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A Tetrachlorethylene Emulsion as an Anthelmintic.

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In a previous paper (1936) the authors reported on anthelmintic tests with tetrachlorethylene against the hookworm *Gaigeria pachyscelis* and other nematodes of sheep. In this connection it was mentioned that a mixture of the drug with mineral oil had two distinct disadvantages, viz. that fumes of the drug tended to cause coughing and choking when the sheep were not drenched very carefully and that the rapid absorption of the drug from the digestive tract produced giddiness and anaesthesia. Brief information was further given on attempts to overcome these difficulties by emulsifying the tetrachlorethylene and some success had been obtained with an ordinary soap emulsion.

In this paper it is intended to report on the further development of tetrachlorethylene therapy against the *Gaigeria* hookworm and other worms incidentally affected.

The soap emulsion beforementioned overcame the trouble of coughing and choking, but it was not satisfactory for several reasons. When shaken, this emulsion formed much forth, which led to inaccuracies in its use. It had to be made and diluted with distilled or very soft water. It broke in the abomasum and so did not prevent giddiness due to rapid absorption. A stable emulsion of tetrachlorethylene alone could not be obtained, so that liquid paraffin had to be added, thus increasing costs and volume for transport.

In the further investigation it was attempted roughly to standardise the technique used, in order to obtain a basis for comparison of the different emulsions made, in regard to their physical properties. A stirring apparatus was made, consisting of a vertical shaft carrying two small propellers, of which the lower one produced an upward and the upper one a downward current. The distance between the two propellers on the shaft could be altered. The shaft was revolved at a rate of about 300-400 r.p.m. by a small electric motor taken from an old fan. The speed would naturally vary with the viscosity of the emlusion. The stirrer worked in a glass cylinder which was slightly wider than the width of the propellers.

It is obvious that the apparatus is not comparable in efficiency to modern emulsifying machinery and that a very high dispersion cannot be obtained with it, but it proved to be a satisfactory instrument for preliminary tests.

When an emulsion had been made a small sample was diluted with water on a slide and the size of the particles was measured under the microscope. Another sample was diluted with 4-10 parts of tapwater (which is fairly hard) to test the effect of such dilution on the emulsion and to observe the rate of precipitation on standing. Further samples were mixed with 1 and 1·5 per cent. concentrations of hydrochloric acid. In general the effect of a 1·5 per cent. hydrochloric acid solution on such an emulsion was found to be similar to the effect of fresh abomasal juice of sheep. The effect of the latter itself was also tested on various occasions. Further samples of the emulsion were left standing in order to determine their keeping qualities and samples were also placed in a refrigerator to test the effect of low temperatures.

It is not intended here to present a full report on all the substances tested as emulsifiers and stabilisers, but it should be mentioned that many were tried, including substances now widely used in emulsification, such as sulphonated castor oil, triethynolamine, silicates, etc. The result of these investigations was an emulsion of tetrachlorethylene, made with a resin soap as emulsifier and casein as stabiliser, described below. In arriving at a decision as to the most suitable form of emulsion, the main factors considered were simplicity, cheapness both in manufacture and transport, keeping qualities and above all suitability for the purpose in view as well as efficacy against the parasites.

Resin soaps have been and are being used in the manufacture of many emulsions. They are easy to make and have one particular advantage that they can be diluted with any ordinary water, since the calcium and magnesium resinates are soluble in water.

Casein as stabiliser is also well known. It is cheap, since only small quantities are required, and it can be readily incorporated in the resin soap. As stabiliser for the tetrachlorethylene emulsion it is quite satisfactory and it plays an important part in retarding absorption of the drug, as will be mentioned later.

Resin soap alone does not produce a satisfactory emulsion, because it is broken by acid, although in other ways the emulsion is not a bad one, as is shown by the following example:—

Emulsifier: Water 800 c.c., NaOH 5 gm., Resin 40 gm.

Emulsion: Emulsifier 50 c.c., Tetrachlorethylene 150 c.c. A moderately thick emulsion (pours well but flows slowly).

Particles: Mostly very small, largest measuring 6·5 μ.

Dilution: No change; precipitation 1/60 in first hour in 1:4 dilution.

Acid resistance: 1·5 per cent. HCl breaks emulsion immediately.

Keeping qualities: After 14 days no change, then discarded.

Series of emulsions with resin soap and casein were made in order to determine the most satisfactory proportions of each of the ingredients, particularly in the emulsifier.

Resin.—The quantity of resin used is of no great importance above a certain minimum. Excess of resin, above the minimum, if it is all saponified, will produce a thicker emulsifier and a thicker emulsion, but no important change in the size of the particles. The emulsion, however, soon becomes so thick that it does not flow readily and is therefore unsatisfactory in practice. If not saponified, excess resin is dissolved by the tetrachlorethylene, also producing a thicker emulsion. The quantity of resin determined as optimal in laboratory tests was 5 per cent. of the emulsifier.

Casein.—The optimum quantity of casein corresponds to the quantity of resin used and is also 5 per cent. of the emulsifier. A smaller quantity produces a thinner emulsion and the particle size increases. A larger quantity produces a thicker emulsion without decrease in particle size.

Alkali.—The quantity of alkali must be exactly sufficient to combine with the resin and casein, leaving no excess of free alkali in the emulsifier. Too little alkali will leave unsaponified resin and uncombined casein and produces a thinner emulsion which does not keep well. Excess alkali also produces a thinner emulsion and, although the particle size is also decreased, the emulsion has poor keeping qualities. The correct quantity of alkali would have to be determined for each sample of resin used, as it would vary with the saponification value of the resin. If a highly saponifiable resin is used, the quantity of alkali required for 5 per cent. resin and 5 per cent. casein is equivalent to 0·75 per cent. NaOH, all in relation to the total quantity of the emulsifier. Ammonia and sodium carbonate were tried as alkalis, but there is no particular advantage in using them and consequently NaOH was decided upon.

The emulsifier was eventually made as follows:—To 500 c.c. tapwater, heated to 70° C., is added 6 gm. NaOH and then 40 gm. casein is rapidly stirred in. The casein should contain as little fat as possible and should dissolve readily. The solution is then heated to 85° C. and 40 gm. ground resin is stirred in rapidly. A highly saponifiable resin should be used, such as is used in most soap factories. It should not be ground to a fine powder, else it will tend to form lumps which dissolve slowly. A coarsely ground resin forms no lumps and dissolves readily. The solution should be kept at 85° C. and stirred for about 15-20 minutes until complete combination of the alkali with the other ingredients has occurred. It is then made up to 800 c.c. with cool water.

In the laboratory, with the apparatus described, an emulsion of suitable thickness and maximum particle size of 5 μ is obtained with 25 per cent. emulsifier and 75 per cent. tetrachlorethylene. Samples of such emulsions made over 2 years ago are still keeping well and appear to be unaltered in all respects.

When the process was repeated on a large scale and using a colloid-mill type of emulsifying machine, it was found that

incorporation of air into the emulsion during the process of mixing must be guarded against, since small air-bubbles will hang on in the emulsion and cause it to break after some weeks.

In order to obtain a moderately thick, stable, stock emulsion the tetrachlorethylene has to be added to the emulsifier in successive small quantities while mixing proceeds, and this is especially important in the initial stages. If the requisite quantities of alkali, casein and water are mixed with tetrachlorethylene which contains the correct quantity of resin in solution, emulsification occurs rapidly simultaneously with saponification of the resin. The emulsion, however, does not keep well, probably on account of the fact that saponification is not complete and some alkali remains free.

The emulsion described above can be diluted with any ordinary water. It causes no coughing if it is properly administered. Mixed with 1·5 per cent. hydrochloric acid or abomasal juice, the emulsion does not break, but the casein is precipitated in floccules which, when examined microscopically, appear like a thick emulsion and contain the particles of tetrachlorethylene in the same finely dispersed state in which they existed in the original emulsion. It is probably this reaction which retards absorption to some extent and prevents giddiness, because the tetrachlorethylene will most likely be released gradually from the floccules of casein as the latter become digested.

The emulsion is issued in the concentrated form and is diluted with an equal quantity of water before use. The dose for an adult sheep is 20 c.c. of the diluted emulsion—i.e. 7·5 c.c. tetrachlorethylene—for lambs of 6-12 months old 15 c.c. and for lambs of 3-6 months old 10 c.c. The remedy is given after a preliminary dose of 2·5 c.c. 10 per cent. copper sulphate, so that it should be swallowed into the abomasum. It is not necessary to starve the animals or to keep them from water, but the treament should be carried out in the late afternoon or early morning when it is cool and the animals should be handled quietly.

Experience so far obtained with this emulsion indicates that it is very safe. Tetrachlorethylene is apparently not very harmful. Beyond anaesthesia, from which animals recover if they are left undisturbed when they lie properly, no harmful effects have been noticed. An unsatisfactory emulsion may break, and, if this is administered by an unsuspecting farmer, coughing and choking with ill results may follow, but a good emulsion can be recommended with safety according to present experience.

The emulsion has been found to be effective against *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus*, *Gaigeria pachyscelis* and *Bunostomum trigonocephalum* in sheep. It has not been tested against *Ostertagia* because sheep infected with these parasites were not available, but it is quite possible that it may be effective also in this case. Against *Bunostomum* the emulsion is not as effective as against *Gaigeria*, possibly on account of the fact that the former parasite is normally located farther back in the small intestine than

the latter and less of the drug reaches it. Against *Oesophagostomum columbianum* the efficacy is low and very variable on account of rapid absorption of the drug from the small intestine. The addition of 1 c.c. of croton oil to each 10 c.c. of undiluted emulsion increases its efficacy against the nodular worm, but it is very debatable whether this is due to tetrachlorethylene or to the purgative action of the croton oil and whether it is desirable to administer croton oil to sheep suffering from oesophagostomiasis, especially if one considers the condition of their intestines.

Summary.

An emulsion of tetrachlorethylene is described, which is effective against hookworms in sheep (*Gaigeria* and *Bunostomum*) and against *Haemonchus*, *Trichostrongylus* and *Nematodirus*. The emulsion does not cause coughing and choking when it is administered with reasonable care and does not produce giddiness since absorption is somewhat retarded. The factor of safety is relatively high.

REFERENCE.

ORTLEPP, R. J. AND MÖNNIG, H. O. (1936). Anthelmintic tests, chiefly with tetrachlorethylene, for the removal of the hookworm, *Gaigeria pachyscelis* from infested sheep, with observations on the effects of this drug on other parasitic nematodes. *Ond. J. Vet. Sc. An. Ind.*, Vol 7, No. 2, pp. 399-417.