During the later part of this work uterine material was also inoculated on to the special media recommended by Edward (1947) for the isolation of pleuro-pneumonia-like organisms from human vagina. The medium consisted of ox-heart infusion broth containing one per cent peptone and two per cent agar; plates were poured after adding 20 per cent horse serum, 10 per cent fresh yeast extract and 1 in 8,000 thallium acetate. The final pH of the medium was 8.0. Just before use two drops of a solution of penicillin containing 1,000 units per c.c. were spread over half the plate and allowed to dry. A semi-solid medium was also used. This contained only 0.3 per cent agar and a 1 in 2,000 concentration of thallium acetate. Immediately before use about 100 units of penicillin per c.c. of the medium were added. The cervical and the uterine mucus was inoculated heavily both on plate and into soft agar.

The plates were incubated in a closed jar at 37°C. After 3-4 day's incubation they were examined for the characteristic colonies of pleuro-pneumonia group. Semi-solid agar tubes were incubated for a week before discarding them as negative.

6. With a view to check up cultures in case of dead bacteria or living organisms which failed to grow in media, smears were made from cervical and uterine mucus or exudates and stained by Gram's method, Giemsa's stain and methylene blue; in suspicious cases Ziehl-Neelsen's stain was also employed.

7. Cervical and uterine discharges from all cases were examined for the presence of trichomonads. Fresh moist smears in warm saline were examined microscopically for the characteristic motility. If the flagellates were not observed, the discharges were inoculated into a special medium which was examined for trichomonads after 24, 48 and 72 hours' incubation at 37°C.

The medium used was that recommended by Kerr (1947) and was prepared as follows:—

Into sterile test tubes about 5 c.cs of sterile bovine serum were run. The tubes were then placed in an inspissator and heated to 75°C. for one hour to coagulate the serum into slopes. Trypsin broth of a pH of 8.0 containing 2 per cent glucose was then run in and this was covered by a thin layer of sterile liquid paraffin. The medium was sterilised by steaming for one hour on each of three successive days, then incubated to test for sterility. The final pH was 7.4 to 7.6.

Before discarding these tubes as negative after examination for trichomonads, sub-cultures were made on blood agar plates for any bacterial growth. It is of interest to note here that *Corynebacterium pyogenes* grew well in this medium. In a number of cases

where no primary growth of *Corynebacterium pyogenes* was obtained on blood agar plates, it was possible to isolate *Corynebacterium pyogenes* from this medium. Moreover its presence was easily indicated by the liquefaction of the serum of this medium. This medium seems therefore to have a special use for the isolation of *Corynebacterium pyogenes* when it occurs in small numbers mixed with other bacteria.

Crystal violet-liver-agar (Huddleson, 1939) was prepared as follows :—

500 c. cs of distilled water was added to one pound of fresh minced liver, mixed well and placed in cold-store for 24 hours. The container was then covered and placed in a steam steriliser for two hours. From time to time the contents were vigorously stirred. The infusion was removed and strained through muslin cloth. The following ingredients were then measured out: Agar 20 grams; distilled water 500 c.c.; liver infusion 500 c.c.; peptone 5 grams and Sodium chloride 5 grams. These were mixed and steamed for one hour stirring to dissolve the agar. Then the pH was adjusted to 7.2 and 1.4 c.c. of a hot 1/10 per cent aqueous solution of Crystal-violet was added. Now the medium was steamed for a further half an hour. Finally the medium was distributed in pour tubes and sterilised at 45 lbs. pressure for 30 minutes. Plates were poured just before use in the usual manner.

Bacto-tryptose-agar was prepared as follows :—

To one litre of bouillon (pH 7.2) 20 grams of Bacto-tryptose, one gram of dextrose, 5 grams of Sodium chloride and 20 grams of Bacto-agar were added. These ingredients were mixed and steamed for one hour stirring to dissolve the agar. The medium was then distributed in pour tubes and autoclaved. The final pH of the medium was 7.0. Plates were poured after adding horse blood to a concentration of 2.5 per cent.

Carbohydrate Media were prepared as follows :—

Peptone (Evans)	10 grams.
Sodium chloride	5 grams.
Distilled water	1,000 c.c.

These were mixed and steamed for 15 minutes to dissolve the ingredients, and then filtered through chardin paper. The pH was adjusted to 7.6 with N/1 NaOH and sufficient Andrade's indicator (one per cent) was added, then autoclaved at 15 lbs. for 15 minutes. When cool the following sterile solution was added aseptically.

Carbohydrates 5 grams.
Water 10 c.c.
Finally distributed into tubes in a sterile manner.

In the study of *corynebacteria* the carbohydrate media were made up in Hiss's serum water.

Fermentation reactions were read daily up to seven days' incubation at 37°C. except in the case of *corynebacteria* where the readings were taken at 2 day intervals up to 10-15 days.

Test for the liquefaction of gelatin was made by incubating the test tubes at 37°C. for four to seven days and examining the tubes after cooling.

All organisms isolated were fully examined for their morphological and biochemical characteristics and in the attempt to identify them the author has closely followed the classification drawn up in the fifth edition of the "Manual of Determinative Bacteriology" by Bergey *et al.* (1939). Standard methods and techniques were used throughout except where stated.

The agglutination test for Brucellosis was performed against *Brucella abortus* antigen, kindly supplied by Dr. A. W. Stableforth, of the Veterinary Laboratory, Waybridge. A titre of 1 in 20 with crystal clear supernatant was taken as a positive test.

The pathogenicity of staphylococci was judged by their ability to form coagulase. Citrated human plasma was diluted 1 in 5 with normal saline solution and 0.5 c.c. of the dilution was pipetted into each of two test-tubes (7.5 cms. × 8 m.m.) One tube was inoculated with five drops of an overnight broth culture of the organism under test. The other tube was used as a control. Both the tubes were incubated at 37°C. for 3 to 6 hours and examined every hour. If no clot had appeared by this time, the tubes were left overnight at room temperature and again examined. The test was recorded as positive if, at either of the readings, the inoculated tube could be inverted without loss of fluid.

In grouping the streptococci, Lancefield's acid extraction method was used. The strain under test was inoculated in 15 c.c. of 1 per cent glucose broth in test tube and incubated overnight, the rest of the procedure was exactly the same as described by Mackie and McCartney (1948).

In the case of *Corynebacterium pyogenes* filtrates of culture grown in litmus milk for 72 to 76 hours at 37°C. were tested for their haemolytic activity in the presence of specific anti-toxin (Lovell, 1939).

Pathological examination. After obtaining material for cultural examination, the genital tract was examined macroscopically and the lesions, if any described. Measurements of the various parts were taken as follows:—

- (a) The cross-measurement of the cervix was taken at about the junction of its middle and caudal third. The length of the canal and thickness of the wall were also noted.
- (b) The body of the uterus was measured from the cranial extremity of the cervix to the point of bifurcation of the horns of the uterus.
- (c) The uterine horns were measured from the bifurcation to their apices. The thickness of the wall was taken about the junction of the middle and the lower thirds.
- (d) The state of the ovaries with regard to the Graffian follicles and corpus luteum was noted.
- (e) Immediately after the macroscopic examination of the genital tract representative slices of tissue were cut from cervix, uterine horns and tubes and preserved in 10 percent formal saline.

After fixation, the blocks were put through dehydrating and clearing reagents in the following manner:—

Methylated spirit (containing 5% phenol) 2 changes, 12 hours each.

Absolute alcohol—2 changes, 3 hours each.

Absolute alcohol and benzol (equal parts)—12 hours.

Benzol—2 changes, 6 hours each.

Paraffin wax—2 changes, 12 hours each.

Paraffin—for embedding.

The sections were stained by Haematoxylin—Eosin; Eosin—methylene blue and van Gieson's stain. Whenever necessary they were also stained by Gram's and Ziehl-Neelsen's methods and other differential stains.

Anatomical features of the uterus of the cow

The uterus has been described by Sisson (1911) as a hollow muscular organ. Anteriorly it receives the Fallopian tubes and opens into the vagina posteriorly. It is situated almost entirely within the abdominal cavity in the adult and is attached to the upper part of the flanks, about a hand's breadth below the level of the external angle of the ilium, by two folds of peritoneum termed the broad ligaments. The uterus is differentiated into a neck or cervix, body and two horns or cornua.

The uterine or Fallopian tubes are thin-walled and flexuous measuring about 20—25 cms. in length. They follow a course over a pouch formed by a folding over of the free edge of the broad ligaments which envelopes the ovary. The fimbria is attached to the free margin of this pouch. The uterine extremity of the tube is funnel-shaped while the ovarian extremity is fimbriated, though the fimbriae are not so extensive as in the mare.

The cervix is about 8—10 cms. long with a dense wall about 2—3 cms. in thickness. The lumen of the cervix i.e. the cervical canal is spiral and is ordinarily firmly closed. It opens posteriorly into the vagina by the os uteri externum and anteriorly into the body of the os uteri internum. The mucous membrane of the cervix is pale and glandless. It forms numerous folds arranged in several series which obliterate the lumen. At the os uteri externum the folds form rounded prominences arranged circularly, which are seen projecting into the cavity of the vagina.

The body of the uterus is only 3—4 cms. in length, although externally it gives the impression of being much longer, owing to the fact that the posterior parts of the cornua are united by connective and muscular tissue and have a common peritoneal covering.

The cornua are very variable in length (25—40 cm.), cylindrical in appearance and taper gradually towards the anterior extremity, so that junction with the Fallopian tubes is not abrupt, as in the mare. The free portion of the horn takes a spiral course by proceeding downward, forward and outward and then turning upward.

The uterine wall consists of three coats, an external serous, a middle muscular and an internal mucous. The serous coat is adherent to the muscular coat and is continuous with the broad ligaments. The muscular coat consists of an external longitudinal layer and an internal circular layer which is in two strata; in between these muscular layers there is a vascular bed in which the blood vessels ramify.

The mucous membrane of the horns and body of the uterus presents a feature characteristic of ruminants but which is unlike that of the majority of other animals. This is the presence of uterine cotyledons, which are round button-like prominences about 80—100 in number, scattered irregularly over the surface or arranged in rows along the longitudinal axis of the uterus; they serve as points of attachment for the foetal membranes. In the non-gravid uterus they are small in size, (15 m.m. x 15 m.m. x 15 m.m.), but during the pregnancy they become greatly enlarged and pedunculated, (10—12 cms x 3—4 cms x 2—2.5 cms). Their surface has a spongy appearance, due to numerous crypts which receive the chorionic villi.

The presence of the cotyledons thus differentiates the mucosa into two parts, viz. the cotyledonary and the intercotyledonary areas. The mucosa of the inter-cotyledonary area is smooth and the uterine glands open on to its surface. Beneath the epithelial layer there is a layer of connective tissue which can be differentiated into two a rather dense superficial layer and a deep layer of loose connective tissue in which the uterine glands ramify. The cotyledons are lined by a single layer of columnar epithelium as in other parts of the uterus, but have no glands opening on to their surface. Beneath the epithelium there is a dense layer of connective tissue richly supplied with blood vessels. The loose connective tissue layer beneath the former has glands as in other parts of the uterus. (Vide Figs. 1, 2, 3, 4, 5).

In order to appreciate the histopathological changes, it is considered necessary to review briefly the sequence of changes which occur in the endometrium of the cow during the oestrous cycle (Hammond, 1927).

The cotyledons:—The connective tissue stroma of the cotyledons appears to be unusually dense three days to 14 hours before heat. There is slight congestion of sub-epithelial blood vessels during heat and twenty-four hours after the beginning of heat. At 48 to 72 hours after the beginning of heat the connective tissues appears to be oedematous, with great congestion of blood vessels and slight extravasation of blood into the sub-epithelial tissues (Fig. 5). By eight days after the beginning of heat, the congestion and oedema of the tissues have subsided.

The intercotyledonary area:—The vascular and connective tissue changes which take place in this area are very similar, but less intense than those which take place in the cotyledons. The capillaries become slightly enlarged during heat, but do not appear to reach their maximum congestion with extravasation of blood until 72 hours after heat. Sub-epithelial extravasation of blood is followed by pigment formation eight days after heat, but not to the same extent as in the cotyledons. About 3 days to 14 hours before heat there appears to be an accumulation of hyaline matter between the strands of loose connective tissue of the uterine mucosa, it disappears in 48—72 hours after the beginning of heat.

No great difference could be observed in the number and distribution of the uterine glands during the oestrous cycle. During the middle of the cycle and then just before the onset of heat the changes appear most marked in the deep glands, with an increase in size which is followed by a rapid diminution to a minimum 24 to 48 hours after heat; thereafter a gradual increase in size again occurs from 3 days after heat until the middle of the next heat period.

Bovine Endometritis



Fig. 1



Fig. 2

Figs. 1 & 2. Sections of normal uterine wall showing the serous, muscular and mucous coats. A cotyledon is visible on the right side of each photograph. Haematoxylin-eosin. X-10.

Bovine Endometritis



Fig. 3

Section of a normal endometrium showing dense superficial and loose deep connective tissue with uterine glands.

Haematoxylin-eosin. X-10.

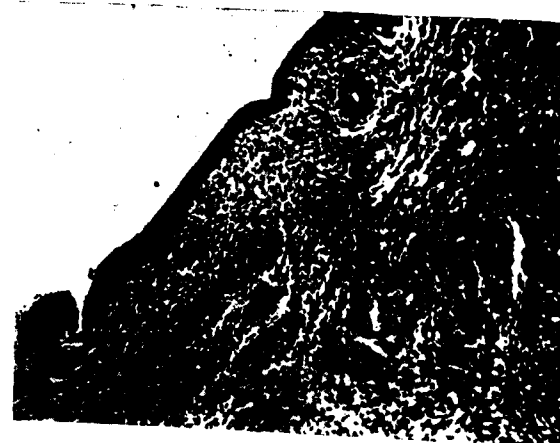


Fig. 4

Section as for 3 showing the columnar epithelial lining of the mucous surface.

Haematoxylin-eosin. X-50.



Fig. 5

Section through a normal cotyledon showing dense superficial and deep connective tissue. Note the congestion of blood vessels (about 48 to 70 hours after the beginning of heat).

Haematoxylin-eosin. X-10.

BOVINE ENDOMETRITIS

387

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CHRONIC BOVINE HAEMATURIA*

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VII. (b) The Parasite from different sources in cultures.

As already explained, attempts were first made to isolate the fungus flora from as many haematuria cattle originating from as well separated areas, as possible. Different pure isolations thus became available. These were maintained to see whether with a sufficiently comprehensive number of isolations having accumulated, cultures of significance would not automatically sort themselves out as the commonest or the invariable strains. Any medium that happened to be readily at hand, whether liquid or solid, was utilised, the object being to effect successful pure isolations only. In the absence of any clue as to the nature of the fungus to be especially sought for, no standard medium was used. Twenty-three cultures thus resulted from eight clinical cases of haematuria then available at Mukteswar. The isolations were repeated and while, for instance, the same type appeared from an animal more than once, additional cultures of what superficially appeared to be different were obtained and this complicated the issue. To take extreme cases, while one animal repeatedly produced a yellowish-green culture, another produced as many as four cultures then described as (1) slightly yellowish white downy growth, (2) greenish yellow powdery growth, (3) dirty brown granular scanty growth, and (4) slightly black downy exuberant growth. The last mentioned was later found to be *Aspergillus niger*, while the first three were, to all intents and purposes, cultures of the same *Aspergillus* sp. The considerable variation in growth characters and pigmentation shown by the different isolations became perplexing. Sooner or later most cultures appeared to pass through a greenish or a greenish yellow colour. Microscopic examination of the morphology was found to be equally complicated. Characteristic aspergillar heads were present, but no uniform shape or size of the specific structures could be fixed for the cultures, to enable an opinion being formed of their identity. The cultures were sometimes white and downy, at others definitely but only mildly pigmented green or yellowish green, at others again they tended to become powdery and brown. It became difficult at that stage to decide whether one or more species of *Aspergillus* was involved, and whether the cultures belonged to one or

several of the groups—*A. glaucus*, *A. flavus-oryzae*, *A. tamaris* and *A. versicolor*. In the conidophores, various gradations in shape and size of vesicle from a perfectly globular to a dome-shaped semi-circular head, or a columnar elongated head were present. The foot-cells were sometimes not recognisable. What was more complicating was the presence of atypical heads. The vesicles were sometimes considerably abbreviated, or even appeared to be absent. The sterigmata were generally in one series, but there were heads with distinct secondary sterigmata or conidiophores. Were the cultures then a *Sterigmocystis*, or a monoverticillate *Penicillium*? Comparatively large and globular masses of conidia, apparently adherent together were often present. Was there a *Gliocladium*? The characteristic aspergillar heads were there, though the heads were somewhat dissimilar, but subcultures after repeated plating and decimal dilutions, left no possibility of doubt about the purity of the isolations. The finding of abnormal conidial heads with a green aspergillus in this case remained inexplicable. The possibility that organisms, belonging to other genera, related to the *Aspergillus* and *Penicillium* (*Penicillopsis*, *Gliocladium*, *Hormodendron*, *Citromyces* etc.) might be involved, was examined, but the confusion continued. The presence of contaminating fungi of the bacteriological laboratory, including *A. niger*, *Mucor*, *Penicillium*, *Rhizopus* made the situation worse. The burden of maintaining subcultures was becoming more and more time consuming, and the fear of contaminating one culture with another was looming large. The difficulty of making any satisfactory study of so many cultures at one time, in the absence of some clear indication of their botanical relationship, was keenly felt, and an opportunity for the drastic curtailment of the cultures presented itself. The writer was transferred from the Headship of the Pathology Department to organise the newly built Protozoological and Entomological Department of the Institute. Before moving to the newly built laboratory buildings, it was considered advisable to discard all the old cultures. Clinical cases were still present. If a mould-fungus was the causative agent of haematuria, the cultures should repeat themselves constantly and invariably. If they did not repeat themselves it was just as well not to waste any further time over them.

At this time a valuable clue suddenly presented itself. Tubes of culture media, inoculated on the spot directly with fresh bladder material from two haematuria animals specially destroyed for the purpose were received back from Kalimpong. On arrival at Mukteswar, more than one tube from each of these cases were found to contain practically pure growths of a yellowish-green *Aspergillus*, looking extraordinarily like the cultures which had been encountered so many times previously in Mukteswar cases. Those two cultures were subcultivated on several

* Continued from Page 301 of *J.V.J.* Vol. XXX, No. 4, January, 1954.

media, and appeared to be identical in the colony characters, pigment production and in minute morphology. In order to have the opinion of an expert as to whether the two cultures were completely identical, they were forwarded to the Imperial Mycologist (Mr. L. D. Galloway) at New Delhi. The report received from him reads as follows:—"The two cultures have been given a preliminary examination. They appear to be identical, and a species of *Aspergillus* which does not fit in with the described species but comes fairly close to *Asp. tamaritii*. If I can get a closer identification, I shall let you know". In a later report it was stated that the two strains were handed over to a friend in London, who placed them in *Aspergillus glaucus* group, somewhere between *Aspergillus ruber* and *A. scheelei*.

Later another culture isolated directly from the bladder tissue of a cow (Cross-Ayrshire No. 4/27) at Mukteswar in March 1938 was forwarded to the same office then held by another officer, who reported:—"The fungus is a species of *Aspergillus* belonging to the group *Versicolor*, and is quite distinct from any previous cultures you have sent. The group *Versicolor* contains pathogenic forms". Later still two further cultures were similarly isolated from the bladder and heart blood of two other cases sent on 17-6-38. A third officer (Mr. G. Watts Padwick) who held the post then reported: "Culture No. 1 appears to be of the series *flavus* of the group *A. flavus-oryzae*. Culture No. 2 contains the same fungus but also a *Penicillium* which we have not attempted to identify".

On his way to Europe, the present writer forwarded a set of eleven cultures of *Aspergillus* sp. recovered from haematuria cases and from the bracken fern and lichen to Mr. George Smith at the London School of Tropical Medicine, for favour of looking after the cultures till the writer arrived in London. A second set of the same cultures were brought in person with a view to make a more detailed study of the cultures with Mr. Smith, who was known to be personally interested in the genus *Aspergillus*. As the writer commenced his studies at Edinburgh and did not have the opportunity of returning to London to undertake the proposed study with Mr. Smith, he enquired if the latter had formed any opinion on the series of cultures that were sent to him from India. The reply reads: "The cultures which you sent to me in July were all strains of *Aspergillus flavus* Link. As I did not hear from you again after your visit in September I concluded that you had no further interest in the cultures and so scrapped them all before Christmas". It may be added here that the cultures sent to Mr. Smith included the isolations already examined and reported upon by the Mycologists mentioned above, besides those that were recovered from

lichen and bracken, and a few other cultures from haematuria animals.

It may now be mentioned the writer employed for his morphological and physiological studies, the following twelve cultures of the *Aspergillus* sp., with a view to their classification and identification. It will be noted that with the exception of one culture which was recovered at Edinburgh, the rest were isolated after the writer's transfer to his new laboratory, and that the cultures refer to specimens from Kalimpong, the Kulu Valley, the Nilgiris and Mukteswar:—

- (1) Isolated directly from the bladder of a naturally affected Cross-Ayrshire cow "Gaiety" at Kalimpong, destroyed for the purpose of obtaining cultural material in October 1936.
- (2) Isolated directly from the bladder of a naturally affected Cross-Ayrshire cow "Susan" at Kalimpong, destroyed for the purpose of obtaining cultural material in October 1936.
- (3) Isolated from the jugular blood of Cow 4/26 on 16-6-38.
- (4) Isolated from the bladder tissue of the same animal on 16-6-38.
- (5) Isolated from the bloody urine of a Cow in the Kulu Valley.
- (6) Isolated from the heart blood of an experimental healthy calf (H. C. 38) which died of too frequent administrations subcutaneously of a strain of *Aspergillus* isolated from a natural case.
- (7) Isolated from the bladder tissue of a case of successful experimental transmission (H. B. 143) effected with subcutaneous injections of haematuria urinary sediments on 26-5-36.
- (8) Isolated from a clinical case (H. B. 549) at one of the Out-kraals of the Mukteswar Institute.
- (9) Isolated from a rabbit (No. 9) inoculated intravenously with material from a haematuria cow on 30-4-38.
- (10) Isolated from bracken fern at Mukteswar.
- (11) Isolated from lichen at Mukteswar.
- (12) Isolated at Edinburgh from a specimen of diseased bladder tissue received in 50% sterile glycerine from the Ellington Dairy Farm in the Nilgiri Hills in South India, in December 1938.

VII. (c) Mycological Studies.

Cultivation on various media.—The twelve cultures listed above were planted out in culture tubes and in petri plates containing a variety of solid media. Even at the most superficial view of the cultures one could see that they produced the same invariable colony characters, and passed through similar grades of colour changes. The close relationship of the cultures, and the suspicion that the whole set represented an identical organism emerged from the very outset. Before attempting to identify and classify the cultures, it was necessary to know the range of their variation under each set of circumstances. Different sugars were substituted in each medium and the effect studied. It was found that when the cultures were young and the very commencing stage of growth, they appear to be whitish and practically unpigmented. Later, as the growth developed, pigmentation gradually manifested itself and the colony colour became deeper to some shade of green. Ridgway's colour standards were used to compare the cultures from stage to stage. The vegetative mycelium did not appear to produce any appreciable pigment, and pigment production appeared to be restricted exclusively to the conidia and conidial heads. It was noteworthy that practically identical amounts of transpiration water and calcium oxalate crystals were produced by each member of this collection of cultures. As far as sclerotia were concerned, there appeared to be some minor differences in the amounts of these produced and in the rates at which they appeared. The maximum amounts of the oxalate crystals appeared on the pellicle cultures on liquid media, such as horse digest medium or glucose broth. There was one other type of crystal noticed in these cultures, and they appeared to be those of earthy phosphates. It may be remembered that these two types of crystals occur in the affected bladder tissue and sometimes also in the urine of natural cases of haematuria. More uncertain than the sclerotia were the variations in the brownish pigmentation that appeared on the reverse side of petri plates of different cultures. It seemed possible that these brownish patches were actually connected with the sclerotia.

Cultures on different sugars.—Of all the sugars tested, it was found that the cultures produced the least growth on lactose, taking a longer time to attain the developmental characters comparable to those of other sugars. Glucose produced the most luxuriant growths while saccharose produced almost equally good results.

Cultivated on the routine Lemco Agar, with one or the other sugar being used in the composition, it was found that the cultures attained to a height of about 5/6 m. m. above the surface of the medium. When glucose was used in the medium, the colour produced was some-

what different from those exhibited on other sugars. Glucose gave a Mignonette green, Saccharose yielded a somewhat similar growth but there was nothing very particular to be noted in the cultures on this sugar. The cultures on Maltose-Lemco-Agar were different, with the centre of the colonies being nodular and of a darker colour than the peripheral zone. The colour in the centre approximated to sea-foam yellow and the periphery was distinctly cottony and fluffy. On Lactose-Lemco-Agar, the colony size averaged that of a sixpence. The growths were the least in amount on Lactose and, to start with, no pigmentation was discerned. Later, however, cultures on it developed the identical and complete pigment production as noted in the other sugars.

Cultures on Czapek-Dox-Agar.—This medium has been recommended by previous workers as the best standard medium upon which the comparative and systematic studies of *Aspergilli* should be carried out. In order that the results of various workers may be comparable, the possibilities of pleomorphism to which this particular genus is extremely liable, should be eliminated by the use of synthetic standard media, such as Czapek medium.

In order to study the comparative rate growth of the different cultures, it is essential that the same amount of inoculum be used in each culture tube or plate of medium. The colony characters are best studied in petri plates but cultures have been simultaneously studied in tubes. They were usually incubated at 37°C., which had already been found to be optimum for the cultures. When the cultures were maintained at the room temperature, or at lower incubator temperature, the most important effect noticed was that the rate of growth was substantially delayed. Alterations in the incubator temperature did not appear to produce any significant changes in the morphological characters of the various *Aspergillar* structural forms. Observations on the colony character were started after twenty-four hours and continued at the same interval over a sufficiently long period.

After twenty-four hours of inoculation, the colonies were found to have started their vegetative growth from the central inoculum but nothing noteworthy attracted attention. After another twenty-four hours, the appearance of the cultures was somewhat more marked, but still there was nothing worthy of description. At the end of seventy-two hours after inoculation, the cultural features were more clearly exhibited. The centre of the cultures was raised and a distinct white margin of appreciable width was clearly seen. The cultures were generally velvety. The pigment produced by each culture was exactly identical, as far as careful naked eye examinations could determine. On comparison with Ridgway's standard, the pigment conformed to oil-green. The growths apparently tended to become floccose. The medium itself

retained its natural colour and no tendency to any discolouration was noticed. Crystals started to appear. The next day the above growth characters were more accentuated. On the fifth day the cultures were decidedly greener. The average colonies varied from 1 c.m. to 3 c.m. but occasional single colonies were as large as 6 c.m. Three zones could be distinguished e.g. central, ring of short coremia, and peripheral zones. The colour of the cultures varied from jade green to Carro green. The water of transpiration appeared to be at its maximum. On the reverse side the centre of the larger colonies stood out due to a brownish umbilicated crust. Whitish mycelial strands were seen radiating out from the brown centres. On removing the crusty brown material, it was examined under the microscope and found to be sclerotia.

In the next day or two there were few changes to be noticed in the cultures. The water of transpiration began to dry off about the sixth day, and practically half the cultures did not show any water on the seventh day. In some cultures this water persisted for another day or two. On the seventh day the pigment varied from Kronberg's green to jade green. On the eighth day, most Czapek plates showed white crystals at the junction of the middle and peripheral zones of individual colonies. The crystals began to appear from the third day onwards. The colour of the colonies varied from Calla green to Cerro green. As the growth became older, it was noticed that after a time when the colonies had reached a particular stage no further extension from side to side took place. The conidiophores, however, grew higher to a certain limit. It was found that a process of dispersion of conidia to considerable distances away from the foci of colony development took place. Consequently, with time, a number of minute colonies began to appear in close and increasing succession in different parts of the plates. Besides it was observed that the tendency to any further change of colour of the cultures was practically non-existent as far as cultures on this medium were concerned. The cultures practically came to a standstill. As the moisture content was being gradually exhausted they became extremely powdery, and the powdery material itself was so loose that the slightest tilting of the plates would make it run down to the lowest level in plates. The cultures were kept under observation for long periods up to one year, but no perithecia developed except in three accidental cases to be noted elsewhere. On the basis of the data recorded by workers as being optimum for perithecia production in certain groups of *Aspergilli*, the concentration of sugar was raised and the cultures were incubated at 22°C. No perithecia, however, resulted with the haematuria cultures in the ordinary way.

Cultivated in tubes, the cultures covered the whole surface of Lemco medium prepared with glucose or saccharose in about four days.

Cinnamon brown drops of liquid appeared and showed a tendency to coalesce and flow down to the lower parts of the culture tube. This feature was not so marked on Czapek cultures. The drops were produced on nodular growths which were composed of closely aggregated mycelial strands. The surface of these nodules initially appeared to be white and irregular. Later these became rather hard and resilient, and were recognised as the typical sclerotia. With the progress of time these became brown.

Of the different solid media used, Czapek produced the greenest cultures, more colouration being evident in the thinner portion of the slant, though later the initially white colour in the lower and thicker parts of the slants assumed the characteristic depth of pigmentation. After six days of inoculation the cinnamon brown liquid drops were still present on Czapek. The medium containing lactose as the sugar, showed the least of the green tinge, and the pigment conformed to Ecru-olive. If the growths on Czapek and Lemco-Agar containing different sugars are arranged according to the intensity of colouration from green to Ecru-olive, they could be arranged in the order Czapek with sucrose, Lemco-Agar with each of glucose, sucrose, maltose and lactose. As noticed on petri plates, the best growths were produced on glucose and saccharose and the least on lactose. In regard to the sclerotia which were rather hardened, tenacious and resilient on pressure, they were produced in largest numbers in media containing saccharose. On the media containing maltose, glucose and lactose, they were produced in gradually diminishing numbers in the same order in which these sugars are mentioned here. In colour, the sclerotia on Czapek were more white than on any other sugar-containing medium. On Lactose-Agar slants, the growth of the fungus was most dense in the centre of the colony, and lower down towards the bottom and thicker portion of the slants, the cultures tended to be very fluffy and white.

Culture on Vegetable and Bread Substratum.—With a view to test the behaviour of the cultures with regard to the production of perithecia, several kinds of natural vegetable substratum were used. Of these, boiled tea leaves, sterilised cabbage, lettuce, potato slants, slices of beetroot, carrots, tomatoes, prunes and bread were employed.

Regarding the details of bread cultures, it may be said that portions of both white and brown bread were sterilised in petri dishes. The same thickness of bread was taken. It was found that if the thickness of the bread was not uniform in the different plates a variation in results would naturally follow even with the same culture. This probably depended upon the available space in the petri plates left over for holding the oxygen supply and for occupation by the fungus growths. An interesting observation was that brown bread gave a more luxuriant

growth to start with, and the developmental stages were passed more rapidly on this medium than on white bread. An extremely small inoculum was used and the same quantity was placed on both the varieties, but the brown bread was covered over the whole surface in a much shorter time. In brown bread the fungus penetrated into the depth of the bread and could be seen on the reverse side comparatively soon after inoculation. The conidial heads appear to be restricted to the centre of the colonies. On the subsequent day, the colonies had not only extended in size but the conidial heads were present almost equally at the periphery as at the centre. After three days of growth the pigment produced conformed to Kronberg's green. The reverse of the plate showed at the periphery of the bread practically the same amount of pigment production as on the upper surface. The fungus spreading on the lower surface of the bread was inclined to be white and comparatively unpigmented in the central zones, as opposed to the periphery of the bread.

Turning to the white bread, the culture was much smaller on the fourth day, and restricted mainly to the central area of the upper surface. It had not penetrated through the thickness of the bread, nor could it be seen on the reverse. The colour that was produced was not so intense as that on the brown bread, it being some tint of light green. When the cultures were examined the next day, the growths had extended themselves further and the colour changed to Mignonette green. The conidial heads preponderated in the central area and extended to the middle zone of the colonies. The peripheral zone of individual colonies continued to be cottony. The pigmentation was decidedly more developed than on the previous day. Slowly and gradually the whole bread was covered up with the felted culture, the characters of the culture conforming more and more to that on brown bread. The cultures were maintained for a considerable time to see if any perithecia would be finally developed. It is known that bread forms a particularly favourable medium for stimulating the production of perithecia and that usually they appear within a week or a fortnight at the most.

Regarding the other vegetable media already mentioned, it is not necessary to go into any details. The cultures were carried out primarily with a view to studying perithecia production. The object was not achieved. It may, however, be added that the cultures were found to be able to grow readily on all the different vegetable media so far tried. The cultures enabled a general impression to be gained of the growth characters under natural conditions. It was found that the growths were influenced to a large extent by the degree of moisture conditions available.

Cultures on Liquid Media.—A number of liquid media including glucose broth, 5% peptone, sugar solutions, tea infusion, glycerine and horse digest medium were used. The different cultures were grown on these media in test tubes and in petri plates. The characters of the fungus growing on moist slides in petri plates can be examined directly under the microscope by uncovering the culture. On horse digest medium, a pellicle culture was obtained, and the thickened hyphae were found to climb up the sides of the glass tube above the surface of the medium to about 1½ c.m. Large amounts of calcium oxalate crystals are produced in these pellicle cultures. With the progress of time, some of the surface growth deposits to the bottom, generally in flake-like masses. The rest of the medium, however, remains clear. In bottom cultures, in glucose broth or similar other media, the character and progress of the growth are considerably changed.

Morphology of cultures.—To summarise the growth characters of the cultures on various media :—

- (1) Rate of growth—moderate, greater in the incubator and on certain substratum, particularly on glucose agar media.
- (2) Colony colour and colour changes—uniform in all the cultures. Colony colour exhibited in the central zone. The edges of the growing colonies tend to remain somewhat colourless. Even in old cultures the edges are somewhat paler. On various media the green shades verge from oil-green, Calla green, Mignonette green, Kronberg's green, jade green, Cerro green. On lactose, Ecreu-olive.
- (3) Colour and colour changes on the reverse—reverse not generally pigmented, small circumscribed brownish pigments appear here and there, perhaps connected with sclerotia.
- (4) Colour changes in the medium—colour production restricted to culture, no pigments diffuse into the medium.
- (5) Texture of surface—growths generally compact over the whole surface, but small areas of loose growths appear in old cultures. The surface is plane, due to the aerial hyphae growing to about the same height. Occasionally wrinkling may be seen on prune extract agar. At the edges of giant cultures on Czapek-Dox-Agar in Erlenmeyer flasks, streamers of hyphae are frequently seen. In the latter, on the walls of the flask the streamers form numerous conidiophores radiating out to equal distances from the centre of individual colonies. This striking picture is produced by all the cultures.

- (6) There is no characteristic odour of these cultures. Occasionally a generally mouldy odour is exhibited. Of all the media used the strongest odour was produced on sterile bread medium and reminded one of fermented bread.
- (7) Transpiration fluid appears on the aerial growth. The globules are shiny and transparent on Czapek. On other media sometimes brownish.
- (8) Submerged hyphae—do not produce any pigment, are segmented, approximately of the same diameter as their neighbours immediately above them. Chlamydospores along lengths of hyphal tubes may be seen, under unfavourable conditions.
- (9) Among the fruiting bodies, intercalary and terminal chlamydospores and conidiophores are frequently present. Conidiophores appear comparatively early but a culture does not have its full complement for about four to seven days depending upon the temperature and substratum.
- (10) Perithecia—the most characteristic mature fruiting bodies of *Aspergilli*—are seldom produced. Perithecia may appear accidentally in a week, or sometimes after the complete exhaustion of the medium with odd cultures. The perithecia are borne on the aerial growth. One obscure type of sporing structure is produced by cultures, but this need not be considered at this stage.

Here it may be added that the identification of the cultures in petri plates can be readily made. The naked eye appearance of the various cultures recovered from different sources of haematuria material has been invariable and characteristic. It is therefore possible to see at a glance whether the cultures are pure or contaminated. In case any contamination has taken place obvious changes in the character and rate of growth of the cultures are seen. The gradation of changes through which individual cultures pass from day to day is uniform in all the cultures, the limits of variation being only restricted. The changes in the colour of cultures, and the appearance of such features of transpiration water, sclerotia and crystals, with advancing age has followed the same sequence in all the cultures.

Coming to the finer details, it is necessary to mention a few particulars about the techniques employed. Direct preparations can be made by removing young cultures on to a drop of lactic acid, glycerine, alcohol or water. As the fungal structures are not readily wetted with watery solutions, a previous treatment with a drop of alcohol may be

useful. A cover slip may be applied and the preparation examined microscopically. A drop of cotton blue may be added by the side of the cover slip. Again minute colonies can be scooped out together with some of the adherent solid medium with the help of a hot platinum loop, and planted on a clean slide. If the slide is then gently warmed the medium slightly melts and the culture is flattened out on the slide. Cultures can also be grown directly on the slides or cover glasses, containing a very thin and uniform film of 2% agar medium. Experience shows that special provision should be made for ensuring the presence of sufficiently high amounts of moisture all the time for uninterrupted growth to take place. It is said that the members of *A. flavus* series require about 15% moisture to start germination, the optimum being over 50%. If smears have to be made from cultures, a certain amount of displacement of fungal connections is bound to take place. One of the chief difficulties of making permanent preparations is the readiness with which conidia and even hyphae may be washed away during the processes involved in fixation and staining. It has been found that workable preparations can be obtained by drying and fixing smears made in plain water, while smears in serum or egg albumin are very unsuitable. To prevent the risk of the conidia being washed away, a protective coating of weak collodion may be used. This interferes only slightly with the staining of the fungus. It may be added that a prior treatment with a weak solution of potassium permanganate or osmic acid, or chromic acid improves the stainability of fungal structures. Fleming's fixative is the most suitable and Heidenhain's iron haematoxylin is the best stain for cytological details. Acid thionin solution may also be used for staining. For mounting these preparations chloroform-balsam appears to be more satisfactory than xylol-balsam. Chromic acid produces distortion of the conidial capsules, depending upon the strength that is used.

Like other *Aspergilli*, the haematuria fungus shows the characteristic arrangement of its conidia and conidiophores. The conidiophore is undoubtedly unbranched, but in some aberrant forms the vesicles are particularly abbreviated and an apparent branched system may be simulated. The vesicle is generally always well-developed, and readily recognisable at the origin of the sterigmata. The majority of conidiophores present a single verticil of sterigmata. The enlarged vegetative cell on the hyphae forming the characteristic foot cell may be quite prominent, but when grown in some media or at some early stages of growth the foot cell may appear to be absent. While the presence of the foot cell and the vesicle distinguishes the conidiophore of the haematuria parasite from that of a penicillium, the aberrant forms are such that a confusion is most likely to be caused.

The conidiophore is septate, but when just growing the septation may not be well-developed. The stalks, under certain conditions of old cultures reveal prominent "echinulations". The conidial heads are mostly globose, with fairly large vesicles. The conidial chains may be uniformly and closely arranged radially around the vesicles, but the vesicle may be only dome-shaped with the conidial chains being set on the convex side. The conidial head may be composed of three to six sterigmata. The colonies of cultures contain some conidial heads with markedly columnar appearance. The proportion of the globose to the columnar heads appears to vary on different media. The sterigmata may be loosely arranged in the domeshaped vesicles. The conidial chains of columnar heads are rather closely aggregated into compact masses, and in dried cultures compact globular masses of conidia, simulating those of *Gliocladium*, are often found. The heads are at first unpigmented or green, and finally may become brown, or buff. The sterigmata are usually in one series, but the same head may show occasional secondary sterigmata. The conidia may appear to be smooth but the majority are roughened or "echinulated", and evidence of superficial granulation and crystalline concretions is sometimes found. The conidiophores are occasionally very long. Regarding the secondary sterigmata, the writer feels that strictly speaking, those produced by the haematuria parasite are the original sterigmata which have become a conidiophore giving rise to two or three other conidiophores in series. The vesicles of the secondary conidiophores are generally well differentiated. The length of these secondary stalks may vary considerably. In the denser parts of the mycelial network, the conidiophores may form half coil like structures. This tendency to form coiled hyphae has been seen in several cultures, but though they were observed for a considerable time no perithecia were produced. In spite of the repeated attempts made by employing modifications in cultural conditions and nutritive constituents, perithecia were not produced in any of the cultures. On three occasions, however, during the course of about three years they were accidentally produced under circumstances somewhat obscure. Under the influence of a bacterial contaminant a culture repeatedly produced them, though there was no doubt about the identity of the *Aspergillus* concerned. When the contaminant was separated the property of perithecia formation was again lost. In another instance, bread cultures of the *Aspergillus* were sealed and allowed to remain uninterfered with. When the culture was opened numerous perithecia were found, and subcultivation established that the culture was still pure. The perithecia, as already mentioned, were detected in sections of diseased bladder, and also once in haematuria urine. The walls of the perithecia are rather firm and composed of polygonal cells. The asci were oval and provided with grooves. In some perithecia numerous asci were present, while in others they were few,

the majority having been already discharged. Bodies simulating perithecia to some extent but having no clear asci were also seen. These might have been aberrant and sterile perithecia or only young stages. Attempts to crush perithecia with a view to count the ascospores contained in an ascus failed. With the naked eye, the perithecia appeared, on the surface of the colony, as small dots of orange colour. It is said that pathogenic *Aspergilli* only rarely produce perithecia. When the bacterially contaminated culture was planted out on a variety of media, perithecia were produced in all. Prune extract agar produced the most luxuriant perithecia. In regard to the above morphological features, all the twelve cultures gave uniform results.

In regard to the actual measurements of the various structures forming the *Aspergillus* thallus, the following data have been obtained :-

Conidia—size varies considerably, the smallest measured being 3.8 microns and the largest being 7 microns, the commonest varying between 5 and 6.

Conidial heads—the smallest were about 8 and the largest about 50 microns.

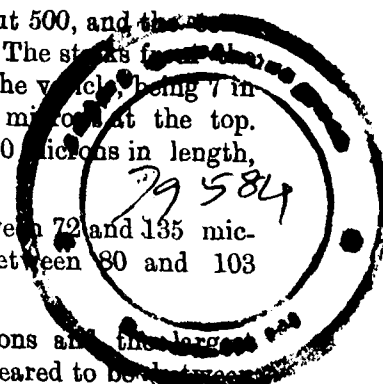
Vesicles—the smallest were 6.6 and the largest 34 microns.

Conidiophores—smallest were 65, largest about 500, and the commonest were between 300 and 350. The stalks from the origin gradually broadened towards the vesicle, being 7 in breadth at the bottom and about 20 microns at the top. The sterigmata were between 7 to 10 microns in length, a few were even between 10 to 12.

Perithecia—measured in their diameter between 72 and 135 microns. Those in sections varied between 80 and 103 microns.

Ascospores—the smallest were about 4 microns and the largest about 16 in length, the majority appeared to be between 6 and 7.

The above measurements were taken from Czapek-Agar cultures, and it was found that considerable variation in size resulted by the use of other media. A dozen measurements were taken in each case. It may be added here that according to Thom and Church in *A. flavus* group heads of certain species show a consistent range of measurements and form, heads of small diameter forming columnar masses, others have globose and large head. Besides in this group, several sizes and shapes of head, as stated by them, are to be found regularly in the same colony. The range of size and shape, however, remain characteristic.



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Ferric Chloride Test.—This test was carried out with all the different haematuria cultures and the blood-red colouration was obtained invariably. Control tests with *A. niger* and *A. repens* from stock cultures were simultaneously carried out and negative results were obtained.

Identification of the Cultures.—A perusal of the many descriptions on Aspergilli which have so far been recorded impresses one with two outstanding facts. Firstly the genus has not been sufficiently studied, and secondly that the naming of the various species has in many cases resulted not so much from any substantial data as from fancy or tradition. The well-known monograph by Thom and Church has no doubt cleared the ground considerably by the broad grouping of all the described Aspergilli, but it cannot be denied that only the foundations of a satisfactory classification has been laid and that not more than a mere building has been started. The biochemical facts which have emerged in connection with some of the groups have not only vindicated the principles of Thom and Church's grouping but have contributed towards a simplified classification, which is amenable to checking by chemical tests. The fact that the colour production is a comparatively stable character in each group notwithstanding the wide range of pigmentation which are produced by different members within the group, allows of even a naked eye identification of some cultures to be made. Sufficient clear evidence exists to show that the pigments produced by the *A. glaucus* series are not produced by any other group. Further, on this character alone, this group is divisible into three sections depending upon whether one or the other of the pigments, flavoglucan, auroglucan, and rubioglucan is produced. Other equally interesting biochemical facts are available in support of the modern classification of this genus. Coming to other groups of this genus, there exists a simple chemical test for identifying members of the groups. *Aflavus-oryzae* and *A. tamaris*, based upon the blood-red colouration which is produced by ferric chloride when added to cultures containing kojic acid, a specific metabolic product of these groups. This ferric chloride reaction indicates the constitutional resemblance of kojic acid to that of a true phenol. The character of pigment production among Aspergilli is thus of diagnostic importance. The other invariable criterion for differentiating the different groups is the morphology of the specific spore-bearing apparatus, the conidiophore.

The identification and classification, however, are not so simple in some cases as might appear from the above remarks. A case in point is well illustrated in the series of cultures now under investigation. It will be remembered that when the same identical cultures were examined by different well-trained mycologists, a considerable difference of opinion regarding the identity of the cultures resulted. The explanation for this

apparent disparity can be considered elsewhere. It is, however, necessary to realise the inherent difficulties and guard against undue reliance being placed upon criteria of value within the groups to extend to the sphere of group separation. It must not be forgotten that Aspergilli are generally highly pleomorphic, and are liable to undergo variations and mutation fairly frequently. Further the invariability of the specific chromogenic and conidiophoric characters of each group is dependent to a great extent upon the use of standard medium. The failure to observe this important precaution has nullified some otherwise very interesting work. Regarding the significance of the gradation in pigmentation seen in stages of growth of Aspergilli, it may be remarked that the green colour of *A. flavus* series is lost on exposure to ammonia vapour but restored again with acetic acid. The intensity of green in the growing colony is a measure of the degree of acid reaction set up, and the fading of the colour to yellow and finally to brown with the advance of the growth indicated the reduction in acidity and increase in alkalinity. The actual colour of a culture thus results from a balancing of these opposing reactions. Thom and Church have rightly pointed out that the wide range of shades of mixtures of yellow and green characterising the *A. flavus-oryzae* group are to be attributed to racial limitation strain by strain in the range of hydrogen ion concentration induced by metabolism. As already pointed out by others and recently emphasised by Smith (1938), when scores and hundreds of strains are examined, the sharp lines of demarcation disappear and instead of a few definite species, there is a perfectly graded series. "For most purposes", therefore, Smith adds, "It is sufficient to be able to identify any particular culture as a member of one of the group series—so-called lumping of strains".

Thom and Church (1926) have provided two short Group Keys, one based on the colour of the heads and the other abbreviated from their larger Synoptical Key. It is necessary now to apply the two Group Keys to the cultures of haematuria Aspergilli. Firstly according to the artificial Key based on colour, the following considerations lead to identification :—

- A. Conidial heads green or yellow green.....B
- BB. Stalks pitted (often rough as seen with low magnification)...H
- H. Stalks pitted (often appearing to be rough or asperulate with low magnifications)...*A. flavus-oryzae* group.

If the other Key is applied, the result is as follows :—

- 1. Heads not clavate.....15
- 15. Stalk walls pitted or rough, sometimes also granular.....220

220. Stalk walls pitted sometimes also granular, often reported as rough or echinulate when observed with low magnifications.....221
221. Colonies some shade of green or yellow green.....290
290. Green yellow-green colonies (rarely with no green colour), without colour bars but with walls marked or grooved with winding pits, often giving an echinulate appearance under low power.....292
292. Sterigmata both simple and double; mostly double in some heads; green colour usually abundant.....*A. flavus* series.

If the grouping is taken down further by the application of the Synoptical Key from 292 on page 250 of Thom and Church's Monograph, the result is as indicated below :—

292. Sterigmata partly simple but commonly in 2 series in the same heads or in different heads of the same colony.....296
296. Sterigmata often partly 1 series, partly 2 series in the same heads, especially the larger heads.....297
297. Sclerotia reported.....305
305. Sclerotia reported.....306

Thom and Church (1926) divide 306 into a, b, and c, thus: (a) Wilhelm adds the observation of "sclerotia minuta nigra tuberosa" to the citation of Brefeld's culture in Rabenhort, Fung. eur. Edit. Nov., Ser. II. 2135..... *A. flavus*.

(b) Colonies fairly green (Kronberg's green on sugar media); stalks usually 400 to 700 microns long, rarely 1,000; conidia pyriform to globose varying from 2 by 3 microns to 5 by 6 or longer.....108 and 3526.

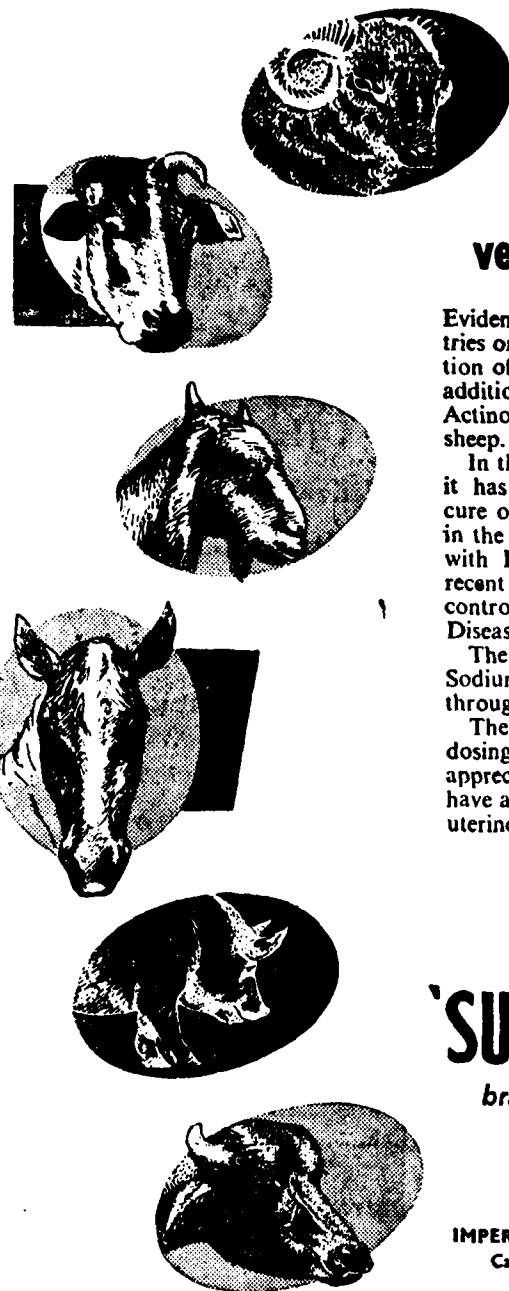
(c) De Bary and Woronin transferred the species to *Eurotium**E. aspergillus flavus*."

The haematuria aspergillus would appear to conform to b. and c. more closely than to a., since the sclerotia examined were not 'nigra'. While the conidial size agrees with that of b. the stalks are somewhat smaller. The colony colour is no doubt Kronberg's green. The only member of the *A. flavus* series which is definitely known to have produced perithecia is c. De Bary and Woronin called the organism *Eurotium aspergillus flavus*, and Thom and Church (1926) appear to have accepted the validity of the species. From the description available in the original publication, which is rather vague, it is not possible to make any detailed comparison. Having studied only twelve cultures, the

writer feels that for the present a more minute classification is not indicated. As emphasised by Smith (1938), it is sufficient to know that the cultures obtained from different haematuria sources belong to the *A. flavus* series.

On pages 307—309 of this treatise, the preliminary reports on the writer's cultures as obtained from different mycologists have been quoted. It will be noticed that the first two cultures, obtained from two different animals were considered to be identical and coming close to *A. tamarii*. The same officer later added that the cultures were to be placed in *A. glaucus* group, between *A. ruber* and *A. scheelei*. The next culture was considered by another mycologist to belong to the *Versicolor* group. When two further cultures were examined by a different officer, the identification was that the two cultures belonged to the *flavus* series of *A. flavus-oryzae* group. Later still Mr. George Smith at London considered that eleven of the writer's cultures belonged to *A. flavus* link. The exact considerations upon which the different mycologists came to apparently dissimilar conclusions are not known, but one may try to explain how this could have happened. Firstly, the ferric chloride test applies to the *flavus-oryzae* and *tamaris* groups, secondly the brown pigmentation and sclerotia formation may be common to both. As Thom and Church have pointed out, aside from colour, *A. tamarii* resembles *A. flavus* in size of colony, habit and appearance. As a differential character, the absence of green colour in the former may be mentioned. Turning to the resemblance of *A. glaucus* to *A. flavus*, it will be remembered that Mangin (1909) suggested that the separation of these groups was difficult. Thom and Church have however placed them at two ends of their group separation. The common bonds between them are the green colour of their conidia, the pitting or echinulation of the conidia and the conidial sizes. Regarding the differentiation, soft-walled quickly ripening perithecia characterise the *glaucus* to the exclusion of sclerotia, while reverse is the case with the *flavus*. Regarding the common bonds of the *versicolor* and the *flavus*, the conidial heads are green or yellow-green in both, and some of the morphological features are common. The character of the stalk, however, differentiates one from the other. Further it is necessary to remember that mutations and variations are a frequent occurrence among Aspergilli. Thom and Church state that the *A. flavus* group is capable of great variation under more or less definable culture conditions. After maintaining a culture of *A. effusus* Tiraboschi they were able to obtain one of the common types of *A. flavus*, and this finding has been confirmed by Burnside. Regarding the classification of individual strains, Funke (1929) makes a very interesting statement, which is typical of others and may be quoted here *in extenso*: "But we must bear in

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THE
Indian Veterinary Journal

(The Journal of the All-India Veterinary Association)

Vol. XXX

MARCH, 1954

No. 5

Editorial

SHORTAGE OF VETERINARY PERSONNEL

Our Editorial in the last issue of this *Journal* on the suggestion to appoint non-veterinarians to be in charge of livestock farms and rural dispensaries with a view to overcome the shortage of veterinary personnel in the country has evoked quite a volume of appreciative communications from the members of the profession all over the country. They have all endorsed what we have written on the subject and they have condemned the fantastic suggestion in no uncertain words. We refer to it only as a pointer to those in power to take note of this popular condemnation and drop the move without any further thought. At the same time, it is becoming more and more clear that the shortage of Veterinary personnel in the country is affecting adversely our national economy. Agriculture is our national industry and it sustains more than 60 per cent of the population. This agricultural industry is entirely dependent upon our livestock, however much one may attempt to mechanise it. Government should, therefore, take a realistic view of things and take immediate and prompt steps to stem the colossal wastage of our livestock resources consequent on paucity of veterinarians. The Welfare State conceived by our administrators will only remain a dream and impossible of achievement if the concept of 'welfare' is not extended to the 'welfare' of the livestock. We have in these pages repeatedly urged that the health of human beings is dependent upon the health and efficiency of our livestock—not only for the building up of the balanced diet of the people but also for improving the fertility of the soil. If this fact is realised by those in power, it will not be difficult for them to build up a corps of veterinarians who will function effectively for developing the livestock resources of the country and for building up the national economy. It was,

mind that the genus *Aspergillus* and especially the group *flavus-oryzae* present great difficulties on this point. I fully agree with Boedijn, when he writes: "While it is not difficult to arrange them under the big groups of this genus classification of the frequently numerous by-forms is practically impossible. Even with the new monograph of Thom and Church, these by-forms cannot satisfactorily be defined "and also" I have relinquished the idea of giving separate names to the races, (though B. admits that: Even slight difference in the races proved to be constant in culture"). It is doubtful whether such a thing would be any good in the case of *Aspergillus* with its numberless forms for every large species. Yet it is not to be denied that it might have been justifiable for a few types in the *Aspergillus flavus-oryzae*."

"There is no reason why any strain, isolated from any spot on earth and from any source of food, should not probably prove to be constantly different from the strains already known. It becomes a hopeless enterprise to give them names. For the present, we have to consider the *flavus-oryzae* group as a collection of hereditary races, the number of which cannot be estimated at this moment. As sexual propagation does not occur in *Aspergillus flavus-oryzae*, we can only imagine that they have come into existence by mutation, as Schiemann suggests." The terms, *Euspergillus flavus* and *Sterigmocystis flavus* have also been previously used for this group.

In view of the statements of Smith (1938) and of Funke (1929), the dominant and invariable associate of haematuria need be called *A. flavus* series, though its relation to *Eurotium aspergillus flavus*, De Bary and Woronin is close. It must be added here that the haematuria cultures have behaved uniformly in biological tests, including optimum temperature of growth and pathogenicity in rabbits.

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we think, Mr. Dalton, when he was the Chancellor of the Exchequer in the United Kingdom, who declared that agriculture was the greatest dollar saving industry in that country. If this were so in a primarily industrial country like the United Kingdom, how much more so should it with a primarily agricultural country like ours? And, therefore, almost the first thing that the Government should do is, as has been suggested already in our last issue, to make the veterinary profession attractive to the young men with high basic training to enable them to take to the study of Veterinary science. It is not often recognised that the course of Veterinary studies is a long and arduous one and is highly expensive. By the time a student completes the preliminary qualifying course of study in the Science Colleges and then the professional studies in the Veterinary Colleges, a good deal of money is expended and, unless there is an adequate return for this amount, very few will think of investing this amount in that education. With the low monetary value of our livestock, it is a practical impossibility for any qualified veterinary surgeon to take up private practice and attempt to make a living, not to speak of a *decent* living. Consequently, the Government is the only agency to utilise his services and this is bound to be still more so when once the concept of Welfare State is developed to its logical conclusion. It would become the duty of the State to look after the health and welfare of our animals in much the same way as they undertake the health and welfare of human beings. As such the employment of qualified veterinarians to look after the livestock of the farm is of paramount necessity and therefore the Government should make it worth while for the young aspirants to take to the study of veterinary science. This is a matter which requires immediate and prompt attention at the hands of the Government because we have lost already quite a bit of ground from past apathy in the matter. The new academic year is about to commence shortly and, therefore, the Governments should immediately announce attractive terms to the members of the Veterinary profession so that when the Veterinary Colleges are reopened they will have sufficient number of right type of candidates for admissions. Failure to do so, will tantamount to ignoring the needs of the 'common man' of the country and equally the economy of the country. Simultaneously, the Veterinary

Colleges must be up-graded in their personnel and equipment to fall in line with the fast growing advances in veterinary science. Why this elementary step to meet this shortage of Veterinary personnel is not taken into account previously is beyond our comprehension. First things must come first. Let us hope that the Government would take this first step immediately and avoid any further loss of time.

A suggestion that has been made now and again by different people to overcome the shortage of veterinary personnel is that retired persons must be re-employed and their services utilised for the benefit of the country. We are afraid we cannot accept this as a general policy to be adopted in toto; but every case can be viewed on its own merits and persons with high equipments and vast experience can certainly be usefully employed to maintain a continuity and standard in certain situations. Such appointments need not interfere with normal administrative promotions of men below. It is always a wise policy to bank on the accumulated experiences of highly qualified officers in special posts whose replacement may not be easily possible. As we said before, such cases are not normal and they can be treated as extraordinary cases deserving of special consideration. Such appointments may also be helpful in training up a good team of workers in different branches of Veterinary Science. The retired men may also be employed on a temporary basis on all Extension schemes, Community Projects, etc., without affecting the interests of the men in service. No one, we dare say, is so bereft of love of his country as to say that these Extension schemes and Community Projects should not begin functioning until the country is able to produce every year all the required number of veterinary men for all schemes. What is required now is more finance to provide greater facilities by way of accommodation, equipment and staff at all Veterinary Colleges to start producing more and more Veterinary men. A beginning has to be made if the country is not to face a crisis in the near future in this field of administration. We fervently hope that the Government would give serious thought to this subject and see that adequate steps are taken early to overcome the shortage of personnel at a very early date.

Notes and News

The General Secretary of the All-India Veterinary Association writes as follows :—

The members of the profession will remember that the All-India Veterinary Association was represented by two Corresponding Members on the Committee appointed by the Pharmaceutical Society of Great Britain, which had undertaken the publication of the British Veterinary Codex. The work is now over and the British Veterinary Codex was published in the last week of December 1953. In reporting about this publication, the Secretary of the British Veterinary Codex Committee writes as follows :—

" May I take this opportunity of expressing once more our grateful thanks to you and your Association for the interest you have shown throughout the compilation of this Codex and for your splendid co-operation. We have all been impressed by the expert nature of the comments received from the Indian Corresponding Members of the Committee and by the thoroughness with which they have examined the many draft monographs we have sent to them. We hope that the British Veterinary Codex, to which your representatives have made so valuable a contribution, will prove a worthy tribute to the work of all concerned with its compilation "

The Corresponding Members of the Association were Mr. M. R. Subramanya Sastry and Sardar Balwant Singh. On the unfortunate premature demise of Mr. M. R. Subramanya Sastry, Sri C. K. Velayudhan Nair, Lecturer in Pharmacology of the Madras Veterinary College was elected as the Corresponding Member.

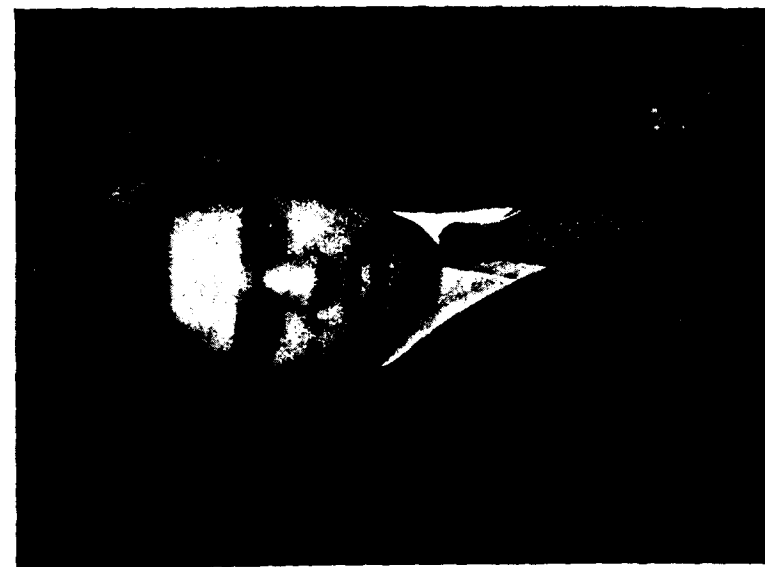
[In publishing the above communication we have great pleasure in publishing also the photos of the Corresponding Members. *Ed. I.V.J.*]

Personal

M. Habib Khan, M.R.C.V.S., Principal, Osmania University College of Veterinary Science and Animal Husbandry, has left for Kansas State under a Fulbright Scholarship. He is likely to be away for about a year. He proposes to study the latest methods of semen preservation and other relative problems.

G. A. Shivnani, G.B.V.C., M.S.—We are glad to learn that Mr. G. A. Shivnani who went abroad in 1952 has taken his M. S. degree in Veterinary Parasitology at Kansas State College on 23rd January 1954. He has further programmed to stay on in the States and take the Ph.D., degree in the same field.

REPRESENTATIVES OF THE ALL-INDIA VETERINARY ASSOCIATION
Who served as Corresponding Members in compiling the British Veterinary Codex.



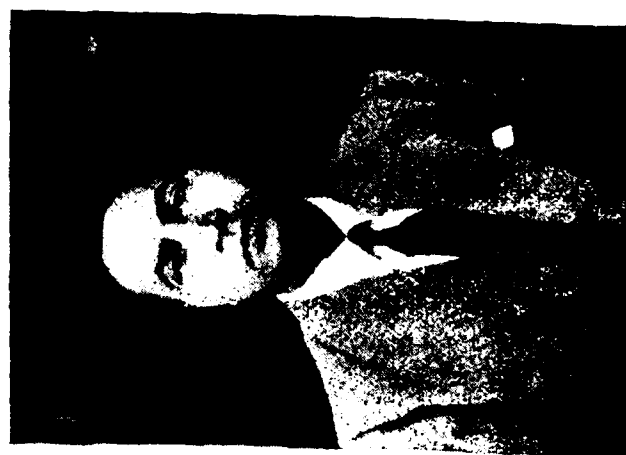
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Sardar Balwant Singh, G.P.V.C.,
Head of Biological Products Division,
Indian Veterinary Research Institute, Mukteswar.



G. A. Shrivani, G.B.V.C., M.S. (Kansas)
who has taken his M.S. degree in January 1954.



Habib Khan, M.R.C.V.S.,
Principal, Osmania University College of
Veterinary Science & Animal Husbandry, Hyderabad-Dn.,
who has left for U.S.A., under a Fulbright Scholarship.

Veterinary and Animal Husbandry Department, Hyderabad-Dn. :—

Commenting on the Budget demand for the Veterinary and Animal Husbandry Department of Hyderabad, the *Deccan Chronicle* has the following from its Lobby Correspondent in its issue of March 3, 1954 :—

"The Veterinary Department which has shown in the past two years remarkable and spectacular progress under its new Director Dr. Shetty is in need of more money if Animal Husbandry which is so necessary for community development is to keep pace with national development plans. Even though this year some 3½ lakhs more have been provided for new schemes and totals to Rs. 23½ lakhs the percentage of expenditure is less than 1% of the State revenues. Treatment and care of animals and poultry is still comparatively negligible in the State and research in animal diseases and allied activities is almost in its infancy. Glancing over the 16 pages of provisions most of the amount is ear-marked for Veterinary education and research and breeding operations. Researches in Dairying, breeding and feeding of animals, in animal and poultry diseases, manufacture of vaccines are among the new activities. About 1 lakh has been increased under hospitals and dispensaries which is a move in the right direction."

We are glad of this appreciative comments on an important Department like the Veterinary Department and we do hope the Government will go on giving more and more money for its expansion and development.

Obituary

We regret to record the death of **Mr. A. Moberly, G.M.V.C.**, on the 15th March 1954. Born on the 10th August 1899, and graduated from the Madras Veterinary College in 1923, Mr. A. Moberly was a Veterinary Assistant Surgeon in the Madras Animal Husbandry Department from 1927 to 1953 after which he went on leave preparatory to retirement. He was a keen student of veterinary science and took a lively interest in his profession. He leaves behind a widow and two sons to whom our sympathies go.

We also regret to record the death of **Mr. C. Madhavan Nambiar, G.M.V.C.**, a Retired District Veterinary Officer of Madras State on the 19th March 1954 after a short period of illness. He was aged 59. Mr. Nambiar is well known to the profession in Madras State as a quiet and unostentatious officer, very keen in his work. He leaves behind his wife, three sons and five daughters to whom we extend our sympathies.

Clinical Articles

TREATMENT OF ASCARIDIA GALLI IN POULTRY WITH (A) CARBON TETRACHLORIDE AND (B) OIL OF CHENOPODIUM

By

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Veterinary Investigation Officer,
Livestock Research Station, Mathura, U.P.

Ascaridia galli is by far the most important helminth parasite of fowls in India. There are probably few poultry farms and rural centres which are free from this large round worm of the intestines, and heavy infestations accompanied by considerable mortality are not infrequently encountered, particularly among chickens. Observations made in Uttar Pradesh show that birds probably pick up infection largely during the spring and early summer, when they are young and when the atmospheric temperature apparently provides optimum conditions for development of embryos inside the eggs. Moreover, nutritional deficiencies are most likely to operate during this period, particularly that of vitamin A which has been strongly suspected by some workers to predispose to infestation with this worm and also to lower the birds' resistance to its pathogenic effects. Further, it is during this season that fresh chicken stock of the most susceptible age is present on poultry farms. Adult birds, as a rule, are resistant and *Ascaridia* infestations in them are known to be either light or absent.

Certain drugs are recommended in literature for treating *Ascaridia* infested birds, but one seldom hears of proper treatment being actually undertaken. This note describes some therapeutic trials which were undertaken in Uttar Pradesh on two organized farms which had suffered much ill-health and mortality in poultry due to this worm year after year, mainly with a view to stressing the great value of at least one fairly cheap drug, viz., carbon tetrachloride, for killing this worm. It is hoped poultry farmers will undertake similar treatment wherever indicated and thereby prevent the economic loss caused by the parasite.

Experiments and Results

Preliminary trials.—The frequent occurrence of *Ascaridia galli*, often in large numbers, in the cadavers of birds dying of various causes

on an organized poultry farm was noted and it was decided to undertake proper treatment. The two drugs selected for the purpose were carbon tetrachloride and oil of chenopodium on account of some good reports in literature. After a preliminary trial with each drug in the birds of two badly infested pens the treatment was extended to the remaining pens.

The two pens included in the initial test contained 30 and 25 chickens, respectively, 3 to 3½ months in age. For three consecutive mornings prior to treatment, droppings were collected in bulk from the two pens separately. The droppings from each pen were mixed thoroughly and randomly selected specimens were examined microscopically for *Ascaridia* eggs by the centrifuge floatation method using saturated salt solution and examining the material from the deposit as well as from the top of the supernatant. Several eggs were found present in these faecal specimens at each daily examination. Carbon tetrachloride was then administered to the chickens of one pen and oil of chenopodium to those of the other, after they had been starved for about 18 hours, water being allowed. Carbon tetrachloride was administered in capsules by mouth at a dose rate of 0.75 cc. per lb. body weight. Oil of chenopodium was given thoroughly mixed in damp mash at a dose rate of one drachm for each group of 12 birds. The chickens were not starved after treatment, nor was any purgative administered. Starting from the seventh day after treatment, the faecal specimens were again collected and examined as before on three successive days, but on no occasion could any *Ascaridia* egg be detected.

Subsequent trial.—After this encouraging preliminary trial, the faecal examination and treatment were extended to the remaining 15 pens which housed a total of 722 more chickens. From the results set out in Table 1 it will be seen that the treatment adopted gave highly successful results. As before, the results were judged by comparing the incidence of *Ascaridia* eggs in bulk faecal samples of the different pens on three successive days preceding treatment and for a similar period commencing from the 12th day after treatment. *Ascaridia* eggs were found in varying numbers in bulk faecal specimens of all the pens on one or more occasions during the three days before treatment, but after treatment they were found in small numbers only in 3 of the 15 pens on one occasion in each case.

These results were further checked by comparing the occurrence of the worm in the intestines of all available cadavers for 30 days before treatment and for a similar period after treatment. The results are set

TABLE I

Ascaridia eggs in bulk faeces before and after treatment.

Pen.	Number of birds	Age group	<i>Ascaridia</i> eggs in bulk faeces on June 17, 18 and 19	Drugs used on 22nd June 1952 for treatment	<i>Ascaridia</i> eggs in bulk faeces on July 2, 3, 4
1	74	5-5½ months	Present	Ol. chenopod	Absent
2	63	" "	do.	do.	do.
3	41	4 "	do.	do.	Present
4	35	4 "	do.	do.	Absent
5	24	5½-6 "	do.	Carb. tetrach	do.
6	45	5-6 "	do.	do.	do.
7	39	5-6 "	do.	do.	do.
8	41	3½-4 "	do.	do.	do.
9	45	do. "	do.	do.	do.
10	30	2½-3 "	do.	do.	Present
11	50	8-10 weeks	do.	do.	Absent
12	38	do. "	do.	do.	do.
13	38	do. "	do.	do.	do.
14	83	6-8 "	do.	do.	Present
15	76	3-4 "	do.	do.	Absent

out in Table II. It will be seen that while *Ascaridia* worms were present in 16 or 50 per cent, of the 32 cadavers examined during the 30 days before treatment, they were found only in 3 or 5.77 per cent, during the 30 days after treatment, adult worms being present only in 1 or 1.99 per cent, of the cadavers. *Ascaridia*, however, started reappearing in fair numbers soon after one month post-treatment period, and treatment with carbon tetrachloride was adopted again with good results. In view of the pens having become heavily contaminated through infestation persisting for several years in the past, the entire poultry stock is now being dosed with carbon tetrachloride at regular intervals in order to completely eradicate the infestation from the farm.

The somewhat larger number of deaths during the 30 days following treatment was mainly due to coccidiosis, which often flares up after elimination of *Ascaridia*.

TABLE II

Incidence of Ascaridia in cadavers before and after treatment.

Before Treatment				After Treatment			
Days before treatment	Cadavers examined	<i>Ascaridia</i> present in	Number of <i>Ascaridia</i>	Days after treatment	Cadavers examined	<i>Ascaridia</i> present in	Number of <i>Ascaridia</i>
30	2	1	10	1	2	1	3 (immature)
29	1	1	11	2	1	nil	—
27	1	nil	—	3	2	nil	—
25	3	2	10, 37	10	7	nil	—
24	2	nil	—	11	4	nil	—
23	1	1	2	15	8	1	25 (immature)
22	1	1	90	16	2	nil	—
20	2	nil	—	17	4	nil	—
19	4	nil	—	18	2	nil	—
18	1	nil	—	19	2	nil	—
17	3	3	20, 27, 33	22	4	nil	—
16	1	1	30	23	1	nil	—
10	1	1	1	24	2	1	50
9	1	1	12	26	9	nil	—
8	2	2	10, 25	27	2	nil	—
6	1	nil	—	28	nil	—	—
4	3	2	1, 55	29	nil	—	—
1	2	nil	—	30	nil	—	—

Administration of Carbon Tetrachloride.—The very successful results obtained on this farm were made use of by applying the treatment on another poultry farm which maintained over 5,000 birds and where *Ascaridia* infestation was noted to be heavy. Repeated dosing with carbon tetrachloride on this second farm also has been of great help in controlling the infestation. Here, carbon tetrachloride is being administered directly into the crop through the mouth by means of a fine-bore rubber tubing attached to a syringe in place of the needle. The technique

is very simple and can be mastered in no time, but some care has to be taken to see that the rubber tubing is inserted into the crop and not into the trachea. This method of administration reduces the cost of treatment considerably as it avoids the unnecessary expense due to capsules, which are more costly than the drug itself.

The writer has recently tried administering carbon tetrachloride by injection into the crop through the overlying skin. This method appears to be the simplest of all for administering anthelmintics (and other drugs for alimentary medication) to poultry, but it can be safely employed only when the crop is partly or completely full. Carbon tetrachloride was injected into the crops of 10 cockerels, in doses up to 4 c.c. with a fine-bore hypodermic needle attached to a 5 cc. record syringe, a few minutes after barley grain feed. The assistant who held the bird also raised the crop by pressing the fingers below it. There was no ill effects either immediately or subsequently following the treatment. This method of administration is so easy and simple that further trials seem warranted.

Toxic effects following treatment with carbon tetrachloride in these flocks have been observed occasionally, but these are mostly confined to very weak birds some of which may die. There is no other hazard to the administration of carbon tetrachloride in poultry, provided the doses employed are not unusually high except some possible loss in egg production in laying birds.

Oil of chenopodium is much more costly than carbon tetrachloride. Moreover, it was found rather difficult to administer satisfactorily because birds did not relish feed medicated with this oil. Further, the few comparative trials have not shown oil of chenopodium to be superior to carbon tetrachloride, which is highly efficacious, fairly safe and cheap, and can be administered easily and without much labour to a large number of birds within a short time.

Recommendations

It is, therefore, recommended that carbon tetrachloride should be administered to the entire flock, directly into the crop, through the mouth with the help of a fine-bore rubber tubing attached to a syringe, wherever *Ascaridia* infestation is known to be present. The recommended dose rate is 0.75 to 1 c.c. per lb. body weight, up to a maximum of 2 cc. or so per bird, though double these dose rates can usually be employed without toxic effects. Very weak birds may be left for treatment on some subsequent date. As immature worms are likely to resist treatment, it is advisable to repeat the treatment after about 4 weeks and again for some months at intervals of 4 to 6 weeks,

as required, in order to destroy any subsequently picked up infection. In the case of badly contaminated grounds, it may be necessary to continue the treatment for many months for completely eliminating the infection from the farm. Alternatively, the pens may be shifted to new ground after all the birds have been treated about twice at one-month interval. Similar treatment should be undertaken after shifting to new grounds. Another measure recommended in literature is the rearing of young chickens, till they attain the resistant age of about 4 months, on clean grounds known to be free from contamination with *Ascaridia* eggs. These eggs can persist alive for several months on wet soil. Consequently, it is important that poultry-raising grounds should be well drained and maintained perfectly dry. Dampness resulting from such sources as water fountains etc., should be avoided, the litter removed daily, and the pens cleaned thoroughly at least once a week. Boiling water is the simplest and one of the best agents for disinfecting metallic and wooden fittings. Feeding of chickens should always receive special care. The basal cereal ration should be rich in proteins and it should be supplemented by such nutritive and protective feeds as meat scrap, soybean meal, skim-milk and greens. When necessary, shark liver-oil may be administered daily.

Acknowledgments

The writer is indebted to Shri R. N. Delilah and Shri H. A. Quraishi for assistance in dosing the birds, to Shri D. K. Murty and Shri S. N. Bakshi for post-mortem records, and to Shri C. S. Mathur for assistance in laboratory examinations. He is also grateful to Shri P. G. Pande, Principal, U. P. College of Veterinary Science and Animal Husbandry, Mathura, for his kind interest in this work and the encouragement and advice received from him.

A SHORT NOTE ON AMOEBIC DYSENTERY IN A LIONESS AT BOMBAY

By

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and

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Asstt. Supdt. Victoria Gardens, Bombay.

Amoebic dysentery due to *Entamoeba histolytica* in man is of common occurrence but its incidence in animals is very low. Much work has been done on the morphology, behaviour and chemotherapy of

amoebiasis in human beings. There are a few scattered reports of the occurrence of this disease in animals. Ware (1916) reported a natural infection, probably due to *Entamoeba histolytica*, in dogs showing pathogenic symptoms. Musgrave and Cligg (1904) observed natural infections in monkeys in Philippines. Many names have been proposed to the different species of amoebae affecting monkeys and it is really an open question whether the different species mentioned by different authors are synonyms of *Entamoeba histolytica* and *E. coli*. Pigs and probably other domestic animals harbour *Entamoeba histolytica* without showing disease symptoms. Hoare (1940) found amoebae in the faeces of goats which were morphologically identical with *E. deblickei* of the pig and were non-pathogenic to goats. It is significant, however, that there is not even a single report of lions acquiring amoebic infections and showing acute dysenteric conditions, although carnivorous animals like the dogs are susceptible to the infection. And, therefore, it is interesting to record a natural infection in a lioness belonging to the Zoological Gardens, Bombay. The amoebae found in the lioness were indistinguishable from *Entamoeba histolytica*.

Morphology.—The vegetative forms when examined fresh in normal saline showed active pseudopodial movement. The activity diminished within three to four hours of time when most of the vegetative forms turned into cysts. The colour of the living amoeba was yellowish red, the redness depending upon the number of R.B.Cs. present in the food vacuoles. The vegetative form in fixed smears measured 18–22 by 35–42 microns. The nucleus when stained with iron-haematoxylin appeared quite distinct. The food vacuoles were numerous, filled with R.B.Cs., tissue cell fragments and bacteria. According to Hoare (1952), the presence of bacteria inside the food vacuoles is indicative of the commensal phase in the life-history of *Entamoeba histolytica*. The cysts measured 14–18 microns.

Symptoms.—Symptoms observed were alternate constipation and diarrhoea, leading to loss of appetite. As the disease advanced the faeces became more liquid, with blood and mucous. It was foul smelling and dark in consistancy. The animal evinced severe colicky pains and restlessness and was greatly emaciated.

Probable source of infection.—The probable source of infection was thought to be from monkeys and bullocks, from the same Zoological Gardens, which were seen suffering from amoebic dysentery at the same time. Infection might have been acquired by ingesting the cysts along with the infected food and drinking water. It may be mentioned that the Superintendent and some of the attendants looking after the lioness were carriers of *Entamoeba histolytica* as they had previously suffered from acute *Entamoebiasis* and were undergoing treatment for the same.

Treatment.—*Sulphaguanidine* 2 dozen tablets, *Enteroquinol* 1 dozen tablets, with one teaspoonful of Sodium bicarbonate were given every day for one week. Parasitological examination of the faeces revealed the presence of vegetative forms of *E. histolytica* along with hook worm eggs. 2 c.c. *Nema capsule* was given orally and repeated once again after 3 weeks. Side by side *Emetin hydrochloride* at the dosage of 2 cc. intramuscularly was tried but had to be given up as considerable amount of difficulty and risk was experienced in controlling the lioness. Instead of *Emetin hydrochloride* by intramuscular injection, *Emetin bismuth iodide*, at the rate of 5 tablets, was given along with the food for 7 days and the lioness got alright within one month. During convalescent period, the diet was restricted to pepsin powder, calcium gluconate, soup, minced meat and liver pieces partly heated and mixed with a few ounces of raw blood. This was given for one month until the lioness regained its original strength and became normal. Subsequent faecal examinations of the lioness carried out once every month revealed cysts of *Entamoeba histolytica* in small numbers. The lioness had evidently become a carrier and the duration of the carrier stage cannot be ascertained with definiteness at present.

Summary

A record of amoebic dysentery due to *Entamoeba histolytica* in a lioness belonging to Zoological Gardens, Bombay, has been made for the first time. The animal evinced acute dysenteric symptoms and showed clinical recovery after suitable treatment.

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LUMBAR PARALYSIS IN A SHE-GOAT

BY

J. SEETHARAMAN, G.M.V.C.,

AND

H. NARASIMHA SHETTY, B.V.Sc.,

Veterinary Assistant Surgeons, Veterinary Hospital, Mangalore.

Subject.—A local-bred, brown she-goat, case No. 2504 I. P., aged 6 years, in milk with two kids aged about 6 months was admitted into the hospital on 16—2—'54 with the following history and symptoms. The goat was said to be a good milker and developed paraplegia 4 days back, almost suddenly, with loss of normal locomotion. The owner reported to us that he had lost two goats previously from a similar complaint. When we examined the animal on 16—2—1954 the animal was unable to walk and, when lifted up and made to walk, was moving with its fetlock flexed and intermittent dragging of the hind limbs. It could hardly move a few yards without support and used to sit on its haunches when the support was withdrawn. There was a slight response to pin-prick. It appeared that the animal had no control at the lumbar region, which was tender to manipulation. The animal was feeding normally and presented almost a normal appearance otherwise. Urine and faeces appeared normal. We examined the blood smear and faeces with negative results. Temp. 102.6°F, mucous membrane of the eye, brick red.

Treatment on 19—2—1954. 30 c.c. of M.F.C. solution (M. & B.) and 40 c.c. of 20% Magnesium Sulphate solution were separately injected subcutaneously at the region of neck and the following medicine was also administered orally twice a day.

Mag carb	...	5 ii
Calcii lactas	...	5 ii
Sodii bicarb	...	3 iii
Treacle	...	Q.S.

Also massaged the lumbar region and the hind legs with Linimentum terebinthinae.

17—2—1954. There was not much appreciable improvement in its locomotion. Temp. 102.4°F.

Injected intravenously 20 c.c. of M.F.C. solution into the ear vein and injected subcutaneously 40 c.c. of Magnesium sulphate solution 20% at the region of the neck. Repeated electuary and massaging with liniment.

18—2—1954. Temp. 102.6°F.

There was a slight appreciable improvement in its locomotion and the animal was able to move with lesser support for some distance, but continued to sit on its haunches or lie down when support was withdrawn. Repeated injections of M.F.C. solution and Magnesium sulphate 20% solution by intravenous and subcutaneous routes as on 17—2—1954. Repeated electuary and massaging with liniment.

19—2—1954. Temp. 102.4°F.

There was much improvement and the animal was able to move with lesser support and greater ease. The flexing of the fetlocks and dragging of the hind limbs were improved. The animal was able to bear some weight on its hind legs. Repeated Intravenous injection of 25 c.c. M.F.C. solution and 40 c.c. of Magnesium sulphate solution subcutaneously. Repeated also the electuary and massaging with liniment.

20—2—1954. Temp. 102.4°F.

There was a still further improvement, and the animal was able to move some distance with a tottering gait. Repeated the treatment of the previous day.

21—2—1954. Temp. 102.4°F.

Very appreciable improvement in its locomotion; was able to walk without support, though with tottering gait still. Repeated treatment.

22—2—1954. Temp. 102°F.

The animal was able to stand squarely on four legs and was able to walk freely showing only a slight weakness of left hind limb. Repeated treatment.

23—2—1954. Temp. 102.6°F.

Animal was walking almost normally and was able to trot. Injections were stopped. Repeated only electuary and massaging with liniment.

24—2—1954. Temp. 102.4°F.

The goat was walking normally. Repeated only electuary and massaging with liniment.

25—2—1954. Animal was normal in its locomotion. Repeated electuary and massaging with liniment.

26—2—1954. Goat was quite normal and discharged from the Hospital.

Discussion

The line of treatment with magnesium sulphate and M.F.C. solution was tried by us in this case, since these injections had given good results in cases of Bovine paralysis in South Kanara District. We were much encouraged by the result in this case and we would record further trials in goats with the same line of treatment.

PARAPLEGIA IN A DOG

By

J. SEETHARAMAN, G.M.V.C.,

AND

K. S. PRABAKER RAO, B.V.Sc.,

Veterinary Assistant Surgeons, Veterinary Hospital, Mangalore.

Subject.—A black dachshund dog, aged about 2 years old, was examined by the authors on 16—12—1953 with the following symptoms: Complete paralysis of both hind limbs with loss of locomotion; the dog was sitting on its haunches and was moving about by dragging the hind limbs; there was involuntary micturition and defecation; there was no reaction to pin-pricks in the quarters and both hinds; feeding was normal and the dog presented almost a normal appearance except a slight depressed expression. It had been under treatment for a week before our examination and had received 2 injections of Vitamin B Complex with no appreciable change.

Treatment and observations.

- 16—12—1953. Injected intramuscularly 2 c.c. of Vitam B Complex TCF and 5 c.c. of Magnesium sulphate 20% solution intramuscularly at the middle third of the neck, (left side B Complex and right side Magnesium sulphate). The idea in giving Magnesium combined with Vitamin B Complex is discussed by us below. Rubbed linimentum saponis on the lumbar region and hind limbs. Advised fomentation. A diet comprising of milk, bread and eggs daily and half boiled liver twice a week was recommended.
- 17—12—1953. 2 c.c. of B Complex intramuscularly and Magnesium Sulphate 20% 5 c.c. intramuscularly at the neck. Diet and treatment same as on 16—12—1953. There was

not much of appreciable improvement except that the dog showed slight reflex to pin pricks.

- 19—12—1953. Rubbed linimentum saponis only.
- 20—12—1953. Repeated injections of B Complex and Magnesium sulphate as on 16—12—53. The dog showed a slight improvement and a slight tendency to bear weight on the hind limbs. The involuntary passing of urine and faeces were not very marked.
- 21—12—1953. Linimentum saponis only.
- 22—12—1953. Repeated B Complex and Magnesium sulphate injections. When the dog was lifted and made to stand, it was able to stand on hind limbs with a slight shivering.
- 24—12—1953. Repeated B Complex only. The dog was able to move about on all four feet with intermittent dragging of hind limbs. Defecation and micturition normal; reflex to pin pricks was becoming more evident.
- 26—12—1953. The dog was walking well with a slight tottering at times. Gave Vitamin B Complex only.
- 28—12—1953. The improvement was maintained and there was only slight abduction of left hind limb. Repeated B Complex injection. The owner of the dog was advised to massage legs and give fomentation. Also advised to continue the feeding with half boiled liver.

We examined the dog on 1—1—1954 and found it to be quite normal in its locomotion. The owner was advised to feed the subject on on a nutritious balanced diet.

Discussion.—Magnesium being an important mineral in animal metabolism with a well-known role in the control of nervous system impelled us to use it along with Vitamin B Complex. Moreover, the success of Magnesium treatment in cases of Bovine paraplegia also induced us to try it with B Complex in canines also. Total B Complex injection given, 2 c.c. each time, 7, and Magnesium Sulphate 20%, 5 c.c. each time, 4.

College News

MADRAS VETERINARY COLLEGE GOLDEN JUBILEE

Further to the notification in the last number of this *Journal*, the members of the profession and *alumni* of this college are informed that it has been decided to mark the Golden Jubilee of this College with a three-day celebration in August this year. A cattle and poultry show and an exhibition will also be held at the same time and the College Day and Sports will form an integral part of the event. A Jubilee Commemoration Volume of the College magazine is also expected to be published. It is hoped that all old students of the College will be able to attend the celebrations and renew contacts with their *alma mater*.

As a lavish programme of meetings and entertainments is envisaged, funds are necessary. Old students, well-wishers of the College and others interested in the profession, who desire to contribute may send their donations, in instalments if desired, to the undersigned, by cheque or Postal order. All cheques and Postal Orders should be drawn in favour of the Principal, Madras Veterinary College, by designation.

I. M. AZIZUDDIN,
Principal
and
President, Madras Veterinary College Association.

PUNJAB VETERINARY COLLEGE, HISSAR

Final B.V.Sc. Examination Results-1952.

The following candidates of the Punjab Veterinary College, Hissar, already holding the L.V.P. Diploma of the Lahore Veterinary College, passed the Final B.V.Sc. Examination of the Punjab University, Solon, held in February and May 1952.

February '52.—Charanjit Lal Vohra.

May, '52

Capt. Taj Singh	Raj Pal Vig
Capt. Dewan Singh	Madan Lal Kalra
Gopal Singh	Prem Dutt Malik
Karam Chand	Kali Charan Kishore
Capt. Mohinder Singh	Jagdish Chander
Capt. Om Parkash Anand	Mahesh Chander Tugnait
Capt. Anand Sarup Thapar	Capt Mehar Singh
Major Gurcharan Das	

PUNJAB VETERINARY COLLEGE RESULTS

425

Final B.V.Sc. Examination results, February 1952.

Raghubir Singh Rana	Amolak Ram Puri
Raghubir Singh Bagga	

Final B.V.Sc. Examination results, May 1952.

Kundan Singh	Lacchman Singh
Suchindar Nath Kaura	Rajinder Paul Singh Bhalla
Surjit Singh Chopra	Om Parkash Narang
Karam Vir Singh	Som Nath Thapar
Jai Singh Sharma. Oct '52.—	

Final B.V.Sc. Examination Results, January 1953.

Dharam Vir	Roshan Lall
Mohd Akbar Malik	Harpal Singh
Sardar Sing Dang	

The following candidates of the Punjab Veterinary College, Hissar, already holding the L.V.P. Diploma of the Lahore Veterinary College, passed the Annual Final B.V.Sc. Examination of the Punjab University, Solon, held in May 1953, under Special Regulations :—

Maj. Hazura Singh, I.R.V.F.C.	Capt. Bal Rama Bakshi, I.V.R.F.C.
Nauratan Perkash Kaushik	Raj Krishan Mehta
Manohar Lall Sharma	Subhan Chander Datt
Maj. Sardari Lall Babbar, I.R.V.F.C.	Sohan Lall Syal
Capt. Ranbir Singh, I.R.V.F.C.	Sant Singh Sethi
Capt. Amar Nath, I.R.V.F.C.	Brahma Nand Kapoor
Capt. William Henry Deane, I.R.V.F.C.	Kartar Singh

The following candidates of the Punjab Veterinary College, Hissar, passed the Bi-annual Final B.V.Sc. Examination of the Punjab University, Solon, held in September 1953 :—

Narinder Singh	Khushdarshan Singh Thind
Harmohinder Singh	Onkar Singh Atwal

Association News

ALL INDIA VETERINARY ASSOCIATION, HYDERABAD BRANCH

*Proceedings of the 1st General Body Meeting held at the
Directorate of Veterinary Department on 20-2-1954.*

The first general body meeting of the All-India Veterinary Association, Hyderabad Branch, was held at the Directorate of Veterinary Department on Saturday, the 20th February 1954, for the election of office bearers for the year 1954 and also to choose a representative to serve on the Interim Veterinary Council. Dr. K. S. Shetty, Director, Veterinary Department, was in the chair. The following were present :

Dr. K. S. Shetty	Dr. Parmanand
„ K. Ragavachari	„ K. S. Reddy,
„ Bavanishanker	„ Y. B. Rajah
„ B. S. P. Rao	„ P. Seetharamaswamy
„ M. R. Reddy	„ Habeeb Ali Khan
„ Singaravelu	„ Madhavakrishnareddy,
„ C. H. Naidu	„ P. Venkatswami
„ C. G. Bhaskaran	„ V. D. Jatkar
„ P. Madhusudhan	„ Raja Rao
„ Muniruddin Khan	„ Shivramkrishnan
„ Ahmaduddin Mohd	„ B. Madhava Rao
„ M. Kishan Rao	

In initiating the proceedings, Dr. Shetty thanked the Government for having accorded sanction to the formation of the Association and appealed to the members to help the Government in all possible ways by dedicating themselves to the service and not to make this Association as a medium to ventilate their grievances and embarrass the Government. He then explaining the aims and the usefulness of forming such an Association, appealed to the members to be duty-conscious and do all their best to uphold the honour and prestige of their noble profession.

He then made a personal appeal to the other Graduates whether retired or in service who have not found it still convenient to join the Association to enroll themselves as members, strengthen its hands and guide it in all its activities, so that they, as members of the Association, might prove themselves to be of service to the dumb creatures and thus help the peasant in improving his economic condition. In emphasising the significant role the veterinarian could play in the achievement of the

above objective he dwelt on the inherent potentialities of the cattle industry of the State and how best they could be canalised towards the betterment of the national prosperity.

The meeting then resolved to form as early as possible a State Veterinary Council on the analogy of the ones existing in other States in order to get all the qualified Veterinary Surgeons registered under the Act.

Election of office bearers was then taken up. In virtue of the recommendations in the constitution that the Director to be ex-officio President and, at the unanimous request of the members present, Dr. Shetty, Director, accepted the Presidentship of the association.

The following were elected as Managing Committee members :

1. Shri Ragavachari	6. Shri T. C. H. Naidu
2. „ Sivramkrishnan	7. „ P. Seetharamaswamy
3. „ B. S. P. Rao	8. „ B. Madhava Rao
4. „ V. D. Jatkar	9. „ Ahmaduddin Mohd.
5. „ Parmanand	10. „ Mahboob Ali Khan

Shri Madhava Rao, Shri Ahmaduddin Mohd and Shri Mahboob Ali Khan were elected unanimously as Secretary, Joint Secretary and Treasurer respectively.

Dr. K. S. Shetty and B. Madhava Rao were elected as representatives of Government and Association respectively to serve on the Interim Veterinary Council.

With a vote of thanks by Shri Madhava Rao the meeting terminated.

B. MADHAVA RAO,
Secretary.

Correspondence

[The views and opinions expressed in correspondence are those of the writers themselves and do not necessarily imply endorsement by the Ed. I.V.J.]

“Use of ‘Lorexane’ Cream in the Treatment and Prevention of Myiasis in Livestock”

Sir,

With reference to the note at the foot of page 287 *Indian Veterinary Journal* XXX 4, 28 7—8 (January, 1954), we would point out that ‘Lorexane’ Antiseptic Cream is a specially prepared oil-in-water emulsion and may be mixed with water to the desired consistency for introducing into difficult wounds, such as that described, without significant loss in efficiency.

In 1953 a wide survey of veterinary hospital treatment records and cost accounts was made to establish the efficiency and cost of treatment by this formulation against those of conventional oily dressings and ointments.

For this purpose those wounds shown by the records to take an average of 20 days to heal were selected and included: accidental incised wounds (average diameter 3"); old maggot-infested wounds; foot ulcers (infected foot-and-mouth disease vesicles); saddle sores, galls and sinuses. The attached table clearly shows the saving in time and money effected by the use of 'Lorexane' Antiseptic Cream.

Dressings	Average quantity per dressing (c.c./Gm.)	Average duration of treatment (days)	Total quantity (c.c./Gm.) per course of treatment	Total Cost		
1. Daily wash (2 pints) Cresol lotion, followed by a Cresol/oil dressing ...	1 drachm (4 c.c.) pure Cresol @ Rs. 0 0 6/oz.	20	80 c.c.	Ra. As. Ps.	Ra. As. Ps.	
Cresol/oil ...	4 drachms (16 c.c.) @ Rs. 0 0 9/oz.	20	320 c.c.	0 1 3	0 8 9	
2. Zinc or Boric Ointments ...	2 drachms (8 Gms.) @ Rs. 0 1 0/oz.	20	160 Gm.	0 7 6		
3. 'Lorexane' Antiseptic Cream ...	3 Gm.	14 (2 applications)	6 Gm.	0 5 0	0 1 7 (100 Gm. pack)	

Calcutta }
16-2-'54 }

G. V. SHORT, M.R.C.V.S.,
Veterinary Service Department,
Imperial Chemical Industries (India) Ltd.

Appointment of Dr. Sampath Kumaran

Sir,

The members of the veterinary profession must be very glad to note that the services of Dr. J. D. Sampath Kumaran, L.V.P., M.A., Ph. D. (University of Missouri, U.S.A.) has been rightly utilised by the Poona University by appointing him as a teacher for imparting instructions in Animal Husbandry to the post-graduates (by research) at Karnal.

We are aware of the fact that Dr. Kumaran has raised the status of Veterinary Profession in India. He is an authority on Endocrinology and Artificial Insemination in India.

I hope that people in this profession will take the opportunity for undergoing the said course.

Jabalpur }
20th Feb. '54 }

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

Hundredth Anniversaries of the Birthdays of Paul Ehrlich and Emil v. Behring at Frankfurt/M, Germany.

Sir,—On March 14th and 15th 1954, celebrations will take place in Frankfurt/M, Germany, in commemoration of the Hundreth Anniversaries of the Birthdays of the two great German scientists Paul Ehrlich and Emil v. Behring. Their names are still familiar to the medical profession as Nobel Prize Winners in their times.

Paul Ehrlich who lived in Frankfurt is known as the founder of chemotherapy. He invented Salvarsan, the first preparation of the arsphenamine series. Paul Ehrlich's side-chain theory and his experimental work in vital staining has opened new ways and his ideas were the basis for the development of chemotherapy in protozoal diseases particularly spirochaetosis, trypanosomal and plasmodial infections. Though antibiotics have substituted arsphenamines in syphilis, treatment with Salvarsan preparations is still much in vogue.

Emil v. Behring was the first to produce sera against diphtheria and tetanus, thus laying the foundation for serum treatment and prophylactic vaccination. The Hundreth Anniversaries of the Birthdays of these research workers to whom humanity owes so much, will be celebrated from the 13th to the 16th of March 1954, in Frankfurt/M and in Marburg/Lahn.

During the memorial service in the St. Paul's Cathedral, at Frankfurt, M. Prof. Dr. Ernst Boris Chain will be awarded the Paul Ehrlich prize for 1954. The celebrations will be concluded by a scientific conference which will be attended by hundreds of scientists, physicians and research chemists from Europe and overseas.

The note given above is an authentic one received from the Behring Institute in Marburg. The news may interest many of your readers particularly those who have practised already before the war, the heydays of arsphenamine treatment and serum therapy.

Bombay }
9-3-54 }

A. G. BROCKE, D.Sc.,
Pharmaceutical Department,
CHOWGULE & CO. (HIND) LTD.,

Abstracts

"Organisation and execution of Anti-epizootic Measures in the U.S.S.R."—By N. LEONOV, Professor, Director of All-Union of Experimental Veterinary Medicine, U.S.S.R.

The imposing program for the development of U.S.S.R. between 1951 and 1955 includes the increasing of their livestock population by almost double and the number of poultry by 3.5 times the present stock. To realize this, they must create a stable fodder supply, build livestock and poultry farms on a large scale and introduce an all-round mechanization of production processes in animal husbandry. A well-organised veterinary service to prevent infections and parasitic diseases of livestock and poultry is needed for achieving the above program. In the U.S.S.R., the systematic and planned efforts to abolish epizootics are ensured through the uninterrupted development and material improvement of the veterinary service.

There are more than 25,000 state veterinary hospitals, in addition to the large number of such hospitals existing in the collective livestock farms. The district officer supervises the execution of the anti-epizootic measures. More than 800 veterinary diagnostic laboratories fully equipped for research are engaged in mass diagnostic examinations and planned preventive inoculations. Special mobile disinfection squads in every region are used for eliminating ticks and other parasites. 13 veterinary research institutes and 30 research stations work out the rational methods of preventing and treating animal diseases.

Every year more than 2000 veterinary doctors graduate from 23 veterinary colleges and 17 more veterinary faculties of other agricultural colleges, and 4000 students graduate with a lower diploma from 19 secondary schools and 30 veterinary faculties. Thus 6000 fresh qualified persons are absorbed every year in the livestock industry. Every year 900 veterinarians take post graduate training to improve their knowledge. The veterinary workers retire at the age of fifty five with 25 years service and receive pension equal to half their salaries even though they continue to work after superannuation. Many of them hold key positions in the administration of the state, in addition to their obtaining high and meritorious awards.

V. MAHADEVAN.

Studies on Fowl Spirochaetosis with special Reference to Penicillin therapy and the Development of an egg-adapted vaccine for its control—By S. B. V. Rao, B. M. THAKRAL and M. R. DHANDA, Division of Pathology and Bacteriology, Indian Veterinary Research Institute, Mukteswar Kumaon U.P.

Literature on the control of Spirochaetosis of fowls has been reviewed.

ABSTRACTS

491

Penicillin, administered as a therapeutic agent at 4000 units per pound body-weight, has been found fully effective in curing the disease at the height of infection.

The minimum infective dose in fowls was determined to be 1 c.c. of a 10-3 dilution of virulent blood obtained from a fowl at the height of a reaction after artificial infection with spirochaetes. This quantity of virulent blood was estimated to contain 46,000 spirochaetes.

An embryonated-egg vaccine has been developed which promises a durable immunity in protected birds. The vaccinated birds have withstood a challenge dose of a thousand minimum infective dose, and have so far proved refractory to three monthly challenge tests with virulent blood.

(*Abs. No. 100, Medl. and Vety. Sec. Ind. Sc. Congress, 1954.*)

Reviews

Animal Nutrition Research in India—By K. C. SEN, D.Sc., F.N.I., Director Indian Dairy Research Institute, Bangalore. Publishers Messrs. MacMillan & Co., Ltd., Calcutta-12. Price Rs. 15/-

Information about nutrition of animals in India has been meagre. It is still scanty about certain classes of stock. Nutritional needs of buffaloes, sheep and goats, to mention only a few, are still not well known. Again we know next to nothing of elephants and their nutritional requirements. We may multiply examples. There was a time when the Agricultural Colleges in India had an "Animal Nutrition" Section wherefrom some results used to be published. But this has completely dried up since the Veterinary Colleges took over the responsibility of teaching animal husbandry in its various aspects to their students. But at the present moment there is no research at all on the animal nutrition side from any of the Veterinary College in India. Under such circumstances it is very refreshing to see a book on Animal Nutrition Research in India.

Dr. K. C. Sen has brought out within the compass of 370 pages of Crown 8vo an excellent book of information on Animal Nutrition Research conducted in India. He is eminently qualified to be the author of a work of this kind, as he had been for several years, the head of the Animal Nutrition Section of the Indian Veterinary Research Institute. Further-more he has himself contributed a good deal to our knowledge of the value of Indian Feeding stuffs. The author has reviewed the literature on the various aspects of animal nutrition available, scattered in various magazines and journals in India; he has also gone through many foreign periodicals and publications for compiling this volume.

The arrangement of the content of the book is pleasing as it takes the reader to the problem facing the country, then through to the technicalities of nutrition and scientific feeding of animals. There are also chapters on fodder conservation, investigations of poisonous plants, the composition of blood of various classes of animals, nutritional diseases and other allied subjects. A good chapter on dairy problems closes the book. Each chapter has a bibliography to help the ordinary reader or the research worker to go to the original source of the information contained in the chapter. The end of the book gives the author index.

As the present curriculum of studies in the various veterinary colleges in India requires a basic knowledge of animal nutrition, this publication fulfills that want. Here is a book that can be confidently recommended to the student, the teacher or the research worker. It is our hope that, in the next edition, chapters would be added on the nutrition of the buffalo and feeding of the poultry etc. Many monographs in Veterinary and Animal Husbandry matters are quite welcome.

K. S. N.

Administration report of the Veterinary Department, Hyderabad Govt., Hyderabad Deccan, for the year 1952-1953—

Dr. Shetty continued as Director, Veterinary Department, during the year under review.

The posts of Assistant Directors of the department were abolished and the resultant savings were utilised to create posts of field workers. A uniform policy with regard to breeding of livestock and management of all State Cattle breeding farms was chalked out with a view to run them with minimum cost without in any way impairing efficiency.

The Veterinary Investigation Officer who was also incharge of the Serum Institute was relieved of the latter work to enable him to concentrate on investigation work. Similarly, the Assistant Disease Investigation Officer, Poultry, was relieved of his additional work, so that he could devote himself entirely to investigation work.

According to the cattle census of 1951, the State has 11.7 million heads of cattle. The important breeds of cattle are Deoni, Red Khandari, Malvi and Krishna Valley. Special measures have been taken at the Government Breeding Farms at Hingoli, Udgir, Himayath-sagar and Karimnagar to preserve and develop these breeds. Moreover, two livestock officers have been appointed, to survey the Malvi and Deoni cattle tracts and assist private breeders in these areas.

The department has suggested the introduction of two bills, one for the improvement of livestock and the other for the control of cattle diseases in the State, and they are under the scrutiny of Government.

Due to the integration of Jagirs and Paighas, the field of veterinary activities has increased and it was found necessary to open more veterinary dispensaries. In 1951-52, 15 touring billets were added; in the coming year it is hoped that a dozen more will be added. There are, in the State, 28 hospitals, 23 touring billets and 86 Veterinary Assistants to cater to the needs of agriculturists.

The year was particularly good from the Veterinary point of view, as the number of cattle dying from Rinderpest and other cattle diseases fell from 21,122 last year to 11,398 under the report year. The total number of animals treated in the Veterinary institutions during the year rose from 2,99,573 last year to 6,23,650 and the total number of major and minor operations done during the year increased from 12,992 to 19,216. In addition to these, the touring staff, while on tour, treated 1,09,749 animals and castrated 25,036.

During the year under review, the department opened Poultry Farms at 6 different places, namely Aurangabad, Nalgonda, Warangal, Karimnagar, Hingoli and Gulbarga. If managed on a scientific basis, poultry farming can become one of the most lucrative cottage industries in the country.

The anti-liver-fluke Scheme in the Nizamabad district has been in operation for over a decade. Work on this scheme has brought great relief to agriculturists by protecting their livestock against Liver-fluke. The work is still in progress and it is necessary that it should continue till the scourge is eradicated.

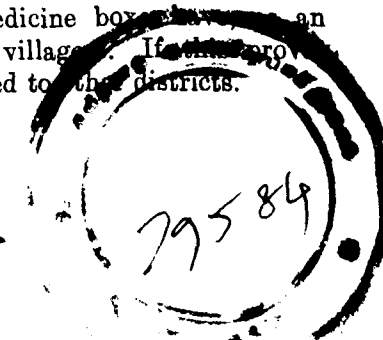
During 1951 to 1953, 9 Veterinary Dispensaries were opened besides the appointment of 15 Veterinary Assistants to give Veterinary aid in the areas which had no facilities for receiving Veterinary Aid.

Three Key Village Centres have been established one in Udgir, the other in Karimnagar and 3rd one at Aurangabad for the development of Deoni and Malvi Cattle and the Maratwada buffaloes respectively.

Under the Rural Welfare programme, officers of the department conducted mass inoculation camps against rinderpest in the Medak District and protected 27,000 animals of 75 villages. In addition to work on inoculation, officers of the department ran farmers' classes to give them lessons on breeding, feeding and management of animals and marketing of animal farm products.

In Nalgonda District a few first aid medicine boxes, as an experimental measure, been supplied to some villages. If this proves successful, work on the scheme will be extended to other districts.

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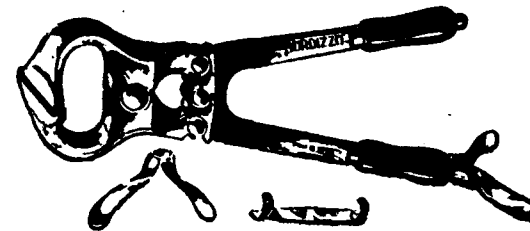
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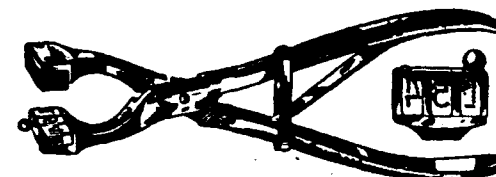
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