SOME EXPERIMENTS WITH FLUORESCEIN AS AN AGENT FOR THE DETECTION OF POLLUTION OF WELLS.

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(1 *Map.*)

Most towns augment their water supply from wells, and in South Africa, where the rivers are said to run underground, Johannesburg is no exception.

Its chief supply is from deep wells in the dolomite some 20 miles to the south-west of the town, but one of the suburbs—Parktown—is not connected to the main supply, but at present draws its water from two wells (the property of the Rand Water Board) which will be subsequently referred to as No. 1 and No. 2, sunk in the shales of the upper Witwatersrand beds. From here the water is pumped to two Water Towers situated at the highest point of the suburb from whence it is distributed by gravitation. For various reasons, the Rand Water Board had decided on replacing the pumping machinery at these wells by electrically driven gear, and advantage was to be taken of the change to properly line and deepen the wells by a steel bore-hole.

Chemical and bacteriological samples regularly taken had shown the water to be of great purity chemically, but *B. coli* had occasionally been found, their presence, however, being attributed to temporary and accidental contaminations due to the entrance of workmen to the wells, etc.

Owing to an outbreak of Plague and other circumstances, no regular sampling was done between March 1904 and May 1905. On resumption, however, the results, both chemically and bacteriologically, were very satisfactory. This condition continued until December, 1905,

when a sample taken from one of the Water Towers showed a marked increase in the albuminoid ammonia figure, and was described as "dirty."

From this time on to March 1905, samples taken from various taps, whilst chemically above reproach, contained *B. coli* in 10 c.c. or less.

Suspicion attached to the Water Towers, which were thoroughly cleansed and disinfected, but on March 19th the number of *B. coli* and other organisms growing in cultures at body-temperature, had increased in No. 1 well, and it was concluded that any pollution must be getting access to the well water itself.

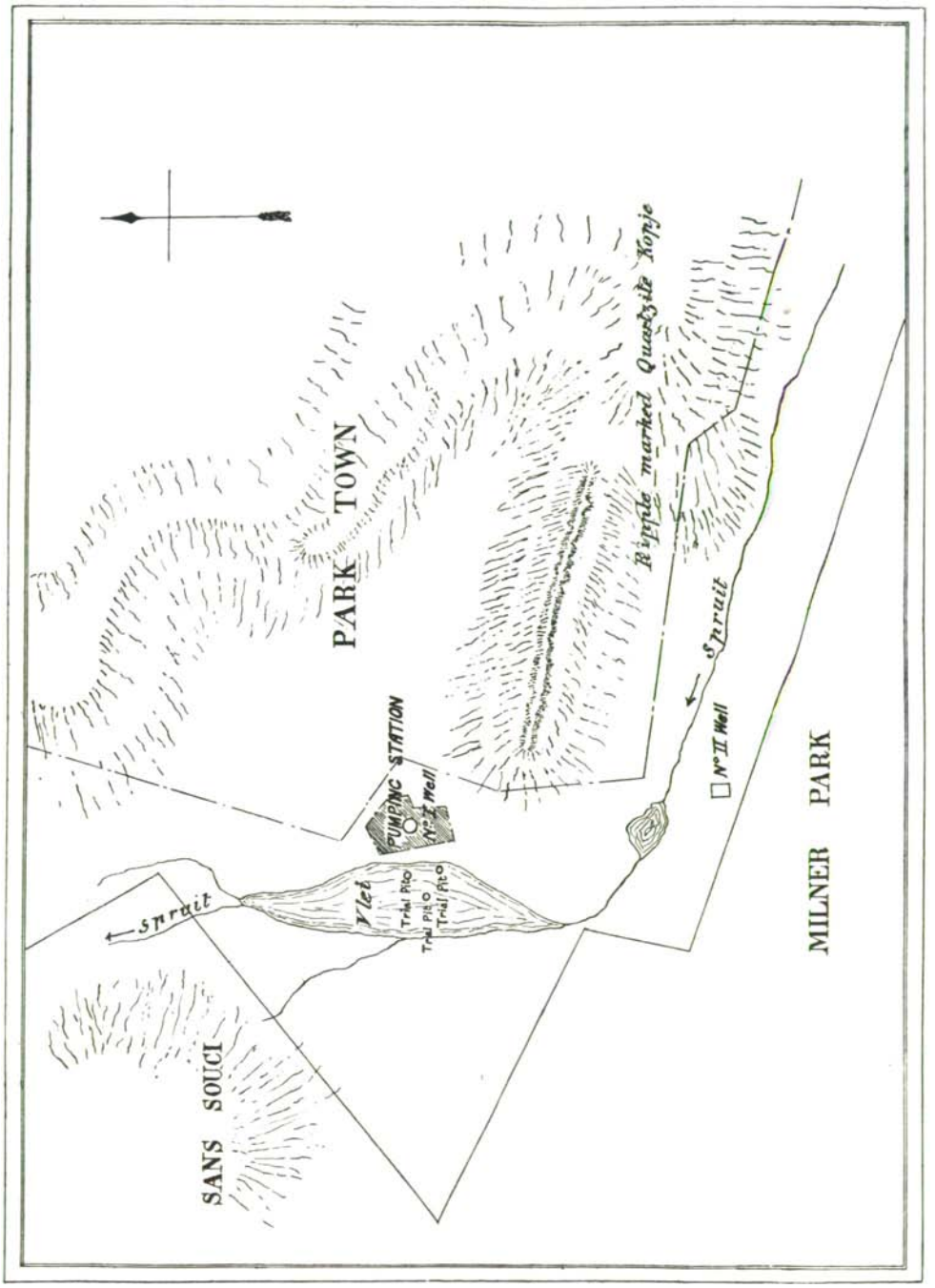
(It is interesting here to note that between April 1905 and May 1906 only two cases of water-borne disease, viz., one of enteric and one of dysentery, occurred in the suburb.)

About this time it came to our knowledge that after long continued pumping from the wells, the level of the water in the adjoining spruit was lowered, but in order that this fact may be better understood, a brief description of the wells is necessary.

No. 1 and No. 2 wells are sunk in weathered red shales—which dip from north to south—through a tract of alluvium, the latter being traversed by the bed of a spruit (or water-course), the water of which is often much polluted, and which courses from Parktown Gates westwards between the ripple-marked quartzite kopjes and the sloping ground on Milner Park. Passing some 50 yards to the north of No. 2 well, the stream turns north and flows about 100 yards to the west of the Pumping Station. Augmented between No. 2 well and the Station by some minor springs in wet weather, it overflows on to a strip of marshy ground, or "Vlei," which lies between it and the Pumping Station. On the eastern, or pump-house side of the Vlei, is an intermittent spring known as the "Peach Tree Eye" from its proximity to a tree of that name.

No. 1 well is situated within the pump-house, and is 75 feet deep, with two tunnels at the lower end respectively 23 and 30 feet long. The yield is about 100,000 gallons per day. There is no proper lining, planks and timber baulks having been used.

No. 2 well is some 200 yards to the south of the Engine House and is also sunk through alluvium in the weathered red shales; the supply is apparently superficial and flows into the bottom of a rectangular basin some 16 feet square by 15 feet deep, with cemented sides and enclosed by a non-dustproof corrugated iron structure. The water enters chiefly near the south-eastern corner, and except to occa-



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sionally augment the supply, is little used for drinking purposes, being used for boilers, etc.

Careful inquiry and inspection had corroborated the statement that the level of the water in the adjoining spruit was lowered after heavy pumping, and it seemed probable that pollution was gaining access to No. 1 well from the Spruit and Vlei, and that it was due to reversed flow or insuction, when the level of the well water was lowered by pumping, along the channel of natural overflow.

It was determined to test this hypothesis, and from some previous work undertaken, fluorescein was selected as the agent. After some preliminary trials, the following experiments were started on April 4th.

The amount of fluorescein at our disposal being small (about 50 grams) the area of operation was restricted to three trial pits, each about 6 or 7 feet deep and 3 feet in diameter, sunk in the marshy ground near the Peach Tree, where the spring had been noticed. To the water *which was present* in the pits a solution of fluorescein was added.

The height of the water in the well and trial pits having been carefully noted, pumping from the former, using extra gear, was started at 12.45 p.m., arrangements having previously been made to collect samples from the well at frequent intervals.

The water in the well having fallen some 45 feet, pumping was stopped at 7 p.m., when it was found that the water level in the three trial pits had fallen $14\frac{5}{8}$, $11\frac{7}{8}$, and $4\frac{1}{2}$ inches respectively.

No doubt this of itself showed a possible connection, but on ordinary examination, fluorescein could not be detected in any samples. About two litres of each sample were therefore concentrated by boiling in a glass dish to about 10 cubic centimetres, and after filtration from the solid deposit, fluorescein was detected in the filtrate of the sample collected on the third day. Possibly the concentration would have been unnecessary had we been able to use more fluorescein in the first place.

The connection between the Vlei and Well being thus established, pumping was discontinued, and the Town Mains connected to the Water Towers.

Arrangements were then made for thoroughly overhauling the well, and on the 18th April, after the pumps had run off the water for some time, a descent was made, when a fall of rock in the western face, some 30 feet down, was discovered, leaving a chasm passing upwards and to the west some 14 feet long. From its appearance, the fall was

of fairly recent date, and no doubt by opening up fissures, etc., had permitted water from the marsh to gain access practically unfiltered to the well.

An opportunity was afterwards afforded of taking samples from the water as it entered the drives, and were found of excellent quality, and since the work undertaken by the Rand Water Board in connection with the well has been completed, all trace of pollution has ceased, and the weekly samples, both chemically and bacteriologically, are quite satisfactory.

Experiments were afterwards carried out with No. 2 well, the surrounding ground being alluvium over the disintegrating shales and a good natural filter.

On April the 20th, fluorescein was put down in a disused well shaft some 80 yards to the east of the well, in what appeared to be a swallow hole about 50 yards north-east, and in a trench 6 feet from the well coping. In the first two the solution was added to the water already standing; in the last, poured into the trench. Pumping was started, but the samples were taken from the basin itself.

On April 26th, the same procedure having been followed with regard to the examination of the samples, it was thought that fluorescein was present in the basin, but as the amount must have been very small, more fluorescein was put down on April 30th. Samples taken the next day showed distinctly the presence of fluorescein. It was still present on May 8th, though in lessened amount, but following a heavy shower the previous day, again appeared on May 21st.

As has been stated before, the sides of the basin were of cement and practically water-proof, the water chiefly entering at the south-east corner. The shortest course for the fluorescein to have travelled would therefore have been about 18 feet, and the time six days.

At this time, apart from the article in the *Standard Dictionary*, the literature at our disposal contained hardly a reference to fluorescein, but the experience gained from the experiments themselves, suggested the need for further investigations, the preliminaries for which were being arranged when, on April 26th, 1906, Dr Porter directed our attention to a copy of Dr Copeman's report "On the outbreak of Enteric Fever at the Fulbourn Asylum," which he had just received through the courtesy of Mr W. H. Power, C.B., F.R.S., of the Local Government Board. While our own conclusions were almost in accord with Dr Copeman's, we freely availed ourselves of the information contained in his report in the following experiments:—

From some preliminary work it had been found that on passing a 1 in 550,000 solution of fluorescein in water through a column of soil, the fluorescein was at first completely removed, but as filtration proceeded, the filtrate was found to contain fluorescein, and ultimately the solution passed through undiminished in fluorescing power. Prolonged percolation of water showed that the fluorescein could be completely washed out of the soil.

By substituting a fine grained sand for the soil, exactly the same phenomenon was observed and further, it was found that precisely the same results were obtained when use was made of a solution of fluorescein made alkaline with sodium hydroxide.

In order to ascertain if the extent of the removal of the fluorescein from solution was proportional to the amount of sand through which the solution filtered, three tubes of the same internal diameter (1·9 cm.) were fitted with stoppers through which passed narrow glass tubes. A layer of coarse gravel—about 3 cm. deep—was placed in each tube, and on this were placed columns of fine sand respectively 7 cm., 14 cm., and 27 cm. in length. These tubes were set vertically, and flasks containing a solution of one part of fluorescein in 200,000 of water were arranged over them in such a way that the solution was delivered into the tubes about 7 cm. above the surface of the sand; the level and hydrostatic pressure therefore being constant throughout the filtration and was approximately the same in each tube.

The following table shows the results:—

Length of sand column	7 cm.	14 cm.	27 cm.
Time taken from starting filtration until 1st drop fell from the narrow tube	17 min.	35 min.	90 min.
Volume of filtrate before fluorescein made its evident appearance	9 c.c.	17 c.c.	36 c.c.
Time taken for the solution to pass through unchanged (estimated approximately)	115 min.	180 min.	350 min.

These results indicate that the amount of fluorescein removed was proportional to the amount of sand with which the solution came into contact, and taken in conjunction with the fact already established, namely, that the removed fluorescein could be washed out of the sand, suggests that the fluorescein is mechanically adsorbed by the sand or soil, and probably the adsorption proceeds until an equilibrium is established between the concentration of the fluorescein in the film adhering to the surface of the grains and that in the bulk solution. This view—that the phenomenon is a surface one—is supported by

the fact that when coarse sand (presenting a smaller surface) was used a smaller amount of fluorescein was adsorbed. The process would appear to be comparable with the dyeing of wool with substantive dyestuffs.

Copeman states that by shaking up ground chalk with a solution of fluorescein, the intensity of colouration was not diminished. As we were unable to procure chalk we could not repeat this, but on shaking up a distinctly fluorescing solution with sand we were unable to satisfy ourselves that any diminution of fluorescein resulted; nevertheless, it is highly probable that if a solution of fluorescein was passed through a column of chalk adsorption would take place.

For the recognition of fluorescence caused by fluorescein we prefer to examine the solution against a dark background, rather than against the white one suggested by Copeman: a good light is necessary, and the solution should not be placed too near the dark surface. Examination of the solution by magnesium light, while a good means of recognising the fluorescence, does not appear to possess any special virtue, or surpass good daylight.

Copeman states that fluorescence is appreciable in a dilution of one in 100,000,000. Obviously the delicacy of the recognition can be increased by concentrating the solution suspected to contain fluorescein provided that no change takes place on evaporation. In order to test this point, a solution of fluorescein, containing one part in 50,000,000, was prepared; in this solution the fluorescence was just recognisable. 10 c.c. of the solution were made up to 2000 c.c. with (1) distilled water, and (2) Johannesburg tap water. At this high dilution (one part in 10,000,000,000) the fluorescence could not be recognised. The dilute solutions were concentrated on the water-bath to 10 c.c., and when examined in an ordinary Nessler tube (giving a depth of liquid of about 2 cm.), the solution showed fluorescence comparable in intensity with the original solution (1 in 50,000,000). When concentrating in this way, a small basin should be used, and filled up with the solution under examination as the bulk is reduced by evaporation: by this means the fluorescein is kept in solution, and does not deposit on the side of the vessel as it would if a large basin was used. Evaporation to dryness on the water-bath although not advisable, does not seem, however, to be prejudicial, for on moistening with water the fluorescein readily passes into solution.

In these laboratory experiments it was not found necessary to render the solution of fluorescein alkaline, probably because sufficient

alkali had been dissolved from the glass—in actual practice, however, it is advisable to do so, since in acid solution fluorescein does not give a characteristic green fluorescence.

One of us has recently had an opportunity of using the concentration method for tracing the course of underground streams over considerable distances, the method proving of great service. In this instance the water at the point of reappearance assumed no obvious colour, but by concentrating, the presence of fluorescein was definitely established.

In connection with the use of fluorescein for detecting a water contamination, it has been suggested that the method is of little use, because it does not follow that bacteria can go where fluorescein can. But this is an objection which, if valid, applies with equal force to all methods wherein the contaminating connection is sought by means of any substance in solution (cf. P. F. Frankland's method with a lithium salt). We are, however, not prepared to admit the validity of this objection; if a solution of fluorescein put into a hole or well in the ground finds its way to another hole or well some distance off, this is definite evidence that a water-connection exists between the two. There is a possibility that the soil between acts as an efficient filter, but this possibility cannot be relied upon, for the connection may be either (1) an underground "lake" of water (as at the Fulbourn District, Cambridge), or (2) a flaw in the rock formation which is so loosely filled with soil, or débris, that it cannot act as an efficient filter.

In so vital a matter as a water-supply therefore, we hold that if a connection can be established by means of fluorescein between a spot known to be contaminated and the source of the water supply, such supply should be regarded as dangerous, and liable at any time to give rise to a water-borne epidemic.

In view of the adsorbing action which soils and sands have been shown to exert on fluorescein, it seemed desirable to ascertain if bacteria would percolate through a column of soil in a similar manner to fluorescein.

A preliminary experiment with an organism of the *B. prodigiosus* group led to no satisfactory result, but more success was obtained with a good pigment-forming strain of a green, very motile organism, closely akin to the *B. pyocyaneus*. A number of trials showed that this organism could be easily recovered both from distilled and tap water, even after some days, and as the presence of fluorescein did

not interfere with its recovery, the organism was suitable for our purpose, and the following experiments were carried out:—

A tube, similar to that previously described, was prepared with a column of fine sand 7·5 cm. in length, and the sand, apparatus, etc., having been found free from the pigment-producing organism, 50 c.c. of a 0·02% solution of fluorescein was made up to 1000 c.c. with distilled water, to which a small quantity of an agar growth of the organism had been previously added and incubated for 24 hours: the solution being fed on to the sand as in previous experiments.

Sixteen minutes elapsed before the first drop fell from the tube, the observations made being as follows:—

	Fluorescence	Green organism
First 0·5 c.c.	None	Present
Next 5·5 c.c. (15 mins. for the collection of 6 c.c.)	Very faint trace	,,
7th c.c.	Faint trace	,,
8th—17th c.c.	Trace	,,
18th c.c. (64 mins. for collection of 18 c.c.)	Very distinct	,,

In the 19th c.c. the fluorescein solution came through apparently unchanged.

	Fluorescence	Green organism
19th—28th c.c.	Unchanged	Present
119th c.c.	,,	,,

Altogether about 500 c.c. of the solution were passed through the sand column. After the fluorescein solution had drained, water was passed through the column, and the green organism was found to be present in the 1st, 50th, 100th, 260th, and 660th cubic centimetre of the wash-water. It was again found that the fluorescein was washed out of the sand.

An experiment carried out similarly with the same material, but with a column 32 cm. long, gave the following results: 69 minutes elapsed until the first drop fell from the narrow tube.

	Fluorescence	Green organism
First ½ c.c.	None	Present
11th c.c.	,,	,,
21st c.c.	Faint trace	,,
22nd to 30th c.c.	Present	,,
31st c.c.	Distinct	,,
41st c.c.	Very distinct	,,
51st c.c.	Apparently unchanged	,,
832nd c.c.	,, ,,	,,

After complete draining, water was added, and the green organism was found in the 7th c.c. of the washing, showing that although the experiment had lasted some days the sand column had not become a *filter* for the organism.

With a column of sea-sand 32 cm. long the first drop came through in $5\frac{3}{4}$ minutes, and showed a very slight (but very greatly diminished as compared with the original solution) fluorescence. After about 4 c.c. had filtered, the fluorescence was apparently as great as in the original solution. The green organism was proved to be present in the 1st, 61st, 110th, and 500th c.c. When a 32 cm. long column of a very sandy soil was used, the first drop fell after six minutes five seconds, and hardly showed any fluorescence. The fluorescence of the first c.c. was not so strong as in the first c.c. which passed through the column of sea-sand. After about 45 c.c. had passed through the intensity of the fluorescence was about the same as in the original solution. The green organism was found in the 1st, 11th and 100th c.c.

A black loamy soil (from Potchefstroom) was next tried. Through a column 32 cm. long the percolation was very slow: 6 hours and 43 minutes elapsing before the first drop fell. Contrary to expectation, however, the first drop showed a very faint fluorescence. The faint colouration persisted for a considerable time without apparently becoming more distinct: after about 20 c.c. had passed through, the intensity of fluorescence began to increase, and after about 50 c.c. had percolated, the intensity was about as great as in the original solution. There appeared in this case to be a slight filtering action with respect to the organism, for on plating the first c.c. the pigment did not show until considerably after 24 hours' incubation. This may, however, have been due to the presence of some substance extracted from the soil (humic acid?) inhibiting the growth or pigmenting power of the organism. The organism was similarly found in the 11th c.c. and in the 30th c.c., showing on agar after 24 hours' incubation.

Conclusions.

These results, and particularly those noted in connection with adsorption, indicate that fluorescein must not be expected to appear in a time proportional to the rate of flow when the water has to percolate through soil, gravel, sand, or detritus. Its appearance will be the longer delayed the finer the material through which the water passes.

Evaporation of the water renders the detection of fluorescein more delicate, but no attempt has been made to show to what extent this is practicable.

The experiments show that $2\frac{1}{2}$ litres can be concentrated for this purpose, and this has proved to be a convenient quantity.

Concentration should not be carried too far, the best results being obtained when the volume was not reduced below 5 c.c. It will usually be found that the concentrate has to be filtered, and in this connection it must be remembered that filter-paper exerts an adsorbing action, consequently, when the filter has drained, it is advisable to wash the paper with 1 or 2 c.c. of water. When the water contains iron, care must be taken not to confuse, in the concentrate, the greenish colour due to the presence of this metal with the green tinge of fluorescein. Until some experience is gained, it is advisable to use, as a delicate control, a very dilute solution of fluorescein.

The Concentration Method has the further advantage of lessening the amount of fluorescein needful, as compared with that necessary to impart an obvious fluorescence to the water as it appears at its point of recovery. This may be of importance when, for aesthetic reasons, it is not advisable to add so much fluorescein that a stream will become visibly coloured—although in the quantity necessary to produce this the material is harmless.

In conclusion, we desire to express our thanks to Dr Porter (Medical Officer of Health, Johannesburg), under whose direction the actual experiments at the wells were carried out, and of whose report on the subject we have freely availed ourselves, for permission to publish the first portion of this paper, and to Mr Louttit (Bacteriological Assistant at the Government Laboratories) for assistance in the bacteriological portion of the work.