munized by three injections given at weekly intervals by a combination of the intradermal, intramuscular, and subcutaneous routes. A total of 10^{-5} grams of formalized virus nitrogen (Experiment 91-2-4 after 12 days) was administered to each of 30 rats in three doses as follows: $10^{-5.6}$ grams of nitrogen intradermally, $10^{-5.3}$ grams of nitrogen intramuscularly a week later, and $10^{-5.6}$ grams of nitrogen subcutaneously at the end of another week. A similar group of animals was immunized by the same method with 10^{-5} grams of nitrogen of the same vaccine in a petroleum oil suspension.

The degree of immunity present in the two groups was estimated by finding the titer of active virus in each in comparison with that of normal control animals of the same age. Concentrations of virus were prepared in steps of 10 from 2×10^{-10} to 2×10^{-5} grams of nitrogen/ml. Each concentration was then injected intracerebrally in a volume of 0.05 ml. into each of five animals from the two immunized groups one week after the last vaccine injection. The control animals were tested similarly with concentrations of virus ranging from 2×10^{-12} to 2×10^{-7} grams of nitrogen/ml. The titer of virus used was calculated from the control animals and, expressed as the ID_{50} dose, was found to be $10^{-11.7}$ grams of nitrogen/ml. Similar titers calculated for the two immune groups were $10^{-6.2}$ for the first mentioned and $10^{-6.5}$ for the group injected with vaccine in oil emulsion.

It is evident from these titers that over 300,000 times as much virus was required to infect 50 per cent of the first group of vaccinated animals as was found necessary for the normal controls. Similarly, in the second group, 150,000 times as much virus was required.

The survivors from both groups were pooled and rechallenged by the same method $10\frac{1}{2}$ weeks after the first challenge. The titer found with the immunized animals as compared with that of their normal controls showed that about 500 times as much virus was now required to infect 50 per cent of the immunized group.

It is possible that the high degree of immunity produced in the experiment cited above was due to the presence of the trace of active virus found in this preparation. Further immunization experiments with the vaccine which showed no virus activity were carried out to test this question. Twenty-four rats immunized with the same amount of completely inactive vaccine by the three routes previously used were each challenged intracerebrally one week after the last injection with about 1,000 ID_{50} doses of active virus. One of the group developed paralysis but recovered, while the remaining 23 showed no signs of the disease. In a similar number of normal control animals receiving the same dose of virus 83 per cent developed poliomyelitis. It is likely, therefore, that the extent of immunity produced by the vaccine after 12 days of exposure to formaldehyde was due to the inactive virus rather than to the traces of activity present.

The 24 survivors from the last-mentioned experiment were rechallenged with 10,000 ID_{50} doses of virus three weeks after the first challenge. A high degree of immunity was still present, as shown by the fact that 67 per cent of the animals survived without showing signs of poliomyelitis as compared to 92 per cent of a similar group of normal controls which developed the disease when injected with the same amount of virus.

The results presented show that cocenntrated Lansing virus from cotton rats can be completely inactivated by formaldehyde and that such inactive virus can be used to produce a high degree of immunity toward the homologous virus. The data show that infectivity as such is not a prerequisite for a vaccine which, in amounts of 10^{-5} grams of concentrated virus nitrogen, produces a highly significant protection against the intracerebral injection of active virus. Whether protection is obtained by neutralization of virus at the site of injection by humoral antibodies or by some other mechanism is not known. The presence of neutralizing antibodies in high titers in the blood of the immunized rats has been demonstrated both by neutralization tests and by the complement fixation test. These results will be presented in an early publication.

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Filaricidal Activity of Substituted Phenyl Arsenoxides[1]

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In the treatment of both canine and human filariasis antimony-containing compounds have been used to the exclusion of almost all other types of therapy. Accordingly, early in our search for an improved form of therapy considerable attention was given to new or hitherto unexplored antimony compounds. The results were disappointing in that we failed to obtain evidence that antimony in therapeutically feasible doses would ever regularly kill the adult filaria, *Litomosoides carinii*, of cotton rats or *Dirofilaria immitis* of dogs. We therefore intensified our search for an entirely different type of therapeutic agent. Consideration was given to the possible advantages of nonmetallic compounds, but in so far as our investigations were carried no such compound was found which appeared to offer any advantages over the antimonials.[3] However, preliminary screening revealed that Mapharsen had an *in vitro* microfilaricidal activity far greater than anything hitherto reported. This led to a study not only of Mapharsen itself but of the whole group of available substituted phenyl arsenoxides.

The attempt to use arsenic in the treatment of filariasis is not new. Among the more recent advocates of its possible value in the therapy of filariasis are King (*4*) and Van der Sar and Hartz (*8*), who obtained evidence of clinical improvement without destruction of the microfilaria following the use of

[1] This work was carried on initially under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and The Johns Hopkins University; it was continued under a contract between the Office of the Surgeon General, U.S.A., and the same university.

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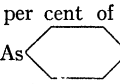
[3] Welch, *et al.* (*9*) have since reported favorable results with cyanines in laboratory animals.

Mapharsen. It is of interest that in Hawking's study (*3*) of the *in vitro* killing of microfilaria of *Wuchereria bancrofti*, 6 of the 7 trivalent arsenicals killed the microfilaria at greater dilution than did any of the 28 other compounds used, including two antimonials (tartar emetic and fuadin). Apparently Goodman and Gilman's statement (*2*) that "micro-filaria can be killed by organic arsenicals" results from Hawking's report, although that worker and Chopra and Rao (*1*) were unable to obtain evidence of *in vivo* killing of microfilaria by these arsenicals.

We have conducted both *in vitro* and *in vivo* studies on the effect of various compounds on microfilaria and adult filaria as well as a study of the toxicity, absorption, and excretion of those offering promise of therapeutic value. However, discussion will be confined here to the filaricidal activity of the substituted phenyl arsenoxides with particular reference to p-[bis-(carboxymethylmercapto)-arsino]-benzamide (Tropical Disease Center #970).

The group of phenyl arsenoxides was shown to have an *in vitro* activity against the microfilaria of *D. immitis* 10–300 times that of the most active of the antimony compounds tested. Thus, within the group of phenyl arsenoxides there was approximately a 30-fold difference between the least active and the most active, whereas there was scarcely more than a 3-fold difference in the acute toxicities for mice. The amide substituted compounds, such as those with the p-$CONR_2$ or p-$SONR_2$ substitution, killed the microfilaria at the highest dilutions. Despite previous reports of the failure of Mapharsen to reduce the microfilaria blood levels in man, this seemed to warrant further study. Accordingly, it was administered to cotton rats infected with *L. carinii* in doses of 0.9 mg. As (3.0 mg. Mapharsen)/kg. b.i.d. intraperitoneally for 45 days and to a dog, infected with *D. immitis*, in doses of 0.6 mg. As (2.0 mg. Mapharsen)/kg. intravenously daily for 35 days. The results were completely negative.

Six other phenyl arsenoxides, kindly supplied by Harry Eagle, of the U. S. Public Health Service, were administered intraperitoneally to cotton rats in doses of 0.9 mg. As/kg. b.i.d. for 25 and 45 days. All showed some activity, and all four of the amide substituted compounds reduced the microfilaria blood levels 70–90 per cent during the course of treatment; three of them killed 90–100 per cent of the adult worms. Only p-arsenosobenzamide (OAs  $CONH_2$), among these compounds, killed all the adult worms in half the above daily dose (0.45 mg. As/kg. b.i.d.) for the same length of time and reduced the microfilaria blood level 50–80 per cent. In lower doses and for shorter periods of time there was some lethal action upon the adult worms but little or no reduction in the microfilaria level. In dogs with daily intravenous doses of 0.45 mg. As/kg. it failed to produce any reduction in the microfilaria count, but in each of the two animals killed at the end of treatment 6 dead, but no living, worms were found in the pulmonary arteries. The microfilaria blood level of the remaining dog was followed for one year and dropped in that time to about one-fifth of its pretreatment level, while the microfilaria count actually rose in the untreated animal. When the animal was necropsied, one living adult was found in the heart. Since this compound is so insoluble (about 1:800 in water), work with it was discontinued and consideration given to the synthesis of a more soluble analogue.

A number of compounds were studied in which organic radicals were substituted for oxygen in the p-arsenosobenzamide. The dithioglycollate substituted compound which was made in our laboratory (*5*)[4] seemed to offer promise of both solubility (2 per cent solutions are practical) and a favorable therapeutic index. This compound, p-[bis-(carboxymethylmercapto)-arsino]-benzamide, killed all the adult worms in cotton rats receiving 0.9 mg. As (4.5 mg. drug)/kg. b.i.d. intraperitoneally for 6 weeks but failed to effect any reduction in the microfilaria level; various shorter courses of treatments killed some of the adult worms. The administration of the drug intravenously to dogs infected with *D. immitis* likewise failed to reduce the microfilaria level during the course of treatment. On the other hand, the drug seems to be consistently effective against the adult worms in daily doses of 0.23 mg. As (1.15 mg. drug)/kg. or more for two weeks or longer. It appears also that effectiveness of the drug may not be measured in terms of the total dose alone, since a total of 9.0 mg. As/kg. given in 5 daily doses of 1.8 mg. As/kg. (dog 11) and 13.5 mg. As/kg. given as 0.9 mg. As/kg. every other day (dog 9) did not kill all the adult worms, whereas smaller total doses, 3.4 mg. and 6.8 mg. As/kg. (dogs 32 and 29), given in doses of 0.23 and

TABLE 1

TREATMENT OF *Dirofilaria immitis* WITH DAILY INJECTIONS OF #970

Dog	Daily dose (mg. As/kg.)	No. of injections	Total dose (mg. As/kg.)	Fate of dog	Adult worms	
					Alive in heart	Dead in pulmonary artery
11	1.8	5	9	D, 5 days	33 (Sluggish)	5
25	1.8	3	5.4	" "	0	3
5	0.9	30	27	Healthy, K	0	11♀ 11 fr.†
27	0.9	30	27	" "	0	2 fr.
28	0.9	15	13.5	" "	0	7♂ 2♂ 2 fr.
9	0.9	15 (alternate days)	13.5	" "	1♀ (Sluggish)	0
29	0.45	15	6.8	" "	0	3♀ 2 fr.
32	0.23	15	3.4	" "	0	4♀ 6♂ 2 fr.
31	0.23	11	2.5	D,* 12 days	0	19♀ 12♂
34	0.115	15	1.7	Healthy, K	2♀ 1♂ (active)	0

* This dog was critically ill before experiment started.
† fr. = fragmented.

[4] We are indebted to C. K. Banks, of Parke, Davis & Company, Detroit, who later supplied the main stock and its precursor (#622) from which we made much of our supply, and to Fitzgerald Dunning and J. H. Brewer, of Hynson, Westcott, and Dunning, Baltimore, who ampouled the compound in 2 per cent solution for use.

0.45 mg. As/kg. daily for 15 days were effective (Table 1). The comparative series is admittedly small, but it is supported by similar experience with the same drug in cotton rats infected with *L. carinii*. More work is needed to determine whether or not the effective dose is lower than 0.23 mg. As (1.15 mg. drug)/kg. daily for 15 days or even to state with certainty that this dose would always be effective. However, 0.23 mg. As/kg. doses of this drug for 15 days appear to be feasible for man. Thus, for the first time, so far as we know, a chemical compound has been shown to kill all the adults of *D. immitis* in doses which appear to be feasible for man.

As pointed out in our brief summaries (*6*, *7*), this compound has approximately the same toxicity for laboratory animals as Mapharsen; it is tolerated by monkeys in doses of 0.9 mg. As/kg. for 20 days. It contains 20 per cent arsenic and is stable either as a powder or as a 2 per cent solution (*5*). Studies are accordingly now in progress to determine its possible value when used against both canine and human filariasis under conditions of routine practice with these infections.

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The Development of Visual Perception in Man and Chimpanzee

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The study of innate visual organization in man is not open to direct observation during early infancy, since a young baby is too helpless to respond differentially to visual excitation. A first attack on this problem has been made by investigating the visual responsiveness of persons born blind and later made able to see by cataract removal. To evaluate the apparent contradictions between these clinical reports and experimental findings with lower mammals and birds, chimpanzees were reared in darkness until sufficiently mature for the testing of visual responsiveness. The results, which corroborate and extend data reported for man by Senden (*3*), may require changes in current theories of learning and perception.

Two chimpanzees were reared in darkness to the age of 16 months.[1] The animals were then brought periodically into the light for a regularly repeated series of observations. By the time of the first observations the animals had developed postural and locomotor skills roughly comparable to normally reared chimpanzees of the same age or approximating in a general way those of a two-year-old human child. At this time the total light experience, received in half a dozen brief (45-second) episodes daily, as required by the routine care of the animals, was approximately 40 hours. At 21 months of age the female was brought permanently into normal indoor illumination. At the present writing the animals are 26 months of age. A full account of their behavior will appear in future publications.

The first tests of visual reactions with both subjects demonstrated the presence of good pupillary responses to changes in light intensity, pronounced startle reactions to sudden increases of illumination, and a turning of the eyes and head toward sources of light. In the darkroom there was pursuit of a moving light with both eye and head movements. The eyes, however, did not fixate steadily on a light. During all tests episodes of a resilient "spontaneous" nystagmus occurred, the quick phase usually toward, and the slow phase away from, the light source. With the subject sitting stationary at the center of a rotating drum marked in alternating black and white stripes, tests for optokinetic responses were made. Characteristic pursuit eye movements with quick jerks in the opposite direction were obtained.

Aside from the reflexes just described, and the pursuit of a moving light, the two animals were, in effect, blind. The acquisition of visually mediated responses proceeded very gradually, with no evidence of any sudden increased responsiveness such as might be expected if, for example, the failure to respond was at first due to a general lack of attention to visual stimulation. No fixation of any object, still or moving, could be elicited in any of the early tests. For a long time there was no eye blink when an object was brought rapidly toward the eyes. An object brought slowly toward the face produced no response until contact was made, when the animal reacted with a quick jerk in the typical startle pattern. With the female this was observed for the last time on the 30th day after she was moved into the daylight room. Her first blink to a threatened blow in the face occurred on the 5th day, but occurred consistently only after 48 days, at which time she had been in the light for a total of 570 hours, was 22½ months old, and had for a month received some pushing around daily in short play periods with a younger but visually sophisticated chimpanzee.

Many repetitions of experience with objects presented visually were necessary before any recognition of such objects appeared in either subject. The feeding bottle, for example, was thoroughly familiar tactually and kinesthetically. If the bottle or nipple touched the hand, arm, or face, either animal promptly seized the nipple in its mouth. First signs of *visual* recognition occurred in the female when she protruded her lips toward the bottle on the 33rd meal, or the 11th day, following her shift into the daylight room. The first reaching for the bottle with the hand (done before 12 months of age by normally reared animals) appeared on the 48th meal, or 16th day in the light. With the male, whose visual experience was limited to mealtime, many more feedings were required before these responses appeared. The first reaching responses of both animals were grossly inaccurate.

A training procedure employing electric shock showed that the learning of avoidance responses was also an extremely slow and gradual process.

These results can best be interpreted in conjunction with the data of Senden. Lacunae in each set of findings, clinical and experimental, are in many respects filled by the other.

[1] The early rearing in the darkroom was arranged by H. G. Birch, whose part in this experiment is gratefully acknowledged.