after section of the carotid sinus nerve or inactivation of the carotid chemoreceptors by $3M$ acetic acid (*13*). Respiratory stimulation, amounting to a 49 percent increase in minute ventilation, was nearly abolished after chemoreceptor denervation.

The concentration of glucose in the blood, measured 15 minutes after intravenous infusions of 1 μg of the peptide per kilogram, increased by about 28 mg per 100 ml ($P < .001$). This increase was approximately one-third the corresponding rise following an equal dose of glucagon (Eli Lilly & Co.) in the same animals.

The diverse and potent biologic effects of this newly isolated peptide suggest possible physiologic roles, particularly in the control of intestinal blood flow and blood sugar. Its hyperglycemic action raises the possibility that it might be related to one of the glucagon-like factors demonstrated in extracts of small intestine (*14*). Since the peptide appears to be inactivated principally in the liver (*12*), it is unlikely that its actions normally extend beyond the splanchnic circulation. The peptide could, however, play an important pathogenetic role in situations where it is excessively released—for example, intestinal ischemia (*15*)—or inadequately removed—for example, hepatic cirrhosis. The changes described here are similar to those in hepatic cirrhosis (*16*)—increased cardiac output, peripheral vasodilation, relative impairment of renal blood flow, and abnormal glucose tolerance.

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Coliform Aerosols Emitted by Sewage Treatment Plants

Abstract. *Development of the science of aerobiology has furnished a tool for the investigation of potential sources of microbial aerosols. An investigation of aerosols emitted by trickling-filter sewage treatment plants revealed that coliforms were indeed emitted and have been sampled to a distance of 0.8 mile (1.2 kilometers) downwind. Factors affecting survival of* Escherichia coli *are presented.*

The association of pathogenic microorganisms with water and sewage has been known since 1855 when John Snow in London traced the source of a cholera epidemic to a sewage-contaminated well (*1*). Since that time human fecal waste has been found to contain the specific etiologic agents of some diseases, many of which are intestinal diseases. Although these are commonly transmitted through the mouth, experimental infection of the chimpanzee by inhalation of large numbers of aerosolized typhoid organisms has been demonstrated (*2*). However, there are other organisms, whose respiratory dosage is comparatively low, which are excreted in the fecal waste of infected persons. Some of these are: various respiratory viruses and the microorganisms that cause brucellosis, encephalitis, hepatitis, poliomyelitis, psittacosis, and tuberculosis.

The development of the science of aerobiology in the last few years provided a tool that has encouraged us to investigate potential sources of aerosolized microorganisms. Schultze (*3*), in 1943, studied the fallout of small droplets resulting from watering crops with liquid raw sewage from an overhead sprinkling irrigation system in Germany. Using a primitive sampling technique, he placed open petri dishes at varying distances downwind from the sprinklers and was able to demonstrate the presence of *Escherichia coli* in the airborne droplets. Spendlove (*4*), in 1956, demonstrated the aerosolization of bacteria from a rendering plant and was able to recover airborne organisms downwind from the plant with the use of Andersen samplers.

Modern trickling-filter sewage treatment plants, because of the nature of their design, may be an exceptional source of aerosolized microorganisms. As we contemplated the spectrum of potential aerosols, it became plausible that the variety of organisms that may be aerosolized is almost unlimited. The trickling filter used in the secondary treatment of sewage sprinkles raw sewage into the open air onto a rock ballast to dose the filter bed. The process of sprinkling the raw sewage into the air would be expected to aerosolize a portion of the material and create micron-size particles (Fig. 1). Sewage varies considerably in its microbial count, but counts of from 10^6 to 10^7 organisms per milliliter are common (*5*). A sewage plant processing several million gallons of sewage per day has the potential, therefore, of providing a microbial aerosol source of considerable magnitude on a continuous basis.

Two municipal sewage plants, ranging in treatment capacity from 6 to 25 million gallons (1 gallon = 3.7 liters) of sewage per day, were studied. The plants were located in the Intermountain West and the studies were conducted during May 1970. Andersen samplers (*6, 7*), connected to a portable field vacuum source, were used to collect the aerosols near and downwind of sewage treatment plants. The Andersen sampler aspirates at the rate of 1 cubic foot (28.3 liters) per minute and impinges the collected organisms on a nutrient medium placed in petri plates positioned within stages of the sam-

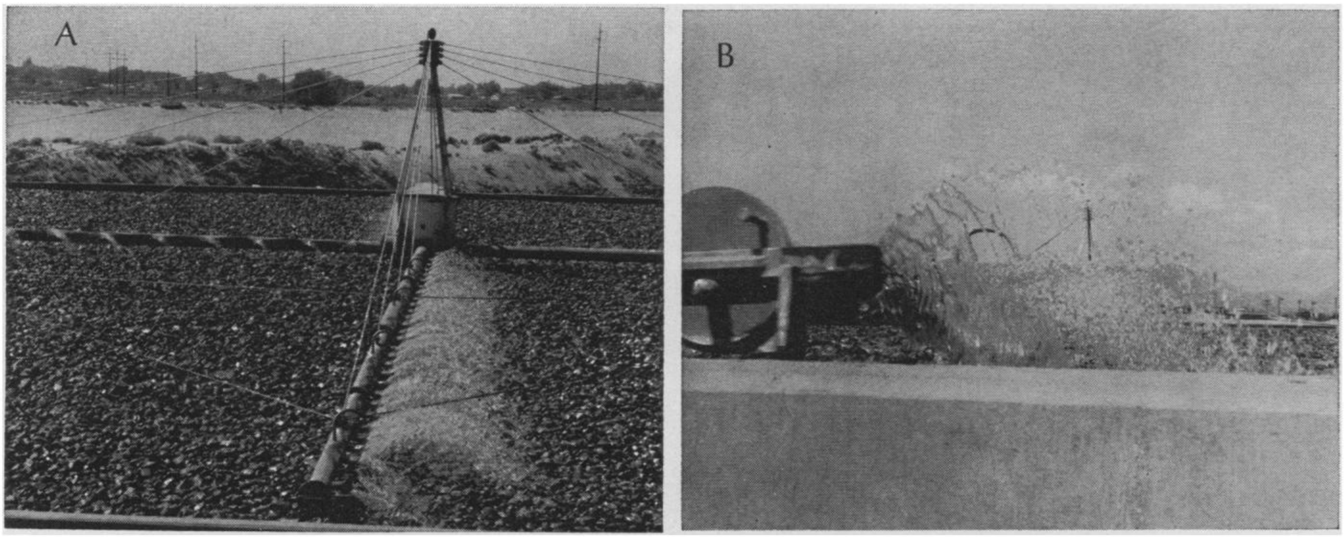

Fig. 1. Trickling filter bed from above (A) and looking along the boom (B). Note the droplet formation.

pler. Each stage collects particles of a different range of sizes, stage 1 collecting the largest particles and stage 6 the smallest.

Three different mediums were used in the studies. Casitone agar was used for the collection and growth of the general microbial population; Endo's medium and eosin–methylene blue medium, for the selective growth of the coliform organisms. Samples were taken from points in the immediate vicinity of the trickling filters and up to distances of 0.8 mile (1.2 km) downwind. Collections of aerosols were made during daytime and nighttime periods, with sampling periods varying from 5 minutes to 1 hour. Upwind controls were collected on each test, and the same types of mediums were used for these controls as for the downwind samples. All plates of nutrient mediums were incubated for a minimum of 24 hours at 37°C.

Substantial numbers of coliforms were aerosolized from the trickling filters. The concentration of aerosol particles collected near the source seemed to be most affected by the size of the source and the velocity of the wind. Plant 1 had only two small trickling filter beds separated by some distance. Plant 2 had two sets of four beds each, with each set of four located in close proximity to each other. As the emitted particles traveled downwind, the relative humidity became more important. If the test was conducted during daylight hours, solar radiation had a deleterious effect. Overcast skies could be expected to reduce somewhat the effect of solar radiation. Generally speaking, high wind velocities, high relative humidity, darkness, and low temperatures would be expected to give the

Table 1. Coliform and total bacterial aerosol particle count from trickling filters (1 foot = 0.3 m, 1 yard = 0.9 m).

Plant	Distance from source	Test conditions				Coliform particles per cubic meter		Total viable particles per cubic meter	
		Wind speed (mile/hr)	Relative humidity (%)	Temp. (°F)	Time of day	Downwind	Upwind control	Downwind	Upwind control
1	50 feet	2–4	25	70 (21.1°C)	10:00 a.m.	364		3,911	
1	50 feet	1–3	25	70	10:30 p.m.	300		19,737	
1	0.25 mile	1–3	25	70	10:30 p.m.	5			
1	110 feet	10–15	70	50	11:00 a.m.	867	0	3,692	51
1	200 yards	10–15	70	50	11:00 a.m.	30	0		
1	130 feet	8–10	65	46	8:30 p.m.	490	0	2,435	574
1	300 yards	8–10	65	46	8:30 p.m.	183	0	3,396	574
1	0.5 mile	8–10	65	46	8:30 p.m.	109	0	622	574
1	130 feet	5–10	25	65	8:30 p.m.	105	0	2,493	1,676
1	200 yards	5–10	25	65	8:30 p.m.	42	0	1,400	1,676
2	300 yards	5–10	25	65	8:30 p.m.	193	1		
2	600 yards	5–10	25	65	8:30 p.m.	26	1		
2	0.8 mile	5–10	25	65	8:30 p.m.	4	1		
2	100 yards	5–10	15	68	9:00 p.m.	159	1	914	607
2	300 yards	5–10	15	68	9:00 p.m.	70	1	817	607
2	600 yards	5–10	15	68	9:00 p.m.	7	1	389	607
2	0.8 mile	5–10	15	68	9:00 p.m.	3	1	856	607
2	140 feet	3–7	55	59	10:00 p.m.	934	1		
2	300 yards	3–7	55	59	10:00 p.m.	73	1		

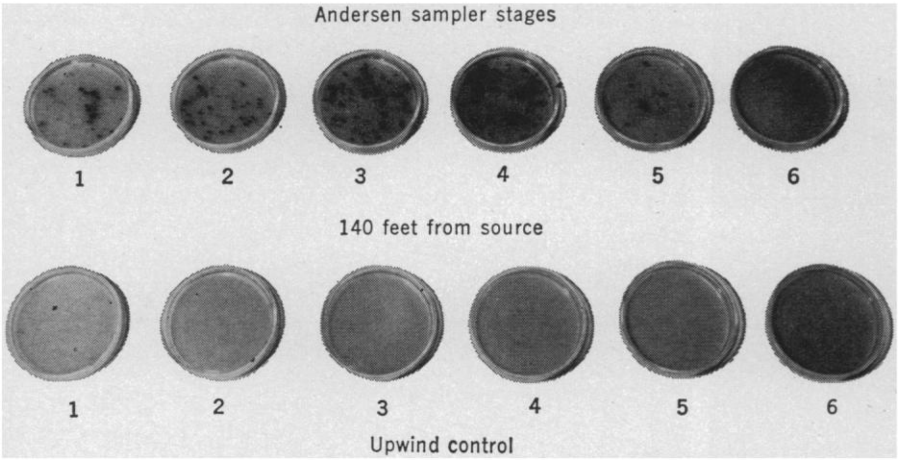

Fig. 2. Petri dishes containing Endo's medium, from various stages of the Andersen sampler. Note high concentration of coliform colonies on the upper set which were located downwind from the trickling filter bed shown in Fig. 1 (1 foot = 0.3 m).

greatest recoveries, both close in and at greater downwind distances. Relative humidity is known to have a pronounced effect on the survival of airborne *E. coli*; usually, the humidity during these studies was low. Low humidities were shown by Brown (*8*) to have a strongly adverse effect on survival of aerosolized *E. coli*. Positive recoveries of coliform organisms were made at night up to a distance of 0.8 mile from the source (which was the maximum distance sampled). Greater distances of downwind travel may be expected under more ideal conditions. Only a qualitative investigation of aerosol emission has been made to date, and Table 1 shows the number of coliform colonies and total number of bacterial colonies that were recovered under the various conditions of the study.

The counts presented in Table 1 are corrected for positive hole count as reported by Andersen (*7*). It should be noted that counts reported are derived from aerosol particles collected on the various stages of an Andersen sampler. Each particle collected theoretically gives rise to one colony; however, most of the particles collected contained more than one bacterial cell. Andersen (*7*) estimated that particles on stage 5 contained 1 to 4 cells; stage 4, 3 to 10 cells; stage 3, 9 to 25 cells; stage 2, 22 to 200 cells; and stage 1, 150 or more cells. In any event, the particle count presented is probably only a fraction of the total cell count. The heaviest counts were observed on stages 2, 3, and 4, with lower counts on stages 1 and 5. Few if any colonies were observed on stage 6. Particles recovered on stages 3 and below are known to be in the respirable size range; hence, if pathogens were present, they would be most infective in this size range. Particles larger than 5 μ in diameter (that is, those collected on stages 1 and 2) would be deposited in the upper respiratory tract but also may be swallowed and enter the gastrointestinal tract where many enteric pathogens are effective.

Since *E. coli* and other coliforms are the universal indicator of fecal pollution, it is apparent that the discovery of aerosolized coliform organisms arising from sewage treatment plants may portend a public health concern. Investigations should be conducted to attempt to identify other bacterial, fungal, and viral aerosols generated by sewage treatment facilities.

Note added in proof: After our report was submitted for publication, it was called to our attention that C. R. Albrecht had performed research of a somewhat similar nature. Albrecht submitted a thesis to the University of Florida in 1958 entitled "Bacterial Air Pollution Associated with the Sewage Treatment Process." We hereby acknowledge Albrecht's work.

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Temperature-Sensitive Neurons in the Brain of Brook Trout

Abstract. *The brain temperature of trout* (Salvelinus fontinalis) *was altered by perfusion of the gills with warm or cool water. Neuronal activity was recorded with microelectrodes. Twelve neurons responded to an increase in temperature with increased activity, and five neurons responded to a decrease in temperature with an increase in activity.*

There is substantial experimental evidence which indicates that ectothermic vertebrates can sense temperature (*1*). For instance, when tested in suitable temperature gradients they have been observed to select a definite range of temperatures within that gradient [for example, fish (*2*), amphibian larvae (*3*), and reptiles (*4*)].

Hammel *et al.* (*5, 6*) have shown in two ectotherms that at least part of the temperature-detecting mechanism is centrally located. They found that alteration of the anterior brain temperature of the Australian lizard (*Tiliqua scincoides*) affected its thermoregulatory behavior. Warming the brain delayed exit of the animal from a cold environment until the colonic temperature was 1° to 2°C below that at which the animal would normally have moved if the brain had not been heated. The converse was also observed when the brain was cooled while the animal was in a warm environment. Similarly, the Artic sculpin (*6*) responded to experimental alteration of anterior brain temperature with a change in thermoregulatory behavior.

Temperature-sensitive neurons have been found in the preoptic area of the brain of the Australian lizard (*7*) and in the hypothalamus of some endotherms (*8, 9, 10*). We have evidence for temperature-sensitive neurons in the brain of the brook trout (*Salvelinus fontinalis*).

The experimental fish were anes-