

to the medium in which the tissues were suspended. The following organs were examined: Uterus and Fallopian tube, bladder and ureters, and vas deferens and seminal vesicle. Aqueous saline extracts of the ram's, dog's, bull's, steer's and human prostate glands were used. It was found that all of the above organs are stimulated *in vitro* by prostatic extracts, provided a sufficient dose is used; but that different organs require different doses of the glandular extract. The uterus and tubes were found to respond to the smallest quantities of prostatic extract; the bladder and ureters came next in the order of their response to such treatment; while the vas deferens and seminal vesicles required the largest doses of the extracts to give evidence of any physiological effect. As a result of the experiments, the authors conclude that the prostatic extracts cannot be regarded as having any specific or marked influence on the tonus and contractions of the bladder *in vitro*. Fuller data will appear in due time in the *Journal of Urology*.

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Phenol elimination in the dog after intravenous injection of neoarsphenamine.

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Although Ehrlich himself recognized the importance of discovering the fate of salvarsan in the animal body, it was Sieburg¹ who first approached the solution of this problem. This investigator succeeded in isolating from the urine of a syphilitic patient who had received repeated intravenous injections of salvarsan the following substances: *p*-aminophenol, *o*-acetylaminophenyl hydrogen sulphate, oxycarbanil, an aminohydroxyphenylarsonic acid, $C_6H_8O_4N$ As, and a hydroxyphenylarsonic acid, $C_6H_7O_4As$, besides inorganic arsenates and arsenites. He concludes that salvarsan is broken down in the system in the following way (Chart 1):

¹ Sieburg, E., *Zeitschr. physiol. chem.*, 1916, 97, 53-108. Abstr. in *Chem. Abstracts*, 1917, 2, 270-1. (Original article not available in U. S. A.)

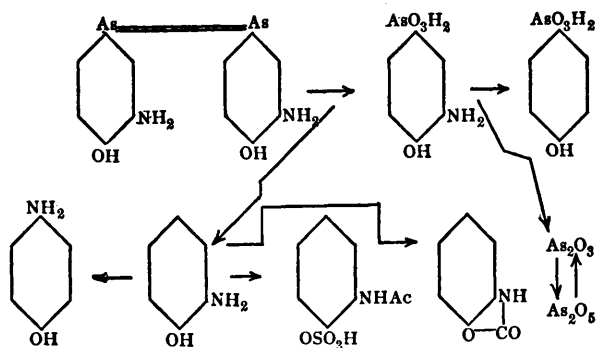


CHART I.

“Apparently *o*-aminophenol is to a comparatively small extent converted into the isomeric, highly toxic *para* compound, while the remainder is conjugated either with H_2SO_4 with the introduction of an acetyl group, or with urea with the subsequent elimination of 2 N H_3 radicals.”

Sieburg also studied the behavior of *para*-arsenobenzoic acid and of 3-amino-arsenobenzoic acid in the body of the calf and concludes that in all cases a cleavage of the toxophoric— $\text{As}=\text{As}$ —linking in ‘arseno’ compounds occurs in the living organism, whereby not only is the toxicity reduced, but transformation takes place into compounds which are more soluble in water and less easily soluble in other media. The characteristic effects are due to the small proportion of the substance which escapes oxidation to the arsenic acids, and to the subsequent liberation of the As_2O_3 and As_2O_5 .”

Sieburg’s discovery of conjugated phenol derivatives resulting from the metabolism of salvarsan as well as our independent investigations on the causes of the reactions following intravenous injections of arsphenamine and neo-arsphenamine¹ led us to direct our attention to the mechanism of detoxication which the body possesses,—the power of rendering innocuous various aromatic toxic substances by conjugation with sulphuric acid, *d*-glucuronic acid, urea, glycocholic acid, bile acids, etc. It seemed plausible to venture the hypothesis that in those patients in whom severe reactions are observed within a few hours or days after intravenous injection of arsphenamine or neo-arsphenamine,

¹ Schamberg, J. F., Kolmer, J. A., Raiziss, G. W., and Weiss, Charles, *Arch. Dermat. & Syph.*, 1920, 1, 235-256.

detoxication is subnormal, owing to injury sustained during the course of the infection by the hepatic, renal and other cells concerned in this mechanism. Of course, normal variations in this process cannot be overlooked as a possible factor.

We have, therefore, studied, in a preliminary way, the elimination of free and conjugated urinary phenols after the intravenous injection of a single large tolerated dose (50 mg. per kilo) of neo-arsphenamine into the dog.

Method.—A normal healthy female dog (16.82 kilo) was employed and was kept on a constant diet. The technique followed was that of Folin and Denis¹ with the modifications described by Dubin.²

Results.—The results are summarized in Table I and indicate the following:

TABLE I.
PHENOL ELIMINATION AFTER A SINGLE INTRAVENOUS INJECTION OF NEO-ARSPHENAMINE (0.841 Gr.).¹

Daily Elimination	Control Period.		1st Neo Period.		2d Neo Period.		3d Neo Period.		Summary.					
	Free.	Total.	Free.	Total.	Free.	Total.	Free.	Total.	Periods of Exp.	Free.	Total.	Conj.	% Free	% Conj.
1st day	106	156	117	158	95	147	90	127	Normal	528	734	206	72	28
2d "	117	187	96	125	115	173	108	143	1st neo	506	673	167	75	25
3d "	78	110	82	109	105	140	119	173	2d neo	581	875	294	66	34
									3d neo	511	728	217	70	30
4th "	106	149	106	143	152	238	88	138	Total 3 neo periods	1,598	2,276	678	70	30
5th "	121	132	105	138	114	177	106	147	15 days "Extra"					
Total 5 days	528	734	506	673	581	875	511	728	phenols 15 days	14	74	60	19	81

¹ All figures refer to milligrams unless otherwise indicated.

1. The elimination of "normal" free and conjugated phenols is quite uniform from day to day, if the animal is kept on a constant diet. The work of Folin and Denis is thus confirmed.

2. After a single intravenous injection of neo-arsphenamine, no appreciable variation in phenol elimination is observed during the first period of five days. During the next period there is an increase in both the free and conjugated phenols. During the

¹ Folin, O., and Denis, W., *Jour. Biol. Chem.*, 1915, 22, 305-308.

² Dubin, Harry, *Jour. Biol. Chem.*, 1916, 26, 69-91.

third period of five days there is a return to practically normal excretion.

3. The injection of a dose of 0.841 gr. of neo-arsphenamine results in the elimination of a total of only 74 mg. "extra" phenols during a period of 15 days; 81 per cent. of this is in the conjugated state. Of the "normal" phenols only from 10 per cent. to 30 per cent. is conjugated. Since in the colorimeter 1 mg. of neo-arsphenamine reacts like 0.466 mg. phenol, we have the striking observation that only 9.2 per cent of the dose of the drug injected is eliminated in the urine in the form of a phenol or phenol derivative. This suggests the following possibilities: (a) Some of the drug is eliminated as phenols in the feces; (b) a portion of it is burned; (c) a portion is retained or "fixed" by the various tissues, notably the liver, for a period much longer than 15 days; (d) most of the drug is oxidized to the stage of *inorganic* arsenic and eliminated as such; (e) a portion is eliminated as nitro-phenols which do not react with the Folin reagent. The latter suggestion seems the most improbable but not impossible.

TABLE 2.
PHENOL ELIMINATION AFTER INJECTION OF NEO-ARSPHENAMINE AND AMMONIUM PERSULPHATE.¹

Daily Elimination.	Normal Control Period.		Persul. Control Period.		1st Period Neo. and Persul.		2d Period Neo and Persul.		Summary.					
	Free.	Total.	Free.	Total.	Free.	Total.	Free.	Total.	Periods.	Free.	Total.	Conj.	% Free.	% Conj.
1st day...	99	154	137	188	136	190	235	265	Normal period.....	523	775	252	68	32
2d " ...	91	154	128	181	191	272	263	301	5 days with persul. only .	604	834	230	73	27
3d " ...	106	152	106	135	137	188	320	365	1st period after neo and persul...	885	1,163	278	76	24
4th " ...	113	153	124	173	212	265	275	331	2d period after neo and persul...	1,426	1,630	204	88	12
5th " ...	114	162	109	157	209	248	333	368	Total 10 days neo and persul...	2,311	2,793	482	83	17
Total 5 days ...	523	775	604	834	885	1,163	1,426	1,630	"Extra" phenols 10 days.....	1,103	1,123	21	98	2

¹ All figures refer to milligrams.

Since the administration of sulphates is known to counteract the effects of phenol poisoning, and particularly in view of the work of Bufalini,¹ we studied the phenol elimination in the same dog after a single intravenous dose of neo-arsphenamine during the time that she was receiving daily subcutaneous injections of ammonium persulphate.

Method. The animal was kept on the same constant diet. Phenol elimination was studied for five days (control period); ammonium persulphate was now given subcutaneously twice daily in 2.5 per cent. solution in physiological saline in doses of 10 c.c. and phenol excretion was measured during this period. A single intravenous dose of 0.841 g. of neo-arsphenamine was now given and persulphate injections and estimation of urinary phenols was continued for ten more days.

Results are summarized in Table 2, and indicate the following:

1. The subcutaneous injection of ammonium persulphate which theoretically would furnish the organism with additional sulphuric acid for phenol conjugation, does not result in the elimination of any "extra" conjugated "normal" phenols. During a period of five days, only a slight increase in the free "normal" phenols was observed.

2. After the intravenous injection of a single dose of 0.841 g. of neo-arsphenamine and the daily subcutaneous administration of the persulphate, an enormous increase in the output of "extra" free phenols was observed. Simultaneously, a marked diminution in the excretion of "extra" conjugated phenols was noted. The animal also showed definite symptoms of intoxication. We must, therefore, conclude that the "extra" free phenols were due entirely to tissue destruction resulting from the toxic effects of the persulphate.

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¹ Bufalini, G., *Archives ital. de Biol.*, 1903, 40, 131-140.