LETTERS TO THE EDITORS

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Mode of Action of 'Paludrine'

INVESTIGATING the activity of 'Paludrine' against plasmodia in tissue culture, Tonkin¹ and Hawking² concluded that the drug was inactive *in vitro*, but was metabolized to an active form in the serum of the host. In view of these observations, it surprised us to find that 'Paludrine' inhibited the oxidation of glucose, pyruvate and lactate by washed chick red cells parasitized with *P. gallinaceum* more powerfully than did quinine (Table 1).

TABLE 1

Inhibitor	Conc.	% inhibition of oxygen uptake after 2 hours		
		Glucose	Pyruvate	Lactate
'Paludrine' HCl	1/300,000	4.6	Nil	Nil
	1/60,000	20.7	2.5	24.1
	1/6,000	84.5	84.0	89.4
Quinine HCl	1/60,000	10.4	5.6	5.5
	1/6,000	15.8	15.6	5.5

Determinations of glucose utilization and changes in phosphorylated intermediates in parasitized red cell incubates in the presence of 'Paludrine' and of iodoacetic acid showed that 'Paludrine' inhibition was essentially different from that of the iodoacetate (Table 2). While the latter inhibited both oxygen consumption and glycolysis, with accumulation of hexose phosphates, 'Paludrine' showed less than 50 per cent inhibition of glucose utilization, although oxygen consumption was inhibited to a greater extent. Similar observations on quinine indicated that it inhibited glycolysis rather more strongly than oxidations, probably by inhibiting the hexokinase reaction³.

TABLE 2

	Inhibitor	Conc.	μ Mols used up (-) or formed (+) per hour per 100 μ mols glucose used by controls							
			Glucose	A.T.P.	Glucose-6-phos.	Fructose-6-phos.	Phospho-glyceric acid	Pyruvic acid	Lactic acid	Oxygen
Expt. 1	Control	—	-100	-141	-23.8		+91.3	+17.5	+625	-266
	'Paludrine' HCl	1/6,000	-69	-74	-31.3		+26.2	+13.8	+313	-31
	Iodoacetic acid	1/6,000	-11	-55	-7.4		+51.0	+2.9	+31	-58
Expt. 2	Control	—	-100	-66	-3.5	+0.7	-3.4	+3.0	-118	-71
	'Paludrine' HCl	1/3,000	-54	-26	-2.6	+1.0	+0.5	+4.3	-187	-5
	Iodoacetic acid	1/3,000	-13	-97	+8.9	+2.6	-3.1	+0.7	-414	-12

These observations suggest that 'Paludrine' exerts its anti-malarial action, at least partially, by inhibiting the oxidation processes in the parasites, but does not directly effect the anaerobic breakdown of glucose to lactic acid. However, higher concentrations of 'Paludrine' than those used by Tonkin and Hawking were necessary, particularly in the phosphorylation experiments, in order to produce measurable changes, and in the comparative experiments with quinine the greater inhibitory action of 'Paludrine' was more marked at high than at low concentration. It may be, therefore, that inhibition of oxidative processes

does not play such an important part where 'therapeutic' concentrations of the drug are concerned.

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¹ Tonkin, I. M., *Brit. J. Pharm.*, **1**, 163 (1946).

² Hawking, F., *Nature*, **159**, 409 (1947).

³ Marshall, P. B., *Brit. J. Pharm.* (in the press).

 β -Glucuronidase and Tissue Damage

AFTER repeated feeding of menthol to mice, Fishman¹ obtained results which on statistical examination showed an increase in β -glucuronidase in liver, spleen and kidney as compared with organs from untreated animals. Similar results were obtained in dogs fed with borneol. Since the compounds examined are known to be excreted as the glucuronides in certain species, Fishman considered β -glucuronidase to be responsible for glucuronide synthesis *in vivo*, and that he was measuring adaptation on the part of the enzyme in response to the presence of excess substrate. The work of other authors is not, however, compatible with the view that the function of β -glucuronidase in the body is other than hydrolytic^{2,3,4}.

Using biosynthetic phenol β -D-glucuronide⁵ for the assay of glucuronidase⁶, an attempt was made to confirm Fishman's findings with menthol. Twenty-four hours after intraperitoneal injection of *l*-menthol in vegetable oil into mice there was a marked rise in glucuronidase in liver, but not in kidney. It was difficult to decide whether the spleen enzyme was affected because of the great variation in normal values observed with this organ. Average normal values were the same in the case of each organ for mice of both sexes and drawn from three different colonies. The rise in liver glucuronidase was less marked after repeated feeding of menthol than after injection of much smaller doses.

It was observed that menthol caused gross damage to the liver, but not to the kidneys, and it was considered that this property, rather than the one of giving rise to a glucuronide, might explain the

effect on liver glucuronidase. A variety of other substances, known to cause damage to the liver or kidneys, but with one exception unlikely to form glucuronides, were therefore examined for their effects on glucuronidase. Compounds which are relatively non-toxic, but which are known or considered to form glucuronides in the body, were also examined. The substances were injected subcutaneously or intraperitoneally in oil or aqueous solution into groups of 3-9 mice, and the animals were killed after 1-3 days. With several of the substances, the organs were examined histologically