gave a good night's rest but symptoms returned next morning. During the second day nasal douching was carried out three times, but in spite of immediate relief to nasal blockage the cold was not cured. On the afternoon of the third day the cold finally cleared up. On the evening of the third day one small fleck of green pus was obtained by blowing the nose.

Thus the cold was not cured in the early stages but relief from nasal stoppage was obtained; this early period of a cold may be the period of virus infection and if it is, patulin proves very effective. Other observations show that patulin has no effect on the virus. The secondary stage of infection with gram-positive and gram-negative organisms was completely prevented and since it is an almost invariable rule that the purulent process continues with me for at least a week, patulin proved to be of great value.

IV. Biological Properties: Extended Trial in the Common Cold

W. A. Hopkins, M.D. (DUBL, M.R.C.P. I)

Surgeon Commander, Royal Navy

Preliminary experiments have been carried out to estimate the action in vitro of patulin against a number of the more common pathogenic aerobic organisms. For the reasons put forward by Fleming (1938) the bacteriostatic power of the drug has been estimated rather than its bactericidal action. In order that the period of a cold may be the period of virus infection and main, the serial dilutional technique used by Oxford (1942) in a similar investigation.

Serial Dilutional Method.—Standard heart broth at pH 7.2 containing 2% glucose, 1% peptone and 0.5% NaCl was used as the basal medium. The solution of patulin was prepared by weighing 25 mg. of the substance into a 100 c.c.m. flask, adding 90 c.c.m. of sterile distilled water and dissolving by gentle warming. The resulting solution was then quickly brought to boiling-point, cooled rapidly and brought up to the 100 c.c.m. mark with sterile water. This gives a 1:4000 solution of patulin. The sterile basal medium was tubed in 4 c.c.m. quantities and to each tube was added graded dilutions of patulin—e.g., 1:4000 solution plus 0.2 c.c.m. of sterile water a 1:20,000 dilution; 0.8 c.c.m. of a 1:4000 solution plus 0.2 c.c.m. of sterile water a 1:25,000 dilution, and so on, using increasing quantities of sterile water and decreasing quantities of patulin solution. Thus each tube contained 5 c.c.m. of liquid, 1 c.c.m. of sterile water being added to the control tube. Different strengths of patulin solution were prepared as the work proceeded and they became necessary.

Both cultures obtained from the National Collection of Type Cultures and organisms isolated in the laboratory were tested. The investigation was carried out with the test organisms, a standard loopful of each of 24 hours were grown in the basal medium before the test was carried.

The tubes containing the sterile basal medium plus the dilutions of patulin were inoculated from glucose-broth cultures of the test organisms, a standard loopful (0.004 c.c.m.) to each. Except in the case of weakly growing organisms all cultures were diluted 1:1000. One part of the super-natant fluid was diluted with two parts of broth to give a 33% pus broth. After filtration through a Sietz EK filter varying dilutions of patulin were added to this broth, which was then inoculated in the usual way with the test organisms, incubated for 24 hours at 37° C. and examined. Pus broth without the addition of patulin was used as a control and the test was carried out in parallel with 2% glucose broth.

Experimental Findings

From table I it will be seen that patulin possesses bacteriostatic powers against a number of gram-positive and gram-negative aerobic and anaerobic microorganisms. The phenomenon with regard to the anaerobes is still under investigation but preliminary results show that the substance is active against this group also.

One of the mould products which have been isolated during recent years, patulin differs from penicillin, gramicidin and citrinin in that it shows no selective differentiation between gram-positive and gram-negative microbes. In this respect the drug is comparable with penicillin, and indeed the inhibitory figures published for the latter compound (Oxford 1942) are very similar to those shown in table I. Patulin is very much less active than is penicillin against gram-positive organisms, but the position is reversed with regard to the coli-typhoid salmonella group. For example, while patulin gives complete inhibition of Salmonella typhi at a dilution of 1 in 50,000, and complete inhibition of Bacterium coli at a dilution of 1 in 30,000, the figures for penicillin (Abraham et al. 1939) are 1 in 10,000 and less than 1 in 1000 respectively. In the same way, with the dysentery group patulin is a more efficient growth inhibitor than penicillin, the figures for Bact. dysenteriae Shiga being 1 in 50,000 for the former drug and 1 in 2000 (Braham et al. 1941) for the latter.

Effect of serum.—In order to estimate the inhibitory effect, if any, of serum the dilution tests were repeated with the addition of 10% fresh human serum. Staphylococcus aureus, Strep. pyogenes and Strep. pneumoniae were used as test organisms and the results obtained are shown in table II. In no instance was any inactivation observed.

Effect of pus.—In view of the projected trials of the substance in the treatment of common cold, it was important that its activity in the presence of pus should be estimated. This was carried out using "pus broth" as described by Florey and his colleagues (1941), and with Staph. aureus, Strep. pyogenes and Strep. pneumoniae as test organisms.

The pus was first "thinned" by incubation at 37° C. for 3 days, and after centrifugisation one part of the supernatant fluid was diluted with two parts of broth to give a 33% pus broth. After filtration through a Sietz EK filter varying dilutions of patulin were added to this broth, which was then inoculated in the usual way with the test organisms, incubated for 24 hours at 37° C. and examined. Pus broth without the addition of patulin was used as a control and the test was carried out in parallel with 2% glucose broth.

The results in table II show that in no instance was any antagonistic effect observed.

Effect on leucocytes.—The in-vitro effect of patulin on phagocytic activity of leucocytes has been tested using the method described by Thrower and Valentine (1943).

Saline dilutions of the drug were prepared ranging from 0.4% to 0.015%; 0.2 c.c.m. of each dilution was added to an equal volume of reconstituted blood and the tubes were incubated at 37° C. for 3 hours. A tube containing saline
DO NOT INTERVENE WITH BACTERIOSTATIC EFFECT OF PATULIN

TABLE II—DILUTION TESTS, SHOWING THAT SERUM AND PUS DO NOT INTERFERE WITH BACTERIOSTATIC EFFECT OF PATULIN

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Highest dilution producing complete inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staph. aureus</td>
<td>2% glucose broth</td>
</tr>
<tr>
<td></td>
<td>Glucose broth + 10% serum</td>
</tr>
<tr>
<td></td>
<td>Pus broth</td>
</tr>
<tr>
<td>33,000</td>
<td>33,000</td>
</tr>
<tr>
<td>80,000</td>
<td>80,000</td>
</tr>
<tr>
<td>80,000</td>
<td>80,000</td>
</tr>
</tbody>
</table>

without the addition of the drug was used as the control. After 3 hours incubation a standard drop of heat-killed three-hour staphylococcal broth culture was added to each tube; the tubes were then shaken and incubated for 30 minutes. After incubation, films were made from each tube and a count was made, noting (a) the number of cells containing cocci and (b) the number of ingested cocci in 100 phagocytes.

The results showed that at 0.05% of patulin phagocytosis is inhibited; it is reduced at 0.025% but unaffected at 0.0125%.

Acute toxicity for laboratory animals.—Patulin has been administered intravenously to mice weighing between 20 and 25 g.; the results are set out in table III. Deaths occurring within 3 days were recorded. The average lethal dose is in the region of 0.5 mg. per 20 g. With the higher dosage levels (1 mg. and 1.5 mg. per 20 g.) most of the animals appeared ill within 3 hours of the injection and died within 6 hours. In a number of instances death was immediately preceded by convulsions, in one case so severe as to fracture vertebrae. On autopsy the lungs were found to be oedematous and grossly hemorrhagic. On section the capillaries showed acute dilatation and the alveoli were packed with red cells. Similar capillary damage, but to a less degree, was seen in the liver, spleen and kidneys. Histological examination of the brain showed oedema, congestion of the vessels and a round-cell infiltration.

The average lethal dose for a 20 g. mouse when the substance is administered subcutaneously to mice weighing between 20 and 25 g.; the results are set out in table III. Deaths occurring within 3 days were recorded. The average lethal dose is in the region of 0.5 mg. per 20 g. With the higher dosage levels (1 mg. and 1.5 mg. per 20 g.) most of the animals appeared ill within 3 hours of the injection and died within 6 hours. In a number of instances death was immediately preceded by convulsions, in one case so severe as to fracture vertebrae. On autopsy the lungs were found to be oedematous and grossly hemorrhagic. On section the capillaries showed acute dilatation and the alveoli were packed with red cells. Similar capillary damage, but to a less degree, was seen in the liver, spleen and kidneys. Histological examination of the brain showed oedema, congestion of the vessels and a round-cell infiltration.

The average lethal dose for a 20 g. mouse when the substance is administered subcutaneously to mice weighing between 20 and 25 g.; the results are set out in table III. Deaths occurring within 3 days were recorded. The average lethal dose is in the region of 0.5 mg. per 20 g. With the higher dosage levels (1 mg. and 1.5 mg. per 20 g.) most of the animals appeared ill within 3 hours of the injection and died within 6 hours. In a number of instances death was immediately preceded by convulsions, in one case so severe as to fracture vertebrae. On autopsy the lungs were found to be oedematous and grossly hemorrhagic. On section the capillaries showed acute dilatation and the alveoli were packed with red cells. Similar capillary damage, but to a less degree, was seen in the liver, spleen and kidneys. Histological examination of the brain showed oedema, congestion of the vessels and a round-cell infiltration.

The investigation was carried out in two parts: Group 1.—A controlled series in which the patients were treated with patulin and half with a control fluid. Group 2.—In which all patients observed in the hospital were given supplies of patulin and asked to keep careful notes of their progress.

Preparation and Administration
Patulin keeps well in a phosphate buffer solution adjusted to pH 6. This buffer solution is prepared as follows:

27.251 g. acid potassium phosphate ('Analdr') is dissolved in 0.2 c.cm. of water. To 100 c.cm. of the solution is added to 250 c.cm. of the phosphate solution; the resulting solution is well mixed and then made up to a litre with distilled water.

The stock solution of patulin is prepared by dissolving 0.05 g. of the substance in 9 c.cm. of water and diluting to 100 c.cm. with sterile distilled water. This stock solution keeps well; a supply which was kept for 3 months showed no evidence of deterioration. For ready use one part of the stock solution is diluted with 9 parts of water to give a 1:10,000 solution; two parts with eight parts of water to give a 1:20,000 solution of substance; two parts with eight parts of water to give a 1:10,000 solution, and so on. The solutions for use were prepared freshly each day and were used within twenty-four hours. The strengths used were 1:20,000 in the first batch, 1:10,000 in the second, and 1:5000 in the third batch. In group 2 a 1:10,000 solution was supplied.

The solution of patulin was applied locally to the nasal passages and pharynx. Patients in group 1 were treated by a sick-beth attendant who sprayed the nose and throat with a De Vilbiss atomiser. Patients in group 2 either sniffed it up from the palm of the hand or instilled it into the anterior nares with a glass pipette; some of the patients in this group also gargled with a De Vilbiss atomiser. Patients in group 2 either sniffed it up from the palm of the hand or instilled it into the anterior nares with a glass pipette; some of the patients in this group also gargled with a De Vilbiss atomiser.
A large number of the cured patients noticed a great improvement after the first application of patulin, and were completely cured within 24 hours. The patients who had a profuse nasal discharge and a raw throat during the day and a stuffed-up nose on waking in the morning, reported 24 hours after treatment had been begun that they felt quite fit and that their nasal passages were dry and clear.

Other patients improved more gradually, but experience showed that if complete cures took place within 48 hours it was unlikely that patulin would have any apparent effect on modifying the course of the cold.

No ill effects were observed after treatment. Some patients (especially those in batch 3, when a 1:5000 solution was used) complained of a transitory stinging in the nose, immediately after they had been sprayed, and a number noticed that for about half an hour after treatment they had a profuse nasal discharge. That these effects were not due either to the buffer solution or to mechanical action is shown by the fact that none of the patients in the control group reported similar symptoms.

The failures may be divided into two groups—(a) patients who did not improve in any way, and (b) patients who showed transitory improvement which was not maintained or in whom complications developed. The second group is of particular interest and mention may be made here of a relevant personal experience.

For many years I have had at least one severe cold each winter. These attacks may be described as typical common colds; they start with vague malaise, a dry throat and slight pyrexia and are closely followed by sneezing, profuse nasal discharge and complete blocked nasal passages on waking in the morning. The acute symptoms usually persist for 4–5 days and are followed by a purulent nasal discharge which lasts for about a week. A few years ago one of these attacks culminated in a severe pansinusitis, which, however, cleared up with conservative treatment. During the time that patulin was undergoing clinical trials I noticed the premonitory symptoms of a cold, and 12 hours later when the attack had fully developed I started treatment using a 1:10,000 solution of patulin every 4 hours. Next morning—i.e., some 20 hours later—I woke up feeling completely cured and without a trace of nasal discharge or blockage of the nasal passages. The same night, however, symptoms of sinusitis (pyrexia, pain close to the inner canthus of the eye, together with generalised headache and vomiting) made their appearance.

Two of my colleagues had similar experiences; after the use of patulin they noticed distinct but temporary improvement, but they also developed attacks which, like my own, both had had attacks of this type before. It is probable that any focus which the solution cannot reach will keep the infection active in the nasal passages, and some of the failures in the treated group may have been due to the existence of a previous sinusitis. In 2 patients clinical and radiological evidence of such a condition was found; neither of these patients improved, but neither became worse nor complained of symptoms suggestive of an acute sinusitis.

### RESULTS IN GROUP 1

<table>
<thead>
<tr>
<th>Batch and month</th>
<th>TREATED</th>
<th>CONTROLS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Recovered</td>
</tr>
<tr>
<td>January</td>
<td>54</td>
<td>24</td>
</tr>
<tr>
<td>February</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>April</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>95</td>
<td>55</td>
</tr>
</tbody>
</table>

### RESULTS IN GROUP 2

Some 14 patients complaining of colds were given supplies of patulin and asked to keep notes of their progress. Of these, 10 reported a complete and rapid recovery. Of the remainder, one felt much better and 3 noticed no improvement whatsoever. Typical case-histories of the cured cases are as follows.

---

The Lancet, Nov. 20, 1943, 633
The one real failure was in an officer who gets very heavy the respiratory tract, was not affected. Pyrexia parted, but the cold itself did not clear up immediately. Cold, accompanied by aches and pains and usually a rise in considerable relief and made them feel much less heavy these are subject to colds which seem deep-rooted and last has had a series of very heavy colds; not a single attack has after treatment.

Whether any change took place in the bacterial flora gated in a number of patients in the controlled series. The most commonly found microbes were malaise, headache, profuse nasal discharge and sore throat. Treatment consisted of 1:10,000 patulin every 4 hours during the day, and 24 hours after beginning treatment the patient reported that he was completely symptom-free and the cold was getting worse. She woke up on Feb. 4 quite well.

In addition to these cases, 27 WRNS personnel were treated with patulin. The results are given in the following report by the officer in charge of the trials.

The first bottle of the substance was provided for my office staff and myself to try. The results were so successful that other officers asked to be allowed to use it, and have sent their ratings for treatment. As far as possible we have used it, as instructed, before a cold has lasted more than 24 hours. Twelve officers and 14 ratings have reported completely successful results after not more than 24 hours treatment. Included among these was a girl motor-transport driver who has a series of very heavy colds; not a single attack has after treatment.

The fundamental data are given by Hopkins in his paper. The lethal dose for mice is about 0-5 mg. per 20 g. body-weight, whether the substance is given intravenously or subcutaneously. Solutions of the substance were sprayed into the nose or snuffed up from the hand. The results obtained were encouraging, 57% of the treated cases recovering completely within 48 hours, compared with only 9-4% of the controls.

My thanks are due to Professor Raistrick for supplies of patulin; to the Royal Naval authorities, both executive and medical, at the depot where the trials were carried out for their cooperation; to Surgeon Lieut.-Commander H. W. Clegg for help in the animal toxicity tests; to SFO Geoffrey Smith, who assisted me both in the bacteriological work and clinical trials.

I wish to thank Surgeon Rear-Admiral C. F. O. Sankey for permission to publish this paper.

REFERENCES


V.—Statistical Note

PROFESSOR OF EPIDEMIOLOGY AND VITAL STATISTICS IN THE UNIVERSITY OF LONDON

(London School of Hygiene and Tropical Medicine)

The purely statistical question which arises in Commander Hopkins's work is a simple one—assuming that the treated and control populations do not differ in any material way, except in regard to the fact of treatment, which is the one variable of the experiment, how likely is it that such divergent percentages of cure would emerge?

It is evident that if two batches of pennies are tossed, the respective percentages of "heads" are likely to differ from the proportion that would be obtained if the coin were absolutely balanced inasmuch as the proportion that would be obtained if the coin were absolutely balanced inasmuch as the proportion that would be obtained if the coin were absolutely balanced by a 1 in 8000 solution, but inhibited by a 1 in 2000 solution.

The phagocytic activity of leucocytes is unaffected by a 1 in 8000 solution, but inhibited by a 1 in 2000 solution.

For these reasons it is felt that although the results of the trials described in this paper are encouraging, no definite claims can be made for patulin until it has been given more extended trial in different localities.

Summary

Patulin is about equally bacteriostatic to both gram-positive and gram-negative organisms; it is much less active than penicillin against gram-positive organisms but much more so against gram-negative ones.

The bacteriostatic power of the substance is unaffected by the presence of serum or pus.

The fundamental data are given by Hopkins in his paper. The lethal dose for mice is about 0-5 mg. per 20 g. body-weight, whether the substance is given intravenously or subcutaneously. Solutions of the substance were sprayed into the nose or snuffed up from the hand.

The results obtained were encouraging, 57% of the treated cases recovering completely within 48 hours, compared with only 9-4% of the controls.

My thanks are due to Professor Raistrick for supplies of patulin; to the Royal Naval authorities, both executive and medical, at the depot where the trials were carried out for their cooperation; to Surgeon Lieut.-Commander H. W. Clegg for help in the animal toxicity tests; to SFO Geoffrey Smith, who assisted me both in the bacteriological work and clinical trials.

I wish to thank Surgeon Rear-Admiral C. F. O. Sankey for permission to publish this paper.

REFERENCES


V.—Statistical Note

MAJOR GREENWOOD, D SO LOND, F R C P, F R S

PROFESSOR OF EPIDEMIOLOGY AND VITAL STATISTICS IN THE UNIVERSITY OF LONDON

(London School of Hygiene and Tropical Medicine)

The purely statistical question which arises in Commander Hopkins's work is a simple one—assuming that the treated and control populations do not differ in any material way, except in regard to the fact of treatment, which is the one variable of the experiment, how likely is it that such divergent percentages of cure would emerge?

It is evident that if two batches of pennies are tossed, the respective percentages of "heads" are likely to differ from the proportion that would be obtained if the coin were absolutely balanced inasmuch as the proportion that would be obtained if the coin were absolutely balanced by a 1 in 8000 solution, but inhibited by a 1 in 2000 solution.

The phagocytic activity of leucocytes is unaffected by a 1 in 8000 solution, but inhibited by a 1 in 2000 solution.

For these reasons it is felt that although the results of the trials described in this paper are encouraging, no definite claims can be made for patulin until it has been given more extended trial in different localities.