

A table giving reactions on seven samples of cholesterol

The Wassermann antigen made with:—	Fixation of complement with titrated positive controls:—			
	Control I, with 3 MHD		Control II, with 5 MHD	
	I, 1, 1 in 75 dil.	I, 2, 1 in 150 dil.	II, 1, 1 in 75 dil.	II, 2, 1 in 150 dil.
1. New stock, Merck	+	T	+	±
2. New stock, B.D.H.	+	T	+	±
3. Recrystallized (process <i>iii</i>).	+	±	+	—
4. Washed in alcohol (process <i>iv</i>).	+	T	+	±
5. Deodorized under pump.	T	±	T	—
6. Washed in water	T	±	T	±
7. Untreated yellow and odorous.	T	±	T	—

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BACTERICIDAL AND FUNGICIDAL ACTION OF ORGANIC MERCURIALS
WITH SPECIAL REFERENCE TO THE DERMATOMYCOSES

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It has been stated recently (Hunter, 1943) that the ideal antiseptic should possess high germicidal and inhibitory properties over the widest possible range of the commoner pathogenic organisms, a low tissue toxicity, efficiency in the presence of organic matter, power to penetrate, stability, and moderate cost.

In this country the commoner pathogenic organisms include a great number of mycotic agents, these latter being especially common in moist, humid regions and during the monsoon period. It is therefore considered that the ideal antiseptic should possess a well-marked fungicidal as well as a bactericidal effect. This report intends to show that certain organic mercurial drugs possess the above-mentioned properties in the highest degree, and it is felt that, in the latest spate of literature concerning modern antiseptics, this remarkable series of compounds has been greatly neglected.

Before describing the uses and method of application of the above-mentioned drugs, it would be well to explain the rationale governing the use of antiseptics and bactericidal substances whose active agent is a phenol radicle or Hg cation.

As long ago as 1881, Koch drew attention to the toxic action of mercury on bacteria, and Paul and Prall (1907) showed that this effect depends on the free concentration of Hg ions in solution.

That this Hg cation was the most effective of the heavy metal cations was proved by Woodruff and Bunzel (1909) and later by Winslow and Hotchkiss (1922). This toxic action on bacteria was well manifested *in vitro*, but it has been shown that *in vivo* its bactericidal action is greatly diminished, especially in the presence of organic matter. Chick and Martin (1908) consider that this was due to the fact that these cations combine with protein to form an insoluble albuminate; hence the concentration of free ions is greatly diminished. Clark (1940) reported that when a 3 per cent suspension of dried human faeces is added to a solution of an Hg salt effective *in vitro*, its activity is reduced by 80 to 85 per cent. The activity of certain organic mercurial salts under these conditions is reduced by only 15 to 20 per cent, and that of phenol is reduced by only 10 per cent. It follows, therefore, that in the presence of organic matter, these organic mercurial salts possess a far greater bactericidal effect than those of the inorganic series, while at the same time the phenol co-efficient in the former is far greater than that of the latter. The advantage of using these organic mercurials in tissue lesions due to infection is thus clearly indicated, for it appears that the formation of an insoluble albuminate does not obtain to any marked degree in the use of drugs of this group.

It is proposed to show that the type of organic mercurial employed exerts an influence on the results, on account of varying chemical constitution. The simple aliphatic compounds of Hg have proved extremely toxic in their effect on the human organism. These compounds are represented by the series Hg (R) 2 where R represents one of the lower hydrocarbon radicles. Mercury dimethyl Hg(CH₃)₂ and mercury diethyl Hg(C₂H₅)₂, the most elementary compounds in the series, have a special affinity for the central nervous system, causing rapid degenerative lesions in cerebral and cerebellar areas. The latter compound is, indeed, one of the most interesting in human toxicology, for there is a latent symptomless period of about 16 days between its application or ingestion and the development of signs and symptoms in the central nervous system.

The higher compounds of the series of organic mercurials (aromatic aryl or tolyl compounds), mainly phenyl mercuric acetate, phenyl mercuric chloride and phenyl mercuric nitrate, have been investigated chiefly in the U.S., but Biskind (1935) gave a detailed account of their action

and reviewed the literature concerning them in an article in the *Lancet* some years ago. (For convenience the above-mentioned compounds will be referred to as P.M.A., P.M.C., and P.M.N. henceforth in this report.)

The bactericidal and bacteriostatic action of P.M.C. was fully investigated by U.S. workers in 1933 and 1934 (Weed and Ecker, 1938). It was found that the concentration necessary to inhibit the growth of *S. haemolyticus* and *S. aureus* in culture was 1/150,000,000 and 1/125,000,000 respectively.

In England, so far, the above-mentioned compounds have been used chiefly in plant chemistry, and the fungicidal action of P.M.C. was investigated in England by Fitzgibbon using various types of plant fungi. It was shown that this substance is one of the most powerful fungicides known, and acts rapidly and effectively in extremely high dilutions.

Byrne and Fitzgibbon (1932) using 2 per cent P.M.C. on both H.E.B. and diglycol stearate showed that it also exerts a rapid and specific effect on fungi and bacteria pathogenic to man.

In choosing this particular member of the organic mercurial series, the rationale was as follows:—

(1) It was known (Biskind, 1935) that the compound C_6H_5HgCl had a very high bactericidal potency, with a relatively low local and systemic toxicity for animals and for man.

(2) This effect is due to the action of the compound C_6H_5Hg ion, and it is thought on theoretical grounds that the bactericidal power of Hg is greatly increased by using it in the form of a phenol derivative.

Experiments by Reichel (1909) on the dispersion phases of phenol between oil and water suggested that the action of phenol is not so much chemical as physical, the phenol being capable of passing into solution in such substances as coagulated albumin, certain lipoids and the cytoplasm of bacteria. He suggested, therefore, that its action results from its penetration into the bacterial cell in the form of a colloidal solution. Moreover, in the presence of serum, no diminution of the germicidal action of phenol occurs (Browning *et al.*, 1917).

(3) Though the solubility of P.M.C. is extremely low, this does not detract from its effectiveness. The active portion is the C_6H_5Hg ion, and it is considered that this radicle enters into some form of colloidal solution in the cytoplasm of the infecting fungi or bacteria, and hence a continual removal of C_6H_5Hg ions takes place from the vehicle or filler in or on which the drug is presented to the infecting organism. There appears to be something in the nature of a continual bombardment of the organisms with organically combined Hg. It is considered on theoretical grounds that its effect is to cause a rapid disturbance of metabolic function leading to the death of the infecting agents.

P.M.A. has also been used in the investigation, but as this compound has a marked affinity

for Cl, the change from P.M.A. to P.M.C. takes place immediately on contact with NaCl, and as this latter salt is an invariable constituent of tissue fluid, it was felt that the direct application of the ultimate chemical product should be made. This report, therefore, deals mainly with the action of phenyl mercuric chloride.

As in all bactericides, the reaction velocity of P.M.C. depends on its concentration in the area in which it is to exert its action, but owing to its tendency to produce a vesicant effect on tissue or skin, the concentration used must be carefully limited. If a 1 per cent concentration is applied for some hours to skin lesions caused by bacteria or mycotic agents, an extremely rapid bactericidal and fungicidal effect obtains, but the surrounding healthy skin areas become erythematous or vesicated and do not recover for a further 48 to 72 hours. In practice, it has been found that 0.25 per cent P.M.C. or 0.25 per cent P.M.A. represent the maximum that can be used without producing these effects. Five per cent P.M.C., adsorbed on a calamine filler, can be used on moist skin lesions without side effects, as the physico-chemical mechanisms obtaining when the drug is presented in this way are entirely different from those which occur when emulsifying or aqueous vehicles are used.

This latter method (adsorption of a practically insoluble compound on a filler) is thought to mark a new advance in the presentation of a substance (which is almost insoluble in ordinary media) to the infecting agents on which it is to act.

An alternative method is the preparation of a colloidal solution of the drug, but the grave disadvantage in this method is that, in using colloidal solutions of a drug, the bactericidal activity of which is an ionic property, the protective colloid removes the charge from the active cation and hence nullifies its therapeutic effect.

Pharmaceutical Details of the Preparation of P.M.C. and P.M.A. Applications

(By H. Fine, M.P.S., Sergeant, R.A.M.C.)

Experimental work on the preparations of P.M.C. and P.M.A. applications presented several difficulties, owing to the conditions under which the work was carried out. In an area well forward, facilities for acquiring pharmaceutical elegance were often lacking. Apparatus available was elementary and the only method of registering temperatures was by means of crude, though accurate, improvised thermometers.

It was necessary to consider the physical properties of P.M.C. and P.M.A. very carefully in order that their incorporation into suitable bases would satisfy the following requirements:—

(a) To possess the necessary pharmacological and bactericidal action.

(b) The preparations to be easily made in field dispensaries using the least amount of dispensing apparatus.

(c) The final products to be stable, easily applicable and useable in all the various areas in which the infecting agent was present.

Arising out of the third point, it was decided that, for moist infected areas, a drying lotion would be necessary, and for dry infected areas, an emollient (using an emulsifying base) would suit the case.

Accordingly P.M.C. and P.M.A. were incorporated in three types of base :—

- (1) In a Eucerin and distilled water base.
- (2) In an adhesive lotion of the calamine lotion type.
- (3) In simple solution in distilled water.

A. *Eucerin and distilled water base.*

P.M.A. is soluble in hot distilled water up to a concentration of 1.5 per cent.

P.M.C. is practically insoluble in water.

It was found that two methods were necessary in order to incorporate P.M.A. and P.M.C. in this emulsifying base.

(a) Phenyl mercuric acetate (P.M.A.)—

P.M.A.	0.125 per cent.
Eucerin	3 parts.
Distilled water	5 "

The P.M.A. is dissolved in distilled water heated to 85°C. This solution, while still warm, is slowly added to the Eucerin previously melted and kept at a temperature not exceeding 55°C. The mixture is continuously stirred until cool, when a white homogeneous cream is produced, which is easily absorbed into the skin with the minimum of friction.

(b) Phenyl mercuric chloride (P.M.C.)—

P.M.C.	0.20 per cent.
Eucerin	3 parts.
Distilled water	5 "

As P.M.C. is practically insoluble in water, the most efficient method is to incorporate the P.M.C. with the melted Eucerin at 55°C., using careful trituration and keeping the contents of the mortar heated to 55°C. by means of a water-bath. It is essential that the P.M.C. be as evenly dispersed as possible in the melted Eucerin before adding the distilled water (heated to 85°C.) in small quantities, with constant stirring. The whole is now allowed to cool rapidly, stirring until a white homogeneous cream is obtained. This product, too, is quite easily emulsified into the skin.

Even more perfect diffusion of the active constituent could be obtained if the final product could be put through a homogenizer of the 'Empire' hand type. Unfortunately, owing to the limited apparatus available, this could not be done in this dispensary.

B. *Adhesive lotion base*

For moist surface infections, it was decided to use local treatment to the affected areas by means of a lotion which was capable of :—

- (1) Being applied in sufficient concentration to exert the maximum bactericidal action.
- (2) Enabling the area to be gradually dried.

(3) Preventing any aggravation of irritation and, if possible, have the maximum cooling effect.

(4) Remaining in contact with the area for a prolonged period, although the area may be constantly moist, due to perspiration.

(5) Being easily applied.

All these conditions were satisfied in the product of the following formula :—

P.M.C. 0.5 per cent impregnated on			
calamine B.P.	gr. xv
Zinc oxide	gr. xxx
Glycerine	ʒ xxx
Aquam ad.	1 oz.

A supply of calamine impregnated with 0.5 per cent P.M.C. was supplied by the courtesy of Lunevale Products Laboratories.

Probably owing to the method of preparation, the impregnated calamine was found to be very much more dense than calamine B.P., and thus required a large amount of zinc oxide and glycerine to maintain an even suspension when shaken, and sufficient adhesiveness when applied to the infected area. The method of preparation is identical with that of calamine lotion, although it must be stressed that the two insoluble powders must be very carefully mixed and sifted before adding the glycerine and required amount of water.

C. *Solution with distilled water*

For large infected areas of deep seated origin, and for open infections of the 'jungle sore' type, it was found that an application of a solution of P.M.A. had the desired therapeutic effect. Varied concentrations were tried and the following solution found to be the most satisfactory :—

Phenyl mercuric acetate	..	0.05 per cent.
Sterile distilled water	..	ʒi

Solution is effected by heating up 2 grammes of P.M.A. in 250 c.c. of distilled water and pouring the almost boiling solution into 750 c.c. cold distilled water. Stir well and allow to settle. Filter from sediment of which there will be traces. This stock solution can be regarded as 0.2 per cent W/W or W/V for all practical purposes. The desired strength of solution may then be obtained by further dilution with the required amount of cold distilled water.

Owing to the extraordinarily high phenol coefficient of P.M.A., conditions for making this solution do not necessitate the use of rigid aseptic precautions, as even in this dilution it is found that the solution is itself self-sterilizing.

A survey of the above methods of making P.M.A. and P.M.C. preparations shows that it is within the scope of any dispensary in the field to dispense these preparations. They are easily and quickly made, and the final products are quite 'elegant'.

Methods of application of the above preparations of P.M.C. and P.M.A.

At the beginning of the investigation, the cases were treated as follows :—

Cases from the Convalescent Wing of the C.C.S. where this work was carried out were

treated as out-patients, attending at the M. I. Room twice daily, where thorough inunction of P.M.C. ointment was carried out. Though results were very satisfactory, it was found that the method was wasteful in the use of the limited amounts of ointments at our disposal, and so all the convalescent cases were admitted for 1 to 2 days to the Skin Section of the C.C.S., where the ointment was applied on lint and bandaged on to the affected areas.

As the reaction velocity of P.M.C. depends both on the concentration applied and the duration of application, it was found that the latter method mentioned above effected a great saving in the amount of ointment used, and produced a much more rapid curative effect. It was later found that if the method of application outlined below is carefully adhered to, hospitalization of infected patients is quite unnecessary.

The types of mycotic infection observed, affecting the trunk and limbs, varied enormously, presumably due to the nature of the infecting fungus, and the skin reaction of the patient. It is considered that variants occur in the strain of fungi akin to those occurring in bacterial infections in man. Dry, superficial, circinate infections of large extent and rapid spread alternated with those producing agminate folliculitis; and maculo-papular lesions of both guttate and punctate varieties were found. Epidermophyton infections, *i.e.* *E. inguinalis* and the so-called 'foot-rot', are among the commonest lesions met with. As stated above, the type of application varied with the nature of the lesion and in general the following principles of treatment were adopted:—

(1) 'Agminate folliculitis'.—One application of 0.20 per cent P.M.C. was used on lint, after thorough cleansing of the affected part. The ointment was left in contact with the lesion for 3 to 4 hours after inunction.

(2) *Dry spreading, circinate lesions.* A four hours' application of 0.20 per cent P.M.C. on lint was carried out, and for the raised maculo-papular type of lesion, this treatment also proved eminently successful.

Occasionally, some small lesions of the guttate type proved resistant to treatment. 0.25 per cent P.M.A. in chloroform applied to these caused a rapid resolution, though in a few cases a temporary vesication was produced. In view of the extreme rapidity of the control of the infection, this latter effect (which was quite mild) was not considered to have any detrimental action, and all side effects cleared up in 24 to 48 hours.

For moist open lesions, preliminary treatment with (0.20 per cent) P.M.C. on lint for 3 hours was used, followed by the P.M.C. lotion (0.5 per cent adsorbed on calamine). Clearance of the lesions occurred in 2 or 3 days.

Epidermophytosis of the feet

The interdigital spaces were thoroughly cleaned and all dead skin was removed. 0.5 per cent

P.M.C. on calamine was used in powder form where moist lesions occurred and when the area was dry (usually 24 hours after the powder application) 0.20 per cent P.M.C. ointment or 0.125 per cent P.M.A. ointment was used. It is essential to keep a careful check on the state of the lesions, and to vary the powder, lotion or ointment in order to keep the skin in its normal condition.

Epidermophyton inguinale (dhobi itch)

This type of infection was treated by one application of 0.20 per cent P.M.C. After thorough inunction into the affected areas, with special attention to the infected spreading edge, strips of lint coated with the ointment were applied to the lesion and fixed in position by means of pads of cotton-wool and a double T-bandage. Four hours' application was usually sufficient to cause complete disappearance of the lesion. Regarding epidermophyton infections of the toes, recurrences are mainly due to the following causes:—

- (i) fresh infection from an outside source and
- (ii) re-infection from the socks or the footwear of the patient.

The spores of the causative fungus remain in or on the patient's socks or footwear, and are not destroyed in washing (by the usual dhobi method). It is essential to steep the socks overnight in 1/1,000 perchloride of mercury or, better still, 1/2,000 P.M.A. solution to ensure thorough destruction of spores. The socks can then be rinsed and washed in the usual manner. Some spores remain in the boot or shoe and, as treatment in a disinfectant renders these articles unserviceable for further use, it is suggested that thorough treatment with 1/1,000 P.M.A. solution will rapidly render them free from spore contamination.

'Jungle sores'

Preliminary local cleansing with removal of all dead organic matter from the affected part was carried out. 1/2,000 P.M.A. compresses were applied for 6 hours, to thoroughly disinfect the lesions; elastoplast was then applied. Extremely rapid healing occurred, as both bacterial and mycotic infecting agents had been destroyed and the sores rendered practically aseptic.

For open infected wounds of all varieties, application of dressings soaked in 1/2,500 P.M.A. gave rapid and excellent results, the infected area being completely disinfected in 24 hours. All dead organic matter must be removed as far as possible before applying this solution.

The number of open infected wounds treated was small, but results were rapid and spectacular. A fuller investigation of the effects of P.M.A. on these conditions is now proceeding, and a report will be published later, when a number of cases sufficient to show results of significant statistical value has been treated.

In some of the dermatomycoses recorded above, 0.125 per cent P.M.A. ointment was used. The results differed in no way from those effected

by P.M.C. The method of application and period of contact employed was the same for both compounds.

Following application of the above ointments, slight and temporary vesication may be seen in some cases, especially round the spreading edges of the lesion, but this disappears after 24 to 48 hours, leaving a clean healthy skin beneath. Secondary infection does not occur, as the minute vesicated areas remain aseptic in view of the powerful antiseptic action of the compound used.

Number of cases treated

Classification—

(i) Ringworm of trunk and limbs ..	115
(ii) Tinea cruris ('dhubi itch') ..	62
(iii) Epidermophytosis of feet ..	20
(iv) 'Jungle sores' ..	32
(v) Infected wounds of various types	21

In groups (i) and (ii) a single application of 0.20 per cent P.M.C. or 0.125 per cent P.M.A. for 3 to 4 hours caused complete healing of the mycotic lesions. In group (iii) 2 to 5 days was sufficient to bring about clearance of the disease. In group (iv) after preliminary application of 1/2,000 P.M.A. disinfection of the lesions occurred and the application of elastoplast gave rapid and satisfactory results. In group (v) the small number of cases so far treated have shown extremely promising results, and further investigation in an extended series of cases is now being carried out.

As regards group (iii) it was found that when the infection was seen at an early stage, 24 to 48 hours' treatment with 0.5 per cent P.M.C. powder was sufficient to effect cure. Where a superimposed cheiropompholyx is present, somewhat slower curative effects obtain.

It must be emphasized that the rapid cure of epidermophytosis of the interdigital spaces is only possible when the lesion is seen at a reasonably early stage. Many cases have been seen where this infection has been present for years; in these cases hyperkeratosis and fissures are present, and it is quite impossible to effect permanent cure by the above methods of treatment, the reason being that while a temporary cure is effected by the destruction of the vegetative form of the fungus, the resistant spores remain below the hyperkeratotic areas. No matter how effective the fungicidal agent used and how efficient the emulsifying base in which it is presented, the conditions obtaining in the chronically infected areas do not permit of the effective destruction of the spores of the responsible mycotic agent, and relapses are bound to occur.

X-ray therapy represents the only efficient method that can effect permanent cure in these cases.

As regards the types of application mentioned above, it is essential that the method of application and duration thereof be strictly adhered to in order to effect that rapid cure which has been described.

Summary and conclusions

(a) Phenyl mercuric chloride and phenyl mercuric acetate in plant mycology are recognized as possessing specific and rapid fungicidal effects in high dilution.

(b) Similar effects obtain in the case of the fungus infections occurring in man.

(c) The bactericidal potency of these drugs is extraordinarily high, and work is being carried out on their action as surface antiseptics. Preliminary results are extremely satisfactory.

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A METHOD FOR DETERMINING THE TEMPERATURE OF AUTOCLAVES DURING STERILIZATION

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WITH the starting of the Blood Bank in Calcutta, the responsibility for sterilizing daily a large variety of material fell on us. As we did not have any large industrial type of autoclave suitable for the purpose, we had to work with a number of small autoclaves that were available. These were of different sizes, makes, and patterns, and it was felt that, before putting