FINDINGS FROM LINGUAL SCRAPINGS TAKEN DURING TREATMENT WITH CHLORAMPHENICOL AND AUREOMYCIN

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In a previous paper (Tomaszewski, 1951) a survey was made of the side-effects of chloramphenicol and aureomycin treatment. These were divided into general, gastro-intestinal, genito-rectal, and oral manifestations. Changes in the oral cavity were found more frequently than in other parts of the body. They consisted of dryness of the mouth, throat manifestations, angular stomatitis, and changes in the oral mucous membranes. Striking changes were seen in the dorsum of the tongue; the most common was the disappearance of the normal whitish coating with some degree of atrophy in the filiform papillae, and in extreme cases marked atrophic glossitis with redness and soreness of the tongue. However, cases were also observed in which there was hypertrophy of the mucosa (hypertrophic glossitis) with overgrowth of the filiform papillae and brown discoloration. This discoloration was also sometimes seen in cases with some degree of atrophy of the papillae.

The present paper describes in detail the microscopical examinations of the lingual scrapings. The findings are correlated with the pathological changes which develop in the tongue during chloramphenicol and aureomycin therapy. The investigations were made on 126 patients treated with these antibiotics for various infective conditions in the wards of the Royal Infirmary, Edinburgh.

Method

Scrapings from the tongue were made at two- to three-day intervals, before, during, and for some time after treatment. The tongue was scraped with a sterile slide from as far back as possible, and the material ground with another slide in order to obtain an even film. After heat-fixation the film was stained with Gram or, in certain cases, with other stains. The scraping procedure is simple and makes it possible to follow the action of antibiotics on microflora and on the epithelium of the tongue. Microflora can thus be studied in their natural environment.

Results

Scrapings from the tongue usually contain epithelial cells, microflora, occasional leucocytes, and amorphous debris.

Changes in the Tongue.—In the majority of patients undergoing treatment a typical series of changes can be seen. The intensity of these depend very much on the

dose of the antibiotics and the duration of treatment. The previous use of penicillin or streptomycin must be considered. The usual dose used in the study was 2 g. daily. In certain cases small doses, 1 g., or higher doses, 3-4 g. daily, were used. Vitamin B complex was given to a group of patients simultaneously with the antibiotics to study the effects of these vitamins on the onset and development of the oral lesions.

In a few days, in some instances 24 to 48 hours after the start of antibiotic treatment, the bacterial flora disappear in the majority of cases and are replaced by fungous flora. At the beginning of treatment there is usually an increased shedding of epithelial cells. Leucocytes often appear in increasing amounts, especially in cases with marked atrophic glossitis. A typical series of changes, occurring during aureomycin treatment in a patient aged 20, who developed atrophic glossitis with soreness and redness of the tongue, is shown in Figs. 1-6. These illustrations refer to a patient who showed a normal tongue before treatment. In a few days shortening of the papillae was noted, and was followed by definite denudation, redness, and soreness of the tongue, and by angular stomatitis. At the end of the three weeks' treatment a slight, brown discoloration was visible on one side of the tongue. The surface of the tongue did not change with the sudden reappearance of the bacterial flora, which took place while the patient was still continuing treatment. After stopping treatment the angular stomatitis and denudation persisted for about two weeks.

Before treatment the tongue scrapings showed a predominance of Gram-positive cocci and also some Gram-positive bacilli (Fig. 1). Five days after the start of treatment (Fig. 2) a few yeast cells (*Candida albicans*) were seen showing the development of some mycelium elements; bacteria apparently were completely absent. At a later stage (Fig. 3) the yeast and mycelium elements were more abundant; bacteria were absent. At that stage large numbers of leucocytes were present (Fig. 4). Clinically, denudation, redness, and soreness of the tongue had developed. By the twelfth day, while the patient was still undergoing treatment, bacterial flora had developed on the tongue, and appeared uniformly diplococcal in character (Fig. 5). At this stage a diminished amount of yeast cells could still be seen. On the last day of treatment (Fig. 6) the tongue scrapings showed very rich, practically uniform coccal flora and a few chains of bacilli. The daily dose was 2 g., the total dose 44 g.

Epithelial Cells.—The number of epithelial cells alters during treatment. Epithelial cells are usually found alone, but sometimes they are seen in plaques of two, three, or more cells. The nucleus is usually well defined, but sometimes it is absent from cells showing degenerative changes.

In addition to the usual epithelial cells other cells are occasionally found. Some are small and navicular and stain deeply. Another type shows peculiar deep, red-violet blotches of various sizes, which accumulate and give to these cells an extraordinary appearance, resembling the spots of a leopard skin (Fig. 7). These "blotchy cells" appear normally only in a certain percentage of persons, but sometimes increase in number in the course of antibiotic treatment. Differential staining with the Shorr method (Shorr, 1941) shows that they are keratinized epithelial cells. However, the nature of these "blotchy" changes is unknown.

Shedding of the epithelial cells is most marked during the first days of treatment. As the tongue becomes denuded and the filiform papillae shrink, the epithelial cells

FIG. 1.—Scraping of the tongue before aureomycin treatment; profuse mixed bacterial flora ($\times 1,000$). Clinically normal appearance of the tongue.

FIG. 4.—Same scraping as in Fig. 3; absence of bacteria; many leucocytes ($\times 500$). Clinically redness and soreness of the tongue.

FIG. 2.—Fifth day of treatment; absence of bacteria; first yeast-like fungi to be seen ($\times 1,000$). Clinically denudation of the tongue.

FIG. 5.—Twelfth day of uninterrupted treatment; sudden appearance in 24 hours of a rich, uniform coccal flora, probably an aureomycin-resistant strain; disappearance of fungi ($\times 1,000$). Clinically still redness of the tongue.

FIG. 3.—Seventh day of treatment; absence of bacteria; rich growth of fungi, mostly mycelium; few ovo yeast cells ($\times 1,000$).

FIG. 6.—Twenty-first day of treatment; bacterial flora continuing, now more of a mixed type ($\times 1,000$); absence of fungi. Clinically some improvement of the tongue surface. Treatment stopped on that day.

FIG. 8

FIG. 7

FIG. 7.—“Blotchy” epithelial cell; nucleus clearly visible ($\times 1,000$). Gram-stained. Treatment with chloramphenicol.

FIG. 8.—Broken fragment of filiform papillae; many epithelial cells ($\times 65$).

become scarcer, and it is increasingly difficult to obtain sufficient material for investigation. Occasionally broken fragments of filiform papillae may be seen as long bodies pointed at one end (Fig. 8). Their number usually increases with treatment in cases showing hypertrophic glossitis.

Bacterial Flora.—Bacterial flora of the tongue, observed during antibiotic treatment, nearly always show a change in character and in number. In the majority of cases (59%) the bacterial flora rapidly diminish in number, and usually disappear completely in a few days. In a number of patients, usually those who receive smaller doses of the antibiotics, bacteria are found throughout the course of treatment, but are markedly reduced in number and frequently change in character. In a few instances there is a change from mixed flora to a growth which is predominantly coccal.

In the majority of cases the absence of bacteria persists for several days after treatment has been stopped. The first bacteria to reappear in large numbers after the discontinuation of treatment are usually cocci. The bacterial flora are not always of the same type as before treatment, and are often more uniform in character.

An interesting observation was that scrapings might show complete absence of bacteria one day and new rich bacterial flora of a uniform character 24 hours later, while the patient was still undergoing treatment (Fig. 5). In three cases (two treated with aureomycin, one with chloramphenicol) a pure rich culture of cocci developed. This phenomenon is most probably due to the development of a chloramphenicol- or aureomycin-resistant strain.

Fungous Flora.—In the majority of cases yeast cells are first found 24 to 48 hours after the start of treatment. Their number increases with the progress of treatment and with the rapid disappearance of bacteria. The growth of fungi is sometimes very profuse, dominating the whole picture.

Fungi were found in 67% of tongues examined, mostly in cases with complete absence of bacteria, though in about one-fifth of the cases fungi coexisted with bacteria. There were cases with no visible fungi. However, if fungi are not seen in several consecutive scrapings it is not an absolute proof of their absence, since their presence may be confirmed on culture. All stages of fungus development may be seen: at first a few scattered yeast cells, and later mycelium and oval yeast cells, which often appear as large clumps (Fig. 9). After treatment is ended fungi usually persist for several days, and sometimes for as long as several weeks. In all cases in which the type of fungus was identified, it proved to be *Candida albicans* (*Monilia*). This agreed with the observations of other authors (Harris, 1950; Williams, 1950; Woods, Manning, and Patterson, 1951).

FIG. 9.—Clump of fungi; mycelium and yeast cells matted together; absence of bacteria ($\times 500$). Treatment with chloramphenicol.

Leucocytes.—A few white cells, scattered among the epithelial cells, are often found in normal scrapings. They are usually dead cells, showing marked degenerative changes, the contour of the nucleus being hardly recognizable and only the outlines of the cells visible.

During treatment with chloramphenicol and aureomycin the number of leucocytes frequently increases, and their nucleus is usually well defined (Fig. 4). The increase of leucocytes is most marked in those cases which show atrophic glossitis with soreness and redness, but it is also encountered in patients whose tongues are either normal in appearance or have a brown coating.

Amorphous Debris.—Masses of amorphous debris are commonly seen in the films. They are composed of particles of food and of decomposed cellular material. There is sometimes a large quantity of mucus, and an excess is found in patients who complained of marked dryness and a "sticky" feeling in the mouth.

The Relation of the Microscopical Findings to the Lingual Changes

A number of persons showed an apparently normal tongue during the antibiotic treatment. In such patients the bacterial flora were present, but diminished in number and changed in character. In a number of cases the coexistence of bacteria and fungi was noted. A high percentage of patients in this group had received vitamin B complex simultaneously with the antibiotics. The tablets used in this study contained aneurin, nicotinic acid, and riboflavin. It must be emphasized,

however, that in a few cases acute oral lesions, resembling avitaminosis B, especially ariboflavinosis, developed quickly in spite of vitamin treatment.

The denudation of the tongue, which took place in the first few days of treatment, was associated with a rapidly diminishing number of bacteria. Once the whitish coating has disappeared, atrophic changes may follow.

In atrophic glossitis there were usually a few fungi, although in a few cases there was a substantial growth. Bacteria were mostly absent or very sparse. An increase in leucocytes was common.

In hypertrophic glossitis there were nearly always many fungi, no doubt because of the better conditions of growth. Bacteria as a rule persisted in diminishing numbers during treatment, but occasionally disappeared, to reappear in a few days.

Brown discoloration of the tongue (melanoglossia) was usually associated with hypertrophic glossitis. In a few cases, however, it could be seen in a partly atrophied dorsum. The scrapings from brown ("black") tongues usually revealed large numbers of fragments of the papillae, which appeared brown in unstained films. No particular type of bacterial flora was found, nor any fungi, other than of the *Candida* type. It must be emphasized that there were a few cases of melanoglossia where no fungi were detected.

The results of examination of the lingual scrapings and their relationship to the macroscopical appearance of the tongue are shown in Table I.

As there was no significant difference in the development of the oral changes in cases treated with chloramphenicol and aureomycin, the results are given jointly.

TABLE I
MICROSCOPICAL CHANGES IN LINGUAL SCRAPINGS IN 126 CASES TREATED WITH
CHLORAMPHENICOL AND AUREOMYCIN

Appearance of the Tongue	Total No. of Cases	Absence of Bacteria (No. of Cases)	Percentage* of Total Cases	Presence of Fungi (No. of Cases)	Percentage of Total Cases	Increase in Leucocytes (No. of Cases)	Percentage of Total Cases
Normal	30	5	16	13	43	9	30
Denuded or atrophic	83	68	81	60	72	38	45
Brown, and hypertrophic glossitis . .	13	2	15	12	92	4	30
Total	126	75	59	85	67	51	40

* There was a considerable overlap in the incidence of absence of bacteria, presence of fungi, and increase of leucocytes. Hence the sum of the percentages in each column is more than 100.

Discussion

The devastating effect of the antibiotics on the bacteria can be seen not only in the oral cavity, but also in the upper respiratory tract (Gray, 1950 ; Meads, Rowe, and Haslam, 1951), in the intestines, and in the external genitalia. An upset of balance between bacteria and host occurs, and as a result certain functional and anatomical changes develop in the tongue and are reflected in the lingual scrapings.

It is possible that the bacteria exert some influence on the shedding of the squamous epithelial cells which cover the filiform papillae. In the absence of

bacteria the shedding becomes scanty, the normal whitish coating disappears, and denudation and redness of the tongue follow. The filiform papillae shrink and sometimes actually atrophy.

Antibiotics may exert a chemical action on cell metabolism. Recently Lépine, Barski, and Maurin (1950) investigated the action of chloramphenicol and aureomycin on the proliferation of normal cells (fibroblasts, epithelial cells, tissue from mesencephalon) in tissue cultures. They found that chloramphenicol and aureomycin, in doses which are within the range of blood concentration in treated persons (10 γ /ml.), could retard the rate of growth and exert an injurious effect on proliferating normal cells. Cells from cultures containing 100 γ /ml. showed vacuoles and fatty degeneration. Fibroblasts were more readily inhibited than epithelial cells. Although it has not yet been proved that these observations are applicable to mature cells *in vivo*, nevertheless such a possibility must be taken into account in attempting to explain atrophic changes in the lingual mucosa. It is of interest to note that aureomycin produces fatty changes in liver (Yesner and Kunkel, 1951; Lepper, Wolfe, Zimmerman, Caldwell, Spies, and Dowling, 1951). Whether the appearance in increasing numbers of "blotchy" cells is connected with such a chemical action of antibiotics is an open question. It may be significant that when these antibiotics (and penicillin) are given as lozenges the number of these cells is sometimes greatly increased in persons normally showing small numbers of such cells (unpublished data).

It has been mentioned before that some patients were treated simultaneously with vitamin B complex and antibiotics. In these patients the tongue retained its normal appearance during the treatment more often than those who did not receive vitamin B complex. However, in a few cases a red, sore tongue and angular stomatitis developed rapidly in spite of vitamin treatment. Similar results were observed by Leitner (1950), Hobson and Rice-Oxley (1951), and others. The striking similarity of the oral changes associated with antibiotic therapy to those of vitamin B deficiency (especially ariboflavinosis) has inclined some authors to the view that these lesions are in fact signs of vitamin B deficiency (Harris, 1950, and others) produced by the destruction of the intestinal flora by antibiotics. As the mucous membrane manifestations appear sometimes within 48 to 72 hours after the start of combined antibiotic and vitamin treatment, it is somewhat difficult to explain this sudden and acute vitamin B deficiency. There is some doubt as to the interpretation of these oral changes as signs of true vitamin B deficiency. It is known (Gilder, 1950) that it takes a considerable time to produce definite deficiency symptoms in well-nourished and healthy subjects. It is also puzzling, as Leitner (1951) states, that 10–20 times the daily requirements of vitamin B complex are necessary to prevent or alleviate the deficiency symptoms after a short course of antibiotic treatment in well-nourished persons.

It is possible that a more complicated mechanism might perhaps play a role in the production of the oral (and recto-genital) changes; for instance, some chemical action of the antibiotics on the tissue (? action on enzymes), monilial infection, or anti-vitamin (Kodicek, 1949). Recently a suggestion has been put forward (Gewin and Friou, 1951) that aureomycin and chloramphenicol might exert a competitive blocking action on intracellular metabolism and thus produce changes resembling

vitamin deficiency. Studies of the metabolism of the various vitamins of the B group during antibiotic treatment might shed some light on this problem.

There are so far some indications that penicillin at least may produce in man certain alterations in the content of vitamins C, B, and riboflavin, resulting in some deficiency state (Mosonyi and Oblatt, 1948).

The invasion of the oral cavity by *Candida* (*Monilia*) *albicans* during treatment is an undesirable side-effect, which has been reported by a number of workers (Zimmerman, 1950 ; Harris, 1950 ; Williams, 1950 ; Woods *et al.*, 1951 ; Moore, 1951). *Monilia*, although usually harmless, may in certain conditions acquire pathogenicity. In some of our patients monilia were found not only in the lingual dorsum but also in the crusts of angular stomatitis, in rectal and vaginal discharges, and in large amounts in the stools.

In healthy persons, even before treatment, single yeast cells are occasionally present in scrapings of the tongue. A competitor of bacteria is therefore constantly present in the oral cavity, and takes the place of the bacterial flora when the latter disappears. It seems that some kind of antagonism exists between oral bacteria and oral fungi. The presence of rich bacterial flora prevents the growth of fungi. In some cases, however, bacteria and fungi have been found to coexist, for a certain time at least. Although Zimmerman suggests some stimulating action of the antibiotics on the growth of monilia, Woods and his colleagues deny it on the strength of experiments *in vitro*.

The increase of leucocytes in the scrapings during antibiotic treatment suggests some inflammatory process in the tongue. It was nearly always associated with the presence of fungi and with the absence of bacteria. In very few instances where the number of leucocytes was increased were fungi found coexisting with bacteria, and only in one case were bacteria present in the absence of fungi. The association of an increased number of leucocytes with fungi may be regarded as evidence of an inflammatory reaction to the fungous invasion.

The results indicate that the existence of normal bacterial flora is necessary for the healthy structure and function of the mucous membranes. Experiments on germ-free animals do not prove that the absence of normal bacterial flora actually provokes any pathological changes in the mucous membranes (Reyniers, Trexler, and Ervin, 1946, and Reyniers, Trexler, Ervin, Wagner, Luckey, and Gordon, 1949a and b). It may therefore be that the bacteria exert a protective action, defending the mucous membranes against invasion by fungi.

Summary and Conclusions

Examination of scrapings from the tongue has been carried out in 126 patients receiving treatment with chloramphenicol and aureomycin.

In the majority of patients these antibiotics cause the disappearance of the normal bacterial flora. In the remainder there is a marked diminution in the number, and sometimes also in the character, of the bacteria. Occasionally the appearance of the new strain of bacteria, suspected to be chloramphenicol- and aureomycin-resistant, was noted in the course of treatment.

Epithelial cells are abundant in the first days of treatment. Peculiar, " blotchy " epithelial cells are sometimes seen in increased numbers during the treatment.

In a great number of patients fungous flora (yeast-like fungi, usually *Candida albicans*) replace the bacterial flora. The fungi are profuse in hypertrophic glossitis, and scanty in the atrophic type. In a few cases absence of bacteria as well as fungi has been noted. An antagonism between bacterial flora and fungi in the oral cavity is suspected. The increased number of leucocytes, which is particularly marked in atrophic glossitis, is most probably a sign of inflammatory reaction to the fungal infection.

The similarity of the oral lesions produced by the antibiotics to those of vitamin B deficiency is discussed, and the role of the bacterial flora in protecting mucous membranes is emphasized.

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