IN VITRO MICRO ASSAY SYSTEM FOR IDENTIFYING ANTIMALARIALS THAT ACT THROUGH THEIR METABOLITES

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CERTAIN antimalarial drugs (*e.g.* proguanil) are active only after metabolic transformation *in vivo*[1]. Since *in vitro* methods so far reported are not suitable to identify compounds that act through their metabolites, there is clearly a need for developing a test to study such compounds[2]. An *in vitro* micro-technique is presented here which may meet this requirement. In this method the inhibitory activity of a compound on the growth of *Plasmodium falciparum*, is tested after metabolic conversion by the hepatic microsomes. Proguanil hydrochloride was used to standardise the test.

FAN-5 strain *P. falciparum* was cultured *in vitro* using RPMI-1640 complete medium[3]. Hepatic microsomal fraction, S_9, was prepared from livers of male Wistar rats pre-treated with phenobarbital[4]. This was mixed with co-factors NADP and G-6-P to get freshly prepared S_9Mix. Employing essentially the procedures described before[5] the inhibitory activity of proguanil on the growth of *P. falciparum* was studied in polystyrene microtitre plates by the following methods.

Parasites were exposed to different concentrations of proguanil with or without S_9Mix. Table 1 shows that parasite counts in normal medium and S_9Mix controls were comparable. Further, growth of parasites was not affected by the addition of different concentrations of proguanil alone. On the other hand, the drug in all concentrations in the presence of S_9Mix produced reduction in parasitaemia by 48 hr and discernible inhibition by 96 hr.

Equal volumes of proguanil and S_9Mix (test) or medium and S_9Mix (control) were incubated in tubes at 37°C for 4 hr followed by heating at 100°C for 20 min. After removal of coagulated proteins by centrifugation, the supernatants were diluted serially in RPMI-1640 medium and tested against the parasites in the plates. From table 2, it may be seen that the parasite growth in supernatant from control remained unaffected, while the supernatant from test was inhibitory. These results indicated that the drug pre-incubated with microsomes was also effective and suggested that the products after bioconversion were probably heat stable.

The above findings clearly indicated that proguanil was active only if pre-incubated with or in presence of hepatic microsomes. It is now known that proguanil *per se* is inactive and that its antimalarial activity is due to metabolites, chiefly cycloguanil[6]. Therefore, our findings suggest that activity of the drug was due to metabolite(s). It may also be noted that a very low concentration of the drug was effective indicating high sensitivity of the technique.

The assay system presented here may therefore be useful for identifying antimalarial activity of atleast

Table 1 *Growth of Plasmodium falciparum (FAN-5) exposed to proguanil hydrochloride with or without hepatic microsomes.*

Dilution of proguanil hydrochloride[a]	Test in	Percent parasitaemia 48 hr	96 hr
10^{-9}	RPMI	1.20	4.65
	S_9Mix[b]	0.40 (66.6)	0.35 (92.4)
10^{-10}	RPMI	1.20	4.80
	S_9Mix	0.60 (60.0)	0.90 (98.1)
10^{-11}	RPMI	1.25	4.85
	S_9Mix	0.75 (40.0)	0.30 (93.8)
10^{-12}	RPMI	1.75	3.65
	S_9Mix	1.00 (42.8)	0.25 (93.1)
Control	RPMI	1.50	5.00
	S_9Mix	1.50 (0.0)	5.60 (−12.0)

Inoculum: 0.9% a, from stock, 5 mg/ml in RPMI; b, S_9Mix was prepared by the addition of 400 μl of S_9 + 200 μl each of NADP (13 mg/ml) and G-6-P (6.5 mg/ml) in 1 ml of RPMI-1640 and adjusted to pH 7.2. *Total volume in each well was 130 μl.* Wells in S_9Mix series contained 10 μl each of parasitized blood, S_9Mix and drug, and 100 μl of medium. Wells in RPMI-1640 series contained same as above except that S_9Mix was eliminated. Appropriate RPMI medium and S_9Mix controls were included. Each figure represents mean values of two wells. Figures in parentheses indicate percentage reduction.

Table 2 *Growth of Plasmodium falciparum (FAN-5) exposed to proguanil hydrochloride pre-incubated with hepatic microsomes.*

Reading at	Control 10^{-1}	Percent parasitaemia, Supernatant dilutions, Activated proguanil hydrochloride 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
48 hr	3.25	0.55	0.65	0.85	1.10	1.20
96 hr	7.50	0.30	0.45	0.75	1.00	1.30

Inoculum: 1.2%. Each well received 10 μl of parasitized blood and 100 μl of different dilutions of supernatant in RPMI-1640.

those compounds that are metabolically transformed in the liver.

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ORIGIN AND GEOLOGIC SIGNIFICANCE OF THE CHAVARA PLACER DEPOSIT, KERALA.

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THE origin of the beach placer minerals like ilmenite rutile-zircon-monazite-garnet and sillimanite of the famous Chavara deposit in Kerala is still a controversy. Krishnan[1] suggested that the placer minerals originated from the pegmatites and gneisses of the interior of south Kerala, while Subrahmanyan and Rao[2] opined that monazite-bearing granites gave rise to the placer deposit. Based on a comparison of mineral ages of monazite and zircon from the placer deposit and granites from south-western India, as also the rare-earth content of a pegmatite from south Kerala, Soman and Nair[3] concluded that the placer deposit originated from monazite-bearing granites or granitic pegmatites of late Precambrian–early Paleozoic age. However, wide disparity in ages of the gneisses (around 3000 m.y.)[4] and pegmatites (445–474 m y.)[5] as well as the absence of similar placer occurrences in the vicinity of Archaean gneisses and granites of late Precambrian age in other parts of Kerala are arguments against the above mentioned sources.

Recent investigations on rocks of the khondalite-migmatite complex in areas south of the Achankovil shear zone (figure 1) provide evidence of their prograde metamorphism with the formation of sillimanite, one of the key minerals of the granulite facies khondalite suite of rocks from biotite (figure 2a, b). *Disintegration of Ti-rich biotite into sillimanite and* ilmenite under the influence of alumina-rich solutions is a process suggested to have been related to the migmatization, associated with granulite-facies metamorphism, involving mostly the metasediments of the south Kerala aulacogen and culminating in the emplacement of S-type granite of quartz-garnet-feldspar composition. This can be schematically expressed as: Biotite + Al-rich solutions → sillimanite + ilmenite + K^+. Presence of garnet in the granite is indicative of the 'dry' nature of the granite-forming fluids, caused by the pervasive influence of CO_2 as evidenced from fluid inclusion studies[6]. S-type granites are generally rich in monazite[7] and zircon[8].

Thus, ilmenite, sillimanite, monazite and zircon formation is linked to the migmatization phase associated with metamorphism. Garnet is a constituent part of the khondalite as well as of the granite, and rutile is an alteration product of ilmenite. These evidences would suggest that the placer minerals (ilmenite-rutile-monazite-zircon-garnet and silli-

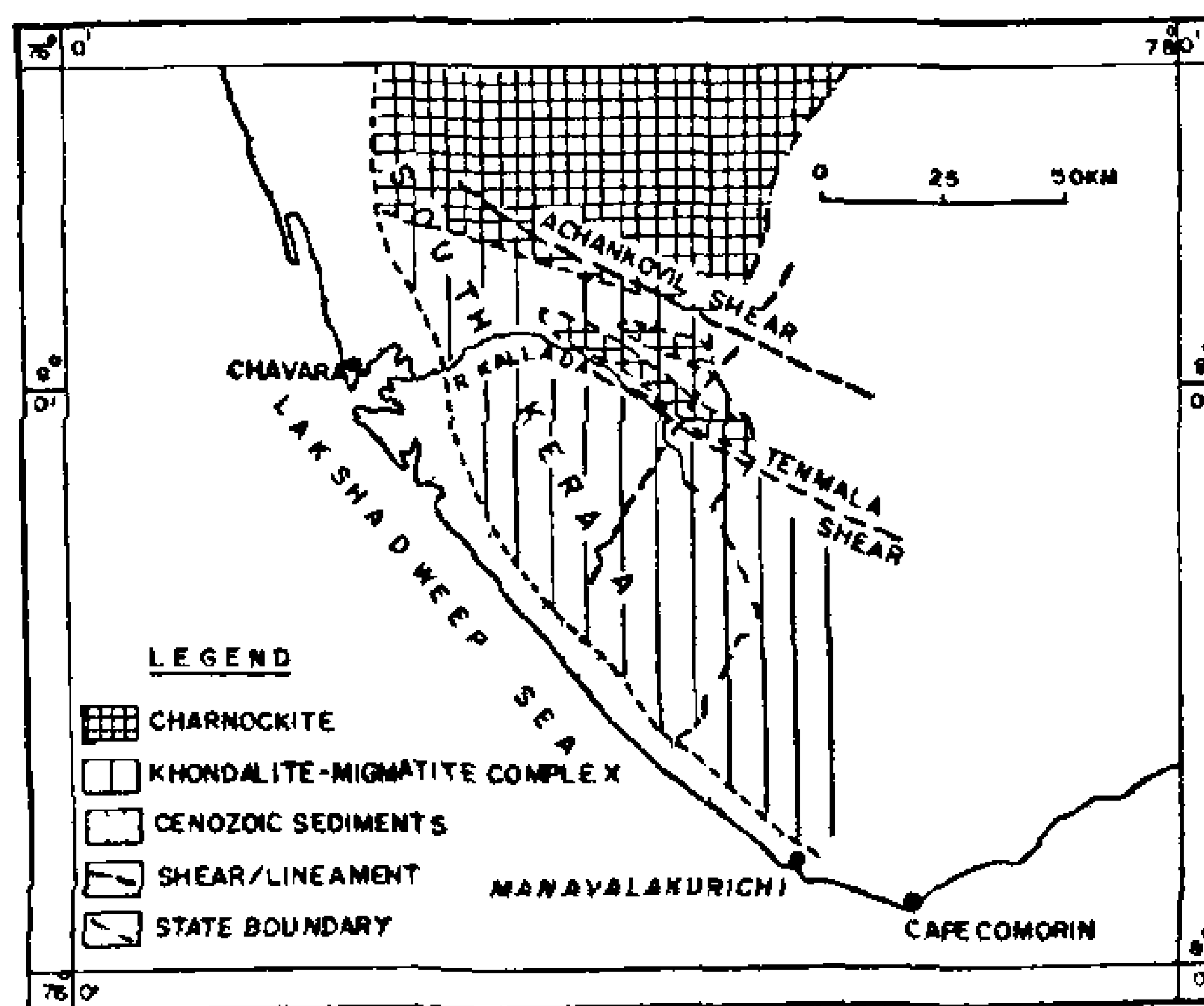

Figure 1. Location map of the Chavara Placer deposit (modified after Rao[12])