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

I

II

III

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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REFERENCES

- Baker, Z., Harrison, R. W., and Miller, B. F. (1941). *J. exp. Med.*, **74**, 611.
 Barnes, J. M. (1942). *Lancet*, **1**, 531.
 Bernheim, Frederick, Bernheim, Mary L. C., and Michel, Harry O. (1937). *J. Pharmacol.*, **61**, 311.
 Berry, H. (1944). *Lancet*, **2**, 175.
 Fleming, A. (1930-1). *Proc. roy. Soc. Med.*, **24**, 808.
 Gough, J., Berry, H., and Still, Bess M. (1944). *Lancet*, **2**, 176.
 Iland, C. N. (1944). *Ibid.*, **1**, 49.
 McIntosh, J., and Selbie, F. R. (1944). *Ibid.*, **1**, 591.
 Williams, R., Clayton-Cooper, B., Duncan, J. McK., and Miles, E. M. (1943). *Ibid.*, **1**, 522.
 ———, Faulkner, H. C., and Thomas, H. E. (1944). *Ibid.*, **1**, 787.
 Williams, R. T. (1938). *Biochem. J.*, **32**, 878.
 ——— (1943). *Ibid.*, **37**, 329.

MEDIASTINAL TERATOMA SUCCESSFULLY REMOVED BY OPERATION

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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

and outwards along the sixth rib. The costal cartilages were cut on the bevel to prevent overlapping when the chest was closed. The right pleural sac was stripped away and a window raised of

bulk by removing a series of wedges of tissue until it had reached reasonable proportions. It was finally removed by further digital dissection. As the upper pole seemed to be the most likely place for a blood supply, since none had been encountered, a clamp was placed across the last piece of tissue which held the tumour. To avoid infection the window in the chest was closed without drainage and the air aspirated out of the cavity to prevent surgical emphysema. Sero-sanguinous fluid collected during the next few days, which I aspirated on one occasion, but, as is so common in all chest operations, could not find any fluid on a subsequent attempt. The fragments of tumour when collected were found to weigh 12 oz.

My colleague, Dr. L. C. D. Hermitte, undertook the examination of the specimen, and his report is as follows:

"Sections from various parts of the tumour show the structure of a multipotential teratoma. The following structures are present: (1) fatty areolar tissue; (2) cartilage; (3) ossifying cartilage; (4) bone marrow; (5) thymus gland tissue complete with epithelial pearls; (6) branching tubules lined by semi-stratified ciliated epithelium with plain muscle, cartilage, and numerous glands in the walls suggestive of bronchial epithelium; (7) tubular collections of melanin pigment; (8) cystic spaces lined by cubical epithelium forming papillary processes, the nature of which is not clear: they resemble the pink-celled cyst of the breast; (9) areas of connective tissue containing calcospheres."

The child made an uninterrupted recovery, and was discharged on April 6.

A radiograph taken three months after operation showed a perfectly normal chest. There appeared to be a tendency to form keloid in the scar, so x-ray therapy was instituted.

In a review by Lloyd Rusby (1944) of dermoid cysts and teratomata of the mediastinum based on a study of the world's literature, it is stated that only

some 245 cases, exclusive of six original cases, had been published between 1827 and 1939.

REFERENCE

Rusby, N. Lloyd (1944). *J. thorac. Surg.*, **13**, 169.

chest wall. This revealed a large yellowish-white tumour, which was found to be relatively free on blunt dissection below, but was more firmly attached towards the upper pole. Owing to its large size it would have been necessary to enlarge the opening in the chest; so, to overcome this difficulty, the tumour was reduced in

Medical Memoranda

The Incomplete Antibody in Rh Immunization

The recognition of an Rh antibody which combines with homologous red cells *in vitro* but does not agglutinate them was announced independently in June of this year by Wiener and by one of us.

The "blocking" antibody (Wiener, 1944) or "incomplete" antibody (Race, 1944) is specific for one part of the antigen complex of Rh-positive cells. If this antibody could agglutinate, it would be of the type which has been variously called anti-Rh standard, anti-Rh 85%, anti-Rh₀, Δ, and rho. It reacts with the antigen common to the genes Rh₁, Rh₂, Rh₀, and the rare Rh_z. However, the complexities of antigenic components, genes, antibodies, and nomenclatures need not be gone into here.

Wiener has pointed out that this "blocking" antibody can be detected in some cases in which immunization is suspected and the mother or recipient is Rh-negative, but in which no antibody can be found by the ordinary means. The purpose of this note is to draw attention to this latest method of detecting immunization of an Rh-negative person and particularly to Wiener's technique, which is more sensitive than that described by Race. The following is Wiener's description of the test.

"The technique of the test is simple. First, one drop of a 2% suspension of Rh-positive cells and a drop of the patient's serum are mixed in a small test-tube and allowed to react in a water bath at 38° C. for 30 to 60 minutes. Then a drop of a suitable dilution of an active anti-Rh serum is added, and after an additional incubation period of 30 to 60 minutes the reactions are read. If blocking antibodies are present no agglutination will occur, or the clumping will be markedly weakened."

The example given of a suitable dilution of an anti-Rh serum is 1:5, when the original titre was 60.

We have tried Wiener's technique on a number of sera collected from Rh-negative women over about two years and stored at minus 20° C. Whereas Wiener placed the tubes in a water bath we used an incubator at 37° C., re-suspending the sedimented cells when adding the active anti-Rh serum after the first period of incubation. Of 19 cases in which the history sounded typical of haemolytic disease and yet in which no anti-Rh had been detected the incomplete antibody was found in 6. Of 26 cases in which a trace of anti-Rh had been detected the incomplete antibody was found in 19.

These results confirm the importance of testing for the presence of the incomplete antibody in any serum from an Rh-negative woman in which no anti-Rh can be found. The less sensitive the technique for detecting anti-Rh agglutinin the more useful will be the test for the incomplete antibody. In some cases it will yield the only evidence of immunization, and in others will strengthen that given by the finding of only a trace of agglutinin.

A fairly extensive search involving more than 100 mothers with histories suggesting haemolytic disease has failed to give any evidence of incomplete forms of the other three types of anti-Rh agglutinins.

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REFERENCES

Race, R. R. (1944). *Nature*, **153**, 771.
Wiener, A. S. (1944). *Proc. Soc. exp. Biol.*, N.Y., **56**, 173.