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(The Journal of the Indian Veterinary Association)

*A Monthly Record of
Veterinary Science*



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[THE JOURNAL OF THE INDIAN VETERINARY ASSOCIATION]

PUBLISHED MONTHLY



A Monthly Record of Veterinary Science
Devoted to the cause of the
Veterinary Profession



EDITOR

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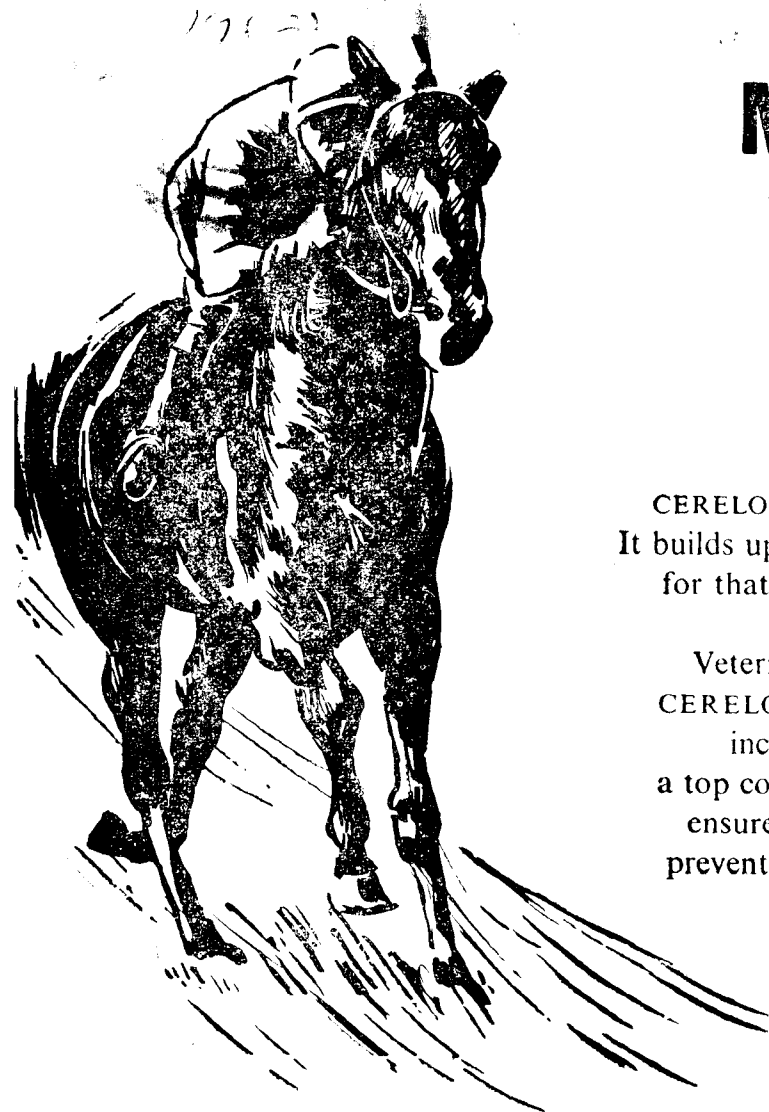
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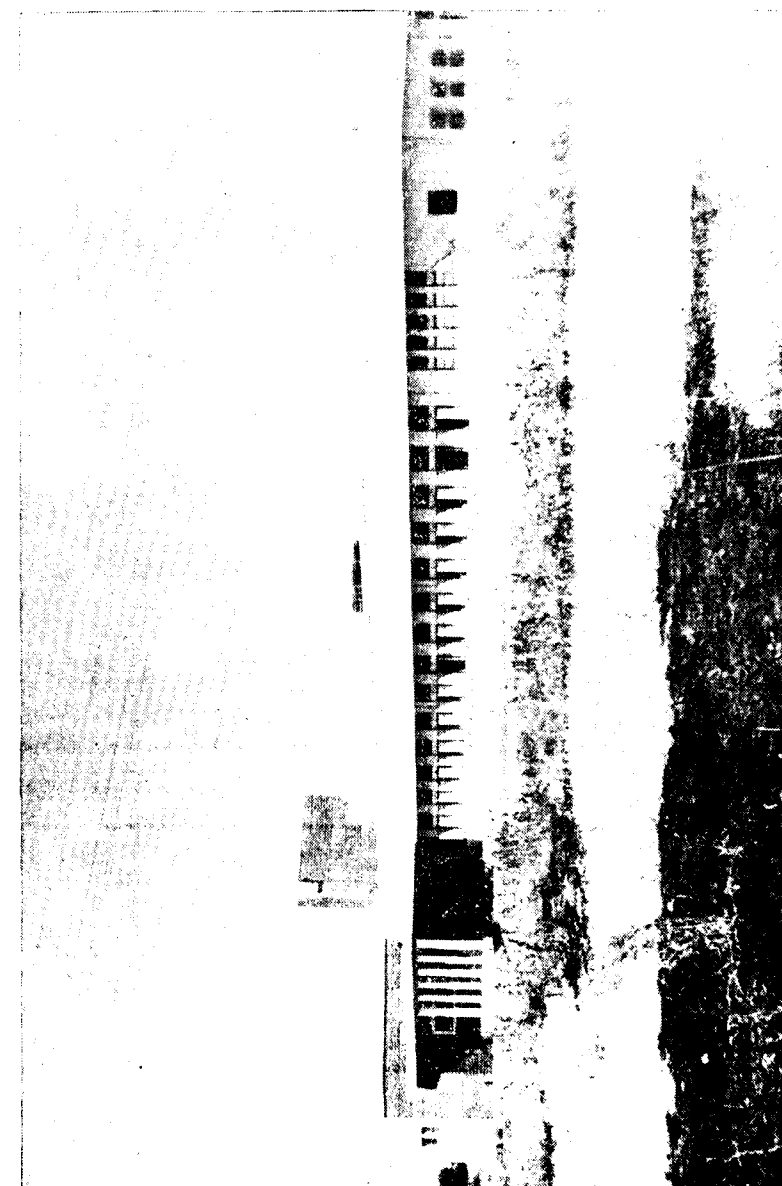
CONTENTS

	PAGE
I. Editorial	
The I. V. J. and the Government of Madras ...	E-9
II. General Articles	
Studies on the Enzyme systems in <i>Pasteurella Multocida</i> Part I. Demonstration of Amino Acid Decarboxylases in virulent and avirulent strains by simple tests.—By S. S. Negi, M. R. Dhanda, P. C. Sekariah and K. K. Baxi ...	165
Bioassay of Freeze Dried Goat Adapted Rinderpest Virus Vaccine.—By K. C. Sinha and C. Seetharaman ...	173
Studies on Surface Tension of Milk.—By Dr. V. Venkataswami and N. Krishnamurthi ...	178
Studies on the constituents of blood of buffaloes. Part I.—By K. J. Simon and Mrs. Annamma Jacob ...	183
A case of Infertility in a bull.—By C. K. S. V. Raja and D. V. P. Nair.	188
Treatment of Ascariasis in young calves with Piperazine Citrate.—By N. Dass, S. N. Panda and G. Biswal ...	194
Some Nutrients for Optimum Hatchability.—By B. Panda ...	197
Prevalence of Lungworms - <i>Dictyocaulus Viviparus</i> among cattle in Hes-sarghatta Farm, Bangalore.—By A. Malaki ...	201
III. Clinical Article	
Inguinal hernia in a dog—A case report.—By Prof. M. N. Menon, A. Venugopalan, P. O. George, and K. V. Rappai ...	203
IV. Abstracts	
Tissue therapy in animal husbandry ...	207
The discovery of a new method of diagnosing the African Swine Fever	208
Feeding of early-cut silage increases milk yield ...	208
Balantidiosis in Cattle (<i>Bos indicus</i>) in Orissa ...	208
Chick mortality and the contamination of yolks by members of the Enterobacteriaceae ...	208
V. News and Views ...	209
VI. Correspondence ...	212
VII. Association News	
The Indian Veterinary Association —Proceedings of the meeting of the Managing Council held on 26-3-61 ...	212
„ The I. V. J. Reserve Fund—List of contributors ...	215
„ Statement of receipts and payments of the Indian Veterinary Journal, Vol. 37, 1960 ...	216
VIII. Review	
The Dog Lovers' Manual:—By Lt. Col. A. C. Aggarwala ...	217

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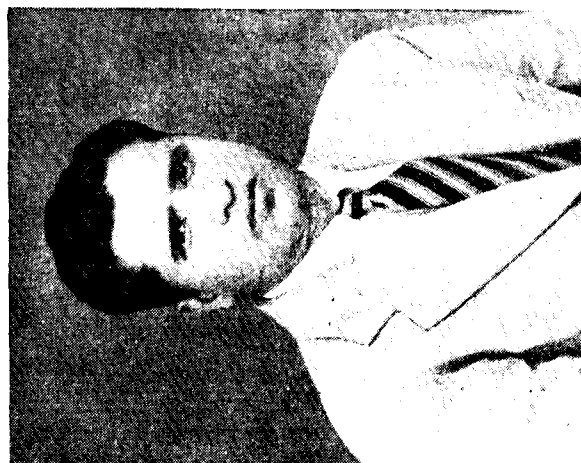
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Editorial

THE I. V. J. AND THE GOVERNMENT OF MADRAS

Yet another land mark in the history of this interesting journal has taken place recently, quite unobtrusively. It is a landmark which will ever stand to the credit of the Government of Madras. At a time when we were beginning to despair of the future of this journal, after its glorious record of work of over 37 years, the Government of Madras have come to its rescue. For sometime now we have been indicating in these pages that the present Editor was expressing his desire to retire from his office, because of his advancing age and indifferent health. The Managing Council was hard put to take a decision and in conformity with the best traditions of such Councils in the past, the present Council acted with great vision and wisdom in approaching the Government of Madras to permit two of the Members of the staff of the Madras Veterinary College, to serve as an Associate Editor and as an Assistant Editor, in a honorary capacity, to give the much needed relief to the Editor to grapple with the increasing volume of work. They will restrict themselves entirely to the scientific side of the work and will not in any way be responsible for the Editorial policy of the Journal. The Government of Madras considered this question as any other human problem on a realistic basis and evidently came to the conclusion that Scientific Journalism should be allowed to thrive in this land without any let or hindrance. They gave their consent readily and G. O. Rt. No. 196 (Food and Agriculture) dated 3—2—61, marks this milestone in the career of this journal. The Council met once again as the proceedings published elsewhere will show, to place on record its deep sense of appreciation and gratitude to the Government of Madras, for what a friend from Bengal has rightly described as "a noble act of theirs". It is such human touch in administration that

earns the everlasting gratitude of the public at large. We merely say "Thank you, Sirs."

The part played by the Director of Animal Husbandry, Madras, Dr. D. Pattabhiraman, as a co-opted Member of the Council at this hour of our crisis cannot be too highly praised. We are equally indebted to the Principal, Madras Veterinary College, Dr. Bertie A. D'Souza, who readily agreed to spare the services of two of his staff as requested by the Council. We are deeply thankful to both of them.

The honour for the first time of sharing the responsibility of running this journal has fallen on

(I) Dr. V. S. Alwar, G.M.V.C., B.V.Sc., M.Sc., Reader in Parasitology.

(II) Dr. M. S. Jayaraman, G.M.V.C., B.V.Sc., M.V.Sc., Lecturer in Bacteriology.

We welcome them and express our thanks to them for so readily consenting to serve in this great cause and in such a selfless manner.

We are sure the profession will realise the full significance of this move on the part of the management. In brief, the future of this journal is assured. The journal is bound to go on with or without Editorials, with the help of these two Officers or their successors.

The next move ought to be to house this Journal in a building of its own, worthy of our country and our profession. We have asked the Government of Madras for a piece of land and we are eagerly looking forward to their favourable orders.

LONG LIVE THE I. V. J. !

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General Articles

STUDIES ON THE ENZYME SYSTEMS IN *PASTEURELLA MULTOCIDA*, PART I. DEMONSTRATION OF AMINO ACID DECARBOXYLASES IN VIRULENT AND AVIRULENT STRAINS BY SIMPLE TESTS

By

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Division of Pathology & Bacteriology, I.V.R.I., Mukteswar.

Since the decarboxylation of amino acids yields amines, some of which have marked pharmacological activities in animals, a study was undertaken on the decarboxylase activities in *Pasteurella multocida*, Robert's type I, the causative organism of haemorrhagic septicaemia (Bain, 1954; Dhanda, 1958; Hudson, 1954). It was considered that such an investigation might be helpful in analysing the biochemical determinants of virulence and immunogenicity in this species. As far as we are aware, such studies have so far not been carried out in *P. multocida*. In the comprehensive enquiry on the enzyme make-up and metabolism of the aetiological agent of haemorrhagic septicaemia, therefore, experiments were carried out on the decarboxylation of amino acids by virulent and avirulent strains and the results have been reported in the present paper. Data on some of the factors affecting decarboxylation have also been included.

Methods and Materials

Cultures and culture media :—The virulent strain of *P. multocida* (accredited Roberts' type I) I.V.R.I. stock No. 52 and the avirulent strain stock No. 25 maintained on nutrient agar slants were inoculated in glucose-yeast-peptone broth to which was also added, in a 1% concentration tryptic digest of casein in order to obtain as wide a range of activities as possible (Gale, 1940). The inoculated medium was incubated for 16–20 hours and a drop of this growth was used as the inoculum for study of the decarboxylation system in the organism. A portion of the same growth was boiled to kill the organism and a drop thereof used for inoculation of the control.

Plain nutrient broth with or without glucose (0.5%) was used as basal medium. The experimental media for demonstration of the

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decarboxylase activity were prepared by incorporating in the basal medium the following amino acids at a 1% level.

- (i) L Arginine monohydrochloride — BDH
- (ii) DL Ornithine monohydrobromide — BDH
- (iii) DL Lysine dihydrochloride — Merck (German)
- (iv) DL Glutamic acid — BDH
- (v) DL Histidine hydrochloride — BDH
- (vi) Tyrosine — BDH

The media were distributed in 1 ml. volumes giving columns about 2 c.m. high. The tubes were autoclaved before inoculation with killed or live culture. After inoculation 3 to 4 drops of sterile paraffin oil were added to each tube. The tests with basal media served as blank observations.

Technique:—According to Gale (1940) bacterial decarboxylases were found in large quantities only when the organisms were grown in an acid medium. The enzyme activity is assayed by the conventional working technique by measuring the pressure change resulting from the liberation of carbon di-oxide from the reaction mixture provided the pH is 5.3 or below (Najjar, 1958). Moller (1955) pointed out the difficulty encountered in case of organisms which failed to grow at lowered pH values and thus precluded the possibility of elaboration of a fair degree of decarboxylase activity. The difficulty in such cases was got over by lowering the pH of the culture by acidification after growth. Since this study deals with the demonstration rather than the assay of the decarboxylase activity, the procedure followed here is a modification of the simplified tests adopted by Moller (1955), who cultured the organism at pH 6.0 in nutrient media incorporating the particular amino acids under study and a combination of indicators. The decarboxylase activity manifested itself by a raised pH of the medium made visible by a change of colour. As a very small rise in the pH of the medium might not bring about a change in the colour of the indicator, here a direct determination of pH of the culture media has been made by Beckman's pH meter-model G before and after incubation at 37°C. Further a more direct evidence of the decarboxylase activity was obtained by culturing the organism at 37°C in Thunburg tubes with lime water kept in bent hollow glass stoppers and the tubes sealed air tight with sterile grease under aseptic conditions. The fall in the pH of the lime water was taken as evidence of absorption of carbon di-oxide liberated from the culture media and hence of the decarboxylase activity. All the results were read at least in duplicates.

Results and discussion

(a) The control and experimental media included glucose at 0.5% level and the reactions of the different amino acid media were adjusted at pH values from 5.7 to 6.2. The pH estimations after 24 hours' incubation showed a fall in all cases. Under the conditions, the decarboxylation activity did not manifest itself.

(b) Media including glucose were used as above. The pH values were adjusted from 6.2 to 6.4. The change in pH was read after incubation for four days. There was evidence of the ornithine decarboxylase activity as shown by the distinct rise of pH in the ornithine media from 6.2 to 6.6. The pH of the control changed from 6.2 to 6.25 only. Results were inconclusive with other amino acids.

(c) Glucose media as above were used with pH adjustments from 4.6 to 4.8. Incubation of the media for 44 hours after inoculation with killed and live cultures did not give any growth response as shown by absence of turbidity in the media. There was no change in the pH of the media.

(d) It appeared from the above that there was little or no growth of the organism at low pH values of around 4.7 in glucose media. So it was decided to give a trial to plain broth media without glucose.

Plain broth without glucose was used for preparation of experimental media the pH values of which were adjusted from 4.45 to 4.65. The pH of the media before inoculation, after incubation for 44 hours without inoculum and with inoculum from killed and live cultures are given in the table below:—

Amino acid medium	Original pH	pH after incubation with inoculum from				Change in pH over control
		Nil	Killed Culture	Live Culture		
Arginine	4.55	4.6	5.10	5.35		+ 0.25
Ornithine	4.55	4.5	4.95	5.40		+ 0.45
Lysine	4.40	4.7	4.65	5.05		+ 0.40
Glutamic acid	4.65	4.6	4.60	4.60		—
Histidine	4.45	5.05	4.85	5.20		+ 0.35
Tyrosine	4.60	4.7	5.2	5.40		+ 0.20
Blank	4.60	4.6	4.65	4.6		-- 0.05

The growth of the organism at pH values used was very low as evidenced by very slight turbidity of the live culture medium. Nevertheless from the rise in the pH of the media it appeared that at pH values of around 4.5, the decarboxylases for arginine, ornithine,

lysine, histidine and tyrosine were elaborated in *P. multocida*. There was no rise of pH in the glutamic acid medium. It is likely that this was inhibited because of the buffering capacity of glutamic acid around pH 4.6, an indication of which has been given by Moller (1955).

It appeared that the decarboxylase activity might not be elaborated strongly because of the poor growth response of the organism at low pH values. The observations were, therefore, repeated at pH values of around 6.0 allowing an incubation period of 24 hours and (e) at lower pH values of 4.7 to 5.6 allowing a long incubation period upto 16 days (f).

(e) Experimental media were prepared by incorporation of amino acids in plain broth without glucose and the pH of various media adjusted at 6.0. After 24 hours' incubation of the inoculated medium it was observed that the pH of the ornithine media was raised from 6.1 to 7.35 confirming the strong ornithine decarboxylase activity observed in (b) above. The observations are reported in the table below :—

Amino acid medium	Original pH	pH after incubation with inoculum from			
		Nil	Killed culture	Live culture	Change in pH over control
Arginine	6.0	6.1	6.2	6.1	—
Ornithine	6.0	6.1	6.2	7.35	+ 1.15
Lysine	6.0	6.1	6.2	6.1	—
Glutamic acid	6.0	6.1	6.25	6.05	—
Histidine	6.0	6.15	6.2	6.1	—
Tyrosine	6.0	6.1	6.2	6.05	—
Blank	6.0	6.1	6.2	6.0	—

In this test there is no evidence of the decarboxylase activity for amino acids other than ornithine.

(Under the condition of the above test, decarboxylase activity in *Salmonella abortusovine* (I.V.R.I. stock No. 95) was also studied in a simultaneous run. Strong decarboxylases for arginine, ornithine and lysine were evidenced along with a weaker decarboxylase for tyrosine. Glutamic acid and histidine did not appear to be decarboxylated).

(f) Experimental media, as above, adjusted at pH values of 4.5 to 5.6 were used. The media were tubed in Thunburg tubes and inoculated with live culture. The addition of paraffin oil was omitted. In the bent hollow stoppers, was placed 1 ml. of lime water at a pH of 12.5 and the tubes were sealed airtight with sterile grease. The tubes were kept in the incubator for a period of 16 days. The observations are recorded in the following table :—

Amino acid medium	pH of media at the time of inoculation	After 16 days incubation	pH of lime water* after 16 days incubation	Appearance of lime water after 16 days incubation
Arginine	5.3	7.3	7.4	Milky
Ornithine	5.0	7.0	7.2	Milky
Lysine	5.6	7.0	6.95	Milky
Glutamic acid	4.7	7.1	6.9	Milky
Histidine	4.9	7.1	7.0	Milky
Tyrosine	4.5	7.2	7.25	Milky
Blank	4.7	5.0	12.3	Clear

* pH at start 12.5.

The rise in pH of the media is indicative of the decarboxylase activity for all the amino acids studied. This observation is also supported by the more direct evidence of the elimination of carbon di-oxide from the media which rendered the lime water in the hollow stoppers milky. The pH of lime water also showed a sharp drop in all the cases except the blank. The observations made under the conditions of this test are particularly important in so far as these amply show that the overall effect of the induction of decarboxylases is to help in the maintenance of the pH of the medium near about the neutral value in case it tends to lower to the acid side.

The findings, while lending support to the observations recorded in (d) above, also gave an indication of glutamic acid decarboxylase activity in *P. multocida*.

From the foregoing observations it appeared that the ornithine decarboxylase activity in *P. multocida* was elaborated strongly even at a pH value around 6 and that given suitable conditions at lower pH values, the decarboxylases for all the other amino acids studied might also be elaborated. It was desired to see if the ornithine decarboxylase activity would be evident at pH values higher than 6 and hence the test in (g) below was undertaken.

(g) Experimental media as above were used with pH adjustments at values around 6.6. In case of the ornithine media the pH of the control tube inoculated with killed culture rose from 6.6 to 6.8 during 20 hours' incubation while, during the same period, that of the experimental tube inoculated with live culture rose from 6.6 to 7.75. In case of the other amino acid media there was no evidence of a rise of the pH in experimental tubes over and above that observed in the control tubes.

Evidently, the ornithine decarboxylase activity in *P. multocida* was very strong and manifested itself even at pH values slightly below neutral.

(Under the conditions of the test the decarboxylase activity of *Escherichia coli* (I.V.R.I. stock No. 20) was studied in a simultaneous run and evidence of decarboxylases for all the amino acids studied except tyrosine was obtained).

(h) Evidence of the strong ornithine decarboxylase activity of *P. multocida* was also obtained by inoculation of the organism in Thunburg tubes fitted with hollow stoppers containing lime water as in (f) above. The observations are recorded in the following table:

Amino acid medium	pH of the medium			pH of lime water*		Decarboxylation activity
	At the time of inoculation	After 22 hours incubation with culture		After 22 hours incubation with culture		
		Killed	Live	Killed	Live	
Arginine	6.55	6.7	5.95	12.2	12.0	?
Ornithine	6.55	6.7	7.5	12.15	7.25	+++
Lysine	6.55	6.75	6.0	12.1	11.9	?
Glutamic acid	6.6	7.0	6.4	12.05	11.85	?
Histidine	6.65	6.9	6.35	12.2	12.2	—
Tyrosine	6.55	6.4	5.9	12.1	12.2	—
Blank	6.55	—	—	12.2	12.2	—

*pH at start 12.2

The ornithine decarboxylase activity was very strongly indicated not only by the fall in the pH of lime water but also by the rise in pH of the culture medium. Weak decarboxylases for arginine, lysine and glutamic acids were also indicated by the fall in pH of lime water.

(i) The above experiment was repeated with media adjusted at pH values of 5.2 and incubation carried out for 48 hours. The following observations were recorded:

Amino acid medium	pH of the medium		pH of lime water		Decarboxylation activity	
	at the time of incubation	after 48 hours incubation with culture	after 48 hours incubation with culture			
		Killed	Live	Killed		Live
Arginine	5.2	5.25	5.4	12.1	8.1	+++
Ornithine	5.2	5.4	6.8	11.9	7.4	+++
Lysine	5.2	5.3	5.7	12.0	7.5	+++
Glutamic acid	5.2	5.4	5.4	12.05	11.9	?
Histidine	5.2	5.15	5.5	11.75	7.9	+++
Tyrosine	5.2	5.25	5.3	11.9	7.7	+++
Blank	5.2	5.2	5.3	12.2	11.6	—

The rise in pH was conspicuous in case of ornithine media again showing that ornithine decarboxylase activity was the strongest. The

fall in pH of lime water was indicative of the decarboxylase activity for all the amino acids studied except glutamic acid.

(j) Further evidence was sought for the decarboxylase activity in *P. multocida*. As the organisms did not grow well at lowered pH values, the experimental media were adjusted at pH 7.0 and the control and experimental tubes inoculated with killed and live cultures respectively and incubated for 24 hours when the pH of all the tubes was lowered by addition of two drops of an arbitrary concentration of hydrochloric acid (approximately 0.01 c.c. of N HCl) and the tubes incubated for another 18 hours such that the decarboxylases could be fully elaborated at the lowered pH values. The pH values of the uninoculated media, and media inoculated with killed and live cultures were determined at the end of 18 hours incubation. The average values obtained in two separate trials are presented below:

Amino acid media	pH of the medium after 18 hours incubation			Decarboxylation activity
	inoculated with			
	Nil	Killed culture	Live culture	
Arginine	5.25	5.25	5.50	+
	4.35	4.10	4.10	—
Ornithine	5.25	5.35	7.15	+++
	3.95	4.30	4.80	++
Lysine	5.40	5.25	5.85	—
	4.50	4.50	4.35	+
Glutamic acid	5.40	5.55	5.45	—
	4.60	4.50	4.7	+
Histidine	5.10	5.50	5.45	—
	5.05	5.10	4.85	—
Tyrosine	5.35	5.60	5.50	—
	4.70	4.70	4.80	+
Blank	5.15	5.25	5.30	
	5.20	5.25	5.25	

The strong ornithine decarboxylase activity of *P. multocida* received added support. The decarboxylases for amino acids other than histidine were also indicated under the conditions prevailing in the tests, though no quantitative estimation was attempted, evidence of the strong ornithine decarboxylase activity was also confirmed by the working of manometric technique.

An exploratory study was also made of the decarboxylases of an avirulent strain of *P. multocida* (I. V. R. I. stock No. 25) Roberts'

type I and it appeared that the decarboxylation activity in this avirulent strain might closely follow the trend shown by the virulent strain.

Conciusion

The ornithine decarboxylase activity in virulent and avirulent strains of *P. multocida* was very strong and manifested even at pH values only slightly below that at neutrality. Given suitable conditions the decarboxylases for the other amino acids could also be demonstrated. Next in order were the arginine and lysine decarboxylase activities. There was also an indication for weak decarboxylase for tyrosine, histidine and glutamic acid. There seemed to be no correlation between virulence and the presence of amino acid decarboxylases.

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K. C. SINHA and C. SEETHARAMAN,
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The relationship between the biological activity, titre or infectivity pattern of a living virus vaccine expressed by the term "minimum infective dose" for a particular species of animals or breed and its therapeutic or prophylactic efficacy is extremely difficult to define. In cattle, especially in rinderpest, this had always been a complex problem, primarily because pure antigen preparations, free from gross tissue particles, is difficult to obtain and the susceptibility pattern of various breeds of cattle and buffaloes in different areas in India would hardly yield uniform response to the vaccine. Although a gross dose expressed in terms of dilution of a particular batch of vaccine, in a measured volume, as a vaccinating dose may serve the requirements of a clinician but it is far from being a satisfactory standard, useful for a virologist, attempting at assessing the minimum effective dose unit.

It may be pointed out that although at the present time, a large number of international standards and references have been evolved especially for antisera, the difficulty in the bioassay of majority of the viral vaccines, in use in human and veterinary medicine, is still experienced keenly. The effectiveness, potency or activity of most of these vaccines is expressed in "animal units" determined through "animal—response effectivity/infectivity" tests; with the aid of observations carried out on survival or death pattern. The undesirability of this test has been pointed out but the practice still continues. The undesirability of such a standard lies in that the response of a group of animals is dependent on the design of the experiment, weather conditions, breed of animal or the species or the stock obtained from a particular tract. These factors may vary from time to time, in different places, in different zones where the laboratories may be located and reliable and reproducible estimates of the potency in "animal units" may be difficult to attain.

Bioassay on stocks of laboratory animals in extremely large number, especially in inbred colonies maintained for long years may yield a sufficiently constant and continuous trend of experimental data, but limitations of such a test are that this would be a very theoretical ideal ever to be achieved and that even carefully planned bioassay of a product with the aid of animal response test, is usually not a "planned" test but is the outcome of years of control assays none of which is

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highly accurate in the statistical sense, but all of which, taken together, may show a highly significant trend. At the most what can be expected is a suggestion. (Jerne and Perry, 1956).

Although prior to 1953, live virus vaccines against rinderpest and ranikhet disease had been in use on an extensive scale, the dose rates recommended were fixed largely on the basis of cumulative experience gathered from the use of the production of immune response in a definite dilution at a particular dosage level. However, with the adoption of the practice of freeze drying and large scale production of live modified virus vaccines against rinderpest, and adoption of freeze dried goat adapted virus vaccine as a product of choice for the vaccination of plains cattle and buffaloes in India to implement the "Rinderpest Eradication Programme" in 1954, the necessity of a suitable standard for the vaccine and bioassay procedure was keenly felt.

Before the initiation of large scale production of freeze dried goat tissue vaccine at this Institute in 1953, bioassay of this product had been carried at laboratories in East Africa and Egypt (Daubney 1949). The infectivity of the desiccated goat tissue virus was estimated at 0.0002 gram and some times at 0.00004 gram. Titrations consisted of animal response tests in two bovines one receiving 0.002 gram and another 0.0002 gram. Based on this, 5 to 20 m.i.ds. were being injected. The limitations of such a test are evident. However, the dosage level was increased (Daubney, 1953) later to 40 minimum infective doses in India when preliminary details of the vaccination campaign were being worked out. It was felt that severe and prolonged reactions are more frequently seen in animals receiving doses approaching the minimum infective doses and that something resembling "excessive dose phenomenon" operates with larger doses to provide milder reactions. It was felt desirable that production establishments will usually be employing as test animals the most susceptible type of cattle available in the area, whereas the issued vaccine may be used to immunise animals with considerably greater resistance to attack. However, for such bioassays, the number of animals for any dosage level was limited to two since the susceptible bovines had to be employed and cost limited the number. This method had had its own limitations.

Titrations of virus in material to be used for vaccine are of great assistance in maintenance of a standard. The estimation of dose on the basis of 100 percent end point using small number of animals may result in gross errors. With 100 percent end point, ten fold dilutions may reveal ten fold differences (Brotherston, 1957) which may be apparent but ultimately is significant statistically. Scott and Mac-Leed (1955) had been able to get the same response in cattle using 1000 ID₅₀ as with 50,000 ID₅₀. Inbred rabbits which are more resistant

required 100 times more, the infective dose of Lapinised rinderpest virus than randomly bred rabbits (Brotherston and Brown 1955).

In India, little work was carried out in this line prior to 1953. Since 1954, the bioassay of the freeze goat tissue vaccine was initiated in susceptible bovines (hill cattle of Mukteswar-Kumaon Hills) and in some other centres in yearling buffalo-calves. The authors carried out a series of bioassays to find out if there is a close correlation between the end titres of the virus required for development of a good grade of immunity in hill cattle and buffalo-calves. In this context observations were also carried out to find if there is any correlation between the infectivity titre of virus in cattle, buffalo-calves *vis-a-vis* goats.

Materials and Methods

Hill bulls between 3-6 years of age and buffalo-calves between 1-2 years were used. Goats, either male or female, between 1-3 years of age were included in the experiments. Hill bulls weighing between 200-300 lbs. were procured through suppliers who brought these animals from distant areas in the Kumaon hills, and these are considered highly susceptible to rinderpest. Buffalo-calves weighing between 150-220 lbs. and were purchased from contractors at Bareilly. Goats represented non-descript type, not conforming to any particular breed. These weighed 40-50 lbs. and represented both sexes.

These animals were kept under observation for at least a week before experiments. In the general scheme, desiccated (freeze dried) spleen pulp in sealed ampoules constituting different batches were weighed accurately and one gramme quantity was taken as sample for making different dilutions. The dilutions consisted of 1: 8000, 1: 12000 and 1: 16000 and in some cases 1: 20000 in 1 ml. quantities injected subcutaneously in two hill bulls and two buffalo-calves. These animals were kept under observation for 10-14 days, body temperatures being recorded twice daily. At the end of this period, these were challenged with 1 ml. of a 1: 100 suspension of the virulent bovine rinderpest (spleen tissue) virus subcutaneously. In goats the infectivity titre of the viruses was assessed by injecting 1 ml. of the virus (vaccine) in dilutions ranging from 1: 8000 to 1: 40,000. In this case the survival/death ratio was worked out at a particular titre *vis-a-vis* immunising titre in bovines. In the event of mortality among any animals, postmortem examination was carried out to determine the cause of death.

Results

In this series of experiments, 40 hill bulls, 24 buffalo-calves and 80 goats were used. Bioassay of six batches of freeze dried goat tissue vaccine was carried out to establish the suitability of the species and the correlation. Results are summarised in table I and II.

TABLE I
Bioassay of freeze dried goat tissue vaccine in hill bulls and buffalo-calves.

Species of animals	Batch number	Dilution of the vaccine				Titre at 100% end point
		1:8000 Survival	1:12000	1:16000	1:20000 (number of animals in brackets)	
Hill Bulls	74	(2/2)	(2/2)	(2/2)	—	1:16000
	79	(2/2)	$\frac{1}{2}$ *	(2/2)	(2/2)	1:16000—1:20000
	84	$\frac{1}{2}$ *	(2/2)*	$\frac{1}{2}$ *	$\frac{1}{2}$ *	1:16000—1:20000
	94	(2/2)	(2/2)	(2/2)	—	1:16000
	104	(2/2)	(2/2)	(2/2)	—	1:16000
Buffalo-calves	109	(2/2)	(2/2)	$\frac{1}{2}$ *	—	1:16000
	74	(2/2)	(0/2)*	$\frac{1}{2}$ *	—	1:16000
	79	(0/2)*	(2/2)	(2/2)	—	1:16000
	84	—	$\frac{1}{2}$ *	(2/2)	—	1:16000
	94	(2/2)	(2/2)	(2/2)	—	1:16000
	104	Not done	—	—	—	—
	109	—	—	(2/2)	—	1:16000

*Mortality due to other causes.

TABLE II
Infectivity of the freeze dried goat tissue vaccine in goats (4 goats were used for each dilution).

Batch Number	Dilutions	Mortality %
74	1:8000	75.0
	1:16000	50.0
	1:24000	75.0
	1:32000	25.0
79	1:8000	100.0
	1:16000	25.0
	1:24000	25.0
	1:32000	50.0
84	1:8000	75.0
	1:16000	25.0
	1:24000	100.0
	1:32000	100.0
94	1:16000	25.0
	1:24000	75.0
	1:32000	100.0
	1:40000	100.0
104	1:16000	100.0
	1:24000	75.0
	1:32000	50.0
	1:40000	50.0
109	1:16000	100.0
	1:24000	50.0
	1:32000	75.0
	1:40000	100.0

Particulars of the immunising titre of the different batches of freeze dried goat tissue vaccine at 100 percent end point level in hill bulls and buffalo-calves *vis-a-vis* infective titre at 100 and 50 percent end points in goats are summarised in table III.

TABLE III
The immunising titre of goat tissue vaccine in bovines and the infective titre in goats.

Batch Numbers	Immunising titre at 100% end point in hill bulls (HB) and buffalo-calves (BC)	Infective titre in goats	
		ID ₁₀₀ level	ID ₅₀ level
74	HB 1:16000	1:8000—1:24000	1:16000
	BC 1:16000		
79	HB 1:16000—1:20000	1:8000	1:32000
	BC 1:16000		
84	HB 1:16000—1:2000	1:24000	1:8000—1:16000
	BC 1:16000		
94	HB 1:16000	1:40000	1:16000—1:24000
	BC 1:16000		
104	HB 1:16000	1:16000	1:32000—1:40000
	BC 1:16000		
109	HB 1:16000	1:40000	1:24000
	BC 1:16000		

Discussion

From the data presented in tables I, II and III it will be seen that the response of hill bulls and buffalo-calves to freeze dried goat tissue vaccine is comparable. This was also established while assessing their susceptibility to virulent rinderpest virus (Uppal, Sinha and Seetharaman, 1959). Although it is desirable to evolve a suitable method of bioassay of this vaccine in goats, the infectivity titre in these did not yield any uniform and comparable level for working out a suitable ratio. Although goats in general, should react to goat adapted virus, their susceptibility may vary, depending upon breed, age and condition. There are evidences that a comparison of the infectivity titre with immunising titre may be rendered difficult, since active multiplication of the virus in the system of goats may take place without thermal response (Sinha and Seetharaman, 1959, 1960).

Although hill cattle are extremely susceptible to rinderpest and are considered suitable animals for bioassay, buffalo-calves can be used.

Summary

Bioassay of freeze dried goat tissue vaccine is discussed in detail. It has been shown that while the immunising titre of the vaccine at 100

percent end point level could be assessed in hill bulls and buffalo-calves with comparable results, the infective titre of the virus in goats reveal little correlation. These titers bear little relationship with these obtained in bovines. This may be due extreme variations in thermal response and differences in susceptibility due to factors viz., breed, age and the state of health.

Acknowledgment

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STUDIES ON SURFACE TENSION OF MILK

By

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Surface tension which is one of the important and interesting physical properties of milk is closely related to its quality. It is affected by such factors as surface-active substances, temperature, lipolysis, ageing etc.

Aschaffenburg and Ogston (1946) found the normal values of surface tension of milk to vary from 46.6 to 52.7 dynes/cm. Chandan and Dastur (1959) reported that it varies with breeds, ranging between 51.93 dynes/cm for cows and a lower value of 50.40 dynes/cm for buffaloes at 20°C.

Dunkley (1951) observed that fat tends to decrease surface tension. Whitnah (1958) noticed that the cream with 36.5% fat to have a lower value than skim milk or whole milk, whole milk having lower value than skim milk. Burri and Nussbaumer (1909) attributed the fall at low temperature to solidification of fat, while Sharp and Krukovsky (1939) attributed it to adsorption of agglutinins on fat globules.

Chandan and Dastur (1959) noticed ageing to decrease surface tension with marked fall during the first 48 hours of storage. Henning and Dahlberg (1925) observed ageing to decrease surface tension slightly. Tarassuk and Smith (1949) reported the inhibiting effect of rancid milk on the growth of *Streptococcus lactis* to be due to lower surface tension. It subsequently increases following gradual utilisation of free fatty acids by the slow growth of *Strep. lactis*.

In the present study the normal values of surface tension and the effect of fat and ageing on it is reported.

Experimental Procedure

Milk samples collected from individual local cows and Murrah buffaloes were transported in ice-chest to the laboratory. Surface tension estimations were made with DuNuoy Tensiometer at 20°C. Initial estimations were made soon after production and then the samples were stored at 5°C for the study of the effect of ageing on surface tension. Fat content was determined by Gerber's method.

Results and Discussion

Normal values of surface tension with 30 milk samples of local cows is found to vary from 46.0 to 49.8 with an average of 47.55 dynes/cm. with 30 samples of Murrah buffalo milk, it ranges from 44.0 to 48.6 with an average of 46.21 dynes/cm. The values observed are lower than the values reported in the literature. However, the lower values of buffalo milk as compared to that of cows' is in agreement with the report of Chandan and Dastur (1959).

Effect of Fat on Surface Tension

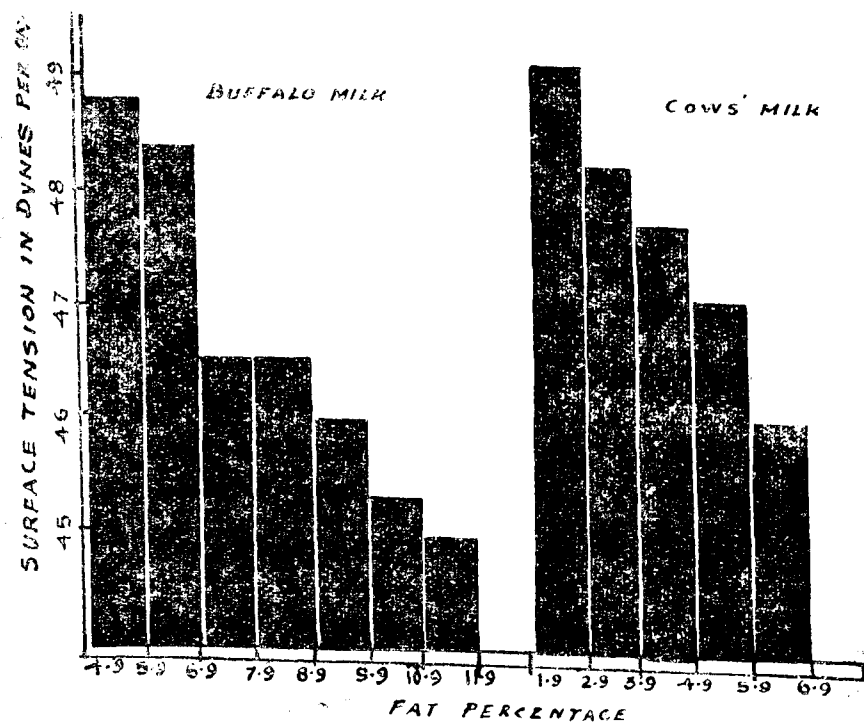
Fat in milk tends to reduce surface tension and hence the relationship between the two is inverse both in cows and buffaloes as shown in table I and Fig. I.

TABLE I
 Showing the effect of fat on surface tension of milk of cows and buffaloes.

Range of fat percentage	Cows		Buffaloes	
	Number of samples	Surface tension in dynes/cm	Number of samples	Surface tension in dynes/cm
1.9 to 2.9	2	49.1		
2.9 — 3.9	8	48.2		
3.9 — 4.9	8	47.7		
4.9 — 5.9	9	47.0	2	48.8
5.9 — 6.9	3	46.0	3	48.4
6.9 — 7.9			3	46.5
7.9 — 8.9			6	46.5
8.9 — 9.9			6	46.0
9.9 — 10.9			7	45.3
10.9 — 11.9			3	45.0

As the fat percentage in cows increases from 1.9 to 6.9 the surface tension decreases from 49.1 to 46.0 dynes/cm. and as fat percentage increases from 4.9 to 11.9 in buffaloes it falls from 48.8 to 45.0 dynes/cm.

Histogram showing the relationship between Fat percentage and Surface tension.



The rate of fall in surface tension for each one percent rise of fat is indicated in table II. There is a slight decrease of 0.581 dynes/cm. for cows and 0.562 dynes/cm. for buffaloes for each one percent rise of fat as calculated by linear regression. However, the fall in cows is slightly higher than in buffaloes.

TABLE II
Indicating the rate of fall in surface tension for every one per cent rise of fat.

Animal	Fat percentage		Fall in surface tension for each one per cent rise of fat in dynes/cm
	Maximum	Minimum	
Cows	2.0	6.2	0.581
Buffaloes	5.6	11.5	0.562

Effect of Ageing of Surface Tension

Ageing at 5°C reduced surface tension of both cows' and buffalo milk as indicated in Table III and IV.

TABLE III
Showing the effect of ageing on surface tension of cows' milk.

Range of fat percentage	Surface tension on ageing in dynes/cm			
	0 hr.	24 hrs.	48 hrs.	72 hrs.
1.9 to 2.9	49.1	45.6	44.6	47.6
2.9 — 3.9	48.2	45.8	44.7	46.3
3.9 — 4.9	47.7	45.6	44.7	46.8
4.9 — 5.9	47.0	46.0	45.1	47.9
5.9 — 6.9	46.0	45.3	44.1	46.1

It is observed from tables III and IV that there is a consistent reduction in surface tension on ageing and it continues upto 48 hours. This observation is in agreement with that of Chandan and Dastur (1959). However, it is interesting to note from the tables that it tends to rise after 48 hours. This is in agreement with the observations of Tarassuk and Smith (1940).

TABLE IV
Showing the effect of ageing on surface tension of buffalo milk.

Range of fat percentage	Surface tension on ageing in dynes/cm			
	0 hr.	24 hrs.	48 hrs.	72 hrs.
4.9 to 5.9	48.8	45.8	44.0	47.5
5.9 — 6.9	48.4	47.4	46.2	48.2
6.9 — 7.9	46.5	45.0	43.4	45.4
7.9 — 8.9	46.5	44.8	42.6	46.9
8.9 — 9.9	46.0	44.2	42.6	46.0
9.9 — 10.9	45.3	42.7	41.4	47.8
10.9 — 11.9	45.0	42.5	41.9	45.1

The rate of fall and rise in surface tension of cows and buffalo milk on ageing is given in table V.

TABLE V
Giving the rate of fall and rise in surface tension of cow and buffalo milk on ageing.

Animal	Number of samples	0 hr.	24 hrs.	48 hrs.	72 hrs.
		dynes/cm	dynes/cm % fall	dynes/cm % fall	dynes/cm % rise
Cows	30	47.55	47.79 3.7	44.80 2.2	46.89 4.7
Buffaloes	30	46.21	44.20 4.4	42.90 2.9	45.20 5.4

The above table reveals that the average initial surface tension of cows' milk is 47.55 dynes/cm. and it is 45.79, 44.80, and 46.89 dynes/cm. after 24, 48, and 72 hours of storage. While in buffaloes the average initial surface tension is 46.21 dynes/cm. and it is 44.20, 42.90 and 45.20 dynes/cm. after 24, 48 and 72 hours of storage. At 24 and 48 hours the percentage fall is 3.7 and 2.2 for cows and for buffaloes it is 4.4 and 2.9. Although the fall continues upto 48 hours, it is marked most in the first than in the second 24 hour storage period in both cows and buffaloes. The fall in cows is 1.76 dynes/cm. or 3.7% in the first and is 0.99 dynes/cm. or 2.2% in the second 24 hour period. For buffaloes it is 2.01 dynes/cm. or 4.4% and is 1.03 dynes/cm. or 2.9% during the first and second 24 hour storage period. However at 72 hours it increases in both cows and buffaloes by 4.7 and 5.4 percent over the values at 48 hours.

The pronounced reduction in the first 24 hour period may mainly be due to solidification of fat (Burri and Nussbaumer, 1909) and the continued fall at a much slower rate in the second 24 hour period may chiefly be due to the liberation of free fatty acids (Tarassuk and Smith, 1940). The effect of solidification of fat is more pronounced in the fat-rich buffalo milk than in cow's milk. The surface tension consistently rises after 48 hours and this trend may be attributed to the utilisation of free fatty acids by the microflora of milk (Tarassuk and Smith 1940).

Summary

- (i) Normal values of surface tension are 47.55 dynes/cm. and 46.21 dynes/cm. for cows and buffalo milk respectively.
- (ii) The relationship between fat percentage and surface tension is inverse. The surface tension falls by 0.581 dynes/cm. in cows and 0.562 dynes/cm. in buffalo milk for each one percent rise in fat.
- (iii) Ageing reduces surface tension with greater fall in the first than in the second 24 hour period. It tends to rise after 48 hours. The possible explanations for these phenomena are discussed.

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STUDIES ON THE CONSTITUENTS OF BLOOD OF BUFFALOES

Part I

CELLULAR ELEMENTS AND HAEMOGLOBIN

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A knowledge on the physiological norms constitutes an essential aid in the diagnosis of many disease conditions. Physiological variations in the content of blood cellular elements due to factors such as age, sex, etc., have been frequently observed to occur in various species of animals (2, 3 and 9). But very little information is available regarding cellular elements of blood of buffaloes, excepting for some data collected by Kehar and Murthi (5). Since buffalo has been recognised as an important milch and work animal in India, studies to determine the normal levels of their blood constituents were undertaken, and the results obtained are incorporated in the present paper, furnishing data on the normal erythrocyte and leucocyte counts, haemoglobin values, packed cell volume, erythrocyte sedimentation rate, and

differential leucocytic count in the blood of animals of different age and sex.

Materials and Methods

Buffaloes in good health from the Government Livestock Farm, Ollukkara, and some others belonging to private owners formed the subjects for the present investigation, and a total number of 90 animals of different ages and sex (*vide* Tables I and II) were utilised for the purposes. Blood samples for study were drawn from the jugular vein.

The erythrocyte and leucocyte counts were made by means of Neubauer Counting Chamber and Thoma Zeiss Haemocytometer. Wong's method (10) was used for estimating haemoglobin initially but since the method was found to be not rapid enough, later estimations were made by Sahli's Haemoglobinometer after standardization against Wong's method. Packed cell volume was estimated in Wintrobe tubes by centrifuging for 30 minutes at 3000 r.p.m. Erythrocyte sedimentation rate was determined in Wintrobe tubes. Blood smears for differential counts were stained by Leishman's.

The data collected were statistically analysed according to the method of Snedecor (8).

Results and Discussion

The results obtained are furnished in Tables I and II.

Haemoglobin:—Coffin (2) has reported 11.7 gms. of Hb per 100 ml. blood in buffaloes, while Kehar and Murthi (5) have given values as low as 7.7 gms. The results obtained during the present studies (*vide* Table I) are in keeping with the values reported by Coffin (2). The investigation further reveals that factors like age and sex have very little influence on the haemoglobin content, the slight difference that is shown in the haemoglobin values for various groups being not significant enough.

Erythrocytes:—Studies by previous workers (3 and 6) have shown that the number of erythrocytes both in the human as well as in animals decrease with age, and this appears to hold good in the case of buffaloes also as revealed from the data obtained during the present investigation. The mean erythrocyte counts for calves under one year averaged 5.88 millions per cubic millimeter, and the value decreases as the age advances, calves of the age group between 1 and 2 years giving an erythrocyte count of 5.10 and the animals of the two adult groups, *viz.*, working animals and lactating animals a count of 4.20 and 3.22 respectively. It is further interesting to note that there exists a considerable difference in the counts between the two groups of older

TABLE I
Blood cellular elements.

S. No.	Animal	No. of samples examined	R.B.C. in millions/Cmm.		W.B.C. per Cmm.		Hb. Grams/100 ml.		P.V.C. per cent		E.S.R./mm. per hour	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	Buffaloe calves under one year	30	5.88	+ 0.29	12917	+ 936	11.84	+ 0.57	36.96	+ 1.12	1.94	+ 0.11
2	Buffaloe calves between 1 and 2 years	20	5.10	+ 0.86	14620	+ 824	11.58	+ 0.55	36.88	+ 1.26	1.76	+ 0.09
3	Working Buffaloe bullocks	20	4.20	+ 0.2	10460	+ 866	11.33	+ 0.47	35.72	+ 1.02	1.69	+ 0.07
4	Lactating she buffaloes	20	3.22	+ 0.30	11220	+ 1102	11.55	+ 0.39	35.58	+ 1.05	1.74	+ 0.06

TABLE II
Differential leucocytic count.

S. No.	Animal	No. of samples examined	Monocyte %		Polymorph %		Basophil %		Lymphocyte %		Eosinophil %	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
1	Buffaloe calves under one year	30	6.30	± 0.80	29.10	± 1.60	0.57	± 0.15	62.60	± 1.76	1.70	± 0.33
2	Buffaloe calves between 1 and 2 years	20	7.40	± 0.85	35.15	± 1.09	0.75	± 0.32	53.40	± 1.23	0.55	± 0.19
3	Working Buffaloe bullocks	20	6.25	± 0.89	39.55	± 1.17	0.65	± 0.70	49.90	± 1.56	3.60	± 0.55
4	Lactating she buffaloes	20	5.20	± 1.12	38.60	± 5.23	1.10	± 0.48	52.00	± 2.34	2.60	± 0.67

animals, the values for lactating buffaloes being lower than those for the working bullocks. The erythrocyte counts reported for buffaloes by Coffin (2) and Kehar and Murthi (5) are much higher than the data obtained during the present studies.

Erythrocyte Sedimentation Rate:—Ferguson (4) reported that in ruminants there is usually little or no settling in one hour. A sedimentation rate of 0.9 mm. for the first hour has been reported in camels by Bhatt and Kohli (1). Raghavan and Gaffer (7) have reported a sedimentation rate in buffaloes ranging from 0.4 to 7.0 mm. per hour, while, during the present investigation, the rate is found to be 1.8 mm. the first hour on an average.

Total White Blood Cell:—Coffin (2) has reported a mean W.B.C. count of 8200 per cmm. for buffaloes. Comparatively higher values were obtained in this study (*vide* Table I) with essentially no variation between the different age groups.

Packed Cell Volume:—The values obtained on the packed cell volume during the course of this investigation are comparable with the data reported by Kehar and Murthi (5).

Differential Count:—The data are set out in Table II. The results are more less the same as those obtained by Coffin (2).

Summary

A systematic investigation to determine the normal constituents of blood of buffaloes of different ages and sex was carried out and the results obtained on Hb, RBC, WBC, ESR, PVC and Differential Counts are reported. It is found that while values for Hb and PVC are more or less in agreement with the data furnished by previous workers, the RBC count is lower and WBC count higher than the reported values. In comparison with other ruminants, a slightly higher ESR is noted in buffaloes. A significant difference is noticed between the working buffalo bullocks and the lactating animals in their erythrocyte counts.

Acknowledgment

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A CASE OF INFERTILITY IN A BULL

By

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Infertility or lowered fertility in bulls had been frequently overlooked as this condition is not often readily revealed in them as it is in the case of females. Consequently the incidence of infertility in males had long been considered to be of a rare occurrence. During the course of an investigation into the probable causes of early disposal of 2,313 bulls belonging to the Swedish bull Societies, it was found that nearly 24% of the animals were discarded for "reasons of sterility of one form or other", (Lagerlof, 1936). Infertility appears to be as common in the males as in the females.

This report describes studies carried out on an apparently normal Sindhi bull maintained in the Livestock Farm, Ollukkara, which had shown a decreased fertility rate during the preceeding years.

History of the Bull

Name	... Chidambaram, No. 183.
Date of birth	... 30-10-1950.
Sire	... Sekharan No. 46.
Dam	... Yasodhara, No. 15.

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The bull was born and brought up at the Government Livestock Farm, Ollukkara. It was issued to the Fruit and Vegetable Farm, Ollukkara on 4-2-1952 and subsequently transferred to the calf-rearing centres at Nelliampathi. It was brought back to the Fruit and Vegetable Farm, Ollukkara on 9-3-1954 and was transferred to the Government Livestock Farm on 10-8-1954. On 16-4-1956 it was transferred to the Key Farm Centre, Ollukkara, and was again brought back to Government Livestock Farm on 4-7-1959. Details of the breeding performance of the bull immediately prior to the present investigation are recorded in Table I.

TABLE I
*The percentage of conceptions effected by the Bull No. 183
during 1957-59.*

Year	No. of Inseminations	No. of Conceptions	Percentage	Dilution ratio of semen.
1957-58	1287	469	36.52 %	1:10 — 1:15
1958-59	518	169	32.62 %	1:10 — 1:15

Observations

The bull under study was docile with growth normal to the age. It showed no sign of any disease of digestive disorders, and its general condition was satisfactory. It lacked exercise and though not fatty, was sluggish. Gait was normal and no ectoparasitic infestation was noticed.

Secondary sex characteristics were observed to be not well developed. The bull was said to have had sex desire but at the time of investigation it showed poor sex drive and serving ability was found to be far from satisfactory. Its interest in cows even when they were in oestrus was markedly poor and at times it took more than 30 minutes for mounting. The thrust also was sometimes very poor.

On clinical examination, the right scrotum was found to be empty. The left testicle was normal in size with consistency slightly harder than normal. No adhesion could be detected in the left scrotum and the testicle was freely movable in its tunica vaginalis. The epididymus was normal. No abnormalities were noted in the prepuce and the penis.

On rectal exploration, the accessory sexual glands were found to be normal. The right seminal vesicle and ampulla were slightly larger than those of the left though this observation was of no significance. The animal seemed to evince no pain during the rectal examination.

Examination of Semen

Semen was collected for examination in a Swedish pattern Artificial Vagina maintained at a temperature of 45-48°C. Whenever possible two ejaculations were collected, in which case the same artificial vagina was made use of with separate cones and collection vials.

Results

The results of examination of semen samples of bull No. 183 made during the present studies are set out in Table II. Results obtained in an earlier investigation in respect of the same animal are given in Table III.

Discussion

The history of the bull under investigation reveals that the animal was showing a low fertility rate during 1957-58 and 1958-59, the conception percentage during the two years being 36.5 and 32.6 respectively. Even after giving allowance for the defect in the technique of artificial insemination, so low a conception rate should not ordinarily be expected from a normal bull. Besides another bull used along with this for artificial insemination under identical conditions had returned a conception rate of 56.3% during 1957-58 and 55.6% during 1958-59. It is thus clear that it was the bull that was responsible for the low conception rate and not the artificial insemination technique.

According to Lagerlof (1936) lowered fertility in bulls occurs in three forms ;—

- (1) non-existence of sex desire,
- (2) incapacity to copulate, and
- (3) incapacity to fertilize.

As far as the bull under investigation is concerned, the first two possibilities can be ruled out as it had served the cows, although with a slightly diminished sexual desire. The lowered fertility rate shown by this animal is probably due to the 3rd factor viz., incapacity to fertilize (*Impotentia generandi*) and this may be due to insufficient number of functional spermatozoa passing through the ejaculate (Lagerlof, 1936) or due to a low concentration of sperm (Haq, 1949 & Lagerlof, 1934). As the bull had only one testicle descended, decreased number of sperm was likely due to the nonfunctioning of the undescended testicle (Roberts, 1956).

Bell (1956) has observed that unilateral cryptorchidism is a condition more commonly encountered than bilateral cryptorchidism in all species of animals, and in cattle, as reported by Roberts (1956), it is due to an inherited nonlethal factor.

TABLE II
Data on collection and examination of semen of bull No. 183 during the present investigation.

No.	Collection Date of collection	Volume in c.c.	Colour	Density	Mass acti- vity	Motility	pH	Concen- tration of sperms per Cmm.	Sperm abnor- malities (%)	Comments
1	10-7-1959	(i) 3.1	Yellowish white	DD	++	50% shows progressive movement	6.2	4,84,000	6%	A white country heifer in heat was used for the bull to mount and it took 20 minutes for the first mount and an interval of 10 minutes before the 2nd. Both the thrusts were satisfactory.
		(ii) 3.4	do.	DD	++	do.	6.2	4,95,000		
2	15-7-1959	(i) 2.5	do.	DD	++	do.	6.4	4,82,500	8.3%	A brown country cow not in oestrus was used for the bull to mount and it took 35 minutes for the mount. Thrust was not satisfactory.
3	22-7-1959	(i) 3	Yellow	D	+	25% shows progressive movement	6.4	2,54,000	5.7%	A local cow in oestrus was used for the bull to mount and it took 30 minutes for the mount. Thrust was very weak.
4	29-7-1959	(i) 4.2	Creamy white	DD	++	60% shows progressive movement	6.2	6,10,000	7.5%	A brown Jersey Sindhi cross cow in heat was used, and it took 15 minutes for the bull to mount. Thrust was forceful; interval between the 1st and 2nd ejaculation was 5 minutes.
		(ii) 4.5	do.	DD	++	do.	6.2	5,80,000		
5	5-8-1959	(i) 3.2	Yellowish white	D (D)	+(+)	30% shows progressive movement	6.0	3,10,000	6.4%	A country heifer not in heat was used for the bull to mount, and it took nearly 35 minutes for the mount. Thrust was satisfactory.

TABLE III
Data on collection and examination of semen of bull No. 183 in an earlier investigation.

Date	Collection		Volume in C.C.	Density	Mass activity	Motility	pH	Sperm concentration per Cmm.
	Num-ber	Ejaculate						
20-12-1953	1	(i)	2.1	DD	++	50 % shows progressive movement	6.5	6,00,000
		(ii)	2.5 c.c.	DD	++	60 % shows progressive movement		
26-12-1958	2	(i)	0.5 c.c.	DD	+	25 % shows progressive movement	6.5	5,75,000

In unilateral cryptorchidism, the sperm concentration is reduced to half, the fertility usually remaining normal especially when natural breeding is attempted (Roberts, 1956). The non-functioning of the undescended testicle is supposed to be not due to any inherent defect, and spermatogenesis occurs if the testicle is brought to the scrotum surgically before puberty, as reported by Bell (1956).

Even though fertility can sometimes be normal in unilateral cryptorchid, it is not advisable to use such animals for breeding purposes as there appears to exist a definite inheritance of the condition in domestic animals. The undescended testicle is also prone to tumours. It is also reported (Roberts, 1956) that the occurrence of this condition increases difficulty in castration, reduces the quality of carcasses, and decreases the value of pure bred animals. Treatment for the correction of the condition in animals is not desirable in view of its hereditary tendency.

Bull that is responsible for the 50% of the destiny of the future herd can, if of low fertility, cause a great deal of loss by producing disturbances in the periodicity of calving of a number of cows. When the lowered fertility is of a hereditary nature, the loss sustained is severe and far extended. Hence, before selecting a bull for breeding purposes, especially by Artificial Insemination, a detailed investigation into its breeding efficiency as judged by its semen qualities should also be carried out.

Summary

Studies carried out with the semen samples collected from an apparently normal Sindhi bull which had shown a decreased fertility rate during 1957-59, are reported and the importance of examining a breeding bull for fertility has been discussed.

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TREATMENT OF ASCARIASIS IN YOUNG CALVES WITH PIPERAZINE CITRATE

BY

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Since several years, treatment of young calves especially buffalo calves against ascariasis, has been a very serious problem amounting to tremendous losses of valuable young stock of private farms as well as different Livestock Breeding Farms of this State. The position in other States of India, it is believed, might be more or less the same and the inquiry in the field indicates that calf ascariasis is pretty common. It has also been observed that most of the buffalo calves in different Livestock Breeding Farms as well as in villages of Orissa, succumb to ascariasis within 2 to 3 months after birth, so that the rearing of these calves becomes a very difficult problem besides the loss of valuable progenies and losses through decreased milk yield. Treatment with several indigenous as well as well-known chemotherapeutic agents like *Scandipsus indica* (Oriya name - Gajapepla), *Butea frondosa* seeds (Oriya name - Polasmanji), Bland oil, oil of terebinthinae, Creosote, Tetrachlorethylene and 1: 8 dihydroxyanthraquinone, either alone or combined with one or more of these drugs, has not yielded any encouraging result in this country or elsewhere to the knowledge of authors. During recent years, considerable success has been achieved by use of 'Hetrazan' (diethylcarbamazine) against several parasitic diseases, including ascariasis, following its widespread use in man.

Das and Singh (1955) gave a trial to this therapy in young buffalo and cow calves in this State and reported very good results on the efficacy of this drug. Simpler piperazines (citrate and adipate) are now used widely to treat ascariasis in sheep (Gordon, 1957 a), horses (Solan *et al* 1954; Behrens, 1957) and dogs and cats (Solan *et al* 1954; Gurlap, 1957; Sprent and English, 1958). In calves, piperazine adipate has been claimed to be 100% effective in eliminating mature and immature ascarids when administered at a dosage of 100 mg. per lb. body weight and good results have been obtained from prophylactic administration of the same drug in a single dose to calves aged 21 days (Lee, 1955, 1956). This paper puts on record the results of treatment of calves against ascariasis with a simple piperazine *viz.*, *Piperazine citrate*.

Materials and Methods

Twenty nine buffalo and 9 cow calves, one to three months old, of the State Livestock Breeding Farm, Khapurja (Cuttack), were

selected for the trial. These calves had very heavy infestation with *Ascaris* as judged by repeated examination of faecal samples using the direct as well as sugar floatation techniques. In some instances, the egg counting technique described by Lapage (1956) revealed about 350,000 to 400,000 ova per gram of faeces. Clinical signs in the form of loose motion, anorexia and progressive emaciation were apparent in all the calves. Piperazine citrate (Antepar Elixir proprietary product of Burroughs Wellcome) was administered after a light feed in the morning in a single dose of 100 mg. per lb. body weight on the lines described by Lee (*op. cit*) followed by a full dose of Mag. sulph. 24 hours after medication. The calves were kept tied individually in a shed and were allowed to take their food as usual. Daily examination of the faecal samples was carried out for 10 days on the lines described earlier. Further, the voiding of worms through faeces, side effects of the drug, if any, and the general condition of the calves were carefully observed keeping another 20 untreated calves of the same farm as control.

Results

Antepar Elixir could be administered with ease to all the calves. Following treatment, large number of *Ascaris* were found to be expelled in 48 hours after medication and continued upto the 4th day. Examination of faecal samples proved positive for *Ascaris* ova; maximum upto the 7th day, though in 14 calves they were negative on the 5th, in 4 calves on the 6th day and the rest of the samples revealed rare ova on 7th day, but were negative on the 8th day. Repeated examinations for another two days resulted in negative findings with all the faecal samples. Following expulsion of worms, the calves looked much brighter, the appetite was improved and the rugged skin coat was looking more glossy and shining. There was no mortality among the calves treated and by about 21st day, these had gained sufficient weight well comparable to the normal body weights of calves of their age. It was interesting to find that side effects so commonly seen after administration of several classical antinematodial drugs in the form of colic, anorexia, alopecia, etc., were absent in all the calves under trial.

Discussion

The results of the above trial against ascariasis in young calves, including buffalo calves, have convinced the authors to adopt as a routine measure the single dose therapy with Antepar Elixir at the dosage of 100 mg. per lb. body weight with unequivocal results and it is strongly felt that the drug should be given an extensive trial in all places where calf ascariasis is often encountered as a serious menace.

In a similar trial with Hetrazan, Das and Singh (1955) contended that the drug proved to be the most efficacious in their hands, when compared with other drugs available then, resulting in 96% recovery of affected calves. The present investigation, has, however shown that with Antepar Elixir, the percentage of recovery may go even upto 100%, a finding that is in accordance with earlier observations of Lee (1955) who used *Piperazine adipate* whereas *Piperazine - citrate* was used in the present study. This is substantiated by the fact that, surprisingly, the piperazine base and its simpler salts appear to be more effective than the complex and substituted derivatives (Meyer Jones, 1957). The complex piperazine-1-carbodithioic acid, which breaks down in the stomach to *Piperazine* and *Carbon bisulphide*, has anthelmintic effects similar to the simpler compounds (Gordon, 1957). But if this breaking down process is either incomplete or absent, the results will not be comparable with those of simpler salts, which are quickly absorbed from the stomach of the animal. A single dose therapy has the advantage of less effort on the part of the physician or veterinarian and, the drug being relatively nontoxic, can be easily administered by the average calf owner himself, when necessary, while with Hetrazan, divided doses for 4 to 5 days have been recommended. Some of the side effects seen in Hetrazan therapy (Das and Singh, 1955 *op. cit.*) have not been encountered with *Antepar*. From the average calf owners' point of view and for the treatment of large number of calves under farm conditions, treatment with *Antepar* is decidedly cheaper (approximately Rs. 2/- with *Antepar* as compared to Rs. 3/- with Hetrazan for a full course), a fact that has to be considered in treating animals.

Summary

Treatment of 38 young calves under controlled conditions with *Piperazines citrate* (*Antepar* Elixir) against heavy infestation of *Ascaris* has resulted in 100% recovery as judged from faecal sample examination and improved general condition in all the calves under treatment. A single dose therapy of 100 mg. per lb. body-weight has been found to serve this purpose. The administration of the drug requires no prior preparation of the calves nor its use was followed by systemic disturbances. The treatment was found to be cheaper than Hetrazan therapy. The role of the simpler *Piperazine* in the treatment of ascariasis in young calves, has been discussed and the usefulness of *Antepar* as a potent ascaricidal drug has been confirmed.

Acknowledgment

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SOME NUTRIENTS FOR OPTIMUM HATCHABILITY

By

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Today we know that the efficient conversion of feed into muscle, fat, blood, and bones is an extremely complex process. For a successful business, the chickens must be fed adequately to be able to produce and reproduce, and at the same time maintain good health through the avoidance of pathological conditions resulting from nutritional deficiencies.

A feed may contain the necessary level of protein and energy (carbohydrate, fat and be sufficiently low in fiber), yet still be nutritionally deficient. If it does not contain adequate amount of certain vitamins and minerals, feed utilization may be greatly impaired,

growth rates reduced, and egg production and hatchability poor. Since birds eat only what they are given or are allowed to find, poultryman should have the responsibility for making sure that the breeding hens get the necessary amount of critical vitamins and minerals for optimal hatching.

Differing from mammals, which nourish their embryos inside their bodies, give birth to the living young, and nurse them, hens produce eggs which should contain sufficient nutrients for the embryo to develop outside the body with very little preformed food. It is, therefore, desirable to see that following critical nutrients are present in the food for breeding hens in order to support optimum hatchability.

Vitamins

The greatest difference between the ordinary laying feed and feed for breeders is in the vitamin levels. Of the vitamins which are essential to good hatchability, riboflavin is usually the most critical. The minimum requirement of the riboflavin for the laying hen is one milligram per pound of feed.

For the breeding hen, the requirement increases by 70%, to 1.7/mg. lb. of total feed. A level of two milligram of riboflavin per pound of breeders' diet provides a slight safety factor and is considered satisfactory. Thus the requirement for hatchability is considerably higher than that for maintenance of health and egg production.

In breeding birds, a deficiency of riboflavin results in poor hatchability. The embryos that fail to hatch as a result of riboflavin deficiency are dwarfed and show a high incidence of edema, degeneration of the Wolffian bodies, and a characteristically defective down development, termed "clubbed" down. On a ration moderately deficient in riboflavin, many embryos die during the second week of incubation. The mortality usually reaches a peak about the eleventh day of development.

Vitamin A is another critical vitamin required for hatchability. Ample vitamin A in the breeders' diet is of great practical significance. The reason is the inter-relationship between vitamin A and parasitic infestations such as coccidiosis, round worms, and capillaria worms. A number of cases have been reported in which hatchability has been low, and even more important, young chick viability has been poor. The factors responsible for these troubles are the intestinal parasites which have been cited above. The birds generally respond to high level of vitamin A in the diet. Levels of vitamin A up to 8000 I.U. per pound of feed have been very effective in reducing mortality and morbidity. The researchers at the University of Maine reported that capillaria worms are responsible for platinum or light colored egg

yolks. In most cases, the capillaria infested flocks do respond to a high level of vitamin A treatment. The N. R. C. requirements and the suggested levels of Vitamin A and other nutrients have been shown in the following table.

Table showing N.R.C. Requirements and suggested Levels of certain Nutrients in the feed of laying and breeding hens.

	N.R.C. Requirement		Suggested Level	
	(National Research Council, U.S.A.)		(Univ. of Maryland, U.S.A.)	
	Layers' ration	Breeders' ration	Layers' ration	Breeders' ration
Riboflavin, mg./lb.	1.0	1.7	1.3-1.6	1.7-2.0
Vitamin A, I.U./lb.	2000	2000	3500	4000
Vitamin D ₃ , I.C.U./lb.	225	225	400	500
Pantothenic acid, mg./lb.	2.1	4.2	3-4	5-6
Pyridoxine, mg./lb. (Vitamin B ₆)	1.3	1.3	1.5	1.8
Vitamin B ₁₂ , mcg./lb.	?	2.0	1.5	3.0
Vitamin E, I.U./lb.	?	?	?	5-6
Manganese, mg./lb.	?	15	15-25	25-30
Zinc, mg./lb.	?	?	5-10	5-10
Calcium, %	2.25	2.25	2.25-4	2.25-4**
Phosphorus, %	0.6	0.6	0.7	0.7

**To get a good estimate of the requirement of calcium, use of the following formula is advisable:

$$\text{Percent of calcium required in feed} = \frac{12}{\text{Pound of feed per doz. of eggs}}$$

Vitamin D, also known as "sunshine" vitamin, helps animals absorb minerals such as calcium and phosphorus which are necessary for good hatchability. Chickens can utilize vitamin D₃ only. Deficiency of this vitamin in breeders' diet will reduce hatchability. In mature laying birds, the first symptom of deficiency is the laying of thin-shelled eggs, followed by other disorders due to such deficiency. Such eggs are not good for hatching; thus hatchability is markedly reduced.

Pantothenic acid plays an important role in chick hatchability. Also, it is necessary for livability in case of young chicks that hatch out. A field case of a pantothenic acid deficiency in breeders' diet has been reported by Dr. Hans Fisher, Rutgers University. He observed that the chicks hatched from the pantothenic acid deficient dams, responded rapidly to pantothenic acid supplementation.

Vitamin B₆ deficiency in mature birds is characterised by loss of appetite, followed by rapid loss of weight, loss in egg production and

marked reduction in hatchability. However, field cases showing the deficiency of this vitamin have not yet been reported.

Biotin has also been found useful along with other vitamins. In the mature fowl, hatchability is decreased by its deficiency.

The vitamin B₁₂, the "youngest" of our vitamins, is also critical in securing top hatchability. Practically all modern breeder formulas are supplemented with a high level of vitamin B₁₂. Deficiency of this vitamin in the breeders' diet, causes marked reduction in growth and hatchability. There is a marked carry over of this vitamin from the breeding hen to the young chick and mortality may be high among chicks deficient in this factor at the hatching time.

Vitamin E, sometimes called "the reproductive vitamin" is also known to play an important role in hatchability. It is especially necessary to the turkey breeder. It has been observed that a vitamin E deficiency greatly slowed down the rate of embryo development. It is especially important for the early embryo development. In cases where this deficiency exists, the rates of growth and differentiation during incubation are slow, and many embryos die during the first two days of development because of a circulatory failure. A definite critical period in the development of the embryo occurs about the fourth day. Late research at Washington (Pullman) has shown that vitamin E is essential in the normal development of the chick embryo to the stage of hatching and is involved in viability of the chicks once they are hatched. In some studies, it has been found that antioxidants were able to overcome part of the deleterious effects of unsaturated fat on hatchability. It is probable that antioxidants were functioning by preventing the oxidation of natural vitamin E in the diet. The presence of vitamin E, an antioxidant itself, in the feed lessens the destruction of vitamin A.

Minerals

Certain minerals are very important to hatchability. Manganese tops the list. In laying and breeding birds, a manganese deficiency results in lowered egg production, egg shell strength and hatchability. Chick embryos which die from a manganese deficiency can sometimes be spotted by close examination. They have a parrot-like beak. The "down" is wiry. The long bones are shortened.

It has been found at the University of Maryland very recently that zinc also plays a vital role. Zinc deficiency in the diet of breeding hens resulted in lowered hatchability. Many of the dead embryos were found on examination to have a variety of malformations. The chicks which hatched out were found to be very weak.

Calcium and phosphorus are also required for good hatchability. We know that the laying hen requires about 2.25% calcium and about

0.6% phosphorus to form a good sound egg shell, and to hatch good healthy chicks. Late research shows that the requirement of calcium varies markedly. Presence of 3.4% calcium in the breeders diet has given good results. To get a good estimate of the calcium requirement, one may use the formula shown in the table. A whole egg contains about 3.5% of calcium mostly in the shell and about 0.2% of phosphorus, mostly in the yolk. Deficiency of these minerals would result in lowered egg production, thin shelled eggs, and consequently reduced hatchability.

Despite this already rather imposing list, there is reason to believe that other factors might remain to be discovered which are required critically for optimum hatchability.

PREVALENCE OF LUNGWORMS—*Dictyocaulus viviparus* AMONG CATTLE IN HESSARAGHATTA FARM, BANGALORE

By

A. MALAKI, B.Sc., M.V.Sc.,

Lecturer, Mysore Veterinary College, Hebbal, Bangalore-6.

Introduction

Hessaraghatta is a small village situated at a distance of sixteen miles to the North of Bangalore, where Government have established a Composite Livestock Farm and Research Station. There is a tank in the middle of the farm. It enjoys a cold and humid climate and seems to be highly favourable with the optimum temperature and moisture requirements for the development of the larval stages of lungworms.

Epizootiology

It is interesting to note here that lungworms which were hitherto believed to be confined to certain hilly tracts of Northern India have successfully adapted themselves to this new environment, causing heavy mortality both among young and old stock. Attempts have been made to know the incidence of infection prevailing round about in the villages. Parasites though enzootic in their nature, can become endemic, the prerequisite factors being warmth and moisture conditions with plentiful supply of oxygen. Thus well aerated waters particularly are favourable. The optimum temperature ranges for the development of lungworm larvae is 20 to 30°C, i.e., a temperature lower than that for larvae of gastrointestinal nematodes. Lungworm larvae withstand colder temperatures better than gastrointestinal worms. The infective larvae, protected as they are by a couple of extra sheaths

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derived from the two preceeding moults are quite resistant to many an adverse condition, such as, heavy drafts, cold currents and dessication. Cobbold (1875) was able to keep the larvae alive in moist earth for more than five months. In the course of routine post-mortem examination of the Farm animals we came across a few animals infested with lung abscesses, whose investigation revealed the presence of a few lung worms, hence the report from this locality.

Clinical Symptoms and Lesions

A few young and old emaciated animals died in the Farm. Their history revealed that the animals died of acute shooting diarrhoea and persistent cough. On post-mortem examination the lungs showed varying degrees of consolidation and collapse of the lobes of the lung tissue. Bronchi and bronchioles were foamy in appearance giving an indication of the presence of lungworms. Bronchial abscesses with a mass of highly strangulated silky white worms were recovered from the lesions and the worms were identified on morphological features as *Dictyocaulus viviparus*. On teasing them in petridishes containing saline solution, the whole dish became flooded with white granular material. On examination they were found to be embryonated eggs of the species as the worms were all oviparous. The male worms were less in number as compared to female ones.

Conclusion

Bhalerao (1935) described *Varestrongylus pneumonicus* in sheep and goat from Mukteswar and *Dictyocaulus unequalis* in Tibetan Sheep. Srivastava (1940) described *Protostrongylus* from Indian buffaloes. Mohan (1948) recorded *Dictyocaulus viviparus* in cattle, *Dictyocaulus filaria* in sheep, *Varestrongylus pneumonicus* in goats and *Metastrongylus pneumonicus* in pigs from Bengal. Khera (1954) also recorded *Varestrongylus pneumonicus* from sheep and goat from Mukteswar. Though often they have been reported from different parts of Northern India, it is still considered to be a parasite of hilly tracts of Himalayan border. But it is quite clear that the parasites in question have become endemic in their nature as the climatic factor seems to be more highly favourable for their existence in this locality. Therefore they necessarily warrant further investigation in the direction of their bionomics, treatment and control.

Acknowledgment

Thanks are due to Sri G. A. Hardiker, G.B.V.C., P.G. (Muk.) present acting Director of Animal Husbandry and Veterinary Services in Mysore, Bangalore for kind permission to carry out the work in Mysore Serum Institute, Hebbal and to Dr. R. D. Nanjaiah, B.Sc., G.M.V.C., B.V.Sc., Ph.D. (U.S.A.) Principal, Mysore Veterinary College for his kind advice in preparing this article.

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Clinical Article

INGUINAL HERNIA IN A DOG—A CASE REPORT

BY

PROF. M. N. MENON, SRI A. VENUGOPALAN, SRI P. O. GEORGE
and

SRI K. V. RAPPAL,

Department of Surgery, Kerala Veterinary College, Trichur.

- Subject:** Dog (Rollo) Alsatian - three-year-old.
- Date of admission:** 20th December 1959.
- Clinic:** Veterinary Hospital, Trichur.
- History presented:** Health indifferent - appetite erratic; bowels constipated - faeces passed in pellets with severe straining efforts - was under treatment at Munnar, for a swelling on the right side of the anus - An attempt was made earlier to cut open the swelling mistaking it for an abscess.
- Observation:** The swelling on the right side of the anus was persistent and the anal sacs were found to be distended. Digital examination per rectum revealed enlargement of the prostate.

The left side of the sheath in front of the pubis presented a fluctuating soft swelling of a reducible nature, with a rupture at the level of the inguinal ring. The animal was observed to make unsuccessful attempts at defaecation making severe straining efforts.

- Diagnosis :** Left lateral inguinal hernia with prostatic enlargement and cystic distension of the anal sacs.
- Treatment :** A glycerine enema was given. Impacted masses of foul-smelling faecal matter had to be removed manually before the enema was effective. The distended anal sacs were evacuated by pressure on the sides of the anus. The emptying of the rectum caused the swelling on the side of the anus to disappear almost completely.
- A dose of 15 mgms. of stilboesterol dipropionate in oil was administered intramuscularly, which was repeated thrice on alternate days. Glycerine enema was repeated daily. Later though defaecation became voluntary, proper cleansing took place only after enema. As the general condition of the patient had since improved, radical treatment was decided upon and the operation was posted for 2nd January 1960.
- Anaesthesia :** A basal anaesthetic dose of Morphine $\frac{1}{4}$ grain in 1 c.c. with Atropine sulphate 1/100 grain in 1 c.c. was first administered subcutaneously. This was followed by an intravenous injection of Nembutal as a 4% solution in sterile distilled water administered at the rate of one c.c. per minute. The patient was safely under with only 7 c.c.s. of solution. Endotracheal intubation was carried out as usual to ensure a patent air way.
- Site of operation.** Left side of the sheath and the area in front of the scrotum was shaved, washed, dried, and painted with 1% solution of acriflavin in 70% alcohol, and the area properly draped with surgical towels.
- Operative Procedure.** An incision, three inches long, was made parallel to the sheath over the hernial swelling and sac to expose the contents. The intestinal loops which formed the hernial contents were seen to be adherent to the hernial sac. It was found that the external inguinal ring was torn antero-laterally. The tear had to be enlarged and the adhesions released before the contents could be restored to the abdominal cavity. The defect which gave passage for the prolapse of viscera was closed by a continuous 00 chromic catgut suture, after dressing the site with crystalline penicillin powder. The suture line was reinforced in addition by interrupted relaxation sutures. The skin incision was closed with a series of interrupted vertical mattress sutures of silk, leaving the

anterior angle free for drainage. A protective bandage was applied for safety and the endotracheal tube was removed before the patient was transferred to the kennel. There was a certain amount of blood loss by capillary oozing, chiefly consequent on the teasing and release of adhesions.

Protective treatment with Calcium gluconate 10% - 10 c.c. i/v, Glucose saline 10% - 20 c.c. s/c, penicillin 4 lakhs i/m, and Coagulen Ciba 5 c.c. s/c were given. The animal recovered from the anaesthetic effect by the evening only. Normal Saline 100 c.c. per rectum, and 40 c.c. s/c, and glucose saline 5% - 30 c.c. i/v, were then administered. For three days subsequently 4 lakh units of Penicillin was given intramuscularly with Vitamin C 100 mgm., orally at one tablet T. I. D. Temperature remained normal during this period and the animal was fed on milk and eggs. Slight post-operative oedema was noticed at the site of operation. Mag. Sulph in glycerine as a paste was applied daily over the oedematous region with a dressing of Penicillin ointment. On the fourth day of operation, the temperature showed a sharp rise to 104°F. and the pulse rate to 152. Motions were dark in colour with clots of blood and mucous and faecal samples revealed severe ancylostome infection. With a view to obviate secondary complications oral treatment with Achromycin and B Complex was given.

On the eighth day of operation, temperature came down to 103°F. Examination of blood smears did not reveal any protozoan infection. But the differential count showed pronounced neutrophilia (83%). To counteract any allergic haemorrhagic enteritis, Phenergan was administered by mouth thrice a day.

On the following day i.e. on the ninth day of operation, a temperature of 102°F, was recorded. The faeces was semi solid without blood. A few ancylostome eggs were still present. Even though the general condition of the animal was far from satisfactory it was decided to risk treatment for ancylostomiasis with Tetrachlorethylene followed by a dose of Mist Alba. As the condition of the animal was found to be deteriorating and as it showed pronounced anaemic symptoms, further medication with Osteocalcium 2 tablets, Ferroncum 1 tablet, and B.

Complex 2 tablets in addition to glucose intravenously was resorted to.

On the tenth day of operation the colour of the faeces still continued to be dark and it was mixed with clots. The skin wound, on account of constant movement did not heal properly and the sutures were also bitten off by the patient. This gaping wound was closed by quill sutures after scarifying the edges.

The next day, temperature again shot upto 103.8°F. accompanied by acceleration of pulse and respiration. The mucous membrane continued to be pale and anaemic. Blood examination revealed an R.B.C. count of 2.29 million/cmm. and a haemoglobin content of 6.9 gm./100 c.c.

Phenergan was repeated on the subsequent days. Dicrysticin injection 1 vial i/m and Ferronium tablets orally were also given daily, combined with Liver extract with Vitamin B - Complex 2 c.c. injections i/m. The diet consisted of soup and cooked liver. The wound was now slowly beginning to granulate satisfactorily.

Marked improvement was noticed by the sixteenth day of operation, the temperature becoming normal and the animal alert. Appetite had also improved and it was allowed rice, meat and fish as diet.

The quill sutures were then removed. Injections of Liver extract 2 c.c. i/m were continued for two more days. By now the swelling at the site of operation had almost disappeared. Defaecation was voluntary though slight straining was still noticeable. The animal was discharged as cured on 19-2-1960 at the owner's request. In order to prevent constipation, a small dose of salines was prescribed as a daily routine for some days. From subsequent reports it is understood that the dog is well as ever.

Summary: A neglected case of inguinal hernia in a dog and its subsequent treatment is reported.

ADVERTISEMENTS

Abstracts

Tissue Therapy in Animal Husbandry:—If we take a plant and keep it in the dark for several days, it will produce substances more active biologically than those in the leaves of a plant left in the daylight. If a patient is given a dose of the preparation made from such leaves, his activity or resistance to diseases will increase. The same applies to living tissues of man and animals.

It was Academician V. P. Filatov who, some thirty years ago, came out with the idea of using tissue preparation for treatment purposes. He came to the following generalizations: any tissue of man, animal or plant, when detached from the parent organism and kept under conditions adverse to it but not killing it, goes through a biochemical transformation and produces specific agents—biogenic stimulants which increase the vital activities of the organism into which they are introduced. This was the beginning of using biogenic stimulants in medicine. They are effective against many diseases. From the Soviet Union biogenic stimulants have found their way into other countries of the world.

Significant successes have been scored in the experimental application of biogenic stimulants in animal husbandry. In the Chernovitey Region, for example, tissue preparations were administered to thousands of pigs and cows. As a result, the growth of the swine herd went up by 15 to 20 per cent. The use of tissue preparations during the past year brought in additionally 800 tons of meat in the Odessa Region, 572 tons in the Crimean Region, and over 200 tons in the Kharkov Region.

Last year injections were given to about 1,000,000 pigs and over 90,000 cows in the Ukraine. Biogenic stimulants are also used on a large scale in the other republics—the Russian Federation, Georgia, and the Baltic Republics. In Moldavia tissue preparations helped to produce additionally 32,000 centners of pork during a year, which is an equivalent to over 80,000 head increase in the pig herd.

The miraculous stimulants also enhance the immunity of livestock to various epidemic diseases.

Biogenic stimulants have started to be produced on a mass scale for animal husbandry. Some 80 tons of them have been made in the Ukraine alone. They are injected into animals five or six times at intervals of 8 to 10 days.—Information Bureau, USSR Embassy, New Delhi.

A. C. AGGARWALA.

The Discovery of a New Method of Diagnosing the African Swine Fever:—A major contribution to more successful control or eventual eradication of African Swine Fever (ASF) is the recent discovery of a laboratory method of diagnosing this deadly disease. The method of diagnosis has been developed during continuing United States Dept. of Agriculture research in Africa to find more effective control for ASF. The studies showed that ASF virus will grow on swine blood culture or bone marrow, and cause red corpuscle clumping and cell death. Swine cholera virus does not produce a like effect. Previously there was no positive laboratory method to differentiate between the two viruses. Although the scientists have not yet been able to develop an effective vaccine against ASF, but their efforts in this field are very much encouraging.

(Ag. Research, February, L. 1961).

B. PANDA.

Feeding of early-cut silage increases milk yield:—Professors S. T. Slack and Keith Kennedy of the New York State College of Agriculture at Cornell University found in three years of experiments that cows produce an average of 16 lb. more milk a day if fed the early-cut silage diet. Their experiments were carried out on pure-bred Holstein and Brown Swiss cows. Cows fed the early-cut silage gave slightly more than 50 lb. of milk a day. Those fed early-cut barn dried hay produced 44 lb. and those on the late-cut field cured hay diet produced less than 35 lb. daily.

(Feed Age, Dec., 1960).

B. PANDA.

Balantidiosis in Cattle (*Bos indicus*) in Orissa:—BY PATNAIK, B. (1960). *Indian J. Vet. Sci.*, 30, 215-218.

The author detected trophozoites of *Balantidium coli* var *bovis* in the dung of 60 cattle suffering from chronic dysentery. The animals were passing foetid diarrhoeic stools with mucus and blood. The absence of any helminthic or other protozoan infection and the presence of a large number of these ciliates pointed out the association of dysentery with balantidial infection. Oral administration of Carbasone (Burroughs Wellcome product) in doses of one gram per animal twice daily for 6 days resulted in a complete cure of all the cases.

V. S. A.

Chick Mortality and the Contamination of yolks by members of the Enterobacteriaceae:—BY R. C. PATHAK, C. M. SINGH, and R. P. TANGRI. *Brit. Vet. J.*, 116, No. 2.

Salmonella were not isolated from the tissues of 146 specimens of chicks submitted for post-mortem examination.

From the study of the yolk flora of 100 dead chicks showing unabsorbed yolk sac, it was found that 43 were infected with *Escherichia*, 25 with *Klebsiella*, 6 with *Aerobacter*, 3 with *Proteus* and 19 with mixed infection while 4 gave no growth.

The importance of disinfection of eggs, incubators, brooders and other equipment concerned with chick raising is stressed. (*Authors' summary*).

News and Views

A new Abode for an old institute:—Starting as a Virus Producing Unit against Rinderpest in 1932 at Bombay and later on adding another Unit at Poona for production of Ranikhet Disease Vaccine, the first sings of a combined and a full fledged Institute for Biological Products made their appearance in 1947-48 at the Bombay Veterinary College. Cribbed and cabined within the space available there, the Institute could not make much head-way and it was therefore decided to shift it to Poona and a new building at a cost of nearly eleven lakhs was put up and it was officially inaugurated on 18-3-61 by the Hon'ble Minister for Agriculture, Shri P. K. Savant, who gave expression to some home-truths on the occasion.

* * * * *

Shri P. K. Savant said "It may also be well remembered that any activities towards breeding side by way of establishment of various farms, key villages, artificial insemination centres, etc. must have to be adequately supported by an organised disease-control programme. In the absence of such an organised programme, it is quite likely that all the good work done by way of breeding improved cattle, suitable to the needs of the state, can be undone in no time due to these ravagious diseases." Very few people realise, as the Hon'ble Minister has done, the integrated nature of livestock development work. Artificial barriers are sought to be created and they expect the scientists to work in water-tight compartments of breeding and disease control!

* * * * *

The Hon'ble Minister further added "Your efforts in maintaining a high standard of quality control would have no meaning if the products deteriorate in quality in transit and lack of proper preservation and storage at the destination. Government do not like to discard valuable sera and vaccines for loss of quality merely for lack of proper transport and preservation. The Standardisation Committee set up by the Government of India has laid down the minimum standards

required of the Veterinary Biologicals. I do not know why we should be content with the attainment of the minimum standards laid down rather than going ahead to reach the maximum standards so that the products of this Institute may be compared with the best produced anywhere in the world."

* * * * *

Stressing on the necessity of Research, the Hon'ble Minister said "Nature does not yield her secrets unless compelled to" and he commended sincere and persistent efforts in the field of research.

* * * * *

Animal Husbandry in Politics.—Mr. C. Rajagopalachari said recently in an electioneering campaign at New Delhi that "People visualised the Congress as a cow which would give them milk, but after independence the Congress had turned out to be a dangerous bull, which could not be allowed to have its own way!"

* * * * *

Livestock Census in Madras.—A livestock census was conducted in Madras State from March 15 to April 14. We await the result with interest.

* * * * *

Meat Breeds in Poultry.—The I. C. A. R. is financing a Poultry Scheme in Hyderabad, under which day-old chicks of suitable meat breeds are imported from abroad and reared with special feed mixtures. The rearing and dressing of the mature birds are to be taught by T. C. M. Experts.

* * * * *

Indian Officers Leave for Australia.—Thirteen Indian Agricultural and Animal Husbandry officials have left for Australia in March 1961 under a Colombo Plan project to help boost India's wool and sheep industries. The officials are the second batch of Indian officials to be trained in Australia under an All-India developmental programme initiated by the Indian Council of Agricultural Research.

The programme includes the development of regional sheep and wool centres, the establishment of three sheep breeding farms, three wool testing laboratories, three wool utilization centres and nearly 400 sheep and wool extension centres.

The first batch of 14 officials were in Australia for most of 1959. The officials who leave to-day will be in Australia for the rest of this year.

The training course has been arranged by the Australian Commonwealth Public Service Board and the Commonwealth Wool Research Committee. It is under the direction of Professor P. R. McMahon, who heads the School of Wool Technology, University of New South Wales.

Professor McMahon spent a year in India as an F. A. O. expert and revisited this country at the end of last year to confer with the Government of India about wool and sheep developmental problems.

Besides technical training at Universities and research establishments the trainees will visit woolen mills, wool auctions, regional centres and leading sheep stations in Western Queensland where climatic factors are similar to those in parts of India.

The ultimate aim of the programme is to evolve and develop suitable types of sheep for improved wool production partly by cross-breeding with well-known foreign breeds and partly by selective breeding among indigenous flocks.

India has a total sheep population of more than 39,000,000 and Indian wool, of a hairy type suitable for carpet weaving, is exported to Australia, the United States and Europe.

The project seeks to improve carpet wool, which already has a high reputation and to provide more fine quality wool for cottage industry as well as textile mills.

The Australian Government are providing the training free of charge under the Colombo Plan and paying an allowance equivalent to Rs. 8,000 a year to each trainee. (Australian High Commission, New Delhi). 15th March 1961.

* * * * *

A Generous Donation:—M/s. May and Baker have sent us a generous donation of Rs. 250 towards our Reserve Fund. It is a glorious piece of fellowship and goodwill towards a profession which they have been serving so well and which in turn was not slow to appreciate their products. We did not send them any special appeal even. They merely said "We have seen your appeal for the Reserve Fund" in the I. V. J. This gesture on the part of this noble Company must serve as an eye opener to many a member within the profession. It has come as an agreeable surprise to us, more particularly at a time when many members of the profession are still hesitant to unloosen their purse strings. We tender our thanks to them.

Correspondence

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

Sir,

With reference to the article on "A Method of Nephrectomy in canines" by Dr. B. N. Patra, B.Sc. (Hons.), M.R.C.V.S., published in No. 9, of Volume 37 of the *Indian Veterinary Journal*, I wish to point out that this method had already been described in *Veterinary Bulletin* (Cyanamid), special edition on Operative Surgery of the dog (1957) by Joseph M. Miller, M.D., F.A.C.S., and Jeannette M. Whitehorn, A.B., on pages 50 and 51, with good illustrations.

Since the author has not acknowledged this reference in his article, I feel both the readers of the journal and the author should be informed of this earlier publication.

Yours faithfully,
F. D. WILSON,
Professor of Surgery,
Madras Veterinary College, Madras-7.

Association News

The Indian Veterinary Association

Proceedings of the meeting of the Managing Council held on
26—3—1961 at 11 A.M., at Hotel "Ashoka", Madras.

Members Present:—

- Dr. T. Vinayaka Mudaliar, President I.V.A., and Editor I.V.J.
(In the Chair).
,, V. S. Alwar, General Secretary, I.V.A.
,, C. I. John, Jt. Secretary and Treasurer, I.V.A.
,, D. Pattabhiraman, Director of Animal Husbandry, Madras,
(Co-opted).
,, Bertie A. D'Souza, Principal, Madras Veterinary College,
(Co-opted).
,, A. R. Vedanayakam, Secretary, Madras Veterinary Association,
(Co-opted).

1. Minutes of the last meeting held on 14—8—1960 at Hotel "Dasaprakash" Madras were read and confirmed.

2. Read G.O. Rt. No. 196 dated 3—2—1961 of Food and Agriculture Department, Government of Madras. The President thanked the Madras Government, the Director of Animal Husbandry, Madras and the Principal, Madras Veterinary College for sparing the services of Messrs. V. S. Alwar and M. S. Jayaraman to the *Indian Veterinary Journal*. The following resolution was moved from the Chair and adopted unanimously.

- (a) This Council places on record its deep sense of appreciation and gratitude to the Government of Madras for permitting Sri V. S. Alwar and Sri M. S. Jayaraman to serve as the Associate Editor and Assistant Editor respectively of the *Indian Veterinary Journal* and conveys its respectful thanks to them.
- (b) This Council further places on record its grateful thanks to Dr. D. Pattabhiraman, Director of Animal Husbandry, Madras and to Dr. Bertie A. D'Souza, Principal, Madras Veterinary College for the help rendered in the matter. The President was empowered to utilise the services of Messrs. V. S. Alwar and M. S. Jayaraman, Associate Editor and Assistant Editor respectively of the *Indian Veterinary Journal* in an honorary capacity from 1—4—1961.

3. Read the correspondence with the Indian Council of Agricultural Research on the question of an annual publication grant for the *Indian Veterinary Journal*. As the chief impediment for the grant of a subsidy to the I.V.J., appears to be due to the journal not running on a deficit basis year after year, the Council was of opinion that a further attempt should be made and the following resolution was unanimously adopted:—

Resolved to request the Indian Council of Agricultural Research to place the matter before the Publications Committee for consideration and relaxation of the existing rules for the grant of subsidies to Scientific Journals of National importance.

4. Reviewed the question of supplying the I.V.J., at a concessional rate to the students of Veterinary Colleges. Even though the response so far has not been encouraging, it was decided however to continue the concession.

5. As no reply has so far been received from the Government of Madras to our request for alienating a Government site, to put up a building for the *Indian Veterinary Journal*, the President was requested

to approach the Government with a definite proposal for a site in the Government House Estate, Mount Road, Madras.

6. Read the reply from the Special Officer and Ex-officio Assistant Secretary to the Government of West Bengal, justifying the transfer of the Animal Husbandry Section from the Veterinary Department to the Directorate of Dairy Development in the State of West Bengal and the note on the same subject from Dr. Anupam Chattarjee, Secretary, West Bengal Veterinary Association and Member of the Managing Council of I.V.A. The matter was duly discussed and the President was empowered to send a suitable reply to the West Bengal Government on the lines indicated during discussion.

7. As no reply was received from the Government of India on the resolution of the previous meeting about the appointment of persons other than Veterinary Specialists, as Livestock Development Advisers to the Ministry of Agriculture, the President was requested to reiterate the above resolution.

8. Read the reply of the Government of Madras on the resolutions passed at the 13th All-India Veterinary Conference held at Bangalore and resolved to request the Madras Veterinary Association to address the Governor for the nomination of a Member of the Veterinary Profession to the Legislative Council.

9. Read the correspondence with the Indian Parliamentary and Scientific Committee. After a thorough discussion it was decided to defer the question of enrolling this association as a Member of the Committee.

10. Read letter from Rao Bahadur V. R. Phadke, resigning his Membership of the Interim Indian Veterinary Council. As Dr. V. R. Phadke represented the Indian Veterinary Association in the Council, it was resolved to recommend to the Secretary of the Indian Council of Agricultural Research the name of Sri V. S. Alwar, General Secretary, Indian Veterinary Association for nomination in the vacancy caused.

11. Resolved to institute a medal from the interest accruing from Rao Bahadur R. Swaminatha Iyer Memorial Fund for the best Clinical Article published in each year in the *I.V.J.*

The following committee was appointed to draft the necessary rules for the purpose.

- (1) Dr. Bertie A. D'Souza, Principal, Madras Veterinary College, (Convener).
- (2) „ M. N. Menon, Principal, Kerala Veterinary College, Member.
- (3) „ P. S. Rajulu, Principal, Andhra Veterinary College, Member.

(4) Dr. D. R. Marwaha, Professor of Clinical Medicine, Bengal Veterinary College, Calcutta, Member.

(5) „ V. S. Alwar, General Secretary, I.V.A. (Secretary).

12. It was resolved to affiliate the Indian Veterinary Association with the World Veterinary Association. The General Secretary was requested to do the needful in the matter.

The President thanked Dr. D. Pattabhiraman and Dr. Bertie A.D'Souza for the readiness with which they accepted to attend the meeting and help the Council in its deliberations.

With a vote of thanks to the Chair, the meeting came to a close.

Madras, }
30th March 1961.

V. S. ALWAR,
General Secretary.

THE RESERVE FUND

An event of great importance to the Veterinary profession in India, took place in August 1924. It was the birth of the *Indian Veterinary Journal*. Circumstances were positively hostile and yet the profession took that bold step and the lion-heartedness of the early pioneers cannot be too highly praised. Ever since then, the *I.V.J.* has toiled hard under very adverse circumstances. The *I.V.J.* lives to-day so that the profession could prosper and science advance.

And now the hat is sent round so as to place this journalistic venture on a sounder and firmer basis. The *I.V.J.* is a priceless possession of our profession. It has placed the Indian Veterinary Surgeon, on the map of the scientific world. Just pause and ponder over, how much we owe to the Journal both individually and collectively. Is it too much then to expect the profession to respond to the appeal issued for a Reserve Fund? We hope not.

Some enquiries have come to us asking us to indicate the extent of help expected. We do not want to put the members of the profession to any undue financial strain in these hard days. At the same time, a certain amount of sacrifice is needed to make this fund a great success, so as to make it worthy of our profession. So we feel that the following scale of contribution will be quite reasonable. It could even be paid in two or three instalments.

Class I Officers	...	Rs. 100/- and above.
Class II Officers	...	Rs. 50/- and above.
All others	...	Rs. 25/- and above.

It is our ambition that no Veterinary Surgeon in this land should remain without contributing to this fund. Brick by brick, this great edifice of ours should begin to rise higher and higher. On the success

THE INDIAN VETERINARY JOURNAL, MADRAS-17.

Statement of receipts and payments for the year ended 31st December '60.

RECEIPTS		PAYMENTS	
To balance as on 1-1-1960 :—		By Journal Printing charges	19241 32
Petty Cash	13 84	" Postage	1760 17
Postage account	11 93	" Rent	1627 50
With A.I.V.A. General Secretary	40 00	" Salaries	2702 47
With Bank	4325 68	" Honorarium to Editor	1500 00
		" Bank charges	67 17
" Subscriptions	4391 45	" Telephone	194 62
" Advertisements	29359 10	" Repairs to Cycle	45 48
" Grant-in-aid from National Institute of Science	4729 49	" Repairs to Typewriter	184 25
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" Other Receipts :—		" Newspapers and Periodicals	87 01
Reprints, Back Volumes & Blocks	1000 12	" Subscription to Indian Science Congress	12 30
Sale of old newspapers	42 47	" Advertisement Commission	35 00
Affiliation & Application fees	75 00	" Audit Fees	50 00
Reserve Fund Collections	153 30	" M. S. Shastry's Photo expenses	187 15
Interest on Bank account	50 00	" Reserve Fund Transfer	130 00
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		" Balances as on 31-12-1960 :—	
		Petty Cash	0 97
		Postage	14 64
		With Bank	12685 72
Total Rs.	40800 93	Total Rs.	12701 33
			40800 93

Madras,
6-4-1961.

EXAMINED AND FOUND CORRECT.

P. GOPALAKRISHNA RAO,
Chartered Accountant.

or otherwise of this venture, the solidarity of our profession depends. "Pay and Prosper" is the slogan we pass on to the members of our profession.

Jai Hind!

T. VINAYAKA MUDALIAR,
Editor, The Indian Veterinary Journal.

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Review

The Dog Lovers' Manual.—By Lt. Col. A. C. AGGARWALA, B.Sc. (Hons.), M.R.C.V.S., A.I.R.O., P.V.S.Cl.I. (Retd.), RADHEY MOHAN SHARMA, L.V.P., C., Bact. (Eng.), P.V.S.Cl.I., and S. D. SHARMA, L.V.P., F.Z.S. (Lond.), 1st. Ed. Feb. 1961. Medium 8 vo. Pp. xvi + 320 + 12 Plates. Publishers: S. Nagin & Co., Adda Hoshiarpur, Partap Road, Jullundur City (Punjab-India). Also available from: The New Book Depot, Block "B", Cannaught Place, New Delhi-1 and Lt. Col. A. C. Aggarwala, 4 Jangpura Extension, Birbal Road, New Delhi-14. Price Rs. 12.00 Nett.

This comprehensive new book on keeping of dogs especially under conditions obtaining in India, is the first ever written in the country by eminently qualified Indian Veterinary Surgeons. It is designed to serve not only as a very highly informative guide and an interesting reading, but also as an excellent reference for dog lovers on almost all conceivable aspects of keeping, feeding, breeding, rearing and diseases of dogs, and yet it is restricted in its scope in as much as, that, while it puts the keepers of dogs on the right track in their dog problems, it does not aim at making them veterinary surgeons. The

book admirably combines the qualities of a text-book and a First-aid treatise, just as its authorship is shared between a teacher and the taught.

The three authors of this book are well-known in the profession as highly qualified and experienced persons with a masterly way of putting highly technical matters across to the average reader in easily intelligible and popular language. They have spared no pains to present to dog lovers a work in simple, straight and distinctive style, which will appeal to all who have the welfare of the dog at heart. It does not take a great mind to make things complicated, but a very great mind to make complicated things simple. The seniormost author, Lt. Col. A. C. Aggarwala, enjoys the reputation of being 'the father of veterinary literature in India'. He has written several books on different animal husbandry and veterinary subjects that have passed through many editions and are eagerly read and much prized. He had been a talented professor and a capable Principal of three veterinary colleges, besides holding many other honourable positions. The junior-most author, Dr. S. D. Sharma, is a well-known clinician, being Incharge of the different S. P. C. A. Hospitals, Race-Course and the Zoological Park in Delhi. He is the most popular veterinarian amongst lovers of pet animals, especially dogs. The third co-author, Dr. Radhey Mohan Sharma is a top-ranking Disease Investigation Officer in the Country, and had been associated with Col. Aggarwala's literary works for the last quarter of a century.

The Manual comprises of 14 Chapters on breeds, norms and choice of a dog; on aging and dentition, kennelling, feeding, breeding, rearing, exercise, training, grooming and washing of dogs, and on diseases, ailments and injuries of dogs and their treatment, in addition to a chapter on the origin, growth and rules and regulations of the Indian Kennel Club. The Chapter devoted to 14 stories of the most famous dogs of the world provides a very light and pleasant reading of good literary merit. The book is profusely illustrated and the cartoons given are highly amusing, interesting and instructive. On the whole, the lay-out of the Manual is well-organised with a distinctive style and well produced. A comprehensive bibliography and an adequate index at the end further enhance credits to the book. It is at once a text-book a reference book, a guide and a light story-book. Mankind owes a great debt to pet animals, especially dogs, who give us much comfort by their affection, loyalty, companionship and help. We, in turn, should be able to render them assistance, should they have a hurt or a problem, for it is to us they must look in all their troubles. This book will help dog lovers to solve their dog problems.

P. N. N.

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