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Comparative Toxicity and Protozoacidal Action of Acetarsone, Carbarsone, and Certain Related Pentavalent Arsenical Compounds.*

N. A. DAVID,† H. H. ANDERSON, D. A. KOCH AND C. D. LEAKE.

From the Pharmacological Laboratory, University of California Medical School, and the Pacific Institute of Tropical Medicine, Hooper Foundation for Medical Research, San Francisco.

We have already shown[1] that 4-carbamino-phenyl-arsonic acid ("carbarsone") is biologically more active than acetarsone, N.N.R., ("stovarsol," 3-acetyl-amino-4-hydroxy-phenyl-arsonic acid). On the basis of oral toxicity in animals and amebacidal concentration *in vitro,* carbarsone has a therapeutic index about 8 times as great as acetarsone. This fact warranted, we believe, a thorough study of carbarsone and related pentavalent arsenicals from the standpoint of their possible application to the treatment of amebiasis. The following compounds were obtained for such an experimental evaluation:

1. $NH_2CONH \cdot C_6H_4 \cdot AsO_3H_2$, 4-carbamino-phenyl-arsonic acid,‡ "carbarsone".
2. $NH_2COCH_2NH \cdot C_6H_4 \cdot AsO_3HNa$, Sodium N-phenyl-glycinamide-p-arsonate, "tryparsamide".
3. $NH_2COCH_2CH_2NH \cdot C_6H_4 : AsO_3HNa$, Sodium phenyl-b-amino-propriona-mide-p-arsonate,§ "proparsamide".
4. $NH_2CO \cdot CH_3 \cdot CH_3 \cdot C \cdot NH \cdot C_6H_4 : AsO_3H_2$, Phenyl-a-amino-isobutyra-mide-p-arsonic acid,‡ "isobutarsamide".
5. $NH_2CO \cdot CH_3CH_2 \cdot CH \cdot NH \cdot C_6H_4 \cdot AsO_3H_2$, Phenyl-a-amino-n-butyramide-p-arsonic acid,‡ "n-butarsamide".

In Table I, data are presented on the toxicity of these substances in comparison with acetarsone when given in single doses as a powder in gelatine capsules by mouth. With increase in the number of carbon atoms in the side chain of the carbarsone series there seems to be the usually associated increase in toxicity. It is noteworthy that carbarsone, although having the highest arsenic content, is the least

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† Lilly Research Fellow.

[1] Leake, C. D., Koch, D. A., and Anderson, H. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 717.

‡ Supplied through the courtesy of Dr. H. A. Shonle, Lilly Research Laboratories, Indianapolis.

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TABLE I.
Oral Toxicity of Certain Arsenical Compounds.

Drug and Actual Arsenic Content of Sample Used		Mortality Ratio at Single Oral Doses in Guinea Pigs	Mortality Ratio at Single Oral Doses in Rabbits	Mortality Ratio at Single Oral Doses in Cats
	%	mg./kg.	mg./kg.	mg./kg.
Sodium Acetarsone	25.3	4/10 at 100 9/10 " 200	2/ 4 at 150	5/7 at 150
Carbamino-phenyl-p-arsonic acid	28.3	6/10 " 200 7/10 " 300	4/10 " 200	3/6 " 250
Sodium N-phenyl-glycinamide-p-arsonate	24.6	7/10 " 150 10/10 " 250	4/ 5 " 200	1/5 " 200
Sodium phenyl-b-amino-proprionamide-p-arsonate	26.0	6/15 " 100 10/10 " 200	4/ 6 " 200	3/6 " 200
Phenyl-a-amino-iso-butyramide-p-arsonic acid	26.5	4/10 " 100 7/10 " 150	——	——
Phenyl-a-amino-n-butyramide-p-arsonic acid	24.0	4/10 " 100 8/10 " 200	4/ 5 at 50	——

toxic of the series. If death occurred it was ordinarily from 3 to 14 days after giving the doses indicated. Post-mortem gross and microscopic examination revealed for these drugs the same general pathological picture which has already been described for acetarsone poisoning.[2] No evidence of injury to the optic tract was noted in any of these toxicity studies, although it might be anticipated in the carbarsone series where a substituted amino group occupies a position para to arsenic.[3]

In Table II one may find certain data on the protozoacidal activity of some of these compounds. The methods used have been previously described.[4] Proparsamide and n-butarsamide were too restricted in amount to permit satisfactory study. Iso-butarsamide was not available for this phase of the effort. Tryparsamide did not seem to be sufficiently active *in vitro* to warrant extensive study in naturally infested animals.

[2] Heitzmann, O., *Arch. f. Derm. u. Syph.*, 1926, **152**, 344; Anderson, H. H., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 267.

[3] Young, A. G., and Loevenhart, A. S., *J. Pharmacol. Exp. Therap.*, 1924, **23**, 107.

[4] For estimation of amebacidal concentration *in vitro:* Koch, D. A., *Univ. Calif. Pub. Zool.*, 1926, **29**, 241; Leake, C. D., Koch, D. A., and Anderson, H. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 717. For estimation of balantidicidal action in naturally infested guinea pigs: David, N. A., and Leake, C. D., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 196. For study of effect in natural monkey amebiasis; Anderson, H. H., and Koch, D. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 838.

TABLE II.
Protozoacidal Activity of Certain Pentavalent Arsenical Drugs.

Drug and Actual Arsenic Content of Sample Used	%	Amebacidal Concentration *in vitro* at 48 hours	Total Balantidicidal Dose on Divided Oral Administration to Guinea Pigs, harboring *Balantidium coli*	Notes on Effects in Monkeys (*M. rhesus*) Naturally Infested with *E. histolytica*
Sodium Acetarsone	25.3	1:600	75% cleared at 150 mgm./kg. with 50% mortality	Tolerate 400 mgm./kg. at 30 mgm./kg. daily with clearing of stools during administration only
Carbamino-phenyl-p-arsonic acid	28.3	1:4,000	80% cleared at 120 mgm./kg. with 20% mortality	Tolerate 500 mgm./kg. at 100 mgm./kg. daily with clearing of stools through post-treatment observation period of 3 months in 3 animals, but with recurrence within 30 days in 4 animals
Sodium N-phenyl-glycinamide-p-arsonate	24.6	1:1,000	100 mgm./kg. (Sweeney[5])	——
Sodium phenyl-b-amino-proprionamide-p-arsonate	26.0	1:10,000	——	Tolerate 800 mgm./kg. at 50 mgm./kg. daily with clearing of stools during administration only
Phenyl-a-amino-n-butyramide-p-arsonic acid	24.0	—	60% cleared at 150 mgm./kg. with 40% mortality	——

Comparative evaluation of the protozoacidal properties of the drugs noted is difficult because the data is inadequate for all except acetarsone and carbarsone. For these 2 drugs it is obvious that carbarsone is the better, not only *in vitro,* but in natural infestations in guinea pigs and monkeys. The latter can not tolerate, without undue gastro-enteric distress, daily doses of acetarsone in amounts great enough to clear the stools except during the treatment period. But carbarsone can be tolerated by macaques in doses large enough to achieve more lasting success. Tryparsamide requires a stronger concentration for amebacidal action *in vitro* than carbarsone. While proparsamide appears better than carbarsone *in vitro,* it can not be tolerated by naturally infested monkeys in large enough daily doses to be more than temporarily effective. N-butarsamide seems too toxic to be seriously considered.

Summary. Comparative oral toxicity studies on guinea pigs,

[5] Sweeney, M. A., *Am. J. Hyg.,* 1929, **9**, 544.

rabbits, and cats, and a comparative protozoacidal evaluation on amebae *in vitro* as well as on natural balantidial infestations in guinea pigs and on natural monkey amebiasis indicates that 4-carbamino-phenyl-arsonic acid ("carbarsone") has definite advantages from the standpoint of application to anti-amebic therapy over acetarsone ("stovarsol"), tryparsamide, "proparsamide," "iso-butarsamide," and "n-butarsamide." It is definitely less toxic on oral administration than these compounds and seems generally more protozoacidal in tolerated doses.

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Effects of Ovaries in Various Stages of Activity and of Pregnancy Upon Adrenalectomized Rats.

JESSE L. CARR. (Introduced by C. L. Connor.)

From the Department of Pathology, University of California.

According to Stewart and Rogoff,[1] the survival period of adrenalectomized dogs is prolonged by pregnancy. H. A. Stewart[2] finds this same mechanism exists in the cat, although E. Z. Corey[3] concludes that the presence of corpora lutea have not this influence in the pregnant and lactating female. Mulon and Vincent[4] suggest that the corpus luteum acts as a temporary adrenal cortical body, thus accounting for the increased survival period which does occur apparently in dogs. Herring[5] on the other hand, points out that the rat liver is 27.5% heavier in the pregnant rat due to glycogen storage, and that this storage accounts for the extended survival period. Harding[6] adds that protein economy in pregnancy may extend this period.

In the white rat, fresh corpora lutea are constant in the ovary and exist in 3 forms, those of ovulation, pregnancy, and lactation. According to Evans, each of these may have certain characteristics and these may be distinguished both grossly and microscopically. The following experiments are the results of an effort to determine

[1] Stewart and Rogoff, *Am. J. Physiol.*, 1927, **79**, 508.

[2] Stewart, H. A., reported by MacCallum, *Int. Cong. Med.*, London, Sec. 3, 173.

[3] Corey, E. Z., *Phys. Zool.*, 1928, **1**, 147.

[4] Vincent, S., cited by Britton, "Internal Secretion of the Ductless Glands," (Monograph), London.

[5] Herring, P. T., *Brit. Med. J.*, 1920, **2**, 886.

[6] Harding, V. J., *Physiol. Rev.*, 1925, **5**, 279.