

429

The Indian Veterinary
Journal

1942 - November

VOL. 19 NO. 3.

JL
KX m2, N24
N42.19.3
79804

THE ⁴⁷²
INDIAN VETERINARY JOURNAL

Published Bi-monthly

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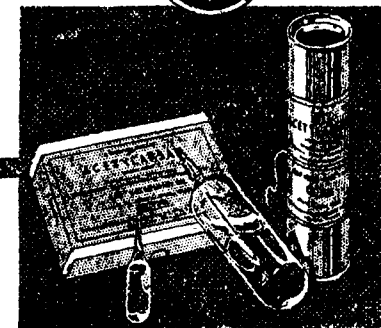
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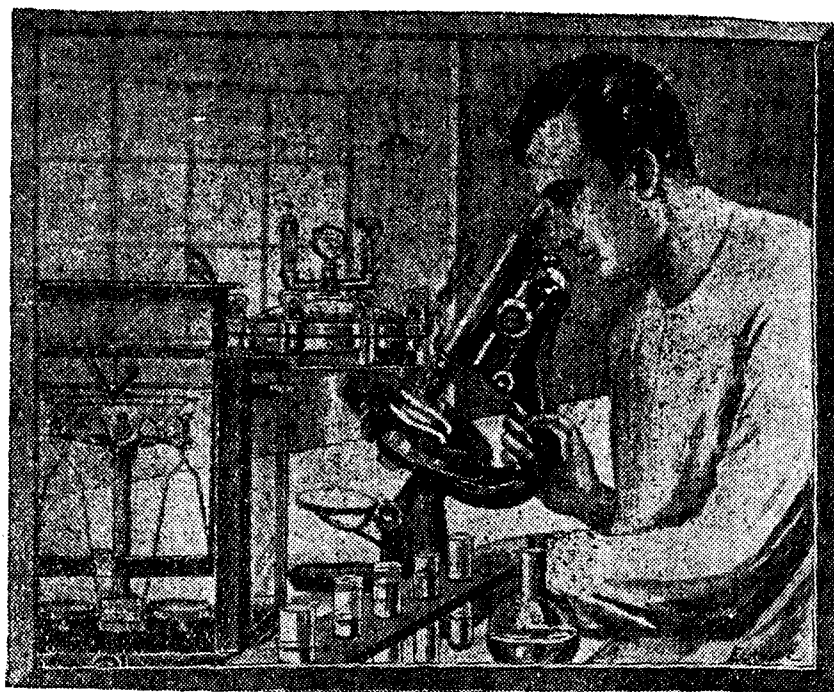
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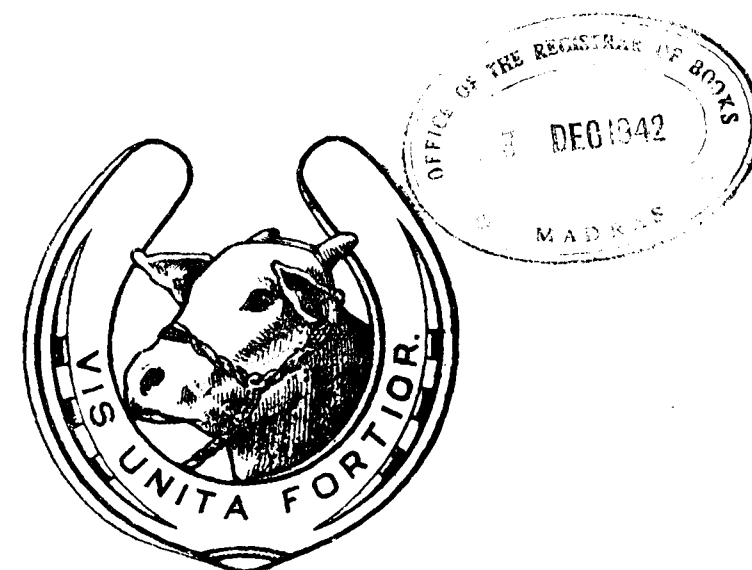
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Published Bi-monthly
 Vol. XIX

November 1942
 No. 3

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



Editor :

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Office : 26, WALLAJAH ROAD, MOUNT ROAD, MADRAS (S. INDIA)

PRINTED AT
 CENTRAL ART PRESS, WALLAJAH ROAD, MOUNT ROAD, MADRAS
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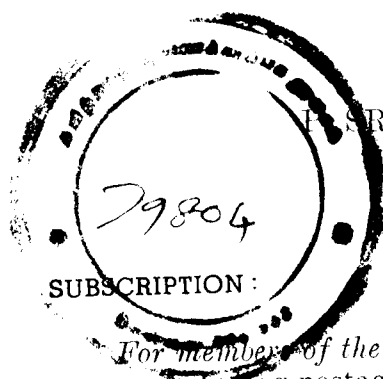
The Indian Veterinary Journal

A Bi-monthly Record of Veterinary Medicine and Surgery
Devoted to the Cause of Veterinary Profession

PUBLISHED FOR THE ALL-INDIA VETERINARY ASSOCIATION

EDITOR:

H. SRINIVASA RAO, G.M.V.C.



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THE Indian Veterinary Journal

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CONTENTS

General Articles	PAGE
Methods for the Local Application of Sulphanilamide.—By F. Hawking, D.M. ...	111
The Common Sulphonamides : Nomenclature, Structure and Uses.—By D. G. Ardley, M.P.S. ...	116
Agriostomum Vryburgi Railliet, 1902.—By S. Vaidyanatha Mudaliar, G.M.V.C. ...	125
The Clinical and Microscopical Diagnosis of Rabies.—By A. V. De Croos, G.V.Sc. ...	128
Pregnancy Diagnosis in Equines.—By P. R. Krishna Iyer, G.M.V.C. ...	132
Editorial	
The Punjab Veterinary College ...	139
Provincial Veterinary Conference, Madras ...	141
Clinical Cases	
Bovine Piroplasmosis in Coonoor and Surroundings of the Nilgiris District.—By S. Ramanarayanan, G.M.V.C. ...	143
A Peculiar Case of spotted condition of the skin in a Buffalo.—By K. S. Patnaick ...	147
Sulfarsenol in the Treatment of Nervous Localisations of Distemper in Dogs.—By P. Abraham, G.M.V.C. ...	148
A Strange Case of Rabies in a Dog.—By S. Ramanarayanan, G.M.V.C. ...	150
A Successful Case of Ruminotomy in a Bullock.—By N. Subramanyan, G.M.V.C. ...	151
Hepatozoon Canis and its Treatment.—By Muhammad Rahimuddin, G.M.V.C. ...	153
Pus in the Guttural Pouch—Treatment with Sulphanilamide.—By V. Venkataraman, G.M.V.C. ...	155
Correspondence	
Rupture of the Bladder in a Bull-calf.—By S. Ramaswamy, G.M.V.C. ...	156
—Reply from V. V. Venkatachala Iyer, G.M.V.C. ...	158
A Note of Corroboration on the article "Care of Horns."—By M. Ramakrishna Pillai, G.M.V.C. ...	159
College News	
Madras Veterinary College—Panikkar Memorial Fund—List of Subscribers from 1-9-42 to 3-11-42 ...	163
Abstracts	
A study of the possible relationship between non-specific mastitis and streptococci infection of the bovine udder ...	164
A filtrable virus isolated from mastitis cows ...	165
Obituary	
G. Avudanayagam, G.M.V.C. ...	166
Rao Sahib C. Suryanarayana Murti, G.M.V.C. ...	166

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General Articles ⁴²⁹

METHODS FOR THE LOCAL APPLICATION OF SULPHANILAMIDE *

BY

F. HAWKING, D.M.,

(From the British Medical Journal, Vol. II, 15th November 1941, p. 685).

Earlier papers have described the anti-bacterial effects of local applications of the sulphonamides to experimental wounds infected with the organisms of gas gangrene, and the concentrations in the blood and in the tissues around the wound resulting from such local applications. During this experimental work various practical methods have been devised, and these are here described in the hope that they may be helpful to those employing local applications of sulphonamide compounds to human lesions. For the sake of conciseness most references to other workers have been omitted. The compounds used have been either sulphanilamide or sulphathiazole.

Powder

In shallow open wounds this is the simplest form in which to apply the substance. It can be distributed by means of a spoon, a pepper-pot or an insufflator. Whichever method is used, enough powder should be applied to cover the whole surface liberally, and yet not enough to form large concretions, which take a very long time to dissolve. Gently massaging the powder into the tissues with a spatula will help to get it effectively distributed and dissolved. For use in the operating theatre on fresh wounds it is convenient to have a supply of the powder in 5-gramme

* "The Indian Medical Gazette," July 1942.

lots, put up either in paper packets or in screw-capped bottles. In order to obviate the very slight risk of fortuitous contamination of the powder, during its manufacture or afterwards by tetanus or other spores of pathogenic bacteria, it can be sterilized in such packets or bottles by dry heat at 150°C. for one hour. (If sterilised in the autoclave with the cap unscrewed the powder is apt to become crystalline and is no longer suitable for use in an insufflator). For routine ward work the use of unsterilized powder would seem to be justifiable.

Gauze

In narrow deep sinuses which are packed at regular intervals with ribbon gauze the powder can be inserted into the depths on the packing. This can be done most simply by pouring the powder on to the gauze as it is pushed in, but a better way is to incorporate the powder on to the gauze beforehand. For this purpose small glass pots with a metal screw cap, such as are used for ointments, are taken. A wide slot is cut in the lid. The pot and the gauze are sterilised separately. The gauze is then passed through a thick paste of sulphanilamide and water under sterile precautions and is placed in the pot, the end of the ribbon protruding through the slot. The top of the pot is covered with a piece of sterile oiled silk. For use in the wards the ribbon is pulled out as required. If the gauze is autoclaved after smearing with the paste most of the sulphanilamide forms powdery crystals, which cease to adhere to the ribbon.

Tablets

It is difficult to transfer powder to the bottom of a deep sinus. The best method in these cases is the use of small tablets made of the compressed compound—*e.g.*, tablets containing about 0.2 gramme of material and measuring about 8 mm. across. Such tablets were first suggested by Dr. A. S. Parkes; they were kindly prepared by the British Drug Houses, Ltd., and May and Baker Ltd. The tablets can easily be inserted into the desired position by sinus forceps, and they are then crushed to hasten disintegration; eventually they are completely absorbed and leave no residue which might act as a foreign body. With sulphanilamide, which is fairly soluble, tablets of the pure compound will probably be dissolved in a reasonable time, although it is advantageous to crush them in order to accelerate the process. With sulphathiazole, tablets of the pure compound (uncrushed) were found to persist in a sinus for over two days without much visible change. It is better to use tablets consisting of 0.1 gramme of sulphathiazole and 0.1 gramme of glucose, the glucose being added in order to promote the disintegration of the tablet; when these are crushed after insertion most of the material is dissolved in twenty-four hours. Tablets could also be used in a wound when it was desired to secure a steady supply

of sulphonamide over a prolonged period; by choosing tablets of an appropriate size the duration of action could be varied as required. The surface of the tablets can be sterilized by dry heat at 85°C. for one hour; heating to 115°C. causes the tablets which contain glucose to turn brown. This degree of sterilization will not kill spores, but the position is the same as that described for the powder.

Pastes

The results described in this section from the preliminary part of a wider programme, which will be described in more detail when it has been completed. Briefly, the ideal sulphanilamide paste for application to wounds, burns, etc., should have the following properties:—

1. It should be satisfactory pharmaceutically—*i.e.*, stable, non-odorous, non-rancid, etc.
2. Its physical consistency should be suitable for the required purpose; burnt surfaces might require a thinner preparation than that used for plugging wounds.
3. It should comprise only materials which are available under present war conditions. Thus, since cod-liver oil, which forms the base of two of the pastes described below, is now in short supply, a search for a suitable substitute forms part of the wider programme now in progress.
4. Sulphanilamide should be liberated from the paste at the optimum rate. Presumably the concentration of sulphanilamide in the fluid in contact with the paste should be as near saturation as possible, provided that:
 - (a) The concentration is not so high as to injure growing tissues or to paralyse leucocytes. Jacoby, Medawar, and Wilmer found that fibroblasts and macrophages are killed by a saturated solution of sulphanilamide (concentration 1 : 60), are temporarily depressed by concentrations of 1 : 150, and are not affected at all by concentrations of less than 1 : 1000. According to these findings, it might be advantageous if the paste exerted a slight restraint on the rate of solution of the sulphanilamide.
 - (b) The amount absorbed is not so great as to produce a toxic concentration in the blood; probably this will not occur except when the areas to be dressed are large; it can be decided only by blood estimations of treated patients. (In a private communication Colebrook reports that one patient who was treated by sulphanilamide paste for a very extensive burn became cyanotic, and the concentration of compound in the blood was 8 mg. per 100 ml.)
 The whole question is under investigation.

It may here be noted that mixtures of sulphanilamide and liquid paraffin appear to be in use in some hospitals. According to experiments in this laboratory sulphanilamide is insoluble in paraffin and cannot diffuse

across it. In mixtures of paraffin and sulphanilamide however, the particles of sulphanilamide usually sink to the bottom of the paraffin layer until they come into contact with the underlying watery fluids, in which they dissolve. Hence, if sulphanilamide suspended in paraffin is injected into rabbits subcutaneously it is absorbed almost as quickly as if it were suspended in saline. This occurs because the mixture is unstable and in the body it separates into its component parts; if this separation were prevented—for example, by using petroleum jelly, little or no absorption would presumably take place.

5. The paste should be non-irritant to the tissues and innocuous to leucocytes. It should not delay healing; rather, if possible, it should stimulate it.

The complete solution of all these problems will take a considerable time; and meanwhile casualties occur which require treatment. Therefore this short description is given of the best pastes which have been found to date so that they may be available in hospitals, and clinical experience may be gained which may help to orientate the further researches in hand. For the pharmaceutical side of the present work I am indebted to the British Drug Houses Ltd, who kindly co-operated with their experience in these matters and devised pastes of different properties for my trial. The physiological action of these pastes was tested by injecting them between the muscles of rabbits and into the peritoneum of rats; the irritation which they caused was not appreciably greater than would have been produced by any foreign body in these positions. Three pastes are provisionally recommended to suit three different types of lesion:—

1. *Sulphanilamide*, 30 per cent.; *oleic acid*, 1.4 per cent.; *triethanolamine*, 0.35 per cent.; *cetyl alcohol* and *beeswax*, each 1.4 per cent.; *water* to 100 per cent.—This is a thin paste to spread over inflamed skin surfaces, e.g., impetigo—in order to apply sulphanilamide with as little other material as possible. It can be used in granulation tissue instead of powdered sulphanilamide, but if inserted into deep wounds it leaves a residue. The sulphanilamide is absorbed from this paste almost as rapidly as when the powdered compound is applied as such. This paste was kindly tested by Dr. A. Glucksman of the Strangeways Laboratories, who applied it every other day to standard wounds in rats. He found that it caused very slight irritation, as shown by stimulation of collagen regeneration and slight retardation of epithelial regeneration: but this irritation was so slight as to be unimportant compared with bacterial infection.

2. *Sulphanilamide*, 30 per cent.; *cod-liver oil* 49 per cent.; *oleic acid*, 3.5 per cent.; *triethanolamine*, 1.05 per cent.; *cetyl alcohol*, *beeswax*, and other ingredients to diminish rancidity, 3.5 per cent.; *water* to 100 per cent.—This is a paste of medium consistency. The absorption of sulphanilamide from it

proceeds slightly more slowly than that which occurs when the compound is applied as a simple powder. Besides supplying a large depot of sulphanilamide, which will be taken up by any serous exudate to form an almost saturated solution a paste of this kind provides an oily protective layer and will also prevent sticking of dressings. This paste was designed especially for the treatment of burns. Colebrook (personal communication) has observed the rapid disappearance of haemolytic streptococci following its use in such injuries; he also found that it is unnecessary to change the dressings more often than every two or three days. Robson and Waalce have reported very satisfactory results in burns treated with similar paste made up with albucid (*p*-amino benzene sulphonacetamide), which is considerably more soluble than sulphanilamide and which accordingly may exert a greater effect upon the less sensitive organisms such as staphylococci and *Ps. Pyocyanea*.

3. *Sulphanilamide*, 30 per cent.; *cod-liver oil*, 60 per cent.; *beeswax*, 10 per cent.—This is a stiff paste, to be used on gauze for packing wounds, in the same way as vaseline gauze is used. Its consistency can be modified by varying the proportions of cod-liver oil and beeswax. It provides a non-irritant, non-adhesive mechanical support to the sides of the wound, and at the same time gives off a supply of sulphanilamide, liberation of the compound being slow. It tends to become rancid, however, unless preventive measures are taken.

Summary

Methods are described for the local application of sulphanilamide: (a) as a powder to open wounds; (b) as tablets or on gauze to deep sinuses; (c) as various kinds of paste to treat inflamed surfaces, burns, and wounds which must be packed.

Details are given of three pastes which may be recommended for use in three different types of lesion.

Acknowledgments are due to Colonel L. Colebrook for inspiration and advice; to the surgical staff of the New End Hospital, Hampstead, for facilities; to Miss M. Kuck for technical assistance; and to the British Drug Houses Ltd., for co-operation in devising and preparing the pastes.

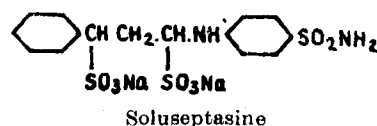
THE COMMON SULPHONAMIDES: NOMENCLATURE, STRUCTURE AND USES*

BY

D. G. ARDLEY, M.P.S.

(From the *Lancet*, Vol. II, 22nd November 1941, p. 625)

With the inevitable increase in the number of compounds introduced into clinical practice for the chemotherapy of bacterial infections the need of an accurate and uniform nomenclature becomes urgent. Few modern British books on general medicine or the specialities make any attempt to achieve accuracy in the names used for these compounds, and the medical journals and encyclopaedias tend to pander to the whims of their contributors instead of exerting strict control over the names employed. As a consequence, for example, all the names which happen to have been employed for the same chemical compound by various contributors to a text-book may appear in different parts of the index. Abstracts of articles from foreign journals often used for drugs names which are unfamiliar to readers in this country and in this way the chief point of the abstract may be lost. Conversely, proprietary names and erroneous nomenclature in English articles duly find their way into foreign abstracts to the mystification of readers. The practitioner who writes a prescription



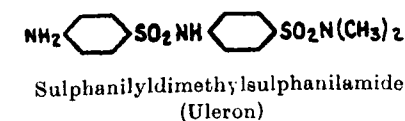
for 'sulphonamide', $\frac{1}{2}$ gramme tablets, or the surgeon who orders a wound to be dressed with sulphonamide powder, does not realise that what he has ordered is meaningless. The writer of an article who describes the treatment of a patient with "Prontosil" given by mouth in such and such a dose overlooks the fact that there are three distinct chemical compounds to which this name is applied, and that all are given by mouth. The limit appears to have been reached by the author of an article on the use of various sulphonamide compounds who calmly announced at the outset that a particular brand name for sulphanilamide would be used to describe all the different compounds employed! It therefore seems opportune to suggest a few rules for general guidance. First, about dosage. The common practice of describing oral dosage in terms of number of tablets or volume of a suspension is inadvisable. A discussion once arose concerning an article

* "The Indian Medical Gazette", July 1942.

COMMON SULPHONAMIDES: NOMENCLATURE, STRUCTURE AND USES 117

on the treatment of gonorrhœa in which the dosage of sulphanilamide employed was in dispute until it was realized that the author was expressing dosage in drachms of a proprietary suspension. Dosage should always be expressed in terms of the active ingredient.

Most of the sulphonamides in common use have three kinds of name—a chemical name often occupying a line of print, a scientific synonym or pet-name, being the chemical name in a shortened form, and a various number of trade names. The synonym is the one intended for use in scientific articles, but unfortunately not all the compounds have one, and in some cases the trade name is at present unavoidable. Whenever possible only the full chemical name or its shortened form should be used, though it may



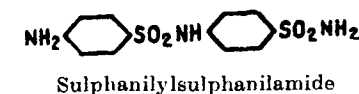
sometimes be convenient to indicate once in an article which particular brand was employed.

Sulphanilamide and its earlier derivatives

Para-aminobenzenesulphonamide or para-aminophenylsulphonamide is the simplest and most widely used of these drugs, and being the amide of



sulphanilic acid the name now commonly adopted of sulphanilamide is at once

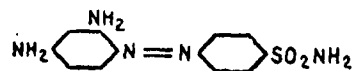


both accurate and brief, and the employment of proprietary names for this compound in medical literature is inexcusable. Of the forty or so trade names indicating proprietary brands of this compound the commonest ones in this country are Colsulanyde, Prontosil album and Streptocide, and sometimes the non-proprietary designations, sulphonamide-P or P.A.B.S. are used. A proprietary brand much used in America is known as Prontylin. Some clinicians still do not realize that these names all stand for the same substance, and it is not unusual to hear a patient's treatment being changed from one make of sulphanilamide to another, because he did not respond to the brand first ordered. Many other proprietary names for sulphanilamide, less commonly used than the four already given, are encountered, chiefly in foreign medical literature. In the case of a few trade names encountered in foreign medical journals there has been no clue to the composition of the drug, or the composition has been variously described in different articles so

that it has been impossible to be certain what the compound was. Sulphanilamide was included in the Fourth Addendum to the B.P. 1932.

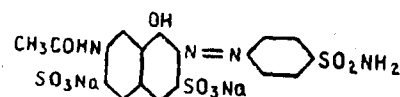
The simplest and most convenient classification of other sulphonamide drugs is to regard them as derivatives of sulphanilamide and as falling into two groups according as to whether substitution takes place in the amino (NH_2) or in the amide (SO_2NH_2) portion of the molecule. All the earlier compounds belong to the first class and are split up with release of sulphanilamide in the body. The more active substances introduced subsequently, such as sulphapyridine and sulphathiazole, fall into the second category, and these compounds do not respond for their action on the production of sulphanilamide.

The first compound to be employed was sulphonamido-chrysoidin, which is 4'-sulphonamido-2:4-diaminoazobenzene, introduced into commerce under



Sulphonamido-chrysoidin

the name prontosil after having been called Steptozon. The name prontosil strictly refers, therefore, to this azo dye-stuff but most English clinicians use it to designate sulphanilamide. If the word is used at all it should never be employed without qualification, so that there is no doubt as to meaning. Sulphonamidocrysoidin is also known as Prontosil flavum or Prontosil rubrum. A less common trade name is Pronzin rubrum. A more soluble compound was later introduced for parenteral administration, which consists of a solution of the disodium salt of 4'-sulphonamidobenzene-2-azo-1-hydroxy-7-acetyl-aminonaphthalene-3:6-disulphonic acid, usually known in this country as Prontosil soluble, and sometimes as Prontosil-S or Prontosil II.



Azosulphamide (Prontosil soluble)

Occasionally the name Prontosil is applied to this compound instead of to sulphonamido-chrysoidin, and in America, where it is given by mouth in place of the latter, it is known under the proprietary name of Neoprontosil, the 'descriptive non-proprietary name' proposed for this substance by the Council on Pharmacy and Chemistry of the American Medical Association being azo-sulfamide. The name streptozon has been given to both prontosil rubrum and prontosil soluble, the latter having sometimes been distinguished as Streptozon II or S, and curiously enough the designation Prontosil red is sometimes used for prontosil soluble rather than prontosil rubrum. The Rubiazols include the French equivalents of the German azo-sulphonamides and rival

them in confused terminology. It is, however, sufficient to remember that in this country the name rubiazol is applied to a compound which differs from sulphonamido-chrysoidin in containing a carboxyl (COOH) grouping and hence is conveniently described as carboxysulphonamido-chrysoidin.

In common with most other compounds of the first group in which substitution takes place in the amino radical of sulphanilamide, these substances owe their activity mainly to the release of sulphanilamide itself in the body tissues, the coloured compounds being split at the azo linkage. Endeavours have been made to show that the azo linkage *per se* may play a role in their action, but all the important recent advances concern colourless compounds, and the dye-stuffs cannot but be regarded as being chiefly of historical interest.

Of the sulphanilamide derivatives belonging to the first group which are not dye-stuffs, the most commonly used in England is that formed by substituting one of the hydrogen atoms in the amino group by a benzyl group.



Benzylsulphanilamide

This compound is para-benzylaminobenzene-sulphonamide or benzyl sulphanilamide. It is known in England as Proseptasine or M. & B. 125, and in France as Septazine or 46 R. P. The drug is soluble in water only to the extent of about 1 in 25,000, compared to sulphanilamide which has a solubility of about 1 in 100. The sodium salt of the corresponding succinyl derivative of sulphanilamide—sodium para-succinylaminobenzene-sulphanilamide or sodium succinyl sulphanilamide—has been issued for parenteral administration under the name Ambesid soluble. There is also a product Ambesid, and it might reasonably be expected that this would be succinylaminobenzenesulphonamide itself, but in fact this is another proprietary name for sulphanilamide.

A widely used compound which is much more soluble than sulphanilamide and consequently more suitable for parenteral administration is Soluseptasine or M. & B. 137, known in France as Soluseptazine or 40 R. P. It is disodium-p-(y-phenyl. prophlamino)-benzene-sulphonamide-a-y-disulphonate, the sodium sulphonate part of the molecule being the solubilizing groups. No convenient non-proprietary designation for soluseptasine has come into use.

A soluble sulphanilamide derivative for parenteral administration, and one having a high sulphanilamide content, has recently been placed on the market under the name sulphanilamide L. S. F. It is described as sulphanilamide lactoside sodium formaldehydesulphoxylate. Reference

has also been made to a compound called Sulphonamide E. O. S. and described as the sodium salt of the ethylsulphonic acid of sulphanilamide. It therefore appears to be sodium 4-sulphonamidophenyl-aminoethane sulphonate. It has been tried clinically in cattle and man. Mutch (1941) has described some investigations on its pharmacology and activity and has given details of its use in 34 cases of cerebro-spinal fever.

Later Derivatives

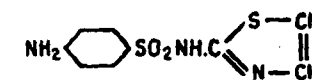
The first drugs of the second class were three disulphanilamide compounds, known originally as Diseptal A, B and C, and introduced from Germany. Of these the first, and the only one to come into use in England, is known as Diseptal or Uleron. It is also called Uliron, Ulirone, D. B. 90 or D. B. 373, and is para-aminobenzene sulphonamido-benzenesulphondimethylamide or sulphanilyldimethylsulphanilamide. Diseptal B is the corresponding monomethyl derivative, and is now referred to in German medical literature as Neo-Uliron. Diseptal C contains no methyl groups, being sulphanilylsulphanilamide. Sulphanilylsulphanilamide is available in America and on the Continent under the name Disulon, where it is used in the treatment of gonorrhoea. It is also known as "disulphanilamide," a name which, unfortunately, has also been used for the dimethyl derivative (uleron). Claims that uleron is valuable for the treatment of gonococcal, staphylococcal and anaerobic infections have not been substantiated, and the subsequent use of sulphapyridine for the treatment of gonorrhoea and the activity of sulphathiazole in staphylococcal infections have led to its falling into disuse. In common with most other derivatives of the second class uleron is not broken down with the release of sulphanilamide but is eliminated partly unchanged and partly in a conjugated form.



Acetyl sulphanilamide

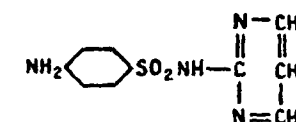
An event of outstanding importance in chemotherapy was the introduction of the compound derived from sulphanilamide by substitution of one hydrogen atom in the amide portion of the molecule by a basic pyridine group, giving 2-(*p*-aminobenzenesulphonamido) pyridine. This was first known as T. 693, later introduced as M. & B. 693 and subsequently called Dagenan, a mutation on an Essex place name. To a non-chemist sulphanilylaminopyridine should not be too long to remember but the Americans christened the compound sulphapyridine, which name, although not accurately descriptive chemically, has been generally accepted. Sulphapyridine proved to have a much higher degree of activity than sulphanilamide, thereby extending the range of chemotherapy far beyond infections by sulphonamide-susceptible strains of streptococci. Pneumococcal infections

were brought under control, a more potent weapon against gonococcal, meningococcal and gas-gangrene infections provided, and the effective chemotherapy of plague and staphylococcal infections brought nearer. A calcium compound of sulphapyridine has been placed on the market in Germany under the trade name Orsulon. It is issued in tablets for oral administration in the treatment of pneumococcal and other infections. In Stockholm a compound of sulphapyridine and salicylic acid, known as salicylazosulphapyridin, and marketed as Salazopyrin, has been used with success in rheumatoid arthritis and ulcerative colitis.



Sulphathiazole

An even more active compound than sulphapyridine is the corresponding thiazole derivative 2-(*p*-aminobenzenesulphonamido) thiazole or 2-sulphanilylamino-thiazole, usually called sulphathiazole, and also known in England as Thiazamide or M. & B. 760, in Switzerland as Cibazol or Ciba 3714, and in France as R. P. 2090. It has proved its value in the treatment of infections with *Staphylococcus aureus*, some of the clostridia, *Pasteurella pestis*, and various organisms found in urinary-tract infections, including *Streptococcus Faecalis*. Various compounds obtained by replacing one of the hydrogen atoms in the thiazole ring have been tried in therapeutics, and of these the name sulphamethylthiazole indicates the 4-methyl derivative—namely, 2-(*p*-aminobenzenesulphonamido)-4-methylthiazole, or 2-sulphanilyl-amino-4-methylthiazole (M. & B. 838). Owing to the danger of peripheral neuropathy from the use of these derivatives (*cf.* uleron which also contains methyl groups), and their greater liability to deposit crystals in the urinary tract compared to sulphathiazole itself, little more is likely to be heard of them.

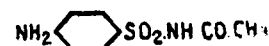


Sulphadiazine

Another heterocyclic derivative of sulphanilamide, the pyrimidine analogue of sulphapyridine, has recently been introduced in America; this is 2-(*p*-aminobenzene sulphonamido) pyridine or 2-sulphanilyl-amino-pyrimidine. Sulphadiazine, as it is called, appears from the preliminary studies of American workers such as those of Finland, Strauss, and Peterson (1941) and of Long (1941) and his associates at Johns Hopkins Hospital to have about the same degree of activity as sulphathiazole and sulphapyridine on pneumococci and meningococci, to have an activity on *Staph. aureus*, and to

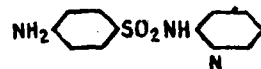
have a high degree of activity on *Klebsiella pneumoniae* (Friedlander's pneumobacillus). It has been tried in pneumococcal pneumonia, cerebrospinal fever, staphylococcal, streptococcal, gonococcal and other infections but the data at present available concerning its activity, toxicity, absorption distribution and elimination are inadequate to predict its ultimate place in chemotherapy.

Sulphapyridine and sulphathiazole being of low solubility in water are unsuitable for parenteral administration in solution, for which purpose their sodium salts are employed. The latter being compounds of weak acids with strong base yield solutions which are highly alkaline. If one of the amide hydrogen atoms of sulphanilamide is replaced by an acetyl radical the compound *p*-aminobenzenesulphonacetamide is formed, which is known as sulphacetamide and was introduced under the proprietary name Albucid. It has been advocated for use in gonorrhoea, for which purpose



Sulphacetamide

it is less effective than sulphapyridine but rather strikingly free from toxic effects, owing probably to its low solubility and consequent poor absorption from the alimentary tract. Its sodium salt differs from the sodium salts of sulphathiazole and sulphapyridine in not yielding a highly alkaline solution. Sodium sulphacetamide is issued as Albucid soluble or Sulphacetamide soluble. It is employed intramuscularly and as a 30 per cent solution for intravenous injection, and a 2.5 per cent solution has been advocated for application to the eyes in the prophylaxis of bacterial infection following injury by vesicant war gases. Albucid and Albucid



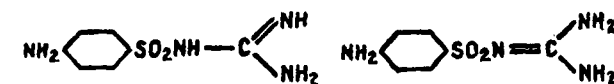
Sulphapyridine

soluble have been placed on the market in Canada under the names Sulamyd and Sulamyd soluble. Sulphacetamide differs from the acetyl derivative of sulphanilamide, into which the latter is partly converted into the body, since in sulphacetamide it is a hydrogen of the amide group which is replaced by the acetyl radical, whereas with acetyl sulphathiazole substitution takes place in the amino group. Analogous conjugation takes place with sulphapyridine, sulphathiazole, sulphadiazine and a number of other sulphonamide drugs. Unlike these compounds sulphacetamide appears to be partly broken down in the body of sulphanilamide.

Chemotherapy inside the Bowel

The absorption of sulphonamide from the alimentary tract or from a wound chiefly depends on its solubility in water at body temperature. Thus

the solubility and absorbability of sulphanilamide, sulphathiazole and sulphapyridine decrease in that order. Other factors, however, have an influence on degree and rate of absorption, and some compounds much more soluble than sulphapyridine or sulphathiazole have a low degree of gastrointestinal absorption. The idea arose, therefore, of using a sulphonamide which is bacteriostatically active, fairly water-soluble and of low absorbability to combat infections which are largely confined to the alimentary canal. Marshall, Bratton, White and Litchfield (1940), who studied various compounds from this point of view, found that sodium sulphanilyl sulphanilate, *N*' (2-hydroxy-ethyl) sulphapyridine, *N*' diglycosulphapyridine and sulphanilylguanidine fulfilled the requirements of water-solubility and limited absorption from the intestine. Since sulphanilylguanidine appeared to be the most active of these compounds against a number of pathogens it was selected for pharmacological and therapeutic investigation. (It may be recalled that sodium sulphanilyl sulphanilate once achieved a short-lived but world-wide interest for a supposed activity in virus infections.)

 α -sulphanilylguanidine β -sulphanilylguanidine

Sulphaguanidine

Sulphanilylguanidine is *p*-amino-*N*-guanyl-benzene-sulphonamide. Marshall and his colleagues suggest that the formulae may both be considered correct for the compound as they are probably resonance isomers, easily transformed one into the other.

The blood levels reached after the administration of sulphanilylguanidine are low as a consequence of its limited absorption, and the concentration in the gastro-intestinal tract is correspondingly high. It is suggested for trial in bacterial infections which are chiefly localized in the intestines, such as bacillary dysentery, cholera and the early stages of the enteric fevers, and for reducing the hazards of intestinal surgery. In acute bacillary dysentery it will have to be established that sulphanilylguanidine has advantages over sulphathiazole and sulphapyridine, those 'veritable Excaliburs, with which dysentery apparently may be vanquished without fail' [(Ravenel and Smith (1941))]. Other sulphonamides have proved inactive on *Vibrio cholerae* at ordinary therapeutic concentrations but Marshall and others have found sulphanilylguanidine bactericidal *in vitro* in a concentration of 200 mg. per 100 c. cm. so that at the high levels reached in the gastro-intestinal tract it is possible that the administration of this compound may be of benefit in cholera. Its use in intestinal infections bears some analogy with the use of sulphonamides in infections of the urinary tract, where the concentration in the urine is, of course, enormously greater than in the blood and tissues.

Various sulphonamides have given unquestionable benefit in cases of ulcerative colitis, although the mechanism of the effect must necessarily remain obscure while the aetiology of this condition is unknown and a few experiences in the use of sulphanilylguanidine in ulcerative colitis are encouraging. The drug is being known as sulphaguanidine, by analogy with sulphapyridine and sulphathiazole.

Other Sulphur Compounds

Not all the sulphur compounds which are active anti-bacterial agents are sulphonamides; a number are sulphones, sulphoxides and sulphides. The sulphones which have been investigated experimentally as well as clinically may be regarded as derivatives of 4:4'-diamino-diphenylsulphone. This substance has proved excessively toxic to mice but not to some other laboratory animals, and its toxicity probably precludes its clinical employment in man. McEwen, Pizer, and Paterson (1941) have reported the rather unexpected finding that the compound is of relatively low toxicity for cattle, producing no toxic effects in therapeutic doses. The di-acetyl derivatives of 4:4'-diamino-diphenylsulphone is of low toxicity and has been used in France for the treatment of gonorrhoea under the proprietary name Radilone (1399 F). The glucoside of 4:4'-diamino-diphenylsulphone has been studied for its activity in experimental bacterial and virus infections, and has been used clinically in the treatment of cerebro-spinal fever, trachoma and lymphogranuloma inguinale. A compound known as Promin and described as the sodium salt of *p.p'*-diamino-diphenyl-sulphone-N,N'-di-dextrose sulphonate has been tried in artificially induced animal infections with various coccal and bacillary organisms, and used clinically by mouth and intravenously. This drug has been found to possess a retarding influence in experimental tuberculous infections of guinea-pigs apparently greater than that conferred by sulphonamides. At present, however, the effective chemotherapy of clinical tuberculous infections remains, with that of brucellosis, and the introduction of virucidal agents, probable achievements of the future. Despite the number of compounds in clinical use and the far greater number which have been studied experimentally, the tremendous strides made during the past few years constitute but the beginning, though a highly successful one, of a new era in therapeutics.

AGRIOSTOMUM VRYBURGI RAILLIET, 1902

BY

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It was Lane (1923) who first recorded this parasite in India and he got his specimen from the gut of *Bos indicus* in Darjeeling. With this material, he was in a position to amplify the original description of the worm by Railliet (1902) quoted by Ware (1925) who obtained his specimen from a zebu (*Bos indicus*) in Sumatra. Ware (1925) got his collection consisting of three males and four female worms, from the intestine of a calf in the Nilgiris, South India. He described the parasite and included figures of the head end and spicules. It was possible for the present author to study the parasite in greater detail, the outcome of which is this short paper.

The worms were received from Ootacamund (the Nilgiris), a hill station in South India and collected from the intestines of a heifer calf. Ware's material was obtained from the same place and it is noteworthy that this worm has been met with by Lane, Ware and the present author from places which happen to be hill stations in India.

A table showing the various features of the worm described by previous workers and the author is appended for comparison.

The male worms measure on the average 10.2* in length and 0.3 in width while the females measure 12.2 and 0.3 respectively. The cuticle is very finely striated. A ventral cervical groove is present, 0.4 from the anterior end of the worm. The cervical papillae are not easily seen, while the pre-bursal papillae are small and rounded. The head end (Fig. 1) of the worm is directed antero-dorsally and has a circular oral aperture leading into a shallow buccal capsule. This capsule bears four pairs of teeth, resembling those in Ancylostomes, arranged along its inner border and is continued into a crater-shaped oesophageal funnel posteriorly. In between the fourth pair of teeth, is a bi-fid tooth-like structure, at the mid-dorsal line. The oesophageal funnel is 0.2 long and no sub-ventral lancets, described by Monnig (1934) are traceable in the specimens examined here. The funnel is continued into a club-shaped oesophagus 1.2 long and provided with valves, prior to its attachment with the intestine.

The male bursa (Figs. 2 and 3) especially, the dorsal rays are characteristic. The externo-dorsals arise from about the middle of the main trunk and are short and stout. The termination of each of the dorsal rays is bi-fid, the inner branch of which is long and pointed, reaching the rim of the bursa, while the outer is short, stout and rounded. The laterals spring from a

*All measurements are in millimeters.

Statement showing the comparative details of the worm *Agriostomum Vryburgi* according to the different authors

Names of different authors	Average Measurement of		Length of Oesophagus	Length of Spicule	Length of female tail	Distance of vulva from hind end of tail	Measurement of ova	Cuticular striations, if present or not	Ventral cervical groove, if present or not	Remarks
	Male worm	Female worm								
Baylis, H. A., 1936	10.1 x 0.32	14.2 x 0.42	1.1	0.85	0.25	0.53	0.16 x 0.075	Present	Present	
Lane, C., 1923	9.2 x 0.3	15.0 x 0.45	No mention	0.84	0.15	0.47	0.18 x 0.07	Absent	No mention	
Monnig, H.O., 1934	9.2	14.5	No mention	0.85	No mention	No mention	0.16 x 0.076	No mention	No mention	
Ware, F., 1925	10.75 x 0.35	13.5 x 0.45	1.1	0.86	0.25	0.55	0.14 x 0.08	Present	No mention	Two subventral lancets in Oesophageal funnel & rudimentary leaf — crown present
Mudaliar, S. V., 1941	10.2 x 0.3	12.2 x 0.35	1.2	0.86	0.2	0.45	0.13 x 0.08	Present	Present	not seen

AGRIOSTOMUM VRYBURGI RAILLIET, 1902

BY
S. V. MUDALIAR

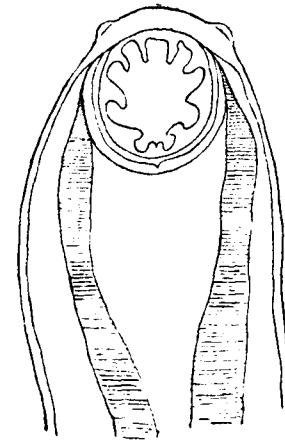


Fig. 1.
Agriostomum vryburgi
Head end.

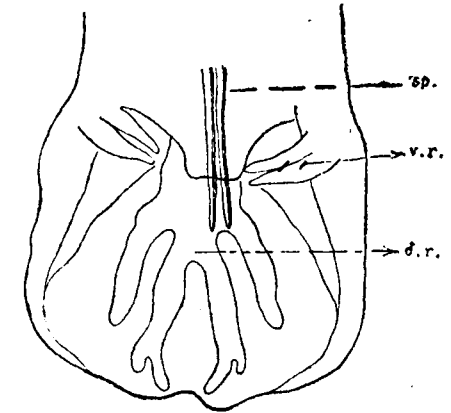


Fig. 2.
Agriostomum vryburgi, male.
Caudal end showing dorsal and ventral rays.

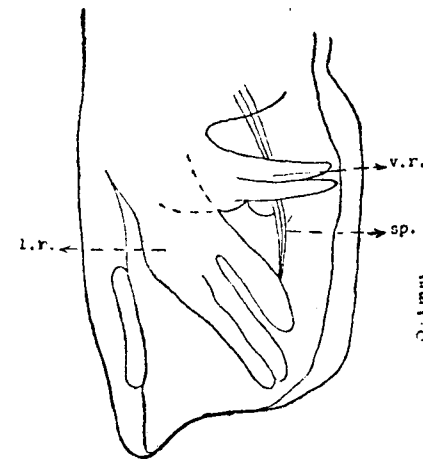


Fig. 3.
Agriostomum vryburgi, male.
Lateral and ventral rays of bursa.

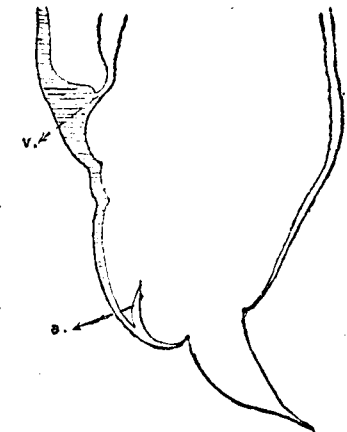


Fig. 4.
A. vryburgi, female.
Caudal end.

common trunk, the externo-lateral being stout and short. There is a deep cleft between this and the medio-lateral, while the dorso-lateral and medio-laterals are apposed. The ventrals are thick and apposed.

The spicules are strong, equal, alate, except at the tip and measure 0.85 long. An accessory piece is present and resembles a shield, viewed dorsally.

The tail of the female is short, being 0.2 long and ends abruptly in a point. The vulva (Fig. 4) is close to the anus and placed on a prominence. It is located 0.45 from the hind end of the tail. The eggs are elliptical, provided with a thin shell and measure 0.13 by 0.08.

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EXPLANATION TO LETTERING

a=anus.	l. r.=lateral ray.	v. r.=ventral ray.
v=vulva.	d. r.=dorsal ray.	g. c.=genital cone.
	sp. =spicules.	

THE CLINICAL AND MICROSCOPIC DIAGNOSIS OF RABIES

BY

A. V. DE CROOS, G.V.SC., COLOMBO

Modes of Infection

Rabies is transmitted from one animal to another through saliva which is injected into the animal by means of a bite or in some cases by the saliva of an infected dog or other animal coming in contact with a broken surface of the skin. The rabid dog roams in the streets biting anything that comes in its way.

Sources of Infection

The dog is the chief source of infection in rabies, but it would surprise many dog-owners to be told that the musk rat and bandicoot so common in any garden is another source of infection. Many people wonder how their pet, that never leaves the compound and wanders along the streets, could have contracted rabies, when to the best of their knowledge, the dog

has never come in contact with another. Most dogs will chase a bandicoot or rat on sight and sometimes succeed in catching it. In the struggle that ensues the dog is bitten and if the bandicoot or rat is a reservoir of rabies, then infection follows.

For a long period of time, rabies was thought to be of seasonal occurrence and even to-day one hears people say that the hot season brings on outbreaks. Rabies is not seasonal in this way. Climatic conditions have nothing to do with the disease. The reason why there are more cases during the dry season than in the wet, is that in dry weather animals go in search of water and thus may come in contact with infected ones.

The forms in which Rabies manifests itself are—the Furious, the Dumb and Pseudo.

The furious form :—In this form there is the tendency to bite anything within reach of the dog—the chain, the bars of the kennel, and even its own body. Abrasions are found on the gums and lips.

The dumb form :—Here there is a marked paralysis of the muscles of the lower jaw—though there may be an inclination to bite, owing to this paralysed condition, the animal is unable to do so.

The pseudo form :—In this there is a slight paralysis of the hind limbs which can be cured if proper treatment is given early.

The symptoms common to all forms are a preference for dark corners, hallucinations, vacant, staring look, photo-phobia, inability to swallow and in the latter stages paralysis of the hind limbs. Death occurs within 6-7 days. Photo-phobia is the most important symptom and is diagnostic to the experienced eye, in the early stages.

Potency of Rabies Virus

Dr. Nicholls in one of his lectures on rabies stated that only 30% of the bites of rabid animals is infective. The reason for this is that in its mad career the dog bites many objects, both animate and inanimate and with every bite the potency of the virus is reduced, until a condition arises when it has lost all its power to infect. Of course, it is impossible to say in what state of virulence the virus is, so that it is imperative that pasteur treatment should be taken at the earliest opportunity after the bite. Probably the many "Quack" cures claimed by various people can be attributed to this loss of virulence at the time the bite was inflicted.

Infection in a case of dumb rabies is more dangerous than from one in the furious state. In the dumb form though the dog shows a tendency to bite, the paralysis of the lower jaw prevents much damage or the biting of many objects with the result that the saliva retains its virulence for a long period.

In the pseudo form the saliva is not infective.

In all cases it is always best to keep a suspected case under control and proper observation. This helps the clinical and microscopic examination to yield better and more reliable results. Negri bodies develop in the brain with the progress of the disease and are fully developed at time of death when the malady has run its full course.

Microscopic Diagnosis

The virus of rabies, is, like all viruses—ultra microscopic. It travels from the seat of infection to the brain via the nervous system. Some authorities state that the blood stream is also one route, but this is not generally accepted. The only way of diagnosing rabies through the microscope is by examining sections of the brain and spinal cord.

Negri bodies are round or oval in shape and are found in hippocampus of the brain, the cerebellum and spinal cord.

A section of the tissue is run through ascending and descending grades of alcohol. It is placed in running water for some hours after which it is embedded in paraffin wax. Sections are then cut with the microtome, stained with haemotoxin and examined under the $\frac{3}{2}$ " lens of the microscope.

There are other quicker methods of examination but these are not very reliable.

I use the following method :—

A saturated solution of Methylene Blue is mixed with a saturated solution of Basic Carbal Fuschin in Methyl Alcohol. The slide is pressed on the brain to be examined. The slide is then stained and dried afterwards. Negri bodies are shown bright cherry red. (Clinical Pathology by Raske).

The Biological Test is that in which the virus is inoculated sub-durally into the brain of a white mouse or rabbit. The inoculation of white mice gives a more reliable result than the inoculation of rabbits. The mice die in ten days time but paralysis comes on, on the 5th or 6th day and this is diagnostic (American Journal of Health, Volume 28, No. 2).

In one instance, recently, the Microscopic Report of the Director of the Bacteriological Institute was negative for a case that clinically showed all the symptoms of rabies. At the request of Mr. Little, the M.V.S., Dr. Nicholls carried out the biological test using a rabbit. The result was positive for rabies. This goes to show that a clinical diagnosis is more reliable than the microscopic for in the former a Veterinary Surgeon sees the animal in a live state.

Experiments were carried out on a number of dogs which were artificially inoculated with brain emulsion. The emulsion was given sub-durally into the first dog A. This animal showed all the symptoms of rabies and

died on the 10th day. The microscopic result was negative. An emulsion from the brain of this dog was inoculated into the brain of the second dog B. This dog again showed all symptoms of rabies and died on the 8th day. The microscopic examination was the same as for dog A. An emulsion from the brain of B was tried on a third dog with similar results. Fixed virus was also tried on a dog. This dog died on the 7th day and the microscopic result was negative. These experiments go to show that artificially infected dogs, although they manifest all the symptoms of rabies, give negative results on microscopic examination of the brain.

Some Observations on Dogs developing Rabies after Single Dose Injections

On occasions, dogs given the single dose inoculation develop rabies. The single dose method consists of one inoculation of a 5% suspension of brain matter in emulsion and is prophylactic. If a dog has been infected with street virus, a single dose inoculation will probably bring on the disease, this is due to the tendency of all vaccines to lower the power of resistance in the animal body. I had a case of a dog so infected, I advised destruction or pasteur treatment. The owner insisted on the single dose. I gave it very reluctantly, but with the warning the dog might develop the disease within a few days. On the 14th day after inoculation the dog showed signs of paralysis of the hind limbs. Two days later it died. A Veterinary Surgeon, who saw it alive, diagnosed the case as one of rabies. The microscopic test was, however, negative.

The next case I had, was when a dog was sent to me for Anti-Rabic injection (one dose). When I questioned the owner, as to whether it was bitten by a rabid dog or not—the usual procedure with me—he said, it was not. I then had no scruples about giving the injection. Within 23 days the dog developed rabies. I had it under my observation and had no doubts about the disease. When the dog died, the owner, after much cross-examination, admitted that the dog was bitten by a "stray dog" some days before inoculation. The microscopic result was again negative.

It is interesting to observe that in both these cases no Negri bodies were found, either in the brain or spinal cord, although the disease was allowed to run its full course. This may be due to the inoculation. I ruled out protein shock because I gave full doses of calcium without effect.

The main object in administering Anti-Rabic Inoculation is to protect the human being by protecting the dog. There are many difficulties in our way, the chief one being the reluctance of the public to take the fullest advantage of the facilities offered. Larger countries than ours have stamped out rabies and there is no reason why a small island like ours should not free herself from rabies in the near future.

PREGNANCY DIAGNOSIS IN EQUINES*

BY

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Horse-breeders and veterinarians well know that, in a large percentage of mares,* it is absolutely impossible to diagnose pregnancy during the first three or four months as they show no obvious physical signs of this condition. In some cases, pregnancy cannot be diagnosed until six or seven months after conception. In fact, a small percentage of mares show no physical signs of pregnancy until a few weeks before the birth of the foal.

The sexual behaviour of mares subsequent to service is not a reliable guide to the existence or otherwise of pregnancy, since the absence of oestrus (heat) cannot always be regarded as evidence of conception in these animals, as in most other mammals. Some mares do not exhibit a return of normal oestrus even after an unsuccessful service. Some thoroughbred mares in milk exhibit only one oestrus in a breeding season, and therefore, the absence of oestrus in them following a service should not always be taken as a sign of pregnancy.

Methods for diagnosing pregnancy in mares are said to have been practised from time immemorial in Arabia by certain Arab tribes who keep the details a secret and hand them down from father to son. There is, however, no authentic information regarding their reliability. Until recently, therefore, most people have had to depend entirely upon the clinical symptoms, history and sexual behaviour of the mare for pregnancy diagnosis. During the last decade, however, the widespread demand for a means of recognizing the existence of pregnancy in its early stages has been successfully met and a spectacular advance has been made in the early diagnosis of this condition in mares.

Ascheim and Zondek (1927) laid the foundation stone for the diagnosis of human pregnancy by biological methods, *i.e.*, methods involving the use of living animals as test subjects. This work was taken up by scientists all over the world and eventually extended to animals, so that to-day there are over half-a-dozen different biological tests for the diagnosis of pregnancy in equines alone. Blood serum or urine samples of pregnant mares can be tested on mice, rabbits, rats or caponized fowls. Pregnancy diagnosis stations have been set up in almost all civilized countries and their usefulness has become generally recognized.

* Reprinted from the "Indian Farmer," July 1942.

Pregnancy Hormones

The tests are based upon the detection of two different 'hormones,' one in the blood serum and the other in the urine of pregnant mares. The term 'hormone' is applied to certain substances which are produced by various ductless glands of the body such as the pituitary, the suprarenals, the thyroid and the thymus. In other words, hormones are the internal secretions of these glands and these secretions are poured by them directly into the blood stream. Recent investigations have established the fact that all the physiological functions of the reproductive organs are entirely controlled by the internal secretions of the anterior part of the pituitary gland. The two hormones referred to above, on which the test depends, are (a) gonadotropic or sex-gland stimulating hormone found in the blood serum and (b) oestrin, a heat inducing or oestrogenic hormone found in the urine of pregnant mares.

The amounts of gonadotropic hormone and oestrin present in the blood serum and urine respectively of mares vary from mare to mare and from time to time. Thus the gonadotropic hormones are found in the blood serum from the 45th to the 100th day after conception. After the 100th day, they gradually decrease in amount and disappear entirely from the blood stream by about the 120th day. From the 40th to 60th day after conception the amount of oestrin present in the urine of many mares may not greatly exceed the amount excreted by non-pregnant mares. From the 60th day the oestrin content steadily increases until, in the advanced stage of pregnancy, there are present about 200,000 mouse units per litre. Indeed, urine from pregnant mares is nowadays used commercially for the production of oestrin for physiological and therapeutic purposes.

The sex-gland-stimulating hormones are responsible for the growth of the sex-glands and for the production of those changes which are associated with sexual activity, particularly those affecting the ovaries of the female and the testicles of the male. Oestrin when administered to a mouse from which the ovaries have been removed, provokes certain characteristic reactions normally associated with oestrus in that animal and it is these reactions which are looked for in making a pregnancy diagnosis.

Tests with Blood Serum

Mice test:—The test requires batches of immature female mice which are injected with small amounts of mare blood serum, *e.g.*, 5 or 6 drops injected twice a day for three days. The mice are killed on the fourth day and their ovaries are examined. If the concentration of gonadotropic hormone in the serum has reached the level expected in pregnancy, white spots and blood splashes are discernible in the ovaries of mice.

Friedmann's test (Rabbit test).—In rabbits, liberation of eggs from the ovary normally occurs only after mating. Therefore, to avoid errors in this test, it is necessary to keep the female rabbits isolated from the male for three weeks before use. So necessary is this precaution that some workers open up the abdomen before injection to make certain that the sex apparatus has not been naturally stimulated.

About 100 drops of blood serum are injected into the ear veins of two rabbits. Twenty-four hours after the injection the ovaries of one of these rabbits are examined. If the reaction is positive, ruptured egg sacs and blood spots will be seen in one or both ovaries. If no reaction is observed in the ovaries of the first rabbit, the second rabbit is injected intravenously with another 100 drops of blood serum and similarly examined. If a negative reaction is again obtained, the result may be regarded as reliable.

Xenopus test:—In this test, the South African clawed toad is employed as the test animal. In these animals, an injection of serum induces egg liberation.

Bitterling test:—In this test, egg liberation is induced in a small fish.

It should, however, be noted that neither of these two tests is extensively used in determining pregnancy in mares.

In view of the fact that the gonadotropic or sex-gland stimulating hormones are present in the blood serum of pregnant mares only from the 42nd to the 100th day of conception, a negative result is of significance only if serum obtained during this period is used. Batches of immature mice may be difficult to obtain unless mice-breeding station is close at hand. The rabbits used must have been kept isolated for about three weeks. In India, the bleeding of pregnant mares may arouse a certain amount of prejudice.

Tests with Urine

Mice test (cornification test).—The Imperial Veterinary Research Institute carries out this test for the diagnosis of equine pregnancy to meet the requirements of horse-breeders in this country at a nominal charge of Rs. 8/- per test. The test consists in demonstrating the presence of oestrin in the urine of mares by the reaction resulting from injection of such urine into mice from which the ovaries have been removed. The ovaries are easily removed and once this operation has been performed, the mice can no longer spontaneously exhibit oestrus but will only do so if injected with oestrogenic substances (*i.e.*, those capable of producing oestrus, such as urine from a pregnant mare).

When sending a sample of urine for this test, one collected in the early morning is to be preferred, since urine, like all other body secretions, is subject to violent fluctuations in composition. The quantity and concentration of urine are closely related to the intake of water, to exercise, to the state of body metabolism and to climatic conditions. Strictly sterile urine, though not essential for the test, is desirable. In order to prevent deterioration of the hormones during transit by bacterial multiplication, a trace of cresol (1 drop to every ounce of urine) should be added. About one ounce of urine is required. Details regarding the name or number of the mare, its breed, age and the date of its last service should be sent along with the sample.

Treatment of Urine

The injection of untreated urine frequently proves fatal to the test mice, as mare's urine is rich in mucin and protein bodies. Therefore, to avoid protein shock in test mice and to prevent bacterial contamination, the sample is treated to remove these harmful factors. One gram of sulphosalicylic acid is added to 25 c.c. of urine, the mixture is then well shaken and left for about 15 minutes. This brings about the precipitation of the mucin and protein bodies and the destruction of bacteria in contaminated samples. The sample so treated is filtered and then neutralized by the addition of sodium bicarbonate. It is then diluted with twice its volume of normal saline and this dilute urine is injected into the mice.

Vaginal smears are taken from each mouse before injection. Four mice are injected subcutaneously morning and evening for three days with this dilute urine until six injections in all have been given to each mouse. The doses given to the four mice are respectively 0.2, 0.3, 0.4 and 0.5 c.c. (1 c.c. represents about 17 drops). Vaginal smears are taken on the fourth day, *i.e.*, 96 hours after the first injection, fixed with methyl alcohol, stained by Giemsa's stain and examined microscopically.

A positive reaction is indicated by certain chemical and morphological changes in the cells lining the vagina. These changes, however, can only be detected by an expert.

Quantity of Oestrin in Mouse units

To enable one to give a diagnosis of pregnancy in a given sample of urine, the sample should contain over 1000 mouse units per litre (1000 c.c.) of oestrin. Normal non-pregnant mares do not show more than 200 to 300 mouse units per litre of their urine. Some mares in oestrus may show up to 800 mouse units and a concentration of anything over 1000 mouse units indicates pregnancy. A mouse unit of oestrin is the minimum quantity that is required to indicate oestrus in a mouse from which the ovaries have been removed. If 1 c.c. of urine can effect this change, it means that 1 litre of urine (1000 c.c.) contains 1000 mouse units.

Miller (1935) tested over 2,000 samples of equine pregnancy urine and found that a considerable number of pregnant mares from the 55th to the 80th day after service gave very weak positive reactions during the early part of the dry season. He observed that during an excessively dry summer grass is deficient in moisture and in proteins and that hormones, particularly oestrin on which the test depends, suffer in consequence. He was able to show that the frequency of such weak reactions disappeared once the rains had broken. Another difficulty in the interpretation of results may sometimes be met with in cases of virgin mares and in some old mares which have missed two or three previous seasons. Many workers are of opinion that a good many mares do not show a sufficient amount of oestrin in their urine to give a definite reaction until the 60th day after conception. Where a negative result is obtained with a sample taken before the 60th day, a second sample should invariably be submitted for test. For such second tests this Institute charges a fee of Rs. 4 only.

Capon Test

In this test a diagnosis of pregnancy can be obtained as early as 48 hours after injection of urine. The test animals used in these tests is the brown leghorn cock from which the testicles have been removed. Fowls of other breeds cannot be utilised for this test, because other breeds of fowls do not exhibit the characteristic sex dimorphism of the characters in the plumage as the brown leghorns. In the female of this species the breast plumage is salmon coloured. In the male, the breast plumage is of a solid black colour. It is the ovarian hormone (oestrin) that is responsible for the maintenance of the characteristic pattern and colour of the female plumage in the normal female of this breed. The assumption of typical female plumage ensures in the male of this breed as a result of successful implantation of the ovary into them. The reaction following the injection of urine containing oestrin consists in the change of the plumage type from male to female. Growing feathers are necessary for this test. Castrated birds have the faculty of rapidly regenerating plucked feathers. A dozen or so feathers are removed from an area on the breast, care being taken to ensure that the plucked feathers are solid black. In about seven days the feathers can be seen pressing the skin outwards, but not yet protruding through it. From this stage until about seven days later, the feathers are considered suitable for the test. A subcutaneous injection of 10 c.c. of the prepared urine is made on each of two consecutive days. On the third day two or three feathers are removed for examination. The feather is then pressed between a glass slide and a cover glass and examined microscopically. When the concentration of hormone has been sufficient to induce a female reaction in feathers an entire absence of black pigmentation and its replacement by red or yellow pigment occurs in the feathers. This test is rarely used.

Workers abroad have sometimes found that mares which gave positive pregnancy results on test were eventually found not to be 'in foal'. On further investigation into such cases, they obtained strong evidence that abortion had taken place in some of these mares. In a few other cases, cystic ovaries were found to be responsible for the increase of oestrogenic hormone in the urine and it was shown that any irritation or inflammatory condition of the ovary or uterus may be associated with the excretion of urine rich in oestrin. In one case a mummified foetus was found and in another the disintegrating remains of a foetus. In such cases, where there is neither abortion nor any ovarian abnormality, Miller suggests that vitamin E deficiency in the diet of the mare may cause the death and atrophy of the foetus, since it is known that deficiency of vitamin E may cause miscarriage in some, particularly working animals.

None of the biological tests enumerated above can be said to be invariably reliable, since 'invariability is not a biological phenomenon' and a certain percentage of error must be allowed for. It is certain, however, that in the great majority of mares an early diagnosis of pregnancy can be made by the use of one or another of these methods.

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THE
Indian Veterinary Journal

(The Journal of The All-India Veterinary Association)

Vol. XIX

NOVEMBER, 1942

No. 3

Editorial

THE PUNJAB VETERINARY COLLEGE

We are very much gratified to learn that proposals are afoot for affiliating the Punjab Veterinary College to the University of Lahore and for instituting in that College a Degree in Veterinary Science. These proposals are made none too soon; in fact, they are long overdue. It is admitted on all hands that the Punjab Veterinary College is one of the best equipped Veterinary Institutions in India and that the clinical material available there is of a very wide and extensive variety. As such it should not at all be difficult for the proposals to go through and end in an early affiliation of the College to the University.

While the proposals are still in the formative stage, we desire to urge on the authorities of the College to have one, and only one, course of unified study and not commit the blunder of having two courses of study as in Madras. As is well known, the Madras Veterinary College which is the only one Veterinary Institution so far affiliated to a University, offers two courses of study, viz., a Diploma and a Degree. The preliminary educational qualification necessary for admission to either of these courses is the same, viz., Inter-in-Arts. The Diploma course extends over 4 years while the Degree course is for 5 years. Only those who take the Diploma can sit for the Final Degree Examination. The students whether for the Diploma or Degree

are given the same kind of training, until they take the Diploma, except a few additional hours of practical training in certain subjects of the Degree. The fifth year of study is a period of practical training in Preventive Medicine, Animal Husbandry and Hygiene and the Final University Examination is confined only to these three subjects. A peculiar feature of the syllabus is that while all the subjects of Veterinary Science are included for the Diploma course, many important subjects are excluded from the Degree course. Subjects like Anatomy, Surgery, Pharmacology and Therapeutics do not find a place there. The explanation offered for this arrangement is that these subjects are taught in the Diploma course and, as every student has to take a Diploma before he can sit for the B.V.Sc. Examination, no harm is done by the omission of these subjects for the latter course. Those who parade the above explanation forget the simple fact that the other subjects also are taught for the Diploma course and as such they have no special claim to be included for the Degree. It cannot possibly be contended that Physiology, Bacteriology, Pathology and Medicine are the only important subjects in Veterinary Science and that Anatomy, Surgery and Therapeutics are minor subjects not fit to be considered for inclusion in an University Curriculum. The explanation is simply too puerile even to merit notice and we would not have mentioned it but for the serious way in which it is often put forward. It is quite possible other reasons may be advanced for this exclusion of vital subjects; but whatever they are, we are sure they cannot stand any serious scrutiny. As a result of this queer disposition of subjects, the Degree course which was intended to be an improved course has lost much of its charm and prestige. But what it has definitely done is to create a class distinction in the Veterinary profession in the country. People who take the Degree course claim, and rightly too, a higher status: while those who obtain the Diploma, knowing the syllabuses of the two courses of study, are not prepared to accept a position of inferiority. A distinction has thus arisen without much of a difference. Naturally, this leads to difficulties and complications in service questions—all these seriously affecting *esprit de corps* in the profession. And this at a time, when the

sister Medical profession had abolished all distinctions in it and initiated a unified course of study. The Madras Government must have taken note of this fact before instituting the two courses of study in the Madras Veterinary College but, unfortunately, the powers that were then responsible, chose to act with singular lack of foresight in this matter with the result we see before us.

In addition to the above complications, the course of study is made as irksome and difficult as possible to the students. They have to face a number of examinations in the course of a year, separately for the Diploma and Degree and this ordeal is, as one of them expressively put it, nerve-racking.

With this experience before us, we are extremely anxious that such arrangements should not come into existence in other places where Veterinary Education is imparted. We, therefore, plead with all earnestness that in the proposals for affiliation to the University, the Punjab Veterinary College would profit by the lessons learnt in Madras and arrange to institute only one course of study in that College and that, too, preferably of an intensive nature. The University Degree course is admittedly of an improved kind of education and there need not, and should not, be any necessity for another course of study which is likely to have the mark of inferiority stamped on it.

PROVINCIAL VETERINARY CONFERENCE, MADRAS

Just a reminder to our readers.

As announced already in the last issue of the *Journal*, the Madras Provincial Veterinary Conference will be held in Madras on the 25th, 26th and 27th December 1942. Sri Rao Sahib M. Anant Narayan Rao, G.M.V.C., Lecturer in Parasitology (on leave preparatory to retirement), Madras Veterinary College, has kindly consented to preside on the occasion and conduct the deliberations of the Conference. We renew our appeal to the members of the profession to muster strong and make the

Conference a great success. Professional articles of general and clinical interest and notices of resolutions to be moved at the Conference should, as notified already, be sent so as to reach the Secretary by the 1st of December 1942.

There is a point of added importance on the occasion. We understand that the A. I. V. A. Madras Branch has requested the A. I. V. A. to hold in Madras a business meeting of that body during any of the days of the Conference so that steps may be taken to renew its activities as of old. We are confident that this request will be complied with and an announcement made shortly about the date of the meeting.

The organisers of the Conference are keen and enthusiastic about awakening the interests of the profession in all possible ways and we therefore hope and trust that members of the profession will co-operate with them in an ample measure and so strengthen their hands.

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PREM NATH KAK, *Captain*,
Senior Veterinary Officer,
Srinagar (Kashmir).

Clinical Cases

BOVINE PIROPLASMOSIS IN COONOR AND SURROUNDINGS OF THE NILGIRIS DISTRICT

BY

S. RAMANARAYANAN, G.M.V.C.,
Veterinary Assistant Surgeon, Coonoor, Madras

Piroplasmosis in cattle is of common occurrence in the Coonoor Taluk and several cases have come under my observation since June 1941. The disease has been more prevalent during the cold months, though it has occurred throughout the year. The Piroplasmosis has been mostly due to *Babesia mutans* though occasionally infection of the *B. bigeminum* type has also been encountered. The disease has been met with in Coonoor as well as the outlying villages.

Altitude does not seem to make any variation in the severity or the course of the disease. In general the infection has invariably been of a mild type and I know of no fatalities as a result of Tick Fever with *Babesia mutans*.

The disease has been manifested in several of the cross-bred cows after serum simultaneous inoculation as a reaction to the inoculation, when the vitality of animals is at a low ebb.

Margopas annulatus and *Hyoloma aegyptinum* are the two varieties of ticks that have been met with.

Symptoms:—The first thing observed by the owner is a reduction of milk yield in milch cows, a general lassitude and an impaired appetite. Dung is invariably on the loose side, sometimes diarrhoea is present. On examination visible membranes are observed to be icteric to a lesser or greater extent. Urine is practically normal but for the high colour. The coat is staining in some cases and in some, there is a thin staining of saliva flowing from the mouth. Temperature rises from 103.0° to 106.0°F. However in Tick Fever of the *Bigeminum* type temperature rises from 104.0° to 107.0°F. These are more aggressive symptoms and when the animal has been sick for a day or two, there is prostration to a considerable extent; the urine is retained for a long time and when passed, very large quantities are passed while in the advanced cases it is coffee coloured. The animal completely goes off feed and rumination suspended, the ears drooping down with a tendency to lie down on the part of the animal is observed.

Diagnosis:—Piroplasmosis—provisional by clinical symptoms and confirmation on microscopical examination of blood smears.

Treatment:—In all cases of mutans infection a single injection of 1 per cent. solution of Acriflavine, doses from 50 to 80 c.c. intravenously, say, at 10 c.c. per 100 lbs. body weight has given very beneficial results. The temperature comes down in 24 hours, when the animal begins to feed and ruminate normally the normal milk yield regaining in 2 or 3 days. No special after-treatment has been found necessary. The infection with *Bigminum* does not so well respond to the above treatment. Bayer's Acaprin has been found very efficacious if the case is taken up early.

With the injection of Acaprin 4 to 6 c.c. subcutaneously according to size of the animal the temperature comes down gradually within a few hours after the injection. The urine of the animals if coffee coloured cleans up gradually from the second day of injection, when the animal is inclined to take conjee and tries to bite grass. There is progressive recovery but unlike the cases of *B. mutans* the animal needs toning up by a course of tonics. Among the cases of Red Water in cattle met with by me where the above treatment was carried there was no necessity to repeat the injection.

Relapses presumably as a result of fresh infection often occurs when the above line of treatment has to be repeated.

Prevention:—Elimination of ticks. A proprietary preparation by name "Pip" an insecticide has been used as a fine spray which immediately destroys all ticks on the body. It does not blister or injure the skin of the animal. For walls and crevices of cattle shed where no actual fire could be used on account of inflammable materials of shed the same "Pip" is sprayed in good quantity.

Spraying has to be repeated both for the animals and sheds and places at least 3 or 4 times at an interval of a week to 10 days.

An abstract of cases treated is furnished separately.

Abstract of Cases of Babesias in Cattle treated in the Coonoor Veterinary Hospital from June 1941 to end of December 1941

No.	Kind of Animal	Age	Date of Admission	Temperature on Admission	Result of Blood Smears	Treatment	Temperature after a day	Result	Remarks
1	Cross-bred Cows	6 years	23-6-41	104.6°F.	<i>Babesia bigeminum</i>	1% Acriflavine 30 c.c. intravenously. 24-6-41. Trypan blue 80 c.c.	105°F.	Died	Treatment at a very late stage.
2	"	7 "	3-7-41	106°F.	<i>B. mutans</i>	30 c.c. of 1% solution of Acriflavine intravenously. 6-7-41 60 c.c. "	105°F. 102.2°F.	Cured	The dose given on the first occasion was insufficient for the size of the animal which was about 700 lbs. Hence the second dose. The animal was perfectly normal in two days. Milk yield normal in 2 days.
3	"	7 "	31-7-41	103°F.	"	60 c.c. of Acriflavine 1% Aqua solution 60 c.c. "	101.3°F.	Cured	
4	"	4½ "	2-8-41	103.2°F.	"	19-8-41. 50 c.c. "	Normal.	"	
5	Heifer Cross-bred	2½ "	18-8-41	103°F.	"	1% 60 c.c. "	101°F.	"	
6	Bullock Cross-bred	6 "	13-12-41	105°F.	"	1% 50 c.c. "	102.2°F.	"	
7	Cross-bred Cow	5 "	12-11-41	105.6°F.	<i>B. bigeminum</i>	1% 50 c.c. "	Normal.	"	
8	"	5 "	10-12-41	106.4°F.	"	Acaprin 6 c.c. subcutaneously	102.2°F.	"	Took a considerable time to pick up condition.
9	"	6 "	16-12-41	106.2°F.	<i>B. mutans</i>	Acaprin 5 c.c.	Not seen.	"	Owner reported that the animal was well on the 2nd day itself.
10	" bred	6 "	16-12-41	107°F.	"	60 c.c. Acriflavine	102.4°F.	Cured	
11	Country bred	Aged	16-12-41	104°F.	"	Acaprin 6 c.c.	102°F.	"	
12	Cross-bred Bullock	Aged	17-12-41	104.8°F.	"	50 c.c. Acriflavine 1% Acaprin 6 c.c.	105.4°F.	"	
13	" Cow	6 years	18-12-41	105.2°F. (20-12-41)F.	"	20-12-41 60 c.c. of 1% Acriflavine	102.4°F.	"	As there was no improvement with the injection of Acaprin, Acriflavine was given on 20-12-41 and the animal was better the next day.

No.	Kind of Animal	Age	Date of Admission	Temperature on Admission	Result of Blood Smears	Treatment	Temperature after a day	Result	Remarks
14	Cross-bred Cow	Aged	20-12-41	105.4°F.	<i>B. mutans</i>	60 c.c. Acriflavine 1% solution intravenously.	102.2°F.	Cured.	
15	" Heifer	2 years	21-12-41	105.6°F.	"	40 c.c. Acriflavine intravenously 1% solution.	101°F.	"	
16	Cross-bred Cow	5 years	21-12-41	106.4°F.	"	60 c.c. Acriflavine 1% solution	102.4°F.	"	
17	Cross Sindhi "	1 year 8 months	22-12-41	106.4°F.	"	40 c.c. "	103.2°F.	"	The calf was normal on 24-12-41. Recovery was a bit delayed on account of the violent symptoms exhibited by the animal.
18	Cross-bred Cow	5 years	22-12-41	105.2°F.	"	70 c.c. 1% aqueous solution of Acriflavine.	102.2°F.	Cured.	
19	Country-bred Bullock	Aged	26-12-41	103°F.	<i>B. bigeminum</i>	Trypanblue 1% 80 c.c.		Died.	The animal was worked until the previous evening and was in a state of great prostration at the time of admission.
20	Cross-bred "	6 years	27-12-41	105.4°F.	<i>B. mutans</i>	Acriflavine 1% solution 80 c.c.	"	Discontinued attending the institution.	Owner reports that the animal recovered next day itself.
21	Cross-bred Bullock	Aged	28-12-41	105°F. 103.8°F. (31-12-41)	"	Acriflavine 1% 100 c.c. " 1% 60 c.c.	102.2°F.	Cured.	The first dose was insufficient for the size of the animal weighing nearly 900 lbs. hence the 2nd dose.
22	Cross-bred Cow	Do	31-12-41	107.2°F.	<i>B. bigeminum</i>	Acaprin 6 c.c.	"	"	The urine which was bloody cleared gradually from the 2nd day and was normal on the third day.

A PECULIAR CASE OF SPOTTED CONDITION OF THE SKIN IN A BUFFALO

BY

K. S. PATNAICK,

Veterinary Assistant Surgeon, Amalapuram

Subject.—A local-bred buffalo bull, aged about five years, was brought to the hospital on 8-11-1941 with fracture of the right femour bone and to my great surprise, the animal had white patches all over the body giving the appearance of a spotted deer.

History and symptoms.—The animal had this condition of the skin for the last one year. These patches first made their appearance on the hind quarters and slowly extended all over the body and head as far as knee and hock joints. The patches were more prominent on the head and the neck. They were on a level with the surrounding parts of the skin. But they looked like patches of ring-worm having the size of a pie at a distance of one inch from one another. After some time, grey hairs grew over the patches giving the appearance of a spotted deer. The grey hairs were longer than the ordinary black hairs of the surrounding parts. The owner had many other animals and none of them in the herd had this condition.

I submitted blood smears and scrapings from the lesions to the Principal, Madras Veterinary College, for microscopical examination. But the blood was negative for microfilaria or any other organisms and the scrapings were negative both for mange and ringworm.

Treatment.—Liquor Arsenicalis 2 drams was given every day for one week. But as the fracture for which the animal was brought to the hospital was slightly better, the owner removed the animal from the hospital and discontinued treatment.

I request my professional colleagues to give their experience if they have come across such a case at any time and if so, what could be the cause of the condition. If the condition was due to the loss of pigment and consequent white appearance of both the skin and the hair on the affected parts, whether the condition of depigmentation on certain portions of the skin, can be called "Leuco.Derma".

SULFARSENOL IN THE TREATMENT OF NERVOUS LOCALISATIONS OF DISTEMPER IN DOGS

BY

P. ABRAHAM, G.M.V.C.,
Touring Veterinary Assistant Surgeon, Penukonda, Madras

Recently when I was in charge of the Veterinary Dispensary, Anantapur, I treated two cases of Nervous Localisations of Distemper in dogs with Sulfarsenol and the results obtained induce me to record my observations as follows:—

Case No. 815:—A big sized Spaniel pup in good condition, aged about 6 months; admitted on 13-9-41.

Symptoms:—Temperature 106·2° F. Anorexia, not eating properly for the previous two days. Choric spasms of head, neck and both forelimbs (A single pustule was noticed on the abdomen on the 8th day after admission).

Diagnosis:—Distemper—Nervous form.

Treatment:—For the first three days symptomatic treatment for the relief of pyrexia and its accompanying symptoms was adapted. But the presence of chorea indicating grave prognosis created a problem whether it was worthwhile administering symptomatic treatment which gives no relief for chorea, or advise the owner the most merciful treatment—immediate destruction.

But remembering an interesting article on the treatment by means of Sulfarsenol of a case of Myelitis Meningitis—the result of distemper complication by M. E. Mondon published in the *Indian Veterinary Journal*, Vol. 9, No. 1, I thought that I should avail myself of this opportunity and try this medication.

In consultation with the owner on the clear understanding that it was only an experiment but not a guarantee for cure, treatment with Sulfarsenol was commenced on 16-9-41. The following injections of Sulfarsenol were administered in addition to the daily symptomatic treatment:—

16th September 1941	3 centigrammes
18th "	6 "
22nd "	9 "
24th "	6 "
26th "	6 "
28th "	12 "
2nd October 1941	15 "

Progressively increasing dose of the drug as thought necessary could not be given owing to its non-availability. All the injections were given subcutaneously for the sake of convenience. Each dose was dissolved in 2 to 3 c. c. of freshly boiled and cooled distilled water.

Up to 26th September 1941, no perceptible improvement was noticed in the choreac spasm of head, neck, etc. But after the 6th injection they gradually disappeared and with the 7th injection cure of chorea—cure if I can call it—was complete. But however, it was resolved to give some more injections to avoid further relapse of the complication. But at the request of the owner, they had to be stopped.

Pyrexia ranging from 106·0°F. to 103·0°F. and its accompanying symptoms did not seem to have been affected in any way with the administration of Sulfarsenol, because they continued for several days afterwards. As such, the animal had to attend the dispensary up to 25-10-41 till which date symptomatic treatment had to be continued and the animal was discharged cured on 25-10-41.

The pup has tolerated all the injections well except for a slight weakness noticed a few hours after every injection. The appetite also was maintained well throughout the course of treatment. Blood smears of the pup examined at the Madras Veterinary College revealed no organisms.

II. Case No. 835. A big size Spaniel pup in good condition aged about 6 months was admitted on 16-9-41.

Symptoms—Temperature 105·2°F. Mucous membrane injected, dull, anorexia and occasional appearance of rythmical movements of both forelimbs.

This pup though owned by a different person was one of the same litter as the first one and was moving with the first pup for some time almost every day.

Diagnosis: Distemper.

Treatment: Injections of Sulfarsenol were given as follows, in addition to the symptomatic treatment:

16th September 1941	3 centigrammes
18th "	6 "
22th "	9 "
24th "	6 "
26th "	6 "
28th "	12 "
2nd October, 1941	15 "

Here also progressively increasing dose of the drug as thought necessary was not given owing to its non-availability. Chorea which was less marked

in this pup began to vanish with the third injection and with the fourth injection complete cure was effected. But temperature ranging from 105.0° to 103.0°F. continued up to 11.10.41, till which date symptomatic treatment had to be continued. Though this animal also has tolerated all the injections well, except for a slight weakness noticed a few hours after each injection, it developed an abscess at the seat of the fourth injection behind the right elbow joint, which had to be opened and treated antiseptically till 20.10.41 after which date it was discharged completely cured.

Blood smears of the pup examined at the Madras Veterinary College revealed no organisms.

Though the administration of Sulfarsenol has not brought about any changes on temperature and its concomitant symptoms, its beneficial effects on chorea were undeniable.

The striking peculiarity of the two cases of distemper under reference was that chorea which would generally appear as a complication during convalescent stage was noticed right from the beginning of the attack.

A STRANGE CASE OF RABIES IN A DOG

BY

S. RAMANARAYANAN, G.M.V.C.,

Veterinary Assistant Surgeon, Coonoor, Madras.

Subject:—A Cocker Spaniel dog, age 3½ years.

History:—He was described by the owner as of a very gentle temperament but rather nervous with strangers. When disturbed had a tendency to snap at persons including the owner. He had bitten in the course of the year four persons but it is asserted that he snapped only on provocation. On 30th July 1942, i.e. on the last occasion, he had badly bitten a boy on the face; this time also it was stated that he bit only when the boy attempted to disturb the dog.

The dog was admitted for observation on 30.7.1942 at the Veterinary Hospital, Coonoor.

During the course of his stay in the hospital, for observation for rabies, he was perfectly normal and fed well except on 7.8.1942 when he was in a state of frenzy—nobody could go near his kennel, even to serve food. He frowned and attempted to bite anybody approaching, but took his evening feed well that day. He was perfectly normal from that day forwards. Even though the period of observation was over on

A SUCCESSFUL CASE OF RUMINOTOMY IN A BULLOCK 151

9.8.1942 he was detained till 12.8.1942 under instructions from the Director, Pasteur Institute, Coonoor. With the consent of the owner the dog was put to sleep on 12.8.1942.

The brain of the dog was forwarded to the Director, Pasteur Institute, Coonoor, for examination. The Director in his B. No. 231 dated 15.8.1942 declared that the brain was positive for Rabies.

Remarks:—It is interesting to note that a dog that was clinically normal to the very minute of being put to sleep except for a state of frenzy observed 6 days previously, had shown positive signs of Rabies on microscopical examination. It is not known at what stage of infection degeneration of the nerve cells of the sympathetic ganglia occur and the Negri bodies are discernable.

A SUCCESSFUL CASE OF RUMINOTOMY IN A BULLOCK

BY

N. SUBRAHMANYAN, G.M.V.C.,

Veterinary Assistant Surgeon, Mayavaram, Madras

Subject:—A middle aged country-bred bullock, fair in condition.

History:—The animal was brought to the dispensary on 10.3.1942 for treatment with the history that it was getting frequent attacks of tympanitis for about a fortnight or more and that the treatment given in the village had not effected any relief.

Symptoms:—The animal looked dull and listless, the abdomen was bloated and the swelling on the left side being more pronounced, was very resilient and gave a drum like sound on tapping. Temperature was 101.8°F. Bowels were much constipated and the animal often attempted to defecate and passed at times hardened pellet-like masses of dung with considerable difficulty.

Diagnosis:—Chronic Tympanitis.

Treatment:—The animal was given a warm soap water enema. The use of the probang and massage to the left flank did not do much good. An oleagenous mixture containing acid Carbolic, Ol. Terebinthenae and Tr. Asafoetida was given, resulting in slight relief by about the evening, which was only temporary. The condition recurred again the next morning with more distressing symptoms and necessitated the use of the trocar and canula. Though repeated doses of moderate saline purgatives and medicaments to tone up the gastric walls for about a week had given some temporary relief and the animal had begun to graze in the compound voluntarily and ruminate a little, the progress was not marked and permanent. Further recurrent attacks led to suspect the presence of some foreign body in the

stomach, and so it was finally decided to perform the operation of rumino-tomy with the consent of the owner who gave it readily.

The site of operation was shaved and washed well with antiseptic lotions and neatly prepared. The animal was placed on the right side against a wall with one man at the head and two other assistants to steady the animal. The hind legs were secured just above the hock with a slip knot and a pole passed in between them and held pressing back over the left thigh. An incision about 5" long was made beginning from the usual seat of puncture downwards on the skin, abdominal muscles, peritoneum and the wall of the rumen. As soon as the stomach was opened there was a rush of foul smelling gas and undigested food materials. Enlarging the opening in the rumen so as to allow the passage of the arm, the sides and the lower angle were secured along with the skin and muscles of the abdomen by means of tapes (with one end tied to a piece of tow to serve as a knot or button) passed from outside in and held up by the attendants and a clean cloth stiped in antiseptic lotion and rung out was spread over the sides to prevent food materials falling into the peritoneum. The hand after thorough cleansing was passed into the stomach and its contents thoroughly examined. Huge masses of undigested straw coming in the way, having been removed, the fingers came in contact with a large deposition of sand at the bottom of the stomach. After removing the same little by little about two pints of warm water containing two drams of Formaldehyde were poured inside. Then the edges of the wound were cleaned and the opening in the rumen was stitched with sterilized catgut (Lambert's suture), the abdominal muscles with silk ligature (continuous suture) and the external skin with interrupted sutures. The wound was then dressed with Tr. Iodine and powder dressing. The animal was allowed kanji and libidum and also allowed to graze as it pleased in the compound. It was put on a course of tonic powder and the operation wound daily dressed for about ten days when it had healed beautifully and the animal was discharged cured. There was no further recurrence of the disease.

Conclusion:—From the above case, it is quite evident that when animals are fed on impure foodstuffs, it is very likely that the impurities such as sand, etc., contained in them get accumulated and paralyse the motor power of the stomach causing tympanitis. Further the remarkable power of resistance of the animal to the operation and subsequent healing of the operated wound without any sequelae are noteworthy.

REFERENCES

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2. Encyclopaedia of Veterinary Medicine, Surgery and Obstetrics by George H. Wooldridge.

HEPATOZOON CANIS AND ITS TREATMENT

BY

MUHAMMAD RAHIMUDDIN, G.M.V.C.,

District Veterinary Officer, Vizagapatam, Madras

Introduction:—Hepatozoon Canis is a protozoal disease of dogs transmitted by ticks '*Rhipicephalus Sanguineus*'. According to certain authors, it is said that this protozoon causes no disease in dogs. But 9 cases confirmed for the disease and admitted for treatment in the veterinary hospitals of Vizagapatam, Tekkali and Parvatipur during the last ten months have all died except one.

Confirmation of the disease:—Blood smears from the auricular vein stained with Leishman's Stain and examined will show, in positive cases, the organisms having the resemblance of a sausage imbedded in certain white cells of the blood.

Symptoms and Treatment:—Symptoms manifested by these animals were fever, dullness, inappetance, marked emaciation, blanched condition of the buccal mucous membranes and conjunctiva, exhaustion and death.

It will be worthwhile to mention here two interesting cases. One a cross-bred spaniel male pup, about 6 months old, was apparently looking healthy, but one day it showed some symptoms of lumbar paralysis and was sent to the Veterinary Dispensary, Parvatipur, for treatment. The Veterinary Assistant Surgeon in charge of the institution successfully treated the paralytic attack but as it had a temperature he sent its blood smears from the auricular vein for examination. These revealed a large number of parasites (*Hepatozoon Canis*). A few days after the incident the writer had to visit Parvatipur and learnt that the pup was alright with Quinine Sulphate treatment. On inspection, it was found to be in good condition and had no temperature; still, examination of blood smears revealed a large number of the organisms. Thinking there will be a relapse sooner or later, the owner of the dog was personally advised to submit the dog for further treatment for which he agreed, but unfortunately, two days later, the pup suddenly showed symptoms of lumbar paralysis again and died within a few hours.

The second case was a cross-bred terrier owned by a gentleman in Tekkali. In this case, it was reported by the Veterinary Assistant Surgeon in charge of the Veterinary Dispensary, that he used "Solu Stibum No. 3" (Central Laboratory, Calcutta) intramuscularly in dose of $\frac{1}{2}$ c.c. A second dose was repeated after a fortnight and third and fourth after a week's interval. The patient also received Quinine Sulph internally.

Soon after the completion of the treatment the blood of the patient was examined and the result was negative. During the inspection of Tekkali dispensary on 4th May 1942, *i.e.*, about two months after by the writer the animal was sent for and its blood examined. No organisms could be found. It maintained apparently normal health. Hence it would appear that "Solu Stibum" in conjunction with Quinine Sulph possess curative action on *H. Canis*.

Being a blood parasite it was thought that arsenical and antimony compounds, such as Sulpharsephenamine and Antimony Tartaratum would do some good but they had no effect. Quinine Sulph internally and "Solu Stibum" intramuscularly may be given extensive trial and the results published. Maphariside (Park Davis & Co.) may also be tried, as this drug is said to give curative results in Black Head in Turkeys. Further investigation into this disease is necessary, as the mortality is very high.

Summary:—(1) The disease affects all breeds of dogs including the local bred ones.

(2) The prominent symptoms of the disease are progressive emaciation, blanched condition of the buccal mucous membrane and conjunctiva, fever of an intermittent nature and lumbar paralysis.

(3) Quinine is reported to have relieved the condition only temporarily: Antimony and Arsenic compounds did not cure the disease.

(4) Quinine Sulph with "Solu Stibum" is reported to have given a complete cure in one case.

(5) Percentage of mortality is very high—probably 90 per cent.

Before concluding the article, I wish to offer my thanks to the Principal, Madras Veterinary College, for kindly confirming the diagnosis and to the Veterinary Assistant Surgeons Messrs. K. Mallikarjuna Rao, Sheik Chengishah and B. Benjamin for furnishing necessary information on the subject.

PUS IN THE GUTTURAL POUCH—TREATMENT WITH SULPHANILAMIDE

BY

V. VENKATARAMAN, G.M.V.C.,
Veterinary Assistant Surgeon, Dindigul, Madras

Subject:—Colt, local bred, in fair condition.

History:—Owner observed a swelling near the left parotid region which began increasing in size daily, with mucopurulent discharge from the left nostril to begin with, then from both, the discharge being greater when the head was lowered, inability to feed properly, respiration difficult, bowels constipated. Period of illness before admission to the hospital was about 12 days.

Symptoms:—A fluctuating painful swelling on the left parotid region, mucopurulent discharge from both the nostrils—profuse when the head was lowered and also when the swelling was pressed; respiration stertorous with mouth open; temperature 103°F., visible mucous membrane slightly congested, slight inflammation of the sub-maxillary gland.

Examination of the discharge revealed numerous streptococci and a few staphylococci.

Treatment:—Irrigated the nostrils gently with warm saline, pressing the parotid region. Injected 2 c.c. Liquor Adrenaline Hydrochloride subcutaneously. Applied dehydrated Mag. Sulphas saturated with glycerine over the parotid region, gave steam inhalation with Oleum Eucalyptus and Tr. Benzoin Co. and electuary containing Pot. Chloras, Hexamine, Creosote, Camphor and Pulv. Glyceyrrhiza Co., Mag. Sulphas; Ammonium Chloride and Pot. Nitrates in drinking water. 6 Tablets of Sulphanilamide were also given orally (3 in the morning and 3 in the evening).

Second day:—Temperature 103°F. Condition of the animal slightly better, respiration improved, repeated treatment.

Third day:—Temperature 102.4°F. Condition better, respiration markedly improved, swelling and discharge less, feeding well. Treatment repeated.

Fourth day:—Temperature 101.6°F. Condition much better than in the previous day. Respiration normal. Treatment repeated.

Fifth day:—Temperature 101.6°F. Animal showed decided improvement, swelling of the parotid region and discharge from the nostrils had considerably reduced. Patient looked brighter and took the normal feed.

have passed urine for the past 2 or 3 days and the dropsical condition was due to accumulated urine in the abdominal cavity.

This case was on the next day tapped on my representation to the District Veterinary Officer, and the abdominal content found as ascitic fluid. The heifer was given ascitic mixtures with Potass Iodide and Digitalis, injections of extract of liver and was discharged temporarily cured. The case was again asked to be brought to the hospital for further examination and the animal was found fit. Subsequently I learnt that the animal was sold away in a fit condition.

The object of writing the note on this article is to press home the fact that in treatment of cases involving major surgical interference, arriving at as conclusive a diagnosis as is possible by using all methods of examination at our disposal is necessary. In this case a diagnosis of rupture of bladder has been rather hastily arrived at, but as luck would have it, the result of the operation has been successful as seen from the concluding portion of the article.

I shall be obliged if the authors of the article will comment on my note and enlighten me.

S. RAMASWAMI, G.M.V.C.,
Veterinary Assistant Surgeon.

Trichinopoly.

The above criticism was forwarded to the author who has replied as follows :—

SIR,

I have for the past many years examined many calves belonging to milkmen, but I have seen only 2 or 3 calves suffering from ascites. For the past two-and-a-half years that I am here, I have not seen any case of ascites in calves in this town.

In cases suffering from ascites, I have not seen retention of urine, and it is not one of the symptoms recorded. Some of the other symptoms as kicking at the belly and straining to urinate and peculiar anxious look are not usually present in animals suffering from ascites.

Diagnosis of rupture of the bladder was only provisional and it was confirmed as such only after operation; this diagnosis was made only with reference to a study of three previous cases of this type. Question of "jumping at conclusion" does not therefore arise. Further, I had no reason to suspect the veracity of the owner's statement.

As regards the opinion of the critic "that the bladder must be operated on in situ" there is no such anatomical impossibility as the critic imagines. The bladder can be brought out and replaced, as was actually done by us.

V. V. VENKATACHALA IYER,
Veterinary Assistant Surgeon.

Madura City.

A Note of Corroboration on the article "Care of Horns"

By T. N. Kulkarni, G.B.V.C., Veterinary Assistant Surgeon

(Indian Veterinary Journal, Vol. XVIII, No. 1, July 1941)

SIR,

While I completely agree with Mr. Kulkarni in his observation that most of the cases of horn fractures in bullocks are due more to paring of horns than due to natural accidents, I wish to offer the following corroborative evidence on the subject.

Paring of horns seems to be a very common practice in this part of the presidency also, namely, Chittoor District. Owners of cattle take a fancy to shave the horn indiscriminately, evidently with a view to make the animal look smarter and younger. Not satisfied with this, they go to the extent of branding all round the base of the horn, with a firing iron to an extent of even $\frac{1}{4}$ to $\frac{1}{2}$ inch deep, so that it may give a clean cut appearance. When I had occasion to observe some of the newly pared horns, I found that they were thinned to such an extent that I could see the purple vascular structure inside through the semi-transparent horny wall. I have also observed that congestion of the part can be easily effected at the slightest irritation such as a knock or a hit against a hard object, when the horns are so thinned.

It is therefore no wonder that about 60 per cent of horn affections, ranging from a slight fissure to a complete fracture are due to this malpractice.

Animals affected are mostly working bullocks of prime age and good condition. The affection is occasionally met with in cows and rarely in buffaloes.

Symptoms.—Symptoms vary according to the severity of the affection ranging from a slight crack or fissure over the horn upto deep sinuses involving sometimes the whole horn. Generally the affection starts with a contusion as a result of external violence over the unduly thinned horny layer. The extravasation can be seen through the transparent horn. A minute slit in the outer wall of the horn is enough to set up infection. When once the infection sets in, the horn begins to bulge, the slit becomes enlarged

Due to an urgent call from home, the owner had to go back to his village and he took the animal with him. Advised him to give sulphanilamide 3 tablets a day orally for 3 more days with electuary and Mag. Sulphas packet to be given orally and Pigmentum Belladonna to be applied externally to last for 30 days.

The owner reported after a month that the animal was completely alright in about a week after he left the hospital.

Conclusion:—The successful non-operative treatment appears to me to be due mainly to the administration of Sulphanilamide. No untoward effects were seen when the animal was given magnesium sulphate along with sulphanilamide.

Correspondence

Rupture of the Bladder in a Bull-calf

SIR,

The article on the above subject contributed by Mr. V. V. Venkatachala Iyer and Mr. A. V. Hariharan in the *Indian Veterinary Journal*, Vol. XVIII, No. 4, is interesting, in that the subject of the article underwent a successful operation of the bladder.

Abdominal sections in the bovine, in spite of their resistance to septic infection, are undertaken only when all other remedial measures have been tried and found unavailing.

Cases of rupture of the bladder in bovines in general and calves in particular though not rare are certainly not common. Many cases of ascites in calves go unrecognised and pass for parasitic pot-belly. A few cases of rupture of the bladder pass for ascites which in turn go off as pot-belly in debilitated conditions in young calves.

Calves born of cows under milk-vendors are usually subjects of this complaint (ascites). Milkmen allow calves to suckle only for a minute or two just to stimulate the gland and strip off all milk clean. Young calves whose main food is mother's milk are fed on straw and concentrated food as a substitute for mother's milk. Thus all protein is denied to them resulting in their becoming weak, hide-bound, shaggy and pot-bellied. An examination of these calves will reveal almost 50 per cent. of them ascitic.

Rupture of the bladder cannot be definitely diagnosed until the nature of the fluid in the abdomen is ascertained. In very patent cases an examination of the bladder per rectum will reveal the condition of the bladder. A ruptured bladder will lie flat on the floor of the pelvis.

Generally in difficult cases for diagnosis elimination of doubtful diseases with similar symptoms should be the first attempt.

Symptoms observed in the present case on arrival at the hospital are common and nothing definite in them to arrive at a diagnosis of the rupture of the bladder. Cannot one find these symptoms in a case of ascites? Ascites in calves is common, at any rate, more common than rupture of the bladder and in ascites in bovines, kidney affection would seem to be more usual, than that of the liver as in the case of dogs.

The "kicking at the abdomen and straining to urinate" may be due to a distended bladder, may be due to the pressure of fluid in the abdomen, not necessarily urine pressing against the bladder. In such a case the abdomen could have been easily tapped and the fluid recognised either as urine or as a peritoneal transudate.

"No micturition for the previous five days" is a statement of the owner which should have been very cautiously swallowed. A calf which had not voided urine for more than 48 hours should have attracted the attention of the most careless and irresponsible owner or servant.

It is said that the bladder was brought out "gently" though and "replaced in the abdominal cavity." This is an anatomical impossibility. I think the bladder must be operated upon in situ. Unless the urethra is a loose tube lying in the midst of the male generative organ it cannot be pulled back to admit of the bladder being taken out of the abdominal cavity.

It is said there was a puncture in the inferior aspect of the bladder about 1/12 of an inch. How was any urine retained in the bladder with a puncture like this at the time of the operation? It is said that the bladder was full. It may be explained that the small gritty calcareous deposits found in the bladder blocked up the slit. This is rather difficult to accept when urine has flown out into the peritonium through this slit. The force of gravity and the force of the distended bladder should have forced down urine displacing the obstruction of deposits and all fluid should have drained out into the cavity. There is only one occasion when the bladder with a slit in it can be found to be full and that is when the fluid in the abdomen is so full that the bladder is floating in it.

In this connection, I like to mention a case which came under my notice recently in my hospital work. It was a heifer calf about ten months old. It came with the history that it had diarrhoea, was off its food and not passing urine for the past 24 hours with a pendulous abdomen. I suspected the case to be one of ascites and was examining the dung for parasites. The then second assistant told me that the case was one of rupture of the bladder and the statement of the attendant that the animal had not passed urine for the past 24 hours could not be relied upon, suggesting thereby that it should not

and inflammatory exudate begins to ooze out. It is at this stage that the owner begins to take notice of the case. Either he brings it immediately to the hospital or has recourse to crude treatment by which means he makes it worse. I have seen cases wherein the horns, which appeared normal at the first sight, have on closer examination been found to be either cancerous or become very highly inflamed and infected—the whole of the internal structure having become completely destroyed and the whole cavity filled with inflammatory exudate.

Site of injury.—Injury may occur at any part of the horn from the base to the tip. In cases that have been branded at the base, there is an inflammatory ulcer in the form of a fibrous band of about $\frac{1}{2}$ inch thickness all round the horn over the skin at the base, which has no tendency to heal but keeps on throwing out exuberant granulations. The portion of the wound just near the base of the ear is very persistent to heal as the ear keeps on rubbing the wound by shaking. One bullock (case No. 200) received treatment for 12 days but stopped away since no improvement was noticed.

Cracks or fissures occur generally at the middle of the horn. Healing is equally difficult in this also. One case for instance, bullock O. P. 222, which was admitted for a small crack of about 1" by $\frac{1}{4}$ ", received treatment for 72 days and the horn had to be amputated before cure was effected. Sinuses start generally from the tip in the great majority of cases, both as a result of excessive shaving and capfixing.

Treatment.—Treatment adopted varied with the nature of lesions. From mild astringent like the white lotion upto strong caustics like chlorinated lime and zinc-chloride have been used. In freshly amputated cases white lotion bandage was supplied for the first two or three days and then it was substituted by hydrargeri perchloride of varying strength (1 in 500 to 1 in 2000). In unhealthy granulations copper sulphate was used. If the growth persisted eusol paste or zinc chloride gauze (5 to 10%) was used. Amputation had to be done in seven cases of which three were broken horns, two fissured and two sinus horns.

Percentage of cure effected.—In all, twenty cases were treated for the various affections of horns of which only four got completely cured. Hence the percentage may be roughly put to twenty.

The following table gives the details of horn affections treated at the Veterinary Hospital, Madanapalli, for twelve months from January 1940 to December 1940:—

S. No.	Case No.	Kind of animal.	Ulcer around the base of the horn	Cracked or fissured horn.	Sinus horn	Broken horn.	Cancer horn.	Whether medically treated.	Whether surgically amputated.	Duration of treatment in days	Result
1	2	3	4	5	6	7	8	9	10	11	12
1	3794	Cow				+			+	25	C
2	3830	"				+			+	26	C
3	4072	Bullock			+			+		1	D
4	4137	"			+				+	1	D
5	4157	"	+					+		1	D
6	4379	"				+		+		4	D
7	4667	"					+	+		3	D
8	4746	Buffalo				+		+		4	D
9	46	Bullock				+		+		4	D
10	77	"		+				+		1	D
11	78	Cow				+		+		1	D
12	200	Bullock	+					+		12	D
13	210	"			+			+		1	D
14	222	"			+				+	72	C
15	271	"					+	+		7	D
16	352	"					+	+		38	D
17	398	Cow				+			+	15	D
18	584	Bullock		+					+	10	D
19	955	"		+					+	22	C
20	1065	Cow				+		+		1	D
			2	3	4	8	3	13	7		4 C 16 D

N.B.—C denotes cured and D denotes discontinued.

Observations.—From the above table it would appear that, out of twenty cases treated, fourteen were bullocks, five were cows and one was a buffalo. While the affection among bullocks varied from slight ulcer around

the base of horn to either broken horn or cancer, it is very interesting to note that all the five cows and one buffalo were all cases of broken horns—other affections like ulcer, fissure, or cancer being totally absent, thereby indicating that manual interference with the horn in these animals is very rare. Again, out of a total of fourteen bullocks affected, there are only two broken horn and three cancer cases—the remaining nine being cases of minor injuries like ulcer, fissure of sinus horns, all of which are purely due to excessive paring of horns. Even the three cases of cancer, since they have occurred in bullocks and not in cows or buffaloes, it may not be wrong to presume that the same manual interference is the predisposing cause of this affection also.

Thus, it is proved that out of a total of twenty cases treated at this institution, only eight are due to pure accidents while the remaining twelve are due to preventable causes.

Findings.—1. Horn affections seem to be very common in Chittoor district.

2. Animals affected are mostly bullocks of prime age and good condition, occasionally cows and rarely buffaloes.

3. While minor injuries like fissures, ulcers and sinuses are purely confined to bullocks, cases of accidental broken horns are comparatively few and they are mostly met with among cows and buffaloes.

4. Minor affections of horn invariably prove to be major and serious in spite of the best attention possible.

5. Percentage of cure in the treatment of horn affections seems to be small.

Conclusion.—Since about sixty per cent of horn affections appear to be due to manual interference, owners should be strongly advised not to interfere with the horn of their cattle.

M. RAMAKRISHNA PILLAI, G.M.V.C.,
Veterinary Asst. Surgeon i/c Veterinary
Hospital, Madanapalli.

College News

MADRAS VETERINARY COLLEGE

PANIKKAR MEMORIAL FUND

List of Subscribers to the Fund from 1-9-42 to 3-11-42
(Continued from Page 108, September 1942 Issue of 'I. V. J.')

		Rs.	A.	P.
	Brought forward	...	327	1 0
		...	3	0 0
77.	Sri C. V. Padmanabhan	...	2	0 0
78.	" P. S. Narayanan	...	1	0 0
79.	" N. Balasubramanian	...	2	0 0
80.	" R. Avudayappa Chettiar	...	2	0 0
81.	" K. Narayanaswamy	...	2	0 0
82.	" B. Manavalasawmy	...	2	0 0
83.	" B. Ramayya	...	1	0 0
84.	" C. Sriramulu	...	10	0 0
85.	Dr. F. C. Minnet	...	2	0 0
86.	Sri A. A. Subramanian	...	10	0 0
87.	" M. A. Rangasawmy Ayyar	...	2	0 0
88.	" V. Ramalingam	...	1	0 0
89.	" R. S. Rangan	...	2	0 0
90.	" S. Seshadhri Iyengar	...	2	1 0
91.	" M. V. Sankaranarayanan	...	1	0 0
92.	" T. Patnaik	...	5	0 0
93.	" P. Srinivasa Rao	...	5	0 0
94.	" S. Vaidyanatha Mudaliyar	...	15	0 0
95.	" R. Swaminathan	...	5	0 0
96.	" V. Mahadevan	...	5	0 0
97.	" L. S. Parameswara Iyer	...		
	Total	...	407	2 0

R. SWAMINATHAN,
Lecturer,
Madras Veterinary College.

For tissue cultures and animal inoculation both blood as well as milk were used. For details of the methods adopted to obtain filtrates and technique of transfer for the passage, see the original.

Method employed was the same as in the cultivation of measles and scarlet fever viruses. The tissues used were chiefly fibroblasts, epithelium and white blood cells from chick and mouse embryos; one additional series of human lymph node cells was carried to the fifteenth passage only. Nutrient medium consisted of homologous blood plasma and embryo extract. A minute drop of the virus filtrate was added to the experimental cultures with identical controls lacking the filtrates maintained for each series.

The cultures were fixed in Carnoy's solution (3 parts absolute alcohol, 1 part glacial acetic acid) and stained. Pyronin methyl green was found very satisfactory for the tissue cultures and gave a good differentiation, showing blue nuclei, pale pink cytoplasm and red inclusion bodies.

Storage of the filtrates at 5°C. for four weeks did not inactivate them.

Injected tissue cultures transplanted into mice cause inflammation of the lymph nodes and mammary glands of mice and from them the virus has been re-isolated.

The virus has been carried through 32 serial transplants in tissue cultures.

Cornell Vet. 29, No. 3, July 1939, pp. 261-270.

Obituary

We regret very much to record the demise of Mr. G. Avudanayagam, G.M.V.C., District Veterinary Officer, Vellore, on 12th September 1942, and Rao Sahib C. Suryanarayana Murti, G.M.V.C., Retired Superintendent, Madras Serum Institute, at Ramarowpeta, Cocanada, on 2nd October 1942.

We offer our heartfelt condolences to the members of the bereaved families.

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Abstracts

A study of the possible relationship between non-specific mastitis and streptococci infection of the bovine udder—By E. H. PETERSON AND E. G. HASTINGS.—There appears to exist some relationship between streptococci and chronic mastitis, exhibiting chronic subclinical symptoms as contrasted with the less common acute or clinical type.

The evidence is conclusive that in these mastitis the portal of entry is galactogenous and yet attempts to produce the disease under natural conditions have resulted in a relatively large number of failures.

Since the findings through recent developments in mastitis research, of the existence of non-specific mastitis, characterised by udder inflammation in the absence of streptococci or any other recognised pathogen, the authors undertook to determine, whether or not, this abnormal condition could predispose the udder to invasion with streptococci.

Typical strains of *Streptococcus agalactiae* causing no observable systemic reactions, and appearing to be entirely typical of the low grade, subclinical type of streptococci mastitis found the world over were isolated from host cows; 8—12 hour dextrose broth cultures were used in all experiments on udders. 10 cows showing varying degrees of "non-specific mastitis," in one or more quarters were subjected to these tests.

Out of 34 quarters to which streptococci were applied externally, the maximum number of times applied to any one teat not exceeding four, 9 became infected; out of 17 quarters inoculated with small numbers of streptococci by inserting small glass capillary rods previously dipped into the prepared cultures through the sphincter, 15 became infected on the first attempt, one upon the second. No further attempt was made on the remaining one. All these indicated of being permanent in nature as the organisms persisted in milk in every case until the cows were slaughtered.

A discussion on the pathology of the udder in streptococci mastitis with the findings of other workers follows and the authors conclude that non-specific mastitis apparently lowers the resistance of the mammary gland to infection with *Streptococcus agalactiae*. *Streptococcus agalactiae* can be quite easily transmitted to quarters affected with non-specific mastitis by experimental infection of the teat. Quarters affected with non-specific mastitis are readily susceptible to internal infection with small numbers of *Streptococcus agalactiae*. The physical changes produced in milk as a result of the super-imposed bacterial infections are quite proportional to the degree of involvement of the quarters with non-specific mastitis.

The pathological changes produced in mammary tissue by non-specific mammary inflammation are identical to the changes observed to be associated with chronic streptococci mammary infections with the exception that a discontinuous infiltration of polymorphonuclear leucocytes, not observed in the former case, is a characteristic of chronic streptococci infections of the mammary gland.

The suggestion is made that non-specific mastitis is a primary or predisposing condition and *Streptococcus agalactiae* a secondary invader in chronic bovine mastitis.

Cornell Vet. 29, No. 1, January 1939, pp. 11-24.

A filtrable virus isolated from mastitis cows—By JEAN BROADHURST, GLADYS CAMERON AND M. ESTELLE MACLEAN.—While mastitis may be caused by a variety of organisms, each capable of invading the udder and producing identical conditions, according to the authors, the probability of an initial or primary agent, such as a virus, which itself unrecognised, favours the development of those organisms as secondary infections cannot be completely disregarded.

The diagnosis of mastitis by plating the suspected milk (conducted as an enrichment method) would fail to eliminate the possibility of a virus which is not culturable under laboratory conditions and which does not form colonies on agar plates. This is a situation not unlike that in scarlet fever where tests made on blood agar plates show only the attendant streptococci and never the filtrable virus which can be isolated by special methods only. Again, the ineffectiveness of sulphanilamide and like preparations in scarlet fever when compared with their results in streptococci infections in general and the place of serum used to act against the virus read with the ineffectiveness in many cases of mastitis leads to the logical thinking of a virus etiology in mastitis.

The authors tested the filtrable virus hypothesis by (1) microscopic study of the cells present in fresh mastitis milk for inclusion bodies and (2) tissue culture inoculation with filtrates of the blood and milk of mastitis cows.

Milk collected in 10-15 c.c. amounts in sterile test-tubes at the end of the milking process were iced, rapidly transported to the laboratory, and the examination conducted in 2—4 hours. The tubes were centrifuged to precipitate the udder and blood cells. A drop of material, pipetted from the bottom of the tubes and delivered on a slide was spread by a platinum needle in a thin smear, dried promptly usually in a 37°C. incubator, fixed with heat or 95% alcohol and stained.

The most differential results were obtained with a modification of Seller's rabies stain by bleaching the slides in alcohol (one dip rinsing again in water). The white corpuscles stain more or less pinkish, the streptococci are dark or purple blue. Inclusion bodies may not be demonstrable in samples kept in the refrigerator for 24 hours although they had been fairly numerous in the same iced sample examined the day before. In a sample received late after collection due to delay in transit inclusion bodies could not be demonstrable but typical ones developed in tissue cultures inoculated with filtrates from it. In another sample of abnormal milk (greyish in colour and without a cream line) 5 or more % of the cells in a smear contained one or more inclusion bodies. The white cells were mainly mononuclears, the low polymorphonuclear incidence being very marked.

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