THE GERMICIDAL ACTIVITY OF THE EUCALYPTUS OILS. Part i.

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(With one Text-figure).

Eucalyptus oil is generally considered to have some disinfecting properties, but, as a disinfectant, it has gone out of fashion. The reason for its scanty recognition at the present time is probably to be found in the greater ease with which solutions or emulsions of the coal-tar products and of other disinfectants can be made. Eucalyptus oil does not dissolve or emulsify readily, and, on standing, gives a floating film, all of which militate against its use. Added to this, there is the uncertainty about its activity. It finds a place in certain ointments, such as Unguentum Eucalypti and the paraffin-preparation recently devised for spraying burns; but, in these, its action as a cutaneous stimulant is probably more considered than its value as a bactericide. Again, in ailments of the respiratory system, Eucalyptus oil is used in combination, as, for example, with menthol. The popular idea is that it acts as a disinfectant, but the medical opinion is that it acts as an indirect expectorant.

The chemistry of the Eucalyptus oils has been extensively investigated by Baker and Smith, who possess a large number of oils of undoubted botanical origin, and, in view of the uncertainty of the disinfecting action of the oils, it seemed opportune to examine their activity while the material was available. The possibility of finding a trade oil of good quality, with a high disinfecting power, was worthy of some investigation; and, upon consulting Mr. H. G. Smith, he kindly offered to supply me with specimens from the material collected by Mr. Baker and himself.

The Eucalyptus oil of the text-books upon Materia Medica is

referred to as the oil of *Eucalyptus globulus*. This oil was originally sent to Europe, and is still distilled in Tasmania; but there is reason to believe that many oils of mixed and doubtful origin were exported in the past under the name of *E. globulus*. At the present time, much of the oil can be trusted to be true to species-name. *E. globulus* is also found in the South-Eastern States of the Commonwealth, but the trees are usually too scattered to be profitably collected, while the yield is poor; and as other species give higher yields of good quality, these furnish the oils to the trade.

In New South Wales, the chief sources of oil at present are *E. polybractea* (the "Blue Mallee" of the Wyalong district), *E. cinerea* (the "Argyle Apple"), *E. australiana* (the "Narrow-leaf Peppermint"), and *E. dives* (the "Broad-leaf Peppermint"). The three first species yield cineol (eucalyptol) oils for pharmaceutical purposes The oil of *E. dives* has the terpene, phellandrene, as the chief constituent, and is mainly used for the flotation of minerals.

The action of the oils when used in ointments was given first consideration, and, as these are generally used for skin-ailments caused by the white and yellow staphylococci, it was thought that much useful information would be gained by testing dilutions of the Eucalyptus oil in an inert oil, such as olive oil, against the yellow staphylococcus suspended in serum. An exposure of two hours was deemed sufficient.

The test-organism was grown on nutrient agar overnight at 37°, and, in the morning, a loop was smeared over the surface of an agar-slope, which was incubated at 37° for three hours, during which the dilutions were prepared. Blood-serum was obtained in the manner used in opsonic work, and a loop of the agar-growth was thoroughly mixed with some of the serum. In preliminary experiments, the infected serum was smeared within the lower ends of glass-tubes, but, as it subsequently fell away in flakes, the method was altered, and a loop of the serum was absorbed in strands of cotton attached to wire-loops. These loops were made by bending a seven-inch length of wire, B.W.G. No.21, round a thick wire, and thickly coating the loop and the

two inches of wire further up with tin by means of a soldering iron. This extra tinning was found to be necessary to prevent the wire rusting and vitiating the tests. A strand of thick darning cotton (Chadwick, No.10) was looped on the eye of the wire with a wire hook, and the loose ends were cut, leaving two strands about a centimetre in length. The wires were sterilised, the cotton was touched with a loop of infected serum and put into the diluted oil. The wire-loops could be used over and over again, but the cotton had to be renewed after every test.

The infected cotton remained in the diluted disinfectant for two hours at 20°, when the wire was withdrawn, and the excess of oil was removed from the cotton by twirling the wire against the inner sides of the tube. The cotton and wire were then dropped into a tube containing three c.c. of meat-extract broth,* after passing the stem of the wire through a flame. The tubes of broth were incubated at 37°, and were observed daily for four days.

The Eucalyptus oils do not appear to have previously been tested by means of their dilutions in a neutral oil. Some years ago, Cuthbert Hall examined the activities of the undiluted oils. His paper was presented as a thesis for the doctorate of Sydney University, and was subsequently issued as a private publication.† From the booklet, I have in the following paragraphs abstracted the main items which bear upon this investigation.

The action of the oils upon the staphylococcus and the colon bacillus was investigated, and it was found that the constituents of the oils possessed individual activities. Aromadendral was most active, as it destroyed the staphylococcus in 15 minutes. The other constituents took much longer—phellandrene 2½ hours, piperitone and dextro-pinene each 4 hours, lævo-pinene 5½ hours,

* This was war-time broth; it had no peptone. A litre of tap-water contained 20 grams of Lemco meat-extract and 5 grams of common salt; the reaction was made decidedly but not strongly pink to phenolphthalein. It gave very satisfactory growths.

† On Eucalyptus Oils especially in relation to their Bactericidal Power. By Cuthbert Hall, M.B., Ch.M. [Little and Co., The Argus Printing Works, Parramatta, 1904].

aromadendrene 6 hours, and cineol (eucalyptol) 2 days.* It took less time to destroy *Bac. coli communis*, but the lethal times ran in much the same order.

The crude oils were found to be very variable in their activity, and this was considered to be caused by a greater or less proportion of free acid because the more toxic oils were markedly acid, and the less toxic were neutral or very faintly acid. From this generalisation, and bearing in mind that acid media are unfavourable to bacteria, the conclusion was come to that the activity of the acid oils was due entirely to the acid. A definite test, however, showed that when the crude oil of *E. Smithii* was neutralised with soda solution, the lethal time was lengthened from thirty minutes to three and a half hours.

The refined oils were found to be much less active than the crude oils. In their case, the variability in bactericidal power was traced to the ozone dissolved in them. The oil of *E. Smithii*, for example, took six and a half hours to destroy the staphylococcus, but when a small amount of ozone was present, it took less than two hours. Cineol took two days, but, when it contained ozone, the time was reduced to three hours. Thus the variation in the refined oils was traced to the ozone, and, in the crude oils, to the acidity, from which we must infer that the refined oils were neutral or faintly acid, and that the crude oils did not contain ozone. Both the acid and the ozone were considered to be strongly bactericidal, in fact, when speaking of aromadendral, it was said that "as compared with the other constituents of the oils, it is a strong bactericide, being only exceeded by acetic acid and ozone."

Some forty or fifty specimens of crude and refined oils were obtained from Mr. H. G. Smith, and dilutions in olive oil were tested upon serum-suspensions of the yellow staphylococcus, *Micrococcus aureus*, absorbed in cotton. The results are summarised in Table i.

* Aromadendral is a lævo-rotatory, high-boiling, aromatic aldehyde, and is the characteristic constituent of most of the "Mallee" oils and those belonging to the "Box" group of Eucalypts. Piperitone is a ketone, and is the characteristic constituent of the oils of the large group of Eucalypts known as "Peppermints."

TABLE i.

M. aureus, suspended in serum and absorbed on cotton.

	Percentage dilution, bactericidal in 2 hours at 20°.		Acidity of oil in degrees.
	As determined.	Probable	
Group iii*a*.			
E. cinerea, rect.	20—20	20	96
,, ,, ,, ...	20	20	95
,, ,, ,, ...	000—100—100	100	18
,, ,, crude	60—50—50	50	81
,, ,, ,, ...	000—000	000	12
E. Smithii, rect.	50—50	50	—
,, ,, ,, ...	50	50	37
,, ,, crude	000—000	000	35
E. sp. "Ribbon Gum," crude, 10 yrs. old	30—30—30	30	127
,, ,, ,, ...	100—100—100	100	18
Group iii*b*.			
E. polybractea, rect.	000—000	000	10
,, ,, ,, ...	000—000	000	10
,, ,, crude	50—40	45	76
,, ,, ,, ...	30—30	30	—
,, ,, ,, ...	100—100	100	4
E. cneorifolia, crude	70—70	70	41
Group iii*c*.			
E. australiana, rect., 1st hour ...	20—20—20—10	20	108
,, ,, ,, ...	100—90	100	5
,, ,, ,, ...	100	100	5
,, ,, crude, 1st hour ...	60—60—50	60	—
,, ,, ,, ,, ...	100—100	100	10
,, ,, ,, 2nd hour ...	30—30—10	30	150
,, ,, ,, 3rd hour ...	30—30—30	30	116
,, ,, steam residue ...	40—40	40	—
,, ,, commercial residue ...	70—60—60	60	—
E. Considenana, rect. ...	60—50—50	50	29
,, ,, ,, ...	40—40	40	—
,, ,, boiling above 190° ...	000	000	31
Group iv.			
E. albens, crude	100—100	100	25
Group vi*a*.			
E. amygdalina, crude	000—000	000	7
E. linearis, rect., thickened... ...	10—10—10	10	160
E. australiana (Braidwood), crude ...	60	60	—
,, ,, ,, ...	000—000	000	6
,, ,, ,, ...	100—90	95	7

"000" means that the oil was inactive.

TABLE i. *(continued).*

	Percentage dilution, bactericidal in 2 hours at 20°.		Acidity of oil in degrees.
	As determined.	Probable	
Group vi*b*.			
E. dives, crude	70—70—60—60	65	17
,, ,, ,, ...	70—60	65	12
,, ,, ,, ...	50—50	50	52
E. radiata, rect., 1st hour	80—80—60	80	—
,, ,, crude	80—80—70	80	18
Miscellaneous.			
E. nova-anglica	100	100	33
E. citriodora	60—60	60	140
Melaleuca Maidenii, crude	70—70	70	49
Prostanthera cineolifera	40—40	40	63
Cineol (H. G. Smith)	000—000	000	—
Cineol (G. I. Hudson)	000—000	000	2
Ol. Eucalypti, P.B. (purchased) ...	000—000	000	12
Essential Eucalyptus Oil (purchased)...	100—90	95	34
Piperitone	60—50	55	—
Geranyl Acetate (*E. Macarthuri*) ...	40—30	35	—
Phenol, crystals at 28° with 0·33% water	6	6	—
Phenol, liquid at 22°, coloured ...	6—6	6	—
Ol. Olivæ	000	000	28

"000" means that the oil was inactive.

The Eucalyptus oils have been classified by Baker and Smith* according to the relative amounts of cineol (eucalyptol), pinene, phellandrene, piperitone, or aromadendral which are contained in the crude oils. The groups or subgroups, containing the oils that were examined, are as follows:—

Group iii*a*.—The oils consist principally of cineol, which is over 40%, and of pinene. Phellandrene is absent. *E. globulus* belongs to this subgroup.

Group iii*b*.—Similar to the last, but aromadendral replaces some of the pinene.

Group iii*c*.—Similar to iii*a*., but phellandrene replaces some of the pinene.

* The Eucalypts and their Essential Oils. By R. T. Baker and H. G. Smith.

Group iv.—The oils never contain more than 30% of cineol, the other constituents being pinene and aromadendral.

Group vi*a*.—The oils never contain more than 30% of cineol. The chief constituent is phellandrene, and there is some piperitone.

Group vi*b*.—Cineol is almost absent. The chief constituents are phellandrene and piperitone.

Some of the oils were, at first, tested several times to determine the accuracy of the method, and it was found to be quite trustworthy when the dilutions were made in steps of 10, *i.e.*, falling by 10% progressively from the undiluted oil through 90%, 80%, and so on to a 10% dilution.

Several samples of the same kind of oil gave the same toxicity-numbers, and it was afterwards learned that they had been taken from one original specimen. The rectified oil of *E. cinerea* is a case in point; the identity of the toxicity and the acidity-numbers brings this out clearly.

The oils of *E. australiana* were extensively tested, because it was one of the first to be examined, and the high toxicity of the first sample compared with the other oils and with the crude oil led to the belief that the disinfecting power might be due to a constituent which accompanied the cineol during rectification, and which might prove to be more efficient than phenol. A second specimen of the rectified oil dispelled the idea. *E. australiana* was once known as *E. amygdalina*, but, owing to the difference from the Tasmanian tree bearing this name, it was renamed by Baker and Smith. The quality of the oil differs according to the location of the tree. When growing on the ridges of the Dividing Range at a high altitude, the quality of the oil is poor compared with that obtained from trees growing at a lower elevation.*

The cineol had been obtained by freezing the rectified oil

* In the latter case, the phellandrene (which is in fair amount in the oil from the species growing at the higher altitudes, such as the Braidwood district), has practically disappeared, and the cineol has correspondingly increased. The alteration in the percentage amount of the constituents is only shown between the cineol and the phellandrene.

obtained from *E. polybractea* or *E. sideroxylon*; one sample came from Mr. H. G. Smith, and the other from Mr. G. I. Hudson.

The Ol. Eucalypti, P.B., was purchased from a chemist, who put it up in ounce bottles at 1s., with a label which, after enumerating the various ailments for which the oil is recommended, continued with "The extract is a thorough deodorant and disinfectant. A few drops on a cloth in a sick-room renders the air refreshing. In fever-rooms the floor should be sprinkled with it."

The Essential Eucalyptus Oil was purchased from the same druggist. It was sold in four ounce bottles at 2s. The oil was of a pale straw-colour and was apparently a crude oil. The label, *inter alia*, affirmed "The oil is a thorough deodorant, disinfectant, and an antiseptic of great value."

The most striking point brought out by the investigation is the irregularity in the action of the oils from the same tree. Two specimens of *E. australiana*, rect., for example, gave widely different bactericidal activities; one was almost inactive, while the other was among the most active of the oils. *E. polybractea*, crude, was almost a similar case. Since there is so much difference between specimens of the same oils, it is not surprising to find that there is no regularity among the groups. One cannot group the oils by their bactericidal activities. If aromadendral* is the most toxic of the components of the oils, those members of the groups which have it as a typical component should be the most toxic, but such is not the case. The aromadendral oils of groups iii*b* and iv are no more bactericidal than the oils of the other groups; indeed the oil of *E. albens*, the only member of group iv. tested, is a very poor disinfectant.

Cineol was inactive when tested in oil, and if an ointment were made with Ol. Eucalypti P.B., which would be used by any druggist dispensing it, the preparation would have no disinfecting action towards the ordinary pus organism.

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* I did not have enough aromadendral to test by the method adopted in this research, but as I shall show in a following paper, it is a strong disinfectant in aqueous suspension.

The oils as a whole were weak compared with phenol, although the thickened oil of *E. linearis* was very nearly as good.

While a 10% solution of phenol in oil is used as a disinfectant, the statement occurs in Hale White's Materia Medica that "The solution in oil has no antiseptic properties." The staphylococcus was destroyed in two hours in a 6% solution in oil.

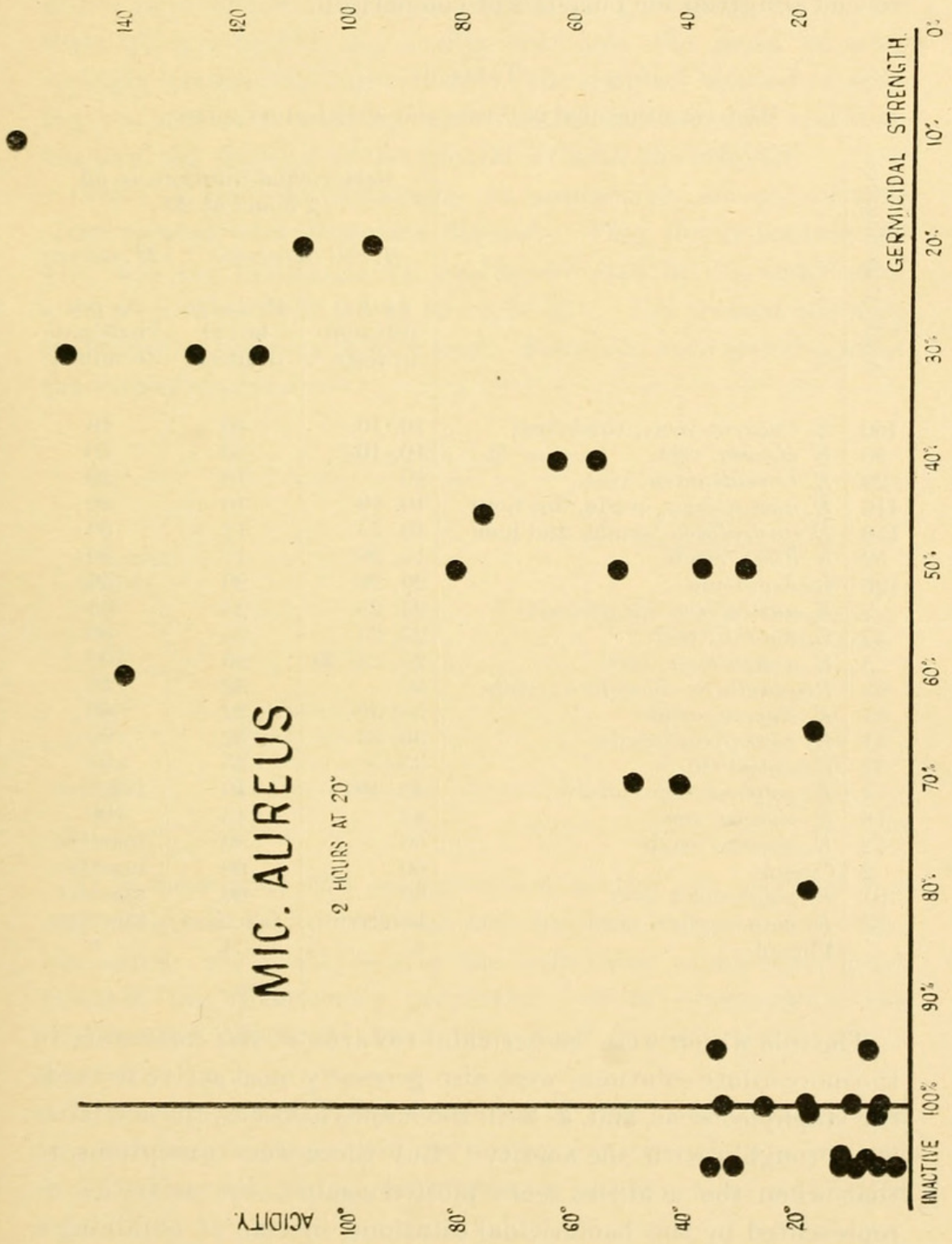
The irregularity of the disinfecting action of the oils leads to the belief that any power which they possess is not occasioned by any of the chief constituents. The oils which have most cineol or most phellandrene or most pinene or most aromadendral are no better than others with less. We must therefore look to the minor constituents which may be occasionally absent. The oils which were most active were pronouncedly acid to litmus paper, as Cuthbert Hall also found, and this led to the determination of the acidity of such samples as had not been completely used up.

The method consisted in taking 20 c.c. of methylated spirit, adding a few drops of phenolphthalein and running in N/100 sodium hydrate until a faint pink colour was produced. One c.c. of Eucalyptus oil was added, and the solution titrated with N/100 sodium hydrate. The numbers in the Table represent c.c. of normal acid per litre of oil, *i.e.*, the degrees of acidity.

The acidity of the oils of each group is as variable as the toxicity, and while the most acid of the oils are also the most toxic, no definite relation between the acidity and the bactericidal power could be detected until the acidities were plotted against the toxicities, and, but for the comparatively large number of oils examined, a relationship would not even then have been capable of demonstration. The curve is of the nature of a broad band within which most of the oils fall. Some are exceptional, such as the oil of *E. citriodora*, which is itself an exceptional oil. The case of *E. australiana*, crude, 2nd hour, cannot at present be explained.

It was shown by Cuthbert Hall, that the undiluted oils were more active towards the colon bacillus, *B. coli communis*, than towards the staphylococcus, *M. aureus*. Tests were made to show that this would also hold for dilutions in oil. Several of

the oils with varying activities towards the staphylococcus were tested with the colon bacillus, and the results are summarised in



Text-fig. 1.

the Table. With *B. coli communis* the dilutions were made in

steps of five, *i.e*, 5%, 10%, 15%, and so on up to 50%, then they rose by 10 to 100%. The numbers obtained with the staphylococcus are given for purposes of comparison.

TABLE ii.

Bacteria suspended in serum and absorbed on cotton.

Acidity in degrees.		Bactericidal dilutions in oil, 2 hours at 20°.		
		B. coli communis.		*M. aureus.*
		Lethal dilution in tests.	Probable lethal dilution.	As previously determined.
160	*E. linearis*, rect., thickened ...	10, 10	10	10
95	*E. cinerea*, rect.	10, 10	10	10
29	*E. Consideniana*, rect.	10	10	50
116	*E. australiana*, crude, 3rd hour...	10, 10	10	30
150	*E. australiana*, crude, 2nd hour...	10, 15	12	30
52	*E. dives*, crude	15, 20	17	50
126	Ribbon Gum	20, 20	20	30
7	*E. australiana* (Braidwood) ...	25, 25	25	95
37	*E. Smithii*, rect.	25, 25	25	50
5	*E. australiana*, rect.	25, 25, 30	25	100
63	*Prostanthera cineolifera*, crude ...	30	30	40
81	*E. cinerea*, crude	30, 35	32	50
41	*E. cneorifolia*, crude	30, 35	32	70
34	Essential Oil	35	35	95
4	*E. polybractea*, crude	40, 40	40	inactive
18	*E. cinerea*, rect.	45	45	100
12	*E. cinerea*, crude	50	50	inactive
2	Cineol	60	60	inactive
10	*E. polybractea*, rect.	60	60	inactive
33	*E. nova-anglica*, crude ...	inactive	inactive	inactive
—	Phenol	4	4	6

The oils which were bactericidal towards *B. coli communis* in the more dilute solutions, were also generally most active towards the staphylococcus, and, as with the staphylococcus, the activities went roughly with the acidity. But there were exceptions, so that when the acidities were plotted against the activities, as represented by the bactericidal dilutions, instead of obtaining a band, as in the staphylococcus, the numbers fell into a wedge-shaped area.

When it had been shown that the acidity of the Eucalyptus oils was a measure of their bactericidal power, the question arose, as to whether the acids were entirely responsible for the toxicity, or whether the acidity was only the index of some strongly bactericidal constituent. The simplest method of testing the matter seemed to be to neutralise the acid, and then compare the toxicity of the neutral oil with the original.

Some of the oils containing an appreciable amount of acid were treated with lime and filtered. They slowly became less acid, and the neutralisation was accelerated by the addition of a drop of water (0·03 c.c. to 10 c.c. of oil). The treated oils were either neutral or very faintly acid. Four oils were tested against the staphylococcus.

TABLE iii.—*M. aureus*.

Original acidity.		Dilutions in oil, bactericidal at 20°.	
		Untreated.	Neutralised with lime.
95°	*E. cinerea*, rect.	20	45
81°	*E. cinerea*, crude	60	100
52°	*E. dives*, crude	50	65
127°	Ribbon Gum, crude, 10 yrs. old	30	80

It is clear that the neutralisation of the oils resulted in a diminution of the bactericidal activity, but it is also clear that the acid or the acidity is not the only thing which contributes towards the disinfecting properties. Were it otherwise, the lime-treated oils would have been inert towards the staphylococcus.

The crude oils of *E. cinerea* and of *E. dives* were treated with dry sodium carbonate until the acidity was, in each case, reduced to 7°. They had the same disinfecting power as the lime-treated oils.

If the alkali does nothing but remove the acid from the oils, it is reasonable to suppose that the addition of acid will render

them more toxic. A preliminary experiment with acetic, propionic, isobutyric, and valeric acids, gave a certain amount of promise that the addition of acetic acid would increase the bactericidal properties of the oils, and a further test was made. In this, acetic acid* did, in some cases, increase the toxicity, but the higher acids were without effect upon the rectified oil of *E. polybractea*. The results are shown in the following Table.

TABLE IV.—*M. aureus*.

Acidity in degreees.		Oil and date of acidification.	Bactericidal percentage dilution, 2 hours at 20°.							
Original.	As prepared.		Original.	16/7	26/7	29/7	5/8	12/8	22/8	4/9
2	128	Cineol 11/7	000	000	90	—	—	—	—	—
34	129	Essential oil 11/7 ...	95	60	50	—	50	40	—	50
52	150	*E. dives*, crude 26/7 ...	50	—	30	—	30	—	—	—
12	110	*E. cinerea*, crude 29/7 ..	000	—	—	000	000	—	100	000
10	112	*E. polybractea*, rect. 29/7	000	—	—	90	70	60	60	50
10	95	*E. polybractea*, rect. 12/8	000	—	—	—	—	000	000	000
52	109	*E. dives*, crude 12/8 ...	50	—	—	—	—	40	40	40

000 = inactive.

An increase in the bactericidal power, as indicated by the reduction in the lethal percentage of oil, was obtained with the crude Essential Oil purchased from the druggist, with *E. dives*, crude, and in one test with *E. polybractea*, rect. *E. cinerea*,

* This is the most pronounced acid in the Eucalyptus oils. It is derived largely from the acetic acid esters they contain, and also from the oxidation of the corresponding aldehyde. Practically all Eucalyptus oils contain low boiling aldehydes which are more pronounced in the oils of some species than in others. Butaldehyde and valeraldehyde are quite common, and in many oils of one class, the ester, butylbutyrate, is a common constituent. The oxidation of the aldehydes to acids and the alteration of the esters are responsible for the presence of the acids in the oils, and, naturally, the older the oils the more acid they become.

crude, was unaltered, and the same was the case with the second test of *E. polybractea*, rect. The higher acidification of *E. dives*, crude, gave an enhanced effect, and it seemed as if the slightly higher acidity of the altered *E. polybractea* might have been responsible for the increased activity of the oil. With the phellandrene oil of *E. dives*, the increase of the toxicity took place at once, while it was slowly developed in the Essential Oil and in *E. polybractea*. The irregular behaviour of the oils led to a further test, in which progressive amounts of acid were added.

TABLE V.—*M. aureus*.

Acidity in degrees.		Oil acidified on 9/9.	Bactericidal percentage dilution, 2 hours at 20°.				
Original.	As prepared.		Original.	9/9	24/9	1/19	14/10
2	104	Cineol ...	000	000	000	—	000
2	151	,, ...	000	000	000	—	000
10	97	*E. polybractea*, rect.	000	000	100	000	000
10	119	,, ,, ,,	000	100	100	000	000
10	136	,, ,, ,,	000	000	000	000	000
12	93	*E. cinerea*, crude	000	70	60	50	50
12	118	,, ,, ,,	000	60	50	50	50
12	133	,, ,, ,,	000	60	40	50	40

000 = inactive.

In this experiment, cineol and *E. polybractea*, rect., were not affected by the addition of considerable amounts of acetic acid. *E. cinerea*, crude, did have its germicidal activity increased, but not definitely in proportion to the acid added.

The two experiments are at variance, and there is apparently some unknown condition, which regulates the action of the acid in producing bactericidal substances in the oils.

The acid itself must have a certain germicidal action, and several tests were made to see how much was necessary to prohibit growth under the conditions of the previous experiments. A quantity of olive oil was acidified with glacial acetic acid, and dilutions of this were made with the neutral oil.

TABLE vi.—Acetic acid dissolved in Olive Oil.

	Acidity in degrees.	
	Growth.	No growth.
11/9	139	186
24/9	163	172
14/10	154	168
4/11	154	168

The amount of acid which checks growth under the conditions of the experiments appears to be about 165°. Although this was never reached in the acidified oils, one cannot but believe, that a high acidity will have a certain influence in assisting the germicidal power of the Eucalyptus oils

There is some foundation for this belief, because the simple neutralisation of the oils by lime or by sodium carbonate removes only a certain amount of the toxicity. The residual germicidal activity must be assumed to belong to the neutral oil.

E. dives, crude, with an acidity of 81°, was toxic in two hours, when in 60% dilution. Upon neutralising the acidity, the same effect was obtained with the undiluted oil, that is to say, the germicidal power was reduced by the neutralisation. In another sample of the oil from the same species, the oil with an acidity of 12° was inactive, and when the acidity was raised to 93°, the oil became toxic when in 50% dilution. Thus an amount of acid, insufficient by itself to destroy the test-organism, caused an inactive oil to become bactericidal under the conditions of the experiment.

THE IODIDE REACTION.

It has been noted that the Eucalyptus oils contain a certain amount of ozone, that is to say, they show, when tested with potassium iodide and starch, the blue reaction. The potassium iodide is oxidised, with the liberation of free iodine which combines with the starch, forming the well-known blue iodide of starch. The reaction is characteristic of an oxidising agent and

many substances, such as peroxide of hydrogen or chromic acid, would produce the blue colour. The test shows that the oils contain an oxidising constituent and whether it is ozone, peroxide of hydrogen, or another oxidising substance, is open to proof. If it were peroxide of hydrogen, it should give off oxygen in contact with binoxide of manganese, but no gas is evolved when manganese dioxide, previously digested with Eucalyptus oil to eliminate occluded air, is added to a fresh specimen of a strongly reacting oil. This test was made under the microscope, and also in a bent tube with a closed capillary end. Mr. H. G. Smith pointed out that this does not negative the presence of peroxide of hydrogen, as the nascent oxygen would remain dissolved and immediately oxidise the constituents of the oil. It, however, appears reasonable to suppose that if the constituents of the oil are so easily oxidised, the dissolved peroxide of hydrogen would itself, in the course of time, be consumed in doing the same work and would not be evident in the oils. The same would apply to ozone.

The amount of this oxidising substance in the oils can be roughly judged by means of iodide and starch paper, but, as the differentiation between the oils, by the method, is very crude, and as it appeared to be advisable to obtain exact numbers relating to the oils, the following method was devised.

A drop of the oil under examination was put into a depression of a porcelain plate, where it diffused more or less over the surface. On the top of this a drop of reagent was allowed to fall. It remained in the middle of the depression and, after a time, became blue, first at the edge and later throughout the drop. By means of a mirror behind the plate, the light from the window above the bench was so arranged that, upon looking down upon the plate, one could not see the edge of the drop of reagent until the margin became tinted. The time required for the edge to become visible was quite definite for each oil, and duplicate tests, made at different times upon the same day, were generally exact to a second. The method was therefore quite reliable. The reagent was a 1% solution of potassium iodide in a 1% starch emulsion. Some 37 oils which were available at the

time were tested. In the Table, the toxicity of the oils towards the staphylococcus is given so that they may be compared.

TABLE vii.

Toxicity %		Iodide of Starch, reaction time in seconds.		Acidity in deg.
		a	*b*	
20	*E. cinerea*, rect.	7	5	96
65	*E. dives*, crude	7	8	17
65	*E. dives*, crude	9	8	12
10	*E. linearis*, rect.	11	9	160
45	*E. polybractea*, crude	13	13	76
50	*E. Consideniana*, rect.	15	8	29
60	*E. australiana*, rect.	15	4	—
100	*E. australiana*, crude, 1st hour ...	15	11	10
40	*E. Smithii*, rect.	16	13	56
30	*E. australiana*, crude, 3rd hour ...	18	16	116
000	*E. polybractea*, rect.	20	8	10
30	Ribbon-gum, crude	23	14	126
100	*E. australiana*, rect.	24	—	5
40	*Prostanthera cineolifera*, crude ...	25	18	63
50	*E. Smithii*, rect.	25	14	37
30	Ribbon-gum, crude, 10 years old ...	27	10	127
100	*E. australiana*, rect.	30	15	5
60	*E. australiana* (Braidwood), crude...	32	15	—
50	*E. dives*, crude	37	14	52
30	*E. australiana*, crude, 2nd hour ...	42	12	150
95	*E. australiana* (Braidwood), crude...	45	12	7
70	*Melaleuca Maidenii*, crude ...	50	43	49
000	*E. amygdalina*, crude	50	23	7
100	*E. albens*, crude...	54	8	25
100	Ribbon-gum, crude	55	50	18
80	*E. radiata*, crude	85	60	18
000	*E. polybractea*, crude	80	30	4
000	*E. australiana* (Braidwood), crude...	120	40	6
70	*E. cneorifolia*, crude	130	75	41
50	*E. cinerea*, crude	000	000	81
95	Essential Oil	000	000	34
60	*E. citriodora*, crude	000	000	140
100	*E. nova-anglica*, crude	000	75	33
000	*E. Consideniana*, over 190° ...	000	000	31
000	Ol. Eucalypti, B.P.	000	000	12
000	Cineol	000	000	2
000	*E. polybractea*, rect.	—	000	10
000	*E. cinerea*, crude	—	150	12
100	*E. cinerea*, rect....	—	25	10
000 = inactive.		000 = over 180″.		

The second column, with the lower numbers, was obtained 48 days later, and although variations were made in the strength of starch and of iodide, the high numbers of the earlier tests could not be obtained. It must be concluded that the activity of the oils had increased. But even with the new numbers, no relation between them and the toxicity of the oil could be traced.

Some of the oils showed a marked decrease in the reaction time, and in order to see if the toxicity had increased in the interval, one of them was tested. This was the crude oil of *E. polybractea*, which had decreased from 80 to 30 seconds. It was found to be inactive to the staphylococcus when tested in oil just as it was at an earlier date.

When the iodide numbers are plotted against the disinfecting powers of the Eucalyptus oils as determined in dilutions of olive oil, no close relation can be noted between the two. The Eucalyptus oils may be divided into two groups, one taking less than 60 seconds to develop the iodide reaction, and this contains two-thirds of all the oils. The other, which took more than 60 seconds, contains thirteen oils, of which seven are inactive to the staphylococcus, and two are slightly active. The two most active oils towards the staphylococcus are certainly among those which show the most vigorous iodide reaction, but they are not sufficiently differentiated from the others to enable one to say that the iodide test is any indication of the disinfecting properties of the oils.

There does not appear to be any relation between the acidity of the oils and the iodide test, as a glance at the acidity column will show. When they are plotted against the acidity, no relation of any kind can be deduced, a fact which could not have been proved in the absence of a rigid test for the reaction. By simply testing with starch-iodide paper, one obtains strong reactions with the oils which are strongly acid, and generally a feeble reaction with those that are weakly acid. It does now, however, take long to make one realise that the paper test is very unsatisfactory.

When the oils are neutralised, the colour takes longer to develop. This was seen in a few oils that had been neutralised about the time that the iodide tests were made.

TABLE viii.—Iodide reaction of neutralised oils.

	Time in seconds.			
	Untreated.	Neutralised with lime	Neutralised with Na_2CO_3	Treated with sodium bisulphite, then with lime.
E. dives, crude ...	37	300	180	over 3,600
E. cinerea, rect. ...	7	15	—	—
E. australiana (Braidwood)	45	—	—	540

The lengthening of the time of reaction certainly points to the acidity having some influence upon the test. If, however, the problem is attacked in another way, viz., by adding acid to a feebly-reacting oil, it is found that the acid has no influence. The crude oil of *E. polybractea* had an acidity of 4°, and gave the reaction in 80 seconds. A quantity had been acidified with acetic acid seven days previously and had increased in its toxicity towards the staphylococcus. The acidity was 95°, but the time of reaction was the same as with the original oil, viz., 80 seconds. We must conclude that the lime or the sodium carbonate, used in neutralising the oils, removes much of the substance which gives the iodide reaction.

When considering the activity of the oils, there is no reason for separating the rectified from the commercial. They are no less germicidal than the crude oils, and indeed some of the rectified oils were among the most active. Acidity may be developed in the oils by aëration during a considerable period of time, and concomitantly an amount of iodide-reacting substance may be formed, but no relation was found to exist between the acid and the ozone-like oxidising substance. Neither was there any relation between the iodide reaction and the bactericidal activity, so that the iodide test is of little importance in determining the bactericidal power of the oils.

THE ACTIVITY OF THE VAPOURS OF THE OILS.

The vapours of the Eucalyptus oils are supposed to possess a disinfecting action, and some experiments were made with them

to see how far this was justified. Wide-mouthed ounce bottles were used, and each received 2 c.c. of the Eucalyptus oil, which was sufficient to cover the dome-shaped bottom of the bottle. The bottles were then heated in the incubator at 37° for half-an-hour to get them warmed up, and so ensure the temperature being constant during the test. Threads of cotton, attached to wires similar to those used in the tests with olive oil, were impregnated with loops of a 20-hour broth-culture of the test organism. Each received the charge of one loop made by twisting a thin wire around a thicker wire (B.W.G. 12). The infected thread was suspended in the middle of the air-space of the bottle, the wire being held in place by the cotton plug. During the test, the bottles were kept in the incubator at 37°.

A few tests were made with the ordinary oils, but, as there was the possibility that the acid in them might have some influence upon the bactericidal power, three of the oils were treated with lime before testing. For the sake of simplicity, all the tests are grouped in one Table. The time when the bacteria were destroyed and the time just short of this are given in order to show the spacing between the times in each case. Growth is indicated in the usual manner by a "+" and absence of growth by a "O" in the headings of the columns.

TABLE ix.—Germicidal Effect of the vapour.

Acidity of the oils in degrees.	Kind of Oil.	Seconds at 37°.							
		B. coli communis.				*M. aureus.*			
		untreated.		limed.		untreated.		limed.	
		+	O	+	O	+	O	+	O
2	Cineol	24	25	—	—	210	240	—	—
4	*E. polybractea*, crude ...	28	30	—	—	80	100	—	—
7	*E. australiana* (B'dwood), cr.	18	20	—	—	40	45	—	—
52	*E. dives*, crude ...	20 25	25 30	15 20	20 25	70	80	over 100	—
81	*E. cinerea*, crude...	20	25	30	40	60	80	over 100	—
96	*E. cinerea*, rect.	15	20	20	25	40	45	100	120
—	Phenol	4	6	—	—	4	6	—	—

The vapours of the oils have an undoubted disinfecting action

at 37°. When neutralised, they certainly are less active, but there does not appear to be any relation between their acidity and their activity. Towards *B. coli communis*, they were all bactericidal in from 20 to 30 minutes, and the acidity appeared to have little or no influence. So far as the yellow staphylococcus is concerned, the possibly exceptional case of the oil from *E. australiana* (Braidwood) prevents a generalisation from the comparatively few tests that were made. At the same time, the results point to the toxicity being proportional to the acidity, and it would appear that the relationship is shown more clearly with *M. aureus* than with *B. coli communis* in the cases of the vapours and of the dilutions in oil.

Summary.

When a serum-suspension of *M. aureus* was absorbed in cotton and placed in dilutions of the Eucalyptus oils in olive oil for two hours at 20°, it was found that the bactericidal power was proportional to the acidity of the oils.

The germicidal effect was not caused by the acidity, but was assisted by it.

The effect upon *B. coli communis* was of much the same nature, although the action of the acid was not so clearly shown.

The iodide reaction was no criterion as to the germicidal value of the oils.

The vapours of the oils had a decided bactericidal action.

I have to thank Mr. H. G. Smith for the many specimens of oils and for his kindness in reading the manuscript and supplying certain useful notes upon the chemistry of the oils, most of which appear as footnotes. I am also indebted to Mr. W. W. L'Estrange for much kindly assistance.