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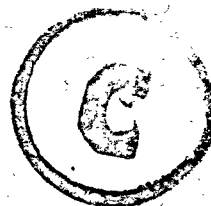
Vol. 37, No. 12

DECEMBER, 1960

THE INDIAN VETERINARY JOURNAL

(The Journal of the Indian Veterinary Association)

*A Monthly Record of
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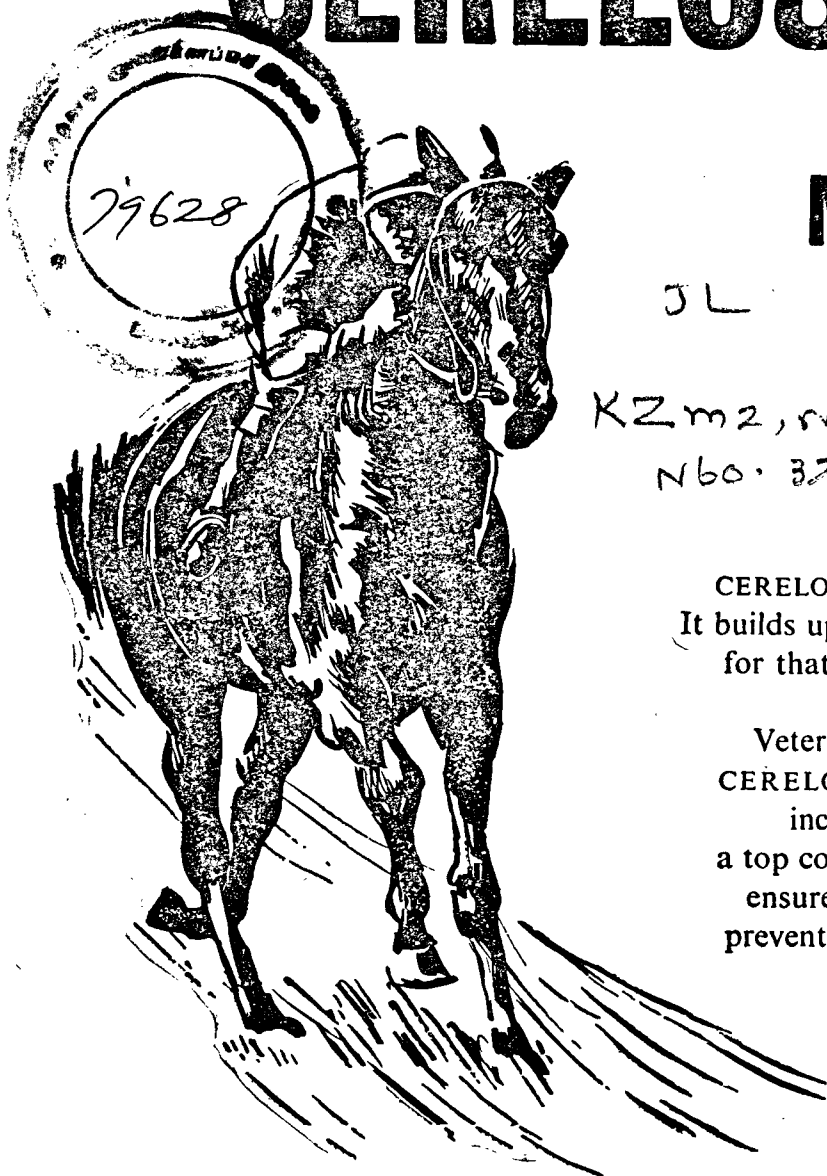
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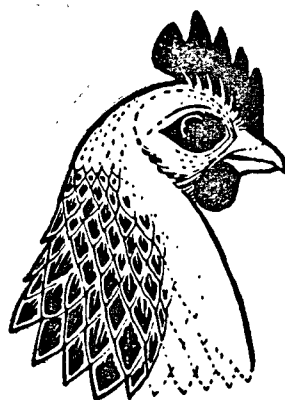
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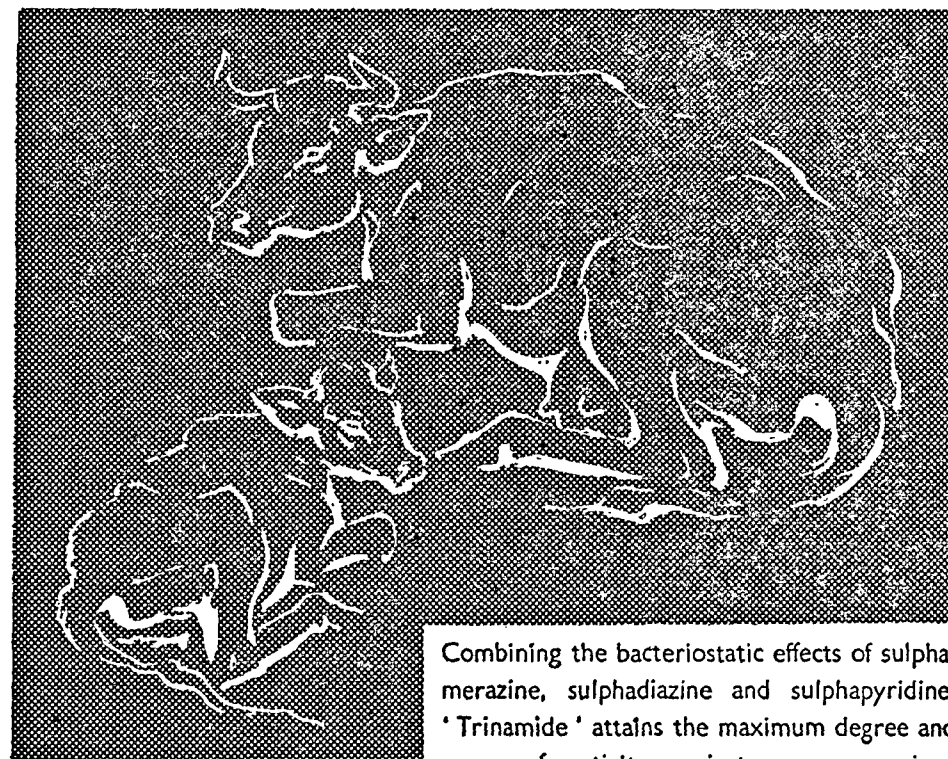
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THE Indian Veterinary Journal

(The Journal of the Indian Veterinary Association)

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No. 12

We Wish Our Readers A Merry Christmas!

Editorial

THE PRINCE OF PEACE

"Tell me the old, old story of Jesus and His love" is a psalm that comes to our mind as we sit down to write the editorial of this month.

The month of December is hallowed by the memory of the birth of Jesus Christ. Not another prophet of his stature has ever walked on this earth. He came to serve and not to be served. He came to save the erring humanity from its abysmal follies. Even though his religion had spread all over the world, it is a matter for regret that humanity in general is still so far away from his ideals. Perhaps they are far too high to be easily followed by the ordinary run of humanity. The present turmoil in the world is in direct contrast to his basic teachings. Can the Western world call itself truly Christian in its present mood of an eye for an eye and a tooth for a tooth? If there is one religion that has taught utmost forbearance and love between man and man, it is the religion of Jesus Christ. But the world has not in the least benefited by it. It is this fact that has brought out the oft-repeated statement, that the one Christian that the world has produced died on the Cross two thousand years ago.

But it is becoming more and more clear that unless and until the world takes to the path of 'peace on earth and goodwill amongst men', there is absolutely no chance of the present world surviving. We are on the brink of destruction and the sooner we begin to learn and

practise Christ's golden rule "Thou shalt love thy neighbour as thyself" the better it would be to all concerned - individuals and Nations. "For what is a man profited, if he shall gain the whole world, and lose his own soul?". Let it be remembered that "All they that take the sword shall perish with the sword".

Jesus has poured out his heart in all his teachings. He was forced to cry out "Ye serpents, ye generation of vipers, how can ye escape the damnation of hell?" Is the world of to-day any better than in the days of Jesus? Let us hope it is never too late to mend. Let us hope that such a great soul did not die on the Cross in vain. The humility and indignity to which he was subjected and the manner of his crucifixion bear no parallel in the history of mankind. It is a blot on mankind which will continue to disgrace the humanity at large through all ages. The only way of wiping out that blot is by following the path of righteousness so clearly enunciated in his teachings.

Mahatma Gandhi, the father of our Nation, often reminded the world of the teachings of Jesus. He practised those teachings in his life time and demonstrated how effective they are. But yet the world, 'having ears hears not' and 'having eyes sees not'. It is high time another incarnation of God makes His appearance now and sets this world aright. For man has lost his bearings and is a positive danger to all good causes.

Lord Krishna has said "When irreligion prevails and religion decays, the Power that upholds the cause of righteousness will manifest itself in the form of a human being". That is the limit set for *Avatars* or incarnations to appear. We have reached that limit and man feels helpless. It is for the Gods to come to our rescue.

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Editor.

General Articles

IMMUNOLOGICAL STUDIES ON *PASTEURELLA SEPTICA* V. FURTHER OBSERVATIONS ON THE IMMUNISING EFFICACY OF ADJUVANT VACCINE*

By

M. R. DHANDA¹ P. C. SERARIAH, J. M. LALL
and

PREM PRAKASH,²

*Division of Pathology and Bacteriology, Indian Veterinary Research
Institute, Mukteswar-Kumaon., U.P.*

The evolution of an oil adjuvant vaccine against haemorrhagic septicaemia and the results of extensive laboratory and field trials undertaken to assess its protective value have been described in earlier communications from this laboratory. The results of these investigations culminated in laying down a procedure for the manufacture and standardisation of haemorrhagic septicaemia adjuvant vaccine that has since been adopted for routine production at the Biological Products Division of this Institute (Dhanda *et al.*, 1956, 1958; Dhanda and Lall, 1958). Suffice it to say that large scale field and laboratory tests have shown that the adjuvant vaccine is absolutely safe for cattle and buffaloes of all ages and that in its immunising properties it is far superior to the commercial broth vaccine. The immunity following vaccination with the adjuvant vaccine was found to be solid for a period of 12 months, the maximum period for which the tests were carried out and results published. Tests on its keeping quality showed that the vaccine did not deteriorate in its protective properties for at least one year when stored at low temperatures. It was also found to withstand fairly high temperatures and could be safely transported under ordinary conditions of rail and bus journey, to far off places covering a journey period of about 10 days during the hot summer months. Studies on the antibody response in vaccinated animals by various indirect tests such as slide-agglutination, agar-agglutination, 50% haemolysis, complement fixation and passive mouse protection tests resulted in the development of reliable and sensitive *in vitro* methods of adjudging the immunological

* Part of the data were presented at the 47th session of the Indian Science Congress held at Bombay, Jan. 1960.

Present address:—1. Dean, College of Vety. Medicine, U. P. Agricultural University, Phoolbagh, P. O., District Nainital.
2. Assistant Professor of Anatomy and Histology, College of Vety. Medicine, U. P. Agricultural University, Phoolbagh, P. O., District Nainital.

status of healthy and vaccinated animals and also in the coincidental delineation of the phenomenon of naturally acquired active immunity independent of previous vaccination (Dhanda *et al.*, 1959a). Further work (Dhanda *et al.*, 1959b) on the same lines led to the preparation of a high titre haemorrhagic septicaemia antiserum employing adjuvant vaccine for immunisation of serum donors followed by challenge with virulent culture or administration of repeated doses of formalin-killed suspensions of *Pasteurella septica*, I.V.R.I. stock strain 52 (accredited Roberts' type I).

Immunochemical studies on yeast-extract agar-grown acetone-dried cells of *P. septica* (Dhanda, 1959 a, b, c) rendered available four soluble antigenic fractions which could be used profitably for determining the nature and significance of antibody response following vaccination, with the whole-cell adjuvant vaccine to facilitate comparative evaluation of immunogenicity.

It is the object of the present paper to place on record results of a comprehensive study, on the protective value of oil-adjuvant vaccine, with special reference to extended observations on development and duration of immunity as well as the pattern of antibody response following vaccination.

Materials and Methods

1. *Vaccines*:—The procedure and standardisation of the manufacture of the adjuvant vaccine have already been described in detail (Dhanda *et al.*, 1958). The dose employed was 2 ml. and the vaccine was given intramuscularly. Two brews of adjuvant vaccine kept for over 2 years at Mukteswar room temperature were also tested for their immunogenicity.

2. *Animals*:—Adult hill cattle, rated as fully susceptible by the various indirect tests (Dhanda *et al.*, 1959a), were used throughout the experiments to serve either as controls or as test animals to compare the protective value of the different bacterins.

3. *Sera*:—Sera were obtained from a large number of hill cattle prior to vaccination as well as at varying periods after vaccination both under laboratory and field conditions with a view to follow the immunological response. Later, these animals were subjected to a challenge with virulent culture of *P. septica*, I.V.R.I. stock 52 and the results of challenge were compared with those of the different serological tests. In a few animals that survived challenge, sera were again collected to screen them for the presence of antigens.

4. *Antigens*:—The method of preparing antigens for slide agglutination has already been described (Dhanda, *et al.*, 1959a.)

Soluble antigens for complement-fixation, indirect haemagglutination and haemolysis tests were the four fractions obtained from acetone-dried encapsulated cells of *P. septica* type I (Fractions IB, II, III and IV) dissolved at appropriate concentrations as indicated at the relevant portions in the text below.

5. *Serological procedures*:—(a) Agglutination (both slide and agar) and passive mouse protection tests were carried out on the lines indicated earlier Dhanda *et al.*, 1959a). Tube agglutination tests were done in round bottom tubes with live antigen adjusted to match opacity tube 2 on B.W. scale. Results were read after incubation for 2 hours at 37°C.

(b) The antigens for complement-fixation were either (i) live encapsulated cells of broth-grown *P. septica*, adjusted to match opacity tube 6 on the Burrough's Welcome tube scale and diluted suitably to obviate the anticomplementary effect or (2) the different antigenic fractions used at concentrations as indicated at pertinent places in the text. Barbitol buffer of Mayer *et al.*, (1946) as used by Brooksby (1952), was employed as diluent throughout. Guinea pig complement collected on the lines of Boyd, (1956) containing 2 M.H.D. in 0.25 ml. was employed for purposes of studying its fixation and the volume of the constituents in the test were: 0.25 ml. antiserum, 0.25 ml. of antigen (whole cell or cell-products) and 0.25 ml. of guinea pig complement. The primary fixation was carried out overnight at $\pm 4^\circ\text{C}$ in the refrigerator and 0.25 ml. of sensitised cells was added to each tube (sensitisation was accomplished by mixing equal volumes of 6% cells and barbitol buffer containing 8 units of amboceptor and incubating at 37°C in a water bath for 15 minutes) and the set incubated at 37°C for 30 to 40 minutes with occasional shaking. The results were recorded no sooner the control tube showed complete lysis of cells. The titres of the sera were recorded as numbers which represent the reciprocal of dilution that gave complete fixation.

The controls included:

(1) 0.25 ml. of 1/10 positive serum, 0.25 ml. complement and 0.25 ml. haemolytic system.

(2) 0.25 ml. antigen, 0.25 ml. buffer and 0.25 ml. haemolytic system.

(3) 0.75 ml. buffer and 0.25 ml. haemolytic system.

(4) 0.5 ml. buffer, 0.25 ml. complement dilution and 0.25 ml. haemolytic system.

Haemolysin was produced in rabbits by a modification of the standard method employing incomplete Freund's adjuvant and stroma

of erythrocytes obtained by lysing with distilled water. Complement and haemolysin were titrated by a modification of the method of Kabat and Mayer (1948) described in an earlier paper (Dhanda, 1959c).

(c) *Indirect bacterial haemagglutination and haemolysis tests*

(i) *Tannic acid modification*:—In the case of fractions IB and II, the tannic acid-modifications (Boyden and Sorkin, 1955) of the haemagglutination was carried out as described below:—

Sheep cells were stored at 5° to 10°C in a sterile modified Alsever's solution (Whitman, 1947) for a minimum of 3 days before use. The cells could be used over a period of 4 to 6 weeks without demonstrable changes in results.

Tannic acid:—A 1% solution in distilled water of E. Merck's tannic acid was sterilised by passage through a Seitz filter and stored $\pm 4^\circ\text{C}$ in the refrigerator. Dilutions from this were made for use in buffered saline, pH 7.2.

Buffer saline:—The pH 7.2 and 6.4 buffers were prepared by mixing equal volumes of 0.85% sodium chloride with the proper mixture of 0.07 M. KH_2PO_4 and 0.07 M. Na_2HPO_4 . The pH of the final solution was checked with a meter and adjusted if necessary.

Antigens:—The sheep red cells were washed three times in 0.85% sodium chloride solution (hereafter called saline) and resuspended to a concentration of 2.5% in buffered saline pH 7.2. An equal volume of tannic acid diluted 1:20,000 in buffered saline was added to the 2.5% cells and incubated in a water bath at 37°C for 10 minutes. The cells were washed once with buffered saline pH 7.2 and once with plain saline, then suspended at 2.5% concentration in buffered saline, pH 6.4. Fractions IB and II were dissolved in buffered saline at a concentration of 10 mg./100 ml., which served as stock solutions. Sensitisation of tannic acid-pre-treated erythrocytes was carried out by mixing equal volumes of 25% tanned cell suspension and a dilution of the stock antigen (titrated earlier for minimal erythrocyte-sensitising activity) calculated to contain at least 4–6 minimal erythrocyte-sensitising units and incubating the mixture at 37°C for 15 minutes. After incubation, the tanned cells with antigen absorbed were washed twice with normal rabbit serum (NRS) diluted 1:200 in saline. The cells were finally resuspended to 0.25% in 1:200 NRS for haemagglutination test and to 0.5% for haemolysis test. The centrifugation of tanned cells throughout was accomplished in round tubes at 1,000 r.p.m. If centrifuged at higher speeds, it became difficult to resuspend them adequately.

Titration was performed on two-fold dilutions of serum (absorbed earlier with sheep cells to deplete them of their heterophile

antibody content) in NRS diluted to 1:100 in saline on Perspex haemagglutination plates (0.5 ml., in the case of haemagglutination test or 'lytic' test). Approximately 0.5 ml. of 0.25% of antigen-coated cells was added to each butt by a tuberculin syringe.

In the case of haemagglutination test, pattern readings were made after incubation for 1 hour at 37° followed by standing for 2 to 3 hours at room temperature. Readings were recorded as +, $\pm$ and negative, with the tube showing complete '+' agglutination being considered the titre of the serum. In the case of lytic test, to two-fold serial dilutions of serum (0.5 ml.) were added 0.25 ml. of guinea-pig complement diluted suitably to contain 2 M.H.D and 0.25 ml. of 0.5% antigen-coated cells. The set was incubated at 37°C for 1 hour when readings were made, the butt showing complete lysis being considered as the titre of the serum.

Controls carried out with each experiment included (i) antigen-treated erythrocytes in N. R. S. diluent (plus complement in the case of lytic test.)

(2) normal untreated erythrocytes in NRS diluent. A known positive and a known negative serum were included with most of the experiments. All titrations were repeated if the controls became unsuitable.

(ii) In the case of fractions III, and IV, sensitisation of sheep erythrocytes was carried out by mixing equal volumes of 2.5% cell suspension (prepared as described previously) and a dilution of the stock antigen solution (10 mg./1,000 ml.) estimated to contain 4 to 6 minimal erythrocyte-sensitising units, (titration carried out earlier) and incubating the mixture at 37°C for 1 hour with occasional shaking every ten minutes. These sensitised erythrocytes were washed 3 times with 10 volumes of 1/250 dilution of NRS to remove excess antigen and resuspended at 0.25% (v/v for haemagglutination and lytic tests). Serial two-fold dilutions of antiserum were prepared in 0.5 ml. volumes using a 1/100 dilution of NRS as diluent. An equal volume of the appropriate cell suspension (0.25%) was added to each dilution of serum on the Perspex plate. The contents were well mixed, incubated at 37°C for 1 hour and held at room temperature for 2–3 hours before results were recorded.

In the case of the lytic modification, to each dilution of serum were added 0.25 ml. of guinea-pig complement suitably diluted to contain 2 M. H. D. and 0.25 ml. of 0.5% antigen-coated (III and IV) cell-suspension. The whole set was incubated for 1 hour at 37°C, when the results were noted. The dilution in the slot showing complete lysis was recorded as the end-titre. Controls were set up in each test, namely, serum dilution with unsensitised erythrocytes and sensitised

erythrocytes with serum diluent. When any agglutination or lysis in the additional presence of complement was detected in the control tubes, the test was discarded.

Later simultaneous and consecutive modification of erythrocytes was tried to explore the possibility of developing a polyvalent test.

(iii) Passive Cutaneous Anaphylaxis Reaction (P.C.A.) in guinea-pigs, designed to detect the presence of skin-sensitising antibodies, was done on the lines of Ovary (1951, 1952, 1953) modified as described by Rosenberg *et al.* (1958). All the sera were heated to 60°C for 15 minutes before use. Guinea-pigs received intracutaneously into the flank 0.1 to 0.3 ml. of different sera samples and these were subjected to a challenge with 0.5 ml. of antigen (whole cell or cell products) mixed with 0.5 ml. of 0.5% aqueous solution of Evans Blue dye T1824. Fifteen minutes after challenge they were sacrificed by a blow upon the head and exsanguinated. The skin was reflected and sites of injection were examined for localisation of the dye. The diameter of the dye leakage was recorded in m.m. and interpretation of the results were made on the lines of Rosenberg *et al.* (1958) and Dray and Young (1958).

(iv) Analysis of these sera with precipitin tests in both liquid and solid media, employing the quantitative micromethod of Heidelberger and MacPherson (1943) for determination of antibody content, was attempted, the results of which are being presented in a separate communication (Dhanda *et al.*, 1960).

Results And Discussion

(I) *Studies on the development of immunity*:—With a view to determine the earliest period when animals vaccinated with the oil adjuvant vaccine developed a good grade immunity, a large number of hill bulls were immunised with the adjuvant vaccine and subjected to a challenge in groups of 4, after 3, 5, 7, 9, 12, 19 and 26 days following vaccination along with controls. In the case of a few hill bulls representative of each group, sera were collected daily until the date of challenge and all the above mentioned in-direct tests were run on the samples in order to follow the antibody response. The results of the immunity tests are presented in table I, which would indicate that hill cattle developed a good grade of immunity even within 5 to 7 days following vaccination with the new adjuvant vaccine. Phase I-agglutinating antibodies appeared between 6th and 10th day, complement-fixing antibodies on 4th to 6th day and mouse-protective antibodies by the 14th day. But, skin-sensitizing antibodies were detected as early as 3 to 4 days by the highly sensitive passive cutaneous anaphylaxis reaction in guinea-pigs.

Of particular importance was the observation that the four antigenic fractions consecutively as well as simultaneously modified erythrocytes, one antigen not blocking the absorption of another, rendering possible simultaneous detection of antibodies against all/or one of the antigenic fractions in the sera, by this polyvalent haemagglutination method or its lytic modification.

It was interesting that, though antitoxin appeared in the circulation as early as 10th day, it disappeared rapidly and by the end of 4th week no such antibodies became demonstrable. Surprisingly they appeared again in the sera of vaccinated hill cattle after challenge and persisted thereon, in one case, upto 6 months and 20 days, the maximum tested so far.

Anti-fraction IB antibodies became discernible as early as 4th and reached a maximum titre by 18th day. The titres of antifraction IV and anti-fraction III antibodies appeared by 6th to 7th and reached a maximal value by 12th day. There was significant correlation between the antifraction IB titre and the results of direct challenge of vaccinated animals, pointing out to the possibility of evaluation of immune status of vaccinated animals in the field by this method.

(II) *Observations on the duration of immunity*:—Dhanda and Lal (1958) reported that the immunity following single dose immunisation with the new adjuvant vaccine was found to be solid up to 12 months. Further immunity tests were carried out after 537, 544, 687 and 752 days following vaccination and the results of all challenge exposures carried out so far are mapped out in table II. A perusal of this table would show that the immunity was solid upto 752 days (2 years and 22 days) the longest period for which tests were carried out so far.

The results of various serological tests on the serum samples collected before challenge showed that, in the majority of the animals, diagnostically significant levels of phase I-agglutinating, complement-fixing, skin-sensitising and mouse-protective antibodies persisted as long as 2 years, indicative of the degree and kind of antigenic stimulation afforded by the adjuvant vaccine. An interesting finding that emerged out of these experiments was the lack of correlation between persistence of skin-sensitising antibodies and immunity status, as demonstrated by challenge.

With a view to study whether the same pattern of antibody response was engendered in animals under field conditions, sera samples were collected at random from vaccinated and unvaccinated stock at Aarey Milk Colony and on Military Farms at Kirkee, Manjri, Pimpri, Bangalore and Secunderabad after a period of 11 months and 22 days

following vaccination. Serological survey of these vaccinated animals showed that as many as 193 out of 200 tested i.e. 96.5% had diagnostically significant levels of phase I-agglutinating, mouse-protective and complement-fixing antibodies, on the basis of which they were expected to survive a direct challenge. Fifty samples of sera tested by the newly developed haemagglutination test showed very high titres of anti-fraction IB antibody (1:5120-1:10,240) but, surprisingly high levels of antibodies against murine toxin (F. II). This persistence of antitoxin led to the belief that these animals might have suffered from a silent infection with *P. septica* prior to or following vaccination, as it was not found to be the case with vaccinated animals kept under laboratory conditions.

Further observations were made on the keeping quality of the adjuvant vaccine. It was reported by Dhanda *et al.* (1958) that the vaccine did not deteriorate in its immunogenic properties by holding for at least one year at room temperature. Two brews of adjuvant vaccine filled in 300ml. quantity in amber-coloured bottles of 350 ml. capacity, were lying at Mukteswar room temperature for over two years. To find out the protective value of such a product, batches of two hill bulls were immunised intramuscularly with 2 ml. of the pooled sample of the vaccine and subjected to a challenge about 4-5 weeks later. Unexpectedly all the four hill bulls survived challenge while both the controls died of pasteurellosis. Thus, it would appear that the adjuvant vaccine possessed an unusually long shelf-life provided it was stored at a suitable temperature.

TABLE I

I.V.R.I. Oil adjuvant vaccine—Development of immunity.

Sl. No.	No. of animals (Hill bulls)	Details of vaccination	Interval between vaccination and challenge	Challenge dose	No. of animals survived total	Percentage survival
1	4	2 ml. intra-muscularly	3 days	1 ml. of 10 ⁻¹ dilution of 11 hour old broth culture	0/4	0.0
2	4	do.	5 days	do.	4/4	100.0
3	4	do.	7 days	do.	4/4	100.0
4	4	do.	9 days	do.	4/4	100.0
			Unvaccinated controls		0/4	0.0
5	4	do.	12 days	do.	4/4	100.0
6	4	do.	19 days	do.	4/4	100.0
7	4	do.	26 days	do.	4/4	100.0
			Unvaccinated healthy hill bulls	do.	0/4	0.0

TABLE II

I.V.R.I. Adjuvant oil vaccine—Duration of immunity.

Sl. No.	No. of animals (Hill bulls)	Details of vaccination	Interval between vaccination and challenge	Challenge dose	No. of animals survived total	Percentage of survival
1	4	2 ml. intra-muscularly	19 days	1 ml. of 10 ⁻¹ dilution on of 18 hour old broth culture	4/4	100.0
2	5	"	21 days	"	5/5	100.0
3	2	"	28 days	"	2/2	100.0
4	37	"	30 to 58 days	"	36/37	97.3
5	32	"	60 to 83 days	"	31/32	96.9
6	9	"	3 months to 3½ months	"	8/9	88.8
7	7	"	4 months	"	6/7	85.7
8	14	"	22 days	"	12/14	85.7
9	9	"	5 months	"	7/9	77.7
10	6	"	6 months	"	5/6	83.3
			8 months to 18 days	"		
11	5	"	9 months	"	5/5	100.0
			21 days	"		
12	4	"	305 days (10 months)	"	4/4	100.0
13	4	"	369 days (12 months)	"	4/4	100.0
14	4	"	544 days (17 months)	"	*3/4	75.0
			22 days	"		
15	4	"	537 days (17 months)	"	*3/4	75.0
			15 days	"		
16	6	"	687 days (22 months)	"	*5/6	83.3
			18 days	"		
17	4	"	752 days (2 years and 22 days)	"	4/4	100.0
			Unvaccinated healthy hill bulls	"	0/30	0.0

*The sera of the three vaccinated hill bulls, that died on challenge, when tested prior to artificial infection, did not show diagnostically significant levels of phase I-agglutinating, complement-fixing and mouse-protective antibodies, on the basis of which they were predicted not to withstand the challenge.

Summary

(1) A good grade of immunity was found to develop within 5 to 7 days and last at least 2 years (752 days), following single dose vaccination with the new adjuvant vaccine. The product was found to possess an unusually long shelf-life when maintained at room temperature in the dark.

(2) Studies on serological response by several indirect tests indicated the appearance of antibodies as early as 3 to 4 days and their persistence as long as at least 2 years.

(3) The anti-fraction IB antibody titre bore a good correlation with immune state, as ascertained by direct challenge.

(4) The level of antitoxin appeared to be a good indicator of natural or artificial exposure to *P. septica*.

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ANIMAL PROTEIN IN POULTRY FEEDING

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The present series is a continuation of the previous efforts to evolve a suitable chicken mash. The effect of different animal proteins and their levels in chick rations has been studied in the present series. Three animal proteins namely fish meal, Liver residue meal and Fresh blood at three different levels and in different combinations have been tried. This study has been made in 3 stages (1) The effect of each of the 3 animal proteins at 3 different levels (2) Effect of the combinations of 2 animal proteins at 2 different levels and (3) Effect of the combinations of all the 3 proteins at 2 different levels.

Fish meal is commonly used as a source of animal protein. Blood though available in slaughter houses, practically free of cost, is not being used to any extent except at big slaughter houses where it is being dried and sold as manure. The residue left after expressing liver extract is also being wasted. The manufacturing concerns incur an extra expenditure in its disposal. Thus blood and the liver residue meal should be cheaper sources of animal protein than fish meal.

Material

Day old White Leghorn chicks of a common strain were obtained from the Central Poultry Farm, Nagpur and were brooded in electrically heated battery brooders at the Nagpur Veterinary College. The composition of the two basal rations used is given in table No. 1. Basal ration No. 1 was used in trials No. 1 and 2 while Basal ration No. 2 was used in trial No. 3.

Basal ration No. 2 has been slightly modified (1) to express the ingredients in percentage, (2) for the want of dried tarota leaves, and (3) the necessity of increasing the carbohydrates to compensate increased level of proteins used. Sharkliver oil at 1% level was incorporated in all the mashes as the chicks were raised in confinement.

TABLE I

Table showing the composition of basal rations.

	Basal 1	Basal 2
Maize ...	20	25
Wheat Bran ...	10	10
Rice Bran ...	20	20
Groundnut oil cake ...	32	30
Penicillin mycelium waste ...	5	5
Dried Tarota leaves ...	3	—
Dried berseem ...	—	5
„ cow dung ...	3	3
Mineral mixture ...	2	2
Total ...	95	100

Trial No. 1 :—Nine different mashes were prepared by the addition of $2\frac{1}{2}$, 5 and 10 parts of fish meal and liver residue meal and 10, 15 and 20 parts of fresh blood to 95 parts of basal ration No. 1. Level of raw blood was selected at 10 & 20 parts as it compares favourably with $2\frac{1}{2}$ & 5 parts of fish meal on protein basis. Still higher levels of blood have not been tried due to the fear of mash becoming unpalatable. A middle of the two levels namely 15 parts of raw blood has been tried. Raw blood collected from slaughter house was mixed with wheat bran and rice bran of the basal ration and then air dried. It was then incorporated with rest of the constituents of basal ration. The nine different mashes used are shown below :—

Mash No. 1	95 parts of basal ration No. 1 + $2\frac{1}{2}$ parts of fish meal
2	do. 5 do.
3	do. 10 do.
4	do. $2\frac{1}{2}$ parts of liver residue meal
5	do. 5 do.
6	do. 10 do.
7	do. 10 do. of fresh blood.
8	do. 15 do.
9	do. 20 do.

The mashes were fed *ad-lib.*

30 chicks were fed each mash. The chicks were weighed initially and at weekly intervals for a period of 4 weeks. Table No. 2 shows the average weekly weights.

In the 4th week of the experiment there was a large mortality under mash Nos. 2, 3, 4 and 5 due to Vitamin E deficiency. The chicks which suffered from Vitamin E deficiency were not from a high or low growing group. They were from different growth rates. The total mortality during the experimental period was 10 per cent.

Statistical analysis :—Gain in weight has been taken as the criterion for growth and results have been analysed accordingly. The chicks which suffered from Vitamin E deficiency have been omitted from Statistical analysis and one way classification of analysis of Variance has been carried out.

Analysis of Variance Table :—A. V. Table showing response of chicks to different animal proteins.

Source of variation	S.S.	D.F.	M.S.	'F' ratio observed
Treatment ...	25803.36	8	3225.42	7.41**
Error ...	100153.50	230	435.45	
Total ...	125956.86	238		

The 'F' ratio is highly significant at 1% level meaning thereby that treatments differ in their effects significantly. The 't' test for two mashes at a time has been applied to find out the best mash. The results are indicated in the following manner :—

Mash	4	1	5	6	7	8	2	9	3
Mean gain weight	136.43	138.23	139.44	142.79	147.86	151.37	157.39	160.86	168.80**

The mashes underlined are insignificant amongst themselves.

Discussion

Fish meal at 5 and 10 parts appears to be good in its effect on growth. Maximum growth however is with 10 parts of fish meal though the growth is insignificant with that obtained with 5 parts of fish meal. $2\frac{1}{2}$ parts of fish meal appears to provide insufficient animal protein. $2\frac{1}{2}$, 5 and 10 parts of liver residue meal are insignificant amongst themselves though they compare favourably with $2\frac{1}{2}$ parts of fish meal or 10 parts of fresh blood. Liver residue meal thus appears to be an incomplete protein.

10 and 15 parts of blood are statistically insignificant in their effects. Similarly 15 and 20 parts of blood are also insignificant amongst themselves. But blood at 20 parts is significantly better than 10 parts of blood. It is also equivalent to 5 or 10 parts of fish meal in

its effect. Being the cheapest amongst the 3 proteins tried, blood at 20 parts could be substituted for fish meal, which is commonly used at present.

As the mortality with mash Nos. 2 and 3 containing 5 and 10 parts of fish meal was high, it was thought worthwhile to repeat the mashes to confirm the results. The following table shows the comparison of the two trials.

Initial (wt. in gms.)		Wt. on 28th day in gms.	Gain in wt. in gms.
Basal ration No. 1 + 5 parts of fish meal...			
<i>Original trial</i>			
Mean	34.21	191.83	157.39
S.D.	2.09	22.63	22.67
S.E.	0.38	4.71	4.72
do.	...	<i>Repetition trial</i>	
Mean	37.93	191.40	153.47
S.D.	2.67	24.70	23.24
S.E.	0.51	4.73	4.47
Basal ration No. 1 + 10 parts of fish meal...			
<i>Original trial</i>			
Mean	31.43	203.08	168.80
S.D.	2.35	20.25	19.54
S.E.	0.43	4.05	3.91
do.	...	<i>Repetition trial</i>	
Mean	37.70	204.73	167.03
S.D.	2.84	32.34	32.09
S.E.	0.50	5.90	5.86

Deficiency of Vitamin E was also observed in the repetition trial but was timely corrected by the addition of Alpha-tocopherol in drinking water. Gain in wt. has been practically the same for both the trials. It thus rules out the possibility of the results of Trial No. 1 being vitiated by the deficiency of Vitamin E and confirms the earlier findings.

Summary

1. 5 parts of fish meal is an adequate animal protein.
2. Liver residue meal alone is an incomplete protein.
3. 20 parts blood is as good as 5 parts fish meal and is therefore an economical source of animal protein.

Trial No. 2:—11 different combinations of 2 animal proteins at a time, at 2 different levels, were studied along with a control mash without any animal protein. Composition of the mashes is given below:—

Mash No.	
1	95 parts of basal Ration No. 1 + 5 parts fish meal 2½ parts liver meal
2	do. 5 " 5 " blood
3	do. 5 " 20 " blood
4	do. 5 " 10 " "
5	do. 2½ " 20 " "
6	do. 2½ " 2½ liver meal
7	do. 2½ " 5 " "
8	do. 2½ liver meal + 20 parts blood
9	do. 2½ " 10 " "
10	do. 5 " 20 " "
11	do. 5 " 10 " "
12	do. alone (without any animal proteins)

In order to reduce dispersion and provide equal opportunity to different mashes, eggs were weighed to the nearest gram, sorted out according to weight and incubated separately so that chicks hatched out from eggs of the same weight could be distributed evenly amongst the different mashes. As in the previous trial 30 chicks were kept under each mash. The chicks were weighed initially and at the end of each week for the period of 4 weeks. The average weekly weights are given in Table No. 3.

Statistical Analysis:—In order to enable two way classification of analysis of variance, equal number of chicks were taken from each of the mashes for further analysis. As the minimum number of chicks surviving at the end of the 4th week was 27, the chicks above this were dropped using table of random numbers. The following table gives two way classification of analysis of variance.

A. V. Table:—Analysis of variance table showing response of chicks to different combinations of animal proteins.

Source of variation	S.S.	D.F.	M.S.	'F' ratio observed
Treatment ...	80489.58	11	7317.23	15.28**
Individuality ...	5340.67	26	205.41	0.34
Error ...	171613.59	286	600.05	
Total ...	257443.84	323		

F ratio for treatment is highly significant. while the ratio for individuality is insignificant. Variation of growth between different

mashes is thus not due to individual variation in chicks but due to intrinsic variation in the mashes tried.

The critical difference at 5% level is 12.74 gms. The mashes in which the difference between the mean gain in weight is less than the critical difference have been bracketed to indicate insignificance amongst themselves. For brevity the combinations of animal proteins are indicated by their levels in the order of fish meal, liver residue meal and blood. Mean gain in weight is shown in bracket for ready reference.

Mash No.	Mash combination	Mean gain in wt.
	FM. L.M.B.	
12	0 -0 - 0	127.85
5	2½ -0 -20	160.15
4	5 -0 -10	163.22
3	5 -0 -20	171.56
9	0 -2½ -10	174.85
11	0 -5 -10	176.30
10	0 -5 -20	178.22
6	2½ -2½ - 0	180.81
8	0 -2½ -20	186.37
1	5 -2½ - 0	192.96
2	5 -5 - 0	194.19
7	2½ -5 - 0	194.33

't' test for two mashes at a time confirms the above findings.

Discussion

Trial Nos. 1 and 2 have been conducted at two different times. But the two trials can be compared, as the results of repetition trial, with 5 and 10% fish meal conducted during this period, tallies with the results obtained previously thus serving as a control.

Mash 5-0-20 (171.56 gms.) when compared with Mash 5-0-0 (157.39 gms.) and Mash 0-0-20 (160.86) does not indicate much improvement. The combination of fish meal and blood therefore appears to be superfluous. The same is confirmed by comparing Mash 2½-0-20 (160.15) and 5-0-10 (163.22) with 5-0-0 (157.39) and 0-0-10 (147.86) of the first trial.

2. Mashes 5-2½-0 (192.96 gms.), 5-5-0 (194.19 gms.) and 2½-5-0 (194.33 gms.) are insignificant amongst themselves though they are the best amongst the mashes studied. But when these results are compared with 5-0-0 (157.39) and 0-5-0 (139.44 gms.) of the first trial a highly significant difference is observed proving thereby that fish meal and liver residue meal are complementary to each other in their effect on growth. But mash 2½-2½-0 (180.81 gms.) does not give as

much good results as 5-2½-0 or 2½-5-0. Difference in mean gain in weight of mashes 5-2½-0 and 2½-2½-0 is 12.15 gms. which is slightly less than the critical difference of 12.74 gms. This indicates that the combined level of fish meal and liver residue meal at 2½ parts each is insufficient. Similarly higher levels of fish meal and liver residue meal at 5 parts each also appear to be superfluous. The level of 5-2½-0 or 2½-5-0 appears to be adequate. The levels of fish meal and liver residue meal are thus interchangeable and can be altered depending upon price and availability.

3. Mashes 0-2½-10 (174.85 gms.), 0-5-10 (176.30 gms.) and 0-5-20 (178.22 gms.) are insignificant amongst themselves. But when compared with 0-5-0 (139.44 gms.) and 0-0-20 (160.86) the combination of liver meal and blood shows better growth thus indicating complementary effect of liver residue meal and blood.

4. Mash 0-2½-20 is insignificant with the best of the mashes namely 5-2½-0, 5-5-0 and 2½-5-0. It may prove to be the cheapest on account of the low cost of blood.

Summary

1. Liver residue meal is complementary to both fish meal and blood for growth.
2. Combination of fish meal and blood does not show statistically significant difference though the combination showed some increase in growth.
3. 5 parts of fish meal and 2½ parts liver residue meal or 2½ parts fish meal and 5 parts liver residue meal give best growth. As the levels of fish meal and liver residue meal are interchangeable they can be adjusted taking price and availability into consideration.
4. 2½ parts of liver residue meal and 20 parts of blood give as much good result as the combination of fish meal and liver residue meal and may therefore prove to be most economical.

Trial No. 3 :—Combinations of fish meal, liver residue meal and blood each at 2 different levels have been tried. The 8 different combinations of animal proteins are shown below :—

1.	100 parts of basal ration No. 2	+ 2½ parts fish meal	+ 2½ parts L.M.	+ 10 parts blood.
2.	do.	+ 2½ do.	+ 2½ do.	+ 20 do.
3.	do.	+ 2½ do.	+ 5 do.	+ 10 do.
4.	do.	+ 2½ do.	+ 5 do.	+ 20 do.
5.	do.	+ 5 do.	+ 2½ do.	+ 10 do.
6.	do.	+ 5 do.	+ 2½ do.	+ 20 do.
7.	do.	+ 5 do.	+ 5 do.	+ 10 do.
8.	do.	+ 5 do.	+ 5 do.	+ 20 do.
9.	do.	alone (without any animal proteins)		

As in trial No. 2 eggs of the same weight were incubated separately and the chicks hatched out were evenly distributed amongst all the lots. 30 chicks were allotted to each mash. The chicks were weighed initially and at weekly intervals for a period of 4 weeks. The average weekly weights for each mash have been given in Table No. 4.

Statistical Analysis :—The combination of animal proteins though arranged on factorial basis factorial analysis would be erroneous as the mashes are neither isoproteinic nor isocaloric. The object however is merely to find out the best combination of animal protein. Simple two way classification of Analysis of Variance has, therefore, been applied to find out the best mash. As in the previous trial table of random numbers has been used to eliminate chicks above the minimum number of 27 in each lot. The following shows the two way classification of Analysis of Variance.

A. V. Table.

Source of variation	Sum of squares	D.F.	M.S.	'F' ratio observed
Treatment ...	69660.80	8	8707.60	10.92**
Individuality ...	19243.68	26	740.14	0.93
Error ...	165846.76	208	797.34	
Total ...	254751.24	242		

The F ratio is highly significant at 1% level for treatments but is insignificant for individuality.

The critical difference at 5% level is 15.05 gms. The mashes in which, difference between the average gain in weights is less than critical difference are underlined, they being insignificant amongst themselves.

Mash No.	9	1	3	2	8	7	4	5	6
Mean gain in wt.	148.26	192.44	193.81	205.59	206.30	209.30	212.81	214.33	214.89

The above results are collaborated by the application of 't' test for two mashes at a time.

Consumption

The following table shows the efficiency of different mashes as indicated by their requirement per unit gain in wt.

Mash No.	Consumption in gms. in 4 weeks*	Total gain in wt. in gms.	Efficiency-requirement per unit gain
9	20773	4637	4.48
1	21700	5186	3.97
2	21595	5808	3.72
3	21855	5896	3.71
8	21670	5840	3.71
7	20675	5651	3.66
5	21740	6093	3.57
4	21505	6180	3.48
6	22430	6467	3.47

* Consumption includes wastage.

Discussion

1. The control mash No. 9 (148.26) of the present trial when compared with the control mash of the previous trial (126.39) indicates significant difference, better growth may be due to change in the basal ration. Results of trial No. 2 cannot therefore be compared with the results obtained in the present trial.

2. When mash No. 1, $2\frac{1}{2}$ - $2\frac{1}{2}$ -10 (192.44) is compared with mash No. 5, 5 - $2\frac{1}{2}$ -10 (214.33) a significant difference in growth is observed. This may be due to the increase in the percentage of fish meal from $2\frac{1}{2}$ to 5%, liver meal and blood being at the same levels. Similarly when mash No. 3, $2\frac{1}{2}$ -5-10 (193.81) is compared with mash No. 7, 5 -5-10 (209.30) a significant difference in growth is again observed confirming the above finding.

But when mash No. 8, 5 -5-20 (206.30) is compared with mash No. 4, $2\frac{1}{2}$ -5-20 (212.81) an adverse effect on growth is noticed. There has been a decrease though not a significant decrease in growth. This is likely to be due to a high percentage of protein in the mash, all the three animal proteins being at their highest levels.

3. When mash No. 1, $2\frac{1}{2}$ - $2\frac{1}{2}$ -10 (192.44) is compared with mash No. 3, $2\frac{1}{2}$ -5-10 (193.81) or when mash No. 5, 5 - $2\frac{1}{2}$ -10 (214.33) is compared with mash No. 7, 5 -5-10 (209.30) no significant increase in growth is observed by increasing the percentage of liver meal from $2\frac{1}{2}$ to 5% keeping fish meal and blood on equal levels. But when liver meal is decreased from 5% to $2\frac{1}{2}$ % when the other two animal proteins are at the highest levels compare Mash 8, 5 -5-20 (206.30) with mash No. 6, 5 - $2\frac{1}{2}$ -20 (214.89), an increase though not a significant increase in growth

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is observed thus confirming the above findings that a high percentage of protein has an adverse effect on growth.

4. When mash No. 1, 2½-2½-10 (192·44) is compared with mash No. 2, 2½-2½-20 (205·59) an increase in growth though not a significant increase is observed. Similarly when mash No. 3, 2½-5-10 (193·81) is compared with mash No. 4, 2½-5-20 (212·81) a significant increase in growth is observed.

But when mash No. 8, 5-5-20 (206·30) containing all the three animal proteins at the highest levels is compared with mash No. 7, 5-5-10 (209·30) an improvement though an insignificant one is observed.

Conclusions

1. Considering the effect on growth and efficiency measured by requirement per unit gain in weight combination of 5-2½-20 or 2½-5-20 appears to be best.

2. A high level of protein has a depressing effect on growth.

Summary

Trials have been conducted at the Nagpur Veterinary College to compare the efficacy and requirements of fish meal, liver residue meal and fresh blood. In the first trial the three animal proteins have been tried individually at 3 different levels, in the second in the combinations of two at 2 different levels and in the third in the combinations of all the three at 2 different levels.

When used alone, 5% fish meal appears to be adequate. Response of liver residue meal even at higher levels has been poor indicating its incompleteness for growth. Fish meal and liver residue meal however appear to be complementary to one another, much better growth being obtained through their combination. 5 parts of fish meal and 2½ parts liver residue meal when added to 95 parts of basal ration gave best growth. The levels of fish meal and liver residue meal are interchangeable and can be adjusted depending upon price and availability.

20 parts of fresh blood when mixed with 95 parts of basal ration gave statistically as good result as 5% fish meal. Blood and liver residue meal also appear to be complementary to one another though the effect is not as good as the combination of fish meal and liver residue meal.

When used in combinations of three the combination of 5-2½-20 or 2½-5-20 appears to be good. Further trials however are essential to see (i) if there is any complementary effect when all the three animal proteins are combined together and (ii) if the level of groundnut oil cake could conveniently be reduced without affecting the efficacy of the combination of animal proteins.

Acknowledgment

Thanks are due to (i) Shri V. N. Sharma Principal and Professor of Animal Husbandry, Nagpur Veterinary College, for his guidance and critical analysis (ii) Shri R. P. Asthana, Principal, College of Agriculture, Nagpur, for providing battery brooder (iii) Shri H. V. Kulkarni, Deputy Director of Animal Husbandry, Nagpur Division, Nagpur, for providing chicks and feed ingredients and (iv) Shri M. N. Jogleker, Professor of Economics, College of Agriculture, Nagpur, for the use of calculating machine in statistical analysis.

Special thanks are due to Shri P. O. Katker and Shri Bondre, Laboratory Assistants and Shri Chhanu Sing, Laboratory Attendant, for their invaluable help in conducting the trials.

TABLE II—(Trial No. 1)

Table showing average weekly Weight of Chicks in grams.

Mash No.	1	2	3	4	5	6	7	8	9
1st Day									
Mean	34·07	34·21	34·43	34·37	34·23	34·07	34·45	34·40	34·97
S.D.	1·99	2·09	2·35	2·34	2·37	2·28	2·85	2·75	3·04
S.E.	0·36	0·38	0·43	0·45	0·44	0·43	0·52	0·50	0·56
7th Day									
Mean	54·76	58·66	60·50	54·85	56·38	57·18	51·59	52·23	53·52
S.D.	5·53	5·39	4·47	4·28	5·39	5·80	6·71	5·64	6·19
S.E.	1·02	1·00	0·81	0·82	1·00	1·09	1·24	1·03	1·15
14th Day									
Mean	86·41	95·24	100·53	84·63	89·24	88·79	79·34	88·33	85·88
S.D.	9·72	11·24	9·52	7·08	10·83	8·44	9·71	11·42	12·03
S.E.	1·80	2·08	1·72	1·36	2·00	1·59	1·80	2·08	2·23
21st Day									
Mean	127·90	141·72	150·33	122·88	124·59	130·54	119·52	126·93	132·83
S.D.	12·34	16·51	14·64	11·48	14·42	17·63	17·01	16·42	17·81
S.E.	2·89	3·06	2·67	2·20	2·67	3·33	3·15	3·00	3·12
28th Day									
Mean	172·34	191·83	203·08	170·81	173·60	176·79	182·31	185·77	195·83
S.D.	15·04	22·63	20·25	18·05	19·58	22·85	23·28	22·45	23·30
S.E.	2·79	4·71	4·05	3·93	3·92	4·32	4·34	4·09	4·32
Gain in Weight									
Mean	138·28	157·39	168·80	136·43	139·44	142·79	147·86	151·37	160·86
S.D.	14·66	22·67	19·54	17·87	19·93	22·51	23·05	21·05	24·12
S.E.	2·72	4·72	3·91	3·90	4·07	4·25	4·28	3·84	4·48

TABLE III—(Trial No. 2)
Table showing average weekly weights of chicks in grams.

Mash No.	1	2	3	4	5	6	7	8	9	10	11	12
Animal Protein Combination.*	5-2½-0	5-5-0	5-0-20	5-0-10	2½-0-20	2½-2½-0	2½-5-0	0-2½-20	0-2½-10	0-5-20	0-5-10	0-0-0
1st Day—Mean	35.22	35.11	34.76	34.03	34.03	35.07	34.89	37.62	36.88	37.93	37.96	38.89
S.D.	1.45	1.07	2.75	2.62	2.48	1.47	1.61	2.68	2.12	2.55	3.06	3.12
S.E.	0.25	0.20	0.51	0.48	0.45	0.28	0.29	0.50	0.42	0.67	0.59	0.59
7th Day—Mean	55.15	60.93	54.10	63.66	53.17	59.29	59.41	58.52	53.31	54.64	56.52	53.71
S.D.	5.85	5.00	6.77	7.58	5.47	4.13	5.99	6.51	7.74	5.50	5.44	6.27
S.E.	1.10	0.96	1.25	1.41	0.99	0.79	1.11	1.21	1.52	1.04	1.05	1.18
14th Day—Mean	97.74	104.07	90.83	87.55	87.60	90.74	100.88	99.14	92.65	94.36	96.63	84.86
S.D.	10.05	10.51	10.16	11.52	8.89	9.49	11.14	11.61	12.91	11.46	8.16	11.62
S.E.	1.93	2.02	1.88	2.13	1.62	1.82	2.06	2.16	2.53	2.17	1.57	2.20
21st Day—Mean	148.00	154.26	138.93	134.93	133.70	137.81	151.34	156.52	145.81	148.75	159.04	125.89
S.D.	14.59	16.60	17.03	15.50	14.47	16.32	16.90	16.97	19.43	19.58	17.11	16.41
S.E.	2.80	3.19	3.16	2.87	2.64	3.14	3.13	3.15	3.81	3.70	3.29	3.10
28th Day—Mean	228.22	239.29	205.79	197.21	194.53	215.88	229.34	223.79	211.73	216.32	214.26	164.84
S.D.	17.06	25.12	25.73	17.68	21.91	27.04	24.84	22.99	29.07	26.19	22.36	23.45
S.E.	3.28	4.83	4.77	3.28	4.00	5.20	4.61	4.27	5.70	4.95	4.30	4.43
Gain in weight	192.96	194.18	171.03	163.17	160.50	180.81	194.45	186.17	174.85	178.40	176.80	126.45
S.D.	16.32	24.66	25.39	18.44	21.68	26.86	22.93	22.58	29.17	25.92	22.18	22.19
S.E.	3.14	4.75	4.72	3.43	3.96	5.17	4.38	4.21	5.72	4.90	4.27	4.20
Mean gain (27 chicks)	192.96	194.19	171.56	163.22	160.50	180.81	194.33	186.37	174.85	178.22	176.80	126.89

*Indicates the level of fish meal, liver residue meal and fresh blood respectively.

TABLE IV—(Trial No. 3)

Table showing average weekly weights of chicks in grams.

Mash No.	1	2	3	4	5	6	7	8	9
Animal Protein Combination*	2½-2½-10	2½-2½-20	2½-5-10	2½-5-20	5-2½-10	5-2½-20	5-5-10	5-5-20	0-0-0
1st Day—									
Mean	38.74	38.57	38.60	38.73	38.46	39.10	38.73	38.36	38.76
S.D.	3.08	2.89	2.69	2.70	2.78	2.75	2.91	8.06	3.05
S.E.	0.59	0.54	0.49	0.50	0.52	0.50	0.57	0.67	0.56
7th Day—									
Mean	58.59	60.36	61.03	60.38	60.79	62.83	60.12	60.43	57.14
S.D.	6.94	7.13	6.81	5.85	4.51	5.00	6.67	7.18	5.28
S.E.	1.34	1.32	1.24	1.84	0.85	0.91	1.11	1.35	0.98
14th Day—									
Mean	101.59	105.32	104.40	105.52	106.82	109.37	108.92	107.61	92.47
S.D.	12.33	12.86	13.83	12.97	9.56	14.17	12.26	14.50	10.62
S.E.	2.87	2.43	2.52	2.40	1.80	2.59	2.40	2.75	1.97
21st Day—									
Mean	157.93	156.04	163.70	170.10	169.29	175.57	166.23	172.46	141.66
S.D.	22.03	19.09	20.01	21.64	13.25	23.56	19.98	22.68	16.26
S.E.	4.24	3.60	3.65	4.00	2.50	4.30	3.91	4.28	3.02
28th Day—									
Mean	231.19	241.39	232.13	251.83	250.96	254.66	248.04	244.25	187.66
S.D.	30.71	25.60	23.58	31.13	21.03	36.41	26.08	28.18	21.04
S.E.	5.91	4.83	4.30	5.78	3.97	6.65	5.11	5.32	3.91
Gain in weight—									
Mean	192.44	205.82	193.53	213.10	212.50	215.57	209.30	205.93	148.90
S.D.	30.90	24.99	25.00	31.98	20.54	36.89	25.83	29.22	21.58
S.E.	5.95	4.72	4.56	5.94	4.00	6.74	4.97	5.52	4.01
Mean gain (27 chicks)	192.44	205.59	193.81	212.81	214.33	214.89	209.30	206.30	148.26

*Indicates the level of fish meal, liver residue meal and fresh blood respectively.

LISTERIOSIS AMONGST SHEEP AND GOATS IN UTTAR PRADESH

By

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Listeriosis is a disease of considerable economic and public health importance. *Listeria monocytogenes* is now known to be present almost throughout the world. It causes disease in a wide variety of animals (sheep, goats, cattle, dogs, horses, chickens and rabbits) including man.

In 1926 Murray, Webb and Swan isolated a small Gram positive non-sporing bacillus from rabbits suffering from a natural disease characterised by mononuclear leucocytosis. They named the organism as *Bacterium monocytogenes*, for which, Pirie (1927) created a new genus called *Listerella*. It was later changed (1940) to *Listeria*.

Listeriosis seems to occur in three distinct clinical types, the nervous type, the uterine type, and the septicaemic type of which the nervous type is the most common.

The nervous type of the disease amongst the sheep of New Zealand was first studied and described by Gill in 1931, who, two years later in 1933, succeeded in establishing its etiology due to *Listeria monocytogenes*. He gave the name "circling disease" to the encephalitic form of Listeriosis observed by him, because of the characteristic feature of moving in circles manifested by the affected animals. Five years later (1936), Burn isolated *Listeria monocytogenes* from two prematurely born human infants and also observed that man may get infection through drinking of milk of cattle affected with Listeriosis. *Listeria monocytogenes* was isolated by Biester and Schwarta (1939) from meningo-encephalitis of sheep in Iowa.

In 1939 Graham observed abortions in cattle due to *Listeria monocytogenes*. Abortions in sheep due to listeriosis have been recorded by Hindmarsh and Blumber (1932), Paterson (1940) and Moule (1953). Further studies of this disease in sheep have been carried out by several workers in the United States.

Listeriosis was first described in India by Mahajan (1936-37) under the name "circling disease" amongst the sheep of Hyderabad State. He did not succeed in isolating the causal organism to establish its etiology conclusively as due to Listeriosis. Viswanathan and Ayyar (1950) studied the disease in detail amongst the sheep of Madras State

and reported the isolation of *Listeria monocytogenes* with complete description of morphological and cultural characters, and biochemical, biological and serological reactions. They also observed that goats were not affected though they were present in large numbers along with the sheep affected with Listeriosis. In 1959, Dhanda and Lall reported isolation of a culture of *Listeria monocytogenes* from the uterine pus swab from an aborted ewe and from several cases of broncho pneumonia in lambs. For all these isolations the materials were supplied by the author.

Reports of the septicaemic type of Listeriosis amongst sheep and goats appear to be very infrequent in veterinary literature. The present article describes the clinical symptoms, post-mortem lesions and other laboratory examinations of septicaemic type of Listeriosis amongst sheep and goats of Rishikesh.

History :—An outbreak of Listeriosis occurred amongst the goats of a private flock comprising of 85 Beetal graded desi goats and 150 sheep belonging to Sri Parash Ram of Rishikesh, which is situated at a height of about 1,000 feet above sea level at the Himalayan foot hills of Uttar Pradesh. The climate of this area is hot and humid. The annual rainfall varies from 50 to 70 inches. The maximum and minimum temperatures recorded during the last two years are 112°F and 32°F, respectively. The relative humidity varies from 70 to 97% during the months of July to October.

Sri Parash Ram is a professional sheep and goat breeder and has been in this line for the last 30 years. His goats yield about 2 to 3 lbs. of milk. The milk is sold in Rishikesh for human consumption. The first cases of illness were encountered amongst his goats during the month of October, 1955. The disease spread slowly and lingered on till December, 1955. The sheep were not affected though they were kept along with the diseased goats in the same shed during the nights. Out of 85 goats, twenty five were affected with Listeriosis and succumbed to it.

Eight sporadic cases of Listeriosis also occurred during the years 1955-56 amongst the Polwarth sheep and their pure bred progeny at the Central Sheep and Wool Research Station, Pashulok, which is situated on the southern bank of the river Ganga about 3 miles from Rishikesh.

Symptoms :—The clinical syndrome was characterised by sudden onset of the disease with marked elevation of the body temperature (106° to 107° F), loss of appetite, congestion of visible mucous membrane, excessive salivation, accelerated pulse and respiration and watery nasal discharge which later on turned into thick muco-purulent.

Keratitis and conjunctivitis were equally present. Occasionally the affected animals developed pneumonia characterised by marked respiratory distress and cough. In the last stages the sick animals went down and were unable to rise. The duration of illness was short in acute cases which ended in death from 2 to 3 days. In subacute cases the course of disease extended as long as 7 days. The disease invariably proved fatal as all the affected animals died and none recovered. Not a single case of "circling disease" the encephalitic form of Listeriosis was observed.

Post-mortem findings :—The author conducted ten post-mortems on 6 goats and 4 sheep which died of listeriosis.

Subcutaneous and sub-mucous haemorrhages were numerous. The lymph glands were frequently swollen and haemorrhagic. Deep red patchy congestion of the mucous membrane of the abomasum and small intestine was usually observed. Approximately in 50 percent cases the lungs were affected and showed consolidation of the dependent parts of the apical, cardiac and diaphragmatic lobes. Few cases showed petichae on the endocardium. There was a slight enlargement and congestion of liver and spleen. The brain and spinal cords did not show any gross lesions.

Laboratory Examination

The laboratory examination of the morbid material was carried out in collaboration with the Indian Veterinary Research Institute, Mukteswar.

The microscopical examination of smears prepared from lungs exudate and heart blood invariably showed gram positive rod shaped organisms resembling *Listeria monocytogenes*.

Cultures were made on blood agar, liver agar, plain agar and glucose broth from heart blood and lungs exudate. These cultures on morphological and biochemical examination resembled *Listeria monocytogenes*.

Pipettes of lung exudate and heart blood were forwarded to Mukteswar, where these resulted in the isolation of *Listeria monocytogenes*, which was found pathogenic for experimental animals.

Histopathological examination of the pieces of lungs forwarded in 10% formalin to Mukteswar, showed characteristic lesions of broncho-pneumonia.

Treatment :—Treatment of the affected cases was tried with Procaine Penicillin and Sulphamezathine without any avail.

Summary

1. Cases of septicaemic type of listeriosis amongst sheep and goats were investigated and described.
2. The clinical syndrome consisted of a sudden onset of the disease with high rise in the body temperature, keratitis, conjunctivitis, accelerated pulse and respiration, nasal discharge and anorexia.
3. The post-mortem lesions were suggestive of acute septicaemic conditions.
4. The cultural examination of lungs exudate and heart blood resulted in the isolation of *Listeria monocytogenes*.
5. The culture of *Listeria monocytogenes* proved pathogenic to the laboratory animals.

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LOCALISED PERITONITIS IN A GOAT DUE TO *SETARIA CERVI*

BY

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During routine collection of materials from goats showing lesions of peritonitis, slaughtered for the manufacture of goat tissue vaccine at the Indian Veterinary Research Institute, Izatnagar, two nematode parasites were encountered entangled in the omentum of one goat which were identified as those belonging to the species *Setaria cervi*.

Setaria cervi is a normal inhabitant of the peritoneal cavity of bovines. In India, Bhalerao (1933) described *Setaria buxi* as a new species from a goat. Ochi (1953) reported *Setaria marshalli* in the peritoneal cavity of sheep and goats from Korea. Emadudin (1954) reported *Setaria cervi* from the eye of a goat and Thapar (1956) identified *Setaria labiatopapillosa* as a normal inhabitant in the peritoneal cavity of goats from Bengal, Orissa and Uttar Pradesh. Baylis (1936) considers all the above species of *Setaria* refer to the same parasite and are synonyms of *Setaria cervi* (Rudolphi, 1819).

Very scant attention has been paid by pathologists to the omentum of animals, and the pathological changes encountered in this organ have not been reported so far, (Florey 1958). Probably, the only reference to histological changes occurring in the peritoneum around these parasites characterised by congestion, presence of endotheloid cells and fibrous tissue formation around the parasite is due to Sarwar (1945) who studied this condition in bovines.

Microscopical examination:—The parasite under reference was an adult one, seen entangled in the omentum where it had undergone degenerative changes, characterised by necrosis and calcification in some parts of the parasite on histological examination (Fig. 1).

The parasite either during its life or immediately after its death appears to have released certain irritants giving rise to an acute inflammatory reaction of the structures around the parasite with an acute congestion of the vessels in the sub-serosa and a large number of eosinophilic leucocytes around the parasite. The histological examination at different levels of the parasite revealed the cross section embedded on the serosa with evidence of necrosis and calcification of the structures within the degenerated parasite, desquamation of the mesothelial cells of the omentum and infiltration of the omental structures

by a large number of eosinophiles and the mononuclear cells which also contained a few polymorphs (Fig. 1). The mononuclear cells appear to have been derived from the "milky spots" of the omentum due to the hyperplasia of these follicles and eosinophiles and neutrophils from the congested blood vessels. However, no epitheloid cells were seen surrounding these parasites as noticed by Sarwar (1945) in bovines. The parasite may also have acted as a foreign body and has produced severe reaction in the sub-serosa and in the loose connective tissue folds of the omentum, which had reacted with the formation of angioblasts and fibroblasts from these structures replacing the inflammatory cells described above in an effort to dislodge and ultimately replace the parasite by granulation tissue as evidenced by the presence of these granulation tissues invading the degenerated parasite at places in some of the sections. The amount of granulation tissue produced was in such a quantity in some areas that the structures situated between the two loose connective tissue folds were seen completely replaced by granulation tissue. The other normal structures of the omentum situated very near the parasite showed varying degree of degenerative changes and even necrosis.

Some of the cross sections of the parasite revealed the presence of microfilaria within the uterus (Fig. 2) while other sections revealed both uteri filled with ova in the same parasite.

Discussion

S. cervi is a filaroid worm, the natural host of which is the cattle residing as adult worms in the peritoneal cavity without causing any damage, though the complete range of hosts is unknown. The microfilaria produced by *Setaria* sp. invade the blood stream and are transmitted to susceptible cattle by *Stomoxys calcitrans* (Gonzalez Alvarez, 1946) and by mosquitoes (Ishii *et al* 1953). The microfilaria mature in two weeks in the arthropod hosts and are then transmitted again to the cattle.

According to Innes (1957), when the parasites are transmitted to unnatural hosts like sheep, goat and horses, they do not develop into adults in the peritoneal cavity but they emerge as immature worms in the central nervous system to cause malacic foci and damage to the brain and spinal cord. Victor (1958) also failed in his transplantation experiments of *Setaria digitata* in experimental hosts like a female goat, a male goat and a rabbit by introducing the adult parasites of both sexes directly into the peritoneal cavity.

However, various species of *Setaria* like *S. buxi*, *S. marshalli*, and *S. cervi* have been recorded from the peritoneal cavity of goats from various parts of India. The authors were only able to obtain two dead

and degenerating parasites which were separated from the omentum with great difficulty from a single goat after examining near about a thousand goats, while the other goats were entirely free from these parasites in the peritoneal cavity. Is it likely that these parasites are brought to the peritoneal cavity as erratic parasites and some mechanism had prevented them from completing their life-cycle in the peritoneal cavity of an unnatural host like goat with the appearance of microfilaria in the blood stream? Probably, further investigations in this direction may throw some light whether the goats are naturally immune to the development of *Setaria* in the peritoneal cavity or whether some other mechanism is playing a part from preventing the completion of life-cycle of the parasite in the peritoneal cavity. For the present, the opinion is very conflicting, since Williams (1955) had considerable success in establishing *S. cervi* in a rabbit whereas Victor (1958) failed in his attempts with *S. digitata*. It appears quite probable that *S. cervi* may be encountered in the peritoneal cavity of goats as an erratic parasite under similar conditions as they are seen in the central nervous system.

Summary

Localised peritonitis of the omentum of a goat caused by *Setaria cervi* is described together with histological changes encountered in the organ surrounding the parasite.

Acknowledgment

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Localised Peritonitis in a Goat due to *Setaria Cervi*

By

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Fig. 1



Fig. 2

Localised Lesions in the Omentum of Goats due to Taenia Species

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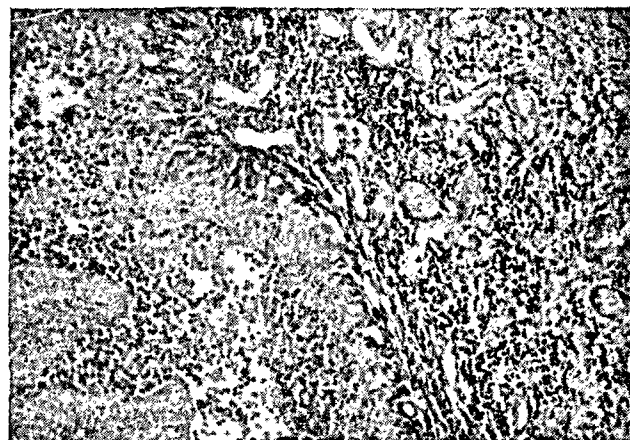


Fig. 1

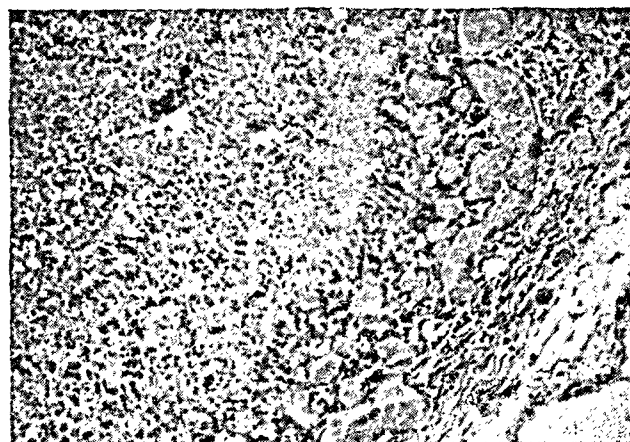


Fig. 2

LOCALISED LESIONS IN THE OMENTUM OF GOATS DUE TO TAENIA SPECIES

By

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and

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During routine collection of peritonitis materials from goats at the Biological Products Division of this Institute, a number of goats revealed the presence of *Cysticercus tenuicollis*, an intermediate stage of the tape worm, *Taenia hydatigena*. The adult parasite is found in the intestine of dog and other related wild carnivora like jackal, fox, etc. The intermediate stage occurs normally in the peritoneal cavity of sheep and goats. The well-developed bladder worm may be found anywhere in the abdominal cavity, enclosed in a delicate cyst formed by the peritoneum. The following table will show the extent of infestation with bladder worms among healthy goats slaughtered for preparation of Rinderpest goat tissue vaccine.

Table showing incidence of bladder worms.

Months	No. of goats observed	No. of goats with bladder worm	Percentage of infection
July, 1959	695	109	15.7
August, 1959	544	99	18.2
September, 1959	608	129	21.4
October, 1959	538	115	21.7
November, 1959	481	93	19.3
December, 1959	411	71	17.2
January, 1960	329	54	16.4

Thus, among 3596 goats examined over a period of seven months, 670 revealed the presence of hydatid cysts in the abdominal cavity, showing an incidence to be 18.6 per cent.

On examination of the peritoneal cavity, in addition to the bladder worms, a few goats showed the presence of a few rounded millet-sized nodules situated in the omentum, the mesentery being free from such lesions. These nodules were collected in 10 per cent formal-saline for histological examinations.

Gross pathology:—The nodules, in general, were bluish-red in colour weighing 0.3 to 0.5 gram which on cut surfaces revealed a

Localised Lesions in the Omentum of Goats due to *Taenia* Species

By A. N. TEWARI and P. K. RAMACHANDRA IYER

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whitish irregular area with serrated margins extending very close to the outer connective tissue border of the nodule. The centre of these nodules had a frank bluish-red colour with a slight depression with interspersed areas of calcification and a few had a thick fibrous tissue capsule surrounding them. A small number of nodules were seen suspended from the omentum by means of a short pedicle.

Microscopical Examination:—The lesions were seen in varying stages of development and they can be conveniently described as follow:—

- (a) *Early lesion*:—From within outwards, the lesion showed the following changes. In the centre, was the cross-section of the parasite, *Taenia*, characterised by the presence of two scolices, unarmed suckers, hooks on the rostellum and calca-roid masses within the body of the parasite. This was surrounded by a dense zone of cellular infiltration mostly made up of monocyctic cells and eosinophiles. Majority of these cells, however, were showing degenerative changes evidenced by pink staining of their cytoplasm with eosin, and shrunken and pyknotic nuclei, which in some cells were completely absent. However, within the cytoplasm of these cells, the eosinophilic nature of the granules was quite distinct. These zones of inflammatory cells were surrounded all around by a clear zone of epitheloid cells or reaction cells having large basophilic vesicular nuclei, limiting the spread of the lesion, and were probably derived from the reticulo-endothelial cells of the neighbouring omental structure, and not from the delicate cyst wall of the bladder worms, since the latter, when examined revealed only a myxomatous structure lined by a layer of low cuboidal cells. These epitheloid cells may also represent a host tissue reaction to the presence of the parasite. Surrounding this eosinophilic-epitheloid zone of cellular reaction was a wide zone of granulation tissue consisting of a large number of minute blood vessels lined by a single layer of endothelium and fibrous connective tissue cells. Distributed, in this granulation tissue, particularly around the blood vessels were a large number of monocyctic cells, probably lymphocytes. This granulation tissue in turn was surrounded by a thin connective tissue capsule and the serosa of the omentum.

The taenia contained in the centre of the lesion showed varying degrees of degenerative changes and infiltration with a few monocular cells. Other area of the omentum, neigh-

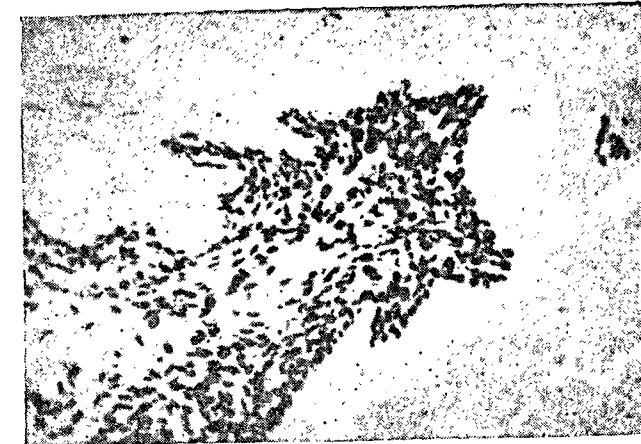


FIG. 3



FIG. 4



FIG. 5

They Go Abroad



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bouring the parasitic nodules failed to reveal any significant change, except an acute hyperaemia.

In other nodules caused by the parasite, it is interesting to note that histological examination revealed the cellular infiltration around the parasite was completely absent or nil, the parasite being enclosed in a very well developed and thick connective tissue capsule, the serosa being absent from such lesions. This may represent the stage wherein the zones of cellular infiltration had been replaced by connective tissue originating from the sub-serosa and loose connective folds of the omentum. The parasite itself showed varying degrees of degenerative changes, and even calcification at places. From the nature of the lesion it appears that the parasite on reaching the omentum had stimulated the sub-serous cells of the omentum to form a fibrous tissue capsule all around it and surrounding the lesion. Compared with the previous case, the zone of cellular infiltration around the parasite, the zone of epithelioid cells and the granulation tissue were completely absent.

- (b) *Late lesions*:—The late lesions were characterised by complete necrosis of the parasite contained inside the nodule. The dead parasite and the necrotic lesion associated with it took intense eosinophilic stain and revealed discrete areas of calcification. The lesion was found surrounded by immature fibrous connective tissue intermingled with a large number of mononuclear cells. Some of these nodules were entirely made up of frank connective tissue which is produced probably as a result of organization of the central necrotic mass with remnants of the degenerated parasite. The advanced lesions showed complete necrosis and calcification of the parasite, and were surrounded by a zone of lymphoid cells, epithelioid cells and a very large number of foreign body giant cells. These giant cells were either seen singly or arranged in multiple groups lineally along the borders of the dead parasite. These cells and the parasite were enclosed in a fibrous tissue capsule, infiltrated, at places, by mononuclear cells. These connective tissue cells themselves revealed mild degenerative changes.

Discussion

From the nature of the lesions and from the microscopical appearances, it appears, that the parasite under discussion is an intermediate stage of *Taenia hydatigena* whose development has been

arrested in the early part of life itself due to a mild torison of the growing parasite on itself which is insufficient to cause immediate death of the contained parasite, and thus acting as a foreign body. The histological structure of the nodules varied according to their age, time of their death and the stages of development from a mild inflammation with eosinophilic and round cell infiltration to complete hyaline necrosis of the central area of inflammation, the parasite undergoing degenerative changes starting from caeseation necrosis to calcification. When the parasite attaches itself to the omentum, it produces certain irritants in common with other parasites, which brings about migration of a large number of eosinophils around the parasite. Simultaneously the sub-serosa and the loose connective tissue folds which carry the blood vessels react to form a fibrous capsule around the parasite. In certain cases, the inflammatory cells are unable to dislodge and remove the parasite, and the resulting lesion contained epitheloid and giant cells around it. The histological appearance was suggestive of progressive inflammation terminating in a granulomatus structure, with central zone of necrosis or calcification which was usually surrounded by a thick belt of granulation tissue consisting of fibroblast, blood and lymph vessels, which appear to grow towards the central necrotic mass.

Summary

Histological changes, encountered in the omentum caused by the intermediate stages of the *Taenia hydatigena* in goats whose development has been arrested, are described.

Acknowledgment

Thanks are due to Shri P. G. Pande, M.Sc., M.R.C.V.S., Director, Indian Veterinary Research Institute, Izatnagar, for providing all laboratory facilities and to Dr. S. C. Dutt, Helminthologist of this Institute for the identification of the parasite.

LEGENDS TO ILLUSTRATIONS

1. Parasitic nodule showing the scolex of taenia surrounded by a zone of inflammatory cells, "epitheloid cells" and granulation tissue; Haematoxylin and eosin. $\times 500$.
2. Lesion showing zone of degenerating eosinophils and mono-nuclear cells, epitheloid cells, granulation tissue surrounded by serosa of omentum. H. and E. $\times 500$.
3. Calcaroid bodies of the taenia, Von Kossa $\times 750$.
4. Degenerated taenia with necrosis and early calcification surrounded by a dense zone of granulation tissue. H. and E. $\times 500$.
5. Granulomatous structure which had replaced the parasite. Foreign body giant cells can be identified in the lesion. H. and E. $\times 750$.

RHODODENDRON POISONING IN ANIMALS

By

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Introduction

There are a number of poisonous plants in India and our knowledge regarding poisoning of livestock by them is very limited. There is a large scope for research to be taken up about this vital problem of livestock industry. Each year a number of animals are lost due to plant poisoning, which remains unattended. It is therefore very necessary that attention of those engaged in research should be diverted towards this end so that complete knowledge about distribution, chemical composition, mode of action, etc., of such poisons is acquired. This can be done by investigation and then suggesting measures to prevent and lessen their deleterious effects.

An attempt is made here to point out the poisonous nature, symptoms of poisoning by *Rhododendron* which I came across during June 1960. People from Kapkote (3,500') side who had brought their buffaloes to higher elevations for grazing lost three buffaloes in the last week of June 1960 due to above mentioned plant.

General

In Kumaon hills according to Osmaston (1927) there are 4 or 5 varieties of *Rhododendron*. Some of them are a serious problem for shepherds and other livestock owners in the Himalayas. The *R. campanulatum* is particularly very poisonous to all classes of livestock although youngleaves of *R. arboreum* are also poisonous. These leaves are not ordinarily browsed by livestock but when the animals are introduced to new surroundings or face drought conditions then they do browse them invariably with fatal results.

Botanical Characters

Rhododendrons are shrubs or trees, often scaly or aromatic. Leaves are alternate, entire. Flowers fascicled or sub-corymbase, terminal, rarely solitary or axillary. Corolla tube long or short, stamens 5-18 usually 10, ovary usually 5 celled. Capsule more or less woody, seeds numerous.

Rhododendron arboreum:—(Locally known as *Burans* in Uttar Pradesh hills). A small tree. Bark pinkish brown, somewhat rough, exfoliating in thin flakes. Young shoots clothed with white scales. Leaves lanceolate or oblong narrowed at both ends, glabrous and glossy

green above, pale beneath. The mid rib and nerves are prominent beneath. Flowers deep red or pale pink, have sweetish sour taste.

R. barbaratum :—Flowers are deep red.

Rhododendron anthopogon :—Flowers very pale yellow almost translucent. Flowering takes place in June-July. The leaves turn purple brown above in autumn.

Rhododendron Campanulatum :—(Locally known as *Chimula*, *Chimul*, *semru*, *Chimru* in Uttar Pradesh (hills). An ever green shrub, stems spreading and nearly always procumbent towards the base. Bark smooth, pinkish brown, exfoliating in thin papery flakes, leaves—crowded towards the ends of the branches, elliptic or elliptic oblong rounded at both ends, mucronate, glabrous and glossy dark green above, clothed beneath with dense buff or cinnamon coloured tomentum which obscures the nerves, mid rib prominent beneath. Flower tinged mauve or lilac and spotted inside anteriorly with pinkish purple. Corolla campanulate Flowering in May-June. Fruit in October—November.

Distribution

R. arboreum occurs between 4000—11,000' above sea level in Oak and Kharsu forests. *R. anthopogon* occurs between 10,000—14,000' and *R. campanulatum* between 9,500—14,000, in Himalayas.

Constituents, toxicity and uses

They have not been properly isolated and assessed but it is stated that *R. arboreum* contains glucoside ericolin. The honey collected at its flowering time is believed to be poisonous. The flowers are sometimes eaten and are known to cause intoxication if used in large quantities.

R. barbaratum : is known to contain andromedo-toxin.

R. campanulatum :—The most poisonous of all species is stated to contain and romedo-toxin which belongs to Veratrine group of substances and paralyses the motor nerve endings of striped muscles. It has narcotic effect and the death seems to be due to respiratory failure. The wood makes a good fuel but the smoke is irritant and acrid. The leaves of this plant mixed with tobacco are used as remedy for rheumatism, sciatica etc., by local people.

Symptoms

The mortality in buffaloes occurred after browsing the *R. campanulatum* leaves which are in abundance in Dhakuri (about 11,000') ridge area when the animals were introduced from lower altitudes (3,500') during June 1960. The buffaloes were lost within 1-2 days after onset of symptoms. The animals manifested intense abdominal pain. There was severe constipation followed by severe

diarrhoea and straining. Grinding of teeth was also noticed, salivation was copious, trembling was noticed from the very beginning. The animals gradually got exhausted and died.

The postmortem could not be conducted as the carcasses had been thrown by villagers before anything towards that end could be done.

Treatment suggested

Cases of poisoning occur in remote places where practically no veterinary aid is available at present. So far no particular attempts have been made in this respect but according to Chopra *et al* (1949) oleginous purgatives may be of some use. According to Newsom (1958) oleum lini may be beneficial. It has been found useful in America.

Summary and discussion

Rhododendron poisoning in buffaloes is recorded. The plant is also known to be poisonous to sheep and goats, who usually migrate to upper hills in summer but the author has not seen any case of poisoning by this plant in them so far. Attempts to treat the poisoned animals are of very little use and villagers also do not believe in treatment. The plants paralyse the motor nerve endings and induce narcosis.

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EFFECTS OF IONIZING RADIATION ON FERTILITY

By

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Atomic energy holds great promise for a better and happier life for this and the succeeding generations. The numerous applications of atomic energy in different branches of science and technology, have been reviewed by Maqsood (1957a). Further, Maqsood (1957b) has also discussed the role of atomic energy, in solving the present food problems of the world especially in the under developed and over populated

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countries. On the other hand during the process of disintegration, radioisotopes give off radiation and the same radiations, which make radioisotopes as one of the most useful tools in the study of Science and Technology, can also make them hazardous to handle. However the radiation problems can be eliminated or minimized to a point where the materials can be handled by proper shielding and instrumentation. Maqsood (1957c) has outlined the biological effects of ionizing radiation on various tissues and organs in different species of animals. The effects of radiation may be local or general depending upon the exposure time and degree of radiation. The physicists consider that 1/100 to 1/10 part of the molecules of a cell must be affected before a change in a cell becomes visible. There is no minimum amount of radiation which may be exceeded before mutation occurs. Any amount, however small, that reaches the reproductive cells can cause a correspondingly small number of mutations and the more the radiation, the more the mutations. In brief, the harm is cumulative and the genetic damage done by radiation builds up as the radiation is received and depends upon the total accumulated reproductive organs dose received by people from their own conception to the conception of their last child. Despite the existing gaps in our knowledge, it is clear that radiation is by far the best understood environmental hazard and in this article, the available literature on the effects of radiation on gonads and fertility in man and different species of animals is broadly reviewed to serve as a basis for subsequent intensive studies.

II Male

(a) *Spermatogenesis*:—In the male, the spermatogonia are the most sensitive cells to the effects of radiation. Inhibition of mitosis and destruction of spermatogonia, and differentiation of these cells following irradiation contribute to their disappearance from the seminiferous tubules. Following reduction or depletion of spermatogonia, the later germ cell generations undergo maturation-depletion and disappear in the order in which they are formed until a point of maximum hypoplasia is reached. The time for the development of maximum hypoplasia of the seminiferous epithelium is about 3–4 weeks, depending upon the species. This time appears to be close to that required for the development of a spermatozoan from a spermatogonium.

The testicular effects of irradiation are qualitatively similar in all mammals including man but vary quantitatively according to differences in testicular radiosensitivity and recovery capacities among species. The effect of irradiations on seminiferous epithelium is direct in that irradiation of the body with testes shielded does not produce them. When spermatogenesis is partially arrested the number of sperm produced decreases and the percentages of sperm motile, alive, and normal tend to

decrease also. Sertoli and interstitial cells are relatively radioresistant. In the case of cells having the capacity both for division and differentiation, irradiation tends to diminish the number of resting cells and dividing cells and by inhibition of division to increase the number of differentiation cells. Although cases of testicular atrophy in humans following irradiation have been observed since 1904 and were commonly observed soon after the Hiroshima and Nagasaki bombings during World War II, little is known at present of the ultimate fate of the lesions produced in survivors and the effects of the lesions on fertility. There are reports of azoospermia or oligonecrospermia or sterility in radiologists. The available data show that dog is the animal most similar to humans in terms of radiosensitivity and regenerative capacity of seminiferous epithelium. In general, both dog and man reveal similar sensitivity which is greater than that in other experimental animals. In the case of dog the effects of different doses of radiation are shown in Table I.

TABLE I
Effects on Fertility of chronic exposure to x-ray of dogs.

Dose/week (r)	Total Dose (r)	Duration of exposure (yrs)	Observations
0.6	124	4	No significant change in sperm count.
0.6	62	2	Little change in germinal epithelium.
3.0	156	1	80% sterile, 20% reduced sperm counts.
3.0	312	2	Atrophy of germinal epithelium.
6.0	312	1	Aspermic
6.0	624	2	Marked atrophy of germinal epithelium.
15.4	477	0.57	Aspermic
15.4	634	0.75	Aspermic

(b) *Onset of Radiation effects*:—Following single dose of irradiation and proceeding with the sterile or subfertile period produced there is a period of fertility, the length of which is much less dependent on low doses than on doses high enough to effect the fertilizing capacity of mature sperm. The first period of continued fertility is due largely to sperm maturity at the time of irradiation and possibly to some sperm in the spermatid stage or even a few in the spermatocyte stage, depending upon the dose and the length of the fertile period. The subsequent period of infertility or sterility is due to decreased numbers of sperm produced, and the fertile period following the period of sterility, if recovery occurs, is due to sperm that developed from cells which were in the spermatogo-

nial stage or were primordial undifferentiated cells at the time of irradiation.

In regard to the efficiency of fractionated versus undivided doses of the same total size in producing testicular effects, there are experimental reports indicating no differences, others indicating less effect with fractionation, and others showing greater effects with fractionation. The effects of fractionation of dose on the testes depends upon the size of the dose fraction, the interval of time between fractions and the total dose. In general, fractionation has less influence on the effect of small total doses than on the effect of large total doses.

(c) *Regeneration after exposure*:—The degree of regeneration, depends upon the dose and duration of radiation, age and species of the animal. In some species of animals active regeneration may be delayed following severe radiation effects much longer than 10 months. For relatively low doses and for certain laboratory animals whose germinal recovery capacity is relatively large, regeneration if it is to occur, begins before the height of depopulation of germinal epithelium is reached or soon thereafter.

III Female

In the female irradiation of the mammalian ovary can cause profound atrophy of the organ with temporary or permanent sterility depending upon the dose and in most mammals, changes in the ovaries may be followed by dependent atrophic changes in accessory genitalia. The ova and follicular cells are the most radiosensitive cells in the mammalian ovary and the cells of the corpora lutea and interstitial cells are relatively radioresistant. The radiosensitivity of the ova and follicular cells varies with their functional states at the time of irradiation. In most laboratory mammals the developing and mature follicles and ova appear to be more radiosensitive than the primordial follicles and oocytes and some primary follicles persist after fairly large doses of radiation and may begin to develop long after irradiation. A dose of radiation which destroys all developing follicles causes failure of development of corpora lutea which may lead to decrease of interstitial gland cells in animals which have these glands, since new cells will fail to be developed from corpora lutea.

It is of interest to mention here that total body irradiation appears to produce greater effects on the ovary and on fertility in female animals than irradiation of ovaries alone with equivalent doses. Irradiation may sterilize the ovary by preventing the development of primary follicles of the ovary and by destroying the ova and follicular cells. Table II shows the effect of single dose irradiation of ovaries on fertility in women.

TABLE II

Effects on Fertility of irradiation of ovaries of women.

Dose (r)	Observation
125-150	Amenorrhea in 50% cases
170	Temporary sterility for 1-3 yrs
500	Permanent sterility in most women
500-624	Permanent sterility 94%
625	Permanent sterility 100%

IV. Embryos and Foetuses

In experimental animals irradiation of the embryos produce cerebral agenesis of malformation. Further exposure of embryos or foetuses to x-ray therapy induces necrotic changes in primitive differentiating non-mitotic cells of the nervous system at a dose level as low as 30-40r. Malformations develop readily after exposure to 100-200r and its nature at a given dose can be determined by the stage of development of the embryo and the capacity of the embryo to recover at that stage. In the first somatic stage, radiation induces severe head and brain defects and later on brain, eye, skeletal and visceral abnormalities. In humans, a variety of defects from microcephaly to eye and skeletal deformities have been observed in newborns following pelvic irradiation during early gestation.

In rats and mice, the embryo is usually resistant to doses up to 400r for the first eight days of its life. When its neural folds and head process begin to form about the later part of the 9th day, its sensitivity increases nearly tenfold, and throughout gestation malformations can be induced. Lower doses of radiation produce lesser defects, greater doses magnify the malformations. Many monstrosities cannot live after birth, while still others, especially those with deficient brains survive into adult life.

V Reduction in litter size (Semisterility)

Irradiation causes a reduction in litter size in different species of animals and the amount of reduction is dependent upon the period of exposure, dose and duration of irradiation. Reduction in litter size is explained on the basis of induction in the sperm and perhaps to some extent in precursors of the sperm, of chromosomal aberrations which

do not interfere with fertilization but which causes death of a zygote or embryo *in utero*. Chronic irradiation at low daily dose rates appears to be much less effective in the production of semisterility than large dose single irradiation. In man, irradiation causes an increase in the incidence of spontaneous abortion following death of embryos or foetuses in the uterus. The size of litter produced in the initial fertile period after irradiation of female animals is reduced and the size of the litter from irradiated females declines more rapidly with rising dose than the size of the litters from irradiated male mice.

VI Sterility doses for Humans

Whether a dose of radiation sterilizes permanently or temporarily depends at least as much on the natural capacity for regeneration of primitive germ cells as on the radiosensitivity of the germ cells existing at the time of irradiation. In the male, Sertoli and interstitial cells are relatively radioresistant, and as such male mammals can be sterilized permanently without prominent histological changes in the interstitial cells and as such without decrease in sexual desire. In the case of female, changes in the ovaries may be followed by dependent atrophic changes in the accessory genitalia in most mammals. Chronic irradiation is relatively of more serious consequence and it is such exposure which may constitute the greatest practical human hazard as far as sterility is concerned.

It seems quite possible that the single doses necessary to cause permanent sterility in 100 percent of men and women may not be far apart. Both male and female humans are among the most radiosensitive of those mammals studied, with respect to gonadal effects of irradiation. A single gonadal dose of 500 to 625r x- and gamma-radiation would permanently sterilize most human males and females. It may be of some interest to workers connected with population problems that sterilization of humans by radiation can be considered as one of the numerous means for controlling population growth under certain conditions.

Summary

The spermatogonia and sperm in the male and follicular cells and ova in the female, are the most radiosensitive cells in different species of animals. A single gonadal dose of 500 to 625r x- and gamma-radiation would permanently sterilize most human male and females. The degree of regeneration in the case of low doses of radiation, depends upon the dose and duration of radiation, age and species of animals. Irradiation of the embryos produces cerebral agenesis of malformation, and also reduces the litter size in animals. In man, irradiation causes an increase in the incidence of spontaneous abortion following death

of embryos or foetuses in the uterus. Chronic irradiation is relatively of more serious consequence and it is such exposure which may constitute the greatest practical human hazard as far as sterility is concerned.

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Clinical Article

STAPHYLOTOMY IN A DACHSHUND

By

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Congenital elongation of the soft Palate in canines was first successfully resected by Farquharson and Smith (1942) and by Schnelle in (1942). According to Munson (1957) the condition is congenital in origin in Brachiocephalic breeds, Bull, Pug and Boston terrier are prone to it. Elongation due to other causes has not so far been reported.

In a normal dog the posterior border of the soft palate comes in contact with the epiglottis. In elongation or hypertrophy the normal air passage is blocked, thus interfering with respiration. This condition is manifested by stertorous breathing, snoring, short breath on exercise and frequently by reflex emesis. Occasional dry cough due to mechanical irritation may be present which worsens with exercise. In advanced cases, cyanosis and syncope may follow severe exercise.

In such cases the resection of the elongated portion of the soft palate brings about immediate and marked relief.

Case Report:—On 16-9-60 a Dachshund bitch aged about nine years was brought to the hospital with the history of chronic intermittent cough for six months and admitted as an In-Patient. The temperature was within the normal range and even on slight exercise symptoms of air-hunger were noticed. The lungs showed no abnormality. Auscultation of the heart revealed cardiac dilatation with normal rhythm. Radiography confirmed the finding. The cardiac dilatation was suspected to be due to the longstanding cough and accentuated by age. Faeces and blood smears revealed no abnormality.

The blood picture was :

R. B. C.	...	8.7 Mill/cmm.
W. B. C.	...	12,400/cmm.
Hb.	...	14 gms per 100 cc.

Differential count :

Neutrophiles	...	75%
Lymphocytes	...	22%
Monocytes	...	1%
Eosinophiles	...	2%

The animal was also having multiple mammary tumours with an enlarged axillary lymph gland. To eliminate metastasis, radiography of the chest and digestive tract were done with negative results. Examination of the throat revealed elongation of the soft palate. In addition bilateral tonsillitis without hypertrophy was noticed. No clinical improvement with medicinal treatment was observed. Hence it was decided to resect the hypertrophied soft palate. The operation "Staphylootomy" was performed on 24-9-60.

Anaesthesia

Atropine sulph was given sub-cutaneously thirty minutes prior to the operation. 3cc. "Napentol" intravenously, produced the required anaesthesia. McGill's endotracheal tube was introduced next and retained in position.

Technique

The animal was fixed in the dorso-recumbant position in an inclined plane with the head at a lower level. The mouth was kept open with a tooth speculum and the tongue was extended to get a good view of the soft palate and adjacent structures. The pharynx and the surrounding area were swabbed well with sterile saline to remove the mucus. The free end of the soft palate was gripped and held with

a pair of Allis tissue forceps and drawn cranially. A pair of curved haemostats was used below the point of removal for perfect haemostasis and two elliptical incisions were made below the clamp with the maximum depth at the centre. The tissue within this area was extirpated. The cut edges were opposed with a row of continuous suture with "OO" chromic catgut so that the pharyngeal and oral mucous surfaces may heal together with a minimum of bleeding.

The clamp was removed and perfect haemostasis was observed. The endotracheal tube was retained till the swallowing reflexes were noticed. The animal recovered from anaesthesia in twenty minutes after the operation. The anaesthesia lasted for a total period of 45 minutes.

After Treatment

The animal made an uneventful recovery without after treatment. No cough was observed from the very next day even with exercise. On 12-10-60 the dog was discharged completely cured.

Discussion

The uvula is not a prominent structure in animals as in man. However the muscle uvula even though slender is better developed in the canine species than in any other animal species. The thin uvula muscles of either side fuse at the free border of the soft palate. In a section, as described by Bradley (1948) the soft palate reveals from before backwards:—

- (1) Mucous Membrane.
- (2) Layer of palatine glands, thickest at the free border.
- (3) Aponeurotic and muscular layer containing pharyngeo-palatine Muscles of the uvula.
- and (4) Mucous membrane of the Nasal cavity.

In congenital cases of elongation of the soft palate the symptoms ought to be manifested before the third year. In the present case, a nine year old dog did not have a history of the condition in youth. The elongation could therefore have taken place only during the later years due to some specific cause. The histopathological study of the excised tissue revealed "degenerative changes in the mucous glands in the lamina propria". This change is possible in senility. Infection does not suggest itself as a cause since general inflammatory changes barring slight tonsillitis were absent and further the blood picture itself was normal. The elongation and hypertrophy can therefore be attributed only to the senile changes. This conclusion is further strengthened by the elimination of diseases as spirocercosis, bronchitis, malignant growths in the thoracic cavity, tonsillitis and by the spontaneous relief obtained by the surgical treatment.

Summary

(1) A case of hypertrophied soft palate is reported in a aged Dachshund.

(2) The operative treatment is indicated in detail.

(3) The condition is discussed completely.

Acknowledgment

We thank the Principal, Madras Veterinary College for all the facilities afforded and the Departments of Pathology and Physiology for their kind co-operation.

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Abstracts

Control of Cattle Grubs or ox Warbles.—By RADHEY MOHAN SHARMA and RAJ PAL VIG (1959). *The Indian Veterinarian*, 2. 135-138.

The authors describe the results of successful trials in the effective control of warbles in cattle, with Neguvon—a Bayer's systemic organic phosphate insecticide. In the first trial 12 heifers were given *per os* at the rate of 75 mgm./kgm. body weight and the treatment was repeated twice at four weeks interval. There was 90-13% reduction in the number of warbles in the treated animals. In the second trial, 12 cows were dressed with 2% Neguvon aqueous solution 3 times at 4 weekly intervals. This mode of treatment also resulted in 97-7% reduction in the number of grubs.

V. S. A.

Cytoecetes ovis vari Deccan (N. Sp.) As the cause of tick-borne Fever in sheep in India.—By RAGHAVACHARI, K. and MADHAVA KRISHNA REDDY, A. (1959). *Indian J. Vet. Sci.*, 29. 69-86.

The authors encountered in the blood smears of apparently healthy sheep in Hyderabad (Andhra Pradesh) *Cytoecetes ovis vari deccan* infecting the leucocytes of granulocyte series-neutrophiles, eosinophiles

and basophiles. With Romanowsky stains, the organism appeared as lightly stained minute cytoplasmic inclusions in the form of delicate rings, solid coccoid elements and aggregations of chromatin stained elements of a variety of shape, size, number and construction. Two modes of development of the parasite were observed. In one, the process of multiplication was both by binary and multiple fission of the coccoid body, while in the other it was strictly by binary division. Bodies similar to those found in sheep were found in 60% of *Rhipicephalus haemophysaloides* recovered from infected animals in tissues connected with the intestines and salivary glands, thus indicating this species of the tick as an efficient vector in nature.

The parasite appeared in the blood of sheep on subinoculation of the infected whole blood or plasma or saline suspension of tick. Goats and cattle were found susceptible to the infection, while the dog, rabbits and guinea-pigs proved refractory. Natural infection in goats was also detected. The organism appeared in the peripheral blood after sub-inoculation of infected blood or plasma either subcutaneously or intravenously in 4 or 7 days respectively, while it took 14 days for the parasites to appear on subcutaneous injection of tick emulsion. The organism persisted in the peripheral blood for 6 days. Thereafter the blood was found infective up to 4 days even though no parasite could be detected in the blood smear.

Blood changes as marked increase in the number of neutrophiles initially and a similar increase of lymphocytes in the declining phase, rise of morning body temperature to 105-106° F for 2-3 days, mucopurulent discharge from eyes and nostrils, slight swelling of superficial lymph glands, diarrhoea and progressive emaciation were the symptoms noticed in the affected sheep during later passages only. No mortality occurred among the diseased ones. Animals which had recovered from a primary infection where susceptible to a subsequent infection and after the second attack, the sheep developed immunity to subsequent infections.

V. S. A.

News and Views

The British Veterinary Journal has virtually brought out a Tuberculosis Number in its October 1960 issue. The articles are mostly contributed by the staff of the Animal Health Division of the Ministry of Agriculture, Fisheries and Food. They cover practically the entire field connected with this disease and must serve as a standard reference and guide whenever required. How far yet India has to go in

such matters! We commend the perusal of this Number by all members of our profession.

* * * *

World Conference on Animal Nutrition:—Over fifty scientists from fifteen countries of Western Europe, the United States and the Near East delivered papers reporting on the latest methods of increasing production of cattle, swine, sheep, poultry fish and milk at the Second International Symposium on Animal Nutrition held recently at Madrid. The symposium was sponsored jointly by the Soybean Council of America and the U. S. Feed Grain Council in conjunction with the Foreign Agricultural Service of the U. S. Department of Agriculture.

—Globe.

* * * *

Political Heads and Education:—Sir A. Ramaswamy Mudaliar delivering the convocation address at the Karnataka University on 24-11-60 said "Nothing could be more disastrous to the development of sound higher education than the ill-advised interference of the political heads in purely academic matters". The subject of education is made a pawn on the chess-board of political parties. The Universities should set their face against all such attempts.

* * * *

Artificial Heart:—Moscow, Nov. 13: An artificial chicken's heart, which functions like a real one, has been produced in a laboratory by a Russian Scientist, *Tass* reported yesterday.

The report said the man responsible for this feat was Prof. Stundnitski of the Soviet Academy of Sciences. His model consists of pieces of real chickens' hearts grafted on to the embryonic envelope of another, and its successful functioning proved that complicated organs such as the heart had the same capacity for biological regeneration as other tissues, the report claimed.—*AFP*.

* * * *

Artificial Kidney:—Bombay, Nov. 23: An artificial kidney, the first ever to be installed in Bombay, arrived here yesterday aboard Swedish ship from Sweden.

The mechanical kidney which is to be installed in the K. E. M. Hospital, is a gift of a Swedish industrialist and shipowner.

A specialist of a medical college in Bombay, who was selected to undergo training in Sweden in the use of the mechanical kidney is expected to return shortly.—*PTI*

* * * *

Misfits:—An agriculturist writes in the Madras Mail:—"Will an experienced compounder or nurse be appointed as a doctor, will a

skilled mason be appointed as an Engineer, will a clerk of 20 years service be appointed as an I. A. S. officer because of his service? But this is happening in the Madras Agricultural Department. Fieldmen, with basic qualifications of Third Form or S. S. L. C. or Intermediate recruited by departmental officers some years back are being appointed as Agriculture Demonstrators, Research Assistants and Farm Managers. These appointments, are made *en masse* without individual merits being considered.

Will not this sort of thing spoil the morale of qualified workers in the department? Food cannot be produced overnight and agriculture cannot be improved or developed overnight by implementing big schemes and that too with unqualified personnel. Let us go slow and steady and win the race of food production".

We learn that such a procedure is adopted as a general rule in the newly formed State of Gujarat and not as an exception. We await further details on this matter. In the mean-while we appeal to the Government of Gujarat not to lower the standard required for advancement of science and for the prosperity of the nation.

Association News

The Indian Veterinary Association

THE RESERVE FUND

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

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

Some enquiries have come to us asking us to indicate the extent of help expected. We do not want to put the members of the profession to any undue financial strain in these hard days. At the same time, a certain amount of sacrifice is needed to make this fund a great success,

so as to make it worthy of our profession. So we feel that the following scale of contribution will be quite reasonable. It could even be paid in two or three instalments.

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

It is our ambition that no Veterinary Surgeon in this land should remain without contributing to this fund. Brick by brick, this great edifice of our's should begin to rise higher and higher. On the success or otherwise of this venture, the solidarity of our profession depends. "Pay and Prosper" is the slogan we pass on to the members of our profession.

Jai Hind!

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

THE I. V. J. RESERVE FUND

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Dr. W. F. Guard, Columbia, U. S. A. \$ 25.00	
" R. P. Krishna Iyer, Badagara, Kerala ...	10.00
" M. R. Dave, Sanand, Gujarat 1st instal. ...	5.00
" N. Satapathy, University of Maryland, U. S. A. \$ 5.00	
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The Year 1960 is coming to a close. We appeal to our subscribers to clear their arrears before the year is out. Subscription is strictly payable in advance and the amount is to be paid "net" without deducting the M.O. commission.

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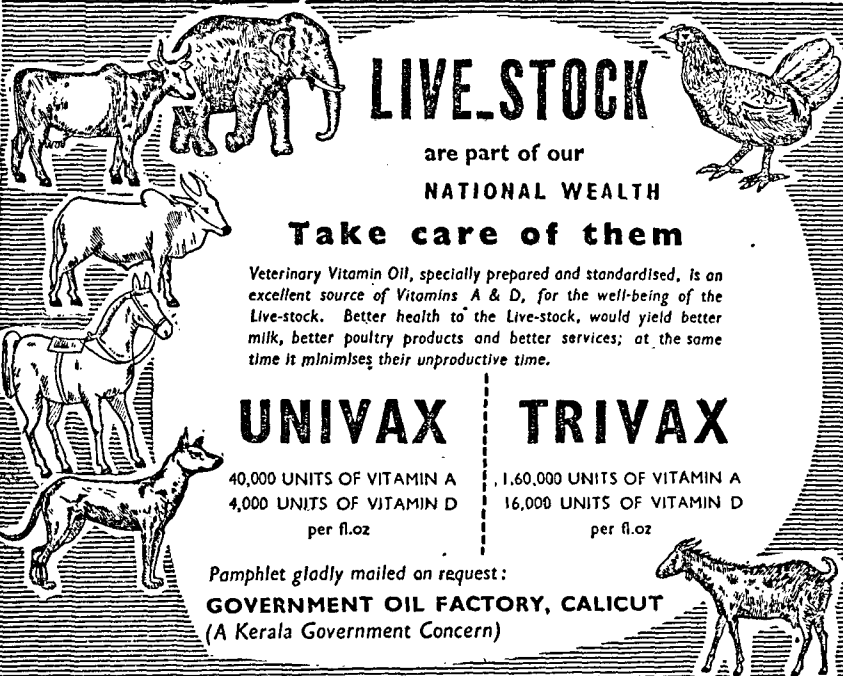
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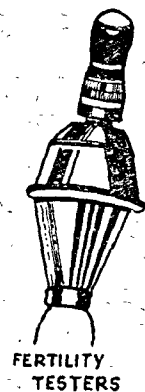
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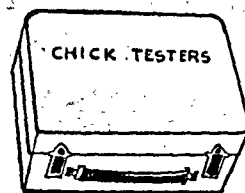
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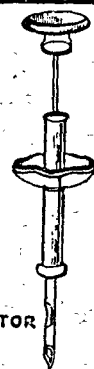
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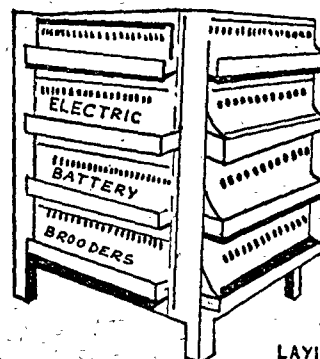
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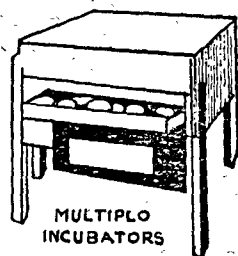
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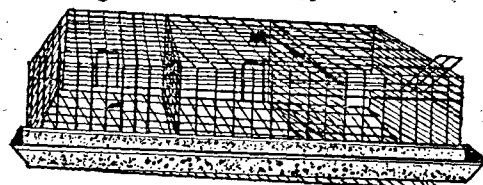
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