ketonuria. When the fat in the diet was increased gradually, glycosuria disappeared without the appearance of ketonuria (except on one day in 8 weeks). The amount of diet eaten containing 70% of added fat provided 90% of the calories available in the amount of normal diet eaten, and maintained body weight.

After successive periods of high-fat diet the glycosuria seen on return to the normal diet steadily diminished.

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ANTIBACTERIAL ACTION OF 2-AMINOPHENOL (O-AMINOPHENOL)

BY

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AND

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In the course of work with a compound between cholic acid and sulphanilamide we discovered that some intermediate substances devoid of sulphanilic groups had a greater activity against Gram-negative bacilli than the final compound. This led to the investigation of a series of compounds for their activity against this group of organisms. Although the investigation is still in progress this paper records the results obtained with 2-aminophenol, as they appear to warrant its trial as a local antiseptic.

The use of sulphonamides and penicillin has brought to light the need for adequate chemotherapeutic treatment of infections due to or kept up by such organisms as *Proteus* and *Ps. pyocyanea*. Acridine derivatives are perhaps the most effective in this respect, although these compounds are toxic to leucocytes and tissue cells. McIntosh and Selbie (1944) recommend a powder consisting of 1 part proflavine and 99 parts sulphathiazole, and claim that it affords protection against pyogenic cocci, clostridia, and Gram-negative bacilli. Recently a group of compounds classified as synthetic detergents have been recommended for local antiseptics. Their action *in vitro* was described by Baker *et al.* (1941), who showed that the cationic detergents had a significant effect on Gram-negative bacilli, although this was less pronounced than their action on Gram-positive organisms. Two of this group—C.T.A.B. and phemeride—have received a clinical trial. The action of C.T.A.B. has been studied by Barnes (1942) and Williams *et al.* (1943, 1944), but unfortunately its antibacterial action is drastically reduced by the presence of organic matter. Phemeride has been shown by Iland (1944) to have an antibacterial action stronger than that of C.T.A.B., to be effective in the presence of 50% blood, and to be relatively non-toxic to the tissues of the body. Unfortunately both these compounds are very much less active against Gram-negative bacilli than against the pyogenic cocci. For the treatment of *Ps. pyocyanea* infections Berry (1944) and Gough *et al.* (1944) have recommended

ethylene glycol monophenyl ether (phenoxetol), which, although a rather feeble antibacterial agent, appears to be particularly active against this organism.

Experimental

In the following experiments proflavine (2 : 8-diaminoacridine sulphate) has been tested in parallel with 2-aminophenol in order that a comparative picture can be obtained. 2-aminophenol is readily soluble in water. Fresh aqueous solutions have a pale-brown colour which deepens on standing, and after a time a slight precipitate forms. In spite of this well-known instability to oxygen and light there is no appreciable difference in bacteriostatic activity between fresh and 7-day-old solutions.

Bacteriostatic Action in Broth.—Serial dilutions were made in broth and then a suitable inoculum of the organism to be tested was added to each tube. Most of the tests were carried out in broth at pH 7.5 containing 1% "lab-lemco," 1% peptone (B.D.H.), 0.5% NaCl, and 0.005% *p*-aminobenzoic acid. Variation of media, however, did not materially affect the results. Table I shows the

TABLE I.—Bacteriostatic Action in Broth at 37° C. (Size of Inoculum less than 500 Organisms in Each Case)

Organism	Limiting Dilution of Chemical causing Complete Inhibition of Growth			
	2-aminophenol		Proflavine	
	1 Day	7 Days	1 Day	7 Days
<i>Bact. typhosum</i> ..	32,000	16,000	64,000	64,000
<i>Proteus vulgaris</i> ..	32,000	32,000	64,000	64,000
<i>Ps. pyocyanea</i> ..	8,000	4,000	8,000	4,000
<i>Staph. aureus</i> ..	64,000	32,000	64,000	64,000
<i>Str. pyogenes</i> ..	64,000	64,000	500,000	500,000

bacteriostatic activity of 2-aminophenol and proflavine obtained with various bacteria when a small inoculum of less than 500 viable organisms was used. Some variation in results occurred in different experiments with 2-aminophenol; the figures given represent an average. The activity of proflavine was rather more constant.

Table II shows the results obtained with *Staph. aureus* when inocula of 3.4 million, 34,000, and 340 viable organisms respectively

TABLE II.—Effect on Antistaphylococcal Action of Varying Size of Inoculum

Size of Inoculum of <i>Staph. aureus</i>	Limiting Dilution of Chemical causing Complete Inhibition of Growth			
	2-aminophenol		Proflavine	
	1 Day	7 Days	1 Day	7 Days
340 viable organisms	128,000	32,000	128,000	64,000
34,000 " "	32,000	32,000	64,000	64,000
3.4 mill. " "	4,000	4,000	64,000	32,000

were used. As will be seen, 2-aminophenol is very much less active against a large inoculum than against a small one, whereas the activity of proflavine is not so greatly affected by the number of organisms.

Bactericidal Tests.—Suspensions of organisms in 2-aminophenol and proflavine respectively were made, such that the final concentration of chemical was 1 : 1000 and the number of organisms per ml. about 20 millions. The tubes were left at room temperature, and viable counts were made after 6 hours and 24 hours. In a typical experiment with *Bact. typhosum* the original concentration of organisms was 17.5 millions per ml. After 6 hours 5.5 millions

TABLE III.—Effect of 2-aminophenol and Proflavine on *Str. pyogenes* in Human Blood in Slide Cells

	Fresh Defibrinated Blood							Blood in 1 : 1000 Liquoid			
	2-aminophenol										
Dilution of chemical:	$\frac{1}{4000}$	$\frac{1}{8000}$	$\frac{1}{16,000}$	$\frac{1}{32,000}$	$\frac{1}{64,000}$	$\frac{1}{128,000}$	None	$\frac{1}{16,000}$	$\frac{1}{32,000}$	$\frac{1}{64,000}$	None
No. of colonies of streptococci ..	0	0	0	0	2	2	2	0	0	32	28
	Proflavine										
Dilution of chemical:	$\frac{1}{100,000}$	$\frac{1}{200,000}$	$\frac{1}{400,000}$	$\frac{1}{800,000}$	$\frac{1}{1.6 \text{ mill.}}$	$\frac{1}{3.2 \text{ mill.}}$	None	$\frac{1}{100,000}$	$\frac{1}{200,000}$	$\frac{1}{400,000}$	None
No. of colonies of streptococci ..	0	0	1	1	2	2	2	0	30	35	28

per ml. had survived in 2-aminophenol and only 300 in proflavine. After 24 hours both compounds had killed all the organisms. In a control tube consisting of a similar suspension of organisms in water slight multiplication occurred.

Antibacterial Action in Blood in Slide Cells.—As Fleming (1930-1) has shown, most of the antibacterial agents used before the discovery of sulphanilamide destroy leucocytic function in dilutions which have no effect on bacterial growth. Many bacteriostatic compounds are also inactivated by blood or serum. The following experiment was carried out to determine whether either of these statements applied to 2-aminophenol. Two series were set up in parallel; in one case fresh defibrinated blood was used, and in the other blood 1:1000 liquid. It was assumed that 1:1000 liquid would destroy all leucocytes. Serial dilutions of 2-aminophenol and proflavine were made in isotonic saline, mixed with equal quantities of blood, and a suitable inoculum of streptococci added. Controls consisting of saline, blood, and organisms only were included. The mixtures were transferred to slide cells, which were sealed and incubated for 24 hours. Table III gives a typical result with a small inoculum of *Str. pyogenes* consisting of approximately 30 viable organisms per slide cell. These figures show that 2-aminophenol has a considerable antibacterial activity in the presence of 50% blood, and the compound does not interfere appreciably with phagocytosis in greater dilutions than it is bacteriostatic.

Action of 2-aminophenol on Penicillin.—The effects of mixtures of penicillin and 2-aminophenol on *Staph. aureus* were studied. A large inoculum of staphylococci will grow in a concentration of 1:8000 2-aminophenol, although much greater dilutions are inhibitory to smaller inocula. Serial dilutions of penicillin were made in plain broth and in broth containing a final concentration of 1:10,000 and 1:100,000 2-aminophenol. An inoculum of approximately 4 million staphylococci was then added to each tube. The results are shown in Table IV. It will be seen that even a dilution of 1:100,000 2-aminophenol increases the antibacterial activity of penicillin.

TABLE IV.—Effect of Penicillin + 2-aminophenol on *Staph. aureus* when an Inoculum of Approximately 4 Million Organisms was used

Penicillin, Units per ml. (approx.)	Plain Broth	1 : 100,000 2-aminophenol in Broth	1 : 10,000 2-aminophenol in Broth
None	++	++	++
1/4	0	0	0
1/8	0	0	0
1/16	0	0	0
1/32	0	0	0
1/64	++	0	0
1/128	++	+	0
1/256	++	++	0
1/512	++	++	0
1/1000	++	++	0

Plus signs indicate degree of growth as observed by turbidity. 0 = No growth.

Toxicity.—(1) *Skin sensitivity*:—This was tested on 6 human volunteers. A 1:500 solution of 2-aminophenol in water was rubbed into the same area of skin every day for a week. No reactions of any kind were observed and no irritation was felt. (2) *In mice*:—A series of 18 mice were given varying doses of 1% solutions of 2-aminophenol and proflavine respectively by subcutaneous injection. Doses of 0.4 g. 2-aminophenol per kg. of body weight were tolerated, but 0.5 g. or more per kg. was fatal. Proflavine was fatal in doses of 0.2 g. per kg.

Activity of Allied Compounds.—The antibacterial activity of 2- and 4-aminophenol (I, II) suggested a further investigation designed to determine the effects of molecular substitution in these

substances. The following compounds were therefore prepared and tested for antibacterial activity: 2- and 3-methyl-4-aminophenol, 5-methyl-2-isopropyl-4-aminophenol (6-aminothymol), 2-methoxy-4-aminophenol (5-aminoguaiacol), 2-aminoanisole (*o*-anisidine), *N*-acetyl-2-aminophenol, 3-, 4-, 5-, and 6-methyl-2-aminophenol, 4-carboxy-2-aminophenol and its methyl-, ethyl-, and *iso*-propyl esters, 4-phenyl-2-aminophenol, and 4-nitro-2-aminophenol (sodium salt, B.D.H.).

As a result of this work the conclusions reached were: (1) The 4-amino- (*p*-amino-) phenols (II) are generally so unstable that their use as therapeutic agents would be seriously limited. Work on them was not pursued, although 4-aminophenol as well as 2- and 3-methyl-4-aminophenol showed antibacterial activity of the order of that of 2-aminophenol itself. The remaining substituted 4-aminophenols were inactive.

(I) Activity among the substituted 2-amino- (*o*-amino-) phenols (I) was limited almost entirely to substances of the class (III), but was absent where R was $-\text{COOH}$, $-\text{COOCH}_3$, $-\text{COOC}_2\text{H}_5$, $-\text{COOCH}(\text{CH}_3)_2$, or $-\text{NO}_2$. The most active substituted 2-aminophenol was 4-methyl-2-aminophenol (3-amino-*p*-cresol, III; R = $-\text{CH}_3$), and the activity of this substance was of the same order as that of 2-aminophenol itself.

So far we have not discovered a substance in which substitution has enhanced the activity of 2- or 4-aminophenol. Compounds of formula III, however, should be worth further investigation, and this is being undertaken.

Discussion

The mode of action on bacteria of 2-aminophenol is not known. Like the sulphonamides, the compound is mainly bacteriostatic in action, and is very much more effective *in vitro* against a small number of bacteria than it is against a large number. 2-aminophenol in strong solutions, however, is slowly bactericidal. 4-aminophenol (*p*-aminophenol) has been shown by Bernheim *et al.* (1937) to have a considerable effect on tissue oxidations. These workers found that this compound, compared with a large number of similar substances, was the most active in inhibiting the oxygen uptake of liver suspensions.

Williams (1938, 1943) has studied the fate of 2-, 3-, and 4-aminophenol in the body. He has shown that 2-aminophenol when fed to rabbits by stomach-tube is excreted in conjugation with both sulphuric acid and glucuronic acid. No free aminophenol was excreted. He gave 1-g. doses of 2-aminophenol to 6 rabbits of 2.5 kg., apparently without toxic effect. He also showed (Williams, 1943) that 2- and 4-aminophenol glucuronides were methaemoglobin-formers *in vitro*.

Further observations on this group of compounds are required, but its low toxicity and its bacteriostatic activity against the Gram-negative bacilli suggest that 2-aminophenol may be of value as a local antiseptic.

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MEDIASTINAL TERATOMA SUCCESSFULLY REMOVED BY OPERATION

BY

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The following rare case is of interest, being that of a teratoma in a child aged 7 which was successfully removed.

Case History

In Jan., 1944, the patient was referred to me by Prof. E. J. Wayne and Dr. Thompson. The history was somewhat scanty, but the child had complained of shortness of breath while playing and on going uphill, and the mother, wondering if her heart was at fault, brought her to the Children's Hospital, Sheffield. Examination of the chest showed that the cardiac area of dullness was considerably increased to the right, and a radiograph disclosed that there was a tumour which occupied a large part of the right hemithorax. Further investigation revealed that it was roughly oval and lying in the anterior mediastinum. Scrutiny of the film showed that the shadow was not of homogeneous consistency, but was an irregular fine one which was very suggestive of bone. A diagnosis of mediastinal teratoma was made. The child was transferred to the Royal Infirmary, Sheffield, and was about to be operated upon when she developed scarlet fever and had to be sent to the local fever hospital.

She was readmitted on March 13, and the operation took place on March 21. The anaesthetic was ether-oxygen administered by an Oxford vaporizer. The chest was opened by an incision running parallel to the second rib, then down the margin of the sternum