inoculated saline solutions raised to the same temperature invariably remain unaltered. . . . we may quite safely conclude that bacteria, vibriones, and their supposed germs are either actually killed or else completely deprived of their powers of multiplication after a brief exposure to the temperature of 158° F. (70° C).'

With the conclusions drawn from these experiments as to the death point of bacteria, I, for the most part, agree, but I shall have to refer to them again presently. I have introduced the facts here, because I believe that they add strong confirmatory evidence of one of the explanations of Bastian's results which I have been trying to establish, viz., that in many cases the organisms which appeared in his fluids after boiling did not arise *de novo*, but were derived from particles on the walls and in the air of the vessel, which had not been deprived of life. For in Gruithuisen's experiments and in Bastian's repetition of them, there was no part of the wall of the vessel nor any air in the interior left to be acted on by more or less dry heat. The vessel was filled with fluid, and all the particles in it were subjected to moist heat. And here the high temperatures required in the other cases were not necessary. A temperature of 158° F. continued for a very short time was sufficient to render the liquid permanently barren.[1]

[1] It is of great interest to mention here the difficulties experienced by Dr. Paul Bert in attempting to preserve meat after subjecting it to high pressure ('La pression barométrique,' p. 880).

'Ainsi, dans mes premières expériences, lorsque je voulais conserver une substance, après l'avoir soumise à la compression, je fermais d'un bon bouchon de liége le flacon où elle était placée : ce bouchon était percé d'un trou, et lorsque j'avais retiré le flacon de l'appareil, j'appliquais sur cet orifice fin une goutte de cire fondue, avec laquelle, du reste, je cachetais tout le bouchon.

'Je ne tardai pas à apprendre que cette précaution était insuffisante. Les bouchons, même neufs, même lavés, même chauffés recèlent trop souvent des germes encore en activité. J'eus alors recours aux matras, ballons, tubes, que j'étirais à la lampe, aprés y avoir introduit la substance en expérience ; le trou presque capillaire de la partie étirée permettait à l'équilibre de pression de s'établir.

'Je m'aperçus encore, à mes dépens, que les germes restés à l'état sec sur les parois du petit récipient suffisaient, surtout quand il s'agissait de la putréfaction, mon laboratoire de dissection en étant bourré, pour troubler les phénomènes. Je ne pouvais me mettre sûrement à l'abri qu'en ajoutant un peu d'eau et en remuant avec soin le récipient, avant de la soumettre à la compression, afin de tuer en même temps et les germes contenus dans la substance, et ceux des parois qui se trouvaient mouillés.'

But was the fluid in these vessels in any special condition which prevented the origin of organisms? It is to be observed that when the heat was not high enough to kill organisms, they developed readily, there was then nothing in the conditions which prevented the development of organisms. The only difference in the two sets of experiments, and it seems to be indicated by Bastian, appears to be that in Bastian's former experiments the fluids were under diminished atmospheric pressure, while here they were not. The conclusion then apparently is that a vacuum is better suited for the spontaneous origin of organisms than the normal pressure; and that such is Bastian's view is openly stated by him, and among facts in support of it we find the admission that turnip infusion, urine, and sometimes hay infusion, may remain for an indefinite time in Pasteur's flasks with open bent necks without any development of organisms in them, while in a vacuum organisms arise in similar fluids, especially if a piece of cheese has been added to them.

We must therefore see if any other facts favour this *in vacuo* idea. As I have already stated, Pouchet, who is largely quoted by Bastian, states distinctly that a vacuum is most unfavourable for the occurrence of spontaneous generation, and he employs a vacuum for repeating some of Pasteur's experiments, in order to show that as soon as air is admitted, spontaneous generation occurs.

And in regarding a vacuum as inimical to life when compared with the ordinary atmospheric pressure, Pouchet was correct, as will be evident from the following quotations from Paul Bert's recent remarkable work, entitled 'La Pression barométrique.'

On submitting seeds to low pressures he found that *germination* was much delayed.

Thus in his 350th experiment he sowed barley in earth in three pots, and placed them :

A under a glass at the normal pressure.
B „ „ at 50c· of pressure.
C „ „ at 25c· „ „

Five days later in A the shoots were numerous, very green and very firm, measuring about 10c·

B less numerous, less green, measuring about 8c·

C still less, measure about 6c·

Next day these shoots were cut off at the level of the grain, dried and weighed :

End shoots of A weighed $8^{mg}\cdot8$.
" " " B " $7^{mg}\cdot1$.
" " " C " $6^{mg}\cdot2$.

A low pressure was also found to be inimical to *vegetation.*

Thus to take his 359th experiment :

A number of sensitive plants about $10^{c\cdot}$ in height were each placed under a bell jar on August 1.

August 1. A at $60^{c\cdot}$ of pressure.
B " $50^{c\cdot}$ " "
C " $25^{c\cdot}$ " "

August 3. Some leaves have fallen from C.

August 6. A leaflets sensitive and open.
B " half open and little sensitive.
C " completely closed.

August 7. All restored to normal pressure.

They are all sensitive, but C much less so than the others. C does not close well this evening.

August 9. A is all right, very sensitive.
B. Little sensitive—sickly, yellowish.
C. Leaves falling off—dying.

A similar result was obtained when putrescible substances were submitted to varying degrees of low pressure.

Thus in experiment 386 the muscles of a dog were placed :

A at the normal pressure.
B at $38^{c\cdot}$ of pressure.

Four days later A was horribly putrid.
B is a little less putrid.

Experiment 392.—On January 17, equal sized morsels of meat were placed :

A in a pressure of half an atmosphere.
B at the ordinary pressure.

Other two, C and D containing increased amounts of oxygen.

January 25. The meat, which is the least altered in appearance, is A. The pieces which are most altered are C and D.

Without multiplying the experiments, I may give his results. To quote his own words :

'Si nous envisageons d'abord celles de nos expériences qui ont porté sur la diminution de pression, nous voyons d'une manière nette que dans l'air raréfié la putréfaction a été notablement ralentie et l'oxydation diminuée.

'Mais ces résultats n'ont rien de bien extraordinaire ; l'on savait depuis longtemps que la putréfaction *n'a pas lieu dans le vide*, et il était tout naturel de penser qu'elle serait d'autant moins active que l'air serait plus raréfié.'

Dr. Bastian does not always obtain these results (growth of organisms) with infusions containing cheese, &c.[1] If he uses the *rind* of the turnip in preparing the turnip infusion spontaneous generation may not occur ! At least such is Dr. Bastian's explanation of his failure to get organisms in one or two instances. An experiment is also narrated in Dr. Sanderson's letter in which the walls of the flasks were thoroughly purified by heat before the introduction of the fluid, in order to see whether the organisms were or were not attached to the walls of the vessels. But this experiment is completely nullified by the mode in which the flasks were afterwards filled, for Dr. Bastian charged them by 'breaking off their points' (they had been sealed when hot) 'under the surface of a neutral infusion of turnips and cheese, freshly prepared for the purpose without employing any of the rind.' Here the previous purification of the walls of the vessels was useless, for they were again soiled by the unpurified fluid passing into the flask.

II. The second series of facts on which Dr. Bastian bases his arguments is, that certain solutions may be exposed in airless and hermetically sealed flasks to a temperature of 270° to 275° F. for 20 minutes, and yet that organisms may subsequently develop in these flasks. Such fluids are chiefly strong infusions of turnip rendered alkaline by liquor potassæ.

Now I have already referred at length to the error that the organisms may not be subjected to moist heat at all, and my remarks apply here also. For 275° F. is not always sufficient as dry heat.

But I would remark—and this may apply to some of the

[1] See letter by Dr. Burdon-Sanderson in *Nature* of January 9, 1873.

first series of experiments—that in only one or two cases were numerous and distinct bacteria found; and I have ventured to think that in some of the other cases the deposit which occurred was simply due to imperfect filtration, and contained the forms described, these forms not having developed since the introduction of the fluid into the flask. For, I would ask, if they had developed anew, why was the fluid not full of them? Why was there only a slight deposit? When organisms are really present in cultivating fluids (as in some of Bastian's experiments) they fill the fluid and render it turbid, often with a scum on the surface.

In some flasks various forms of organisms were found, and fungi were present in the deposit at the bottom, more especially when tartrate of ammonia was used. In some cases Dr. Bastian mixed deal wood with the fluid, and found bodies like vegetable cells, which were undoubtedly portions of the wood. With regard to the fungi, Dr. Bastian has himself pointed out that crystals of tartrate of ammonia, when old, generally contain fungi in their interior.

Professor Huxley, in 'Nature' for October 13, 1871, stated that he had seen Dr. Bastian's experiments and preparations, and expressed his belief that the organisms which Dr. Bastian got out of his tubes were exactly those which he put into them, that in fact he had used impure materials, and that what he imagined to be the gradual development of life and organisation was the simple result of the settling of these solid impurities. For instance, he relates how on one occasion Dr. Bastian showed him a specimen of a fungus developed spontaneously, which Professor Huxley recognised as a fragment of the leaf of a Sphagnum, and that it was so he ultimately, after great difficulty, convinced Dr. Bastian.

On the other hand, it is but fair to Dr. Bastian to admit that these settled impurities were not the only things which he got, and that in reality in some cases undoubted organisms developed. Dr. Burdon-Sanderson, in the letter just quoted, says: 'The accuracy of Dr. Bastian's statements of fact, with reference to the particular experiments now under consideration, has been publicly questioned. I myself doubted it, and expressed my doubts if not publicly, at least in conversation. I

am content to have established, at all events to my own satisfaction, that by following Dr. Bastian's directions infusions *can*[1] be prepared which are not deprived, by an ebullition of from five to ten minutes, of the faculty of undergoing those chemical changes which are characterised by the presence of swarms of bacteria, and that the development of these organisms can proceed with the greatest activity in hermetically sealed flasks, from which almost the whole of the air has been expelled by boiling.' Cheese was used in most of the experiments which Dr. Sanderson witnessed.

Among others who have been unsuccessful in repeating Dr. Bastian's experiments may be mentioned Dr. E. Ray Lankester. In 'Nature' for January 30, 1870, he says, 'In numerous experiments with turnip solution made by Dr. Poole and myself recently in the Laboratory of the Regius Professor of Medicine of this University, we found that under the conditions given in Dr. Bastian's book, no life was developed, a result contrary to that obtained by him in 999 cases out of 1000.'

The fallacy of Dr. Bastian's experiments with saline solutions was well demonstrated as long ago as 1872 by Mr. Hartley.[2] In no instance was he able to confirm Dr. Bastian's statements. In his first experiment he made a fluid consisting of a 5 per cent. solution of tartrate of ammonia and phosphate of soda in distilled water slightly acidified with tartaric acid. Several tubes were filled with these solutions, and were heated for four hours to a temperature of 150° C. They were afterwards kept at a temperature of about 25° C. In none of them did any organisms develop, but in some he found that a slight deposit occurred which apparently was what Bastian had taken for a development of organisms. On examination this deposit was found to be inorganic, and to consist of silica alone. 'The disodic phosphate had attacked the glass, the silica deposited on standing, and hence the jelly-like mass.' He adds further, in reference to Dr. Bastian's use of magenta as a test for fungi, that magenta also stains silica.[3] Hartley does not consider these solutions

[1] The italics are my own.

[2] *Proceedings of the Royal Society*, vol. xx.

[3] Dr. Frankland (*Nature*, January 19, 1871), in whose laboratory Dr. Bastian had performed these experiments, was not satisfied with the results, and repeated some of the experiments, using a solution of carbonate of ammonia

capable of supporting life. In another set of experiments he kept the tubes at a fluctuating temperature, which is another of the conditions which Dr. Bastian considers favourable to spontaneous generation; but here also there was no development. Similar experiments, which gave similar results, were made with turnip infusions and with urine boiled and filtered from mucus. After keeping such fluids *in vacuo* for a long time they were exposed to air, filtered through cotton wool, and kept at fluctuating temperatures without any development; but when they were exposed to unfiltered air under the same conditions, organisms rapidly developed.

Dr. Bastian[1] says: 'The disruptive agency of heat is fairly enough supposed by the evolutionists to destroy some of the more mobile combinations in each solution—to break up more or less completely, in fact, those very complex organic products whose molecular instability is looked upon as one of the conditions essential to the evolutional changes which are supposed to take place.' With regard to this Hartley remarks, 'Before granting such a supposition it would be necessary to know, first, what are the "very complex organic products" of such peculiar "molecular instability" existing in a solution of tartrate of ammonia, sodic phosphate, acetate of ammonia, oxalate of ammonia, in a solution of sugar and calcined yeast, in turnip infusion, or any other putrescible liquid. My experiments show that there is no such disruptive agency in a high temperature; that it does not influence the "more mobile combinations" either in solutions of organic salts or vegetable infusions; . . . Dr. Bastian records the development of organisms in a liquid heated as high as 153° C.; yet the assumed "disruptive agency of heat" is supposed to have influenced the results of Schwann and Pasteur at a temperature of 100° C.! His experience is contradictory to his own theory, and at the same time to the experiments of others to which his theory raises objection.'

and phosphate of soda, as had been done by Bastian in one of his experiments. He also states that the figure of eight particles and bodies which Dr. Bastian had mistaken for living organisms were merely 'particles of glass which had become detached from the inner walls of the tube by the corrosive action of the enclosed liquid at the high temperature to which it had been exposed in the 'digester.'

[1] *Nature*, vol. i. p. 176.

III. The third, and indeed the only series of experiments which can still be held to be worthy of consideration, are those with alkaline fluids which, as is well known, are more difficult to sterilise than acid or neutral fluids. Dr. Bastian states that even though superheated, organisms may develop in them.

This difficulty in purifying alkaline fluids was long ago recognised by M. Pasteur, and was attributed by him to imperfect wetting of the organisms.

However that be, Dr. Roberts[1] has conclusively proved that this is not a case of spontaneous generation, for he has shown that while on the one hand an alkaline fluid is very difficult to sterilise, yet as the same fluid without the caustic potash is very easily rendered barren, and as the caustic potash is pure, if each be sterilised separately and then brought together, without any fresh access of dust, the fluid still remains pure; in other words, the caustic potash does not *determine spontaneous generation.* He shows, in fact, that the potash acts by increasing the resisting power to heat of the particles, which are the forerunners of organisms—not by increasing the abiogenic aptitude of the infusion.

Ten flasks were charged with unneutralised hay infusion. Five of these were simply plugged with cotton wool, and boiled over the flame of a lamp for five minutes. The other five were also plugged with cotton wool, but through the centre of each plug there passed an hermetically sealed glass tube bent obliquely, and containing the quantity of liquor potassæ requisite to neutralise the fluid in the flask. These tubes had been previously heated (after being charged with liquor potassæ and sealed) in oil up to 121° C. in order to destroy any organisms they might contain. The flasks thus prepared were then boiled over the flame for five minutes. At the end of a fortnight their contents were unchanged. The tube was now broken and the liquor potassæ mixed with the fluid. Not one flask germinated; at the end of two months they were still barren. But although these flasks had not acquired the power to germinate, they had acquired *the property of enabling freshly introduced germs to survive a boiling heat,* for when the flasks were unplugged and infected with ordinary air or water and then replugged and boiled five minutes, their contents in every instance germinated in a few days.

[1] *Phil. Trans.* 1874.

I can quite confirm Dr. Roberts's statements, for I have used his method of boiling these fluids separately as an easy mode of obtaining any required degree of alkalinity, and I have never got any results which in the least support the view that the addition of liquor potassæ to any sterilised infusion will make organisms develop in that fluid.

I have already mentioned Mr. Lister's method of procedure in preserving fluids. I have mentioned how successful this was, and how all the difficulties as to tall and small flasks, &c., were overcome simply *by purifying the walls and the air in the flasks; by taking care, in the introduction of the impure fluids, to avoid contact with the neck and walls of the flask so purified* (above the level of the liquid); *and by avoiding spurting or frothing during the heating of the fluid.* By Mr. Lister's method all sorts of fluids may be preserved and transferred from one vessel to another, without the development of any organism, with the same certainty as without the occurrence of any fermentative change (see Chapter I.) Mr. Lister's method has removed a great source of error in all these experiments, and I am confident that if his instructions be strictly followed out, the instances of difficulty in purifying fluids will become fewer and fewer. During four years more or less constant work at such experiments, I have only once met with an instance of difficulty in purifying fluids. This case will be alluded to presently.

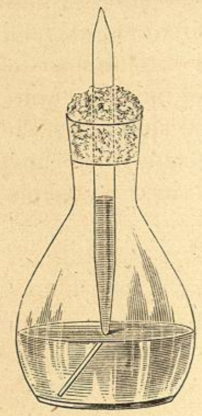

FIG. 66.—DR. ROBERTS'S EXPERIMENT WITH ALKALINE FLUIDS.

The experiments of Dr. Roberts and of Prof. Tyndall[1] as to the absence of fermentative changes in preserved fluids show also the absence of organisms under the circumstances referred to. For their experiments were made with a view to the determination of the question of spontaneous generation. And in my own experience, in order to test various materials, as to whether they contained organisms or not, I have prepared many hundred flasks of cucumber and turnip infusion, and also many of milk, meat, &c., without in any instance obtaining the slightest evidence in favour of abiogenesis. (The case in which I found difficulty in preserving milk has been already mentioned, and will be alluded to presently.) The rapid souring of milk during a thunderstorm is looked on as a change due to electricity. Accompanying this rapid souring there is a rapid increase of bacteria. I have kept flasks of pure milk for a year, through several violent thunderstorms, without any change taking place in it, and without the appearance of any organisms; and at the end of the year the milk was quite fluid and of normal character, though in a few days after the flasks were opened (they were covered with cotton wool caps) it had coagulated, become putrid, and contained numerous bacteria.

[1] For later experiments than those quoted at p. 24, *et seq.*, see Tyndall's paper in the *Philosophical Transactions*, vol. 167, 1877, where some difficulties which he experienced are explained.