drug to rabbits, or immediately after death when it occurred within 24 hours. Organic and inorganic sulfates were determined by the turbidimetric method of Treon and Crutchfield,[7] and glucuronic acid by a quantitative procedure described elsewhere.[8] A 1:1000 solution of adrenalin chloride (Parke, Davis and Co.) was employed. It was injected into an ear vein at a rate of 1 ml/30 min. Subcutaneous injections were given in 1.0 ml doses, once every 2 hours, while the compound was given orally (by stomach tube) in 10 ml doses once every 2 hours until the intended dose had been administered. (Precautions to prevent inactivation of adrenalin by reason of the alkaline reaction of the intestine were not taken.)

Experimental results are summarized in Table I. It is evident from the data that adrenalin is responsible for an augmented excretion of sulfate esters in the urine. The intravenous injection of 1 or 2 mg caused a reduction in the percentage of inorganic to total sulfates from a normal average of about 89, to 74%. Doses of 2 to 10 ml injected subcutaneously caused a reduction to 79 and 64%, respectively, while the oral administration of from 10 to 50 ml adrenalin reduced the percentage of inorganic sulfates to 85 and 50, respectively. The excretion of glucuronic acid remained normal after the injection or ingestion of this drug. It is of interest to note that a far greater quantity of organic sulfates was excreted than could be accounted for by conjugation with the amount of adrenalin that had been administered.

Conclusions. In the rabbit the administration of adrenalin is followed by a markedly augmented excretion of organic sulfates. This appears to indicate that this drug is inactivated *in vivo* by conjugation with sulfuric acid. Conjugation with glucuronic acid does not occur. These observations confirm those reported by Richter in 1940.

[7] Treon, J., and Crutchfield, W., Jr., *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 119.

[8] Deichmann, W., *J. Lab. and Clin. Med.*, 1943, **28**, 770.

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On the Toxicity of Atabrine.*

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Atabrine (Quinacrine) has lately attracted much attention as an effective substitute for quinine. In animals large doses of atabrine administered orally cause gastro-intestinal and central irritation; intravenous administration causes lowering of the blood pressure, and direct action of the drug on the excised heart results in "paralysis." In man the gastro-intestinal symptoms are reported to be most conspicuous; irritation of the central nervous system occurs but is encountered more rarely.[1]

A consideration of chemical configuration and the mechanism of drug action suggested that the toxic symptoms encountered after administration of atabrine may be due, in

* This work was supported by grants from the Joshua Rosett Research Fund and from the Josiah Macy, Jr., and Dazian Foundations.

[1] Findlay, G. M., *Recent Advances in Chemotherapy*, 1939.

TABLE I.
Inhibition of Choline Esterase of the Electric Eel of Human Serum and Red Cells by Atabrine, etc.

Exp. No.	Drug	Concentration molar	γ/ml (free base)	Acetylcholine split per hr, mg	Inhibition %
1				2.81	
				2.82	
	Morphine	1x10–3	303.	1.75	38
	,,	1x10–4	30.	2.46	13
	,,	1x10–5	3.	2.75	2
2	Quinine	1x10–3	324.	1.96	30
	,,	5x10–4	162.	1.96	30
	,,	2x10–4	131.	2.22	21
3				5.40	
				5.60	
	Atabrine	1x10–3	410.	.51	91
	,,	1x10–4	41.	.96	82
	,,	1x10–5	4.	2.43	55
				3.07	
				3.09	
	,,	1x10–5	4.	1.22	61
	,,	5x10–6	2.	1.76	43
	,,	2.5x10–6	1.	2.07	33
4				3.07	
				3.09	
	Eserine	5x10–7	.14	.45	85
	,,	2.5x10–7	.07	.83	83
	,,	1.25x10–7	.035	.68	68
5		Serum (human) dil. 1:15			
				5.2	
				5.0	
	Atabrine	1x10–3	410.	.17	95
	,,	1x10–4	41.	.43	92
	,,	1x10–5	4.	1.8	65
6		Red cells (human) dil. 1:25 hemolyzed			
				3.75	
	,,	1x10–5	4.	1.90	50
		Red cells (human) dil. 1:25 not hemolyzed			
				4.00	
	,,	1x10–5	4.	2.02	50

part, to the inhibition of choline esterase, the enzyme intimately involved in the transmission of nervous impulses. Support for this hypothesis was obtained in experiments described herewith.

Experimental. The activity of choline esterase was determined with the Barcroft-Warburg manometric method.[2] Preparations of choline esterase from the electric organ of *Electrophorus electricus*, from human plasma and human red cells served as enzyme sources. The original extract of the electric organ was diluted 0.6 to 100, the plasma 1 to 20 and the red cells 1 to 25 with buffer. The red cells hemolyzate was obtained by hemolysis of the washed red cells with 10 times the volume of distilled water. Two ml of the enzyme dilution and 1 ml buffer containing the drug to be tested were introduced in the main vessel. Ten mg of acetylcholine chloride in 0.1 ml buffer was added from the side bulb after thermo-equilibrium had been reached. The effects of atabrine, quinine (bisulfate), morphine (hydrochloride), and eserine (sulfate)

[2] Nachmansohn, D., *Bull. Soc. Chim. Biol.*, 1939, **21**, 761.

on the enzyme were compared. In Table I the mg acetylcholine split during one hour are calculated from the rate of hydrolysis during the first 20 minutes.

Discussion. Atabrine inhibits the choline esterase of the electric organ, of human plasma and red cells to 50% in a molar concentration of 10^{-5} corresponding to 4 γ of free base per ml. Quinine and morphine are on the average 200 times less effective whereas eserine is about 100-200 times more potent calculated on molar concentrations. An equal distribution of a daily dose of 300 mg of atabrine in a man of 70 kg would result in a concentration of 4 γ per g. Since such an equal distribution can not be assumed, the actual effective concentration (disregarding the elimination of the drug) must be much higher in certain tissues. The peripheral toxic symptoms after the administration of atabrine resemble a vagal stimulation, an effect which could be expected from the inhibition of choline esterase.

The particular toxicity of atabrine for the intestinal tract may be an example of selective drug action in the intact organism. The action of a drug depends not only on its affinity for some enzyme, but also on blood supply and on the permeation of the drug into cells and on the interaction of the drug with other cell constituents. For example, physostigmine and prostigmine have the same action in the periphery; but the former acts as a stimulant, the latter as a depressant on the central nervous system.[3] Furthermore, strychnine, although 10-30 times less effective than atabrine in its inhibitory action on choline esterase *in vitro,* acts selectively as a stimulant on the central nervous system.[4]

A study of drug action on isolated enzymes is essential in obtaining a clear picture of the relationship between chemical configuration and drug action. The antimalarials, quinine, atabrine, and plasmochin, are strong inhibitors of the tributyrin splitting lipase.[5,6] The continuous administration of such inhibitors of body esterases may conceivably influence fat metabolism in such a way as to be reflected in the chronic toxicity of these agents.

The chemical configuration which is responsible for the inhibition of choline esterase appears frequently to be related to the presence of an alkyl substituted nitrogen atom. A number of compounds with high affinity for choline esterase, such as methylene blue,[7] oxidation products of adrenaline,[8] and acriflavine[9] contain substituted nitrogen atoms. It is noteworthy that the configuration of one ring nitrogen and 2 nitrogen atoms in a similar side chain occurs in atabrine, plasmochin, and in the antimalarials synthesized by Fourneau.[1] It is also interesting in this connection that methylene blue shows antimalarial activity.

Our finding offers the possibility of an experimental approach to the problem of antimalarials and their toxicity. The specific enzyme inhibiting action found with antimalarials may be used as a lead in synthesizing new and non-toxic drugs. Such a study would show whether the chemical configuration responsible for enzyme inhibiting action is also essential for the antimalarial activity.

Summary. Atabrine is a strong inhibitor of choline esterase. This finding is discussed in relation to toxic and antimalarial activity and chemical configuration of drugs.

[3] Schweitzer, A., Stedman, E., and Wright, S., *J. Physiol.,* 1939, **96**, 302.

[4] Nachmansohn, D., *C. R. Soc. de Biol.,* 1939, **130**, 1065.

[5] Stedman, E., and Stedman, E., *Biochem. J.,* 1931, **25**, 1147.

[6] Fulton, J. D., *Ann. Trop. Med.,* 1936, **30**, 491.

[7] Rentz, E., *Arch. Exp. Path. Pharmakol.,* 1940, **196**, 148.

[8] Waelsch, H., and Rackow, H., *Science,* 1942, **96**, 386.

[9] Collier, H. B., and Allen, D. E., *Can. J. Research, B,* 1942, **20** (9), 189.