A Method of Testing the Antimalarial Properties of Compounds *in vitro*

THE rate of hæmolysis produced *in vitro* by the hæmolytic substance isolated from normal blood plasma¹ has been found to be decreased on addition of antimalarial drugs in low concentration (1:5,000 to 1:40,000). Closely related inactive compounds did not affect the rate of hæmolysis. Quinine, 1:10,000, for example, delayed the lysis time from one minute in the control to between 20 and 30 minutes, while a number of inactive quinoline, quinaldine and quinidine compounds failed to affect the rate of lysis.

Altogether, 117 substances, comprising active and inactive compounds, have been examined by the lysis test, as shown in the accompanying table:

	Known antimalarial activity	No. of drugs	Result of lysis test	
			Hæmolysis delayed (= active)	Hæmolysis not affected (= inactive)
I	active	39	38	1
II	slightly active	7	5	2
III	apparently inactive	17	5	12
IV	inactive	54	4	50

In Group I, 38 out of 39 antimalarial compounds were found to be active by the lysis test. Groups II to IV showed a certain discrepancy between antimalarial activity and lysis test: two slightly active drugs did not inhibit the rate of lysis, while a few of the apparently inactive drugs were found to do so. These differences may be due to the following facts: (1) the conditions of the lysis test as applied in these experiments (horse erythrocytes in isotonic phosphate buffer solution, pH 7.3) may as yet be far from optimal; (2) some antimalarial drugs reveal their activity in one host only, as for example in ducks, and are inactive in chicken, canary, monkey and man. Their antimalarial action, therefore, may not have been revealed by the particular biological test used for determining their activity.

Several other hæmolytic substances such as cobra lysocleithin, saponin, sodium dodecylsulphate, sodium desoxycholate, ethylene glycol, ammonium acetate and ammonium oxalate have been substituted for the naturally occurring hæmolytic substance in tests with quinine, mepacrine, paludrine, and a few other active and inactive compounds. The results of these tests were, however, entirely inconclusive, none of the hæmolytic substances giving any useful indication which would help to distinguish between the antimalarial activity or inactivity of compounds.

The marked parallelism between the antimalarial properties of compounds and their inhibitory effect on the hæmolysis *in vitro* produced by the naturally occurring hæmolytic substance makes possible the introduction of an additional test for screening potential antimalarial compounds. This parallelism makes it conceivable that the malaria parasites at some stage of their development produce a metabolite closely related to the naturally occurring hæmolytic substance, and that the effect of antimalarial drugs *in vivo* is bound up with their action on this metabolite. The results of this work, summarized above, constitute a step towards the biochemical approach to the study of the chemotherapy of malaria.

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A detailed report will appear elsewhere.

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¹ Laser, H., and Friedmann, E., *Nature*, 156, 507 (1945).

Penetration of Water into Erythrocytes with Heavy Water as Indicator

THE usual methods of studying the water metabolism of the red corpuscles always change the salt concentration of the surrounding medium. It has not yet been possible to follow the passage of water in and out under normal isotonic conditions. This can be done now by using heavy water.

Blood samples are collected from normal adults or patients with pernicious anæmia; to prevent clotting, sodium citrate or heparin is added. 0.1-0.3 c.c. of a 7.9 per cent deuterium solution in distilled water or 9 parts per thousand sodium chloride are added to about 5 c.c. blood. After a time ranging from 1 minute to 45 minutes, the blood is centrifuged and a small volume of both plasma and packed red cells is drawn. In one experiment the plasma was made hypotonic before the addition of heavy water.

The amount of deuterium is estimated by measuring the density of the water according to the method of Linderstrøm-Lang¹; density is given in 'units', that is, the difference, multiplied by 10⁶, between the density of the sample and that of pure water at the same temperature. The water of the samples is completely distilled off in vacuum, the samples being maintained at room temperature while the water is trapped in liquid air. The water is purified as described by Ussing and Wernstedt².

Even after a very short time, the water of the red corpuscles and that of the plasma have the same density. Water enters the erythrocytes so quickly, both under normal conditions and in a hypotonic medium, that the existence of a continuous exchange of water between

Temp. (° C.)	Time of contact	Density		Anti-clotting	Observations
		Plasma	Erythrocytes		
24°	—	188	188	citrate	normal
24°	6	136	136	heparin	pernicious anæmia
24°	4	129	117	citrate	normal
24°	3	440	407	"	"
24°	3	471	528	"	"
6°	1	153	153	"	"
6°	21	148	153	"	"
6°	3	136	136	"	"
6°	1	191	181	heparin	pernicious anæmia
24°	3	353	353	citrate	normal
24°	3	400	384	"	"
24°	45	295	303	"	"
24°	45	610	610	"	blood diluted with water (1 vol. water + 1 vol. blood)

plasma and red corpuscles must occur. Wolpers³ and Ruska⁴, using the electron microscope, have recently shown that the surface layer of the red corpuscles has a proteic framework, filled up by lipid material.

Our experiments establishing the identity of the water inside and outside the erythrocyte suggest that its surface, although it shows a frame-like structure⁵, must have a large number of very small fissures or holes, through which water passes in and out.

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¹ Linderstrøm-Lang, Jacobsen, O., and Johansen, G., *C.R. Lab. Carlsberg*, 23, 17 (1938).

² Ussing, H., and Wernstedt, A., *Skand. Arch. Physiol.*, 83, 169 (1940).

³ Wolpers, C., *Naturwiss.*, 29, 416 (1941).

⁴ Ruska, H., *Dtsch. Med. Wschr.*, 67, 281 (1941).

Quantitative Assay of Aneurin (Vitamin B₁)

IN the course of an investigation of base-exchange of aneurin (vitamin B₁) on zeolitic materials, it has been found that sands display properties which suggest that they possess a zeolitic surface layer. Using this property for a process of purification, a quantitative method has been developed for the estimation of vitamin B₁ in wheat products.

As might be expected, it has been found that wheat ground with sand and water gives a lower aneurin assay than wheat ground in a laboratory grinder. The importance of this in the grinding of biological material for the subsequent recovery of basic substances is obvious.

Full details of the work done are being published.

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Effect of Antiseptics on the Germination of Pollen Grains

POLLEN grains from many plants can be readily germinated on 30 per cent solutions of cane sugar with 0.8 per cent of gelatine. The rate of germination varies; but in many Labiates it begins within fifteen minutes at room temperature, and growth is obvious under a 2/3 objective with a micrometer eyepiece.

It has been found that many substances with bacteriostatic properties inhibit the formation of pollen tubes, in concentrations similar to bacteriostatic values. For example, phenol inhibits germination at 0.0035 per cent, and formalin at 0.01 per cent with white dead nettle pollen; and these values are similar to those found from many other pollens. Merthiolate is effective at 0.0002 per cent.

Pollen grains germinate, however, in very high concentrations of penicillin. The effective concentration varies slightly from plant to plant; with white dead nettle no inhibition is found at 5,000 units per c.c.; *Eschscholtzia* pollen is inhibited by 250 units per c.c.

In general, then, pollen grains show the same order of sensitivity to antiseptics as bacteria; but their sensitivity to penicillin is negligible, and of the same order as vertebrate cells in tissue culture.

The germination of pollen grains in the Labiates does not involve cellular multiplication. Presumably the development of the pollen tube is due to endosmosis, with distension of an elastic membrane, which becomes immediately inelastic except at the tip. This view is supported by the fact that if the temperature be raised the pollen-tube ruptures at the tip; the same effect follows the lowering of sugar concentrations.

The remarkable rate at which tube development takes place makes pollen grains very suitable objects for biological test. In many species, for example, iris, there is a striking movement of granules in the tubes; this movement may be studied in relation to antiseptic concentrations.

How antiseptics control pollen germination is not known; one reason may be an effect on the elastic membrane of the grain; another an effect on the contents of the grain which determine osmosis. Whatever the cause, penicillin is distinguished from other bacteriostatic agents by its inefficiency.