TABLE 3

INHIBITION OF HEMOAGGLUTINATION BY HUMAN AND SIMIAN CONVALESCENT MUMPS SERUM

		Dilution of serum									
		4(1)	8	16	32	64	128	256	512	1,024	2,048	Virus control
Conval. monk.	(4)	0	0	0	0	0	0	tr?	tr?	++	++	++++
Normal monk.	(4)	+(2)	++	+++	+++	+++±	+++±	nd(3)	nd	nd	nd	
Conval. human	(5)	nd	0	0	++	++	+++	++++	++++	++++	nd	++++
Before dis. "	(5)	nd	++	+++	++++	++++	++++	++++	++++	nd	nd	
Conval. human	(6)	nd	0	0	0	+	+	+	++	++	nd	
Acute human	(6)	nd	+++	+++±	++++	++++	++++	++++	nd	nd	nd	

(1) Reciprocal of final dilution of serum.
(2) *Cf.* table 2, footnote 2.
(3) *Cf.* table 2, footnote 3.
(4) Sera from different monkeys.
(5) Sera from same individual; one specimen taken before exposure to mumps; the convalescent 7 days after onset of symptoms.
(6) Sera from same individual; convalescent specimen taken 15 days after onset.

is only gradually acquired must await further experimentation.

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DISTRIBUTION OF RADIOACTIVE ARSENIC FOLLOWING INTRAPERITONEAL INJECTION OF SODIUM ARSENITE INTO COTTON RATS INFECTED WITH LITOMOSOIDES CARINII[1]

INTRODUCTION

IT has been shown[2, 3] that adult *Dirofilaria immitis* in naturally infected dogs show a specific uptake of antimony following treatment with trivalent antimony compounds. These adult parasites appeared to be normal at the autopsies of the treated dogs, but microscopic examination of the uteri by Ashburn *et al.*[4] revealed significant alteration of the ovaries and uterine contents.

We have found[5] that the adult filarids of dogs have a specific uptake for trivalent arsenic as reported for the trivalent antimony. Having established the fact that both antimony and arsenic in the trivalent form were localized in the adult filarids of dogs in concentrations greater than the majority of the tissues of the hosts and certainly greater than the concentration gradient of the blood, it was believed important to ascertain if the actual location of the parasite in the host would be a factor in this specific uptake. The adult *Litomosoides carinii* are in the pleural cavity, whereas the adult *Dirofilaria immitis* are in the right side of the heart.

METHODS

The radioactive arsenic was prepared by the bombardment of germanium metal with deuterons in the 60-inch cyclotron at the Department of Terrestrial Magnetism, Carnegie Institution of Washington. This arsenic had a sufficiently long half life (16 days) to enable great precision to be obtained in the final measurements.

A chemical separation of the arsenic from the other radioactive and non-radioactive contaminants was made by a procedure recently described by Ness.[6] The arsenic was converted to sodium arsenite, $NaAsO_2$, as outlined in the above reference. Six cotton rats naturally infected with *Litomosoides carinii* were injected intraperitoneally with 1.6 milligrams of arsenic, as sodium arsenite, per kilogram of body weight. Twenty-four hours later the animals were sacrificed and twelve tissues removed, carefully weighed, and then dried *in vacuo* over phosphorus pentoxide. After sixteen hours of drying, the tissues were re-weighed, ground, and the arsenic content determined by means of suitable Geiger-counters, scaling and counting circuits. Each sample was measured in a lucite cup of standard dimensions, each cup being carefully tested for radioactive contamination before being used and no cup was used twice during any experiment. Conversion of the counting data for each determination of the radioactive samples to micrograms of arsenic was made by preparing from the solution of sodium arsenite to be injected a standard solution containing one microgram of arsenic per milliliter. This was treated in the same manner as the unknown tissue

[1] From the Zoology and Chemistry Laboratories, National Institute of Health, and the Department of Terrestrial Magnetism, Carnegie Institution of Washington.

[2] Frederick J. Brady, Alfred H. Lawton, Dean B. Cowie, Howard L. Andrews, A. T. Ness and Glen E. Ogden, *Am. Jour. Trop. Med.*, 25(2): 103–107, 1945.

[3] Dean B. Cowie, Alfred H. Lawton, A. T. Ness, Frederick J. Brady and Glen E. Ogden, *Jour. Wash. Academy Sci.*, 35(6): 192–195, 1945.

[4] L. L. Ashburn, T. Perrin, Frederick J. Brady, Alfred H. Lawton (in press, *Arch. Path.*).

[5] Unpublished data.

[6] A. T. Ness, "Separation of Radioactive Arsenic from Copper and Germanium" (in manuscript).

samples and sufficient counts were taken for all samples to insure an accuracy better than 1 per cent. Whenever possible, equal amounts of all the dried samples were measured for radioactivity at a standard distance from the counter to maintain rigid uniformity in the measuring techniques. Since the average dry weights of the various tissues studied were about 25 per cent. of the wet weights, and equal quantities of the tissues were measured, self-absorption of the radiation by the sample material was considered to be constant and equal for all samples. For the final calculations, individual corrections were necessary for the per cent. of water lost during the drying. No radioactivity was detected in the phosphorus pentoxide after the drying process, indicating no loss of the arsenic during this procedure.

TABLE 1

ARSENIC CONCENTRATION OF TISSUES OF SIX COTTON RATS TWENTY-FOUR HOURS AFTER THE INTRAPERITONEAL INJECTION OF 1.6 MILLIGRAMS OF ARSENIC PER KILOGRAM OF BODY WEIGHT AS SODIUM ARSENITE

Tissue	Micrograms arsenic per gram wet wt. of tissue	Tissue	Micrograms arsenic per gram wet wt. of tissue	Tissue	Micrograms arsenic per gram wet wt. of tissue
Cotton Rat 46		Cotton Rat 49		Cotton Rat 50	
Adult Filarids	1.310	Liver	2.62	Adult Filarids	1.65
Kidney	1.044	Epidermis	1.93	Liver	1.59
Liver	1.022	Adult Filarids	1.83	Kidney	1.53
Lung	0.918	Spleen	1.51	Epidermis	0.956
Spleen	0.753	Lung	1.34	Lung	0.833
Epidermis	0.549	Kidney	1.29	Muscle	0.791
Muscle	0.475	Blood	0.989	Spleen	0.667
Heart	0.422	Muscle	0.866	Heart	0.411
Dermis	0.320	Heart	0.842	Thyroid	0.199
Thyroid	0.267	Thyroid	0.585	Blood	0.196
Blood	0.207	Dermis	0.401	Dermis	0.196
Brain	0.172	Brain	0.299	Brain	0.196
Cotton Rat 51		Cotton Rat 27		Cotton Rat 28	
Adult Filarids	1.738	Kidney	1.566	Kidney	1.791
Kidney	1.18	Spleen	0.879	Spleen	1.484
Epidermis	1.16	Adult Filarids	0.874	Epidermis	1.016
Liver	0.841	Liver	0.775	Liver	0.978
Lung	0.695	Epidermis	0.654	Lung	0.478
Spleen	0.466	Lung	0.593	Muscle	0.440
Muscle	0.352	Thyroid	0.586	Heart	0.345
Heart	0.340	Heart	0.332	Dermis	0.267
Blood	0.180	Muscle	0.285	Blood	0.264
Dermis	0.131	Dermis	0.266	Adult Filarids	0.214
Brain	0.123	Brain	0.201	Brain	0.176
Thyroid	0.000	Blood	0.169	Thyroid	0.000

RESULTS

The arsenic concentration of twelve tissues from each rat twenty-four hours after injection is given in Table 1 in terms of micrograms of arsenic per gram wet weight of tissue. In three of the rats, the adult filarids had the highest concentration of the tissues studied. The filarids in two other rats had the third highest concentration, while in rat No. 28 the adult filarids showed no specific uptake. Rat No. 28 was an old animal with a marked chronic fibrous pleuritis which was not found in any of the other rats.

Contrary to the findings in dogs treated with antimony, the cotton rat thyroid showed no specific uptake of arsenic. The epidermis was found to have a high concentration. This is in agreement with the knowledge that arsenic can be eliminated by the epidermis.[7] Rather significant is the extremely low level of concentration found in the dermis relative to the high concentration of the epidermis. In fact it appeared that the dermis reflected the blood concentration while the epidermis was on an average about five times greater.

Figure 1 is a histogram of the average arsenic concentration in micrograms per gram of wet weight of the tissues shown in Table 1. If the data on rat No. 28 were eliminated from these averages, the adult filarids would contain the highest concentration of arsenic instead of containing the third highest.

DISCUSSION

The high concentration of the kidney and liver are not surprising. It is well known that arsenic is eliminated in urine and bile. The kidney cortex, liver, epidermis, spleen and lung may be said to show a specific uptake of arsenic. Although the dermis does not appear in this list, the appearance of the epidermis here would suggest that trivalent arsenical compounds might offer more promise in the treatment of *Onchocerca volvulus* than antimonial compounds which showed no specific affinity for either dermis or epidermis.

Lymph nodes showed no specific affinity for either trivalent arsenic or antimony. However, this should not eliminate studies on the usefulness of these elements in treating *Wuchereria bancrofti* infection as the filarids residing in the lymph nodes may be able

[7] A. Heffter, *Arch. Internat. Pharm. et Therapie*, 15: 399–417.

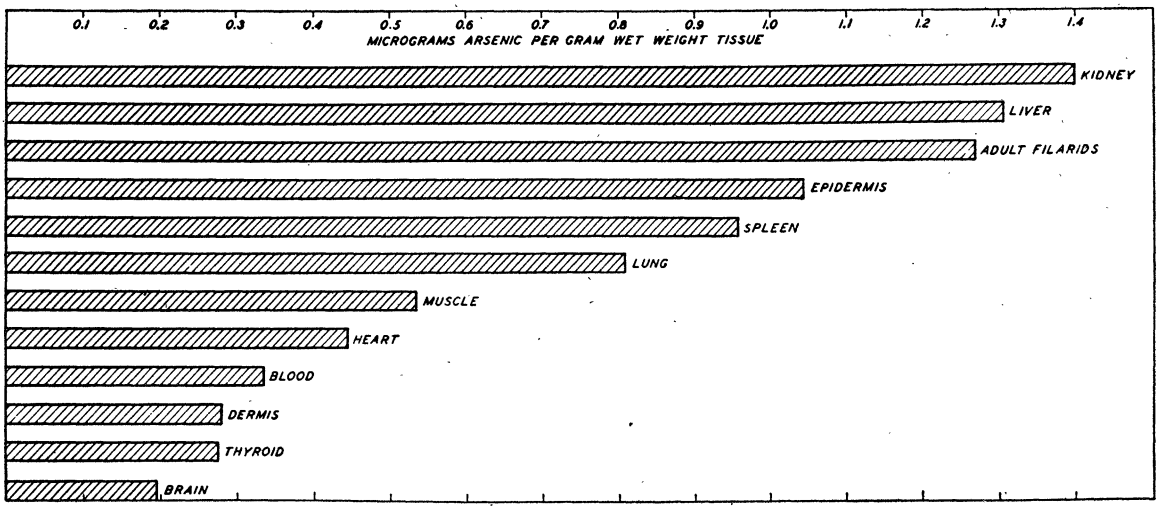

FIG. 1. Average arsenic concentrations of tissues of six cotton rats twenty-four hours after the intraperitoneal injection of 1.6 milligrams of arsenic per kilogram of body weight as sodium arsenite.

to take up sufficient quantities of the elements from the lymph to prove damaging to themselves. The fact that *Dirofilaria immitis* and *Litomosoides carinii* both showed specific affinity for arsenic makes such a hypothesis tenable.

In spite of the fact that *Litomosoides carinii* live in the pleural space, they showed a specific uptake of trivalent arsenic in five of the six cotton rats studied. Although the filarids in the sixth cotton rat appeared to be normal, they showed no specific uptake of the arsenic. In this rat, the only factor apparent to explain this difference was the presence of a chronic fibrous pleuritis. Such results suggest that treatment in human filarid infections might be more efficacious in early stages of the disease before the formation of extensive scar tissue.

CONCLUSIONS

Adult *Litomosoides carinii* of five of six infected cotton rats showed a specific affinity for arsenic after the injection of sodium arsenite. Lack of uptake in the sixth rat is attributed to the presence of a fibrous pleurisy. A specific localization of arsenic was shown in kidney cortex, liver, epidermis, spleen and lung of cotton rats.

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SYNERGISTIC ACTION BETWEEN SULPHONAMIDES AND CERTAIN DYES AGAINST GRAM-NEGATIVE BACTERIA

AS one of a number of possible explanations of the mode of action of substances that inhibit cell division of bacteria Henry[1] has suggested that the bacteriostatic agent may "poise" the oxidation-reduction potential in the immediate environment of the cell at a level which will preclude the normal functioning of one or more desmolytic enzyme systems.

Ingraham[2] has presented evidence in support of this hypothesis with respect to bacteriostatic dyes, a similar concept having been previously propounded by Dubos.[3] Henry[1] has indicated that inhibition of dehydrogenases by sulphonamides may, in part, be due to such a mechanism. More recently, Michaelis and Thatcher[4] have shown that the antibiotic substance, citrinin, depresses the action of certain desmolytic enzymes of susceptible species. These last workers also (unpublished) investigated the possibility of augmenting the "poising" effect of antibiotic substances by the addition of dyes of known E'o values. Various dyes brought about bacteriostasis, but a synergistic effect was not disclosed either between combinations of dyes or between dyes and citrinin.

The work of Soo-Hoo and Schnitzer,[5] however, confirms Ungar[6] in that a synergistic action does exist between penicillin and sulphonamides. Similar studies were therefore undertaken with sulphonamides and eH indicator dyes with respect to their activity against both *Staphylococcus aureus* and *Escherichia coli*.

It became immediately apparent that a pronounced synergistic effect was manifest, as is indicated by comparison of Tables 1 and 2. Moreover, the sulphonamides, which normally have little if any effect against

1 *Bact. Rev.*, 7(4): 175–262, 1943.
2 *Jour. Bact.*, 26: 573–598, 1933.
3 *Jour. Exp. Med.*, 49: 575–592, 1929.
4 *Can. Jour. Res.* (in press).
5 *Arch. Biochem.*, 5: 99–106, 1944.
6 *Nature*, 152: 245, 1943.