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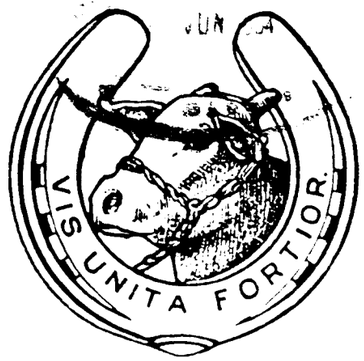
THE INDIAN VETERINARY JOURNAL

(The Journal of the All-India Veterinary Association)

A Bi-Monthly Record of Veterinary Science



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TO OUR SUBSCRIBERS

The next volume commences from the July issue of the *Journal*. Subscribers are hereby requested to remit their annual subscription for the next volume so as to reach the Editor before the 10th of July 1954.

The postage for book packets has been doubly increased in addition to the increased V.P. charges and our subscribers are therefore requested to send their subscriptions in advance by money order.

Those who are in arrears of subscription are also requested to send in their dues immediately so that their accounts for this volume may be correctly adjusted.

Madras,
20th May 1954

EDITOR.

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THE
Indian Veterinary Journal
(The Journal of the All-India Veterinary Association)

Vol. XXX MAY, 1954 No. 6

General Articles

OBSERVATIONS ON THE ACTIONS OF DIETHYLSTILBESTROL ON PULLETS

By

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Much interest in hormones has been aroused since the demonstration in 1902 by Bayliss and Starling of the existence in the animal body of a chemical regulator known as secretin. Of the internal secretions, the sex hormones which control the different phases of the reproductive activity of animals seem to be most fascinating. Male hormones are generally referred to as androgens, while the female hormones, estrogens. The latter are so called because they influence the estrus cycle in females.

There are at present a variety of synthetic products with estrogenic activity. The discovery of synthetic estrogen, stilbestrol, (4, 4'—dihydroxystilbene), by Dodds was of great scientific interest as the compound possesses the physiological properties of natural estrogens. Gilman (4) reported that it was proved that C-alkylated stilbestrol was far more potent than the parent compound and this product was found to be equal in potency to natural estrogen. This very potent synthetic estrogen has been identified as dihydrodiethylstilbestrol. It was obtained by alkaline demethylation of anethol (4-propenylanisol), a natural ether.

This synthetic estrin, diethylstilbestrol, has been used quite extensively in human medicine and seems to have a vast field of application in animal industry.

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CONTENTS

According to Harris and Thimann (6), Riddle and Tange, working on ovarian hormones, found that the injections of estrogenic extracts would induce hypertrophy and hyperplasia of the oviduct of immature pigeon, and that later, Juhn and Gustavson observed that similar effects could be produced in fowls.

Kar (7), in 1947, injected stilbestrol to young brown Leghorn female chickens at different ages and for varying periods. He found that the oviduct responded considerably after the injection. He observed that, in many cases, the injection caused perforation of the oviduct and increase in the secretion of the glands of the duct. He stated that these observations were in conformity with the findings of other workers.

Pincus (11) reported that activation of the infantile ovary with resultant premature puberty could be produced by implantation of the pituitary and by injection of pituitary extracts. According to Fincher (3), Walpole could produce something like estrogen shock in cows with nonfunctioning ovaries, by injecting a single small dose of estrogen; Casida induced multiple pregnancy in ewes by estrogen treatment; and Folley and Malpress restored by large doses of estrogen the estrus cycle of cows in anestrus.

Moore (10) treated with stilbestrol 45 cows suffering from pyometra, 42 of which conceived with an average of 1·62 services per conception and only 1 cow (2·33 per cent) became sterile. Of another 50 cows which were treated with stilbestrol, 14 per cent became sterile. In stilbestrol-treated cases, an average of 86-1/3 days elapsed between parturition and conception and in the control group, 144-4/5 days. Chensney (2) has also reported that out of 200 cows with retained placenta which were given stilbestrol injections, normal expulsion of the placenta occured without any subsequent complication in 50 per cent of the cases.

Burkhardt (1) claimed that diethylstilbestrol had given encouraging results in anestrus in mares. Estrus was induced in from 49 to 66 hours and out of 11 cases of induced estrus, pregnancy took place in 9 cases.

Lewis and Turner (9) administered small doses of diethylstilbestrol for stimulating secretion of milk in virgin goats and reported that the ovaries of all the goats except one were normal after long periods of treatment. They did not observe any adverse effect on growth or health of the animals from the treatment.

Taber (13), treating domestic fowls with pituitary follicle-stimulating hormones, leutinizing hormone and equine gonadotropic hormone, did not observe precocious folliculur maturation and ovulation. In

fowls treated with equine gonadotropic hormone and follicle-stimulating hormone, there was an increase in ovarian weight which was attributed to hypertrophy of the medullary component accompanied by a pronounced distension of medullary tubules. He also observed hyperplasia of vacuolar and lipoidal cells in the medulla and a reduction in their lipoidal contents. He was of the opinion that failure to induce precocious development of follieles might have been due to the absence of other hormonal factors or to an inadequate supply of nutritious substances necessary for yolk formation.

Gonzaga (5) reported that the National Commissariat of Health, U.S.S.R., effected a great increase in egg production through the influence of prolan injections.

According to Koneff, Simson and Evans (8), the rate of weight increase was diminished and the gonads became atrophic in male and in female hamsters implanted with tablets of stilbestrol for 3-1/2 to 8 months. The male accessory reproductive organs atrophied with hypertrophy of muscle and connective tissue. The uteri were hypertrophic and edematous. In some cases, cystic hyperplasia was noted.

Harris and Thimann reported that implantation of estrone tablets greatly reduced the size of the testes of birds. At present, caponization of chickens by diethylstilbestrol implantation is widely practiced.

The object of this study has been to contribute data on the effects of diethylstilbestrol on pullets, especially on egg production.

Materials and Methods

The experiments were conducted on three groups of pullets. The first group consisted of 6 White Leghorns of the same brood, 6 months old, purchased from the Araneta Institute of Agriculture, Calcocan, Rizal, and 5 natives of the same brood, 6 months old, purchased from Alabang, Rizal. The second group consisted of 4 natives of the same brood, 3 months old, purchased from Tondo, Manila; and the third group, of 18 culled White Leghorns of the Del Monte Poultry Farm, San Francisco del Monte, Quezon City. All the birds were provided with numbered leg bands for identification.

The pullets in the first experiment were fed corn grains during the first two weeks. The following week, they were fed laying mash. From the fourth week on, they were given laying mash and corn grains. The birds in the second and third experiments were given laying mash and corn grains as feeds throughout the period of the experiment. All the birds had free access to clean drinking water. The 18 culled pullets were kept in the Del Monte Poultry Farm where observations on them were conducted.

All the pullets in the first experiment were kept in small cages for the first eight weeks, and then transferred to a poultry house with a run enclosed by wire nettings. Nests were provided for laying. Two weeks after the transfer, a native rooster was placed in the pen with the pullets to determine the sexual reactions of the latter.

Lipellets, each containing 15 milligrams of diethylstilbestrol, manufactured by Norden Laboratories, Lincoln, Nebraska, U.S.A., were used for pellet implantation. The pellets were implanted subcutaneously or intramuscularly with a pellet injector.

The solution of stilbestrol used in this study was an oil solution (2 mgm, per mil.) of 4-4' dihydroxy-alphabeta-diethyl stilbene prepared by Jensen-Salsbery Laboratories, Inc., Kansas City, Mo., U. S. A. This was administered with hypodermic syringe and needle.

To find out the effects of Stilbestrol injection and implanation in six-month old White Leghorn and native pullets.

Experiment 1.—In this experiment, 6 White Leghorn and 5 native pullets were used. The birds were weighed at regular intervals once a week from July 24 to October 23, 1948.

On August 2, 5 mgm. of diethylstilbestrol (1/3 lipellet) was imbedded subcutaneously in the neck of each of pullets Nos. 3, 4, 8 and 9. One lipellet was implanted in each of these pullets after six weeks. On the same day (Aug. 2) 1 ml. of diethylstilbestrol solution was injected in the breast muscles of each of pullets Nos. 5, 6, 10, and 11. This treatment was repeated after four weeks. Pullets Nos. 1, 2, and 7, were kept as controls.

Observations were made on the growth of the comb and wattles, size of the abdomen, spreading of the pubic bones and the sexual receptiveness of the pullets before and after treatment. The dates the individual birds started laying and the total number of eggs laid by each bird were recorded.

Pullet No. 3 was killed 11 weeks after the second implantation of the lipellet. On the same day, control pullet No. 1 was also killed. Pullet No. 4 was killed 7 weeks after the second implantation. Pullet No. 5 was killed one week and pullet No. 6, twelve weeks after the second injection of stilbestrol solution. Pullets Nos. 8 and 9 were killed 12 weeks after the second implantation and pullets Nos. 10 and 11, fourteen weeks after the second injection. Autopsy was conducted on all these birds killed to find out the effects of the drug on the internal organs, especially on the reproductive.

To find out the effects of large doses of Stilbestrol on the ovary and oviduct of native chickens.

Experiment 2.—In this experiment, 4 three-month old native chickens were used. On October 10, one lipellet was embedded in the breast muscles of each of the chickens Nos. 12, 13, and 14. Bird No. 15 was kept as control. Three weeks later, chicken No. 13 was killed and autopsied, because it was found to be inactive and sickly. On the same day, birds Nos. 12 and 14 were given another intramuscular implantation of one lipellet each. Chicken No. 12 was killed two weehs after the second implantation and autopsy conducted. Three days later, chicken No. 14 was given another intramuscular implantation of one lipellet. Two weeks after this implantation, the bird and control chicken No. 15 were killed and autopsied.

To find out the effects of subcutaneous implantation of Stilbestrol pellets on the egg droduction of culled pullets.

Experiment 3.—This experiment was conducted at the Del Monte Poultry Farm, San Francisco del Monte, Quezon City. Eighteen culled pullets (White Leghorn) were used. The number of eggs laid by these birds for two weeks were recorded. At the end of this period, they were divided into two groups of 9 birds each. Each group was kept in a separate pen. One lipellet was implanted subcutaneously in each of the 9 pullets in the first group. The birds in the second group served as controls. After the implantation, the number of eggs laid by each group were recorded daily for six weeks.

Results and Discussion

In the first experiment, the test and control birds did not show any appreciable difference in the growth of the comb and wattles, According to Harris and Thimann (6), Regnier found that injections of estradiol benzoate had resulted in regression of the comb of growing hens, but the effect was not marked in adults during laying season. In the present study, the regression of the comb was not noticed even in birds which had not started laying.

Two weeks after the first implantation and injection of diethylstilbestrol, it was observed that the pubic bones of the test birds were spreading more quickly, causing greater increase in space between the free ends of these bones. These pullets showed a definite change in conformation with an increase in the breadth of the back. The size of their abodomen was increasing and their vent was enlarged and moist. On the whole, they had the appearance of more matured birds about to start egg laying.

The sexual receptiveness was increased in test pullets after the administration of stilbestrol. They were observed many times to squat

at the approach of the rooster and even of people, while the controls ran away. According to Harris and Thimann (6), Noble and Zitrin found that young chickens injected with estradiol squated for treading males, and their behaviour was typically that of a sexually receptive hen. The findings in the present study were apparently in agreement with the above observations, showing that the administration of stilbestrol caused early sexual maturity in pullets.

There was evidence of increase in weight in test pullets following the injection and implantation of diethylstilbestrol as shown in Plate No. 20, Fig. 1. This increase in weight was, however, only temporary, as the action of the drug waned out as time went by.

The oviducts of test pullets Nos. 3, 4, 5, and 6 in this experiment, and those of birds Nos. 12, 13, and 14 in the second, showed abnormal enlargement following the administration of stilbestrol. Plate No. 20, Fig. 3 shows the difference between the oviduct of test pullet No. 3 and that of control pullet No. 1.

Table 2 shows that the measurements of the oviduet of the test birds were more than double those of the controls in the second experiment. In this experiment, plenty of mucoid secretion was noted in the uterus of the test pullets. The enlargement of the oviduct and the presence of mucoid secretion in the uterus were observations similar to those reported by Kar (7).

Pullet No. 5 showed, on autopsy, remains of broken yolk in the abdominal cavity and in the uterus. There were also lesions suggestive of peritonitis. The cause of the broken yolk in this pullet was not known. While the condition is generally seen in heavy layers, it was possible that, in the present case, the injection of stilbestrol might have overstimulated the ovaries, resulting in broken yolk.

The autopsy of pullet No. 4 showed fatty degeneration of the heart and enlargement of the liver, and that of pullet No. 13 of the second experiment, enlargement of the liver with adhesion of the left lobe. These changes in the liver of these birds were in conformity with the previous findings of Smith (12) who, working on stilbestrol, found that large doses of the drug will sometimes cause anorexia, hepatitis, etc.

Postmortem examination revealed that the ovaries of the White Leghorn test pullets were firm, while those of the native were not. This seemed to indicate that the ovaries of White Leghorn were more sensitive to stilbestrol. Only large doses could adversely affect those of the native pullets.

Plate No. 20 Vol. XXX, No. 6

Actions of Diethylstilbestrol on Pullets.

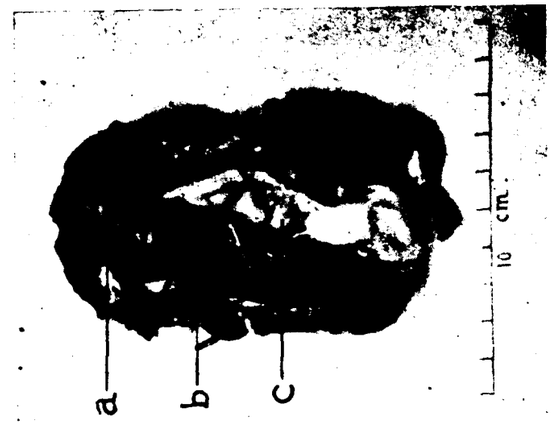


Fig. 2

Reproductive organs of test Pullet No. 6.
a. Ovary containing small ova.
b. Cyst.
c. Enlarged oviduct.

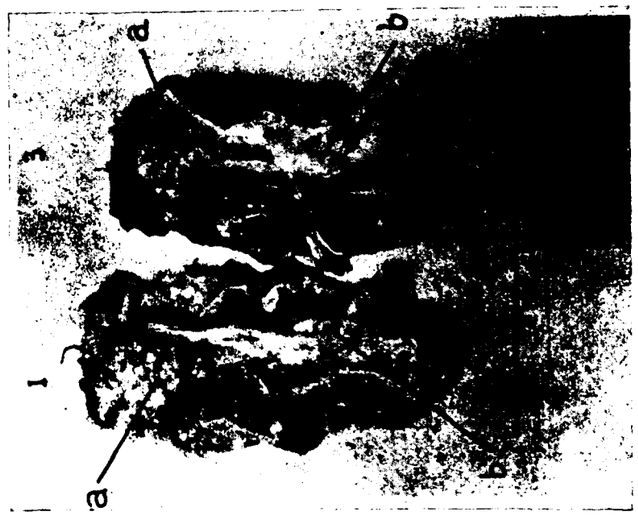


Fig. 3.

Fig. 1.—Reproductive organs of control pullet No. 1.
a. Normal ovary.
b. Normal oviduct.

Fig. 3.—Reproductive organs of test pullet No. 3.
a. Firm ovary with small ova.
b. Enlarged oviduct.

A Nervous Disease in Poultry

FIG. 1.
R.I.R. Chick showing complete paralysis of the legs & wings with head turned backwards.

FIG. 2.
R.I.R. Chick showing Torticollis.

[*Vide article Page No. 471.*]

In the ovary of pullet No. 6, (Plate No. 20, Fig. 2) were found two serous cysts—one as big as a pigeon's egg, and the other, half this size. This was also the observation of Koneff and co-workers (8), showing that stilbestrol treatment may cause cystic formation in some cases.

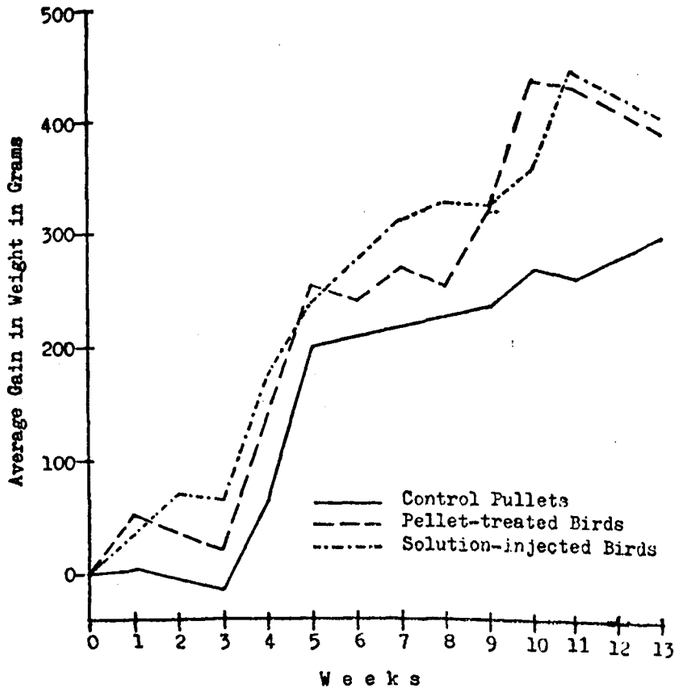
All the test pullets in Experiment 1 started laying before the end of the experiment, with the exception of pullet No. 5. This bird was killed one week after the second injection of stilbestrol solution because it was found to be very sick. Autopsy findings on the case were given above. Out of the three controls, only pullet No. 2 started laying before the end of the experiment.

Table 1 shows the number of eggs laid by each pullet per month. Both the test and control White Leghorn birds produced an average number of eggs lower than that usually observed for this breed. These were culled pullets from the Araneta Poultry Farm. Some of them showed some pathological lesions after stilbestrol treatment. This might have caused their low egg production.

The results of Experiment 3 are given in Table 3. It shows the egg production of the test and control birds. It will be seen that the pullets given subcutaneous implantation of diethylstilbestrol laid more eggs than the controls.

The native pullets are known to mature late and lay only few eggs inspite of good feeding. In the present study, it was observed that native birds treated with stilbestrol matured early and laid satisfactory number of eggs. From a review of the available literature and from the observations in this study, it may be presumed that some relationship exists between estrogens and the function of the ovaries. It is quite possible that proper dosage of estrogen in combination with gonadotropic hormones may yield desirable results.

The results of the third experiment clearly indicated that estrogen has stimulating effect on non-functioning ovary of the hen. Folley and Malpress made the observation that suitable doses of estrogen activated the quiescent anterior pituitary. It has also been reported that the National Commissariat of Health, U.S.S.R., effected tremendous increase in egg production by the influence of prolan injection. From these observations, it may be logical to suppose that stilbestrol injection can also increase egg production in fowls, and that something like estrogen shock mentioned by Walpole can be produced in the hen with non-functioning ovary by injecting diethylstilbestrol.



Summary and Conclusions

A study on the effects of diethylstilbestrol on White Leg-horn and native pullets is herein reported.

The administration of diethylstilbestrol did not produce any appreciable effect on the growth of the comb and wattles in the pullets tested. It caused, however, temporary increase in body-weight and early sexual maturity in treated birds.

In proper doses, the drug increased the egg production.

In large doses, it caused, in some birds, some pathological lesions like hepatitis, firm ovaries, fatty degeneration of the heart, cystic formations in the ovaries, abnormal enlargement of the oviduct and broken yolk.

TABLE I

Showing the number of eggs laid by each pullet in experiment 1 and the autopsy findings in birds killed.

Pullet No.	Number of eggs laid					Autopsy findings
	Aug.	Sept.	Oct.	Nov.	Dec.	
1*						Killed on Dec. 8, 1948. Internal organs apparently normal; ovary had plenty of small ova.
2*			9	7	2	Not killed.
3	10	6	13			Killed on Dec. 8, 1948. Oviduct thickened and enlarged; ovary firm, with small ova present.
4			16			Killed on Nov. 11, 1948. Fatty degeneration of the heart; liver enlarged; ovary thickened and firm; oviduct enlarged.
5						Killed on Oct. 9, 1948. Remains of broken yolk seen in the pelvic cavity and in the uterus; ova small; ovary enlarged and haemorrhagic; oviduct enlarged and hyperemic; peritonitis.
6			14			Killed on Nov. 29, 1948. Small ova in the ovary; 2 cysts in the ovary, one the size of pigeon's egg, the other, half this size; oviduct enlarged.
7*						Not killed.
8						Killed on Dec. 14, 1948. Internal organs apparently normal; many large sized ova in the ovary.
9				12		Killed on Dec. 14, 1948. Internal organs apparently normal; numerous ova in the ovary.
10			4	10		Killed on Dec. 17, 1948. Internal organs apparently normal; many ova in the ovary.
11	15			15		Killed on Dec. 17, 1948. Internal organs apparently normal; numerous small ova in the ovary.

* Controls.

TABLE II

Showing the measurements of the oviduct of the Test and Control chickens.

Chicken No.	Oviduct	
	Width	Thickness
	cm.	*cm.*
12	1.5	0.6
13	1.7	0.8
14	1.5	0.7
15*	0.5	0.3

* Control.

TABLE III

Showing the weekly egg production of the eighteen pullets in experiment 3.

Week	Number of eggs laid		Total
	Test Pullets	Controls	
1st	Test and control pullets were kept together		16
2nd			14
3rd	12	8	20
4th	20	8	28
5th	24	9	33
6th	22	7	29
7th	24	10	34
8th	22	9	31
Total	124	51	205

Acknowledgment

The writers wish to thank Mr. Marcial Sebastian, owner of the Del Monte poultry Farm, San Francisco del Monte, Quezon City, for permitting the use of his birds in this study, and those who, in some way or other, extended help during the progress of the work.

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STUDY OF A PROVEN SCINDHI SIRE AT THE LIVESTOCK RESEARCH STATION, HOSUR, MADRAS STATE

By

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While evaluating the sires of Scindhi breed adopted for breeding at the Livestock Research Station, Hosur, Rajagopalan (1953) observed that Scindhi bull No. 38 was able to produce daughters whose average milk yield performance of the first lactation was more than the respective dams, thus resulting in a highly significant result by progeny testing. This indicated a further investigation for exploring the possibilities of this Sire transmitting the milk character either from its Sires' or Dams' pedigree.

From a perusal of the pedigree records of Scindhi breed maintained at the Livestock Research Station, Hosur, it is found that a small percentage of inbreeding is practised. The scheme of breeding for conspicuous results in livestock improvement comprises, of choice of the

foundation stock, inbreeding to produce homozygous line and the crossing of selected lines. The Livestock improvement actually to be effected in any Livestock Farm is controlled by the meticulous care exercised in selection at one or more of the three stages mentioned above. It will be quite impossible to carry out an in-breeding programme without any selection. There may be a great danger if the animals are selected for a few special qualities instead of for all-round excellence because it may cause degeneration in respect of the neglected points. Inbreeding is the only means of getting pure breeding groups, where as selection gives direction to this process. In any of our breeding policies, it is with great caution that inbreeding can be used and the selection of the male breeding stock especially will have to be done according to the quality of the off-spring.

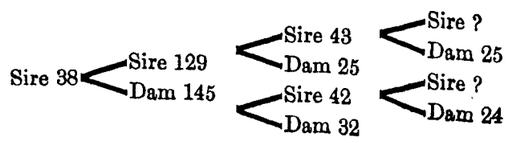
In the present study, it has been our objective to investigate the effect of inbreeding with respect to the milk yield of the daughters of Sire No. 38 as far as the records available will permit.

The co-efficient of inbreeding does not measure the exact state of relationship or the degree of homozygosis or heterozygosis, but only the state of the animal relative to the average of the population from which it comes. The formula, as suggested by Prof. Lush for the coefficient of inbreeding, is as follows :

$$F_X = \tfrac{1}{2} \leq [(\tfrac{1}{2})^n (1+F_A)]$$

where F_X is the inbreeding coefficient of the animal x, n is the number of generations by which Sire and Dam are related; F_A is the breeding coefficient of the common ancestor (A) out of whom the line of descent divides and $\leq$ is the summation sign meaning that each such path of relation between sire and dam is to be evaluated separately and then all the results are to be added together.

The Scindhi Sire No. 38 traces inbreeding with Dam. No. 25 as follows :



The Sire No. 129 which is the father of sire No. 38 was born to Sire No. 43 and Dam No. 25; Sire No. 43 is the son of Dam No. 25, this resulting in Sire No. 129 being an inbred off-spring to Dam No. 25. The coefficient of inbreeding works out to be 0,15625 for Sire No. 38. Hence this Sire is probably

homozygous for $\frac{5}{32}$ of the genes which were heterozygous in the ancestors at the foundation or base date to which the pedigree of this Sire is traced. This amounts to the transfer of $\frac{5}{32}$ of the heterozygous gene pairs into homozygous ones, half of the $\frac{5}{32}$ being added to each of the two kinds of homozygotes. From the study of inbreeding, one fails to understand that inbreeding is powerless to create either good or bad, but it may bring about the good or bad characters to light. In the case of Sire No. 38, it is presumed that the milk genes may be attributed to Inbreeding. Since we cannot see the genes in our animals, perhaps the best way to think about it is in terms of inbreeding, but the fact remains that inbreeding is concerned primarily with the genes. Thus, if one has a really good sire (proved good by his off-spring), it is better that the performance of the sons and daughters are also investigated.

The daughters of Sire No. 38, which have given a high milk yield over the respective dams are taken up for investigation. The co-efficient of inbreeding is calculated in this case of all the available 12 daughters and a correlation co-efficient was set up on the effect of inbreeding with the milk yield of the daughters. The result is tabulated below :

TABLE I

Table showing the effect of inbreeding over the milk yield of the daughters of Sire No. 38.

S. No.	No. of the daughter.	Co-efficient of inbreeding.	Highest milk yield in lbs.	Milk yield of first lactation in lbs.	No. of days in milk.	Milk yield adjusted to 300 days.
1.	115	0.4374	3090	3090	262	3711
2.	123	0.1561	3696	3149	329	2720
3.	126	0.13846	3044	2941	280	3268
4.	129	0.13846	6135	3645	372	2468
5.	131	0.13476	5542	4927	373	3774
6.	134	0.1249	5241	5241	385	3851
7.	136	0.07226	5312	4474	361	3477
8.	138	0.1404	5127	4904	353	4038
9.	155	0.374	5313	5313	374	4103
10.	169	0.344	4158	4158	317	3880
11.	174	0.1873	4966	4966	382	3625
12.	182	0.1873	5331	3838	293	3952

The milk yield of the first lactation yield is reduced to 300 days for each animal by using the Regression co-efficient and in this case the Regression coefficient " b " = 16·35 With the use of the adjusted milk yield reduced to 300 days of the first lactation and the respective coefficient of inbreeding the correlation " r " is calculated with

$r = \frac{\Sigma(x-\bar{x})\,(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2(\Sigma y-y)^2}}$ where x is the coefficient of inbreeding and y is adjusted milk yield.

The correlation "r" in this case is O. 36385. This is not significant for 10 degrees of freedom and thus an indication is seen that the milk yield of the daughters of Sire No. 38 do not appear to be associated with inbreeding co-efficient.

While studying the pedigree particulars of a bull, the performance of the milk yield of the Dam and its ancestors are also considered. Many experienced breeders believe that the dairy qualities are inherited stronger from the sire than through the Dam, but still there is some question concerning this view. Hence further investigation into the transmitting abilities of the sons and daughters of the parents of Sire No. 38 is taken up though it is stated that too much attention should not be given to ancestors prior to three or four generations.

On the Sire's side the bull No. 38 traces the ancestory from Dam No. 25 and on the Dams' side, it has its Dam No. 145, grand Dam No. 32 and great-grand Dam No. 24. The milk-yield records of all these dams are collected from the pedigree records maintained at the Livestock Research Station, Hosur, and a correlational study is undertaken between the first lactation milk yield of the daughters of Sire No. 38, without that of the grand daughters of other dams mentioned above.

With respect to the milk yield records of Dam No. 145 and Dam No. 32 only three grand daughters' milk yield performances in each for comparison is available. As such the averages of the first lactation reduced to 300 days by regression is adjusted and compared with the average of the daughters of Sire No. 38.

1. Average Milk yield of first lactation of the daughters of Sire No. 38. — 3572 lbs.
2. Average Milk yield of first lactation of the grand daughters of Dam No. 145. — 4414 lbs.
3. Average milk yield of first lactation of the grand daughters of Dam No. 32. — 2977 lbs.

The average of the grand daughters of Dam No. 145 (Dam of Sire No. 38) with the grand daughters (i. e. daughters of Sire No. 38) is considerably high, but the average of the grand daughters of Dam No. 32 (i. e. grand Dam of Sire No. 38) is low and thus further investigation into the performances of Dam No. 24 (great grand dam of Sire No. 38) is undertaken. Since there is only one daughter and three

sons for Dam No. 24 the milk yield performances of the grand daughters of Dam. No. 24 are considered and tabulated below :-

TABLE II

Table showing the milk yield of the grand daughters of Dam No. 24 adjusted to 300 days.

S. No.	No. of the grand daughter of Dam No. 24.	Milk yield of first lactation.	No. of days in milk.	Milk yield adjusted to 300 days.
1.	Dam No. 57	3492	323	3249
2.	,, 145	2432	303	2400
3.	,, 196	564	93	2750
4.	,, 160	3166	291	3261
5.	,, 21	3426	391	2285
6.	,, 25	3614	350	3086
7.	,, 26	3217	332	2879
8.	,, 29	3848	296	3890
9.	,, 31	3283	293	3357

The Regression coefficient of milk yield on days = 10·56 for which the adjustment is made for 300 days.

TABLE III

Milk yield comparison between the daughters of Sire No. 38 and the grand daughters of Dam No. 24 of the first lactation adjusted to 300 days.

S. No.	Adjusted Milk yield of daughters of Sire No. 38.	Adjusted Milk yield of the Grand daughters of Dam No. 24.
1.	2468	3249
2.	3774	2400
3.	3851	2750
4.	3477	3261
5.	4038	2285
6.	4103	3086
7.	3880	2879
8.	3625	3890
9.	3952	3352
Mean	3685.33	3016.9

The correlation between the two is – 0·3272 which is not significant for 7 degrees of freedom and thus indicating that there may not be any relationship between the first lactation milk yield performances of the above two.

Thus having eliminated all the possible study on the dams and its ancestors' performances of Sire No. 38, the only grand dam No. 25

on the Sire's side is also investigated. A similar study as in the previous case of Dam No. 24 is also taken up with the grand daughters of Dam No. 25.

TABLE IV

Showing the adjusted milk yield of the first lactation of the grand daughters of Dam No. 25.

S. No.	No. of the grand daughter Dam No. 25.	Milk yield of first lactation.	Number of days in milk.	Milk yield adjusted to 300 days.
1.	Dam No. 5	5103	331	4902
2.	,, 8	3595	316	3491
3.	,, 10	3392	418	2629
4.	,, 12	4377	314	4276
5.	,, 19	3836	351	3506
6.	,, 60	4339	356	3977
7.	,, 180	2414	263	2653
8.	,, 189	2121	154	2886
9.	,, 191	3053	384	2510

The Regression co-officient of milk yield on days is 6·47 for which the adjustment is made.

TABLE V

Milk yield comparison between the daughters of Sire No. 38 and the grand daughters of Dam No. 25 of the first lactation adjusted to 300 days.

S. No.	Milk yield of the daughters of Sire No. 38.	Milk yield of the grand daughters of Dam No. 25.
1.	2468	4902
2.	3774	3491
3.	3851	2629
4.	3477	4276
5.	4038	3506
6.	4103	3977
7.	3880	2653
8.	3625	2886
9.	3952	2510
Mean	3685.33	3425.55

The correlation between the two is -O. 664 which is not significant, though it just reaches the 5 % level of significance.

Collateral relatives in the pedigree also furnishes evidence about the genetypes of their parents or grand parents. Though the grand parent is generally a good indicator of an individual's performance,

as the half-brothers and sisters do, yet, the performance of the half-brothers and sisters may be more accurate, because the number of individuals in the case of half-brothers and sisters will be naturally more than the grand parents which are only four for an individual.

TABLE VI

Showing the adjusted milk-yield of the first lactation of the half sisters of Sire No. 38 by the Regression co-efficient.

S. No.	No. of the half sisters of Sire No. 38.	Milk yield of first lactation of the half sisters of Sire No. 38.	No. of days in milk.	Milk yield adjusted to 300 days.
1.	Dam No. 40	5216	345	4631
2.	,, 41	4754	305	4689
3.	,, 43	7118	399	5831
4.	,, 47	3766	340	3246
5.	,, 49	4973	343	4414
6.	,, 82	4166	385	3061
7.	,, 83	2878	247	3567
8.	,, 85	3778	354	3076
9.	,, 104	4078	383	2999

The Regression co-efficient of milk-yield on days is 13·0 for which the adjustment is made for 300 days.

TABLE VII

Comparison of the milk-yield of the first lactation adjusted to 300 days between the daughters' and half-sisters of Sire No. 38.

S. No.	Milk yield of the daughters of Sire No. 38.	Milk yield of the half sisters of Sire No. 38.
1.	2468	4631
2.	3774	4689
3.	3851	5831
4.	3477	3246
5.	4038	4414
6.	4103	3061
7.	3880	3567
8.	3625	3076
9.	3952	2999
Mean	3685.33	3946

The correlation in this case is - 0·2001 which is also not significant.

In all the above study, the result is not indicative of any significant correlation with the milk yield performances of the grand dams and half sisters of Sire No. 38 with the daughters of Sire No. 38.

The sign of the correlation in these cases is inverse showing that, as one falls, the other rises. The reason for such an association may also be due to some other common factor between the two individuals of measurement, as no definite conclusion can be drawn.

The progeny test starts with the assumption of the sampling nature of the inheritance, where each off-spring gets from the parent a sample half of that parent's inheritance and for every additional off-spring of the parent, another independant sample is received, thereby enabling us to find out the exact nature of these samples of inheritance received by the off-springs from the parent. While studying the sampling nature of these hereditary characters, there are environmental factors which may credit or blame the heredity of the parent. If there is anything unusual about the environmental conditions of the parent and off-spring, proper allowance has to be made.

The statistical calculation of the correlation in this study gives only a measure of the intensity of their association. While interpreting the correlation co-efficient in these cases, there are various possibilities by which the hereditary and environmental factors are associated, as in the case of the daughters of Sire No. 38 and its ancestors. Though the correlational study of the milk yield is attributed to hereditary factors, there is a possibility of the environmental condition checking the positive correlation on the milk yield of the number of individuals and establishes a negative correlation. This can be attributed to the environmental factors which play a conclusive part over successive generations of these animals under the study. Thus, this may be a support of the theory that "hereditarily conditioned tendencies are capable of awaiting their time till a certain degree of environmental level is reached ".

This study gives us an idea of the limitations of such investigation based only on the dam-daughter comparison and emphasises the need for the study of the sisters, with their collateral relatives, in order to equate the environmental condition.

The authors are grateful to the Superintendent and staff of the Livestock Research Station, Hosur, for furnishing the data.

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MICROBIOLOGICAL DETERMINATION OF CYSTINE IN PROTEINS AND FOODS BY USING *LACTOBACILLUS ARABINOSUS**

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Almost all the fundamental amino-acids except hydroxyproline can be estimated by the microbiological method. These are arginine, aspartic acid, cystine, glutmic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, serine, threonine, tryptophane, tyrosine, valine, proline and alanine (2, 3, 4, 7, 8, 9, 10, 11, 13, 16, 17, 18, 19, 21, 26, 27, 31 and 32).

In 1948, E. E. SNELL and L. M. HENDERSON (30) have described a micro method for the acids on a uniform basal medium, employing different micro-organisms. Various micro-organisms have been suggested in the literature for the determination of cystine in proteins and foods. *Lactobacillus arabinosus* 17-5, *Lactobacillus fermentus* and *Leuconostoc mesenteroids* by Max. S. Dunn and *Streptococcus faecalis* by E. E. SNELL and L. R. HAC (28) are recommended for the estimation of cystine.

But no detailed method for determining cystine in proteins has been developed on account of the chief difficulty, viz, that cystine is destroyed to different extent in the hydrolysis of proteins by acids under the conditions employed. It is proposed to describe a microbiological method of estimating cystine in proteins and foods. Besides standardising the conditions for the determination, the optimum factors for the hydrolysis of proteins without destroying the cystine liberated, have also been investigated.

1. Essential Conditions of the Microbiological Method :

In their detailed review of bacteriological assays for growth factors, W. H. PETERSON and M. S. PETERSON (24) state :-

"Theoretically an assay method should be possible for any compound that is required by a micro-organism, but in practice some micro organisms are more satisfactory than others." The following are some of the necessary conditions to be fulfilled :-

* Translated and reproduced with kind permission from "*Bulletin de la Societe de Chimie Biologique*" Vol. XXXI, No. 9-10; PP 1478-1493. (This work was done by the author while he was working as Madras Goverament Deputationist at the Laboratory of Biochemistry of Nutrition, National Centre of Scientific Research, BELLEVUE, FRANCE.)

(1) The medium should contain, all the constituents that are necessary for optimum development and activity of the micro organism other than the factor to be determined. Such a medium is indicated by the production of a low turbidity or acidity in the absence of the factor and optimum growth and formation of products in its presence.

(2) The response of the micro-organism (as measured by cell growth, products, or other index) to increasing quantities of the factor should be regular and preferably proportional.

(3) In the assay of natural materials, the responses at different levels should give the same value when this is calculated per gram or other unit of the material. Irregular values are indicative of another factor or factors in the material which may be stimulatory or inhibitory at different levels.

(4) Repeated assays of the same material should check e. g. within 5 percent.

(5) The sample should contain no inhibitory or toxic substances. If such substances are present, the results will probably be variable as mentioned under 3. Such a substance may be present in the original material, or produced by the treatment of the material, e. g. decomposition products or excess salts.

(6) A standard innoculum should be used. To start with a sturdy and stable micro organism is essential. The stock culture should be carried on a medium that contains the organism in a stable condition, and the innoculum should be developed in the same way each time. Obviously such points as age and size of innoculum are of great importance.

(7) The microorganism should perfectly be non-pathogenic. If the method is to be widely used, a pathogen is dangerous and necessary precautions reduce the number of assays that can be performed in a given time.

(8) The method should be rapid. In research work, the time required for an assay should be one to three days and for control work in industry 5 to 20 hours.

E. E. SNELL and B. S. SCHWEIGERT (29) summarise the advantages and disadvantages of microbiological methods for the assay of amino acids, as follows :—

They are highly specific and sensitive. They eliminate many of the laborious separations previously necessary in protein analyses.

They are applicable in a number of instances where no other method suitable for routine application has been developed and several estimations are little more trouble than a single one.

Several amino acids may be determined in a single prepared hydrolysate with the same micro-organism with only slight modification of the basal medium.

They are not subject to isolation losses since determinations are made directly on the hydrolysates.

Their present disadvantages include the following :—

Unfamiliarity to most chemists of an analytical technique which requires control of a multitude of factors, some of which are imperfectly understood.

Danger of the lack of activity of the unnatural isomers may cause these to be overlooked. For some applications, of course, their specificity is advantageous.

The risk of the possibility that unrecognised factors may combine to alter the specificity of the test organism in a given instance, is always present.

2. Choice of Micro-organism :

A preliminary study for the choice of the micro-organism, which gives the best results under the experimental conditions employed, has been made with *L. arabinosus* 17-5 (A. T. C. C. 8014), *Leuconostoc mesenteroids* (A. T. C. C. 8042), *S. faecalis* (N. C. T. L. 6459) and *L. fermentus* (A. T. C. C. 9338), using the same basal medium in a total volume of 10 c.c. for a range 0 of to 100 gamma (l) cystine during a growth period of approximately 48 hours. The acid production is measured by titration with O.I. N. NaOH. The results are shown in table I and the standard curves are drawn in the Fig. 1.

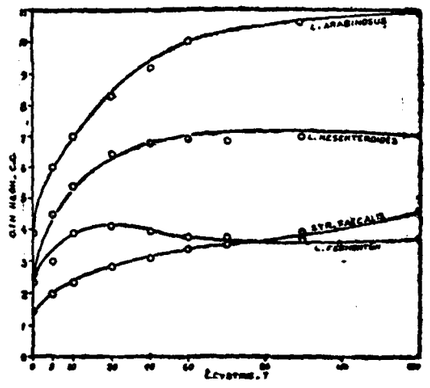

Fig. 1.—STANDARD CURVE FOR CYSTINE ESTIMATION (METHOD OFFICIAL).

TABLE I

Total gamma (l) cystine in the culture tube.	C.C. O.I. N. NaOH required for titration.			
	Leuconostoc mesenteroids.	*Lactobacillus arabinosus.*	*Streptococcus feacalis.*	*Lactobacillus fermentus.*
0.00	2.4	3.9	1.4	2.6
5.0	4.5	6.0	1.95	2.90
10.0	5.4	7.0	2.30	3.90
20.0	6.4	8.3	2.80	4.10
30.0	6.75	9.15	3.10	3.95
40.0	6.90	10.15	3.40	3.75
50.0	6.85	10.40	3.60	3.70
70.0	7.00	10.85	3.90	3.70
100.0	7.00	10.80	4.35	3.80

Leuconostoc mesenteroids gives a uniform curve, though the maxium growth is reached with 40 gamma concentration of (l) cystine. *L. fermentus* has given a curve with irregularities under the conditions employed. *Streptococcus feacalis* has a low blank. It produces a uniform curve though not as steep as *L. arabinosus*. With *L. arabinosus* is obtained a steep and uniform curve over the entire range, though its blank is a little higher.

It is desirable to know the probale cause for the high blank with *L. arabinosus*. Two possibilities are suggested for investigation.

Is there a difference in the blank, if the concentration of methionine in the medium is decreased or increased? The standard curves obtained with different amounts of methionine are shown in Fig. 2. It is found that there is no significant difference.

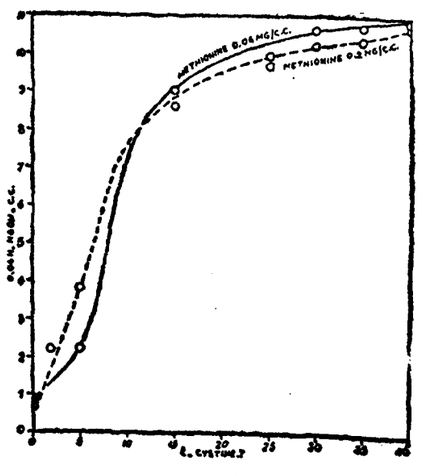

Fig. 2.—STANDARD CURVE FOR THE ESTIMATION OF CYSTINE WITH *L. arabinosus*.

It is desired to examine if any of the amino acids employed is not pure or contaminated with cystine and if the high blank is due to any possible impurities. For this purpose, fresh and pure tested specimens of all the amino acids are received from Prof. Max S. DUNN, in the University of CALIFORNIA. The standard curves are studied with both samples at the same time. The curves are drawn in the Fig. 3 There is no appreciable difference in the blanks of the two curves and it is about 2.8 c.c. of 0.04 N. NaOH for a total volume of 4 c.c. The exact cause for a little higher blank is not known.

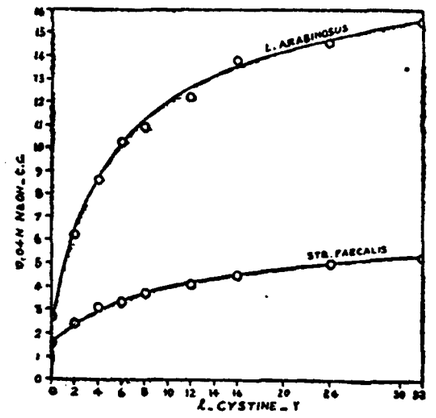

Fig. 3.—STANDARD CURVE FOR THE ESTIMATION OF CYSTINE WITH *L. arabinosus* AND *Str. faecalis*.

By employing *L. arabinosus* and *S. faecalis* with the same basal medium good agreement is observed in the assay values. As *S. faecalis* produces low amount of acid, *L. arabinosus* is better. On account of the steep and uniform nature of the curve also, it is preferred for the assay of cystine. As this organism does not require folic acid as an essential nutrient as *S. faecalis*, it constitutes a practical advantage. Therefore *L. arabinosus* is employed in the determination of cystine in proteins like wool, hair and also in the protein foods.

3. Factors Governing the Hydrolysis of Proteins.

The effects of acid hydrolysing agents on cystine and cysteine in proteins are variable. The amino acids are decomposed during the conversion stage of peptides to amino acids. They are more susceptible to decomposition when combined in the peptide linkage than when they are in the free state. Prolonged acid hydrolysis may cause interconden-sation of cystine and tryptophane. On the other hand short periods of

hydrolysis will not sever all peptide linkage. Thus the liberation of cystine by acid hydrolysis offers two fold difficulty.

The important factors which control the liberation of cystine from proteins by acid hydrolysis are chiefly the concentration, quantity, nature of acid, temperature, time and the absence of reacting substances like aldehyde and humin. Many workers have devoted their attention to this problem in the past and their observations are summarised in the tabular form below:—

Conditions of Hydrolysis and Results.

Author.	*Acid.*	*Strength.*	*Temp.*	*Time.*	*Observations.*
HALWAR *et al.* (14)	HCl	6 N	115°	16-20 h.	Added cystine is lost or converted to cystine.
OLCOTT *et al.* (23)	HCl	6-7 N	100-125°	6-24 h.	Cystine is lost in the presence of pyruvic acid.
BAILEY (1)	HCl	5 N	100°	15 h.	No loss of cystine in the case of keratins, (wools). Recovery of added cystine is 99% but in other cases loss upto 30%.
SULLIVAN *et al.* (33)	HCl	6 N	125-135°	12 h.	Cystine is lost in some specimens of insulin. In some other samples no loss.
do.	HCl 50% Formic Acid.	6 N			
BLOCK *et al.* (5)	HCl H COO H	3 N 90%	100°	20 h.	No loss of cystine in Lactoglobulin.
MILLER *et al.* (22)	HCl HCOO H	6 N 50%	110-120	48 h.	No loss of cystine in the insulin.
LUGG (20)	HCl	5 N	100°	24 h.	No loss of cystine in keratins. In the presence of carbohydrate more cystine loss than cystine. HI protects the loss.
HORN *et al.* (15)	HCl	6 N	100°	24 h.	Methionine and lysine estimated microbiologically without loss.
SNELL *et al.* (30)	HCl	3 N	100°	5-10 h.	For estimating 14 amino acids other than cystine, by microbiological method.
STOKES *et al.* (31)	HCl	3 N	100°	10 h.	Recommended to liberate amino acids without loss.
Method proposed	HCl	3 N	120°	7 h.	For pure proteins (keratins).
				5 h.	For carbohydrate containing foods.

Our studies have shown that it is sufficient to hydrolyse foodstuffs for 5 hours or pure proteins like keratins (hair or wool) for 7 hours to liberate all the cystine. The method consists in hydrolysing 1 g. of the material by 20 c.c. of 3N. HCl in sealed pyrex tubes for 5 or 7 hours as the case may be. Formic acid could not be used for the estimation by micro-organisms. The results obtained (table 5, 6, & 7) confirm the validity of the method.

4. Principle of the method.

The microbiological method is based upon the fact that *L. arabinosus* required *l*-cystine for growth as an essential nutrient. Using a cystine free, but otherwise complete basal medium, growth responses of the organism at different levels of added cystine are compared quantitatively in the standard and unknown samples. The acid produced by the organism is measured to determine the extent of growth in a fixed time and thereby the amount of cystine in the test solution. A standard curve showing the relation between acid produced against the concentration of cystine is drawn. The corresponding concentration of cystine in the unknown solution is read from the graph against the acid measured.

5. Composition of the Stock Solution and Basal Medium.

The concentrations of the nutrients in the stock solution and the basal medium are given in the table II.

TABLE II

Amino acids.	(in mg per c.c.) Stock Solution.	Basal Medium.
Glycine	2	0.2
dl-Isoleucine	2	0.2
l-Leucine	2	0.2
dl-Threonine	2	0.2
l-Tyrosine	2	0.2
dl-Valine	2	0.2
l-Proline	1	0.1
dl-Norleucine	1	0.1
dl-Serine	1	0.1
l-Histidine	0.5	0.05
dl-Phenylalanine	1.0	0.1
l-Hydroxyproline	0.5	0.05
dl-Alanine	5.0	0.5
l-Glutamic acid	5.0	0.5
l-Arginine	2.0	0.2
l-Aspartic acid	5.0	0.5
dl-Asparagine	2.0	0.2
dl-Methionine	1.0	0.1
l-Lysine	1.0	0.1
dl-Tryptophane	1.0	0.03

Carbohydrate, Buffer and Salts.

	in mg per c.c. Stock solution	Basal medium
Glucose.		20
Sodium acetate crystal.		14
Ammonium sulfate.		5
KH_2PO_4	50	0.5
K_2HPO_4	50	0.5
$MgSO_4$ $7H_2O$	40	0.4
$FeSO_4$ $7H_2O$	2	0.02
$MnSO_4$ $7H_2O$	2	0.02
NaCl	2	0.02

Vitamins & Growth Factors in gamma (microgram) per c.c.

Thiamine HCl	10	0.1
Riboflavin.	20	0.2
Nicotinic acid.	100	0.1
Biotin.	0.4	0.002
Pyridoxine HCl (B6)	100	1
P-Aminobenzoic acid.	100	1
Calcium Pentothenate.	100	1
Adenine sulfate.	1.000	10
Guanine HCl	1.000	10
Uracil	1.000	10

Amino acids :—The stock solutions of the amino acids are prepared as three mixtures without cystine, tryptophane, methionine and lysine so that the same solutions can be used for the estimation of methionine or lysine or cystine.

Amino acid mixture I :—500 mgms. each of glycine, *dl*-isoleucine, *l*-leucine, *dl*-threonine, *l*-tyrosine and *dl*-valine is exactly weighed and dissolved the mixture in about 100 c.c. of double distilled water and 4 c.c. of 20% HCl. Heat and stir until the mixture is completely dissolved. Cool and make up to 250 c.c. It is stored in a clean stoppered bottle in the refrigerator.

Amino acid mixture II :—250 mgms each of *dl*-norleucine, *l*-proline, *dl*-serine and *dl*-phenylalanine and 125 mgm each of *l*-histidine and *l*-hydroxyprolines is exactly weighed and dissolved by warming and stirring in double distilled water about 100 c.c. with 1 c.c. of 20% HCl. Cool and make up to 250 c.c. Store it in a clean stoppered bottle in the refrigerator.

Amino acid mixture III :—1·25 gm. each of *dl*-alanine, *l*-glutamic acid and *l*-aspartic acid and 500 mgms each of (*l*) arginine and (*dl*) asparagine, is weighed and dissolved together in double distilled water containing 5 c.c. of 20% HCl by warming. Cool and dilute to 250 c.c. and store it in a clean stoppered bottle in the refrigerator.

Methionine, lysine and tryptophane are weighed exactly at the time of preparing the basal medium (see below).

Adenine-Guanine-Uracil solution (Purine solution) :—0.2 gm. of Adenine sulphate, Guanine hydrochloride and Uracil is weighed and dissolved in about 100 m.l. double distilled water with 8 m.l. of 20% HCl, (6N) by warming. Cool and dilute to 200 m.l. with double distilled water and place it in a 300 m.l. Erlenmeyer flask; plug with non-absorbent cotton wool covered with gauze and wrap with a paper cover. Sterilize it for 20 minutes at 120° C. Keep it, after cooling, in the regrigerator. Take aseptically the purine solution by flaming before and after for making the basal medium.

Salt Solution A :—Dissolve 5 gms each of K_2HPO_4 and KH_2PO_4 in about 50 to 60 m.l. warm double distilled water. Cool and dilute to 100 m.l. Keep it in a clean stoppered bottle in the refrigerator.

Salt Solution B :—Dissolve 4 gms. of $MgSO_4$ $7H_2O$ and 0.2 gms. each of NaCl, $FeSO_4$. $7H_2O$ and $MnSO_4$ $4H_2O$ in 50 to 60 c.c. warm distilled water and add 3 drops of 20% HCl. Cool and dilute to 100 m.l. and store in a clean stoppered bottle in the refrigerator.

Biotin Solution :—Dissolve 40 gamma biotin (2 ampoules of 20 '*r*') in 100 m.l. of 50% alcohol and keep it in a stoppered bottle in the refrigerator.

Niacin stock solution :—100 gamma per c.c.; 10 mgm of niacin is dissolved in 100 m.l. of alcohol and is kept in a coloured, stoppered bottle in the refrigerator.

Vitamin I Solution :—Dissolve 20 mgms. of riboflavin, and 10 mgms. of thiamine hydrochloride in 0.02N acetic acid and dilute and make up the solution to 1 litre with 0.02N acetic acid. Keep it in a coloured stoppered bottle in the refrigerator.

Vitamin II Solution :—Weigh 10 mgms. each of *d*-calcium pentothenate, para-aminobenzoic acid and pyridoxine hydrochloride. Dissolve them in 50% alcohol and make up to 100 m.l. with 50% alcohol and keep it in a coloured stoppered bottle in the refrigerator.

Dilute 10 m.l. of the above solution to 100 m.l. and keep it in a coloured stoppered bottle in the refrigerator for use.

Cystine, Standard Soln. :—100 'gamma' *l*-cystine per c.c. Dissolve exactly 10 mgms. of *l*-cystine in about 50 c.c. warm water with one or two drops of ammonium hydroxide. Boil for 3 to 5 minutes to drive off the excess ammonia. Cool and make up to 100 c.c. and store it in a clean bottle in the refrigerator.

Agar media for stock culture:—Dissolve 1.5 gm. of agar in about 60 m.l. of hot water with stirring and continue boiling. Mix 1 gm. of yeast extract, (Difco), 1 gm. of tryptone (Difco) and 0.5 gm. of Dextrose with 10 to 20 m.l. of water and add this to the boiling agar solution with stirring and add water to make 100 m.l. Stir well and heat for a minute. While hot distribute the agar about 6 to 8 m.l. in each test tube. Plug the tubes with non-absorbent cotton. Sterilize in an autoclave at 120°*C.* for 20 minutes and allow it to cool. Store in a clean almirah or refrigerator.

Stab culture.—Prepare stab culture using a pure culture of the organism and incubate for 16 to 24 hours at 35—36° *C.* and store in a refrigerator or in the shelf in a clean cupboard, free from contamination, both the unused tubes and stab cultures. Prepare a fresh stab of the stock culture every week from a stab made in the preceeding week.

Normal Saline:—Dissolve 0.9 g. NaCl and make up the solution to 100 m.l. with water ; add approximately 5 m.l. to each tube. Plug with non-absorbent cotton and sterilise for 15 minutes at 115 C. Cool and keep in a refrigerator or shelf free from contamination.

Sodium hydroxide solulion, 10 N, N, O·I· N and 0.04N, and (app) 10N, NaOH.

Dissolve 400 g. NaOH in 600—700 m.l. water with cooling and make up the solution to 1 litre.

IN, NaOH	Dilute 100 m.l. of 10 N. NaOH to 1 litre.
O·IN,NaOH	Dilute 100 m.l. of N. NaOH to 1 litre.
0.04 N.NaOH	Dilute 400 m.l. of O.IN NaOH to 1 litre.

Dilute 39% HCl (fuming) with an equal volume of water to make approximately 20% HCl.

Prepare 10% HCl, by diluting 20% HCl with an equal volume of water.

Bromothymol blue indicator (0·1 %):—To 1. g. Bromothymol blue in a mortar, add 25 m.l. of alcohol and triturate well until dissolved. Transfer the solution to a beaker, add 20 m.l. of O.IN. NaOH gradually with stirring, then about 900 m.l. of water and adjust the pH to 6.8, a blue green colour with O.IN.NaOH. Finally dilute to 1 litre.

Sodium acetate Buffer Solution 2.5 M:—Dissolve 68 gms. of sodium acetate ($C_2 H_3 O_2 Na, 3H_2O$) crystals in water and make up to 200 c.c. and store it in a clean stoppered bottle in the refrigerator.

Solid reagents:—Dextrose. Chemically A. R. Grade Dextrose (for bacteriological use) is used for preparing the media for use in a concentration of 0.02 g per c.c.

Sodium acetate crystals (A. R. grade)
0.014 g. per c.c.
Ammonium sulphate (A. R. Grade)
0.0064 g. per c.c.

PROCEDURE

Preparation of the Sample for Assay.

(I) *Hydrolysis of Protein*:—1. gm. of wool, (washed with water and finally with alcohol and dried at 100° C, if necessary) or any other materials as hair, is cut into small pieces, and placed in 50 m.l., Pyrex tubes. 20 m.l. of approximately 10% or 3 N HCl is added and the tube is sealed off. It is hydrolysed in an autoclave at 120° *C.* for 7 hours in the case of pure proteins and for 5 hours for protein containing feeds. After hydrolysis, the tube is broken and the contents with washings, are transferred to 100 m.l. Erlenmeyer flask, marked at 80 m.l. volume.

(2) *Preparation of a neutral extract*:—Add 2 m.l. of 2.5 M.Sodium acetate (buffer) and neutralise with NaOH. Add 5 m.l. 10 N. NaOH at first and complete the neutralisation with N. NaOH (about 4 to 5 m.l.) to pH 6.8 testing externally with bromothymol blue as indicator. Dilute the contents with distilled water to 80 m.l. volume. Filter 20 m.l. and dilute to 25 m.l. Filter if necessary and put it in a stoppered test tube and keep it in the refrigerator. It is 1 in 100 dilution.

Preparation of the basal medium from stock Reagents:—Calculate the number of tubes and the media required for them:—

Example:

Standards in duplicate 11 tubes each	...	22
10 samples 3 tubes each	...	30
	Total	52
Media required for 52 t.ts at 4 m.l. each	...	208 m.l.
Prepare 10 to 12 m.l. more to serve as media for future use in preparing 24 hours culture for innoculation	...	12
Total media to be prepared	...	220 m.l.

TABLE III

Preparation of the Media.

No.	Description.	For 220 m.l. Quantity.
1.	Dextrose	4.4 g
2.	Sodium acetate crystals	3.0 ,,
3.	Ammonium sulphate	1.4 ,,
4.	(dl) tryptophane	11 mg
5.	(dl) methionine	22 ,,
6.	(l) lysine	22 ,,
7.	Amino acid mixture 1	22 ml
8.	,, ,, ,, 2	22 ,,
9.	,, ,, ,, 3	22 ,,
10.	Salts A	2.2 ,,
11.	Salts B	2.2 ,,
12.	Puriness (adenine, guanine, uracil solution)	2.2 ,,
13.	Vitamins I	2.2 ,,
14.	Vitamins II	2 2 ,,
15.	Nicotinic acid	0.22 ,,
16.	Biotin	0.4 ,,

The above reagents are measured exactly and placed in a 200 m.l. cylinder. Dissolve Lysine, Methionine and Tryptophane in the amino acid mixtures, by warming if necessary. Lastly add salts A and B shaking the solution after the addition of each salt mixture to prevent precipitation. The mixture is neutralised to pH 6.8 with N.NaOH, testing externally with bromothymol blue and it is diluted to (3/4 of 220 m.l.) 165 m.l. to be distributed as 3 m.l. per tube and the extract and distilled water to be made up to 1 m.l. so that the total volume comes to 4 m.l. in each tube.

Preparation of the standards and unknowns.

Standard :—The *l*-cystine standard solution for the assay is made from the stock solution of 100 gamma per m.l. Dilute 2 m.l. of 100 '*r*' per m.l. to 10 m.l. and use. This standard solution contains 20 gamma *l*-cystine per m.l.

The exact volumes of the above standard solution are calculated for a range of 10 to 18 gamma (0 to 0.9 m.l.) and distributed in the tubes in duplicate as shown in the table.

Unknowns :—Different volumes (1 to 4 fold) of 1 in 2000 diluted extract (dilute 1 m.l. of 1 in 100 dilution to 20 m.l.) are added (in duplicates) to contain 2 to 10 gamma per tube approximately. The difference between the volume of extract and 1 m.l. is the volume of water added to each tube to make the total volume of 4 m.l. Stopper the tubes with good non-absorbent cotton.

Sterilisation :—Sterilise the standards and unknown, containing tubes for 15 minutes at 115° C, in an autoclave. Cool the tubes before inoculation.

(Note: Do not expose the tubes media to direct light which will destroy the vitamins).

Inoculum preparation: Culture Medium :—Use a spare culture medium prepared before, as follows.

To about 10 m.l. of the above basal medium add about 2 m.l. of the standard *l*-cystine solution and distribute in 2 tubes and sterilise at 115° C for 15 minutes. Cool and store it in a clean cupboard.

With a sterile platinum wire transfer aseptically, the *Lactobacillus arabinosus* (A.T.C.C. 8014) from an agar stab made the previous week, to the above sterilised culture medium.

Incubate at 35·5°C for 18 to 24 hours. This should be done the previous day to carry out the assay next day.

Dilute aseptically about 0.5 m.l. of the 24 hours culture with about 10 m.l. of sterilised normal saline solution with a sterile pipette (micro). Mix well and use a tiny micro-drop of the diluted culture as inoculum for each tube.

Inoculation and incubation :—The standards and unknowns are inoculated with one small micro drop of the inoculum through a sterile pipette, observing the bacteriological technique of flaming before and after inoculation. Replace the cotton plugs immediately after inoculation. Incubate the tubes at 35—36°C (35.5°C) for approximately 48 hours in the dark.

Titration :—After nearly 48 hours, transfer the contents of each tube to a 50 m.l. beaker and rinse the tube twice with about 4 m.l. of distilled water, adding the washings to the beaker.

Add about 2 drops of 0.1% Bromothymol blue and titrate with (app.) 0.04 N, NaOH to a greenish blue colour, pH 6.8. It is important to keep the first beaker as a reference end point, so that all tubes are titrated to the same colour and pH.

Preparation of standard curve :—From the titration volumes of the standard cystine tubes, prepare a standard curve with the duplicate points (which should not differ 0.1 to 0.2 c.c.) plotting the volume of 0.04 NaOH against the total of *l*-cystine in each tube. Values for a typical standard are shown in the accompanying table 4, and the standard curve in Fig. 4.

Calculation :—Using the curve determine the *l*-cystine content corresponding to the titration values of each of the unknown tubes.

Divide the *l*-cystine content per tube by the volume of sample extract added to it, getting the concentration of *l*-cystine in terms of gamma per m.l. extract. Multiply the gamma per m.l. by dilution factor to obtain the mgm. per gram for each tube. Determine the arithmetical and biological average concentration of cystine per sample from the 4 or 3 different tubes of the same extract in different concentrations. The values of a typical estimation are shown in the table 5.

TABLE IV

Standard solution : 20 gamma l. cystine per m.l. Organism. L. arbinosus. Time : 48h. 15m.

S. No.	Vol. of extract c.c.	H_2O c.c ;	Medium c.c.	Total Vol. c.c.	Total 'r' in tt	Original. NaOH 0.04Nc.c;	Duplicate. NaOH 0.04N c.c.
*1.	— Control 0.0	1.0	3	4	0.0	0.40	0.50
2.	+ Control 0.0	1.0	3	4	0.0	3.20	3.20
3.	0.05	0.95	3	4	1.0	5 20	5.20
4.	0.10	0.90	3	4	2.0	6.70	6.60
5.	0.15	0.85	3	4	3.0	7 90	7.75
6.	0.20	0.80	3	4	4.0	8.70	8.70
7.	0.30	0.70	3	4	6.0	9.60	9.80
8.	0.40	0.60	3	4	8.0	10 70	10.70
9.	0.60	0.40	3	4	12.0	11.4	11.70
10.	0.80	0.20	3	4	16.0	12 70	12.70
11.	0.90	0.10	3	4	18.0	13.20	13.20

(*) non inoculated control.

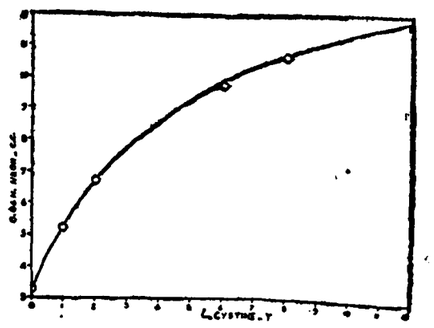

Fig. 4.—STANDARD CURVE FOR THE ESTIMATION OF CYSTINE WITH *L. arabinosus*.

TABLE V

Volume total, 4 c.c.; incubation period, 48 hours.

Sample	Volume of extract c.c.	NaOH 0.04N c.c.	Cystine in the tube 'r'	Cystine per c.c. of extract 'r'	Dilution factor c.c.	Cystine per gm. in the sample mg.
Rat hair (normal)	0.05	7	3	60	2000	120
	0.10	8.25	6	60	2.000	120
	0.20	9.90	12.5	62.5	2000	125
					Average.	122
Rat hair (Abnormal)						
,, (I)	0.05	5.6	1.25	25	2000	50
	0.1	7.3	2.55	25.5	2000	51
	0.2	9.6	5.50	27.5	2000	55
					Average.	52
, (II)	0.05	5.7	1.30	26	2000	52
	0.1	7.3	2.55	25.5	2000	51
	0.2	9.5	5.30	26.5	2000	53
					Average.	52
Wool Normal	0 05	7.5	4.4	88	1000	88
	0.10	9.1	9.2	92	1000	92
	0 20	11	18	90	1000	90
					Average.	90
Wool Kempy.	0.05	5.5	0.7	14	:000	28
	0.10	7.3	1.5	15	2000	30
	0.20	8.7	2.8	14	2000	28
	0.30	10.10	5.25	17.5	2000	35
					Average.	30

The experimental values are concordant to give the significant average in each case.

The recovery values for *l*-cystine added to the sample before hydrolysis are given in Table VI, which show the validity of the method of hydrolysis without any cystine loss.

TABLE VI

Cystine in the wool mg.	Extra-Cystine added mg.	Total cystine present mg.	Cystine estimated mg.	Percentage cystine recovered.
30	50	80	80	100
30	50	80	76	95
30	50	80	80	100
			Average.	98.3

Our results agree well with the values given in the literature, some of which are shown in table VII.

TABLE VII

% of cystine calculated for 16 gm. nitrogen.

Description.	Percentage of cystine found.	Value given in the literature.	Author and reference.
Wool :			
Merinos du Chattillonais	9.5		
Ille de France	9.4		
Berrichon du Cher	11.1		
Merinos d'arbs	11.1		
Sologuote	9.9	11.1 ± 0.9	Block and Bolling (6)
Average	10.2		
Normal rat hair 1	12.12		
,, ,, ,, 2	12.8		
,, ,, ,, 3	14.07		
,, ,, ,, 4	12.8		
,, ,, ,, 5	12.6		
,, ,, ,, 6	12.6		
,, ,, ,, 7	13.1		
Average	12.9	13.4	Block and Bolling (6)
Wheat, whole wheat	1.1	1.3 ± 0.3	Block and Bolling (6)
Wheat Flour	1.4	1.6 to 1 9	,,
Palm Oil cake	1.7	1.7	Roche and Michel (25)

Discussion

(1) Four micro-organisms, *Streptococcus faecalis, Lactobacillus arabinosus, Leuconostoc mesenteroids* and *Lactobacillus fermentus* have been reported in the literature requiring *l*-cystine as an essential nutrient for their growth. Their growth responses are studied with the same basal medium. From the results obtained and the standard curves drawn, it is found that *L. arabinosus* is the most suitable organism for the cystine assay under the conditions employed. Though *S. faecalis* can also be used, it is found to produce low acid production and requires folic acid as an additional nutrient.

(2) After investigating the conditions for a 10 c.c total volume, the method has been adopted to a total volume of 4 c.c. over a range of 0 to 18 gamma *l*-cystine.

(3) The incubation period of nearly 48 hours has been found to produce the maximum growth and acid uniformly as for 72 hours. Thus the assay period is reduced to 2 days instead of 3 days.

(4) The assay has been extended to the analysis of proteins and foods for cystine determination. The hydrolysis of proteins is carried out without destroying cystine as shown by the complete recovery of added cystine. The protein hydrolysate is prepared with 10% HCl instead of 20% HCl employed by Horn *et. al.* for Methionine assay. The removal of excess acid by distillation or evaporation is not found necessary, keeping the salt concentration at a minimum in the final extract.

(5) A uniform media, slightly different from those employed by others, is used for the determination of three amino-acids, cystine, methionine and lysine in protein hydrolysates.

(6) The values so obtained closely agree with those obtained with 10 m.l. volume method and also with the values determined by chemical methods reported in the literature. The values obtained with *L. arabinosus* and *S. faecalis* for the same samples check very well.

(7) Standard curve has been smooth and uniform and is found to be completely reproducible over a long period of six months under the conditions employed.

(8) Large number of proteins and foods have been assayed for by this microbiological method.

Summary

A micro-biological method of estimating *l*-cystine in proteins and foods by employing *Lactobacillus arabinosus* (A. T. C. C. 8014) is described. A semi-micromethod using a total volume of 4 c.c. and titrating the acid produced by the growth of organism is recorded.

Grateful acknowledgement is made to Professor M. S. Dunn of California, for his valuable criticisms and the gift of tested specimens of amino-acids. The author is highly indebted to Professor Raymond, Jacquot Director, for his kind advice, continued encouragement and lively interest in the studies presented. Thanks are expressed also to (Miss) Dr. Redla Cailleau, for the helpful suggestions received during the course of this investigations.

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Intracaudal method of vaccination against brucellosis with strain 19 was as effective as the standard subcutaneous method. (Gregory, T. S. *Aust. Vet. J.* May, 1953).

A mutant of Brucella melitensis from the semen of 45 rams was found to produce abortion in ewes. This was highly pathogenic to guinea-pigs and less so for rabbits. (Buddle. M. B. & Boyes, B. W. *Aust. Vet. J.* June, 1953).

AN OUTBREAK OF A NERVOUS DISEASE IN POULTRY POSSIBLY DUE TO RANIKHET DISEASE VIRUS.

By

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Introduction :— Ranikhet Disease is the most dreaded of all poultry diseases in India and in certain other parts of the world. It was first encountered in 1926 at Newcastle-on-Tyne, England, by Doyle (1927) and later others described the disease in many other parts of the world. In India the disease was first encountered at Ranikhet near Mukteswar and Shirlaw, after some investigation of the disease, reported that the disease bore a great similarity to the one described by Doyle as occurring in Newcastle-on-Tyne (Edwards, 1928 and Iyer, 1943). Later Cooper (1931) showed that the virus responsible for the disease at Ranikhet was indistinguishable immunologically from that occurring in Java, Phillippines and England.

Beach (1942) described, under the term Avian Pneumo-encephalitis, outbreaks of disease in chicken characterised by respiratory-nervous disorder. He reported that the disease had been in existence in U. S. A. for a long time and himself and Stover (1942) described the cause of the outbreak to be a filter passing virus which could be propagated in chick embryo. In the latter, the virus, irrespective of the disease in the chick, produced fatal infection with haemorrhages in the alimentary tract. This finding suggested a possible similarity to Newcastle disease and fowl plague, both of which had not been recognised at that time in United States. To explore the possibility of such an identity, Beach (1944), conducted neutralization tests using cultured Pneumo-encephalitis virus with Newcastle and Fowl-plague anti-sera obtained from England. The result of this test showed that the virus of pneumo-encephalitis identical with that of Newcastle Virus. The findings of Beach were confirmed by Brandly *et al* (1946) by neutralisation and cross-protection tests. However, in view of the great contrast in the symptomatology, mortality and transmissibility under artificial conditions of the disease, as occurring in The United States and in other parts of the world, it may well be doubted whether the disease is caused by one and the same virus or by two different viruses. However, the findings of Beach and Brandly greatly establish the identity and Beach further states "Credulity must be assigned to the concept that the disease in United States is Newcastle disease which has for some undetermined reason occurred in a benign form upto the present time".

In the Indian form of the disease, the course is rather rapid, affected birds usually dying in 3 days from the onset of the disease. There is watery yellowish-white diarrhoea, mucus discharge from mouth and nostrils and characteristic gasping inhalation through half-opened mouth. Chronic form of the disease showing paralysis of legs and wings and twisting of head and neck to one side is seen occasionally in some birds in an outbreak. However, such cases are few and rarely reported from the field. The disease manifesting clinically, purely as a nervous disorder, does not appear to have been reported in this country and as such an opportunity is availed of here to record an outbreak of a purely nervous disorder, possibly caused by the Ranikhet disease virus. The symptomatology, course etc., bore a great similarity to the American form of the disease. The object of this article is to bring to the notice of veterinarians the existence in this country of a nervous disorder in chicken due to the virus of Ranikhet disease and the need to fully investigate such outbreaks.

Materials and methods :— It was reported from the Hosur Cattle Farm (Madras State) that 102 chicks from ages of 1 - 3 months during the months April, May and June 1953 showed symptoms of nervous disorder. In all, two consignments of ailing chicks one consisting of 11 and another consisting of 5 were received for investigation.

Symptoms :—As stated above, altogether 102 chicks from ages 1-3 months showed symptoms of nervous disorder. A large number of these chicks were observed by one of us (K. P. Chandrasekharan) at the farm and further clinical observations were done after they were brought for investigation to the College. All the chicks showed symptoms referable to central nervous system. These symptoms varied in degree in different individual and consisted of paralysis of legs, wings and neck (vide Fig. 1. Plate No. 21.) Tremor of head and body incoordination of the cervical muscles were also noticed. Due to the latter condition, the head was turned backwards or inwards between the shoulders or in some others regular circling movements were noticed (vide Fig. 2. Plate No. 21.) Chicks walked in circles or backwards. Some became completely prostrate in course of time, debilitated and died. The appetite of most of the birds were not impaired and many in spite of their disability to control movements managed to feed themselves sufficiently to keep alive. The disease in almost all cases ran a chronic course. In about 25% of the chicks affected, opacity of the cornea was reported.

Experiments conducted :—As the chicks at the time they were referred to us showed symptoms referable to central nervous symptom, investigations were undertaken with the following possibilities in mind.

1. Nutritional deficiency especially due to Vitamin B complex variety ;
2. Neuro-lymphomatosis ;
3. Infectious encephalomyelitis (viral);
4. Nervous form of Ranikhet disease.

The chicks belonging to the first batch were at random divided into 2 lots of 3 each—I and II. Both the lots were given balanced food consisting of wheat flour 45, wheat bran 10, skimmed milk powder 20, fish meal 5, oil cake 15, bone meal 5, fish liver oil 1·5 and salt 1 part. Lot No. II was kept as control. The chicks in Lot No. I received in addition, Adexolin 4 drops (oral), Vitamin B complex 1 tablet (oral) and Berin 100 mgms/1 c.c. (intra-muscularly). This treatment was followed by Thyroxine 0·25 mgm orally and 1 c.c. of Pyridoxine intramuscularly.

Autopsy was conducted on all birds dying of the disease and materials were collected from brain, spinal cord and liver from all birds for histo-pathological study.

Two ailing chicks (from batch II) were sacrificed to obtain brain material for intra-cerebral inoculation into healthy animals. The material was given in 1% suspension in saline into 5 healthy White Leghorn chicks of ages between 1 to 2 weeks and in 4 white mice each weighing between 15–20 gms. Adequate controls were kept for this experiment.

It was thought that the birds might have been affected with a mild form of Ranikhet disease and to test this possibility a batch of six birds were challenged with (subcutaneously) 0·5 c.c. of a 1% saline suspension of virulent Ranikhet disease virus using two control birds.

Bacteriological examination of brain and heart blood was done from two birds used for transmission experiment.

Observations and results :—The lot I which was given in its feed additional supplement of Adexolin and Vitamin B complex and 100 mgms. of Berin intramuscularly showed no improvement after three days of treatment. Hence this lot was given further Thyroxine orally and Pyridoxine intramuscularly. This treatment though showed slight gain in weight, did not give any relief in the nervous symptoms.

The chicks and mice that were inoculated intra-cerebrally with brain material did not show any symptoms of paralysis nor did they show any symptoms attributable to central nervous system as compared with the controls.

The six chicks that were chronic sufferers from the disease when infected with virulent Ranikhet virus, withstood the virus and showed no evidence of the disease proving thereby that all of them were solidly immune. The controls died showing typical symptoms and lesions of Ranikhet disease.

Post-mortem examination did not reveal any gross abnormality in any organ. Histopathological examination of brain showed meningeal congestion in a few chicks, foci of glial proliferation, sclerosis of medium sized vessels and stray neuronal damage. These neurologic changes were prominent in the cerebellum and medulla. In one case there was histological evidence of Avian leucosis complex.

Discussion:—From the history of the outbreak and the investigation conducted, it will be seen that the disease in question was of a chronic type, the ailing birds usually living long in spite of the nervous disorder and difficulty in getting sufficient food thereby. Avian leucosis complex was ruled out in all cases excepting in one, both on macroscopical and histological grounds. The feeding experiments conducted showed that nutritional factors were not involved in the causation of the disease in these birds. The same conclusion has been reached on pathological examination of the materials from these cases.

Histological examination showed that there was damage of a patchy character to the central nervous system involving mostly the cerebellum and the medulla. These changes were unlike those seen in viral avian encephalomyelitis or in riboflavin or tocopherol deficiency. The limited transmission experiments done did not reveal any specific infectious agent, nor was it possible to reproduce the disease in chicks or mice by intra-cerebral inoculations. However, the transmission experiment did not yield any conclusive result in as much as the birds used as donors were not in the early stages of the disease. Possibly, had the experiments been conducted in the early stages, fruitful results could have been obtained.

The report from the Assistant Disease Investigation Officer (Poultry) that some chronically ailing birds in this outbreak gave positive reaction to haemo-agglutinin inhibition test was significant and further, when a batch of birds withstood actual challenge dose of the virulent Ranikhet disease virus, there was ample proof to conclude that these birds were suffering from Ranikhet disease of a mild type with involvement of the central nervous system. In this connection, it may be recalled that the virus of Ranikhet disease in United States (called Pneumo-encephalitis) produces symptoms not unlike to what has been presented in this outbreak. It is reasonable thus to conclude that the disease in the birds was nothing other than a

mild form of Ranikhet disease involving chiefly the central nervous system. It is proposed to isolate the virus and make further studies should future opportunities arise.

Summary

An outbreak of nervous disorder affecting about 102 chicks of 1 – 3 months age has been investigated.

2. The symptoms shown by the ailing birds and the investigations which lead to conclude that it was Ranikhet disease of mild type similar to what has been described in The United States are described.

Acknowledgment

The authors are grateful to the Superintendent and staff of Livestock Research Station, Hosur, and the Director of Animal Husbandry for furnishing the birds.

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SOME METHODS OF INTENSIFYING FODDER PRODUCTION IN BIHAR

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The last census revealed that the State of Bihar is over populated to such an extent that only 0·7 acres of arable land is available *per capita*. Hence strenuous efforts are being made to bring under the plough more acreage of even the least cultivable land. The cattle population of the State is valuable both for providing the motive power for farming and for the supply of milk, which is essential for the health of the people especially children and vegetarians. Cattle are also keeping pace with human population in multiplication. Thus they share a considerable quantity of agricultural produce from arable land of the State

in addition to what they derive from the so-called grazing areas. The distribution of rainfall of this State is confined mainly to the four months of the rainy season i.e., June to September with one or two showers in winter in some years. Irrigation facilities of some kind are available to about 30% of our arable land but none to the pastures. The seasonal nature of precipitation leads to green herbage production in the pastures during those few rainy months only. But due to the over-stocking of those pastures it is not possible to support all the livestock even during the periods of maximum growth. It need not therefore be further emphasised that our pastures are quite inadequate to meet the needs of our cattle wealth even during a part of the year. The farmers have to meet the defficiency of their fodder requirement from arable lands. As only a very small acreage of such land can be released from human food production it is imperative to adopt most intensive methods of fodder production.

Since 1950, at the college farm at Sabour, we are making a concerted effort to maximise production of green fodder and to make it available through out the year to the farm live-stock. The methods adopted to attain these objectives have been outlined below. Broadly they consist of two practices i.e., introduction of new fodders and adoption of certain changes in the agronomical practices of raising the established fodder crops.

Changes in agronomical methods.

Juar (Sorghum vulgare): Juar is our mainstay as the bulky *Kharif* fodder. The usual method of cultivation is to sow the seeds behind the plough or to broadcast in the month of June or July and harvest in September or October. The average outturn of a well manured field is about 350 - 400 mds. of green fodder per acre. It has been observed that the stems of Juar crop, 4–5 months old, become rather stalky and hence unpalatable to the live stock. Considerable wastage of such fodder occurs in the mangers. Further it has been reported[1] that as *Juar* grows older after 2–2½ months' growth, the protein percentage in the crop goes on diminishing and at the same time the digestibility of the nutrients also goes on decreasing. To counteract these drawbacks in nutrition in the usual methods of Juar cultivation for fodder and also to increase the total yield of fodder the following modifications have been successfully adopted at Sabour.

Ratooning :— It has been observed[2] that if two cuttings of *Juar* at an interval of 2–2½ months were taken,[3] then, the fodder does not become too stalky and at the same time the nutrient content, palatability and digestibility is maintained at a very high level. To achieve this the crop has to be sown in the first week of May with irrigation, in lines,

two feet apart. The first cutting may be taken by the middle of July, and then the lines may be intercultured and fertilised with a maund of ammonium sulphate per acre. The second cutting may be harvested by the end of September. By this method 15–20% more yield than the usual method is obtained and the period of utilisation of the fodder is spread over a much longer period.

In mixture :— Juar sown mixed with a climbing legume like *Meth (Phaseolus ricciardianus)* gives at least 75–100 mds more green fodder per acre than a pure stand. At the same time this legume considerably minimises the depletion in fertillity caused by the *Juar* crop. Where ratooning of *Juar* is contemplated, it is not advisable to sow it in mixture.

Maize :—Maize is a well known quick growing nutritious fodder crop. It may be sown at intervals from January onwards to meet the fodder requirements from March to beginning of June. Irrigation facilities must be provided during the hot weather.

Maize in mixture :—Apart from sowing pure stands of maize fodder crops, it might be possible to sow it in mixture with some crops sown during this period e.g., with sugarcane. Under our conditions, the sugarcane takes about 6 to 8 weeks for complete germination. Maize seeds can be put in the furrows where cane setts have been planted or they can be dibbled on the ridges. It has been found that germination of the cane was not much affected. By the time all the buds had germinated, maize fodder crops could be removed so as not to affect the main crop at all. With only a little extra dose of manure it is possible to get 80–100 mds. green fodder at this time of the year i.e., early summer when green herbage is so scarce. It must be conceded that if maize is left for cobs, the main sugarcane crops would be adversely affected[4].

Kalai (*Phaseolus mungo*) :—Kalai is a pulse crop which is equally important in Bihar as a quick growing fodder crop. The seeds of this crop can germinate under any kind of adverse field conditions[5]. During rainy season this crop can be grown as a catch crop wherever there is any opportunity. It has been established at Sabour that this crop betters the fertility status of the soil instead of depleting it[5].

Early maize plots can be ploughed up and put down in *Kalai* for fodder. But the easier and equally effective method is to sow *kalai* in maize at the time of final earthing up. As soon as the maize crop is harvested, the *kalai* crops start growing vigorously yielding 75–100 mds of nutritious green fodder per acre. Similarly lands which have been green manured in Kharif for crops like sugarcane can be easily utilised for growing *Kalai* fodder inter crop.

Lucerne.—This is a perennial legume fodder needing good irrigation facilities. It has been possible to raise 300–400 mds. of fodder per acre in 4–5 cuts. It does not appear to be so well adapted to Sabour climate. Lucerne was sown in lines 2½ ft. apart for ease of interculture and irrigation. Lucerne is rather dormant between March to September. During this period we can get good amount of forage if some annual quick growing millets like *Kodon* (*Paspalum scorbiculatum*) or *Kheri* (*Panicum frumentacum*) is sown between the lines of lucerne. It was observed that the partial shading effect of these grasses help lucerne to stand the hot winds better during the summer months. Discovery of some perennial grass which can grow in mixture with lucerne with mutual benefit of association should be attempted.

Introduction of new fodders.—Teosinte (*Euchlaena mexicana*) is a relation of maize in origin. This fodder crop can be sown either in the month of March or June. If sown in March, two cuttings can be easily taken; but June sowing proves very useful as it keeps green and palatable as late as end of November when no other bulky grass fodder is available (1). It may be used for such special purpose for specified periods of expected shortage of green feeds.

Sudan Grass (*Sorghum vulgare* Var *sudanensis*).—Under irrigation this crop has a very rapid growth during the hot months of April, May and June when even *jowar* and maize are retarded in their growth. If it is sown in March, first cutting can be taken at the end of May, second at the end of July and the third by September. The land then can be released for growing any *Rabi* crop. Normally Sudan grass yields 300–500 mds. of green feed per acre.

Napier Grass.—It is a perennial grass and can yield upto 1000 mds. of green fodder in 8—10 cuttings under well managed conditions. With adequate irrigation it yields valuable green feed even during difficult hot months of April and May or in early winter like November.

Berseem.—It is no doubt the most prized *Rabi* fodder for dairy animals. This crop needs plenty of irrigation water competing with other money crops of the same season like potato. It should be sown in early October so that the first cutting is obtained by December.

Rape.—This green fodder also yields about 150–200 mds per acre with hardly one irrigation.

Oats and peas.—It has been possible to get 150–200 mds of green fodder in *Rabi* season from this mixture with one irrigation.

Choumoellier (*Brassica oleracae* Var *acephala*).—It is a new *Brassica Rabi* fodder crop introduced from New Zealand. It could be grown without irrigation and could be fed from January to April

without losing its succulence and palatability. Yields nearing 300 mds. per acre were obtained'.

Senji (*Melilotus alba*).—This is a very nutritious *Rabi* fodder. Local collection of seeds grown at Sabour farm yielded up to 250 mds. per acre with 2 to 3 irrigation. This crop is not so exacting in its requirement like Berseem as regards fertility or irrigation. It can be easily sown in October in the plots where *Jowar* or Sudan grass were grown in *Kharif* and harvested in March.

Thus, in a farm of 100 acres, if we utilise hardly 5 acres of land during *Kharif* and *Rabi*, we can obtain quite a good quantity of fodder per year spread all over the year as shown in table below based on average Sabour farm yields.

Kharif fodder	Acreage of crop	Sowing time	Harvesting time	Yield in Maunds.
1	2	3	4	5
1. *Juar* + *Meth*	1.00	June	Sept. (end)	400
2. *Juar*	1.00	May	2 cuts (Jul. & Sept.,	400
3. Maize	0.25	Jan.	March	25
	0.25	Feb.	April	25
	0.25	March	May	25
	0.25	April	June	25
4. Napier (Perennial)	0.50	—	8–10 cuts	400
5. *Kodon* in Lucerne Plot	0.50	Feb.	2 cuts	40
6. Sudan Grass	0.50	March	Sept. (end) 3 cuts	150
7. Teosinte	0.50	March	Nov. 2 cuts	125
Total	5.00			1635

Rabi fodder	Acreage of crop	Sowing time	Harvesting time	Yields in Maunds
1	2	3	4	5
1. Berseem	2.00	Oct.	March 5–6 cuts	600
2. Lucerne	0.50	Oct.	5 cuts	200
3. Rape	0.60	Oct.	Dec.	100
4. Oats and peas	1.00	Oct.	February	100
5. Senji	0.50	Oct.	March	100
6. Napier	0.50	—	—	—
Total	5.00			1100

Normally a 100 acre farm like Sabour grows about 40 acres in maize for cobs, 10 acres in green manuring crop and 5 acres in Sugarcane. If these are utilised for raising catch crop of *Kalai* as indicated, then 2000–2500 mds. of additional green fodder can be easily obtained without

much effort during *Kharif*. Then the total annual production of fodder can be estimated at about 5000 mds. per year.

If we assume that the farm maintains 10 pairs of bullocks of average size then roughly 3600 mds. will be consumed by them during the whole year. The surplus 1400 mds. of green fodder along with other bye-products of the farm like sugarcane tops, maize stalks, cereal straws etc. can support 10–15 heads of milch cattle.

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GLYCOSURIA IN RABIES

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Introduction.—Clinical symptoms of Rabies in cattle are usually well-defined and there is no difficulty in the diagnosis of such cases correctly. But the authors have come across a few cases wherein clinical symptoms were atypical and only suspicious. With a view to get more information on such cases, biochemical tests were carried out on the urine from these cases for pathological constituents in general. The objeet of this paper is to record our observations based on clinical cases of Rabies in herbivora wherein glycosuria was a prominent symptom.

Hutyra and Marek (1949) quoting Nocard, Rabieaux and Redecha state that the urine from cattle showing symptoms of Rabies "often

contains glucose". Redecha (1949) found 9% of glucose in a cow showing clinical symptoms of rabies shortly before death. Krishnamurthy (1946) in his observations on 21 cases of Rabies (16 dogs, 1 cow and 4 calves) concluded that "no glycosuria was detected in any of these cases and albumin was noted only in four of them (19%). It would thus appear that glycosuria and albuminuria are not frequent features, much less symptoms of Rabies". Batia and Mehta (1951) in their investigation on "glycosuria in Rabies due to fixed virus" in dogs detected glycosuria in only 3 cases and albuminuria in one out of 45 cases examined and concluded that glycosuria and albuminuria do not seem to be common symptoms of Rabies due to 'fixed virus'.

Materials and methods.—This study was made on 4 cows and one calf brought to the Madras Veterinary College Hospital showing suspicious symptoms of Rabies and kept under observation.

Urine samples were collected from five cases and analysed for pathological constituents. Quantitative estimation of sugar present were made only in two of the cases.

Results.—It is seen from an examination of Table I that all the animals examined were positive for Rabies. The brain was examined in all the five cases and was positive for negri bodies in all. The sugar content, of the urine samples examined varied from traces to a maximum of 2·5%.

Discussion.—The observations recorded herein were based on the clinical cases admitted in the Madras Veterinary College Hospital. So the question of 'fixed virus' producing glycosuria syndrome may be left out. Regarding the presence of sugar in the urine of cows showing symptoms of Rabies at any particular time, it is seen from the protocol that it is present from the time the symptoms are well advanced. Even on post-mortem, sugar was present in a case (Case No. 1889).

Krishnamurthi's (1946) materials consisted of the urine samples taken for examination from all the cases on post-mortem only and his observations were confined to only one case in cattle, while the findings of Bhatia and Mehta relate to "fixed virus" in dogs.

Conclusion.—In all the five cases examined, there was sugar in the urine and the authors' observations and findings are in agreement with those of Hutyra and Marek (1949) and Redeck (1949). The authors are therefore of the opinion that glycosuria, as a feature, in cases of rabies in cattle cannot be brushed aside and that examination of urine from suspicious cases of rabies will greatly help in the ante-mortem diagnosis of rabies in cattle where symptoms are atypical.

TABLE I

S. No.	Case No.	Date of admission.	Kind of animal.	Date of clinical examination of urine.	Results of examination of urine.	Date of death.	Result of microscopical examination of brain for Rabies.
1.	2494	14—3—46	Cow.	14—3—46	Colour—faint yellow. Sediment—turbid. Reaction—Acid. Sp. gr. 1030. Albumin—present *in traces*. Sugar—*Present*. (quantity insufficient for percentage estimation).	14—3—46	Positive for Rabies
2.	2596	26—3—46	Bull calf.	26—3—46	Pale yellow; clear; alkaline; 1010; Albumin not present. Sugar *present 2.3%* Acetone present.	29—3—46	do.
3.	1619	16—1—44	Cow.	17—1—44	Yellow; clear; alkaline; sp. gr. 1040; Albumin *absent* Sugar *2.5%* Acetone present (++++)	20—1—44	do.
				18—1—44	do.		
4.	1889	12—11—51	Cow.	12—11—51	Glucose *present*. Quantity insufficient for percentage estimation.	12—11—51	do.
5.	3031	4—1—53	Cow.	8—1—53	Sugar traces present; Acetone present; sp. gr. 1016. Acid reaction; clear.		do.

Acknowledgment.—Our thanks are due to the staff in the Pathology and Physiology Departments for making available the records pertaining to the cases cited above.

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AN OUTBREAK OF ANTHRAX AMONG SHEEP EXTENDING TO HUMAN BEINGS.

By

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In the month of August 1950, an outbreak of Anthrax among sheep and goats occurred in the villages of Muddurthi and Bhogapuram in Chodavaram Taluk, Godavari District. The disease was confirmed by getting the materials examined by the Principal, Madras Veterinary College.

Simultaneously a disease broke out among the inhabitants of the village characterised by lymphadenitis and pyrexia. The disease was first suspected to be Bubonic plague, but was subsequently confirmed as one of Anthrax caused by the consumption of flesh of dead sheep and goats. The Director of the King Institute, Guindy, to whom a section of lymphatic gland from an affected person was sent reported that "Anthrax-like bodies were present".

The outbreak among the animals was controlled by preventive inoculation and simultaneously the disease among human beings also subsided.

[On referring the matter to the Director of Public Health, Andhra, the following report on the outbreak by the District Health officer, Vizagapatam, has been kindly forwarded to us. *Ed. I. V. J.*]

I submit that during the month of August 1950 a disease, mistaken to be Bubonic Plague, was reported in two villages Muddurthi and No. 84 Bhogapuram of Chodavaram Taluk among the human beings. The disease was characterised with acute inflammation of lymphatic glands usually of prescapular region associated with febrile symptoms. Suspec-

ting the disease to be plague, preventive inoculations were conducted by the subordinates of the Public Health Department.

The following were the symptoms of the disease among human beings. The signs and symptoms were varied. (1) In all cases there was swelling of lymph glands with rise of temperature varying from 100 degrees to 103 degrees. (2) The enlargement of glands was not confined to one particular region of the body in all cases. (3) Some cases developed swelling of groin glands. (4) Some developed swelling of axillary glands and some showed swellings of pectoral and parotid regions. (5) In some cases the glands were acutely inflamed and oedematous with adenitis and periadenitis and surrounding cellulitis. (6) In some cases there was simple enlargement of glands with no inflammatory symptoms. (7) In some cases there was high fever and in some there was no fever. (8) None of the patients showed (except one in Bhogapuram village) enlargement of spleen. (9) No visible local lesion indicating Anthrax was found among the human patients nor any visible ulcers were found among them.

Later on, from the above history given by the affected persons, the disease was suspected to have been caused by eating the flesh of dead sheep and goats. Post-mortem on a dead sheep was conducted. Heart blood smears and smears of the section of the spleen were sent to the Madras Veterinary College, which proved positive for Anthrax. A post-mortem on a goat was conducted by the Veterinary Investigation Officer and the smears proved positive for Anthrax both microscopically and biologically. Smears of section of the lymphatic glands and one complete lymphatic gland of the affected persons were sent to the Institute, Guindy. The result was as follows :—" Anthrax-like bodies were present ".

It was confirmed that the disease was caused by the eating of the flesh of the dead sheep and goats. The peculiarity of the disease was the characteristic acute inflammation of the lymphatic glands of the body of the affected persons and it was nowhere mentioned about Anthrax among human beings.

Preventive vaccinations were conducted among sheep, goats and cattle and the disease subsided.

Nearly 30 persons were affected with the disease and 5 persons died. The affected were treated with sulphanilamide powders at intervals of 4 hours. In some cases soluseptisine injections were given.

A NOTE ON THE TERMINATION OF THE COMMON CAROTID ARTERY IN DOMESTIC ANIMALS

By

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It is commonly accepted by Veterinary Anatomists that the internal carotid artery is absent in the bovines and that the common carotid artery ends in a trifurcation. The object of the present note is to present the views of the different authors and to focus attention to the existence of the internal carotid artery in bovines also and to emphasise the need to revise the description of the mode of division of the common carotid artery in the domestic animals.

Sisson and Grossman (1953) describe that the common carotid artery terminates by dividing into external carotid, internal carotid and occipital arteries. Of these, the internal carotid artery is the smallest and it usually arises just behind and, not rarely, by a short common trunk with the occipital. In the ox, the common carotid artery ends by dividing into external carotid, external maxillary and occipital arteries and the internal carotid artery is regarded as absent and its place taken up by several branches arising from the internal maxillary artery. In the sheep, the lingual artery takes the place of the external maxillary artery, the latter being absent. In the pig and dog the division is the same as that in the horse. In the domestic fowl, the common carotid artery terminates by dividing into external and internal carotid arteries.

Bradley and Grahame (1947-48) state that in the horse and dog the common carotid divides into occipital, external carotid and internal carotid arteries.

Soares (1946) had shown the existence of the internal carotid artery in the ox and describes the origin of this vessel from the common carotid artery close behind the origin of the occipital.

Habel (1949) writes that Sisson's statement of the common carotid ending by dividing into occipital, external carotid and external maxillary arteries is misleading from a comparative stand-point and also because these branches are not of equal size nor of simultaneous origin. He says that in adult cattle the internal carotid artery is vestigeal and begins just caudal to the origin of the occipital and the external carotid which continues the common carotid gives off the external maxillary artery.

Jewell (1952) in his paper refers to " carotid bifurcation " in the dog and describes the occipital artery as a branch of the external carotid artery.

During the course of my dissection on the ox, I had always observed this internal carotid branch of the common carotid arising just behind the origin of the occipital artery and pursuing a course as described by Soares.

A study of the developmental history of the carotid arteries in vertebrates reveals the fact that the internal carotid artery of the adult is but the persistent cranial part of the primitive dorsal aorta and the external carotid is a later acquisition as a branch of the common carotid, simultaneous with the development of the face. Comparative anatomy thus shows that the common carotid artery ends in all vertebrate species by dividing into external and internal carotid arteries. In the ox type too, the internal carotid artery is present, though much reduced and vestigeal in the adult. Hence, in all the domestic animals it is advisable to describe the common carotid artery as dividing into external and internal carotid arteries and to consider the occipital and external maxillary arteries as collateral branches of the external carotid artery as this will be in conformity with comparative vertebrate anatomy.

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TRANSPARENT SPECIMEN OF THE INTERNAL EAR AS A TEACHING AID IN ANATOMY

By

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The structure of the internal ear is so complicated that a certain amount of imagination is required to understand it. Diagrams, stained sections and paper mash models of the different parts of the ear are made use of as aids in teaching the subject. However, a natural

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specimen would make a clear impression on the mind of the student, of the actual size and location of the labyrinth, the course of the spiral cochlea and the different directions of the semi-circular canals.

The internal ear is enclosed in the petrous part of the temporal bone. The osseous labyrinth is composed of a central cavity, the vestibule. Projecting from above and behind the vestibule are the three semicircular canals viz., the superior, the posterior and the lateral canals. The vestibular aqueduct extends from the medial wall of the vestibule to the posterior surface of the petrous temporal bone. Anteriorly, the vestibule continues in the form of a spirally coiled tube, the cochlea. The osseous labyrinth contains the membraneous labyrinth which is studied by examining the stained serial sections of the petrous temporal bone.

The following technique was found to be very helpful in bringing out clearly the structural details of the internal ear.

The petrous part of the petrous temporal bone was separated from a buffalo-calf which was bled, preserved with formalin and used for dissection in the department. (The tissue is usually fixed in 5 to 10% formalin for 24 hours). The bone was then placed in about four times its volume of a decalcifying fluid (Carleton, 1926) which was prepared as follows:—

5% aqueous formalin	...	100 c.c.
Nitric acid (1.4 Sp. Gr.)	...	7.5—15 c.c.

The fluid was changed daily for about a week by which time the bone became completely decalcified. The acid was removed by treatment with 5% aqueous solution of Sodium Sulphate for 12—24 hours. The tissue was left in water for 12—24 hours and then dehydrated by treatment with ascending grades of alcohol in 12 to 24 hours (for 3—6 hours in each of 50%, 70%, 90% and absolute alcohol). It was then cleared in Xylol for about 24 hours, Xylol being changed every 4 hours till it became transparent. The specimen was left in Xylol. The outline of the osseous labyrinth was clearly visible through the bone surrounding it.

Summary

The natural specimen of the osseous labyrinth is made use of as a teaching aid in Anatomy. The petrous temporal bone is decalcified and rendered transparent. The specimen gives a comprehensive picture of the intricate structure of the labyrinth, much of which is otherwise left to the imagination of the student.

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A REVIEW OF LITERATURE ON ENVIRONMENTAL TEMPERATURE IN RELATION TO REPRODUCTION

By

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Introduction

Studies on factors influencing reproduction have revealed that sex activity in animals is directly or indirectly affected by environmental temperature. Breeding in different species of animals occurs in different calendar months, i.e., at periods most suitable for the birth of the young and its rearing. This means that for reproduction each species has a different physiological timing mechanism for responsiveness to the activating agents. Some species are stimulated to sex activity by increasing temperature (spring) ; others by decreasing temperature (late summer, autumn). Under natural conditions, deer, sheep, goats and, in general, ruminating ungulates tend to come into sex activity when days become shorter ; ferret, fox, field-mouse, wild cat and, in general, carnivores and practically all birds investigated tend to come into sex-activity only when days become longer.

Therefore a review of the available literature on the subject would be of great help to those engaged in livestock breeding work.

Effect of temperature on gonadal functions of different animals :— Guthrie (1922) reported that ovulation in bats will occur any time after the middle of February (in Columbia, Mo) if they are brought into a warm room ; but this varies with dietary and other habits of particular bat.

Witschi (1929) observed that temperature is an effective factor in modifying the cortico-medullary balance of frogs and toads and suggested that high temperatures apparently enhance the production of medullarin,

which inhibits the cortex and thus causes the genetic female to develop testes ; the low temperatures favour the release of cortex which inhibits the medulla and encourages the cortex, thus causing genetic males to develop ovaries temporarily.

Turner (1948) reports that in the newt (*Triturus viridescens)* spermatogenesis normally occurs during the summer. When the animals are subjected to low temperatures (12° c. or less during the summer months), spermatogenesis ceases and testicular involution ensues ; when they are maintained at higher temperatures (21° to 25° c.) during the winter, sperms are matured and discharged. Under natural field conditions, testicular regression is associated with rising summer temperatures and seasonal reactivation of the testis is associated with diminishing temperatures of fall and winter.

Lee (1926) studied the breeding cycle in white rats and found that the length of the cycle varied roughly with the environmental temperature. The results are interpreted as being due to the effect of temperature on ovarian activity and seems likely that, in a state of nature, the estrual rhythm may vary considerably with the environmental temperature.

Cordrelia Ogle (1934) reported some of the effects of change of temperature on reproduction in white mice. The mice subjected to a warm humid environment show a low fertility in three ways, viz., a low percentage of mating that results in pregnancy, small litter size and low viability of the offspring. Most efficient sex-functioning comes with steady cool environment when the greatest number of matings result in conception and large litter of lusty offsprings are born.

Lamoreux (1943) observed that chicks kept at higher temperature had longer caneb growth (68.2 sq. cm) which was associated with intense cellular activity and hypertrophy of seminiferous tubules and those at low temperature (36°F) had smaller growth (22.4 sq. cm.) with less acedrogenic humane secretion and restricted peripheral circulation. Bennion and Warren (1933) reported that the mean weekly egg weight when compared with the mean maximum weekly temperature showed a sharp decline when the temperature was above 85°F. All the components of the egg decreased under high temperature; the shell and albumen decreased considerably more than the yolk in proportion to their weight, which indicates that the oviduct is more sensitive than the ovaries to high temperature.

Phillips *et al* (1943) reported marked decrease in breeding efficiency in terms of fertile matings in bulls during the summer months. Significant differences were observed between seasons in volume, total sperms, abnormal heads, abnormal middle piece and abnormal neck of

the semen of bull. Observation made on rams and by Phillips *et al* (1943) on bucks indicated seasonal differences in sperms motility, number of sperm per c.c., total sperm produced and the proportion of abnormal heads, necks, middle pieces, tails and total abnormal sperm, and Bogard and Mayer (1946) reported summer sterility in rams.

Effect of temperature on microscopic structure of gonads and pituitary glands :—Young (1927) made a study of the histological changes on the testis of guineapigs which were subjected to high temperature of 46° to 47° C and found that degeneration of the germinal epithelium began immediately. The order of germ-cell degeneration was concluded to be in no relation to the order of germ-cell formation, but, presumably, in relation to their state at the time of treatment, the more active cells being the first to undergo degeneration changes.

Some epididymal sperms are very resistant to heat injury and even after 31 days after treatment are capable of taking part in fertilisation which are followed by apparently normal litters. Recovery of spermatogenic function seems dependent upon the survival of spermatogonia. Recovery of spermatogenic function was followed by reproductive function on 16 out of 19 males as characterised by decrease in the proportion of sterile matings.

Bogart Ralph & Mayer (1946) presented evidence that high summer temperatures induce in rams a temporary " summer sterility " in which both spermatogenic and interstial cell activity are markedly affected. The histological studies showed the complete failure of spermatogenesis in the semeniferous tubules which are devoid of spermatozoa and in the lack of spermatogenic activity in the surrounding cells.

Bailif (1938) reported on microscopic changes in the hypophysis of the albino rat following exposure to cold. The rats were exposed to 70° c to 0° c. for a period of time ranging from 9 to 50 hours. Early changes in anterior lobe is dilatation of sinuses and their filling with blood, or with a cell free fluid which is not precipitated by fixing agents. The appearance of this fluid is associated with an increase in cellular activity as exposed by vacuolation of the alpha and beta cells. This primary secretory hyperactivity is never accompanied by pronounced cellular disintegration. If the cold exposure is mild, this phase of secretion may persist and the gland will remain in the apparently hyperactive state for considerable period of time. Under several stimulus of cold exposure, cellular degeneration appears in degranulated centres and a stainable colloid becomes noticeable in the degenerative region. With prolongation of stimulus, these areas coalesce until a large part of the lobe is definitely destroyed. The formation of colloid material lacks behind the cellular transformation. During this time there is disintegration not only of the cells but also of the fibres, and their remnants are apparently incorporated into the forming colloid. This mass may become progressively harder and more brittle. Probably colloid is not associated with specific hormonal secretion of gland but rather with cellular disintegration resulting from long continued or severe stimulation.

Functional inter-relationships between Thyroid, Gonads, Anterior Pituitary gland and environmental temperature :—There are many observations which suggest that the thyroid secretes its active principle more rapidly in cold than in warm environments. Prolonged exposure of the rat to cold results in hyperplasia of the thyroid and in the dimunition of follicular colloid, a response simulating that which follows stimulation by thyrotropin (Bernstein, 1941). During exposure to cold the activated thyroid increases its output of thyroid hormone, and the basal metabolic rate is increased (Starr *et al*, 1940). This reaction to cold does not occur in hypophysectomised animals (Wolf, 1937) or in animals in which the hypophysis is severed from the brain by section of the infundibular stem (Uotila, 1939). Similarly at high temperature the thyroid activity is reduced and the secretion of its active principle is lessened. Dempsy *et al* (1943) reported that a quantity of 9.5 micrograms of Thyroxine was required to maintain a thyroid of normal weight in young male rats kept at 1° c while the requirement was decreased to 1.7 micrograms at 35°C. These values are considered to be quantitatively equivalent to the amount of hormone produced by the normal thyroid gland.

The relation of the thyroid to testicular function in the male reproductive system had been studied in thyroidectomised rats and normal litter mate controls from 18 litters by Smelser, 1934. Thyroidectomy was performed in 4 to 6 months old rats, autopsied 40 to 200 days later. There were smaller accessory reproductive organs and fewer sperm production in the thyroidectomised animals than in controls The gonads of thyroidectomised animals were particularly responsive to gonadotrophic hormone. Potency of the pituitaries from the series of these rats was determined by implanting them into immature mice and there was no evidence of any marked change in the potency of the pituitaries.

Smelser (1937) again made a study of the testicular function in a state of hyperthyroidism. Administration of thyroxin or dried thyroid gland to adult male rats caused a decrease in testis weight, sperm production and accessory weight. Testicular function was affected by amounts of thyroxin too small to produce weight loss although larger doses had a proportional effect. Daily injection of small amounts of thyroxin did not markedly affect testicular structure but the accessories of such animals are like the castrates, and sperm production determined by counts of the sperm in the epididymis, decreased markedly. If thy-

roixn treatment was discontinued a rapid and complete recovery ensues. Normal testicular and accessory function might be maintained in hyperthyroid animals by injection of gonadotrophic preparations. There was a marked decrease in effectiveness of gonadotrophic hormones (glandular or urine extracts) in hyperthyroid immature males. Similarly the amount of testis hormone required to stimulate the accessories of castrated rats increased approximately 5 times in hyperthyroid animals. The gonadotrophic hormone content of blood of castrated male rats was not affected by thyroxine injection, which caused marked body weight loss. There was no evidence indicating either a subnormal production or secretion of gonadotrophic hormones, or an increase in the rate of their excretion or destruction in hyperthyoid animals.

Greenwood *et al* (1939) observed that thyriodectomy in hen did not grossly interfere with the primary and secondary function of the cortex of ovary (i.e., egg and female hormone production) since normal shelled eggs were produced but in all of them the hormonal activity attributed to the medullary region of the gland had been markedly suppressed as growth of comb was inhibited. The testes of the operated males showed not only a suppression of their male harmone activity but also of their primary function of spermatogenesis. Replacement therapy using dried thyroid substance orally, induced growth of comb in both sexes.

Reineke *et al* (1941) reported that thyroidectomy of male goat kids resulted in a reduction in the gonadotrophic content of the pituitary gland and the testis of the thyroidectomised group showed lack of stimulation, averaging lighter than the youngest group of normal controls. P'an (1940) likewise found that the gonadotrophic potency of the anterior pituitary was decreased following thyroidectomy of normal castrated rats and normal rabbits. Evans & Simpson (1929) reported that the gonad-stimulating properties of the anterior pituitary from hyperthyroid rats were increased, while the glands from hypothyroid rats were less effective than normal.

Stein and List (1942) similarly reported a decrease in gonad stimulating potency of the anterior pituitary of the young male rat 21 to 30 days after thyroidectomy. Histological examination of the anterior pituitary of a thyroidectomised young rat showed a decrease in the number and size of eosinophiles with a corresponding relative increase in the number of chromophobes and basophils. Vacuolization was especially marked in the chromophobes and basophils.

Chu (1944) noted that thyroidectomised rabbits contained many more large follicles than normal controls, but ovulation did not take place after coitus. The animals that were operated on readily ovulated after the injection of pregnancy urine extract and the ruptured follicles were

more numerous than in normal estrus animals. He also prepared fresh saline extracts from the pituitaries of normal and thyroidectomised rabbits and assayed their relative amounts of ovulating hormone. The pituitaries from the normal animals induced 60 p.c. ovulation in estrus rabbits whereas the pituitary extracts from the thyroidectomised rabbits caused no ovulation and only induced growth follicles. These results indicate that alterations in thyroid activity may not only affect the gonads directly, but may also change gonadotrophic secretion in anterior pituitary.

Brolin (1946) made a study of the effect of different environmental temperature levels in the pituitary function of rats. Rats were kept at the following temperature ranges: 5—30° c.; 5—10 c, and 25—28° c. At the lower temperatures the pituitary showed a loss in weight, but it was only the anterior and intermediate lobes which were reduced, the posterior lobe being unaffected. At the lower temperature the testes showed no definite changes but both seminal vescicles and prostates were reduced. There was no appreciable effect on the female organs nor on the vaginal smears. The main conclusion was that in cold conditions involving an increase in the basal metabolic rate the anterior pituitary basophils produce increasing quantities of thyrotrophic hormone which stimulates high activity in thyroid gland. There is some evidence in the male that this hypersecretion of the basophils interferes with their normal production of gonadotrophic hormone. Lamoreux (1943) suggested that light and cold together increase the thyrotrophic and adrenotrophic hormones, while causing a decrease in effect of the luteinizing hormone.

Bogart & Mayer (1946) reported that changes in environmental temperature produce variations in reproductive activity in the ram indirectly through the thyroid gland. The thyroid affects the spermatogenic tissues of the testis and has little or no effect upon the interstitial tissue or the accessory organs which are dependant upon androgens produced by the interstitial tissue.

Meites *et al* (1949) reported that in young male rats thyroprotein partially or completely inhibited the response of the seminal vescicles and coagulating glands to a constant dose of P.M.S. while in young male mice thyroprotein increased the gonadotrophic response. These investigators also found that thiouracil increased the gonadal response to P.M.S. in male rats and reduced the response in male mice. Similar findings were reported by Johnson (1949) in immature female rats and mice. The response of the ovaries of immature female mice to P.M.S. given thyroprotein was increased, but there was no significant change when given thiouracil.

The mode of action of environmental temperature:—Generally animals fall into two classes with reference to the type of sexual cycle: those that breed more or less continuously throughout the year, and those that breed only during a particular season of the year. In animals of the latter type the genital complex attains maximal functional capacities for only a limited period and then regresses to a quiescent or almost juvenile state during the remainder of the year. The evidence has already been presented which indicates that seasonal testicular activities are influenced by such environmental factor as temperature. We are in a position now to inquire how these factors operating outside the organism influence the internal mechanism that controls the gonads. Although this question cannot be answered completely, we may call attention to a few suggestive clues.

Surgical removal of the hypophysis or pituitary gland abolishes the normal seasonal cycle and renders the gonads unable to respond to environmental factors which normally activate them. This indicates that the hypophysis is a necessary link in the chain of reactions which lead to sexual activation. By physiological tests it has been demonstrated that the hypophysis is deficient in gonadotropins during the non-breeding portion of the cycle. Through the administration of pituitary and chorionic gonadotropins it has been established that the gonads are capable of responding during most of the non-breeding period. It has been shown also that the gonad stimulating potency of the hypophysis increases during the period of normal or artificial reactivation of the gonad. Thus it appears that the seasonal cycle is conditioned by certain external environmental factors which excite or inhibit the secretion of gonadotropins by the anterior hypophysis.

The evidences have already been shown in both small and large animals that temperature fluctuations influence the sexual cycles of certain species by conditioning the release of hormones from the pituitary gland but the mode of reception of such stimuli is unknown. However, the observation that hypertrophy of the thyroid on exposure to cold does not occur after severance of the infundibular stalk, presumably because the anterior hypophysis cannnot augment the output of thyrotropin after the section of certain nervous pathways from the brain, suggests the possibility that the temperature changes which influence sexual cycles may be mediated also by a nervous mechanism. We have seen that thyroid is one of the most sensitive endocrines to react to changes in temperature and the different effects of thyroidectomy and hyperthyroidism on gonadal function have already been noted. The mode of such actions is not well known; but Reineke (1946) suggested that thyroid could influence reproduction by way of one or more of several mechanisms :—

1. by a specific influence on the pituitary gland,
2. by a specific effect on the gonadal cells, or
3. by the general calorigenic effect on all cells, including those of the endocrine system.

These observations indicate that the sexual cycles are correlated with the secretary capacity of the anterior hypophysis irrespective of the types of environmental stimuli which may be of regulatory importance in particular species. Turner (1948) gives two points of view regarding the manner in which the anterior hypophysis participates in the regulation of sexual cycles. One concept is that this gland is merely a necessary adjunct and that seasonal influences cannot operate in its absence because of the general resulting systemic disturbances; the other point of view is that the environmental stimuli influencing the sexual cycles are mediated directly by this organ.

While sexual behaviour in relation to temperature changes and neural and hormonic mechanisms is such a complicated problem that final conclusion cannot be drawn, there is much to commend the view that at least some of the external environmental stimuli are mediated rather directly through the hypophysis. Accordingly, such a factor as temperature modifies the release of gonadotropins by the anterior hypophysis, and these secretions in turn activate the gonads. An increasing amount of evidence indicates that the secretary activity of the hypophysis is conditioned in part by a nervous mechanism. It is probable that certain environmental factors influencing seasonal changes in the genital tract are mediated by nerve fibres coursing from the brain to the hypophysis. The possibility remains, however, that the environmental changes may be effective in altering the endocrine glands by modifying the general metabolism of the organism. Thus it can be concluded that a variety of external environmental factors as temperature may condition gonadal functions but the exact manner in which these factors are mediated in the organism has not been solved satisfactorily.

Summary

Temperature is one of the important environmental factors for proper gonadal function in both laboratory and farm animals. The alteration of the sexual behaviour of ground squirrel, the lengthening of the estrous cycle of rats and the disorganisation of testicular function of sheep and goat and such other reproductive disfunctions due to changes in temperature have been described. The functional inter-relationship between thyroid and anterior pituitary in relation to changes in temperature have been reported. It seems that the environmental temperature operating outside the organism influences the internal

mechanism that controls the gonad through the anterior pituitary. There are evidences that the secretory activity of hypophysis is conditioned in part by a nervous mechanism and the environmental stimuli influencing seasonal changes in the genital tract are mediated by nerve fibres coursing from the brain to the hypophysis. Accordingly such a factor as temperature modifies the release of gonadotropins by the anterior hypophysis, and these secretions in turn activate the gonads. Although this question cannot be answered completely, but some of the suggestive clues have been reported.

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PARYPHOSTOMUM SUFRARTYFEX (Lane, 1915) Bhalerao, 1931 IN PIGS *(SUS SCROFA DOMESTICA)* In Madras.

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Records of this trematode in the small intestine of man and pig seem to confine to India only. Its first description was by Lane who obtained the specimens in 1915 from an Assamese girl. It was Maplestone who collected this fluke for the first time from pigs in Calcutta during 1930. The only record about the incidence of the above parasite in Madras was by Govinda Reddy and Kerala Varmah (1950) from a boy.

During a recent survey of the helminths of pigs in Madras State, two out of 54 pigs examined revealed infection with this echinostome. Since the infection was very light, we could collect only six parasites. The worms were found firmly attached to the mucosa of small intestine. While fresh they were pinkish white in colour and very active.

Since the life cycle of this helminth has not so far been unravelled, an attempt to study the same was made. A large number of eggs were got by keeping the parasites in normal saline for a day at room temperature in petri dishes. The eggs were collected in a shallow dish containing tap water and cultured at atmospheric temperature changing the water daily. On the eleventh day most of the eggs hatched out into miracidia. About fifty *Limnea luteola* and fifty *Indoplanorbis exustus* were exposed to the miracidia for about six hours and were then removed and kept in an aquarium. Unfortunately all the snails died in about two days leaving us with the only information about the incubation period of the eggs.

While making a study on *Paryphostomum sufrartyfex*, one has essentially to take into consideration the literature available on the other echinostome — *Echinostoma malayanum* considered as its synonym. *Echinostoma (Euparyphium) malayanum* recovered from two tamil workers of Singapore and Malaya respectively was described by Leiper in 1911 itself i.e., four years earlier to the description of *Artyfechinostomum sufrartyfex* by Lane. Quite recently this intestinal fluke was recorded among the inhabitants of Java (Bonne, Bras and Lie Kian Joe, 1947). A closely related species was also found by Bare (1930) in the natives of Sino-Tibetian border. Rao (1933) recovered in Madras echinostomes which tallied with the description of *E. malayanum* excepting for the smallness of their size, from the small intestines of an indigenous dog.

Moreover, by experimental feeding of the metacercariae of *Cercariae indicae XXIII* Sewell, 1922 to dogs and kitten he recovered the same kind of ehinostomes from their small intestines.

Heavy infection with the parasite was invariably observed in human beings, the reason being perhaps the continued consumption of infective material or perhaps to a greater susceptibility than the pigs. The boy in Madras harboured several thousands while the Assamese girl discharged 63 flukes. Such a heavy infection accounts for the protracted and fatal ailment in the human host. The infection in pigs seems to be comparatively light and no clinical manifestations are known. As regards the intensity of infection, the rare records in human beings is in contrast to its fairly wide prevalence in porcine host. Maplestone found 33% out of 49 pigs examined infected while only about 3% out of 54 examined by us revealed the flukes. The human host in Madras hailed from South Arcot district. It is interesting to note that the main source of pigs to Madras slaughter house from where the material for our survey was secured was also from the same district.

The study of the life cycle of this echinostome could not be completed as enough material was not available. But connecting the investigations made by us till the discharge of miracida with the study made by Rao (1933) on *E. malayanum* (Malay fluke) considered as a synonym of *P. sufrartyfex* (Lane's fluke) one can build up the following probable life cycle.

The eggs voided in the faeces discharge miracidia in 11 days and *Linmea luteola* may be incriminated as the primary intermediate host in which the miracidia penetrate for further development. Cercariae discharged from the above mentioned primary intermediate host can encyst either on the gills of the common edible fishes like *Barbus stigma* or on fresh water molluscs as *Planorbis* and *Limnea*. On ingestion by human beings of the infected common variety of fishes uncooked, the metacercariae develop into adult parasites in the small intestine. The pigs may get the infection by swallowing the infected secondary intermediate molluscan hosts along with the tubers and other vegetations found at the edges of the water sources.

The authors are in search of sufficient material to confirm the above suggested life cycle and for describing the developmental stages in the primary intermediate host.

Summary

Paryphostomum sufrartyfex is recorded from pigs for the first time in Madras.

Miracidia are discharged from the eggs in 11 days.

Linnea luteola as the primary intermediate host and the common edible fishes like *Barbus stigma* and fresh water molluscs of the genera *Limnea* and *Indoplanorbis* as the secondary intermediate hosts are suggested.

The probable mode of infection in human beings and pigs is indicated.

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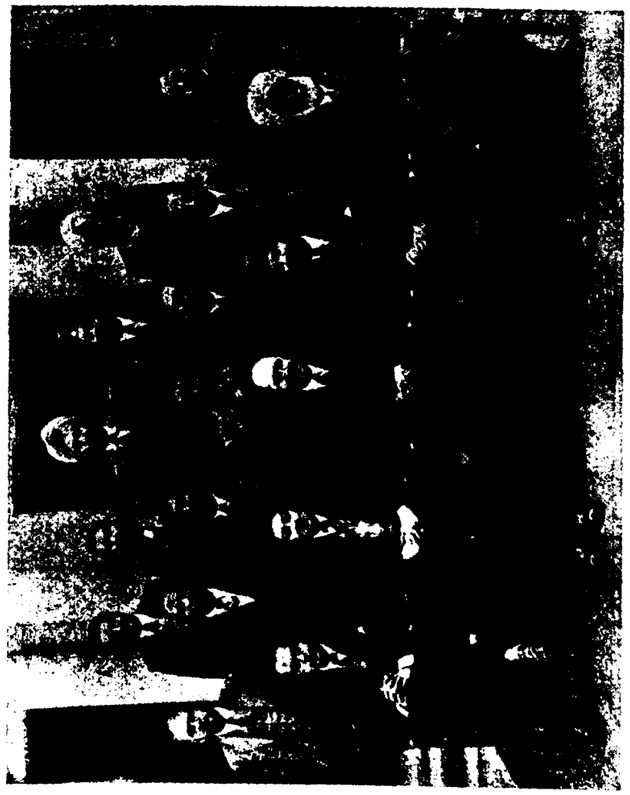

SIR THOMAS DALLING, F.R.C.V.S., M.A., D.Sc., LL.D., F.R.S.E.,
F. A. O. Chief Veterinary Consultant, with the Staff of the
Punjab College of Veterinary Science & Animal Husbandry, Hissar
13th JANUARY, 1954.

Sitting :—Dr. T. S. Gultiani, Ph.D.,(I.V.R.I.) Maj. A. C. Aggarwala, B.Sc., Hons., M.R.C.V.S., A.I.R.O., Principal & Professor of Medicine.
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Professor of Pathology. S. Dalip Singh, L.V.P., Professor of Surgery, Kishore, L.V.P., B.V.Sc., Demonstrator in Physiology.

Standing 1st Row :—Karam Chand, L.V.P., B.V.Sc., Professor of Parasitology, Kali Charan Kishore, Maheshwar Chandra Tugnait, L.V.P., B.V.Sc., Lecturer in Surgery, Daya Ram Saini, L.V.P., Professor of Pharmacology, Tek Chand Sehgal, L.V.P., B.V.Sc., Prof. of Animal Husbandry, Prem Lal Arya, B.V.Sc., Demonstrator in Pathology. Kishen Singh, B.A., L.V.P., Prof. of Anatomy, Lal Arya, B.V.Sc., Demonstrator in Medicine.

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1953–54.

Left to Right :—
Sitting :— Krishan Dev (*Delhi*), M. G. Sinha (*U.P.*), Shri B. R. Ramachandani (*Registrar*), Dr. S. C. A. Datta (*Director*), Dr. N. D. Kehar (*H. D. A. N.*), Dr. H. D. Srivastava (*H. D. P.*), Shri C. Seetharaman (*H. D., B. P.*).
Standing 1st Row :— V. Dasaratha Ramiah (*Andhra*), K. Kotaiya Choudary (*Andhra*), M. R. Sharma (*Rajasthan*), Banke Bihari Lal (*U.P.*), I. S. Kohli (*Punjab*), V. Venkatanarayanan (*Madras*), D. Sanjeevi Reddy (*Andhra*).
Standing 2nd Row :— Parikshit Das (*Assam*), Capt. G. W. Karandikar (*M. P.*), R. D. Mahto (*Bihar*), D. Jayaraman (*Andhra*), A. M. Kazi (*Bombay*).

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Editorial

ANIMAL NUTRITION

With the growing organisation and extension of veterinary services in our country, the incidence of contagious and infectious diseases, as a serious menace to livestock development, may now be said to be fairly within control. Among the deadly diseases that were playing havoc amongst our livestock and levying a heavy toll on them periodically, Rinderpest, Haemorrhagic Septicaemia, Black-quarter and Anthrax are the most important. All these diseases are now under effective check, thanks to the prophylactic vaccines and protective sera evolved in the Veterinary Biological stations in the country. By means of preventive inoculation, effective immunity can be given to our animals against these diseases and consequently they have lost their dreaded nature now. Whenever and wherever they show their ugly heads, it is easy to ring them up and smother them without being allowed to spread out. Having thus successfully tackled these epidemics, it is now necessary to turn our attention to the nutritional problems of our livestock. It is well known that the majority of our livestock are in varying stages of malnutrition. In his report on the Development of the cattle and Dairy Industries in this country, Wright observes as follows: There is no doubt that the majority of Indian dairy cattle are seriously underfed. This is apparent from a superficial inspection of village stock which lacks the sleekness and bloom of well-fed cattle and not infrequently have an emaciated and mal-nourished appearance. It is equally apparent from the slow rate of growth, the late maturity and the long dry periods of Indian cattle which are kept under village conditions. Having described these conditions, Prof. Wright goes on to assure us that these are not hereditary failings and that animals improve in their milk yield by

60 per cent. as a result of better feeding and management. He finally agreed with the Royal Commission on Agriculture (1925) that no substantial improvement in the way of breeding is possible until the cattle can be better fed. These findings have been amply confirmed by further work in Government Livestock Farms and private concerns and, therefore, we must set about tackling this aspect of our livestock problem in right earnest. Unfortunately, we are not self-sufficient with regard to our requirements in the feeding of our livestock. According to Dr. Kehar of the Indian Veterinary Research Institute, Izatnagar, the available supply of digestible crude protein and starch equivalent from our organised sources is only 23 per cent and 38 per cent of the required amount. Consequently, a good deal of planning on a scientific basis must be conducted to utilise the existing feeds and fodders in our country economically to the maximum extent. Human beings as well as animals improve in their general health and condition and show greater vim, vigour and vitality only when they get a balanced ration. It is only then the performance of animals in milk yield, draught power and increased production in meat and wool increases. Equally also, their power to resist diseases. Consequently, to enable one to prescribe a balanced ration to our animals, one must have sufficient data about the composition and nutritive value of the cattle-feed available in the locality. It is a great pity that fundamental research on problems like basal metabolism and mineral and vitamin requirements have not been conducted on any regional scale till now. The pioneering laudable works of Drs. K. C. Sen and Lander gave us some data about the composition and nutritive value of a number of animal feeds. This work has been followed up excellently well by Dr. Kehar and his team of workers at the Indian Veterinary Research Institute, Izatnagar, and they have added greatly to our knowledge in animal nutrition. But the work done so far does not even touch the fringe of the problem of livestock feeding and nutrition. For a vast country like ours, with its varying climates, soil and weather conditions, we want a number of institutions to conduct research on Animal Nutrition. The feeds and fodders available in different parts of the country are of varying nutritive value,

depending on the methods of production and preservation and, therefore, a number of regional Animal Nutrition Research institutes should be established. These should work in close collaboration with the Central Nutritional Institute at Izatnagar and put out for the benefit of our people their findings on nutrition problems as and when any definite finding is reached. Only then, can we improve the Nutritional level of our livestock and make them more and more efficient in their performance. We, therefore, strongly appeal to the Government to view this subject of Animal nutrition as a top priority one and open as many regional research Institutes as possible at an early date. We would also appeal to the Planning Commission to include this subject in their plans and arrange for the establishment of these nutritional institutes without any delay.

Since writing the above, we are glad to learn that the Government of India is planning on these lines and that two Institutes are already in the process of being established. We lack information on the details of these and will publish them as soon as we get them.

The Late Dr. F. C. Minett.

With deep regret we record the death of Dr. F. C. Minett on December 26, 1953, after a long period of illness, at Windybank in Kent. In his death, the Veterinary profession has lost an outstanding research worker well-known in international circles.

Our readers will remember that Dr. Minett was the Director of the Indian Veterinary Research Institute, Mukteswar from 1939 to 1947. During his stay there, he worked on a number of problems and particularly on the development of *B. anthracies* and *B. chauvoei* and also on the climatological influences on the milk yield of animals and their body temperature. He also studied about Panting, the common sequel to Foot and Mouth disease, and published two papers on the subject.

After partition of India, he opted for Pakistan and became Animal Husbandry Commissioner of that country. He stayed there for about two years and then returned to his country to join the Animal Health Trust.

The Late Mr. M. Sunderanathan

We are extremely sorry to record the death of Sri M. Sunderanathan which took place at Tanjore on the 22nd April 1954 after a short period of illness.

The late Mr. Sunderanathan was one of the earliest Graduates of the Madras Veterinary College. After graduation, he worked for some time in the mofussil stations of the State with great credit and distinction. He was then drafted to his *alma mater* where he remained as a member of the staff for nearly two decades until his retirement in 1942.

During his stay in the College, he endeared himself to one and all by his high sense of duty, keen devotion to work and great practical knowledge. He was loved and feared alike by the students who found in him an impressive teacher and sympathetic mentor. As a practitioner, he was highly popular and his death will be felt greatly not only by the members of the profession but also by the public.

Notes and News

Census Bronze Medal:—We are very glad to learn that Mr. Muhammad Jaffer Sheriff, Veterinary Assistant Surgeon, Kulitalai, Tiruchirapalli Dt., Madras State, has been awarded the 1951 Census Bronze Medal by the President of India "in recognition of the outstanding zeal and high quality of the service rendered during the 1951 census of India".

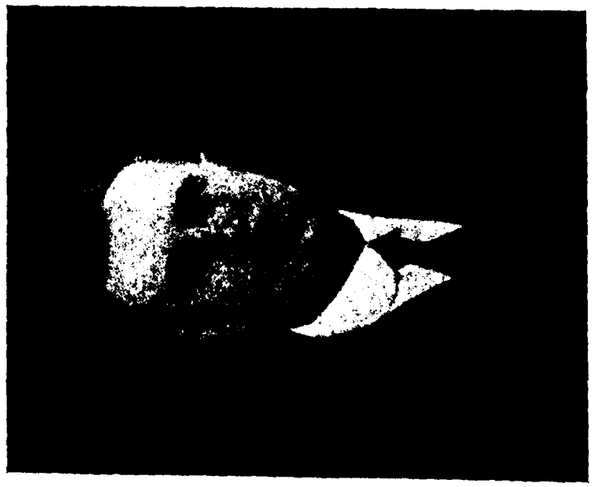
* * * *

Overcoming shortage of Veterinary personnel.—The following news is taken from the Madras Mail for May 6, 1954.

To meet the serious shortage of qualified veterinarians for undertaking schemes of livestock improvement as envisaged in the Five-Year Plan, the Union Ministry of Food and Agriculture has, in consultation with the State Governments, arranged, as an experimental measure, to run double-shifts at the Veterinary Colleges at Hyderabad and Hissar (Punjab), and to increase the number of admissions at the U.P. Veterinary College, Mathura, from the next session beginning in July 1954. This will enable an extra 180 students being admitted to these colleges.

The State Governments, which have no veterinary institutions of their own have been informed of these expanded facilities, and requested

The Late Mr. M. Sunderanathan, G.M.V.C., Retired Lecturer, Madras Veterinary College.



The Late Dr. F. C. Minett, C.I.E.

Plate No. 25 Vol. XXX, No. 6

Luxation of Patella in Cattle

Fig. 1.

Showing Method of control (Tying of affected limb) for operation.

Fig 2.

Showing site of the ligament with the ligament lifted up by the Tenaculam.

[*Vide article Page No. 507.*]

to finalise selection of candidates they would like to sponsor to the Veterinary colleges before the next session commences.

Meanwhile, Orissa has asked for 34 seats, Madhya Bharat for 30, Vindhya Pradesh for 20, Tripura for 10, Bhopal for 10 and Himachal Pradesh for 3. It is hoped that the remaining States will also take advantage of the opportunity and get an adequate number of persons trained as assistant veterinary surgeons. The minimum qualification prescribed for admission to these colleges is Intermediate in Science.

* * * *

Cattle Preservation bill: its legality.—The Attorney-General, Mr. M. C. Setalvad, told the House of the People that Parliament had no legislative competence to enact the non-official bill of Seth Govind Das to preserve milch and draught cattle of the country.

Mr. Setalvad said that in his view the subject matter of the Bill did not fall under any of the items mentioned in the Union List or the Concurrent List, but was covered by several items in the State List.

The pith and substance of the "Indian Cattle Preservation Bill", Mr. Setalvad said, was clearly to preserve both milch and draught cattle by stopping their slaughter in public licensed slaughter houses or in private places, and to improve the milk supply to the country and provide enough draught cattle for agricultural needs.

Scanning the Union and Concurrent Lists, he found no entry under which the subject matter of the bill could be placed or to which it could be related.

Entry Fifteen of the State List related to the preservation, protection and improvement of livestock and the bill had certainly this as one of its objects. It might also be said to be "remotely related" to Entry 27 relating to the production and supply and distribution of goods as the subject of the bill was remotely related to the supply of milk. Thus the subject of the bill came within the purview of these items in the State list.

The Attorney-General said that no doubt the directive principles of State policy referred to prohibiting the slaughter of cows and calves. But this had no application to the legislative competence of Parliament. Under the circumstances, the House would not be competent to pass the Bill.

The Speaker told the members who wanted to discuss the issue that they could do so when the bill next came up a for discussion before the House. When Mr. Govind Das wanted that the bill should be put down for discussion again on some official day, as per the assurance given to him by the Parliamentary Affairs Minister, the Speaker said

that he should approach the Government in the matter. He could not say when the bill would come up.—PTI (1st May, 1954).

* * * *

Punjab Veterinary College:—Our readers will remember that, consequent on the partition of India, the State of Punjab was left without a Veterinary College of its own and that the authorities there very quickly organised a Veterinary College on a temporary basis in 1948 at the famous Hissar Cattle Farm. Thanks to the energy and enthusiasm of the officers concerned, the College has been growing very rapidly and we are very glad to be told that Sir Thomas Dalling, Chief Veterinary Consultant to the FAO of the UNO, visited this institution on the 13th January 1954 and was greatly impressed with the way in which it had been built up. He also took the opportunity, we understand, to address the students and the staff and congratulated the Principal (Major A. C. Aggarwala) for running the institution so well.

* * * *

Personal

Sri C. Seetharaman, B.Sc., G.M.V.C., B.V.Sc., M.Sc. We are very glad to learn that Sri C. Seetharaman of the Indian Veterinary Research Institute, Mukteswar, has been selected by the Union Public Service Commission, as the Head of the Division, Biological Products, and that he assumed charge of the new post on the 10th April 1954. Ever since his graduation from the Madras Veterinary College, Sri C. Seetharaman has been working at the Indian Veterinary Research Institute and latterly was the Liaison Officer to Mr. R. Daubney, the F.A.O. Expert, whose services were requisitioned by the Government of India in connection with the implementation of the All India Rinderpest Eradication Plan which has been accepted by the Planning Commission. In May 1953 he was appointed Research Officer (Pathology) at Mukteswar. His efforts have been to put into the field—freeze dried Ranikhet disease vaccine, freeze dried rinderpest vaccines and the I.V.R.I., is now issuing these products to the field. During February-March 1953 an International Seminar on the Manufacture of Virus Vaccines was conducted jointly by the F.A.O. and the Government of India and he served on the teaching staff of the F.A.O. personnel. In June 1954 he is proceeding to U.S.A., on a Fellowship under the Point Four Programme on a study tour to various Biological Production Laboratories in U.S.A. He is working in collaboration with Mr. Olver A. Bauman, the Research Biologic Advisor of the U.S.A., Technical Co-operation Mission to India who is assigned to the I.V.R.I. for modernisation of the large scale production of Veterinary Biologicals. He has risen to his present highly responsible post by dint of hard work, ability and merit and we are sure he will acquit himself creditably in this new assignment. We wish him all success.

Clinical Articles

RECURRENT LUXATION OF PATELLA IN CATTLE AND ITS TREATMENT BY PATELLAR DESMOTOMY

By

BALLAV NARAYAN PATRA, B.Sc. (Hons,), M.R.C.V.S.,
District Animal Husbandry and Veterinary Officer,
Angul, Orissa.

Introduction:—"Recurrent luxation of patella" is commonly met with in cattle in all parts of India and every village will have at least 2-10 animals suffering from this condition. The money value and the working capacity of an affected animal are considerably reduced and, therefore, this condition is of great economic importance to the livestock owners of our country.

The earliest observations on the condition in India were reported by Rao (1925). He advocated its treatment by injections of Tr. Iodine into the stifle joint. Sankaran (1927) confirmed the success of the treatment. The injection is, however, painful and throws the animal out of work for some time.

Incidence:—The disease has been observed in cows, buffaloes, bullocks and bulls, mostly in animals over two years, and more commonly in working bullocks. Animals below one year may also be affected.

Symptoms:—The characteristic symptom is a particular kind of lameness of one or more hind limbs which appears when an animal starts to walk after a prolonged period of rest. The animal first drags the affected hind limb which is held in a rigidly extended position backwards and then carries it forward with a jerk. In slightly affected cases, the 'drag' is less marked, the patient holding the limb back, and bringing it forward immediately by a sudden jerk which is very characteristic of the complaint. The animal walks sound after a time. In acute cases however, the 'drag' lasts for a much longer period and the animal has great difficulty in bringing the limb forward for the first few steps. In these cases the forward jerk becomes less prominent.

Aetiology:—Among the factors that contribute towards the aetiology of the disease or predispose an animal to this disease may be mentioned the following:

(a) *Nutritional deficiencies*:—This disease is not seen in cattle of Western countries where nutritional standards are high.

It is also not so common in herds kept under a higher level of nutrition in this country. It is seen more in village herds, where the nutritional standards are generally low. However, it is not un-common in very well-fed herd of animals also. In these cases, other contributory factors may be responsible.

(b) *Heredity* :—Predisposition to this disease may be transmitted. Observations in this respect are not yet complete as to hazard a definite opinion. But quite a number of cases have been observed where young ones of affected animals developed the complaint.

(c) *Work* :—As the disease has been observed in both cows and bullocks, work may not be a direct cause. But once the disease starts,' it comes into more prominence in animals which are working and are straining their muscles and joints more than animals which do not work. That perhaps explains the occurence of this disease more commonly in working bullocks.

(d) From a survey of the distribution of this disease it appears to be more common in areas with higher rainfall and clayish soil. At the same time very few cases are recorded in high land areas. Further work on this aspect is indicated.

Treatment :—Patellar desmotomy is advocated as a radical cure for the condition.

Theoretical considerations :—According to Share Jones the mechanism of the stifle joint is as follows. " The interior face of the straight patellar ligaments gives attachment to the common tendon of insertion of the quadriceps extensor cruris muscles. When these muscles contract, therefore, the patella is drawn upwards, and the straight ligaments are made tense, the stifle being extended through the medium of the ligaments. Again, when the femero-tibial joint is completely extended, the patella is placed on the upper part of the trochlea and the straight patellar ligaments are made very tense. The latter are relaxed and the patella descends along the trochlea during flexion of the stifle."

During the ascent of the patella, the tension on the internal ligament is much greater since it is not as powerful as the other two tibio-patellar ligaments. Now when it is congenitally, pathologically or otherwise relaxed, there is a tendency for the patella to be held imprisoned on the upper surface of the inner ridge of the trochlea and, as Merrilat says, " the ligaments and the muscles are too lax to prevent the bone from slipping from its groove." The relaxed state is improved and prevents the patella from remaining in a state of luxation, if

division of the ligament is done. Injection of iodine solution into the stifle brings about inflammation and subsequent thickening and shortening of the ligaments and so acts also to the same end.

According to Sisson " The principal movements of the stifle joint as a whole are flexion and extension. In extreme extension which is accompanied by a slight out-ward rotation of the leg, the patella can be pushed upward and inward so that the fibro-cartillage hooks over the upper end of the medial ridge of the trochlea but will not remain there unless held in position. When pressure is removed the base of the patella tips forward and the cartillage lies on the most prominent part of the trochlear ridge ".

Now if due to pathological, congenital or other changes the gliding ability of the patellar hook on the medial lip of the femoral trochlea is impaired, the hooking may stick momentarily before release. This will cause the limb to be left in extreme extension for a moment and be dragged. By this time the propulsive action of the biceps and other muscles pulls the limb forward, the hooking is released and the limb is thrust forward with a jerk.

Section of the medial straight ligament counter-acts the effects of this hooking and so effectively cures the condition. Injection of Tr. iodine also causes an inflammation and subsequent shortening of the ligaments and so prevents the patella from moving up to the highest point of the condyle (preventing hooking, thus acting to prevent the symptoms). But injection of Tr. iodine causes swelling and pain in the joint in the process of inflammation and keeps the animals out of work for sometime.

Control of the animal :—The animal is cast by any of the suitable methods, on the same side as the affected hind limb. The other three limbs (excepting the affected limb) are tied together. The affected limb is released and tied just above the fetlock joint securely to the middle of a stout pole or a stout piece of bamboo about 8 to 10 feet long so that the bamboo or pole is at a straight angle to the shin bone. The pole or bamboo should be lowermost and the limb tied over it. Three men are necessary. One holds down the head of the animal in the cast position ; the other, sitting at a level of the croup of the animal, holds in place the hind quarters and the extension of the rope by which the three legs are tied together. The third man sits on the pole tied to the affected limbs and keeps the limb in position. The operator for additional security sits with one of his legs on the pole also (vide Plate No. 25). By moving the pole up and down and side ways, the exact position of the limb suitable to feel the ligament can be obtained. This method completely secures any vicious animal from jerking or struggling during

operation. For safety a local anesthetic may be injected over the region of the ligament, but this may not be necessary.

Site of Operation:—After securing the animal as mentioned above, the limb to be operated upon is kept extended in such a way that the axis of the limb makes an angle of 120° to the median line of the animal, at its abdominal region. In this position, the stifle joint is fully flexed. Now the stifle joint is slightly extended by moving the pole securing the limb slightly towards the body line of the animal.

In this position, the medial condyle of the tibia can be clearly felt. Also the attachment of the medial straight patellar ligament to the tuberosity of the tibia can be clearly felt as the ligament is a thick conspicuous band. Now slide a finger along the edge of the medial condyle of the tibia till the highest point is reached. At this point, if the finger is slipped forwards and downwards in the articular surface of the condyle, the medial straight patellar ligament is felt as a prominent cord. Feel and locate the direction of the ligament. The line of incision is alongside the ligament and about half an inch away from the edge of the medial tibial condyle. The distance of half an inch will very slightly from animal to animal, depending on their size and health.

Instruments necessary.

1. Scalpel, preferably a Bard Parker with attached blade.
2. A pair of scissors—thin and blunt pointed.
3. Dissecting forceps—1 pair.
4. A few artery forceps for emergencies.
5. A tenaculum to lift the ligament.
6. Needle and suture material, (Nylon or silk-worm gut).
7. A pair of retractors to open the skin incision.
8. Swabs.

Operation:—A bold incision about 1½ to 2 inches long is made along the long axis of the ligament at the site detailed previously. The lips of the incision are kept apart by the retractors. With the point of the knife the subcutaneous tissue is incised to the level of the ligament. When the ligament (a white glistening structure) is exposed along its long axis, the tenaculum is passed underneath it and lifted. The ligament is then cut right through either with a knife or a pair of scissors. It has not been found necessary to remove a bit of the ligament because, when cut, the tension on the ligament is released and the two cut ends retract considerably, leaving no chance of apposition or reunion.

There is a certain amount of muscular tissue just over the ligament and in most cases some of this tissue is caught in the tenaculum and has to be cut through before the ligament could be exposed. This tissue, which is only a segment of a cutaneous muscle, is prominent in well-conditioned animals and may cause a troublesome haemorrhage interfering with the quick healing of the operated wound.

The structures met with during the operation are:—

1. Skin.
2. Subcutaneous connective tissue.
3. Fascia lata.
4. Aponeurosis of the Gracilis and Sartorius.
5. The ligament.

After the ligament is cut an yellowish mass is exposed which is the fatty pad under the ligament and the wall of the joint capsule.

Sometimes the aponeurosis and fascia have strong bands which remain tense after the ligament is cut. These also have to be lifted and cut through. Unless these are cut the animals will still show symptoms of the complaint even after the operation.

After the operation the wound is dressed with some non-irritant dressings like Penicillin ointment, Sulphonilamide preparations, acriflavin in glycerine and sutured. Acriflavin in glycerin had been used in most cases and the skin sutured by mattress type of sutures. As a suturing material, nylon or silk worm gut should be preferred to silk sutures.

The subsequent treatment consists of fomenting the part two or three times a day for the first two days as there is always some subcutaneous exudation with a slight swelling at the part an hour after the operation. This completely subsides after fomentation which is an important part of the after-treatment. Fomenting can be done by dipping a clean towel in a bucket of boiling water and applying the *dry* towel lightly over the wound avoiding the sutures, as far as possible. No trace of incision is observable afterwards.

Case Records:—Upto date, 85 cases of this complaint have been operated. In all the cases, the symptoms of lameness disappeared immediately after the operation and the animal walked perfectly sound. Using nylon for the skin suturing, healing has taken place within a week varying from 5 to 9 days. Animals operated in September, October, November and December, 1952, are now perfectly normal.

In an organised dispensary or hospital, the whole operation from casting the animal to completion of sutures will take 8-10 minutes at the very most. And, therefore, this operation can be taken up on a mass scale in every veterinary institution and field veterinary dispensaries. Nine or ten operations have been performed in one morning's work

besides the regular duties. The animal can be put to normal work after 6 to 10 days in almost all cases.

Acknowledgment

My work in this disease began in the middle of 1952 when Mr. G. B. Singh, B.A., M.R.C.V.S., Director of Animal Husbandry and Veterinary Services, Orissa, was kind enough to place at my disposal funds for the experimental work. I may say that the surgical success of this operation is due to him and I take this opportunity of conveying to him my gratefulness.

Summary

"Recurrent luxation of patella in cattle" is a common condition found throughout India characterised by a peculiar dragging lameness of hind limbs, one or both, and is of great economic importance to the agriculturist.

The pathological anatomy of the condition and the factors contributing to the aetiology are outlined.

Patellar desmotomy is advocated and it consists in severing the medial straight patellar ligament.

The operation being simple can be taken up on a mass scale and in all veterinary institutions and field veterinary dispensaries.

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AN INTERESTING OUTBREAK OF ANTHRAX

By

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Veterinary Assistant Surgeon, Kovur, Nellore District,
(Now at Kurnool.)

Introduction :—During the year 1952-53, there was rather a heavy incidence of anthrax in Kovur taluk. There were as many as nineteen outbreak reports with 126 attacks and 85 deaths. Among them, there was one particular outbreak report viz. the one at Gandavaram which, I hope, will be of interest to our professional colleagues.

Gandavaram is a village situated about 7 miles from Kovur, Nellore District. This is a paddy producing locality, the lands being irrigated through canals connected to Kaligiri Reservoir. The average rainfall is about 30". Summer is very severe. There was no record of any previous incidence of this disease in this village.

I was called upon to attend the outbreak on 1-3-52. I immediately proceeded to the village and enquired of the owners of cattle regarding the symptoms exhibited by the animals. According to them, the following symptoms were observed. The animals were dull, taking food sparingly, rumination not altogether suspended, recurrent tympany and sometimes colicky pain, bowels constipated and death after 3 or 4 days. So far 10 animals had died. I collected a piece of skin from one dead animal and sent it to the Veterinary Investigation Officer, Ranipet, who later on reported it to be positive for Anthrax.

Animals affected :—Mostly cattle and, even among them, adult animals.

Total deaths :—21 (16 cattle and 5 buffaloes).

Symptoms :—On examining the herd, 22 out of 82 animals showed hyperpyrexia, the temperature ranging between 107°-109°F. In spite of the high temperature, the animals were feeding sparingly and also ruminating. There was slight tympany and the affected animals were dull. As the day was exceedingly hot, I took the temperature of some of the healthy animals and they recorded between 100°-101°F. The age of the ailing ones were between 6 months to 5 years.

Treatment :—The following animals were treated :—Cows and heifers between 3-5 years-16 ; calves below 3 years-6.

N.A.B. (M&B), in doses between 0·9 grams to 0·3 grams, dissolved in 20 c.c. of aqua distillata, was given intravenously, the dose being regulated according to the size and age of the animals. On 5-3-52 I visited the village and inspected the 22 treated ones and found one cow, aged 4 years, dead and the rest appeared better. The temperature also ranged between 101°—102°F. I examined the dead cow and found no typical symptoms of Anthrax. There was no bloating of the abdomen or eversion of rectum or bloody discharges from any of the natural orifices. With all precautions, a postmortem was conducted. Except for the enlargement of the spleen, there was nothing abnormal. An impression smear from the spleen and a piece of skin were sent to the Veterinary Investigation Officer, Ranipet, who confirmed them for Anthrax.

Four more animals viz. 3 cows aged between 4-6 years and one bull calf aged 8 months showed hyperpyrexia. They too were treated

with N.A.B. intravenously. There were no more attacks and deaths after this.

Duration of the outbreak :—The disease prevailed in this village from 20-2-52 to 16-4-52 with an interval of 20 days in the middle when there was a lull in the incidence of the disease.

Prophylaxis :—Two batches of 329 and 365 animals were protected with Anthrax Spore Vaccine on 16-3-52 and 3-4-52 and a third batch of 374 animals were protected with Spore Vaccine on 16-4-52.

Result of vaccination :—Only one animal in the first batch, a cow aged 5 years, died on the fifth day after vaccination. There were no after-effects of vaccinations. The village is free from Anthrax till today i.e., 19-6-52.

Summary

The following peculiarities are noted in this outbreak :—

1. Its fairly long duration viz. nearly 2 months.
2. The disease flaring up in one particular herd in an epidemic form (26 ailing animals at a time).
3. Among the total number of attacks i.e., 46, only 5 were buffaloes and all the affected ones were in good condition previous to attack.
4. Successful treatment with N.A.B. (26 treated and 25 cured).

Acknowledgments

I wish to acknowledge the valuable suggestions given to me by Dr. B. A. Shirazi, B.V.Sc., District Veterinary Officer, Nellore. My thanks are also due to Dr. R. Nagarajan, Veterinary Assistant Surgeon, Sarvepalli and Sri M. Abdul Hakim, Stockman-compounder who helped me greatly in recording the temperature and in treating the cases.

PENETRATING WOUND OF THE CHEST IN A DOG—A CASE OF EXTRAORDINARY RECOVERY

By

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Reserve Veterinary Officer, Sholapur.

History :—On 23—12—53, at about 11 a.m., a dog was admitted in the Veterinary Hospital, Sholapur, with the history that, while it was wandering near a butcher's shop, a butcher hit it with his knife. The dog came running to the house and the owner noticing a wound on its chest brought it immediately to the hospital in a conveyance.

Description of the wound.—A vertical incised wound, about 6" long, in the middle of the left thoracic region. The interior of the thoracic cavity was visible through the opening and air was hissing through with each respiration.

Treatment :—Bearing in mind that the prognosis of such a penetrating thoracic wound was grave, the case was taken on hand. After thoroughly cleaning the part, the pleural layers were secured with great difficulty and sutured with catgut. The sutured portion was coming up like a balloon. Then the muscles were separately sutured with all aseptic precautions. Sulphanilamide powder was freely dusted over the area before and after suturing. Lastly, the skin was brought into a apposition by interrupted and retention sutures and the part bandaged with plenty of cotton wool. No penicillin was given.

As the owner was not willing to keep the animal as an in-patient, it was taken away by him in a tonga. He was instructed to keep the dog quiet and undisturbed.

Next day, to my utmost surprise, the tonga came bringing the dog. It quietly walked to my table with his master. On examination, the temperature was 101°F and there was very little discharge from the wound. The owner informed me that the dog took some milk and rice the previous night. I cleaned the part with spirit, painted the sutures with Tr. Iodine, dusted Sulphanilamaide powder and bandaged. Daily dressing was carried out and the case was progressing very satisfactorily, when, after four days, it was found that four of the skin sutures had been taken out by the dog. The muscle sutures were, however, intact. The skin sutures were put on again and the dressing continued. During this period, the dog was walking along with his master without the help of a conveyance. After a few days, the skin sutures were pulled out again and had to be re-done. Recovery was complete by 10—1—54 and, what was obviously a hopeless case, was cured without much trouble.

I am deeply indebted to Shri N. G. Sahsrabudhe, the present Veterinary Officer, Sholapur, for his guidance in treating this case as well as in writing this article.

Correspondence

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

The role of domestic pigs in the life cycle of *Linguatula Serrata* Frochlich, 1779 (Tongue-Worm)

Sir,

During the routine examination of the lungs of a pig in the course of a survey of porcine helminths, two small lightly pinkish and very active parasites measuring about 5 m.m. in length were seen. On close

examination they were found to be the nymphal stages of *Linguatula serrata*. References about its occurrence in pigs reveal the variety of the infection. Hence, opportunity is taken to record the same as pigs should be thought of as a possible disseminator of the infection. Incidently, this happens to be the first record in Indian pigs.

Dogs and other carnivores and human beings act as the hosts and herbivores as the intermediate hosts for this aberrant arthropod. But, at times, in the herbivores themselves, the nymphs migrate from the cysts found in the organs and lymph glands of the abdominal region to develop as adult parasites in the nasal and respiratory passages. In the hosts as well as in the intermediate hosts the nymphs are said to migrate through the lungs. Hence it is not possible for us to decide whether this pig is the definitive host or an intermediate host.

Department of Parasitology,
Madras Veterinary College,
6th May 1954.

G. RAMANUJACHARI,
V. S. ALWAR.

Reviews

Administration Report of the Coorg Veterinary Department for the year 1952-53.

Sri A. M. Muthappa, the Chief Veterinary Officer, Coorg supervised the Veterinary and Animal Husbandry activities during the report year. The executive staff consisted of one Chief Veterinary Officer, one Dairy Development Officer, one Poultry Development Officer, one Artificial Insemination Officer, five Veterinary Assistant Surgeons and ten stockmen.

The general health of the livestock of the State was satisfactory and the mortality from contagious diseases was very low.

The Livestock Farms and the Poultry Farms have been functioning well.

The breeding schemes continued to work satisfactorily and the Artificial Insemination work has progressed very well. The Department has also paid attention to the propaganda value of departmental activities and a number of shows and calf-rallies have been held with great advantage. Poultry development also is receiving attention at the hands of the Department and several poultry blocks have been opened. In the Kajoor model unit, about which a special mention is made in the report, the grading up of the *desi* birds has been intensively worked with the result that most of the ryots are earning Rs. 3/- to Rs. 4/- a week by the sale of chicks and eggs. This is indeed very gratifying and we hope that this cottage industry will develop further in years to come.

There has been thus a steady progress all round reflecting credit to the department.

* * * *

The Great Dane.— By Virginia Keckler. 1953. Judy Publishing Company, Chicago-16, U. S. A. Price $ 3 50.

What has been often termed the Apollo of the canine race, the Great Dane has been steadily coming into popularity during the last decade. This majestic breed of dog cannot be classified under 'pets'. It should be described more as a guard and dependable companion. The origin of the breed is lost in antiquity but it has been a favourite in the continent for quite a long time. In this book of Great Dane, we find in the first section of 30 chapters the history of the breed in all countries with special emphasis on America. We also get a good description of a typical Great Dane, so that it should not be difficult even for an amateur to appreciate what a good specimen should be like. Detailed information on cropping of the ears of a Great Dane is found in these pages. The latter half of the book contains chapters on the care and management of this breed including feeding and training, dog shows and show ring, bloodlines and pedigrees and mating, whelping and litter care.

The author is a great breeder of a score of champions and judge of leading Dane Shows and this second edition, revised and enlarged over the first, will be readily welcomed by all lovers of Great Danes who will in this finely get up and profusely illustrated book, quite a good deal of information about that breed.

ERRATUM

On page 363 of the March '54 issue of the *Journal*, (I.V.J. Vol. XXX. No. 5) the figures given under T. D. N. in Table I against the percentage of butter-fat represent D. P. and the correct 'T.D.N.' figures are 0·28; 0·32; 0·37; and 0·42 respectively for 4, 5, and 6 percent butter fat content. The figures 0·39 and 0·42 should be mutually interchanged in the last column.

V. MAHADEVAN

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