MEETING HELD AT BREWERS' HALL, ADDLE STREET, E.C., ON MONDAY, APRIL 18TH, 1904.

Mr. A. GORDON SALAMON (Vice-President of the Institute) in the Chair.

The following paper was read and discussed:—

On a Method for the Application of Hansen's Pure Yeast System in the Manufacturing of Well-Conditioned English Stock Beers.

By N. HJELTE CLAUSSEN (Director of the Laboratory of the New Carlsberg Brewery).

AT the present time there hardly exists on the Continent, a brewery of any importance which has not adopted, directly or indirectly, Hansen's pure yeast system. The larger brewing establishments are provided with yeast propagating machines in which they cultivate the yeast proceeding from a single cell, and these breweries, or special zymotechnical laboratories, supply the smaller breweries with pitching yeast. And this is the case, not only with bottom, but also with top-fermentation breweries.

Moreover the benefits of the pure yeast system have long ago been utilised by other fermentation industries besides brewing. Pure yeast of a single methodically selected race or species is also used to a great extent in distilleries, in the manufacture of yeast, and in the fermentation of wines, including those derived from fruits other than the grape. In respect of its applicability no less than in other respects, this system has proved to be remarkably fertile and capable of development, so that, beginning with breweries, it has found its way not only into all the other branches of alcoholic fermentation industry, but also—and with great success—to such fermentations in a wider sense of the word as are used, for instance in tobacco manufacture and in dairies.

Such being the state of things, it seems rather difficult to account for the fact that there is a branch of the brewing industry itself—and a very important one—in which the system has not hitherto been able to gain a footing. I refer to English brewing practice.

At the outset, one would be inclined to take it for granted that the brewers of Great Britain would be sure to derive quite the same advantages as their Continental colleagues from the use of pure yeast. Indeed, during the years immediately following 1883 (when Hansen introduced, with success, his pure yeast system in the bottom-fermentation brewery, Carlsberg, near Copenhagen), the first trial was made at Burton-on-Trent to ferment English beers with single-cell yeast. I refer to the experiments made at that time by the late Dr. Morris in conjunction with your celebrated chemist Dr. Horace T. Brown. The results of these and several subsequent experiments, however, fell short of the expectations.

In the year 1894 Alfred Jörgensen published a communication "On Hansen's System of Pure Yeast Culture in English Top Fermentation" (*Transactions of the Institute of Brewing*, **7**), and he has since then, from time to time, reverted to this subject. Jörgensen comes forward as an ardent advocate of Hansen's principles and Hansen's methods. In his paper, just referred to, he states that he has succeeded in isolating from English top-fermentation yeast, several races which prove to be capable of carrying through the entire fermentation. He is, therefore, strongly of opinion that English top-fermentation brewers ought to adopt Hansen's system in that simple form in which it is in use on the Continent, that is, as single-cell yeast. The same train of reasoning is repeated in a paper written by Jörgensen in conjunction with Mr. Walter A. Riley and recently read before this Institute. But on going through the experiments made by the latter, I have nowhere found it stated, that these experiments relate to stock beers, that is to say to the real type of English beers. Accordingly it seems to me to be doubtful whether Riley agrees perfectly with Jörgensen, in whose opinion it is a positive fact that single-cell yeast is just as well suited to stock beers as to running beers.

On the part of English brewers and brewing chemists it has been repeatedly stated that their attempts at the introduction of single-cell yeast resulted in failures and, accordingly, they deny that single-cell yeast is capable of carrying through the whole work of fermentation.

In the face of these assertions Jörgensen holds that those failures may have been due to peculiar causes which have nothing to do with the single-cell yeast itself. Thus he sets forth a series of surmises and especially that the cultures of single-cell yeast may have been propagated in an unsuitable manner in the breweries, or that they may have degenerated in consequence of the shaking to which they have been subjected before being taken out of the flasks in the laboratory. But as will be shown hereafter, the solution of the question is to be sought at quite another point, and the real truth is that Jörgensen is completely mistaken.

As matters stand at this moment, the great majority of English brewers are doubtful, or prefer to wait and see, which course to take in regard to the pure yeast question, feeling that the latter has not yet been sufficiently cleared up, as regards the conditions obtaining in English breweries.

As the employment of single-cell yeast was found often to entail deficiency of secondary fermentation, the latter was generally supposed to be carried on by a peculiar yeast different from the one which is active in the case of primary fermentation. But no positive proof was ever adduced in support of such a view. With regard to the nature of the so-called secondary yeast, the brewing scientists also confined themselves to mere conjectures. The opinion has been advanced that it was a true Saccharomyces, a wild yeast belonging among the species described under the old designations *Saccharomyces pastorianus* and *Saccharomyces ellipsoideus;* indeed, the only attempt hitherto made at isolating and utilising this yeast was made on this assumption by van Laer (*Transactions of the Institute of Brewing*, 7).

Now, I have succeeded in proving experimentally that the "secondary yeast" exists, indeed, but it is not as was heretofore presumed a Saccharomyces, but a peculiar, non-sporulating budding fungus, which has not been isolated and described up to the present. It belongs to the group Torula. As, however, in contra-distinction to the other hitherto known species of this group, it is one of great practical importance, I have thought proper to propose a particular name for it, and with regard to its close connection with British brewing industry I have called it Brettanomyces.

As to the manner of isolating and propagating this new micro-organism and concerning its specific properties, I shall not anticipate

a complete scientific investigation on the subject which is being made in the Carlsberg laboratory by Professor Hansen's well-known assistant Mr. H. Schiönning, and which will be published before long. In this paper I shall only make some statements relating to the industrial use of the new fungus.

Brettanomyces produces a slow fermentation in wort or in beer fermented with ordinary brewer's yeast. The carbonic acid developed by its action is retained very firmly, and when set free by agitation, forms a copius and lasting foam. In the course of the fermentation rather a considerable amount of acid is formed, accompanied by ethereal substances, the taste and flavour of which cannot fail to attract the attention of any connoisseur by their striking resemblance to the flavour of stored English beers.

Like Saccharomyces, Brettanomyces appears in a large number of varieties. In English breweries as well as anywhere else the primary fermentation is carried on by Saccharomyces, whereas the secondary fermentation of the typical English beers, as being due to Brettanomyces, essentially differs from those secondary fermentations known on the Continent. In other words, *the action of Brettanomyces is absolutely necessary to bring English stock beers into proper cask and bottle condition, and to impart to them that peculiar and remarkably fine flavour which in a great measure determines their value.*

It is very easy to furnish a direct and striking evidence of its being so. If we add to pasteurised beer a slight portion of a Brettanomyces culture in wort (say a few drops to a bottle of the beer), and if we then leave the beer to stand in well-corked bottles at a temperature of 75—85° F. during 10—14 days, a slight deposit will be observable, and at the same time the beer will assume an *unmistakable English character*, both in regard to its content of carbonic acid gas and to its taste and flavour.

Under the influence of Brettanomyces, however, the great majority of bottom-fermentation beers assume a somewhat impure taste, and the same is true of most of the Continental top-fermentation beers. More particularly, attenuation must have proceeded to a certain limit during the primary fermentation if Brettanomyces is to yield a pure flavoured product. The fact is that when Brettanomyces is added to beer fermented with the yeast of very feeble attenuative power in common use in Danish top-fermentation breweries, the beer thus infected possesses a

peculiar impure and sweet mawkish taste, whilst at the same time an "English" character becomes apparent especially to the nose, and a very similar impure taste is the result if the primary fermentation has been partially carried out by English top yeast, but interrupted at an early stage by filtering and pasteurising, and if then the Brettanomyces is added.

On the other hand, by using a suitable English single-cell yeast and adding a pure culture of Brettanomyces after the termination of the primary fermentation, a remarkably good result will be obtained. Thus, for instance, I have fermented with English single-cell top yeast a Danish stout wort prepared by decoction mashing, and after addition of Brettanomyces and 2 or 3 weeks' storage, I bottled the beer which was then left to stand for a fortnight at a temperature of 77° F. According to the verdict of several connoisseurs, the product thus obtained was in no way inferior to the best sorts of London stout, whilst parallel bottles which did not contain Brettanomyces entirely lacked the English character.

Hence it is evident that the *secondary fermentation effected by Brettanomyces is indispensable for the production of the real type of English beers.*

This fact being established it gives no difficulty to account for the contradictory results of the attempts hitherto made at using single-cell yeast in English breweries.

Brettanomyces exists as a general infection in these breweries. I suppose it invariably forms a minor constituent of English pitching yeast, and it may probably be found in all such places in the pipes, utensils, and vessels of the breweries, where such infections may creep in and get fixed. If now, in the experiments on single-cell yeast, sufficiently effective measures have been taken to avoid the disturbing effects resulting from accidental infections, then the Brettanomyces is kept off, and consequently the production of stock beers of the quality wished for is rendered out of the question. As to the running beers, however, which do not undergo any secondary fermentation, they may very well turn out satisfactory. If, on the other hand, the experiments on single-cell yeast are made while using the plant of the brewery in its usual state without subjecting it to any particularly radical cleansing, then a sufficient quantity of Brettanomyces has been present, and consequently the experiments have been able to yield satisfactory results, and, in fact, they have sometimes proved to do so. This has been

erroneously interpreted, as if in practice Saccharomyces were capable of carrying through the whole fermentation.

A state of affairs in which the success of a process is dependent on fortuitous infection, which is beyond control, is obviously unsatisfactory. In fact, in those places in a brewery where the Brettanomyces necessary for the production of typical stock beers lives, there may, of course, at the same time exist a lot of pernicious disease germs, and the ways by which, depending upon an experience handed down from generation to generation, the brewer has learned to introduce, blindly as it were, the indispensable Brettanomyces in his manufacture offer the inevitable drawback of opening an easy access to all sorts of noxious organisms. By way of example, I would only mention the current procedure of dissolving isinglass in returned beers, which by a long exposure to the action of air have become converted as far as possible into acid, which solution is then added to sound beers as finings. If subjected to biological analysis, the solution referred to will doubtless be found to contain a good quantity of Brettanomyces, and in so far it will have a profitable effect and contribute towards bringing the beer into condition, but it is likely to shelter at the same time numerous disease germs.

If once the fact is established that the secondary fermentation of typical English beers cannot possibly be effected by Saccharomyces alone, but that Brettanomyces is quite indispensable for its being carried duly through, you will clearly see how important it is that, in regard to this part of his manufacture, the brewer should no longer trust the chapter of accidents, but walk upon sure ground in making use of methodically selected and prepared cultures of Brettanomyces. By this means alone considerable advantage can already be obtained, but full certainty and constancy is only attainable if the pure yeast system is brought to bear upon both the primary and upon the secondary fermentation.

In what manner then are the principles laid down by Hansen to be applied to the English art of brewing?

In the first place the pitching yeast must be selected single-cell yeast suited to local requirements. I need not here trench on the question of the advantages of single-cell yeast, this question having been so often discussed before the Institute of Brewing, that from a general point of view, there may hardly be anything to add to it. I

only wish to say that, as the secondary fermentation peculiar to English beers is to be effected by other means, there is no reason whatever why the use of single-cell yeast should not yield the very same advantages to the English brewers as those which Continental brewers have attained several years ago in using it.

It is most probable that a suitable single-cell yeast will prove fully to suffice for the fermentations of all sorts of running beers. These beers do not get sufficient time to go through a secondary fermentation, and consequently Brettanomyces will hardly be able to influence upon them to an appreciable extent. But this holds good only for such beers which are drunk very soon after their being racked off. If the beers are kept for a longer period than a few days, either on the premises of the brewery or at the customers, their content of Brettanomyces may have some influence upon their character. This is a question, however, which can only be definitely settled by experiments on an industrial scale.

At any rate, with the various sorts of stock beers the case is quite different. In the case of these beers the action of Brettanomyces is a necessary condition for the production of a beverage possessing the properties wished for, and it is therefore necessary, after the yeast has done its work and has been separated off, to add a pure culture of Brettanomyces. This will give no trouble in practice, because the quantities required are very small. A pure culture of Brettanomyces may conveniently be propagated in wort of 1055 specific gravity at a temperature of 75—80° F. Brettanomyces grows like a bottom-fermentation yeast at the bottom of the vessels, and at the end of about a week it will have formed a deposit which in each pint of wort will be sufficient for at least five barrels of beer. A general rule cannot be given for all cases, but the quantity of Brettanomyces to be added must be regulated by local circumstances, more especially by the time the beer has to be stored and by the temperature of the storing room.

As was said before, there are different varieties of Brettanomyces which attack the fermentable substances with unequal energies and which settle more or less readily and completely in the fermented liquid. Hence it follows that here also a pure culture must be made, starting from a single cell. This is the more necessary because there also exist noxious forms of Brettanomyces, and among them several varieties which form films on the surface of the beer in the bottles.

By means of single-cell cultures of Brettanomyces the secondary fermentation can be effected with the same certainty as the primary fermentation. Most of the difficulties connected with conditioning the beer can doubtless be avoided, as well as the work done for this purpose, such as rolling the casks. But still more important it is that the brewer is enabled to regulate the secondary fermentation and to materially shorten the storage.

Thus, the peculiar character of English stock beers renders it necessary to make two separate pure-cultivations, whereas one pure-cultivation suits the purpose of Continental beers. It is true that the mode of working is rendered a little more complex, but in return insecurity is removed.

It must be admitted that the judgments passed against the applicability of Hansen's pure yeast system to English beer brewing by eminent English brewing chemists were essentially sound, in so far as trials were made to use single-cell yeast, by itself, but through our knowledge of the Brettanomyces and its action such facts have been brought to light as compel a revision of those judgments. I have no manner of doubt that a different judgment will be pronounced as regards the mode of working, the outlines of which I have just described and which is perfectly in accordance with the principles laid down by Hansen. The primary fermentation is effected by putting a selected single-cell yeast to a liquid sterilized by boiling. This is an ideal case where the single race used is allowed to operate without any competition on the part of other ferments. In the secondary fermentation this is, no doubt, not the case to the same degree, in so far as the beer always contains rather a considerable quantity of yeast from the primary fermentation, however bright it may appear to the naked eye. But this yeast has virtually performed the whole amount of work which it is capable of doing in the liquid concerned and, consequently, it will not offer any appreciable competition to the Brettanomyces culture. Thus, the latter is enabled to produce, without let or hindrance, the effect which is characteristic of it and on account of which it was selected. But this is the very principle of the pure culture system: to preclude, as far as practically possible, the co-operation of, or competition between, different organisms and to make exclusive use of the particular species which best serves the purpose in view.

Discussion.

The Chairman said he felt sure that Mr. Claussen would feel that the best way of rewarding him for the trouble he had taken in preparing his paper, would be to discuss it in such a manner as might best assist him in the continuation of the research he had undertaken. There were many present who were well qualified to give an expression of opinion upon this most interesting question. Letters of regret at not being able to attend had been received from several gentlemen, including Dr. Horace T. Brown and Mr. Walter A. Riley. It appeared to him that this paper might be divided into two phases, the one purely scientific, in which they welcomed the acquisition of another form or variety of ferment which it was predicted would be of use in the brewing industry. Knowing that Mr. Claussen had worked for so long in the laboratory of Professor Hansen, it would be at once agreed that the work he had brought forward was sound and valuable. That it was full of interest, went without saying, for to think of this stranger lurking in their midst for these hundreds of years and not to have known that it had this power of imparting its peculiar flavour to the secondary fermentation went far to prove the necessity of continuous research upon the subject. In the second phase the paper seemed to him to be purely industrial and commercial, and the question was, could they in the future avail themselves of the investigation which Mr. Claussen had submitted. If he would kindly throw a little further light on one or two points which appeared to require elucidation he would render the task of judging as to the suitability of his method a little easier. In the first place he referred to the action of this variety of Torula as applicable to black beers, and he noticed that his experiments were made upon black beer worts and not upon ale worts. Of course, what was applicable to an after fermentation of black beers was conceivably applicable to the fermentation of ale, but one would like to know whether these ferments carried, as some of this variety did, a haze—whether, in other words, the action of this variety of Torula would affect the brightness of the beer, and if so, would it do so permanently. Again, he had referred to sediment as being deposited as fermentation progressed, and, of course, one would have to determine the extent and character of that sediment, whether, for instance, it would affect bottled beer by giving a greater

sediment than they had to cope with already, and whether it would by itself constitute the haze to which he had referred as being possible. Then one would like to know the gravity of the wort on which he had operated, and whether this after-fermentation could be produced with light gravity ales which required conditioning before they were sent into consumption, or whether it only applied to what *used* to be known as "stock ale"; in other words, they would like to know what Mr. Claussen really regarded as stock ale and what were the limits of functioning of this ferment in respect of the other ales which he might not include in that category. It would also be interesting if Mr. Claussen could give some account of the action which this organism had upon the carbohydrates in effecting the after-fermentation, whether any selective or preferential action. Did it, for instance, break down some of the carbohydrate combinations which the ordinary *Saccharomyces cerivisea* would not readily break down, and if so, what was approximately the time limit of its action? It seemed to him of importance to know how long this organism must be given to effect its influence, as if it was going to be a question of months or a year, as might have been the case with old stock ales, if it were going to be slowly progressive all that time, then they might have to judge as to its practicability. He would also ask Mr. Claussen if he considered that this Brettanomyces was solely responsible for really satisfactory secondary fermentation. He knew there were other ferments which would bring about a secondary fermentation, and did he consider that as regarded a true satisfactory after-fermentation this new organism could alone account for the result which they desired to achieve? He hoped many members would join in the discussion, and that there might be a useful addition to the stock of knowledge as the result of that evening's work.

Mr. C. H. Babington (President of the Institute) said he was glad to have the opportunity of adding his quota of gratitude to Mr. Claussen for coming so far and giving them so interesting a paper. If there were nothing else in it, it would be easy from the scientific point of view to observe his acumen from two sentences in which he remarked on the attitude of English brewers to scientific questions—the attitude which had hitherto prevailed being that of waiting to see what was going to turn up; and in another sentence where he pointed out how the English brewer was too apt to trust to the chapter of accidents.

He hoped those days were past, and that English brewers were waking up to the importance of science and were less inclined to trust to the chapter of accidents. Speaking as a brewer who had to provide cellarage for stock beers and to meet the requirements and idiosyncrasies of his customers, he could quite realise the enormous importance which attached to the question now raised, and if the research of Mr. Claussen would enable them to decide when the stock beers would go through a secondary fermentation, so that they could suit both their cellarage and their customers, he would be conferring enormous practical benefit on the English brewers of such beer. He was convinced from the paper he had read that he had thrown light from which they would all benefit upon this dark and delicate question round which they and the biologists had been so long groping.

Dr. Moritz said he was afraid he could not say very much on the matters which Mr. Claussen had raised, and it seemed to him that beyond asking him a few questions it was not very easy for him to say much, because everything the author had put before them was entirely new, and no general opinion as to whether it was right or wrong would be worth very much without systematic practical trials. It really lay in the hands of the brewers to put this matter to the only test which was worth anything at all, the practical test. He should like to repeat and to emphasise the importance of the questions which the Chairman had put when he asked for a definition of what was in the author's mind in mentioning the term "stock" as applied to beer. That class of beer became every day less important, because the tendency of modern brewing was against the production of stock beer, and the substitution of what possibly Mr. Claussen might call a running beer, viz.; a beer kept anywhere between 4 and 12 days before it was sent out of the brewer's cellar. A point he should like further information upon was this: supposing that this Brettanomyces was really the essential producer of the conditioning of stock beer, where did that particular organism get into English beer. Under ordinary conditions English beers underwent the secondary fermentation satisfactorily; he did not say there were no abnormalities, unfortunately there were; but the great bulk of these beers, say 98 per cent., did get into a good after-fermentation, and if that were so, and if, furthermore, the Brettanomyces were the cause of that secondary fermentation, where did they get into the beer? Did they enter with the pitching yeast? Did they come off the fermentation when the yeast was removed from the fermentation for pitching purposes, or was it entirely an accidental introduction from dirty plant or anything of that sort? He was bound to say he could not quite accept the second proposition if Mr. Claussen suggested it; because at the present day there was a great number of breweries which were worked under very clean conditions, and it was difficult to conceive any such considerable and regular amount of such organisms getting into the beer, as would account for the very considerable and regular secondary fermentation produced. If, on the other hand, Mr. Claussen considered that it was not an accidental infection, but a normal constituent of a pitching yeast, then the question arose whether they were going to do very much better with a pure cultivation of this than they were now doing. Of course he could quite anticipate Mr. Claussen's answer, namely, that there were at present occasional abnormalities, which he, and every brewer must admit. No doubt the intention was, that by the introduction of a pure cultivation of this particular organism and adding it at the right time, and substituting for the pitching mixture a pure Saccharomyces, they would be able to get over all these variations. It was to be hoped they would, but he should like a little more information upon that particular point as to where, under ordinary practical conditions, this Brettanomyces got into the English beer. In any case, they owed very much to the school with which Mr. Claussen was connected for pursuing these investigations upon the one subject on which English brewers were not quite certain of their ground. They were pretty certain with regard to the preparation of the wort, and to the exclusion of bacteria, but on this question of yeast there was still a good bit to learn.

Mr. A. C. Chapman said, like Dr. Moritz, he felt it was difficult to say much on the matter of the paper. Mr. Claussen had given them something quite new, which would have to be put to the test of practical experience and of many experiments on a large scale in breweries and by the results of these tests it would stand or fall. The author was very much to be thanked in the first place for having given them a matter which appeared to be of great scientific interest, and one which might turn out to be of very considerable practical importance. In the second place Mr. Claussen ought to be cordially thanked because he had done a good deal to do away with what had been for some time a state of deadlock. The Copenhagen authorities had stoutly

maintained that single-cell yeast was capable of carrying through complete fermentation of English beer, primary and secondary. Many very capable and very careful technologists in this country had tried the method with all possible skill and care and they had said, "We have been unable to do so," and there, until that evening, the matter had rested. On the one side they had a distinct statement that the thing was possible, and on the other side they had a number of statements that it had not been found so in this country, and Mr. Claussen was much to be congratulated on having at last relieved this state of *impasse*. There were one or two questions he should like to ask, because, after all, no one could add anything to the discussion except what might be elicited from the author by putting questions to him. In the first place he was struck by the remark that there was no positive proof in support of the view that the secondary fermentation was brought about by true saccharomycetes. He was not at all prepared to say there was, but everyone in that room who had made beer-forcing experiments must have noticed one fact which would on any other grounds be exceedingly difficult to explain. In the case of beers which conditioned well on the forcing tray, and which conditioned well in cask, there was always, or nearly always, a production of one of a few forms—the well-known Pastorianus or Ellipsoideus forms. Usually when these yeasts were absent in the forcing flask there was a deficiency in the cask process of conditioning, even when there was plenty of available carbohydrate matter. He did not say that amounted to proof by any means, but these organisms had always been considered to be the cause of the after fermentation and the evidence seemed very strong indeed. The only other explanation, of course, would be that when these forms were present the Brettanomyces was present also, and when they were absent the Brettanomyces, too, was absent, and he ventured to think that negative results in this case would be more valuable than positive. If it could be shown by a large number of experiments that whenever conditioning was unsatisfactory the Brettanomyces was absent, it would be to his mind far more convincing than to prove that whenever conditioning took place that organism was present. He was speaking now not of beers fermented with single-cell yeast in conjunction with Brettanomyces, but beers such as were produced in this country with the ordinary pitching mixture. There was one other point which it seemed to him was frequently

overlooked in connection with this question of conditioning. In Continental low fermentation beers the conditioning differed entirely from the conditioning of English beers, inasmuch as it might be looked upon in the light of a retarded primary fermentation. All the lager beers were stored at low temperatures, and a slow fermentation went on over a considerable period of time, whereas in this country the fermentation went on rapidly to a point and then stopped; after which it commenced slowly and increased in vigour until the beer was carried into consumption. There was a marked difference in the two processes, and that possibly accounted largely for the difficulties they had experienced. He could well understand that a single-cell yeast might suffice to carry on the slow fermentation which took place in Continental bottom beers, but it was quite another thing to set up a rapid fermentation, which would take 5 or 6 days, and then, in the course of a few days, to expect it to start again. He presumed from what Mr. Claussen had told them that there were different varieties of this Brettanomyces, and that these different varieties were possessed of the property of conferring upon the products of their fermentation different flavours and characters. If not it would be exceedingly difficult to understand how it came about that two English beers which, at the end of the primary fermentation were very much alike, should after a short period of conditioning assume totally different flavours, if the conditioning was due solely to one particular organism. That was a point on which perhaps Mr. Claussen would give a little furthur information if he could. He must add his own personal thanks to him for coming to read the paper.

Mr. W. R. WILSON said he had been very much interested in this paper, and he should like to have a sample of the Brettanomyces. He was working with Hansen's pure yeast, and it would be interesting to have some of this, and see what the effect of it would be on a manufacturing scale, in conjunction with the pure yeast, on English beers. They were perfectly satisfied with the Hansen system as it was, and he thought it would be a pity, unless it were found absolutely necessary, that it should be complicated by using a secondary culture; but, of course, a good deal depended on what were considered stock beers. At the present time, with the exception of a very minute percentage of what were known as old ales, there was practically hardly any stock beer in existence at all in a brewery. The whole of the beers, if not actually

running beers, which were sent out a week after they were racked, were, at all events, primed, so that, even supposing a single-cell yeast was not capable of carrying on a secondary fermentation, you might say almost invariably now that they had priming, and this would enable the yeast to produce the necessary fermentation. They were having pale ales up to about 6 weeks old, but he did not know whether that would be considered stock beer or not, but, up to that time, there did not seem to be the slightest difficulty in getting primary yeast to carry it through. Mr. Claussen would probably say that it was not the primary yeast that was carrying it through. That was a very difficult point to prove. All he could say was, the beer retained the character which the primary yeast gave it, and the ordinary microscopical examination did not exhibit secondary yeast forms. He begged to thank the author very much for his paper.

Dr. L. T. Thorne said he should like to add his thanks to those which had been already expressed to Mr. Claussen for coming over. When he received from Professor Hansen the letter offering this paper and giving the outline of the valuable work being done by Mr. Claussen, he felt it was a paper which they should be only too glad to obtain for the Institute; and as the programme for the session was full at the time, he thought, and the Chairman agreed with him, it was certainly a case where a special meeting would be most valuable, and what they had heard that evening had entirely justified that point of view. It enabled them to obtain for publication in the Journal for the first time particulars of this very valuable piece of work. He had had the advantage of having the paper in his hands for a day or two, so that he had had a better opportunity than others to digest the research, and it seemed to him from the evidence of the paper itself that it had been carried on in a very careful and scientific way, and that when the author made the statements about the action of these Brettanomyces he had very strong grounds indeed to go upon. The question had been raised whether the secondary fermentation that took place under ordinary conditions in the English brewery was always due to the Brettanomyces. It was possible, and he thought very probable, that in some cases, as Mr. Wilson had said, they were not, but that the apparent secondary fermentations were really not true secondary fermentations but were merely the carrying on of the primary fermentations by means of the added priming. This point

was specially dealt with in the paper—the production of a secondary fermentation after the primary had ceased; and this secondary fermentation brought out the characteristic differences in flavouring which they found in English beers as distinguished from Continental beers. That was the point Mr. Claussen laid special stress on, and he thought the question Mr. Chapman had raised, that in many cases two beers of very similar character after primary fermentation had ceased, after the secondary fermentation differed very much, was rather a strong piece of evidence in favour of Mr. Claussen's work, because he referred to the Brettanomyces as a class having several species, and as the different species of that class have different action in the same way as different species of Saccharomyces have different action, they produced a different flavour. Where they had the Brettanomyces introduced accidentally, as they were in the present system, one or other form might happen to preponderate, and the action of that particular form would have a preponderating influence in the flavour of the beer after it was conditioned. The question of stock beers had been raised, but it seemed to him Mr. Claussen used the term stock beers in rather a wider sense than the technical one in which it was used here, and that the ordinary bottled beers, for instance, would come under his definition of stock ales. He thought it was particularly in the bottled ales that this process, if the author's view proved as correct as it seemed to be, would have a very great influence and importance. You might be able, for instance, to Pasteurise the ale intended for bottling, then to seed with pure Brettanomyces, and in that way obtain a beer that would come gradually into true condition without danger of deterioration by bacterial infection as at present. The time Mr. Claussen had referred to was only a question of from 10—15 days, or perhaps a little longer. The Chairman had spoken about the reference being to black beers, but he did not think it was intended to apply only to black beers. Mr. Claussen mentioned one case in which he had examined a special brew of Danish black beer, but he thought he had also included a good deal of work on ordinary ales.

Mr. Julian L. Baker said he was sure they had all listened with the greatest interest to this paper, for it threw an entirely new light on the difficulties which had hitherto surrounded the application of pure yeast cultures in English breweries. Furthermore, they would all

look forward to seeing the results of the biological examination of these organisms. They could not really criticise the statements that had been made that evening, but he should very much like to ask one or two questions with regard to this particular organism. Did this Brettanomyces behave like the other members of the Torulaciæ described by Hansen? It would also be interesting if Mr. Claussen could tell them something about the morphological appearance of the new Torula. He would like to know if it produced much alcohol, or did it, like other Torulaciæ, produce only a small quantity? With regard to the application of pure yeast, there seemed to be a general concensus of opinion that the matter was at a deadlock. Occasionally, satisfactory experiments had been carried out, and, so far as the secondary fermentation was concerned, the success was said to be due to infection with other organisms. Of course, with running beers, that difficulty did not come in, and his own experience had been confined simply to these.

Mr. R. GREY said he had not much to say at present because, like others, he was waiting probably to get a full report when this special research was completed in Professor Hansen's laboratory. He was very glad that this new departure had been taken at the New Carlsberg Brewery, because when working with Mr. Alfred Jörgensen a few years ago he was much interested in Van Laer's paper (*Trans. Inst. Brewing*, 1894, **7**, 5), and advocated research on equivalent lines. So far that matter seemed to have remained in abeyance until now, and he was very glad to see Mr. Claussen come forward. One point he should like to draw attention to which Mr. Chapman had referred to, namely, forcing-tray work, and he would ask whether experiments with forcing trays were carried out in Copenhagen, and if so, were such experiments at a temperature of 15° C. and also at 25° C. carried on for the purpose of comparison; also if those experiments had been made, whether it had been found that primary yeasts, pure culture yeasts, worked at the lower temperature and maintained their vigour in opposition to other organisms, and whether they failed at the higher temperature. He had tried experiments himself at these two temperatures, 15° and 25°, with pure cultures of primary yeasts and wild yeasts, and found that at the lower temperature of 15°, the culture yeast was vigorous and held its own, but at the higher temperature of 25°, which was the common temperature in use for forcing-tray work, it became

more or less exhausted and even died. If those yeasts had been put into flasks and put on the forcing tray with wild yeasts, it would naturally account for the fact of the culture yeast being found to be exhausted and dead, while the wild yeast was still alive. With regard to the actual organism with which Mr. Claussen had carried on his research, the Brettanomyces, he should like to know whether the ordinary tests for sporulation were made, and whether sporulation resulted.

Mr. JEFFERS said he should like to ask if Mr. Claussen actually isolated the Brettanomyces from the English beers themselves, or did he get it from a chance cultivation in his own laboratory, and if so had he attempted to identify the different varieties of these Brettanomyces, say, as developed in the London, Burton, and Dublin yeast, and were they all distinct varieties or were they identical?

Dr. MORITZ said there was one further question he should like to ask, which was a rather important matter from the point of view of those who felt disposed to try the action of this ferment. How did Mr. Claussen suggest that it should be added? Would it be possible to get anything like a trustworthy result by taking this cultivation and adding it to a beer which had gone through its ordinary fermentation, or was it essential before getting any trustworthy result to take the fermentation down with the pure cultivation yeast, and in which case would this new cultivation be added to the cask at the time of racking or altogether with the pure cultivation at the time of pitching. That was important in this way, that he should be glad to try this ferment if he could add it to the cask, but he might hesitate to add it straight off if he had to put in something new from the very beginning. No brewer would mind sacrificing a few barrels of beer in the interests of science, but when it came to dealing with the whole gyle, he would think twice about it.

Mr. BUTLER asked if Mr. Claussen had experimented at all with different types of wort. For example: had he compared Burton, which was probably the only beer one could look upon really as stock beer, with London or with Birmingham beer, because the worts were essentially different, and the construction of the plant alone was almost sufficient to account for the very pronounced after-fermentation or the want of it. In Burton, when the beer was racked the conditions of the plant were such that there was a good deal of loss of carbonic

acid gas, whereas the same yeast and the same wort produced on other brewery lines would rack with a certain amount of gas which would give in a way a fair draught condition. After all, stock beers were not highly conditioned beers. Burton stock beer was not a beer in cask that carried a lot of gas. His experience was that in Birmingham, for instance, beers in certain breweries would develop the same amount of condition after many years as would be acquired in Burton in almost a few weeks.

Mr. E. B. COLLIER said that as a practical man he should like to try putting the pure yeast into the fermentation, and after that another pure type into the cask; but could you make sure that in the cask you were only going to have one yeast. In pale ale finished with dry hops you might have such a thing as a continuous disturbance by the Brettanomyces and subsequently by the *Saccharomyces pastorianus* which might be fatal to the resulting beer. Of course, if you sterilised it and had the beer perfectly free from primary yeast also it might be all right, but in practice it struck him it would be very difficult to apply such a method to stock pale ales. He could quite imagine that if one could be assured of carrying through the pure yeast system, and having no infection in any way it would be an excellent thing, but he should like to be sure whether the Brettanomyces was similar to the Pastorianus, or was it a different type altogether.

Mr. M. J. CANNON asked if Mr. Claussen could give any idea of the function of those organisms found in cask fermentation, which they, in their ignorance, had been terming secondary yeasts under the generic name of Pastorianus or Ellipsoideus. It had been suggested that in all probability in looking at the growth of these yeasts under the microscope either from the cask or forcing flasks they were simply looking at this new species of Brettanomyces, but he did not think that was probable, because he had himself made a number of cultivations of the asco-spore formation, and very seldom failed to obtain the typical spore formation from yeasts obtained from cask or other deposit. The whole paper was supplementary to the old pure yeast system of brewing. He was rather under the impression from the papers contributed to that Institute previously by Mr. Jörgensen and by those who had opposed his views that the pure culture system, as applied to English brewing, was by no means free from difficulty, and those who remembered the last paper contributed by Messrs.

Jörgensen and Riley would remember the difficulties they met with in obtaining from the brewing yeast a yeast which would carry on a fermentation satisfactorily. They behaved most erratically, and he believed the yeast had a great tendency to degenerate. He thought most English brewers would require that the original pure yeast system should be perfected before this new discovery of the Brettanomyces could be properly applied to English brewing.

Mr. GREY said there was one further question he should like to ask: whether the author had received any beer for the purpose of his experiments from Carnegie's brewery at Gottenburg? It was a stout and porter brewery, where very good stout was made on English lines, and it occurred to him that the proximity of Gottenburg to Copenhagen offered facilities if the Carlsberg laboratory contemplated obtaining a stock beer infusion wort for their researches on cask fermentation of English type stock beers.

Dr. P. SCHIDROWITZ asked if any experiments had been made to ascertain whether the special products of the secondary fermentation were formed to a greater extent by this particular species than by ordinary yeast. They knew that in very old bottled beers they got extraordinarily high amounts of compound ethers and higher alcohols, and if this Brettanomyces was really responsible for the specific secondary fermentation, it was conceivable that by laboratory experiments they might be able to exaggerate this effect so as to get these characteristic products in very large proportions as compared with the amounts formed by ordinary yeasts.

Mr. CLAUSSEN, in reply, said he had been working on different kinds of worts, and if he had only given, as an example, the results obtained from stout wort, it was because that had given the best results. In Denmark the stout wort was very similar to the English, but the ale worts were quite different, and he could not therefore obtain very good results from experiments upon them. He had, from necessity, worked only on the laboratory scale, as there was no English brewery at his disposal in Copenhagen, and he could not conduct experiments at the New Carlsberg Brewery on the industrial scale. But he had obtained definite results of which he was able to produce full evidence. As soon as one began to work on the practical scale, one always got infection and could never be sure whether the results were due partly to infection or solely to the added ferments.

Dr. Moritz had remarked that many English breweries were now worked under aseptic conditions, but in practice that was quite impossible. He had been working in a brewery where the cleanliness was very perfect, and yet in counting the germs in the wort when it came into the fermenting tun, he had sometimes found rather large numbers. A distinguished German scientist casually told him he had had the same experience in the German breweries, and that he estimated the average content of germs in their wort at about 10,000,000 per litre. It was quite impossible, therefore, to work without infection, and brewers could not be blamed for its occurrence. One gentleman spoke about wild yeasts in the barrel, and he would say that on the Continent they had wild yeasts in every bottle of beer if not Pasteurised. The action of most of them would be quite harmless, though of course, some would be noxious. But by means of the pure yeast system—by introducing week after week a new portion of absolutely pure yeast into the manufacture, you were able to check the wild yeasts, and keep them within such limits that they were practically harmless. Professor Hansen had shown that the pitching yeast might contain wild yeasts up to 1 in 40 without injuring the product.

The CHAIRMAN asked if Mr. Claussen could say whether the Brettanomyces would exert its action in the presence of these wild yeasts.

Mr. CLAUSSEN said he felt quite sure that it could. As to secondary fermentation, there were two things which went under that name. When he spoke about secondary fermentation in connection with Brettanomyces, he was not thinking about the production of alcohol and carbonic acid alone. There was no doubt that a suitable single-cell yeast would be able to produce a slow secondary fermentation, so long as there was fermentable matter present, but Brettanomyces effected a secondary fermentation of quite another sort. During its progress there was the formation of a considerable amount of acid, which, with the alcohol present, formed the ethereal substances which were essential to English beers, and gave them their peculiar taste and flavour. The secondary fermentation of English beers could not be compared with Continental secondary fermentations. He must admit that he had not quite made up his mind as to how long a beer should be kept in store to be a real stock beer. What he meant to convey was that Brettanomyces would have no effect upon running beers, such, for instance, as were drunk within a week after racking. If they were kept longer it might be possible that it might have some influence. He could not say where the limit was to be drawn, that could only be decided by experience. But he knew that for beers which were kept in store for 4, 5, or 6 weeks, Brettanomyces could be used with great advantage. It must be added after the termination of the primary fermentation in the cask. It would not be a rational method to add it to the pitching yeast. It might be done, but you had to use that yeast for the next brew, and after one fermentation you would no longer know what mixture you had. As he had stated, he believed that Brettanomyces always formed a minor constituent of English pitching yeast, but it must be only a minor constituent, as it worked very slowly and settled to the bottom. It did not behave like other Torulæ, and he had not found any other Torula which formed such a large quantity of acid. Some varieties produced a considerable quantity of alcohol, *e.g.*, 4 or 4½ per cent., but it took a long time. It was a very slow fermentation.

In reply to a further question from Mr. Baker, he said he had not identified the acid produced, but he should say it was not acetic. He had not tried it on the forcing tray, which was not used in Copenhagen. The Chairman had remarked that it would be interesting to try this practically, and it could easily be done on a small scale. Take, for instance, some running beers, say 3 days' old, which would not have the character of stock beers. If you Pasteurised these beers in bottle, and then added Brettanomyces, and then allowed the bottles to stand for a fortnight at a suitable temperature, you would have the proper amount of carbonic acid gas, and the taste and flavour peculiar to stock beers. You had killed all the other organisms, and therefore the result must be due to the Brettanomyces. As he had not experimented with genuine English ale wort, he could not say with certainty if it would or not give, at some points of its action, a slight haze, but if they would take it for granted that the action of this organism was necessary for the production of stock ales, and as stock ales were produced of excellent brightness, they must be able to find the species of Brettanomyces which would give perfectly brilliant stock ale. He had not determined which carbohydrates it fermented, but it was probable that it was able to ferment carbohydrates of the moderate and higher types. There were, however, some dextrines that it did not break down at all.

The CHAIRMAN having proposed a hearty vote of thanks to Mr. Claussen, which was carried unanimously,

Mr. CLAUSSEN, in responding, said he should like to add a word or two with regard to the position of Danish scientists with regard to the applicability of Hansen's system to English breweries, and especially to Professor Hansen's own standpoint. As Hansen had never carried out experiments in the practice of English brewing, his standpoint had been essentially founded upon the results reported by Jörgensen and in the simple form of single-cell yeast. As his system had been adopted with great success in all other countries, he naturally was most inclined to listen to the statements made by Mr. Jörgensen and others as to its successful working in English breweries in the same simple form; but he had always had an open mind with regard to the difficulties which obtained in England, and had always recommended that further experiments should be made to clear up those difficulties. He had, therefore, thoroughly sympathised with these experiments, and had introduced him (Mr. Claussen) to the Institute of Brewing, feeling that he could not have more competent critics of his work than would be found amongst its members.

Dr. MORITZ then proposed a vote of thanks to the Chairman, which was carried unanimously.

Mr. WALTER A. RILEY sent the following contribution to the discussion :—

"I have found that it is absolutely impossible to get a stock ale or in fact any beer which is of an original gravity higher than 20 lbs. into proper condition when fermented with a good selected type of single-cell yeast.

I have made a series of experiments using the same type of single-cell yeast and fermenting the same wort, and have then tested the conditioning power of the beers brewed at different gravities, and found that a beer when brewed at 16 lbs. comes into excellent condition at the end of 2 weeks from racking, a beer of 25 lbs. original gravity comes into condition very slowly and needs a slight addition of priming to help it along, and when it does come into condition makes an excellent beer, but a beer of 30 lbs. will not come into condition, and will only get into condition with difficulty by adding sugar. I have, therefore, found that single-cell yeast is only suitable

for low gravity beers, and, in fact, in my humble opinion the cure for some of the evils of 'Bottle Beer' is to employ a properly selected single-cell yeast, as I have done in our own case.

I am afraid that I cannot at present give any definite reason why this should be the case with worts taken from the same brew, that a length made up to 16 lbs., and a length made up to 30 lbs., and then fermented with the same single-cell yeast, the lighter beer will come into excellent condition, the heavier beer having only a vinous taste and no condition. I still have a 14-lb. beer, which was brewed 2 years ago, and fermented with a selected type of single-cell yeast; that beer is to-day as sound and full of life as it was 6 weeks after having been brewed.

Therefore, as a result of many practical experiments carried out with different classes and strengths of wort, and fermented with different types of single-cell yeast, and after a good deal of thought, I have to reluctantly admit—as I had to admit to Professor Hansen—that the conclusion I have drawn from my own observations and research are as follows :—

That single-cell yeast can only be used with success for the production of light-gravity English beers, the class of beers that are suitable for light bottled beers, and *cannot* and ought not to be used for the production of high stock or high gravity beers."

With reference to the above remarks of Mr. Riley, which were unknown to me when I wrote my paper, I can only say that they are a strong support for my arguments, and therefore afford me great satisfaction. Evidently Mr. Riley's high gravity beers are of the type of stock beers, which can only be conditioned by Brettanomyces.

N. H. C.