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# THE CHEMOTHERAPEUTIC ACTION OF PHENANTHRIDINE COMPOUNDS

## PART VI

## THE PROPHYLACTIC AND TOXIC ACTIONS OF 2:7-DIAMINO-9-*P*-AMINOPHENYL-10-METHYLPHENANTHRIDINIUM CHLORIDE (150C47) AND IODIDE

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In Part V of this work (Goodwin and Unsworth, 1952) it was shown that two new phenanthridinium compounds were active against trypanosome infections in Fulani cattle at a dose of 1 mg./kg. Although the earlier phenanthridinium compounds, dimidium and phenidium, have proved disappointing as prophylactics, it was thought worth while to make a trial with one of the new drugs. No toxic effects had been observed with the 1 mg./kg. dose and we thought that larger doses would be tolerated; also we wished to study the toxic action of the drug when the dose was raised above the limit of tolerance. In addition, we had at our disposal an oily suspension of the sparingly soluble iodide corresponding to 150C47 (2:7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium iodide), which, by analogy with antrycide chloride, we thought might form a depot and exert a trypanocidal action for a prolonged period. Previous work by Unsworth had shown that at Lumu on the borders of the Ribako forestry reserve (Zaria province of N. Nigeria), where there was very heavy infestation with *Glossina morsitans*, untreated cattle became infected with *Trypanosoma vivax* and *T. congolense* in 3–6 weeks and died in 1–3 months. Antrycide prophylactic mixture injected at intervals of 90 days was powerless to prevent infection of the majority of cattle in this area, although it prolonged the survival time of the animals (Unsworth, 1951). The grazing at Lumu was very good; there was an extensive river plain (fadama) covered with good grass along the borders of a tributary of the Kaduna river.

The experiment described below showed that the prophylactic activity of 150C47 was poor, and that at doses of 2 mg./kg. and over the drug had toxic effects.

### MATERIALS AND METHODS

*Drugs.*—150C47—2:7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium chloride was prepared in fresh 5 per cent (w/v) solution and injected subcutaneously on the side of the neck in doses of 2 or 3 mg./kg.

* This work was done while L. G. Goodwin was working as a guest at the West African Institute for Trypanosomiasis Research.

65C51—The corresponding iodide in 15 per cent (w/v) suspension in arachis oil was injected similarly in doses of 3, 5, or 10 mg./kg.

These drugs were prepared by Dr. L. P. Walls.

*Cattle.*—Sixteen Fulani bulls about 12 months old and weighing 102–181 kg. were purchased for us at Bokkos on the plateau by Mr. Erik Krog. They were inoculated against blackquarter and haemorrhagic septicaemia. They then travelled on the hoof to Vom, where blood and lymph-gland examinations were made and injections of the drugs were given. Examination of the blood revealed that two animals were already infected with *T. vivax*. Three bulls were selected at random from among the uninfected animals to serve as untreated controls; the rest were distributed into groups for treatment by drawing numbers from a hat. The cattle were rested overnight after the dose and during the next 6 days walked 160 miles through the bush to Lumu. Riverine tsetse (*Glossina palpalis* and *G. tachinoides*) were encountered soon after leaving the plateau and attacked the cattle at the shady crossings of the watercourses. *G. morsitans* appeared about 40 miles from Lumu. None of the cattle died on the journey; all arrived in fairly good condition and were sent to graze daily in the Ribako forestry reserve.

*Examinations.*—The cattle were left at Lumu in the charge of herdsmen who had had several years' experience of similar experiments. Twice a week, at least one of the authors travelled to Lumu to make blood examinations (fresh preparations and thick and thin smears) and to assess the condition of the animals. Lymph-gland punctures, liver biopsies, and weight assessment were made on several occasions, and the local reactions at the sites of injection were carefully noted and recorded. If an animal fell sick, one of us (R.L.C.) went as quickly as possible to make an examination, and if necessary to slaughter and post-mortem the animal. Samples of the organs were fixed in formol-saline. Frozen and paraffin sections were prepared and stained in England.

*Survey of plants.*—When it was seen that the treated cattle were dying, we made a survey of the vegetation of the area to see if there were any known poisonous plants which might have contributed to the toxic effects of the drug. We attempted to identify as many as possible of the grasses, herbs, shrubs, and trees in the area which were available to the cattle. The plants which were in flower were collected and identified in the fresh state by reference to the *Flora of West Tropical Africa* (Hutchinson and Dalziel, 1928). The plants which were not in flower were more difficult to identify, so we made a collection in the company of a party of African herdsmen who knew the district. The Hausa name of each plant was recorded, together with any information as to its reputed virtues or poisonous properties. With this record, and a careful examination of the plant, it was possible to identify all but a very few with reasonable certainty by reference to the list of vernacular names and the information in the appendix to the *Flora* (Dalziel, 1948). The habitats of the plants collected fell into three distinct types: (*a*) the grassy river-plain, (*b*) the tree-savannah (secondary forest) which adjoins the plain, and (*c*) savannah clearings which had been farmed by the local population. Plants in all of these areas were available to the animals every day during the time of grazing. A list of the species identified is given in Table III. For the whole period of the experiment there was a good supply of green grasses on the river plain.

## RESULTS

*Local reaction.*—The local reaction at the site of injection of the drug was similar to that already described by Goodwin and Unsworth (1952). There was no difference between the effect of the chloride and iodide; large doses of drug produced more severe reactions than smaller ones.

*Prophylactic action of* 2: 7-*diamino*-9-p-*aminophenyl*-10-*methylphenanthridinium salts*

Table I shows that the untreated control animals showed trypanosomes in the blood after 19, 33, and 47 days respectively. Two of them died. Of the 13 treated animals, 10 died from the toxic effects of the drug, and two of the three survivors showed trypanosomes in the blood 72 days and 73 days respectively after exposure

TABLE I

PROPHYLACTIC ACTION OF 150C47 (SOLUBLE CHLORIDE) AND 65C51 (INSOLUBLE IODIDE)

| Drug | Bull No. | No. of days free from trypanosomes | Other notes |
|---|---|---|---|
| Controls .. | 32 | 19 | Killed *in extremis* after 73 days (*T. congolense* and *T. vivax*) |
| | 37 | 47 | Survived 78 days (*T. congolense*) |
| | 44 | 33 | Killed after 66 days, with heavy trypanosome infection (*T. congo.* and *T. vivax*) and abscess of abdominal wall |
| 10 mg./kg. iodide | 46 | Until death | Killed *in extremis* 32 days after dose |
| 5 mg./kg. iodide | 31 | Until death | Died 39 days after dose |
| | 36 | Until death | Killed *in extremis* 37 days after dose |
| | 45 | Until death | Died 32 days after dose |
| 3 mg./kg. iodide | 34 | Until death | Killed *in extremis* 39 days after dose |
| | 39 | Until death | Died 34 days after dose |
| | 43 | 73 | Survived, uninfected |
| 3 mg./kg. chloride | 35 | 72 | Survived, but *T. vivax* present in blood |
| | 40 | Until death | Killed *in extremis* 32 days after dose |
| | 42 | Until death | Killed *in extremis* 39 days after dose |
| 2 mg./kg. chloride | 33 | 73 | *T. vivax* infection; the animal was weak and was killed |
| | 38 | Until death | Died 17 days after dose |
| | 41 | Until death | Killed *in extremis* 27 days after dose |

to the tsetse fly. The remaining animal (No. 43), which had received 3 mg./kg. of the sparingly soluble iodide, remained free from infection for the whole of the 73 days spent in the fly-infested area. The prophylactic value of the compound was not impressive.

*Toxic effects*

A treated animal gave very little previous indication that he was shortly going to die. One day he would lag a little behind the others while grazing; the next morning he would be unable to rise and in a further 1–3 days he would die or be so ill as to require slaughtering. The animal would continue to eat until too weak to do so. On no occasion was jaundice, or dermatitis suggestive of photosensitization, observed, although we examined the animals very carefully with these possibilities in mind. Examination of the urine of bulls 39 and 41, collected shortly before death, revealed no protein, reducing substances, or bile pigments. We had no facilities for studying the amino-acids present in the urine. A record of the sunshine

and rainfall during the period of the experiment was obtained from the Meteorological station at Kaduna airport, 30 miles from Lumu. This showed that there was no lack of sunshine; the light intensity during this period of the year in N. Nigeria is very high. The post-mortem appearances and significant histological findings in the organs of cattle which died from the toxic effects of the drug are summarized in Table II. The animals which died showed lesions in the liver, the kidney, and

TABLE II

SUMMARY OF PATHOLOGICAL FINDINGS IN ANIMALS WHICH DIED

| Drug | Bull No. | Liver | | | | | | Kidney | | | | Heart | | Worms | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 1 | 2 | 1 | 2 | |
| Controls .. .. | 32 | | + | | | | | | | | | | + | | | |
| | 37 | | | | | | | | | | | | | | | Survived |
| | 44 | | + | | | | | | | | | | | | | |
| 10 mg./kg. iodide .. | 46 | | | | | | + | + | + | + | + | + | + | + | + | |
| 5 mg./kg. iodide .. | 31 | + | + | + | + | + | | + | | | | + | | + | + | |
| | 36 | + | + | + | + | | | + | + | + | + | + | + | + | + | |
| | 45 | + | + | + | + | + | + | + | + | + | | | | + | + | |
| 3 mg./kg. iodide .. | 34 | | | + | | | | + | + | + | | | + | | + | |
| | 39 | + | + | + | | + | | + | | | | | + | + | + | |
| | 43 | | | + | + | + | | | | | | | | | | Survived |
| 3 mg./kg. chloride .. | 35 | | | | | | | | | | | | | | | Survived |
| | 40 | | | + | + | | | + | + | + | | + | | + | + | |
| | 42 | + | | + | | | | + | | | | + | + | | + | |
| 2 mg./kg. chloride .. | 33 | | + | | | | | | | | | + | + | | | |
| | 38 | | + | | | + | | | | | | | + | | + | |
| | 41 | + | + | | | | | | + | | | + | | + | + | |

KEY.

*Liver*
1—Thick, dark bile.
2—Deposit of bile pigment in liver section.
3—Slight fatty infiltration.
4—Gross fatty infiltration.
5—Central necrosis.
6—Pink-staining lumps.

*Kidney*
1—Vascular congestion.
2—Protein deposit in Bowman's capsule.
3—Fat in some tubule cells.
4—Casts.

*Heart*
1—Fat droplets in muscle fibres.
2—Sarcocystis.

*Worms*
1—Liver flukes.
2—Stomach worms.

the heart. No significant abnormalities were observed in the gastro-intestinal tract, the lungs, or the endocrine glands. Most of the animals carried very heavy worm infestations.

*Liver.*—The lesion in the liver was a fatty infiltration, which sometimes affected all parenchymal cells (Fig. 1). In some cases there was centrilobular necrosis, and collapse of the reticulin framework in the central areas (Fig. 2). Some animals showed only a slight fat deposit, limited to the cells near the portal tracts) Several livers showed heavy bile pigmentation, and some (Nos. 46, 45, and 38. showed the presence of lumps of hyaline material which stained faintly pink with Sudan III.

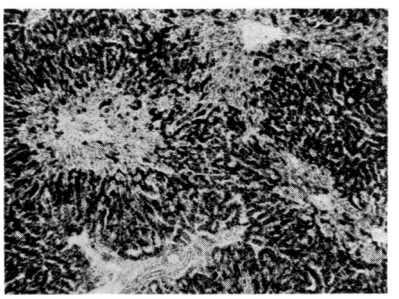

FIG. 1.—Liver of calf No. 31, showing fatty infiltration. Sudan III, haematoxylin, and aniline blue (× 38).

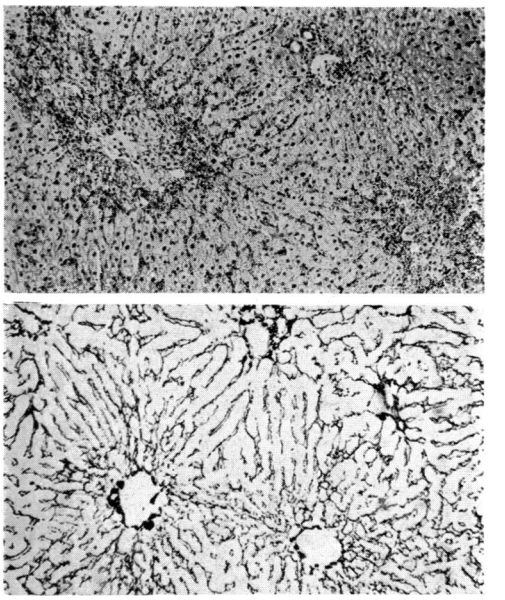

FIG. 2.—Liver of calf No. 31, showing fatty infiltration, centrilobular necrosis, and collapse of reticulum. Upper figure—haematoxylin and eosin; lower figure—modified Foot (× 75).

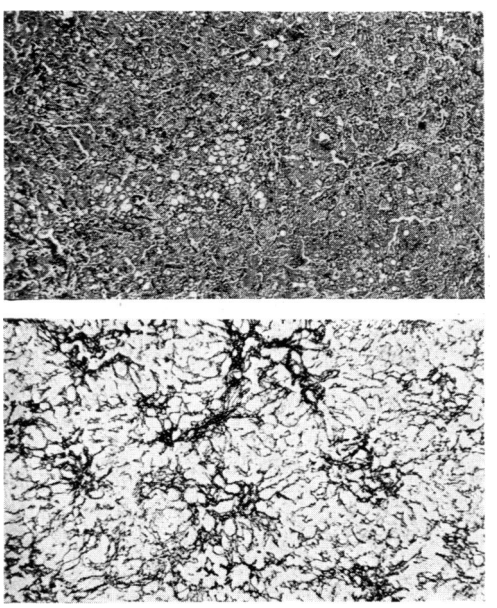

FIG. 3.—Liver of calf No. 43. Biopsy specimen taken 7 weeks after a dose of 3 mg./kg. of 65C51. The liver architecture is destroyed and the reticulum condensed; there are numerous fatty cysts. Upper figure—haematoxylin and eosin; lower figure—modified Foot (× 75).

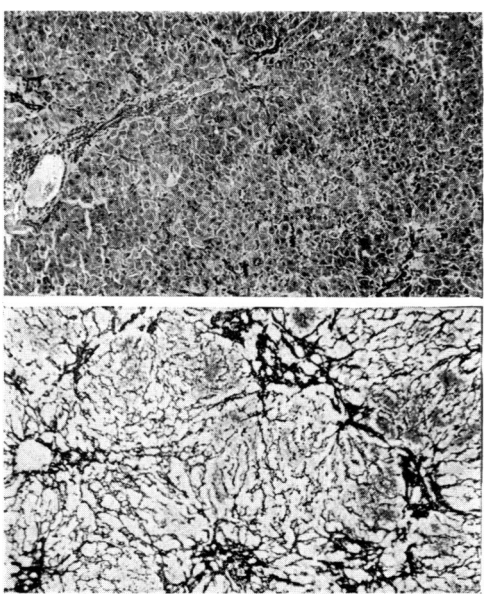

FIG. 4.—Liver of calf No. 43. Biopsy specimen taken 11 weeks after a dose of 3 mg./kg. of 65C51. The liver parenchyma and bile ducts have regenerated, but the architecture is abnormal. Upper figure—haematoxylin and eosin; lower figure—modified Foot (× 75).

The effect of the drug upon the liver in the animal which recovered (No. 43) was well shown by serial liver biopsies. Seven weeks after the dose the liver was heavily infiltrated with fat, and there was formation of fatty cysts similar to those described in choline-deficient rats by Hartroft (1950, 1951). The architecture of the liver had been severely affected (Fig. 3). In a further four weeks there was some regeneration of liver tissue (Fig. 4) although the reticulin stain showed that the architecture was abnormal. The animal may well have been developing a diffuse fibrosis (Himsworth, 1947).

*Kidney.*—In the kidneys which were affected, there was sometimes a slight protein deposit in some of the capsular spaces, and occasional nephrons showed fat droplets in the tubule cells. This may have been secondary to the fatty infiltration of the liver (Hartroft, 1951). Only in No. 46 and No. 36 was there any evidence of cast-formation. On the whole, the lesions in the kidney appeared to be slight, except in the animals which were given very large doses of drug.

*Heart.*—Several animals showed a generalized infiltration of the muscle fibres of the heart with minute droplets of fat. Similar fatty infiltration was observed after a lethal dose of phenamidine and in animals which died from other causes (Goodwin and Unsworth, 1952). It cannot therefore be stated with certainty that the drug was responsible for the condition of the heart muscle. Many of the animals showed extensive invasion of the muscle with *Sarcocystis.*

*Worm infestation.*—Most of the animals which died showed very heavy infestation with worms. Paramphistomes were present in the rumen and trichostrongyles in the abomasum, sometimes in huge numbers, and most livers contained flukes (*Dicrocoelium*). It is interesting that only a few worms were found in the untreated control animals.

*Plants.*—Table III is a list of the plants found at Lumu which were available to the cattle as food. Of course, this list is not a complete one; it may well be that we have missed highly poisonous species with a limited local distribution, plants

TABLE III

I. HERBACEOUS PLANTS

Acanthaceae: *Nelsonia campestris.*
Ampelidaceae: *Cissus populnea.*
Asclepiadaceae: *Glossonema nubicum, Raphionacme Brownii, Asclepias lineolata.*
Cochleospermaceae: *Cochleospermum tinctorium.*
Commelinaceae: *Cyanotis angusta, C. bulbifera, C. lanata.*
Compositae: *Aspilia latifolia, Chrysanthemum procumbens, Echinops longifolius, Gynura cernua.*
Convolvulaceae: *Ipomoea reptans.*
Cyperaceae: *Carex* sp., *Cyperus* sp., *Fimbristylis dichotoma, Kyllinga erecta.*
Euphorbiaceae: *Euphorbia hirta.*
Gramineae: *Acroceras* sp., *Andropogon* sp., *Digitaria* sp., *Echinochloa* sp., *Eragrostis* sp., *Heteropogon* sp., *Hyparrhenia rufa, Imperata* sp., *Jardinea* sp., *Lasiurus* sp., *Paspalidium geminatum, Paspalum scrobiculatum, Pennisetum* sp., *Sporobulus minutiflorus, S. spicatum, Urelytrum* sp.
Iridaceae: *Gladiolus unguiculatus, Romulea* sp.
Juncaceae: *Juncus capitatus.*
Liliaceae: *Anthericum* sp., *Urginea altissima.*
Orchidaceae: *Eulophia adenoglossa, E. cullulata, E. lutea.*
Papilionaceae: *Crotalaria Perrottetii, Tephrosia elegans.*
Rubiaceae: *Mitracarpum verticillatum.*

Solanaceae: *Schwenkia americana, Physalis angulata.*
Sterculiaceae: *Waltheria americana.*
Thymelaceae: *Arthrosolen chrysanthus.*
Verbenaceae: *Stachytarpheta angustifolia.*
Zingiberaceae: *Costus spectabilis, Kaempferia* sp.

II. Trees and Shrubs

Anonaceae: *Anona senegalensis.*
Araliaceae: *Cussonia nigerica.*
Caesalpinaceae: *Bauhinia Thonningii, Daniella Oliveri, Isoberlinia doka.*
Combretaceae: *Combretum sokodense, Terminalia avicennioides, T. glaucescens.*
Euphorbiaceae: *Uapaca guineense.*
Loganiaceae: *Strychnos spinosa.*
Mimosaceae: *Entada sudanica, Parkia filicoidea.*
Moraceae: *Ficus glumosa, F. kawuri.*
Myrtaceae: *Syzygium guineense.*
Ochnaceae: *Lophira alata.*
Olacaceae: *Ximenia americana.*
Palmeae: *Borassus flabelliformis.*
Papilionaceae: *Afrormosia laxiflora, Tephrosia Vogelii.*
Proteaceae: *Protea Elliottii.*
Rubiaceae: *Crossopteryx febrifuga, Gardenia erubescens.*
Sapotaceae: *Butyrospermum Parkii.*
Sterculiaceae: *Sterculia tomentosa.*
Verbenaceae: *Vitex Cienkowskii.*

which were small, or plants which had completed their season of growth and were dry at the time of collection.

A few of the species collected call for comment. The genus *Crotalaria* is well known as a cause of poisoning to stock in S. Africa (Steyn, 1934; Steyn and van der Walt, 1945; Steyn, 1949), and is known to cause liver lesions. *Urginea altissima* is also known to be poisonous (Steyn, 1934, 1945, 1949); this was growing in abundance on the river plain. One of the herdsmen regarded *Gladiolus unguiculatus* as an undesirable food plant for cattle; this too was common on the plain. No published records of the properties of many of the species recorded in Table III are available, but in a personal communication Prof. D. G. Steyn states that, in addition to the plants mentioned above, the following genera contain known poisonous plants in South Africa and elsewhere: *Cissus, Asclepias, Ipomoea, Euphorbia, Tephrosia, Arthrosolen, Terminalia, Strychnos.* Specific inquiry for *Tribulus terrestris* drew the unanimous reply from the herdsmen that this plant was not found in the Lumu area.

## Discussion

The phenanthridinium compound dimidium is known to have toxic effects upon the livers of cattle when given in doses only a little in excess of the therapeutic range. A dermatitis resembling that of photosensitization, and appearing several weeks after the dose of drug, is seen in some parts of Africa (Bell, 1945; Randall and Beveridge, 1946, 1947; Lederman, 1950). The occurrence of photosensitization also suggests toxicity to the liver, because " Geeldikkop," a disease of sheep in South Africa caused by eating poisonous plants such as *Tribulus terrestris*, is characterized by liver insufficiency which allows a photosensitizing derivative of chlorophyll to accumulate in the blood. The whole question of photosensitization has been fully discussed by Clare (1952).

Study of the delayed toxic effects of phenanthridinium compounds is made difficult by the fact that as yet they have not been produced in small laboratory animals. Photosensitization, jaundice, and severe damage to the liver have not been demonstrated in mice, rats, guinea-pigs, or rabbits, even after large and repeated doses of dimidium. It appears that it is essential to perform all investigations of toxicity upon cattle. A careful study in the Belgian Congo by Lederman (1950) led to the conclusion that the drug itself was responsible for the toxic action, but that the presence of other factors might influence the incidence of fatalities.

In the present experiment, although photosensitization did not occur, there is no doubt that 150C47 (which is closely related to dimidium) caused damage to the liver. Burdin and Plowright (1952) recently examined the action of phenanthridinium compounds on African cattle at Kabete, Kenya, and showed that the 10-methyl derivatives dimidium, 150C47, and, to a lesser extent, 621C47 all caused a loss of body weight and a rise in serum bilirubin and alkaline phosphatase. The structure as well as the function of the liver was affected; serial liver biopsies showed infiltration with fat. The present study agrees with the East African results, and it is clear that 150C47 has no apparent advantages over dimidium for the treatment of cattle trypanosomiasis. It has slightly greater activity, but its toxicity is of the same order and of the same type. However, it may be of use to consider the factors which may contribute to the toxicity of the drug, and which may account for the apparently contradictory reports upon the dangers of treating cattle with dimidium which have come from different parts of Africa.

The contributory causes of toxicity which have been suggested may be listed as follows: (1) Variation in the quality of batches of the drug. (2) A poor state of nutrition of the cattle. (3) The presence of toxic plants in the diet. (4) The presence of photodynamic substances in the diet. (5) The " lighting up " of an unspecified hepatotropic virus. (6) The presence of worm infestation or *Babesia* infection.

Variation in batches of the drug are not very likely to occur because the drug is a crystalline chemical compound of fixed constitution. Brownlee, Goodwin, and Walls (1947) re-examined a selection of the original samples of drug sent to Africa and could detect no differences in their toxicity or trypanodical activity in laboratory animals. Also, in the study of a large series of phenanthridinium derivatives by Brownlee, Goss, Goodwin, Woodbine, and Walls (1950) it was shown that the toxicities of all the compounds were of the same order. It is difficult to envisage an impurity which could be present and which would have a greater toxicity than dimidium itself. It is true that from some commercial batches of dimidium a precipitate forms when a sterile solution is allowed to stand for some weeks (personal communication and sample from Mr. S. G. Laws, Entebbe). Examination by Dr. L. P. Walls of the very small amount of material deposited in such a solution showed that it was probably an azo derivative, which is less toxic than dimidium (Walls and Beveridge, 1952).

The rest of the possible contributory causes of toxicity listed above have the common property of being associated with lesions in the liver. The lack of certain dietary factors, notably choline and cystine, is a well-known cause of liver disease in man and animals (Himsworth, 1947; Hartroft, 1951). The long delay in development of the signs and symptoms of liver failure in animals treated with phenan-

thridinium compounds is itself suggestive of deficiency of an essential food factor. Perhaps the drug may block biochemical reactions in which cystine, choline, or methionine are involved, and thus create deficiencies by a mechanism analogous to the folic acid deficiency produced by administration of aminopterin. Under such circumstances, the quality of the diet of the animal may make all the difference between recovery and fatal liver impairment. Evans (1948) showed very clearly that when cattle in the Sudan were transferred to an area of good grazing, the toxic effects of dimidium were very greatly reduced. Burdin and Plowright (1952) suggest that the 10-methylphenanthridinium derivatives may interfere with transmethylation; this would agree with the idea that the metabolism of choline and methionine are involved. Further evidence in support of the hypothesis was that "Ethidium," the 10-ethyl-compound corresponding to dimidium (Watkins and Woolfe, 1952), did not affect liver function or histology in doses of 3 mg./kg. However, this argument is not easy to reconcile with the other interesting observation that the 10-methyl compound phenidium (7-amino-9-*p*-aminophenyl-10-methylphenanthridinium chloride) was devoid of effects upon the liver in doses of 8 mg./kg. (Burdin and Plowright, 1952).

The presence of toxic plants in the diet could also make a contribution to the load upon the liver when a dose of a phenanthridinium compound is given. An animal might tolerate either of the noxious elements alone, but might be thrown into liver failure by their simultaneous action. The amount of photodynamic substances taken in the diet, as suggested by Wilde (1949), may explain the difference between an animal which exhibits the signs of photosensitization resulting from the accumulation of phylloerythrin in the blood, and an animal which does not.

There is as yet no sure evidence for or against the presence of the hepatotrophic virus suggested by Fiennes (1951). Such viruses are known to exist, and the almost epidemic qualities of some of the "outbreaks" of photosensitization in animals treated with dimidium lend weight to the suggestion. Further work is required, of the type already undertaken by Fiennes, to show whether or not a virus can be transmitted from photosensitized to normal animals.

Worm infestation has been observed to be a potent contributor to the toxic effects of dimidium in some parts of the Belgian Congo. It is likely that most intercurrent diseases—and few African cattle are free from them—will lower the resistance of the animals to the toxic side-effects of a drug with a narrow margin of safety.

## Summary

1. A solution of the chloride (150C47), and a suspension in oil of the iodide of 2: 7-diamino-9-*p*-aminophenyl-10-methylphenanthridinium, were given subcutaneously in large doses to cattle in order to study their toxic and prophylactic effects.

2. Ten of 13 cattle treated with 2 mg./kg. or more of the drugs died between 17 and 39 days after the dose because of a toxic action which was mainly upon the liver. Fatty infiltration, centrilobular necrosis, and condensation of the reticulin framework of the liver were shown in varying degrees of severity in the animals which died. In an animal which survived, serial liver biopsies showed considerable regeneration of liver cells at the end of 11 weeks after the dose of drug.

3. The prophylactic power of the drugs was not impressive and the local reaction at the site of injection was too severe for the drug to be of value for frequently repeated doses.

4. The factors which may influence the toxicity of phenanthridinium compounds to cattle are discussed.

We are grateful to Brig. J. S. K. Boyd and Col. H. W. Mulligan for their interest and encouragement, and to Mr. E. Krog, of the Nigerian Veterinary Service, for obtaining the cattle. We wish to thank Dr. L. P. Walls for preparing the phenanthridinium compounds, and Mr. P. E. Nesbitt and Mr. H. Richards for cutting sections of a great many liver biopsy and post-mortem specimens. We also wish to thank our African assistants, especially Mr. E. Ejemuta, Mallam Tanko and his herdsmen, and Mallam Dan Boy Biu. Finally, we acknowledge the courtesy of the Director of the British West African Meteorological Services in giving us access to sunshine and rainfall records.

## References

Bell, F. R. (1945). *Vet. Rec.*, **57**, 449.

Brownlee, G., Goodwin, L. G., and Walls, L. P. (1947). *Vet. Rec.*, **59**, 518.

Brownlee, G., Goss, M. D., Goodwin, L. G., Woodbine, M., and Walls, L. P. (1950). *Brit. J. Pharmacol.*, **5**, 261.

Burdin, M. L., and Plowright, W. (1952). *Nature, Lond.*, **169**, 666.

Clare, N. T. (1952). *Monograph on Photosensitization.* Farnham Royal: Commonwealth Agricultural Bureaux.

Dalziel, J. M. (1948). *The Useful Plants of West Tropical Africa.* London: The Crown Agents for the Colonies.

Evans, J. T. R. (1948). *Vet. Rec.*, **60**, 418.

Fiennes, R. N. T.-W. (1951). *Trans. R. Soc. trop. Med. Hyg.*, **44**, 605.

Goodwin, L. G., and Unsworth, K. (1952). *Brit. J. Pharmacol.*, 7, 581.

Hartroft, W. S. (1950). *Anat. Rec.*, **106**, 61.

Hartroft, W. S. (1951). *Liver Disease: Ciba Foundation Symposium*, p. 90. London: Churchill.

Himsworth, H. P. (1947). *Lectures on the Liver and its Diseases.* Oxford: Blackwell Scientific Publications.

Hutchinson, J., and Dalziel, J. M. (1928). *Flora of West Tropical Africa*, 2 vols. London: The Crown Agents for the Colonies.

Lederman, F. (1950). *C.R. du Congrès Scientifique Elisabethville*, Vol. V, p. 202. Bruxelles: Commission de Medecine Humaine et Vétérinaire; Comité Spécial du Katanga.

Randall, J. B., and Beveridge, C. G. L. (1946). *Vet. Rec.*, **58**, 398.

Randall, J. B., and Beveridge, C. G. L. (1947). *Vet. Rec.*, **59**, 224.

Steyn, D. G. (1934). *Toxicology of Plants in South Africa, together with a Consideration of Poisonous Foodstuffs and Fungi.* Johannesburg: South African Agricultural Series, vol. 13.

Steyn, D. G. (1945). *Farming in South Africa*, **20**, 437.

Steyn, D. G. (1949). *Vergifting van Mens en Dier.* Pretoria: van Schaik.

Steyn, D. G., and van der Walt, S. J. (1945). *Farming in South Africa*, **20**, 445.

Unsworth, K. (1951). Paper submitted to International Scientific Committee for Trypanosomiasis Research at Bobo Dioulasso; summarized in *B.P.I.T.T. Bull. Bibliographique*, 151/T.

Walls, L. P., and Beveridge, E. (1952). *Nature, Lond.*, **170**, 666.

Watkins, T. I., and Woolfe, G. (1952). *Nature, Lond.*, **169**, 506.

Wilde, J. K. H. (1949). *J. comp. Path.*, **59**, 48.