The pituicytes are best demonstrated by the Penfield modification of the Hortega method and are chiefly of the multipolar and bipolar varieties.

9. Distinctions between the powdered, desiccated, anterior and posterior lobes of the pituitary of cattle are presented together with methods of preparing the powdered products for examination.

10. The most diagnostic elements of the powdered, desiccated, anterior lobe are the two kinds of chromophile cells with blue-stained alpha- and beta-granules as observed after treatment with copper hematoxylin and eosin and Weigert's differentiator.

11. The most diagnostic elements of the powdered, desiccated, posterior lobe are the pituicytes (formerly called mossy neuroglia), whose processes were found to be more shrunken and contracted in the powder than in the sections. The second most diagnostic element was found to be the segments of non-medullated nerve fibers with bulbous ends.

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ANTHELMINTICS II. A COMPARISON OF CERTAIN OZONIDES, CHENOPODIUM OIL AND DIHEPTANOL PEROXIDE.*,[1]

BY LEWIS W. BUTZ AND W. A. LA LANDE, JR.[2]

The major component of oil of chenopodium is ascaridole and the anthelmintic action of the oil is due chiefly to this constituent. In a previous paper (1) it was shown that hydrogen peroxide and certain oxygenated terpenes are, like the terpene peroxide ascaridole (Fig. 1), highly toxic to swine ascarids *in vitro*. Since the peroxide function was demonstrable in these oxygenated mixtures, we were tempted to ascribe the anthelmintic effect of all these substances to the peroxide grouping —O—O—. In support of such a theory is the observation (2) that *Ascaris lumbricoides* lives practically anærobically in the intestine and it is, therefore, to be expected that substances capable of supplying a large quantity of active oxygen would seriously interfere with the life processes of the parasite. However, it was shown (1) that the antiascaridic activity of the oxygenated terpenes apparently survived the disappearance of the peroxide function, as indicated by a negative vanadium pentoxide-sulfuric acid

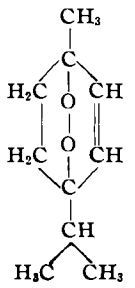

Fig. 1.—Ascaridole.

* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Presented in part before the Section on Medicinal Chemistry, American Chemical Society, New York City, April 1935.

[2] From the Laboratory of Dr. D. Jayne & Son, Inc., Philadelphia, Pa., and the Harrison Chemical Laboratory, University of Pennsylvania.

test. This result is, perhaps, to be attributed to the small sensitivity of this reagent in these terpene mixtures. The present paper, which reports results with ozonides and other peroxides, leaves no doubt that anthelmintic properties are common to many such compounds.

Swine ascarids have now been submitted to the action of a number of organic ozonides and peroxides *in vitro*. Several of these substances have also been administered to dogs harboring ascarids.[1] Organic ozonides are obtained by adding ozone to unsaturated compounds or products containing them, *e. g.*, certain vegetable oils. According to Rieche (3) ozonides are to be regarded as peroxide ethers with the structure $RR'C(-O-O-)CR'R$, where $R' = H$ or an organic radical. On hydrolysis ozonides yield hydrogen peroxide and aldehydes and/or ketones. Peroxides of the type $RCHOH-O-O-CHOHR$ are sometimes intermediate in the hydrolytic degradation of ozonides in which $R' = H$ (4). Accepting Rieche's conception of the structure of the ozonide grouping, there is thus an interesting chemical relationship between ozonized oleates and diheptanol peroxide, $CH_3(CH_2)_5CHOH-O-O-CHOH(CH_2)_5CH_3$, one of the peroxides included in the present study. On strong hydrolysis oleate ozonides yield nonaldehyde (among other products) while the similar decomposition of diheptanol peroxide yields the lower homologue, heptaldehyde, which was found to possess pronounced anthelmintic properties (5). Diheptanol peroxide was selected partly for its inherent stability. It was hoped that this substance might decompose sufficiently slowly in the animal body to exert a maximal anthelmintic action. Many other peroxides are available, *e. g.*, the acyl peroxides containing the group R—CO—O—O— and the alkyl peroxides containing the group R—O—O—, but they are characterized by great chemical instability. Ozonized ethyl oleate, ozonized cottonseed oil and ozonized olive oil were selected as stable and readily available materials.

We have found that the ozonides and diheptanol peroxide are just as effective *in vitro* as is oil of chenopodium. Also a single dose of an ozonide or of diheptanol peroxide effects a complete cure in ascariasis of dogs. In one comparative experiment, extending over a four months' period and requiring about five hundred dogs, the toxicity and therapeutic efficiency of diheptanol peroxide and ozonized cottonseed oil were compared with those of oil of chenopodium. Our work unfortunately had to be discontinued before precise values for the therapeutic indices of the new drugs and for oil of chenopodium, tested under the same conditions, had been obtained. However, the results already secured strongly indicate the usefulness of the new anthelmintics. In view of this and the large expenditure of time and experimental animals, the work is being presented at this time.

Oil of chenopodium (and ascaridole as well) gave rise to pronounced toxic symtoms in the dogs, *e. g.*, convulsions and irritation of the gastrointestinal mucosa, followed in the case of the larger doses by death, whereas toxic and lethal effects were not observed after administration of diheptanol peroxide and the ozonides. In the case of the ozonized vegetable oils, the drug exhibited a favorable action,

[1] One drug, ozonized cottonseed oil, has also been given to a few hogs with ascariasis. The results were uniformly good and one animal was completely cured. Too few experiments, however, have been done up to this time to draw any conclusions regarding efficacy or dosage.

possibly nutritional, aside from the anthelmintic action, since the dogs receiving this treatment thrived better than the controls. At this stage of the work the maximum tolerated doses of the ozonides and of diheptanol peroxide cannot be given since all doses which were mechanically administrable were without harmful effect. A number of animals have been given several doses, each far above the therapeutic requirement, with general improvement of health rather than toxic symptoms following the treatment. It is possible that better results could be realized by substituting a multiple dose for the single dose treatment, but this has not yet been tried.

EXPERIMENTAL PART.

The ozonides were prepared by passing a stream of oxygen containing 4% of ozone into the compound or oil dissolved in ethyl acetate. The reaction mixture was surrounded by ice during the ozonization. The solvent and dissolved gases were removed by distillation at 35–40° in high vacuum. The ozonized products were very viscous oils (except the quite fluid ozonized ethyl oleate) which became solid in the refrigerator. They retained their anthelmintic potency indefinitely and altered little in appearance on standing. Unlike many members of this class of compounds, the ozonides studied were quite safe to handle and showed no tendency to decompose spontaneously or explosively. It was possible to heat them over a free flame in open beakers without causing violent decomposition.

There is some evidence to show that the potency of the ozonides is dependent on the use of a solvent during ozonization, and on the temperature of the mixture during ozonization. Also it appears that incipient decomposition of the ozonized cottonseed oil, induced by heat and moisture, raises the potency. A systematic investigation of the preparation and decomposition of the ozonides under various environmental conditions has not yet been made. It is sufficient for the purposes of this report that a product of constant potency can be obtained by the method described.

The diheptanol peroxide was prepared according to the directions of Rieche (6). As he states, this peroxide can be boiled with water without appreciable decomposition. It is therefore safe to handle and not subject to deterioration in storage. The oil of chenopodium was U. S. P. The ascaridole was obtained from Eastman Kodak and redistilled, the fraction boiling at 97–98° at 8 mm. being used. The oils used were standard commercial products of the best grade. The ethyl oleate was the practical grade from Eastman Kodak.

THE IN VITRO EXPERIMENTS.

The results summarized in Table I were obtained by the assay procedure previously described (1). Since water was an unsuitable dispersion medium, a 1:9 mixture of 95% ethyl alcohol and water was used. Even then the emulsions of the ozonized vegetable oils were not as homogeneous as that of the ozonized ethyl oleate. However, the results of bioassay could be duplicated and therefore the dilute alcohol emulsions can be regarded as satisfactory. This was not true of the diheptanol peroxide which separated from the dilute alcohol to varying extents as a solid. Warm, heavy white mineral oil was used to disperse this compound. The swine ascarids survived twenty-four hours' immersion in the mineral oil to which no peroxide had been added. The 1:9 dilute alcohol alone affects the worms in a very constant manner from season to season, paralyzing forty to fifty per cent in four hours and killing none. It is noteworthy that whereas the dilute alcohol emulsions of the ozonized vegetable oils lose their potency after a few days, the emulsions of ozonized ethyl oleate retain their potency for at least several months.

TABLE I.—EFFECT OF VARIOUS OZONIDES AND OF DIHEPTANOL PEROXIDE ON ASCARIS LUMBRICOIDES IN VITRO.

Material.	Concn. %	Medium.	No. of Worms.	% Paralyzed, Hrs. 1.	2.	3.	4.	% Killed, Hrs. 1.	2.	3.	4.
Ethyl Oleate Ozonide	0.1	10% Alc.	10	...	20	10	60	...	...	30	40
" " "	0.2	" "	20	...	35	10	0	0	45	70	100
" " "	0.2	" "	10	...	30	...	10	...	40	70	80
" " "	0.3	" "	10	...	30	0	0	0	60	90	100
" " "	0.4	" "	20	...	30	0	0	0	50	85	100
" " "	0.5	" "	20	5	30	...	...	...	55	100	...
" " "	0.5	" "	60	10	20	5	...	10	65	100	...
" " "	0.5	" "	20	45	...	...	...	40	90	100	...
" " "	0.5	Mineral Oil	20	...	...	70	...	...	...	...	80
" " "	0.97	10% Alc.	10	10	20	30	...	60	90	100	...
" " "	1.0	" "	20	30	5	...	...	50	90	95	...
Ethyl Oleate	0.5	" "	20	...	10	20	35	5	10	20	25
Oleic Acid Ozonide	0.2	" "	20	...	5	25	35	...	...	10	...
" " "	0.5	" "	20	...	10	25	35	...	30	45	60
Ozonized Cottonseed Oil	0.1	" "	20	...	...	...	20	...	...	...	15
" " "	0.1	" "	20	...	...	10	35	...	...	...	0
" " "	0.1	" "	10	...	10	20	30	...	...	...	10
" " "	0.3	" "	10	10	10	20	30	...	30	40	40
" " "	0.3	" "	40	15	35	35	30	...	10	35	50
" " "	0.3	" "	10	20	10	20	20	...	20	30	50
" " "	0.3	" "	20	15	30	10	40	...	30	60	60
" " "	0.3	" "	10	10	30	0	10	10	60	80	90
" " "	0.4	" "	10	80	70	20	20	10	30	60	80
" " "	0.5	" "	20	80	75	35	15	0	25	65	75
Ozonized Olive Oil	0.1	" "	20	...	...	0	0	...	...	0	0
" " "	0.3	" "	20	...	...	20	30	...	...	0	0
" " "	0.3	" "	20	5	40	40	40	0	5	5	20
" " "	0.5	" "	20	45	30	...	...	45	90	100	...
" " "	0.5	" "	20	15	25	5	5	30	35	75	85
" " "	0.5	" "	10	40	...	...	...	...	50	70	80
" " "	0.5	" "	15	...	25	45	45	...	75	40	60
" " "	0.5	" "	20	20	20	0	5	0	60	90	95
" " "	1.0	" "	20	30	...	...	...	55	95	100	...
Olive Oil	0.5	" "	20	...	...	15	25	...	...	0	0
" "	1.0	" "	20	...	...	25	25	...	...	0	0
Diheptanol Peroxide	0.1	" "	20	...	...	...	0	...	...	...	0
" "	0.15	Mineral Oil	10	...	...	20	20	...	...	...	0
" "	0.2	10% Alc.	20	...	30	20	30	...	0	15	20
" "	0.2	" "	20	...	50	25	40	...	0	40	40
" "	0.25	" "	20	...	0	30	40	...	0	0	10
" "	0.25	" "	20	...	50	70	65	...	0	0	5
" "	0.25	Mineral Oil	10	...	...	50	40	...	...	30	60
" "	0.3	10% Alc.	20	...	65	55	40	...	0	10	45
" "	0.33	Mineral Oil	50	...	40	...	...	20	100	...	...
" "	0.4	10% Alc.	20	...	65	25	35	...	0	45	50
" "	0.4	" "	20	...	35	50	40	...	0	10	25
Mineral Oil	100.0	.....	20	0	0	0	0	0	0	0	0
Alcohol	10.0	H_2O	10	...	...	40	40	...	...	...	0

TABLE I.—(*Continued.*)

Alcohol	10.0	"	10	...	...	40	40	...	...	...	0
"	10.0	"	10	...	0	50	50	...	...	...	0
"	10.0	"	10	...	0	30	30	...	...	10	...
"	10.0	"	10	...	...	60	60	...	...	...	0
"	10.0	"	10	...	...	50	50	...	...	...	0
Chenopodium Oil	0.4	"	85	...	74	...	...	...	...	75	80
Ascaridole A	0.1	"	24	...	100	...	...	...	...	91	...
" B	0.1	"	12	...	0	91	...	...	...	0	0
" B	0.2	"	27	...	44	85	...	...	...	52	89
" B	0.4	"	15	...	87	...	...	...	...	87	...
Hydrogen Peroxide	0.033	"	20	...	0	0	...	...	...	0	0
" "	0.1	"	32	...	91	...	...	...	...	100	0

Two interesting facts emerge from these experiments with Ascaris lumbricoides; all of the peroxides and ozonides paralyze and kill the worms, and those materials containing more peroxide oxygen are more potent. As might be expected, the increase in potency with increase in peroxide oxygen content is not strictly proportional. On the basis of peroxide oxygen content alone, the minimum effective concentrations should be directly proportional to the quantities of the several drugs which contain one equivalent of active oxygen. Hydrogen peroxide was definitely toxic to the worm at 0.1 per cent concentration. Equivalent effectiveness in the case of ascaridole would not have been expected in concentrations under 0.49 per cent, and with diheptanol peroxide, ozonized olive oil and ozonized ethyl oleate, not in concentrations under 0.79, 0.87 and 0.91 per cent, respectively. Actually, in spite of the poor dispersion of the materials low in peroxide oxygen, lower concentrations than those predicted were markedly effective. Thus 0.1 per cent ascaridole, 0.33 per cent diheptanol peroxide, 0.3–0.5 per cent ozonized ethyl oleate, 0.3–0.5 per cent ozonized cottonseed oil and 0.5 per cent ozonized oil were approximately as toxic as 0.1 per cent hydrogen peroxide. These relations are shown in Table II. It would be premature to give an interpretation of these results.

TABLE II.—COMPARISON OF CHEMICALLY EQUIVALENT CONCENTRATIONS AND ACTUALLY EFFECTIVE CONCENTRATIONS.

Substance.	Molecular Weights.	Equivalent Weights.	Ratio of Equivalent Weights.	% Equivalent Effective Concentrations.	Actually Effective Concentrations.
(*a*) Hydrogen Peroxide	34	34	.....	0.1	0.1
(*b*) Ascaridole	168	168	$\frac{b}{a} = 4.9$	0.49	0.1–0.2
(*c*) Diheptanol Peroxide	268	268	$\frac{c}{a} = 7.9$	0.79	0.33
(*d*) Glyceryl Trioleate Triozonide	1029	343	$\frac{d}{a} = 8.7$	0.87	0.5
(*e*) Ethyl Oleate Ozonide	358	358	$\frac{e}{a} = 9.1$	0.91	0.3–0.5

THE EXPERIMENTS WITH DOGS.

For determining the anthelmintic efficiency of diheptanol peroxide and ozonized cottonseed oil relative to that of oil of chenopodium, we used puppies ranging in weight from one to two Kg. Ozonized olive oil, ozonized ethyl oleate and heptaldehyde (6) also expelled large quantities of worms from larger dogs, but these animals were not submitted to autopsy so that the efficiency of the treatment was not absolutely determined. The puppies were usually quite sick when received. They were kept under observation for at least one week before treating, during which time fecal examination for ascarid eggs was made. After segregation in individual cages, the

clinical condition of each animal was observed daily. The puppies were fed three times daily on a well-planned diet. The cages were frequently cleaned and the room temperature maintained constantly at 70–75°. In spite of careful handling the mortality among the puppies was very high. Table III presents conditions as observed over a several weeks' period. It is seen that the mortality among the control puppies was higher than that among any of the treated animals with the exception of those receiving oil of chenopodium.

TABLE III.—COMPARISON OF MORTALITY IN CONTROL AND TREATED ANIMALS.

	% Dead before Day of Treatment.	% Dead after 2 Days.	% Dead after 4 Days.	Dosage Gm./Kg.
Controls	44.7	36.9	48	.....
Chenopodium Oil	..	60	78.3	0.05–0.2
Diheptanol Peroxide	..	20	30	0.1 –0.2
Ozonized Cottonseed Oil	..	13.3	20	1.0 –2.0

The puppies harboring ascarids (*Toxocara canis*) were starved for twenty-four hours before treating. The drugs were administered in gelatin capsules. The administration of purgatives with the anthelmintics was not tried. The number of ascarids passed each day by rectum, before and after administration of the drug, was observed. These worms were always dead when seen but some may have been alive at the moment of passage. Some of the experiments are summarized in Table IV. Only those animals are included which died or which were sacrificed one to ten days after treatment and which had infestations of more than five ascarids. Many of the dogs which showed ascarid eggs in the feces were found later to pass or to have harbored only one or two parasites. It seems undesirable to include these animals whether the worms were expelled or not.

TABLE IV.—THERAPEUTIC EXPERIMENTS—CANINE ASCARIASIS; PERCENTAGE OF TOTAL WORMS REMOVED—ONE DOSE TREATMENT.

	Dose Gm./Kg.	No. of Animals.	Worms Present.	Worms Removed.	% Removed.
Diheptanol Peroxide (solid)	0.2	6	129	48	37
" " "	0.3	2	25	22	88
" " "	0.5	3	36	13	36
" " "	1.0	8	138	128	93
" " (in alc.)	0.2	4	51	28	55
" " "	0.5	4	76	63	83
Ozonized Cottonseed Oil	0.2	2	29	28	96
" " "	0.5	4	64	61	95
" " "	1.0	9	126	106	84[1]
" " "	2.0	4	68	68	100
Chenopodium Oil	0.02	1	14	6	43
" "	0.03	4	55	43	78
" "	0.05	6	72	66	92
" "	0.1	4	71	71	100
" "	0.2	3	46	46	100
Controls	..	30	426	62	14

[1] 8 animals showed 100% removed.

These data show that the minimum therapeutic doses of ozonized cottonseed oil and diheptanol peroxide are higher than that of oil of chenopodium. Diheptanol peroxide dissolved in alcohol is more effective than the solid alone. The somewhat erratic results obtained with the solid peroxide are probably to be explained by poor distribution of the drug in the intestine. Often the peroxide was passed in the feces in a mass of the shape of the capsule and apparently undiminished in size. In view of this circumstance, the positive results obtained are quite startling. Ozonized cottonseed oil was more effective than diheptanol peroxide, doses as low as 0.2 Gm. per Kg. being curative. A few observations for individual puppies from typical experiments are reproduced in Table V. It is seen that in every case the rate of elimination of ascarids was greater after administration of the drug than before.

TABLE V.—THERAPEUTIC EXPERIMENTS—CANINE ASCARIASIS; RATE OF WORM ELIMINATION.

No.	Dose Rate Gm./Kg.	Survival (Days).	Cure %.	Rate Eliminated Worms Day. Before.	After.	No. of Worms Eliminated. Before.	After.	Autopsy.
			Diheptanol Peroxide (Solid).					
161	1.02	8	100	0.2	14.0	1	14	0
69	0.30	12	100	0.2	13.0	2	17	0
43	0.20	12	28.4	1.1	2.1	8	23	58
			Ozonized Cottonseed Oil.					
189	1.00	7	100	0.4	7.0	3	21	0
334	0.48	15	100	0.0	9.0	0	18	0
			Chenopodium Oil.					
118	0.10	4	100	0.4	10.5	2	21	0
258	0.05	8	100	0.7	4.7	5	28	0
			Ozonized Olive Oil.					
	1.0	12	100			2	27	0
	1.0	14	100			1	8	0
	1.0	30	...			0	6	..[1]
	2.0	30	...			0	22	..[1]
	3.0	30	...			0	14	..[1]
	3.0	30	...			0	10	..[1]
			Ozonized Ethyl Oleate.					
	3.0	30	...			0	12	..[1]

[1] A second dose after three weeks did not expel any more worms.

Unfortunately we do not yet have enough experiments to give a precise quantitative picture of the toxicity of ozonized cottonseed oil or diheptanol peroxide. The puppy experiments do not serve us here because of the poor clinical condition when treated. However, the data in Table III and that from many similar experiments do indicate that the new drugs are much less toxic than oil of chenopodium. Some supplementary experiments were carried out using larger (five to fifteen Kg.) dogs in apparent good health with the results shown in Table VI. The animals which received sub-lethal doses of ascaridole and oil of chenopodium invariably showed convulsions and prostration, while the peroxide and ozonide dogs never did. The deaths following the administration of ozonized oil were surprising in view of the beneficial results from this material with the puppies. We are inclined to view these deaths as not due to the drug, although mechanical damage may have been done since the doses of this viscous product became excessively large with the larger animals.

TABLE VI.—COMPARISON OF LARGE DOSES OF CHENOPODIUM OIL, ASCARIDOLE, OZONIZED COTTONSEED OIL AND DIHEPTANOL PEROXIDE ON LARGE DOGS.

	Dose Gm./Kg.	No. of Dogs.	% Deaths.
Chenopodium Oil	0.2	2	0
	0.5	6	33
	0.8	4	50
	1.0	2	100
Ascaridole	0.2	2	0
	0.5	5	60
	0.8	4	75
	1.0	1	100
Ozonized Cottonseed Oil	5.0	3	33
	10.0	4	50

Diheptanol Peroxide	0.2	2	0
	0.5	4	25
	1.0	3	0
	3.0	5	0
	5.0	1	0

These experiments indicate that organic ozonides and diheptanol peroxide generally are toxic to swine ascarids *in vitro* and are valuable in the treatment of dogs infested with ascarids. They show that ozonized cottonseed oil and diheptanol peroxide have a higher therapeutic index than oil of chenopodium in canine ascariasis, although they do not determine the index. The ozonized oil seems superior to the solid peroxide. The experiments suggest that organic ozonides and possibly peroxides may find wide application in the treatment of metazoan and protozoan disease, and offer a new group of organic compounds for exploitation by the experimental therapeutist. This seems timely just now when these diseases are being recognized as important, since the drugs so far put forward for their treatment have been limited to representatives of a few chemical classes, characterized generally by a too high toxicity for the host.

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DRUG EXTRACTION. XI. THE EXTRACTION OF JALAP.*[1]

BY WILLIAM J. HUSA[2] AND PAUL FEHDER.

In an earlier paper (1) a report was made of the effect of solvents in relation to swelling, penetration, imbibition and extraction of jalap. A further study has been made of the extraction of jalap, including such factors as fineness of powder, variation in solvents and methods of assay.

EXPERIMENTAL DATA.

Effect of Fineness of Powder on Percolation.—100-Gm. portions of jalap in Nos. 20, 40, 60 and 80 powders were percolated with alcohol, the percolates being collected in successive fractions of 100 cc., 100 cc. and 300 cc. in each case. Assay results on the percolates indicated that within the limits of No. 20 and No. 80 powder, the fineness of powder has practically no effect on the rate of extraction of resin by percolation.

Effect of Variation in Solvents on Rate of Extraction.—Percolation experiments were conducted using jalap in No. 60 powder and a series of alcohol-water mixtures. The drug used contained 9.4% moisture and the resin content was 7.3% by the U. S. P. X method of assay and 6.0% by Warren's assay method (2). In each case 200 Gm. of drug were moistened with 100 cc. of menstruum, packed in the percolator and menstruum added. After allowing a maceration period of 48 hours, percolation was allowed to proceed at a rate of 10 drops per minute, the percolate being collected in successive fractions of 200 cc., 200 cc. and 600 cc. The various fractions of percolate were assayed for resin and total extractive.

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* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] This paper is based on part of a dissertation presented to the Graduate Council of the University of Florida by Paul Fehder, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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