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THE BACTERIAL FLORA OF THE SEMI-DESERT REGION OF NEW MEXICO, WITH ESPECIAL REFERENCES TO THE BACTERIA OF THE AIR.

By John WeinziirL

The study of the bacterial flora of the semi-desert region of New Mexico was begun about two years ago, the work being undertaken for several reasons.

First, the writer is not aware that any similar attempt has been made in this country, and disregarding occasional experiments, perhaps no similar study has been made anywhere under the same conditions. The results, then, of such a study possess considerable interest from a purely scientific point of view. In the second place, the results may possess some practical bearing, although this point is not especially emphasized. To mention only a single instance of the possible practical bearings, we may cite the practice of promiscuous expectoration of the numerous consumptives who gather in this region. This may be partly due to the general belief that bacteria cannot live at an altitude of 5,000 feet or above.\(^*\) The utter falsity of this idea is made only too evident by the experiments presently to be recorded.

No attempt will be made to review the mass of literature that has been accumulated on the subject of air bacteria, for such a review would be quite as useless as it would be laborious. Reference to a few of the historic land-marks in the problem may, however, prove of interest.

It is quite certain that the ancient Greeks suspected the existence of organisms in air, causing fermentation and decay, but they possessed no means for their actual demonstration. The Greeks loved speculation more dearly than scientific demonstration, and so this truth, surmised by them, was destined to remain a secret until Antony von Leeuwenhock, a Dutch lense-maker with scientific inclinations, discovered the bacteria in putrid solutions, in the saliva of the mouth,

\(^*\) The altitude of Albuquerque is approximately 5,000 feet. This, then, would be practically the upper limit of germ life.
and in tartar from the teeth. This was in 1675. New life was given to the tottering theory of spontaneous generation by this discovery, and two more centuries of experimentation were required for its final overthrow. In this experimentation the bacteria of the air played an important part, for when life was extinguished in fermenting solutions by heating them, new life soon entered from the air.

In demonstrating the role played by air bacteria, the classical researches of Pasteur and Tyndall are most interesting. Pasteur had previously shown that nutrient bouillon contained in glass vessels, and stoppered with ordinary cotton-wool, would not ferment after sterilization. To show that the ferment which spoiled the medium in the previous experiments came from the air, he exposed a large number of tubes containing sterile bouillon, by removing the cotton stoppers for a time and then replacing them again. Nearly all the tubes so exposed underwent fermentation while others kept as controls remained sterile. This experiment was performed near Paris. To show that the ferment (bacteria) varied in quantity in different places, he repeated his experiment in the Alps mountains, and found that only a few of the tubes fermented.

We have then in Pasteur's work the first evidence that the air of high altitudes contains relatively fewer bacteria than that of lower altitudes.

Tyndall was primarily interested in the physics of light, and his contributions to biology were merely incidental to physical problems. In order to obtain air free from dust particles, and which should reflect none of the light passing through it, he constructed an air-tight box and covered its inner walls with glycerin. When the box was allowed to stand for some time, the particles of dust settled upon the sides and were held fast by the glycerin. Having become interested in Pasteur's work on air, he was curious to know whether any organic life remained in the box. Sterile nutrient media were exposed in the interior of the box for some time, but they developed no life. Thus he proved in a novel way that air does contain bacteria, and that the putrefaction of the solutions is not due to life arising spontaneously.

From Miquel's table it is seen that the air in the park contained on the average about one twenty-eighth as many bacteria as the city air—a point of considerable interest. It is also seen that the number is greatest in summer and least in winter, and somewhat less in the autumn than in the spring.

Without further comment on the above at the present time, we will proceed to the experimental part of our work. It was thought that the simple method of exposing petri dishes containing a layer of sterile neutral medium, would give approximate results, and, perhaps, be as practicable as any. Only qualitative data can be obtained in this way, since we have no means of estimating the volume of air in which the bacteria were contained. Later in the work some quantitative determinations were also undertaken. The discussion of the methods and results of the latter will be postponed until after the qualitative work has been recorded.

In general, it may be stated that most of the experiments were performed in the vicinity of the University of New Mexico, which is situated on the "Mesa," an elevated plain, east of the city of Albuquerque. Some exposures have also
been made in different parts of the city itself. A number of exposures have also been obtained in various places in the Territory, and in these instances I have been materially assisted by a number of my students.

The details of the several experiments will now be recorded and discussed in turn.

**Qualitative Determinations of Air Bacteria.**

*Exp. 1.*— September 28, 1898, 4.00 p.m. Three agar petri plates were exposed 100 feet from University building, to the air for 2, 4, and 6 minutes respectively.

Conditions: Stiff southwest breeze; dry; no rain for several weeks.

October 3.—Colonies were counted with the following results:

- **Pl. 2 min.**—6 bacteria (3 spp.) and 1 mold.
- **Pl. 4 min.**—40 bacteria (4 spp.) and 2 molds.
- **Pl. 6 min.**—50 bacteria (4 spp.) and 2 molds.

Average per 10 min., 71 bacteria.

The species of bacteria were A, A₂, A₃, and A₄.

For descriptions of species, see the end of this paper. The letter “A” was arbitrarily given to all the air bacteria to distinguish them from other cultures in the laboratory. The decided and brilliant colors of these colonies may be of service to the reader in keeping them in mind. They may be summarized as follows:

- A₁—salmon-pink.
- A₂—sulphur-yellow.
- A₃—milky-white.
- A₄—Orange-yellow.
- A₅—pink.

Others—some modification of white.

*Exp. 2.*—October 7, 1898, 4.00 p.m. Three agar plates were exposed to air 300 feet east of University building for 2, 4, and 6 minutes respectively.

Conditions: Gentle breeze blowing; no rain for some weeks; a dozen flies interfered with exposures.
Exp. 5.—October 20, 1898. Three plates were exposed 500 feet from main building for 2, 4, and 6 minutes respectively. A moderate breeze was blowing from the west, i. e., from the city toward the University grounds.

October 27:
- Pl. 2 min.—(agar), 10 bacteria.
- Pl. 4 min.—(gelatin), spoiled.
- Pl. 6 min.—(agar), 30 bacteria.
Average per 10 min., 50 bacteria.
Spp.: A₁, A₃, A₅, and A₇.

Exp. 6.—November 17, 1898, 10.00 A. M. Made three agar plate exposures 15 rods northeast of main building for 4, 6, and 12 minutes. Air was clear, calm, and warm. No rain for some time.

November 22:
- Pl. 4 min.—2 bacteria and 1 mold.
- Pl. 6 min.—5 bacteria and 1 mold.
- Pl. 12 min.—5 bacteria and 1 mold.
Average per 10 min., 5.8 bacteria.

Beside other colonies, A₅ and A₇ were isolated. The great relative falling off in number, even with increased time, is remarkable.

Exp. 7.—November 28, 1898, 10.00 A. M. Made three plate exposures about 500 feet north of buildings.

Conditions: Clear and cold with moderate south breeze. Five inches of snow fell on the 26th and nearly disappeared on the following day.

December 12:
- Pl. 5 min.—(agar), 4 bacteria and 1 mold.
- Pl. 10 min.—(agar), 26 bacteria and 2 molds.
- Pl. 10 min.—(gelatin), 1 bacteria and 0 mold.
Average per 10 min., 11.6 bacteria.

This experiment illustrates the weakness of our method of analysis. The variation in the number of bacteria that fell upon the several plates is most remarkable. This is not wholly a disadvantage, however, for it shows the decided irregularity existing in the number of bacteria that different portions of the air may contain at the same time. This difference is even greater when different periods of time are taken. Perhaps an average of the three plates taken gives us a figure that is fairly representative.

Exp. 8.—December 7, 1898. Three agar plates were exposed about 30 rods east of buildings for 30 minutes. Air was clear and cold, with slight breeze from the south.

December 12.—No growth. Plates were kept too cold.

December 22:
- Pl. 1.—75 bacteria (6 spp.) and 7 molds.
- Pl. 2.—113 bacteria and 4 molds.
- Pl. 3.—260 bacteria and 10 molds.
Average per 10 min., 49.8 bacteria.

Again note the variations in numbers. A₃ and A₇ were isolated from above plates.

Exp. 9.—December 20, 1898. Three agar plates were exposed simultaneously as follows:

No. 1.—15 min. in Biological Laboratory on table.
No. 2.—15 min. in open air north of building.
No. 3.—15 min. in private residence near by.

January 1, 1899:
- No. 1.—36 bacteria and 1 mold.
- No. 2.—92 bacteria and 3 molds.
- No. 3.—106 bacteria and 6 molds.
Average per 10 min. (No. 2), 61.3 bacteria.

These results are interesting, since they give us a comparative idea of the number of bacteria found in rooms as compared with open air. More bacteria were expected from the Laboratory.

Exp. 10.—December 31, 1898. Exposed four plates 30 rods east of main building for 30 minutes. Clear and cold with stiff breeze from northwest.

January 13:
- No. 1 (glucose gelatin).—Spoiled by large numbers of molds which luxuriate on sugar media.
- No. 2 (gelatin).—15 bacteria and 5 molds. Colors of colonies are not well defined on this medium.
- No. 3 (agar).—57 bacteria and 2 molds.
- No. 4 (agar).—123 bacteria and 9 molds.
Average per 10 min., 21.6 bacteria.
Exp. II.—February 13, 1899. Four plates were exposed 20 rods north of main building, all for one hour.

Conditions: Partly cloudy with slight breeze from south.

Moderately warm after cold wave.

February 25:

No. 1 (agar).—21 bacteria and 3 molds.

No. 2 (agar).—33 bacteria and 2 molds.

No. 3 (gelatin).—27 bacteria and 8 molds.

No. 4 (gelatin).—10 bacteria and 5 molds.

Average per 10 min., 3.8 bacteria.

Exp. II.—March 28, 1899, 10.20 A. M. Exposed four plates north of University grove for 30 min.

Conditions: Clear, moderate breeze from southwest; slight snow storm on 27th, which makes the ground wet today.

April 7:

No. 1 (agar).—10 bacteria and 4 molds.

No. 2 (agar).—9 bacteria and 3 molds.

No. 3 (gelatin).—18 bacteria and 4 molds.

No. 4 (gelatin).—20 bacteria and 2 molds.

Average per 10 min., 4.75 bacteria.

It might appear from this experiment that the gelatin medium developed more colonies than agar, but this does not hold in other exposures, as in Exp. II, for example.

Exp. III.—October 5, 1899, 3.45 P. M. Three agar plates were exposed north of grove for 28, 40, and 60 min. respectively.

Atmosphere was clear and calm.

October 11:

Pl. 28 min.—51 bacteria (4 spp.) and 6 molds.

Pl. 40 min.—290 bacteria (5 spp.) and 19 molds.

Pl. 60 min.—Covered with molds. No count made.

Average per 10 min., 49 bacteria.

Spp. present: A, B, A, A,

Note.—Plates 20 and 40 minutes were photographed to show the relative numbers of bacteria, as well as the size of colonies, etc. See Figs. I and II, page 219.

Exp. IV.—October 19, 1899, 3.18 P. M. Made three agar plate exposures north of grove.

Atmosphere clear, calm and warm.
October 27:
Pl. 24 min.—161 bacteria and 8 molds.
Pl. 40 min.—242 bacteria and 19 molds.
Pl. 60 min.—187 bacteria and 24 molds.
Average per 10 min., 52.8 bacteria.

The following three experiments may be included here, though they really constitute a single experiment. Their object is to show the effect of rain in clearing the atmosphere. The first experiment (No. 15) as the data show, was made soon after a considerable rain. The other experiments follow on successive days.

Exp. 15—November 15, 1899, 3:53 P.M. Three agar plates were exposed east of Gymnasium after a heavy rain (½ in.) which fell at noon to-day. There were slight previous showers also. The ground was thoroughly wet down. Atmosphere, clear. Slight breeze from southeast.

November 28:
Pl. 20 min.—7 bacteria and 10 molds.
Pl. 40 min.—10 bacteria and 18 molds.
Pl. 60 min.—10 bacteria and 27 molds.
Average per 10 min., 2.5 bacteria.
Three species: A, A, A.

Exp. 16—November 16, 1899, 3:23 P.M. Exposed three agar plates for same time and in same place as in previous experiment. Soil still quite damp. Atmosphere, clear. Quite strong wind from southeast.

November 28:
Pl. 20 min.—51 bacteria and 4 molds.
Pl. 40 min.—Spoiled.
Pl. 60 min.—23 bacteria and 9 molds.
Average per 10 min., 14.65 bacteria.

Exp. 17—November 17, 1899, 3:04 P.M. Agar plates were exposed as before, under following conditions: Very slight dust beginning to appear. Cloudy, with practically no wind.

November 29:
Pl. 20 min.—126 bacteria and 6 molds.
Pl. 40 min.—143 bacteria and 5 molds.
Pl. 60 min.—133 bacteria and 6 molds.
Average per 10 min., 40.3 bacteria.

NOTE.—Large molds on second and third plates undoubtedly prevented the growth of some bacteria.

Since the details of the above experiments are rather numerous to carry in mind, it may be advantageous to cast them into table form. For such a summary the figures have already been reduced to a common basis, that is to a basis of ten minute exposures.

The figures thus obtained for the several plates in a given experiment are averaged for the final figure of the table. The experiment number, date, and number of plates averaged are given, as well as the weather conditions which necessarily are made very brief. The table is as follows:

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Date</th>
<th>No. of Plates Averaged</th>
<th>Atmospheric Conditions, Etc.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Sept. 28, 1898</td>
<td>3</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>Oct. 7, '98</td>
<td>3</td>
<td>102.7</td>
</tr>
<tr>
<td>3</td>
<td>Oct. 13</td>
<td>1</td>
<td>32</td>
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<tr>
<td>4</td>
<td>Oct. 17</td>
<td>3</td>
<td>36.1</td>
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<td>5</td>
<td>Oct. 20</td>
<td>2</td>
<td>50</td>
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<td>6</td>
<td>Nov. 17</td>
<td>3</td>
<td>5.8</td>
</tr>
<tr>
<td>7</td>
<td>Nov. 28</td>
<td>3</td>
<td>11.6</td>
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<tr>
<td>8</td>
<td>Dec. 7</td>
<td>3</td>
<td>49.8</td>
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<tr>
<td>9</td>
<td>Dec. 20</td>
<td>1</td>
<td>61.3</td>
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<td>10</td>
<td>Dec. 31</td>
<td>3</td>
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<td>11</td>
<td>Feb. 13, 1899</td>
<td>4</td>
<td>3.8</td>
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<tr>
<td>12</td>
<td>March 28</td>
<td>4</td>
<td>4.75</td>
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<td>13</td>
<td>Oct. 5</td>
<td>2</td>
<td>49</td>
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<td>14</td>
<td>Oct. 19</td>
<td>3</td>
<td>52.8</td>
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<td>15</td>
<td>Nov. 15</td>
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<td>16</td>
<td>Nov. 16</td>
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<td>14.65</td>
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<tr>
<td>17</td>
<td>Nov. 17</td>
<td>3</td>
<td>49.3</td>
</tr>
</tbody>
</table>
We may observe from the above data that the decrease in the number of bacteria in the air in winter, over fall and spring, is quite plain. Experiments 1, 3, 4, 5, 8 and 9 representing autumn conditions, stand in marked contrast to experiments 6, 10 and 11 of the winter season. Yet experiments 8 and 9 show how great a variation even the winter season may present. It is unfortunate that the conditions of the weather were not recorded in experiment 9, but presumably some disturbing factor entered. Two such factors enter predominantly to modify our results. Wind, frequently resulting in dust-storms in our locality, increases the bacteria in the air, while rain produces the opposite effect. These two factors may neutralize each other wholly or in part and thus modify the final result. The effect of rain is nicely illustrated by the results obtained in experiments 15, 16 and 17. The first exposure was made shortly after a heavy rain, and similar exposures were made on the two following days. The average number of bacteria that fell on the plate in 10 minutes on the three days was 2.5, 45.65 and 40.3. Here we have a constant and material increase as the ground dried off, or as the effect of the rain factor diminished.

The explanation of the effect produced by rain lies in two directions. First, during the rain the atmosphere is literally washed of impurities, including bacteria. Secondly, the laying of the dust and subsequent soaking of the soil prevents for a period of time the rising of dust and the bacteria contained in it. As the dust increases the bacteria again increase in the air.

Ultimately, rain may exert yet another influence. It is well known that bacteria require considerable moisture for reproduction and multiplication. For this reason they do not increase during their journey through the air, but only in moist soil, in decaying bodies, in stagnant pools, etc. It is evident that rain facilitates reproduction by furnishing one of the most essential factors for it, viz., moisture.

As to the workings of the wind, it is plain that its only effect is to carry dust and its bacteria into the air and to transport them for varying distances. This factor is of greatest importance when dust is most abundant and relatively insignificant after heavy rains.

Still another factor may enter more or less prominently, though indirectly, to modify the number of bacteria to be found in the air; this factor is sunlight. That direct sunlight is a powerful germicide is well known. Its effect then is to decrease the actual number of bacteria that might otherwise find their way into the air. It is not possible to make a reasonably accurate estimate of the force of this factor, but some idea may be obtained from a number of experiments made in testing the effect of direct sunlight on two of the air bacteria—A, and A5. Both of these bacteria are micrococci, and hence do not form spores. A, is killed by direct sunlight in about 30 minutes (March 3, 1900, 12.30-1.00 P. M.) and A5, in 20 to 25 minutes (January 21, 1900, 11.30-11.50 A. M.)

The potato bacillus is not materially killed out in less than 60 minutes and the spores survive a much longer period. It is possible that the air bacteria can survive the effect of sunlight much longer than other forms which might be abundant in the air but for this fact. Professor H. L. Russell, in exposing an agar plate of B. campestris (cabbage blight) to sunlight for 30 minutes showed that this organism is completely killed out in that length of time on the sunny portion with heavy growth in the shaded part.* Probably the killing-out time was even less than 30 minutes. Further remarks in this line are reserved for a subsequent paper.

Perhaps further discussion along this line may be postponed with advantage until we have recorded a number of similar experiments made under modified conditions and which were made with special objects in view. Several experiments were made to show the relation between the number of bacteria in the air in the residence and business districts of the city of Albuquerque.

Exp. 18—October 20, 1899, 6.00 P.M. Three agar plates were exposed in private yard on South Arno Street in the residence portion of town for 20, 40, and 60 minutes. Nowind.

October 27:

Pl. 20 min.—529 bacteria and 3 molds.
Pl. 40 min.—575 bacteria and 9 molds.
Pl. 60 min.—Worthless, due to molds.
Average per 10 min., 276 bacteria.

At least 6 species were present, viz: Aₙ, Aₓ, Aₜ, Aₐ, Aₗ, and A₉.

Exp. 19—October 20, 1899, 5.25 P. M. Plates were exposed as in previous experiment but in business portion of town, i. e. on sidewalk on Gold Avenue. Time—10, 15 and 30 minutes. No wind.

October 25:
- Pl. 10 min.—1,197 bacteria, 13 molds and 1 yeast.
- Pl. 15 min.—1,827 bacteria, 15 molds and 5 yeasts.
- Pl. 30 min.—2,036 bacteria, 17 molds and 3 yeasts.
- Average per 10 min., 1,031 bacteria.

Exp. 20—Jan. 25, 1900, 5.00 P. M. Three agar plate exposures were made in same place as for previous experiment, but for shorter time—1, 3 and 5 minutes. Former plates were too heavily seeded.

February 12:
- Pl. 1 min.—725 bacteria or 7250 per 10 min.
- Pl. 3 min.—976 bacteria or 3253 per 10 min.
- Pl. 5 min.—1,228 bacteria or 2456 per 10 min.
- Average, 4,320 per 10 min.

Exp. 21—January 26, 1900, 5.00 P. M. Three agar plates were exposed under same conditions as in Exp. 18, i. e. in front yard of private residence. Time—20, 40 and 60 minutes.

February 12:
- Pl. 20 min.—980 bacteria or 455 per 10 min.
- Pl. 40 min.—1,373 bacteria or 343 per 10 min.
- Pl. 60 min.—Spoiled.
- Average, 394 per 10 min.

Exp. 22—March 17 and 18. Four agar plates were exposed on Gold Avenue as in Exps. 19 and 20, but the first pair of plates were exposed in the evening at the close of business; the second pair the following morning.
- 6.00 P. M., Mar. 17 (Saturday)—Pl. a—2 min. Pl. b—4 min.
- 8.30 A. M., Mar. 18 (Sunday)—Pl. c—2 min. Pl. d—4 min.

March 23:
- Pl. a, 2 min.—150 bacteria and 1 mold.
- Pl. b, 4 min.—208 bacteria and 0 mold.
- Average per 10 min., 685 bacteria.
- Pl. c, 2 min.—41 bacteria and 1 mold.
- Pl. d, 4 min.—37 bacteria and 0 mold.
- Average per 10 min., 139 bacteria.

NOTE. Plates b—4 minutes, and d—4 minutes, were photographed to show the relative proportions between evening and morning conditions. See Figs. III and IV, page 226. Plates were photographed March 28.

Exp. 23—March 17 and 18. Duplicated conditions of last experiment on South Arno Street, i. e. residence district. Time—5 and 10 minutes.

March 23:
- Pl. a, 5 min. (7.33 p. m.)—346 bacteria and 5 molds.
- Pl. b, 10 min. (7.33 p. m.)—626 bacteria and 8 molds.
- Average per 10 min., 664 bacteria.
- Pl. c, 5 min. (7.33 a. m.)—103 bacteria and 7 molds.
- Pl. d, 10 min. (7.33 a. m.)—128 bacteria and 8 molds.
- Average per 10 min., 167 bacteria.

It will be observed that the six experiments just recorded were planned in pairs—18 and 19, 20 and 21, etc. The object was to establish a comparison of conditions between the residence and business portions of the city of Albuquerque. If the data of Table I are borne in mind, we can also establish a comparison between the country air and that of the two parts of the city.

If then we make the first comparison, that is between the residence and business portions, we find that the latter uniformly shows a higher number of air bacteria, the ratios being (per 10 min.) 276:1,031; 394:4,320, etc.

Probably the second ratio of approximately 1:10 is nearer the actual than the first because of the thickly seeded plates in the former—some of the bacteria being prevented from developing; and partly also because of the difficulties in counting the colonies.
If we take Exp. 14, of October 19, 1899, as representative of "mesa" or country air, we have then as compared with the residence district the following ratios: 52.8:276 and 52.8:304 or approximately 1:6; and for the business district the following: 52.8:1,031 and 52.8:4,320. Assuming the latter to be freer from error, we would have an approximate ratio of 1:80. In other terms, as compared with country air, that of the business district of Albuquerque contains about eighty times as many bacteria. Undoubtedly, at times, the difference is much greater than this even.

Experiments 22 and 23 are intended to show the difference between the relatively undisturbed air in the morning and the same in the evening after the disturbances due to business life in the two sections of the town. For this purpose Saturday evening and Sunday morning were selected as showing perhaps the greatest extremes. In the business district we have the ratio of 685:149 or about 5:1 (see Figs. III and IV); and in the residence district 664:167 or about 4:1. This illustrates well the fact that in large part the heavily-laden air of the city is due to the intense activity of business life.

The flora of the city air in the above was not worked out, as no special interest was involved. It was, as might have been expected, more extensive than that of the country air. A few experiments were also made to show for the most part the character of the flora of districts lying at some distance from Albuquerque. They are as follows:

Exp. 24—March 19, 1899, P. M. Belen, N. M., 30 miles south of Albuquerque. Three agar plates were exposed in usual way by Rev. T. A. Bendrat, for one hour. No wind.

March 29:
Pl. 1—96 bacteria and 1 mold.
Pl. 2—126 bacteria and 12 molds.
Pl. 3—178 bacteria and 7 molds.
Average per 10 min., 22 bacteria.

The species were as follows: A₁, A₂, A₃, A₄, and A₅. The yellow colonies predominated, while the red colonies invariably found at Albuquerque were absent. A₁ is new.
Exp. 25—March 30, 1899, 3:30 P. M. Socorro, N. M., 75.5 miles south of Albuquerque. Three agar plates were exposed by Mr. J. B. Terry, in the usual way, about one-half mile west of town. A heavy rain four days previous. Wind very slight.

April 5:

Pl. 30 min.—168 bacteria and 12 molds.
Pl. 45 min.—264 bacteria and 14 molds.
Pl. 60 min.—228 bacteria and 14 molds.
Average per 10 min., 51 bacteria.

Species present: A₁, A₂, A₃, A₄, A₅, and A₆.

This is substantially the same flora as at Albuquerque, but the red colonies were numerous, especially A₅, which at the former place was relatively infrequent. The white colonies predominated.

Exp. 26—April 30, 1899. Belen, N. M. Three agar plates were exposed for 30 minutes by Rev. Bendrat. The exposure was made on the roof of a store-building, height, 15 ft. Very slight breeze.

May 8:

Pl. 1—317 bacteria and 14 molds.
Pl. 2—344 bacteria and 6 molds.
Pl. 3—890 bacteria and 9 molds.
Average per 10 min., 172.3 bacteria.

Species: A₁, A₂, A₃, A₄, and A₅. The flora for this exposure is identical with that for Albuquerque, but the red colonies, A₃ and A₅, are very rare, while A₄ and A₆, predominate.

Exp. 27—May 2, 1899. Clemens' ranch, in San Mateo mountains, 15 miles from Magdalena and 105 miles south of Albuquerque. Altitude nearly 6,500 ft. Two plates were exposed by Prof. F. S. Maltby under the following conditions:

Pl. 1—May 1, 2.25 P. M. Exposed for 30 minutes on watering trough. Considerable wind carrying dust from horse corrals over plate.
Pl. 2—May 2, 6.20 A. M. Exposed for 35 minutes in front of cabin. No wind and no dust.

May 8:

Pl. 1—6,042 bacteria.
Pl. 2—328 bacteria and 4 molds.
Species: A₁, A₂, A₃, A₄, A₅, A₆.
The experiment shows that for this relatively high altitude we still have a considerable number of bacteria. The effect of the various factors incident to an extensive ranch should not be overlooked here. Had the exposure been made some distance out, the results would have been materially different. Nevertheless, we see that bacteria can and do exist here.

Exp. 28—March 8, 1900. Hell Canon, 15 miles east of Albuquerque in Sandia mountains. Three agar plates were exposed by Pres. C. L. Herrick. No wind.

Pl. 1—6.30—8.00 P. M.
Pl. 2—6.30 P. M. — 6.00 A. M.
Pl. 3—6.30 P. M. — 6.00 A. M.

March 16—Pls. 2 and 3 show no growth, the medium having been completely dried up, due to the dryness of the atmosphere. The plates had been placed in the moist chamber for development with the hope of saving the work.

Pl. 1—90 min.—167 bacteria and 8 molds.
Average per 10 min., 18.5 bacteria.
Species: A₁, A₂, A₃, A₄, A₅, and A₆.
The above experiments (24-28) would indicate that the bacterial flora in other parts considerably removed, is very similar to that about Albuquerque. The greatest difference is found in percentages of the species. A given species that is abundant in one place may be rare in another, while the reverse may be true of other species.
The exposure in Hell Canon shows rather a larger number of bacteria for an uninhabited mountainous district than might have been expected.

Experiments with Reference to Altitude.

In order to make a special test of altitude in this connection, several experiments were made at Camp Whitcomb, which is located in Tijeras canon, 18 miles east of Albuquerque, and at a height of nearly 7,000 ft.
Exp. 29—July 28, 1900, 9.00 A.M. "Cliffs" near Camp Whitcomb. About 7,000 ft. Three agar plates were exposed on some large rocks in shade for an hour. Light breeze from over the canon. Location is sufficiently removed from the camp so as not to be affected by it.

Aug. 8:
Pl. 1—Completely dried up.
Pl. 2—106 bacteria.
Pl. 3—111 bacteria.
Average per 10 min., 18 bacteria.
The colonies were all white with only two species present.

Exp. 30.—July 30, 1900. Camp Whitcomb. Six agar plates were exposed as follows:
Pl. 1—15 min.
Pl. 2 and 3—30 min.
Pl. 4—60 min.
Time 11.45 A.M.
They were exposed on large rock of the highest peak of the Sandia Mts., altitude about 10,000 ft. A very slight breeze from the west. Slight rain the previous day.
Pl. 5) 10 min. Time 2.00 P.M.
Pl. 6) These two plates were exposed on a lower peak—altitude about 8,500 ft. Double quantities of agar were used to prevent excessive drying out of medium.

Aug. 8—All the plates contain some colonies of bacteria, and a large number of molds which have affected the results detrimentally. Only one plate was counted.
Pl. 2—30 min.—42 bacteria and 15 molds.
Average per 10 min., 14 bacteria.
Three species: A, A₁, and A₂, the last two being white and gray in color, respectively.

Exp. 31—Aug. 5, 1900, 5.50 P.M.
Repeatecl Exp. 29. Plates contained double quantities of agar to balance evaporation.
Exposure was made in open on large rock, there being no sunlight to avoid. No disturbing influences.

Quantitative Data.
In addition to the qualitative work that has been recorded, a number of determinations were also made quantitatively. For this purpose two methods were resorted to. In experiments 33-38, an 18-liter oil can, filled with water, was used as an aspirator to remove the air from a large (500 cc) Erlenmeyer flask. This flask was fitted with a rubber stopper through which entrance and exit tubes passed. These tubes were plugged with cotton, and a quantity of sterile gelatin poured into the flask. The whole apparatus was then sterilized and used after cooling. Connection was made with the aspirator by means of a strong rubber tube. By referring to the table below, it is seen that the results obtained with the flask method are unsatisfactory, for either no bacteria entered or the number was so large as to lead to a suspicion of contamination.

The flask method was, therefore, abandoned for the filter method, which may be regarded as similar to sugar filters used by Miquel, and also by Sedgewick and Tucker. As sugar is difficult to sterilize, and is also liable to adhere to the

Bacterial Flora of the Semi-Desert Region of New Mexico.
walls of the containing vessel, sodium sulphate and finally fine sand were substituted. It was found that the sulphate exerted an inhibitory effect upon the colonies and was, therefore, undesirable. The sand worked admirably, but leads to some trouble in counting the colonies. Still, where the colonies are well developed this difficulty is very slight. The same aspirator was used as before for drawing air through the apparatus.

The filter was made from ordinary glass tubing of approximately 1/4-inch bore. This was drawn out at one end so as to lessen the bore to 1/10-inch or so. In the neck thus formed a small, loose, cotton plug was fitted. A layer of carefully sifted sand (40-mesh) was placed upon this cotton, and another cotton plug closed up the mouth of the tube or filter. The whole is thus sterilized by dry heat, preferably in a glass box from which the filter can be removed when wanted. When used the filter is fastened in a clamp and attached to the aspirator. It is desirable, also, that the clamps and other closely lying parts be sterilized; this can be effected in a number of ways: e.g., by washing with sublimate solution. When the aspirator is started the cotton plug is removed. The bacteria enter with the air drawn through the filter, but are held back by the sand. The filter material can be added to any desirable medium.

This method worked quite satisfactorily, and its simplicity and cheapness would seem to recommend it for all ordinary work. If care is taken to insure good suction and a steady current, perhaps the results are as accurate as those obtained with the most elaborate and expensive apparatus.

The number of experiments or determinations made are very limited, but perhaps of sufficient interest to warrant their insertion here. It is thought, however, that a table summarizing the data would be sufficient. Such a table follows:

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>No. of Bacterial Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mar. 2, 21</td>
<td>10:15 a.m.</td>
<td>750</td>
</tr>
<tr>
<td>Mar. 18</td>
<td>11:00 a.m.</td>
<td>700</td>
</tr>
<tr>
<td>Oct. 19</td>
<td>3:30 p.m.</td>
<td>300</td>
</tr>
<tr>
<td>Nov. 28</td>
<td>4:00 p.m.</td>
<td>250</td>
</tr>
<tr>
<td>Jan. 14</td>
<td>2:00 p.m.</td>
<td>100</td>
</tr>
<tr>
<td>Apr. 24</td>
<td>4:00 p.m.</td>
<td>50</td>
</tr>
</tbody>
</table>

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The number of experiments or determinations made are very limited, but perhaps of sufficient interest to warrant their insertion here. It is thought, however, that a table summarizing the data would be sufficient. Such a table follows:
TABLE II.

From the above it is seen that in all, seventeen experiments were made, with a total of twenty-five determinations. These extend from October to April, a period of seven months. The determinations were made partly at the University and partly at the residence portion of Albuquerque (S. Arno St.). Those made at the former place were made for the most part by the flask method, and as the results are somewhat doubtful, they will be eliminated from the following discussion. This leaves only experiments 39, 40, 48, and 49 (five determinations) made at the University. These five give an average of only 41.6 bacteria per cubic meter of air. Taking the eleven determinations made by the same method in the residence district of Albuquerque, we have an average of 143 bacteria per cubic meter, or more than three times the number found in the mesa air. It is recognized that these figures are very imperfect, due to the limited number of experiments made, but they may serve, in a measure, to indicate the conditions as compared with other places. If we take for comparison Miquel's table p. 213, we find that for autumn, winter, and spring we have an average of 6,016 bacteria per cubic meter for Paris and 220 for Mont-Souris. That is, the air of the residence portion of Albuquerque contains rather more than half the number found in Mont-Souris park, while the mesa id contains less than a fifth of that number.

While the number of bacteria per volume are undoubtedly less in our arid district than for similar places in more humid climates, the number found is still quite large; larger, in fact, than had been expected. The explanation of this fact is found in the greater facilities afforded by our climate in transporting into the air such organisms as may be able to thrive. The pulverization of the ground and the creation of dust that is readily carried into the air by our relatively high winds, undoubtedly accounts largely for the condition. In other words, while as many bacteria may not exist in a dry climate as in a moist one, the opportunities for carrying them into the air are relatively much greater, and consequently we find the atmosphere, not free, but well laden with bacterial life.

And here, perhaps, a practical application may be made to the conditions found in our locality. While the intense sunlight and dryness may do much to kill off bacterial life in New Mexico, we also have greater facilities for distributing what life remains than do most communities. There is, then, abundant room for the application of practical and hygienic sanitation here as elsewhere. Especially is such sanitation desirable in the matter of expectoration by tuberculous patients, more particularly in cities and towns. The rapidity with which sputa may dry and become pulverized, and finally carried into the air as dust by winds, is remarkable. They may be, and undoubtedly are, carried off by our strong winds into the sparsely settled country, but this cannot entirely eliminate the danger.

From a botanical point of view, our flora is quite interesting. A large number of species show highly colored colonies. Six out of the fourteen species are chromogenic. Four of these chromogens are micrococci, viz.: A1 (salmon pink), A2 (pink), A3 (sulphur yellow), and A4 (orange). Two are bacilli, A6 (yellow) and A10 (pale yellow). The remaining colonies are white or gray-white, and with the exception of A6, all are bacilli.

Among the micrococci the majority form tetrads, though A1 is a sarcina and A2 a diplococcus. The bacilli are usually immotile and sporeless. Bacilli A6 and A10 form spores.

In numbers the chromogenic and non-chromogenic bacteria are about equally divided. Bacillus A1 is probably most numerous, with A2 (yellow coccus) a close second. Of the two red species, A1 was quite numerous, while A2 was somewhat rare though usually present. A3 was not at all abundant except at Belen, but was frequently present. A4 was frequently present and quite plentiful. All other species were occasional and rare.

It is quite remarkable that this flora is apparently quite constant for our region, as is shown by determinations made fully 100 mi. apart. Even the mountain flora, as shown by experiments in Hell Canon and on the Sandia Mountains, contains most of the common species. It would seem that this general uniformity is to be attributed to the strong winds prevailing here, carrying the bacteria for many miles, thus producing a common flora.
As to the source of these organisms, nothing is positively known. Analysis of superficial layers of the soil show their presence, but this might be attributed to their falling upon the ground. At a depth of several inches we find an entirely different flora, which comprises mostly non-chromogenic, spore-bearing, liquefying bacilli. These have never been worked out in detail. The waters from the Rio Grande do not contain our air flora to any material extent. Analysis of milk from a number of dairymen show different results. At times they are quite absent, and this is especially true of the more careful and cleanly dairies. At other times they form a large proportion of the milk flora. In these instances it is believed, however, that they invariably gain entrance from the air through carelessness on the part of the dairyman. It would appear most probable then that our flora is obtained from the superficial soil layers, especially in moist places.

It has been mentioned that many of the air bacteria may be isolated from milk. It may not be inappropriate to record here, the fact that the typical milk flora, as found elsewhere, is also characteristic here. *B. acidi lactici* (Hüppe) and *B. lactis acidi* (Marpman) have been found in all samples analyzed, and coming from a number of dairymen who deliver milk in the city of Albuquerque.

It may also be mentioned that search has been made for *B. tetanus* in garden and other earth, through animal inoculation, but it has not as yet been found.

Search has been made for *B. subtilis* on native hay, but repeated cultures in bouillon have failed to reveal it. *B. mesentericus vulgatus* can be regularly obtained from native-grown potatoes.

**DESCRIPTIONS OF AIR BACTERIA.**

Of the fourteen organisms isolated, nearly all were present on the plates a number of times. These have been quite fully described during the work; the characteristics being corroborated by one or more subsequent cultures. It has been thought worth while to include ten of these descriptions in this place. They are as follows:

**A.**

**Morphology.**—Medium sized micrococcus; single and in pairs; involution forms are found in old potato cultures; size, about 1 μ.

**Gelatin Plate.**—Small colony with regular and clear-cut outline; finely granular. No liquefaction. Surface colonies larger than the deep seated ones. Color, salmon-pink.

**Gelatin Stab.**—Good growth along needle-track, but more abundant toward the surface. A salmon-pink surface growth appears, which spreads with age. No liquefaction. Oxygen is necessary to color production.

**Glucose Gelatin.**—Apparently no growth. No gas.

**Glucose Bouillon.**—No gas and no visible change in medium.

**Potato.**—Exceedingly slight growth after two days; dries up without further increase. Characteristic salmon-pink color. If kept in moist chamber, the growth proceeds slowly, but becomes abundant and granular and shows the typical salmon-pink color. The potato is slightly darkened.

**B.**

**Morphology.**—A medium sized coccus; single, pairs and fours; size, 1 to 1.2 μ.

**Gelatin Plate.**—Colony begins as a small, round, smooth pin-head growth which increases slowly in size. Color, sulphur-yellow. No liquefaction.

**Gelatin Stab.**—Moderately abundant growth about equal along entire track. After some days a slight surface growth appears, which ultimately becomes quite abundant and shows the characteristic sulphur-yellow color. No liquefaction.
**Glucose Gelatin.**—Growth throughout tube but more abundant at surface. No gas.

**Gelatin Slant.**—Rather slight growth at first, which increases slowly. Quite irregular and shiny, but becomes wrinkled with age. No liquefaction. Sulphur-yellow.

**Agar Slant.**—Quite abundant sulphur-yellow growth. Regular, moist and shiny. Spreads slowly.

**Bouillon.**—Uniform cloudiness throughout medium. No surface growth. Yellowish precipitate.


**Milk.**—No apparent change. Sulphur-yellow growth on sides of tube at the surface.

**Potato.**—Slight sulphur-yellow growth which does not increase and soon dries up. If kept in moist chamber, abundant growth takes place producing moist, shiny, raised ridges. Potato is not changed.

A.


**Gelatin Plate.**—A small, round, irregular and white colony, which increases considerably in size with age. After some time very slight liquefaction of the medium.

**Gelatin Slab.**—Abundant growth along track. Most abundant toward surface. Grey color. After ten days a slight pit forms which increases slowly.

**Glucose Gelatin.**—Slight growth. No gas production.

**Gelatin Slant.**—At first slight growth, but this becomes quite abundant with time. White, slightly irregular and shiny. Slight liquefaction after ten days.

**Agar Slant.**—Very abundant, white, spreading, moist and shiny growth. Opaque.

**Bouillon.**—Heavy and uniform cloudiness. Abundant white precipitate.

**Glucose Bouillon.**—No gas.

**Milk.**—No change at either room temperature or blood heat.

**Potato.**—Abundant growth with age. Irregular outline, surface irregular and warty. White, with creamy tint.
Bouillon.—Medium remains quite clear, but flocculi appear after several days. These finally settle to the bottom, forming a pink precipitate.

Glucose Bouillon.—No gas. Moderate cloudiness in open arm. No surface growth. Pink precipitate at bottom.

Milk.—No change during whole month.

Potato.—Exceedingly slight pink growth. This soon dries up. If kept in moist chamber, an abundant growth takes place, which shows granular structure and has a light red color.


Gelatin Slant.—No liquefaction. Growth moderate and decreases rapidly downward. Surface growth increases slowly, and finally becomes dry and wrinkled. Yellow.

Glucose Gelatin.—Media clear. No gas.


Agar Slant.—Growth becomes quite abundant in time. Forms a thin, dry sheet. Yellow, i.e., darker than sulphur.

Bouillon.—Cloudy and flocculent. Finally heavy yellow precipitate.

Milk.—No change.

Potato.—Growth becomes abundant and spreading. Dark yellow color. Potato turns blue.

Morphology.—A large bacillus, resembling the potato bacillus. Single, but usually in chains, which are long and somewhat irregular at times. Ends rounded, but in chains appear square. No spores observed. Immotile. Length, 52 μ; width, 1 μ.

Gelatin Slant.—Abundant growth, decreasing slowly downward. Regular and abundant surface growth. After five days liquefaction takes place, producing a pit which takes in the whole upper portion of the tube. A heavy precipitate falls to the bottom of the liquefied gelatin.

Glucose Gelatin.—Medium remains clear. No gas.

Gelatin Slant.—Abundant white granular growth. Slow liquefaction with the growth sinking into the liquid.

Agar Slant.—Abundant ashy-grey growth. Moist, shiny, and tendency to wrinkle.

Bouillon.—Medium remains quite clear, but a very heavy white precipitate settles to the bottom.

Milk.—Slowly digests the casein without first precipitating the same.

Potato.—Growth slow, but finally becomes quite abundant, producing dome-shaped heaps or mounds of a cream-buff color.
Morphology.—Medium sized micrococcus. Single, twos, and fours. Stains readily. Size about 1 μ.

Gelatin Slab.—Abundant growth, decreasing downward. Abundant yellowish surface growth. No liquefaction.

Glucose Gelatin.—Slight growth. No gas.

Gelatin Slant.—Growth finally becomes quite abundant. White, with yellowish tinge. Shiny and slightly irregular. No liquefaction.

Agar Slant.—Abundant growth, cream-colored, but turns yellow in time. Moist, spreading, shiny.

Bouillon.—Slight cloudiness with considerable precipitate.

Milk.—Casein is digested without previous precipitation, after two weeks.

Potato.—At first slight growth, but this increases slowly. Dry and wrinkled. Color changes from pale yellow to a decided yellow.

Conclusions.

1. The air bacteria of our semi-desert region presents a somewhat limited flora; but this is found to be widely distributed, due undoubtedly to the high winds which sweep uninterruptedly over our wide stretches of nearly barren mesas.

2. The actual number of bacteria contained in the air is not as large as in fertile and cultivated regions, but the number is not as small as is popularly supposed.

3. It would seem to follow from the above that sanitary measures and precautions should receive practically the same attention here as elsewhere. Disease-bearing materials, such as infected clothes, sputum, etc., should be carefully disinfected or burned.

4. Many of the species show highly-colored colonies; these belong mostly to the group of micrococci. The flora is characterized by its inertness toward sugar media, and its failing to peptonize gelatin.

5. Apparently none of the species have been previously described.
Unavoidable delays in the publication of this Volume have made it desirable to issue the articles separately as they may be printed.

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