It must be emphasized however, that some children had had little or no food in the morning when the drugs were administered and that the side-effects noted might have been less frequent if the drugs had been given after a meal.

Summary

Field trials with chloroquine sulphate, pyrimethamine, camoquin, and azacrin (an amino-aza-acridine) were carried out on 120 African schoolchildren with a subclinical natural infection with *P. falciparum* and *P. malariae*.

All four antimalarials have proved to be good schizonticides. The clearance time of parasitaemia due to trophozoites of *P. falciparum* was shortest with azacrin (1.58 days) and camoquin (1.69 days), closely followed by pyrimethamine (1.84 or 1.77 days) and chloroquine sulphate (1.78 or 1.95 days). None of the four drugs shows any direct gametocidal action.

It appears that chloroquine sulphate and camoquin have a more marked action on *P. malariae* than either pyrimethamine or azacrin.

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AN ASSESSMENT OF CHLOROPHYLL AS A DEODORANT

BY

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Between 1943 and 1950 many claims were made in published articles for the beneficial effect of water-soluble chlorophyll in stimulating the growth of granulation tissue and accelerating epithelization of skin ulcers. The work of Moss and his colleagues (1949) cast doubt on the validity of these claims. In 1951 the Council on Pharmacy and Chemistry of the American Medical Association could find no evidence that water-soluble chlorophyll stimulated granulation and healing beyond the normal rate.

Many reports also noted a deodorant property in chlorophyll when applied to skin ulcers. More recent workers have claimed that the alleged deodorant action of chlorophyll is effective against all kinds of body smells when the substance is taken orally. Such claims have received the widest publicity in the lay press. On the other hand, the *Journal of the American Medical Association* (1951) questions whether chlorophyll does in fact have deodorant properties at all.

The present communication presents the results of a number of *in vitro* experiments designed to determine

the effect of chlorophyll on a number of odours. Some clinical experiences with the drug are also recounted.

Full summaries of the earlier work with chlorophyll have appeared in recent leading articles in the *British Medical Journal* (1952) and the *Lancet* (1952). It is therefore unnecessary to review the literature again here. It may be noted, however, that all published findings of the action of chlorophyll as a deodorant have depended on the olfactory sense of the observer. Not only is the sense of smell notoriously inconstant from one observer to another, it is also apt to fluctuate in the one observer in different circumstances. Of all the human senses the sense of smell is the most easily affected by suggestion.

Chemistry

As there are various types of chlorophyll, a brief comment is required on the chemical composition of the substance used in these experiments.

Chlorophyll is extracted from plants (especially nettles, spinach, and alfalfa) with alcohol or acetone. This substance, which has the formula shown below, is oil-soluble

Chlorophyll formula. R=CH₂ (chlorophyll-a) or CHO (chlorophyll-b). Ester groups indicated by dotted boxes.

but not water-soluble. It has been described (*Lancet*, 1952) as "a system of four pyrrole rings pivoted on a central magnesium atom," and the formula is similar to that of haem, in which the pyrrole rings pivot in similar manner on an iron atom. Treatment of this substance with acid removes the magnesium atom. The magnesium atom is also easily displaced by other metals—for example, copper.

Treatment with alkali (hydrolysis) splits off the two ester groups shown in the diagram. The resulting substance is a salt of the dicarboxylic acid chlorophyllin. The sodium and potassium salts of this acid are soluble in water and constitute the active principles of the so-called water-soluble chlorophylls (Mitchell, 1952). The therapeutic substance called chloresium was defined by the Council on Pharmacy and Chemistry (1951) as consisting chiefly of the copper complex of the sodium and potassium salts of chlorophyllin. This is the substance used in those published trials in which the nature of the chlorophyll was disclosed. More often, proprietary preparations were used and no information was supplied about their chemical composition.

In the present experiments the substance hereafter called chlorophyll solution A consists chiefly of sodium copper chlorophyllin, and is made up in a solution containing 1 g. in 10 ml. The substance called chlorophyll solution B is a crude commercial preparation of water-soluble chlorophyll intended for use as colouring matter. Solution C is a proprietary preparation intended for use as an atmospheric deodorant, and is exposed to the atmosphere by means of a wick. Solution D is a proprietary water-soluble preparation sold for dispensing purposes. It is reported to contain 12.5% water-soluble chlorophyll.

Experiments

The principle of the first experiments is the same as that used by Alstead (1942) in assessing the deodorant properties of activated carbon. Methylmercaptan gas (one of the substances contributing to the odour of the gas normally present in the lower bowel) is evolved by adding 5N solution of

sodium hydroxide to crystals of thiourea sulphate (Fig. 1). To obtain as near standard conditions as possible in consecutive experiments, constant quantities of the reagents are used (3.5 g. of thiourea sulphate and 5 ml. of 5N NaOH). The flask containing the reagent is kept in a water-bath at a constant temperature of 65° C. Nitrogen is bubbled through the flask at a constant rate of flow so that the inert gas carries mercaptan through the rest of the apparatus. The gas passes through two bell-jars, securely clamped together, where it comes into intimate contact with a screen of filter paper soaked in chlorophyll solution. The filter paper is arranged around a baffle (Fig. 1).

After passing through this part of the circuit the gas bubbles through test-tubes containing N/10 solution of iodine. Any mercaptan passing over combines with iodine to form colourless hydriodic acid. Thus the time taken to decolorize a standard quantity of iodine solution can be used as a measure of the amount of mercaptan passing over. Alternatively, after some, but not all, of the iodine has combined with mercaptan, a back-titration with N/10 sodium thiosulphate solution indicates the amount of iodine remaining unchanged, and by difference the amount of mercaptan which has reacted with the iodine solution.

Experiment 1

The circuit was set up as in Fig. 1. To the filter paper around the baffle was added 2 ml. of either water or chlorophyll solution A. The whole circuit was filled with mercaptan. New reagents were then added for producing mercaptan. The iodine indicator was added and the gas passed across the circuit for 20 minutes. In this way as nearly similar quantities of mercaptan as could be obtained were exposed to water or to chlorophyll. By back-titrations at the end of the experiment the amount of iodine which had been reduced was noted.

The results were as follows: (1) water, 2 ml. on filter paper—12.6 ml. iodine solution reduced; (2) chlorophyll 2 ml. on filter paper—14.6 ml. iodine solution reduced; and (3) chlorophyll, 2 ml. on filter paper—18.5 ml. iodine solution reduced. These findings show at once that chlorophyll has not removed or altered all the mercaptan exposed to it. Indeed, more mercaptan has passed across when exposed to chlorophyll than when exposed to water.

Experimental error is obviously too great to allow of exact quantitative comparison. The source of error was thought to lie in the existence of a large dead space resulting from the use of bell-jars, and accordingly this was eliminated in experiment 2.

FIG. 1.—A and B=Test-tubes containing iodine, used as an indicator.

FIG. 2.—A=Test-tube containing chlorophyll solution, and used as a trap to collect froth from the burette. B=Test-tube containing iodine, used as an indicator.

Experiment 2

The apparatus is shown in Fig. 2. An ordinary 25-ml. burette is filled with the substance to be tested. It is tilted slightly off the horizontal so that bubbles of gas slowly traverse the column of fluid. The gas then passes to the test-tubes. The first test-tube, which also contains a measured volume (10 ml.) of the chlorophyll solution being tested, acts as a trap. Chlorophyll solution has a low surface tension, and bubbles of gas passing through it tend to carry the solution forward as foam. This is caught in the first test-tube. The second test-tube contains 5 ml. of iodine solution. The time taken for decolorization of successive tubes of 5 ml. of iodine is noted.

The reading from the first tube of iodine is always the highest, as mercaptan is soluble in water. The first reading is therefore a measure of the solubility of mercaptan in the solutions used. These were as follows:

Water	34 minutes
Chlorophyll solution A (a)	34 "
" " (b)	35 "
Chlorophyll solution C	36 "

After decolorization of this first tube of 5 ml. of iodine, fresh reagents for production of mercaptan were added and notes were made of the times taken to decolorize a second and then a third test-tube, each containing 5 ml. of iodine. The results obtained on two different days were as follows:

First Test.—Rate of flow of nitrogen carrying mercaptan across=8 bubbles a minute.

	Time to decolorize 5 ml. iodine
Water (i)	4 minutes
" (ii)	6½ "
Chlorophyll solution A (i)	4 "
" " (ii)	7 "

Second Test.—Rate of flow of nitrogen=4 bubbles a minute. (N.B., reduced rate of flow.)

	Time to decolorize 5 ml. iodine
Chlorophyll solution A (i)	7½ minutes
" " (ii)	22½ "
Chlorophyll solution C (i)	6 "
" " (ii)	22 "

Thus, regarding chlorophyll solution A as a standard, it will be seen that there is no difference in the time taken for a constant amount of mercaptan gas to pass through solutions of chlorophyll A and C and water. Furthermore, the times taken for such solutions and water to become saturated with mercaptan are the same. It is obvious, therefore, that intimate exposure of mercaptan gas to the action of chlorophyll solution, as described above, did not reduce the volume of the gas.

Experiment 3

This experiment involves the use of very much smaller quantities of mercaptan and of other odorous vapours, and exposing these to chlorophyll for much longer periods. This method depends on the reliability of the observer's olfactory sense, and is open to criticism on that account. Such criticism, however, as has already been noted applies equally to all who have made positive claims for the deodorant value of chlorophyll.

The apparatus is shown in Fig. 3. The bell-jar stands on a ledge in a water-bath, so that there is a complete water seal. A is a filling funnel from which N/2 sulphuric acid is run in; B is a shallow dish containing N/2 sodium hydroxide saturated with

methylmercaptan. By mixing equal quantities of acid and base methylmercaptan gas is liberated. The filter paper (C) stands clear of the water in a petri dish soaked with chlorophyll or other substance under trial.

FIG. 3.—A=Filling funnel ($N/2 H_2SO_4$). B=Dish containing mercaptan in $N/2 NaOH$. C=Filter paper soaked in chlorophyll.

with acid liberates very approximately 30 mg. of methyl mercaptan, or 130 ml. of the gas. In two cases tablets of chlorophyll, marketed for oral use, were crushed and suspended in water (one tablet per ml.), and this suspension was exposed to the gas.

The experiment is set up and stands for one to three days. The bell-jar is then opened and the odour of its contents noted. No attempt is made to record the degree of smell present, as the method is too subjective to provide reliable quantitative results. The smell is therefore merely recorded as being present or absent.

These experiments were also carried out using (a) ether, and (b) syrup of garlic to yield characteristic smells.

The results are shown in the Table. One millilitre of $N/2 NaOH$ when neutralized

Source of Smell	Substance under Trial	Quantity	Duration of Exposure	Result
Methylmercaptan:				(Smell +/-)
30 mg. . .	Water (control)	3 ml.	12 hours	+
30 " . .	" " "	3 "	13 "	+
30 " . .	Chlorophyll solution A	2 "	12 "	+
30 " . .	" " "	3 "	12 "	+
30 " . .	" " "	3 "	14 "	+
30 " . .	" " "	3 "	16 "	+
15 " . .	Water (control)	3 "	14 "	+
15 " . .	Chlorophyll solution A	3 "	14 "	+
15 " . .	" " "	3 "	15 "	+
15 " . .	Suspension of "chlorophyll tablets	3 "	12 "	+
15 " . .	" " "	5 "	20 "	+
15 " . .	Water	5 "	20 "	+
15 " . .	Chlorophyll solution A	5 "	22 "	+
15 " . .	" " "	2 "	46 "	-*
15 " . .	" " "	1.5 "	46 "	+
Meth-ether:				
4 ml. . .	Water	2 "	28 "	+
2 " . .	Chlorophyll solution A	0.5 "	22 "	+
1 " . .	" " "	4 "	47 "	+
1 " . .	" " " D	4 "	25 "	+
1 " . .	" " "	4 "	36 "	+
Syrup of garlic:				
5 ml. . .	Water	1 "	12 "	+
5 " . .	Chlorophyll solution A	1 "	12 "	+

* In this case the smell of mercaptan was not detected. There was, however, a very strong smell of chlorophyll, which may have masked it.

This experiment, which involved exposure of small quantities of mercaptan, ether, and syrup of garlic in a small enclosed space for long periods of time to filter paper soaked in various chlorophyll preparations, showed a possible complete deodorant effect in only one case, and because of the presence of a strong smell of chlorophyll on that occasion it can hardly be regarded as being significant. It is concluded from this experiment that chlorophyll when exposed in this way to an odour does not remove the odour from the air.

Experiment 4

(a) Three jars were set up containing, respectively, 4 ml. of concentrated solution of chlorophyll; 4 ml. of dilute solution of chlorophyll; and 4 ml. of water. To each was added one drop of perfume. On smelling the jars subsequently no difference could be detected between them. In each case all the liquid evaporated within 14 days. The smell of the perfume persisted strongly in all the jars even after five months. A similar experiment, using slices of onion instead of perfume, showed no difference after 24 hours. The smell was so offensive that the experiment then had to be abandoned.

(b) Stopped test-tubes containing 10 ml. respectively of water, chlorophyll solution A, and solution C were set up for 72 hours, with 10 ml. of a solution of mercaptan in water added to each. At the end of this time there was a strong smell of mercaptan from each tube. It was also noted that the tube containing solution C (a preparation of chlorophyll which is perfumed) yielded the smell of mercaptan after standing for 72 hours, but on being shaken the smell of the perfume emerged and masked the smell of mercaptan fairly effectively. This was noted on several occasions, and suggests that the apparent deodorant property of such a preparation is due to substitution of a smell which is stronger but more pleasant than that which it is desired to eliminate. This experiment was repeated, using a smaller quantity (1 ml.) of mercaptan solution. Instead of adding chlorophyll solutions, however, plugs of cotton-wool were inserted at the upper part of each tube. These were then saturated respectively with (1) chlorophyll solution A; (2) chlorophyll solution A evaporated to dryness; (3) powdered activated carbon; and (4) a control of plain cotton-wool. After 24 hours the smell of mercaptan was perceptible in all the tubes. After a further 24 hours the smell was perceptible from all tubes except that containing activated carbon.

(c) A series of four stoppered test-tubes was set up containing (1) 5 ml. of syrup of garlic and 4 ml. of chlorophyll solution A; (2) 5 ml. of syrup of garlic and 4 ml. of chlorophyll solution D; (3) 5 ml. of syrup of garlic alone; and (4) 5 ml. of chlorophyll solution A alone. The smell of garlic was present in all tubes except the fourth whenever the contents were smelled—at intervals from one day to one month after setting up. (The test-tubes were left in an ordinary warm room during all this time.)

Clinical Trial

The following simple *in vivo* tests were performed:

(a) A patient incontinent of urine and faeces was being nursed in a special bed which gathered the excreta direct into a receptacle. This patient was given three-day courses of (1) chlorophyll solution A, 400 mg. daily; (2) proprietary chlorophyll tablets, 400 mg. daily; and (3) periods with no chlorophyll. The days on which particular courses were given were not known to the observer. Each morning the odour of the receptacle containing the night's collection of urine was noted. This characteristic and unpleasant odour of urine was present every day no matter whether chlorophyll was being given or not.

(b) *Asparagus Test*.—It is well known that in many people the urine develops a characteristic odour within an hour or two of eating asparagus. One of the earliest and widely quoted observations on the alleged deodorant action of chlorophyll is one by Westcott (1950), who stated that oral administration of chlorophyll before taking asparagus prevented the appearance of this odour in the urine. A colleague in this hospital who consistently detects the asparagus odour in his urine after eating this vegetable decided to test Westcott's findings. In the 24 hours preceding the meal he swallowed 48 100-mg. tablets of a proprietary water-soluble chlorophyll in doses of 6-12 tablets at intervals of four hours. He then took his meal, which included an average serving of asparagus. Some two hours later the urine which he passed smelled strongly of the volatile substances of asparagus, and the odour was readily

detected in further specimens of urine passed about 12 hours later. It is concluded that, in this observer, chlorophyll in doses at least 25 times greater than those usually recommended was valueless in preventing the characteristic smell of the urine following ingestion of asparagus. It was also noted that the smell of the faeces was unaffected by this large dose of chlorophyll.

I have confirmed these findings in similar manner, and have also found that the oral administration of 800 mg. of chlorophyll daily for a week did not remove the odour of urine, faeces, or axillary perspiration.

So much has been made of the alleged deodorant effect of chlorophyll on the urine of persons who have eaten asparagus that it would be helpful if a large number of doctors would themselves repeat this simple experiment. The experience would be in the best tradition of the advice offered to Jenner by John Hunter.

Conclusions

Exposure of methylmercaptan gas in a closed system to water-soluble chlorophyll did not reduce the amount of gas passing over to an indicator, compared with a control.

Exposure of small quantities of methylmercaptan gas to water-soluble chlorophyll in a closed system for up to three days did not remove the smell of mercaptan.

Mixtures of water-soluble chlorophyll and various strong-smelling solutions did not remove the smell of these solutions even after exposure for one or more months.

Clinical trials are reported in which the smell of urine, faeces, sweat, and of urine after ingestion of asparagus was not removed after taking large numbers of chlorophyll tablets.

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The Faculty of Ophthalmologists has for some time been considering the desirability of standardizing reading types used for testing vision. Suggestions from many sources have been studied and the final recommendations of the Council of the Faculty have been embodied in a memorandum by Mr. F. W. Law (*Brit. J. Ophthalm.*, 1952, **36**, 689). The Council's recommendations for a standard test of near vision are that: (1) "Times Roman" type shall be used, with "standard" spacing; (2) suitable passages of literature shall be selected, and a different passage used for each type; (3) specimens of printing in 5, 6, 8, 10, 12, 14, 18, 24, 36, and 48 point type shall be used, starting with the smallest [1 point = 1/72 in.]; (4) a few isolated words in lower-case letters shall be printed in each size; (5) near acuity shall be recorded by the letter N, followed by a number indicating type-face size—for example, N.5, N.8, etc.; (6) the test shall include a facsimile of a Snellen distance chart reduced to 1/17 normal size and used at 35 cm. distance, the result being recorded as a "Snellen equivalent"—for example, N=6/9; (7) the test may also include reproductions or actual portions of familiar types in everyday use; (8) near test types shall be reproduced on sheets about 9 × 6 in. (23 × 15 cm.); (9) the print shall be on dead-white matt-surface cartridge paper or board, and the latter may be varnished to increase durability.

CAPACITY OF A CATIONIC EXCHANGE RESIN ("ZEO-KARB 225") IN VIVO

BY

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Cationic exchange resins are insoluble powders which will exchange cations on the same principle as the familiar water softeners. The exchange obeys the law of mass action, and mixtures of cations combine with the resin *in vitro* in proportions determined by equilibrium constants (MacIntyre and Jordan, 1951). *In vitro* the affinity for monovalent ions follows the order $K > Na > NH_4 > H$. The affinity for divalent ions is greater than for monovalent ions. The resins have been given in the H or NH_4 form to prevent absorption of Na from the bowel in patients with oedema or ascites and in patients with hypertension (McChesney, Dock, and Tainter, 1951; Greenman and others, 1951). The resins give off H or NH_4 in exchange for Na; H or NH_4 is absorbed combined with acid, and Na is excreted in the faeces combined with resin. The resin takes up K and other cations as well, and the depletion of cations may result in acidosis. To minimize these complications the resin has been given partly combined with K.

Most of the information available is on the effects of carboxylic exchange resins. In the course of work on experimental depletion of potassium we have obtained data on the effects of a sulphonated polystyrene resin, "zeo-karb 225," on the absorption of K, Na, Mg, Ca, and P with a normal diet in man. Though these data are incidental to another investigation and give information of limited scope, we think it may be useful to report them now that a pharmaceutical preparation of zeo-karb 225 has become available ("kationium"). Additional information was derived from studies of three patients who were receiving only small amounts of sodium in their diet.

Procedure

There were three experiments—F.1, W., and F.2—on two normal subjects, F. and W. The H form of the resin was given in F.1 and the NH_4 form in W. and F.2. The dose of resin was 60 g. daily for eight days, followed by 90 g. daily for eight days in F.1 and F.2 and 90 g. daily for four days in W. (These doses are two or three times what is likely to be given therapeutically.) The diets contained, per day, Na, 180–220 mEq; K, 31–38 mEq; Mg, 12–22 mEq. The composition of the diet in each experiment was constant. Resin was given with meals; added sodium was given between meals. The faecal excretion of N, P, Ca, K, Na, and Mg and the urinary acidity and ammonia were measured. Other details are available in a previous publication (Fourman, 1952).

In addition three patients were studied: a woman (A.) with intractable cardiac oedema; a man (M.) with cirrhosis of the liver, ascites, and oedema; and a woman (P.) with hypertension but without oedema or other evidence of heart failure. The patients received resin in a dose of 20, 30, or 45 g. daily while taking a diet which contained less than 20 mEq of Na a day. The faecal excretion of Na was measured in periods of seven or twelve days while the resin was given in the NH_4 form and also while it was