Remarks

ON

FURTHER EXPERIMENTS CONCERNING THE ORIGIN OF LIFE.*

BY

H. CHARLTON BASTIAN, M.D., F.R.S.,

HONORARY PROFESSOR OF MEDICINE, UNIVERSITY COLLEGE, LONDON.

My work entitled *The Origin of Life* was based in the main on 196 experiments with hermetically sealed tubes whose contents had been exposed to high temperatures.

Since the date of its publication in the spring of 1911 I have performed a much larger series of experiments, as many as 280, and consequently should be in a position entitling me (1) to pass some judgement on the value of my previous results; (2) to deal more or less authoritatively with the doubts expressed by others as to the validity of my conclusions; and (3) to offer some useful advice to those who may wish successfully to repeat my experiments.

1. THE NATURE OF THE PROBLEM AND THE EXTENT TO WHICH IT HAS BEEN SOLVED.

It has long been widely believed in the world of science that the occurrence of "spontaneous generation" is a myth.

This view gathered strength thirty to forty years ago, seeing that the experiments of Pasteur, and later those of Tyndall, induced them to urge it with much insistence; and because, moreover, it has seemed to be in accord with the practice found needful in bacteriological inquiries of growing importance.

In all their experiments Pasteur and Tyndall, as well as previous experimenters, made use of solutions of organic matter—mostly infusions of different kinds—heated to 100°–105° C. for varying periods, and subsequently protected more or less securely from external contamination. All these solutions or infusions must have been more or less teeming with bacteria of different kinds, as well as with torulae and germs of common moulds. This would have been notably the case with hay infusions, which were so largely used by Tyndall.

These experimenters made no attempt whatever to exclude such organisms and their germs. They trusted to what was known and well established concerning the lethal influence of heat on living matter immersed in fluids, and consequently to their ability to sterilize the solutions and vessels used.

Having extinguished all life in their media and receptacles, they exposed them for varying periods to what were considered suitable incubating temperatures. Then, if evidences of fermentation showed themselves within the experimental fluids, they were seemingly content to account for it as a result of some error; while their numerous negative results impressed them so strongly that they were induced to favour a general conclusion far wider than was warranted by their premisses.

My own experiments have been of the same order, only much more stringent, seeing that instead of infusions of organic matter I have of late been dealing with saline solutions only, always contained in hermetically sealed vessels, and, moreover, often heated to temperatures very much higher than those employed by my predecessors. My saline solutions have also contained some bacteria, toruloid corpuscles, and germs of mould, though probably very much fewer than those existing in the organic

infusions dealt with by Pasteur and Tyndall, whose experiments have been believed to be so decisive.

Thus, if from the basis of their negative results they felt themselves warranted in proclaiming their belief that the present occurrence of "spontaneous generation" was a myth or chimera—a view which has been so widely accepted—how much stronger is my warrant for the opposite belief, looking to the very numerous occasions on which, when opening previously sterilized tubes and solutions, I have taken therefrom undoubted living organisms.

Even one such positive result free from all error is, of course, worth more than any number of negative instances. It will be well to consider, therefore, the various possibilities of error that have been suggested.

2. REPLY TO CRITICISMS.

Some of my critics—mostly not biologists, and presumably not thoroughly acquainted with bacteria and torulae—have supposed that what I have taken from my tubes have been mere pseudo-organisms such as *Leduc*, *Herrera*, *Jules Félix*, the brothers *Mary*, and other plasmogenists are able to produce from various saline solutions—bodies which undoubtedly often simulate organic forms with great exactness. This, however, is an absolute error not likely to be shared by

biologists, and one which is shown to be erroneous by the fact of the free multiplication of my organisms when taken from their own media and introduced into other quite different nourishing fluids.†

Others do not doubt that the organisms found and photographed are real organisms, but cannot believe that they have really been engendered within the tubes.

Some of these suggest that the organisms found have rushed into the tube from the atmosphere when its neck has been filed off. But there is practically no inrush of air at this time; certainly none that would suffice to carry atmospheric germs at once through the fluid (about 1½ in. deep) to the sediment at the bottom of the tube, where the organisms are always found.

Others suggest that the organisms may have been contained in the pipette; but this is always thoroughly heated in a spirit lamp flame just before it is introduced into the freshly-opened tube. And

when a small quantity of the sediment is obtained by the pipette, it is at once transferred to a thoroughly clean microscope slip and covered with a cover-glass.

Then torulae are supposed by some of my critics to fall from the atmosphere on to the drop of fluid on the microscope slip, during the moment or two that it remains uncovered. This supposed explanation, however, of the presence of organisms found by me is mere unsupported theory, as any one would soon find who set himself to discover torulae in the way suggested, and by an examination of Fig. 1. That such formed organisms, and often in such numbers, could have been gathered from the atmosphere during the transit of the pipette for one or two feet from the mouth of the tube to the microscope slip, and before the drop of the solution is covered is, in fact, devoid of all probability, and will be shown to be so by the numerous cases subsequently to be referred to in which, during similar examinations of various series of other tubes (barren), no organisms whatever could be found.

Other critics are content to assume that the organisms found are not living. They believe them to be dead organisms, some of those pre-existing in the experimental fluids, but killed by the heat to which they and the tubes have been subjected.

This objection requires to be looked at in more detail, because it is admitted that some few organisms may be found in the experimental fluids, derived from two of the constituents of such solutions—namely, the sodium silicate and the ammonium phosphate—the dilute phosphoric

Fig. 1.—One of several groups of small torulae beneath the same cover-glass, as taken direct from a tube exposed to light containing a yellow solution, which had been heated seven months previously to 100° C. for twenty minutes on three successive days. $\times 700$.

* Paper read before the Pathology Section of the Royal Society of Medicine on November 19th, 1912.

† Solutions of 3 per cent. glucose and of 5 per cent. cane sugar, as well as the ammoniac tartrate solution referred to in *The Origin of Life*, p. 35.

acid, the liquor ferri pernitratis, and the freshly distilled water being, however, free from them.

Any such pre-existing organisms would, of course, have been killed by the sterilizing process; but seeing that in each tube there would only be one to three drops of the dilute sodium silicate, either alone (in the yellow solutions) or in association with three or four grains of ammonium phosphate, as in the colourless solutions, these pre-existing organisms could never be very numerous within the tubes. It is very important to note, moreover, that

Fig. 2.—Large group of the "elementary organisms" taken from a tube containing a colourless solution which had been exposed to light for nine months after having been heated to 135° C. for five minutes in a colza oil bath. $\times 700$. Another sample of the sediment from this tube was ringed with paraffin, and after four days swarms of torulae were found beneath the cover-glass.

nothing like well-developed torulae are ever to be seen in either of these newly prepared solutions. There are, however, often a few very minute spherical organisms (such as are shown in Fig. 14 of *The Origin of Life*), and, more rarely, a minute mould may be found when a control tube is opened, and some of the sediment is examined the day after the sterilizing process.

But let months elapse, with suitable exposure, before other tubes of the same series are opened, and then these minute "elementary organisms," as Professor Hewlett terms them, may be found in large numbers within the tubes, and staining freely with eosin or carbo-fuchsin. These primitive organisms are very slow and sluggish in their growth, requiring some months for origination and for their development from ultra-microscopic particles to the minute embryo bodies that are often so numerous, but which may not always be easily shown to be living in the way that is open for torulae and bacteria. Still when tubes containing them have been closed, and then left undisturbed for twelve months or so, they have often been found to have given rise to minute moulds, mostly of the *Streptothrix* type

The fact, however, that such minute organisms are either very scarce or not to be found at all in control tubes, and to be abundant, as in Fig. 2, after months in other tubes of the same series which have never previously been opened, is conclusive evidence that they have developed and multiplied within the tubes.

At other times, moreover, these "elementary organisms" are found to be associated with well-developed torulae or with bacteria, which can be shown, in the course of a few days, to be living by the growth and multiplication which they undergo under the conditions indicated at p. 39 of *The Origin of Life*—that is, either (a) by their multiplication on the microscope slip, beneath a cover-glass which has been ringed with paraffin; or (b) after their inoculation into certain nourishing fluids that have been previously sterilized.

The so-called "elementary organisms" are, as I believe, to be regarded either as potential embryo torulae, or as fungus-germs.* At times when they have been inoculated into a sterilized ammoniac tartrate nourishing solution, swarms of full-grown budding torulae have been found after a few days; while when left in their own tubes at ordinary room temperature for many months, minute moulds have, as I have said, been found within the tubes, one of which is shown in Fig. 4.

Finally, other critics, unable from the evidence adduced

Fig. 3.—Bacilli and a minute mycelium, stained with eosin, from a colourless solution which had been heated to 100° C. for twenty minutes on three successive days, and was opened after having been six months in an incubator, first at 27° and then at 37° C. $\times 700$.

to doubt that what I have taken from my tubes have been veritable organisms rather than mere pseudo-organisms, that they have really been taken from the tubes, and that they have been really living organisms, fall back upon the only remaining means of staving off the conclusion that they have been engendered *de novo* within the tubes. They hint darkly that some desiccated germs (hitherto unknown) may have been able to survive in the heating processes to which I have had recourse, though these have included exposures from five to twenty minutes to tem-

peratures ranging from 120° to 145° C., or else the method vaunted by Tyndall as lethal for all desiccated germs, and now universally trusted by bacteriologists as a safe means of sterilization.

Strange that this objection should be raised now, seeing that my earliest results in 1870 were disbelieved in by Huxley, Burdon-Sanderson, and others, because of the strength of their belief that bacteria and torulae could not survive a heat of even 100° C. for a few minutes in fluids, and seeing that Pasteur himself never thought it needful to use a higher temperature than 110° C. in any of his experiments. This objection is, however, a sort of refuge of the destitute, which has always played a large part in discussions concerning the *de novo* origin of life.†

But where are the obdurate desiccated germs imagined to come from, and why have they hitherto eluded discovery?

They cannot exist in the yellow solutions, which contain only about two or three drops of the dilute sodium silicate solution and four drops of the liquor ferri pernitratis to the half ounce of distilled water, yet it is precisely these particular solutions which

Fig. 4.—Mould from a colourless solution which had been heated in an autoclave to 130° C. for ten minutes five months previously to its being first opened, when numbers of the "elementary organisms" were found, but no moulds. The tube was then securely closed, and opened again after the lapse of twelve months, when numbers of small moulds of *Streptothrix* type were found on the flakes of silica, as well as a much larger mass, part of which is shown here. $\times 500$.

* Associated with them there can often be seen a number of particles of different sizes down to the *minimum visible* stage, doubtless representing different stages in their growth and development. Precisely similar associations with growing torulae were seen and described by me in *The Beginnings of Life* (1872), vol. 1, pp. 281-283.

† After what has been stated on p. 58 (*loc. cit.*) concerning the sterilization of the tubes employed, no one has ventured to renew a previous objection and say that the experimental vessels have not been sufficiently *flambés*. All germs on the tubes, when nearly red hot, would have been effectually burned off.

I have found to yield a distinctly greater variety of organisms than the colourless solutions, into which desiccated germs might be introduced by the 3 or 4 grains of ammonic phosphate contained in each tube. Different kinds of mould have often been found in these yellow solutions, two of which are shown in Figs. 5 and 6.

Any such doubt is however easily solved by introducing 5 or 6 grains of this salt into one ounce of the sterilized ammonic tartrate nourishing solution, and boiling it merely for five instead of twenty minutes on three successive days.

Treated in this way ammonic phosphate crystals cannot be shown to contain living organisms capable of resisting even such comparatively low temperatures and short exposures, though the tubes may have been kept under favourable conditions even for seven or eight months. And that the suggestion is groundless in the present case is shown by the fact to which reference is made on p. 1545 touching the barrenness of tubes when their solutions have been made from old samples of sodium silicate or colloidal silica, though these solutions can be easily shown (by inoculating them from a turbid ammonic tartrate solution) to be still capable of favouring the free growth and multiplication of micro-organisms.

Further, it must never be forgotten that torulae and micrococci, which have so frequently been taken from the tubes (and more especially the former), are not known to

Fig. 5.—Mould of *Streptothrix* type, as taken from a tube containing a yellow solution which, after having been heated to 100° C. for twenty minutes on three successive days, was exposed to light for seven months. $\times 300$.

be capable of resisting temperatures even of 60° to 70° C. for two or three minutes.

3. CONCERNING THE NEW EXPERIMENTS AND WHAT SHOULD BE DONE IN FURTHER TRIALS.

(a) *Exposure to Daylight or in the Incubator.*—One of the first points as to which I needed further information was the extent of exposure to direct sunlight that was advisable.

As related (loc. cit., pp. 50, 56), I had found that exposure of the tubes inside a south window during the last three to four weeks before the opening of them (and in not very hot weather) was followed by good results, though I was always rather doubtful whether this was not merely due to the increased heat to which they and the solutions were thus subjected.

In the spring and summer of 1911 a number of tubes were exposed from the first inside a south window, the weather being unusually bright and warm during most of the time. The result was that when the time came for their examination the solutions were found to be nearly all barren. As I subsequently ascertained that the temperature of the window ledge on which the tubes stood rose often daily for some hours to 43° C., or even rather higher, I am disposed to think that the harm was probably more due to the prolonged and repeated high temperatures than to the effect of the mere actinic rays through the window-glass and that of the tubes.

In view of this experience I now advise that all tubes exposed to light should be placed just inside a north, or, better still, a north-east window, unless they can be put into a conservatory, and protected there from the direct rays of the sun.

Tubes exposed to light should not be examined till nearly six months have elapsed in summer, or eight to twelve months when much of the time of exposure has been in colder and duller weather.

During winter months I have, however, of late made use of the incubator, maintained at 27° C. and at 37° C., and I prefer half of the time to be at the lower and half at the higher temperature. I cannot say positively at present whether it would or would not be desirable to use the higher temperature from the first; though I have found that a final temperature approaching 43° C. for two weeks has been distinctly harmful, often apparently killing plenty of torulae found when the tubes were opened.* Exposure to such a high temperature from the first, therefore, would probably prevent the evolution of organisms, and thus would explain the barrenness of the tubes that were exposed from the first to the hot sunlight of 1911.

Tubes exposed in the incubator should not be opened under four to six months.

(b) *New Combinations Tried.*—As the organisms were often so scarce in the experimental solutions, and this

Fig. 6.—Part of a much larger mould of the same type as the last, as taken from a companion tube which had been similarly heated and similarly exposed to light for seven months. $\times 300$.

might be considered due to the scarcity of carbon within the tubes, I made a series of tentative experiments trying to remove this disadvantage, as a result of which I found that the colourless solutions could be varied by the addition (to each fluid ounce containing the ordinary constituents) of 4 grains of neutral ammonic tartrate with 2 of sodic phosphate; and also by the addition to each ounce of 4 grains of ammonic carbonate, or 6 grains of pure glucose.

Trials with the first of these more complex solutions yielded torulae with one sample of sodium silicate, but not with another, though the organisms were not notably more abundant than they have previously been found to be in a similar solution without the additions indicated.

Those in which ammonic carbonate was added have all been barren; and those with the addition of glucose have also all been barren. In the latter case, however, the solutions were unfortunately made this spring with some of an old sample of sodium silicate.† Further trials with the addition of glucose ought therefore to be made.

None of these additions could be made to the yellow solutions without causing their complete decomposition.

Another variation in the solutions with which trials have been made has been the substitution of Kahlbaum's 10 per cent. sodium silicate for one of the ordinary

* On two or three occasions they have really been very numerous, but apparently dead, as no evidence of subsequent multiplication could be obtained. It looked as if growth and multiplication had been at first stimulated, and that the continuance of the high temperature had ultimately killed the torulae.

† Though the tubes were opened after they had been in the incubator for six months, no organisms of any kind could be found. I then inoculated each of the tubes with three drops of a turbid ammonic tartrate solution, and replaced them in the incubator. At the expiration of only two days each fluid had become slightly turbid, and was found to be swarming with bacteria.

commercial preparations. This was used in the proportion of 14 drops to the ounce for the colourless solution, and 7 drops for the yellow solution; the quantities of the other ingredients being in each case those usually employed. But in all these trials the solutions have either been almost barren, or have only yielded bodies of an uncertain nature. Torulae have never been found in either of them, though in the yellow solution what appeared to be large sausage-shaped bacilli were taken from three tubes of the same series.

It seems clear, therefore, that in the ordinary commercial sodium silicate additional elements must be present that are not found in the 10 per cent. solution, and which, in some way, help to favour the evolution of living units.

(c) *Preparation of the Solutions.*—A very important point has only lately been thoroughly brought home to me, which is that too old solutions of sodium silicate must not be employed. I have carefully kept the solutions obtained in the spring of 1910, not only with the view of myself repeating combinations which I had previously found successful, but in order that I might give others desiring to repeat my experiments some of the actual materials used by me.

During the present year solutions prepared exactly as they had been in the previous summer and earlier have no longer been yielding torulae and bacteria as of old. I found first that the yellow solutions made exactly as they had been before became decomposed, and that the colourless solutions, which after the heating process should have yielded a slight amount of deposit, showed none at all. Thus many tubes were spoilt; and when by slight alterations in the number of drops of the dilute sodium silicate for the colourless solutions I succeeded again in producing a scanty deposit after heating, even then, after due exposures either to daylight or in the incubator, no torulae were found when the solutions came to be examined. The deposit itself, moreover, had not the same character as of old.

These irregularities caused much perplexity at first, but I have now come to the conclusion that they must be due to changes which have taken place in the stock supplies of sodium silicate that I have been using since the spring of 1910. That these have undergone some change is now, indeed, obvious, since at the bottom of each bottle there is a white cloudy deposit nearly an inch in depth, which has been gradually increasing. This was comparatively unheeded by me, as, when using either of the solutions, I had been accustomed to withdraw with the pipette only some of the clear supernatant fluid.

Several times during this year solutions that I have prepared exactly like those which formerly yielded a fair amount of torulae have proved, after similar exposures, to be so barren that not a single torula could be found.

I had not suspected this kind of degradation with the ordinary commercial sodium silicate, though I had, nine months ago, recognized that the dilute colloidal silica with which I was so uniformly successful in 1910 had in some way lost its virtues, seeing that the next time I used it, after an interval of eight months, solutions similarly prepared and similarly treated no longer yielded torulae as before. I looked upon this as a well-known unstable fluid, liable to undergo change, but had not suspected that the commercial sodium silicate might also, though after longer periods, undergo changes, and similarly become less productive when used as a constituent of one of the previously fertile solutions.

How soon the colloidal silica undergoes these harmful changes I am at present unable to say, but I am afraid it does so even in the course of a few months. In his original description Professor Graham¹ said: "Another and eminently characteristic quality of colloids is their mutability. Their existence is a continued metastasis. . . . The solution of hydrated silicic acid, for instance, is easily obtained in a state of purity, but it cannot be preserved. It may remain fluid for days or weeks in a sealed tube, but it is sure to gelatinize and become insoluble at last. . . . To the gradual manner in which colloidal changes take place (for they always demand time as an element) may the characteristic protraction of chemico-organic changes also be referred."

*The solution I have been using is so dilute that it never actually gelatinizes.

Dr. Rosenheim was kind enough to prepare and give me a new supply of the very dilute colloidal silica last November, and a few tubes were at once prepared in which it was one of the constituents. Two of these were examined after they had been only five and seven weeks respectively in the incubator, and no torulae were found after these short periods, while two others were opened after thirteen weeks, one of which had been in the incubator and one exposed to light, and in each of these latter tubes a moderate number of typical torulae were found.

Other solutions were made with it barely four months after it had been received. Two of these tubes were examined after seven weeks and no torulae were found, while others were recently examined after four to seven months, and they also, much to my surprise, have been barren. I fear, therefore, that this new supply of colloidal silica had already undergone some harmful changes even after the comparatively short period of four months.

The fact of the barrenness of solutions in which old samples of commercial sodium silicate or not quite fresh solutions of colloidal silica has been used, is of itself a convincing proof that the temperature to which these and other tubes have been submitted has sufficed to kill any organisms that the solutions may have originally contained. While the fact that organisms are much more abundant in them than they are in fresh supplies and that they go on increasing in these old stock solutions shows that their capability of favouring mere growth and multiplication of such organisms is in no way diminished. The remainder of my stock solution of colloidal silica is clear like water, but there is now a slight sediment which on examination is found to be composed of bacteria, toruloid corpuscles, fungus-germs, and minute moulds. Numbers of such organisms may also be found mixed with the white deposit in the bottles in which the old commercial silica has been standing.

I have, moreover, inoculated the barren solutions in many of the tubes of which I have been speaking with organisms taken from the old stock supplies, or with others growing in a contaminated ammoniac tartrate solution, and have found that the organisms grew and multiplied freely in these previously barren tubes—thus showing that it was not the power of nourishing organisms which had been lost in the solutions made from the old supplies, but what, owing to some change, we can only regard as the capacity for engendering them. It also explained what for a time was a great puzzle to me, that is, why solutions precisely alike in their constitution and the conditions to which they had been subjected should at first be fertile, and then, when prepared at a later date, should be barren.

From what I have said, it seems clear that it is better not to work with ordinary commercial sodium silicate which is more than twelve months old, and that the dilute colloidal silica used should be only recently prepared.†

It must be recognized also that no two samples are exactly alike,† so that in order to estimate the amount of the dilute sodium silicate to be used when dealing with a new supply tentative trials must be made, as described at p. 30 of *The Origin of Life*. The aim for the colourless solutions being to obtain only a quite small amount of deposit after the fluid, boiled for ten to twenty minutes,

†Looking to the uniformly successful results that I obtained when working with the freshly prepared very dilute colloidal silicic acid, it is much to be desired that others should repeat these particular experiments. Dr. Rosenheim has kindly sent me the following details concerning the mode in which, by a modification of Graham's method, he prepared the weak solutions samples of which were given to me:

"66 c.c. of Kahlbaum's sodium silicate (10%) were diluted to 200 c.c. with distilled water, and the solution was poured into an equal volume of water containing 20 c.c. of concentrated HCl."

"A specially prepared appendix from a sheep (see *Handbuch der Biochem. Arbeitsmethoden*, E. Abderhalden, Bd. 3, p. 10) was filled with the slightly opaque solution, and suspended in the dialysing apparatus of Wiechowski (see *Ibid.*, Bd. 3, p. 177). Dialysis against running distilled water was carried on for ten days until the dialysate was perfectly free from chlorides."

The first sample given to me was, according to Dr. Rosenheim, "a pure 0.01% solution of colloidal silica" (see *The Origin of Life*, p. 52, note 2). Kahlbaum declined to supply any such solution, partly because of its instability.

†Quite recent experience leads me to think that the commercial product is now more variable than ever, owing, perhaps, to the fact that it is no longer much used by surgeons. Samples that have lately been supplied to me have varied very much, so that it is more than ever important to make use of recently prepared colloidal silica. (November 23rd, 1912).

has been subsequently cooled; while for the yellow solution, when cooled, the aim should be to obtain also a minute amount of deposit from a fluid having a pale port wine colour, or else (when the amount of the silicate is at times only very slightly more) a pale canary yellow colour.

After trials of this kind have been made, the worker will know how many drops of the dilute sodium silicate with which he is dealing should be used to the ounce for the yellow and for the colourless solutions respectively; and he may be fairly certain that the results will be similar for the hermetically sealed tubes after the sterilizing process, if they have been charged with similar solutions.

In the actual preparation of the fluids a certain order should be observed. Thus, for the yellow solution it is important that the liquor ferri should be added to the distilled water first, and then the dilute silicate drop by drop.

Reversing the order generally leads at once to the formation of a dense precipitate. And the changes with different quantities of sodium silicate, added at the proper time, are often very surprising, as may be seen from the following example of the results obtained during the testing of a new supply of sodium silicate. In each trial 8 drops of the iron solution were used in an ounce of water, and when the one or more drops of the sodium silicate had been added the solutions were boiled for fifteen minutes and then allowed to cool. The results with different proportions of sodium silicate were as follows:

- 1:8 Fluid colourless. Deposit red and copious.
- 3:8 Fluid slightly yellow. Deposit red and copious.
- 5:8 Fluid reddish-yellow. Deposit reddish-yellow and copious.
- 6:8 Fluid yellow and slightly clouded. Deposit very minute in amount.
- 7:8 Fluid yellow and not clouded. Deposit moderate in amount.
- 8:8 Fluid of pale drab colour. Deposit drab and copious.

This sample of sodium silicate would not yield a solution of port wine colour such as I had often obtained with previous samples. With another supply, tested on the same day, the results were equally variable but different. In charging tubes with some of the supply the results with which are given above I used a 6:8 combination, as the deposit, with time, always tends to increase slightly.

For the colourless solutions I have of late been dissolving the ammoniac phosphate and filtering the solution first, through the finest (No. 0) Swedish filtering papers, to remove minute impurities that may be found with the crystals, before adding the dilute phosphoric acid and then the dilute sodium silicate.*

Of late, in order to be able to pursue these studies where I live, in the country and away from a laboratory, I have been boiling the tubes for twenty minutes on three successive days rather than heating them as before to higher temperatures in an autoclave or an oil bath.

(d) *Examination of the Solutions.*—When examining the solutions I use a pipette about 8 in. long, having a bore of 3 mm., and provided with a rubber teat at its expanded upper extremity. It is desirable to take up with the sample of sediment only a single drop of the fluid, so that when this sample is transferred to the microscope slip it may be kept together under a carefully lowered cover-glass.

The specimen is then systematically searched through, beginning at one side of the cover-glass and working regularly up and down till the whole field has been carefully examined, at first with a $\frac{3}{8}$ in. objective, or concurrently with that and a $\frac{1}{4}$ in. with No. 6 eyepiece—and all the time using the nose-piece freely, so as to bring the higher power into use when anything like an organism comes into view. This answers well for the detection of torulae, but of course for bacteria only the higher power is of any use.

The light in all cases requires to be most carefully adjusted so as to get the right kind of illumination; and when the organisms are scarce and minute, their recognition may be much facilitated by running under the cover-glass a drop of a freshly filtered solution of eosin or of carbo-fuchsin.

* In some experiments made in conjunction with Sir William Ramsay and Dr. A. C. Stevenson, both colourless and yellow solutions were centrifugized for twelve hours or more in order to get rid of any organisms that the solutions might contain. The tubes charged with these solutions, after having been sealed, were boiled for twenty minutes on three successive days. Several of these tubes, which had been exposed to light or in the incubator for over six months, have lately been examined, with the result that they have been found to contain bacteria, torulae, and moulds just as freely as in other similar solutions which had not been centrifugized.

CONCLUSION.

There is one difficulty of a general character applicable to all experiments of this order, though not more to mine than to those of previous workers, to which it may be well to make reference here.

Many persons find it more or less impossible to believe that bacteria, torulae, and simple moulds should be products of so-called spontaneous generation, and that for two reasons.

In the first place they are not deemed simple enough to rank as primordial living things.

But what has been said on this head by others of late makes it desirable for me to repeat what I have often said before, namely, that new-born living matter, wherever and whenever it appears, must have its first beginnings as ultra-microscopic particles, which as they grow would at last become recognizable by the aid of the microscope as very minute spherical units.† These may in some cases go on to the formation of micrococci, of bacilli of different kinds, of torulae, or of germs which gradually develop into one of the simplest moulds.

When it is said, therefore, that such organisms are not simple enough to be regarded as primordial living things, it must not be forgotten that the real primordial formations are ultramicroscopic particles, and whatever the type produced—whether minute formless masses of jelly like the Monera of Haeckel, or blue-green algaoid corpuscles—they also would in each case have to commence as ultra-microscopic particles.

It seems to me quite possible that in Nature both Monera and blue-green algaoid corpuscles may be constantly appearing *de novo*.‡ But that is a mere supposition devoid of all positive evidence, as it must always remain till someone may be fortunate enough—whether chemist or biologist—to hit upon such combinations of materials as will, under necessarily restricted experimental conditions, suffice to engender either Monera or blue-green algae. For whatever the future researches of chemists may achieve in the way of synthetically building up the bases of protoplasm, when it comes to the demonstration of the production of actual living matter, they could never convince themselves or the world in general that they had succeeded in their quest till they were able to produce it under such restrictive conditions as I have had to cope with in my experiments—that is within hermetically sealed vessels which, with their contents, had previously been sterilized. They, like the biologist, would have to eliminate all pre-existing life and securely guard against contaminations, and what the chemist may then produce no one can say, though he is certainly never likely to produce living matter in tangible lumps, as recent vaticinations in regard to what “the chemist is going to do in his laboratory” would seem to intimate. It may be safely affirmed that living matter, like crystalline matter, must always begin from a collocation of its elements, and then can only, after a time, reveal itself as minutest particles tending to develop in this or that manner in accordance with the extremely varied nature of the initial molecular combinations. And for our sterilized solutions we can only suppose that the conditions are such as merely to permit of the production of ultra-microscopic particles that slowly, in the course of months (rather than in a few days after the manner of infection by pre-existing organisms), develop into bacteria and torulae, though the latter have the potentiality of growing into simple moulds.†

† When it is said, therefore, that a belief in “spontaneous generation” would tend to contradict the experience of all mankind, “My reply is that Archebiosis may be occurring all round us, and that from its very nature it must be a process lying altogether outside human experience, and never likely to come within the actual ken of man” (*The Nature and Origin of Living Matter*, 1905, p. 144). While long previous to this, in *The Beginnings of Life*, 1872, vol. i, p. 267, I described what I then termed “plastic particles” (rather than “microzymae”) as primordial particles of living matter, “between $\frac{1}{10000}$ in. and $\frac{1}{100000}$ in. in diameter”; and at pp. 293-97 I described the mode in which such particles might be seen to emerge in certain solutions from the region of the invisible, and then gradually develop into bacteria.

‡ As long ago as 1868 M. A. Trécul, an eminent French authority, in an important article entitled “Observations sur la levure de bière et sur la *Mycoderma cerevisiae*” (*Compt. rend.*, t. 67, pp. 137, 212, and 1153), brought forward very conclusive evidence showing that *Torula cerevisiae*, *Mycoderma cerevisiae*, and *Penicillium*, as he says, “ne constituent qu’une seule espèce” (since these forms were shown to be mutually convertible); and, moreover, that torulae under the conditions which he described were constantly arising *de novo*, showing themselves first as minutest particles. See also the investigations of Hector Grasset (*Etude historique et critique sur les générations spontanées et l’hétérogénéité*, second edition, J. Rousset, 1912, pp. 157-159) for further light on this subject, and a simple means of proving the *de novo* origin of torulae within the grape.

What real knowledge, however, have we, apart from experimental results, entitling us to come to any definite opinion as to what primordial living things should or should not be like? We know, indeed, that primordial crystals may be either simple or of very complex forms. Rather let us be content carefully to question Nature by way of experiment, and learn from her to think less of the other objection to bacteria and torulae being accepted as veritable products of spontaneous generation—that is, simply on the ground that they are well-known forms, instead of being something new and strange.

This latter one-sided objection, strongly raised by Huxley in 1870 in an address "On the Relations of Penicillium, Torulae and Bacterium,"³ would necessarily disappear with the proof of "spontaneous generation," because then it would at last be realized that what took place in the experimental vessels must be *much more freely occurring in the world outside*, and that the bacteria, torulae, and moulds whose appearance is so ubiquitous must be constantly *originating* as well as multiplying all around us. We should, moreover, no longer have to postulate the existence everywhere in the atmosphere of inconceivably numerous and varied germs, always ready suitably to tenant every new possibility in the way of site, however unusual it may be.* To meet such requirements the atmosphere ought to present itself as infinitely more crowded with germs of the most varied kinds than it has ever been found to be by the many persons who have most carefully examined it from this point of view.

REFERENCES.

¹ Phil. Trans., 1861, p. 183. ² The Nature and Origin of Living Matter, p. 145. ³ Quart. Journ. of Microscop. Science, 1870, p. 355.

Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

SCARLATINAL MEASLES.

A good many cases have come under my notice during the last few years, when epidemics of scarlatina and measles have occurred at the same time, which could be described as above. The symptoms at the commencement depend on which fever has the start; in some of the cases the rash on the first day of my visit resembled scarlatina, but on the second measles. The rash when fully developed has the appearance of both combined, yet from its mottled condition one is inclined to diagnose measles. I find, however, that these cases always develop severe throat trouble, peeling, and very often Bright's disease.

About a fortnight ago I saw a case of undoubted scarlatina, and a week after the rash had disappeared well marked measles started, which proves that the two diseases can be in the system at the same time. I think this is very important, as in these cases the measles rash is often very much more pronounced than the scarlatinal rash, and the after-treatment (especially with regard to isolation and exposure to the weather) is very different.

West Hartlepool. G. F. E. MORGAN, L.R.C.P., M.R.C.S.

NECROSIS OF THE JAW FOLLOWING MEASLES.

In the Epitome of Current Medical Literature (August 24th) Bindi refers to the rarity of necrosis of the jaw following measles. I have not here any access to works of exhaustive reference, so that I can say nothing as to the rarity of the occurrence, but if it is so rare I should like to add to the records a case which occurred in the Refugee Camp, Vredefort Road, Orange Free State, of which I was Principal Medical Officer during the war.

The patient was an infant aged 11 months, which had been attacked with measles eight days previous to admission to hospital. It was getting on, with no lung complications when the camp sister sent it in, fearing that it was developing cancrum oris, of which there had been a few cases. The left cheek was red, shiny, swollen and hard,

but this condition was not confined to the soft parts of the cheek; the swelling was fixed to the maxilla, and evidently there was periostitis extending from the canine socket to between the positions of the first and second molars. An incision was made through the mucous membrane down to the bone, giving exit to a considerable quantity of thin sanious pus. Four days later the mucous membrane was found to be stripped from the alveolar margin to the extent already indicated, and the bare bone protruded. A few days later the bone seemed to be protruding more through the membrane, and was found to be quite loose and easily removed without an anaesthetic. The sequestrum was of exactly the same extent as described by Bindi, but extended a little further up into the body of the maxilla.

Bethulie, O.F.S.

JOHN A. GRAHAM, M.B.

EPIDEMIC JAUNDICE.

HAVING had a good many cases of jaundice in my district during the last few months I have read with interest the notes that have lately appeared in the JOURNAL on the subject. The following four cases in a farmhouse are, I think, worthy of record. The eldest daughter had what appeared to be an ordinary attack of catarrhal jaundice towards the end of August. The attack ran the usual course, and she recovered in about a fortnight. Three weeks from the commencement of the eldest daughter's attack the next daughter (aged 24), a son (aged 20), and the youngest daughter (aged 9), were taken ill all about the same time and practically with the same symptoms—severe pains in the legs, headache, vomiting, epigastric tenderness, great prostration with a temperature of 104°. These symptoms were followed by jaundice, clay-coloured evacuations, and bile-stained urine. No enlargement of liver or spleen, as in the cases narrated by Dr. Lyons, could be discovered. The temperature became normal in about five days, and the jaundice gradually disappeared. Nausea and a great distaste for food continued for some days. In a fortnight all three patients were convalescent. Now comes the interesting part of these four cases. They had all been drinking water from a well, the sole water supply of the farm. This well is situated in the garden, and is 36 ft. away from the parish churchyard. A bacteriological examination of this water showed: *Bacillus coli*, over 1,000 per litre; streptococci, over 10,000. The bacteriologist pronounced the water "contaminated and unfit for drinking purposes." Father, mother, and two other children escaped unharmed.

Milverton, Somerset.

CHARLES RANDOLPH.

A MODIFICATION OF THE BURRI METHOD OF DEMONSTRATING THE SPIROCHAETA PALLIDA.

A DISADVANTAGE of the Chinese ink method of demonstrating the *Spirochaeta pallida* is that unless the ink is prepared by centrifugalizing it, or, as Captain Frost, R.A.M.C., recommends, by the addition of tincture of iodine, the background is too coarsely granular to make the detection of *Spirochaeta pallida* easy.

I have found that a much more homogeneous field is obtained with less trouble by substituting for Chinese ink a suspension of collargol, made by adding one part of the powder to nineteen parts of distilled water, according to the makers' directions. The powder is put into a black bottle (or an ordinary stoppered bottle wrapped round with carbon paper) and the water poured on it. After standing for a few minutes the bottle is thoroughly shaken, allowed to stand a few more minutes, and again shaken.

The suspension, which keeps good for months, is used for the demonstration of *Spirochaeta pallida* precisely as if it were Chinese ink; a drop of the suspected secretion and a drop of the collargol are mixed together at one end of a microscope slide, and, with the end of another slide, a thin film is spread just as in making a blood film. The specimen is examined as soon as it is dry with the aid of an oil immersion lens. The spirochaetes appear as delicate spirals on a reddish-brown field which is almost perfectly homogeneous.

L. W. HARRISON, M.B., Major, R.A.M.C.,
Pathologist, Military Hospital, Rochester Row.

* See Fée, as cited by Hector Grasset (loc. cit., p. 37), and a much longer list given by Pouchet (*Hétérogénie*, 1859, pp. 336-339) of rare moulds found (but with constancy) only in rare and exceptional situations.

¹ Chemische Fabrik von Heyden.