

The Enzymes of B. Coli Communis which are Concerned in the Decomposition of Glucose and Mannitol. Part IV.—The Fermentation of Glucose in the Presence of Formic Acid.

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(Communicated by F. Gowland Hopkins, F.R.S.—Received May 3, 1920.)

In this series of communications the writer is endeavouring to show how, by varying the conditions of the experiment, it is possible to alter the proportion between the products which arise from the fermentation of glucose and allied substances, and to point out how, by a consideration of the manner in which these products group themselves, conclusions may be drawn as to the order in which such products arise during the degradation of the glucose molecule. Substances which can be shown to arise in constant proportions under varying conditions of experiment may be considered as being produced by one and the same enzyme.

In Parts II and III it was shown that the formation of lactic acid by *B. coli communis* ran a separate course to that of the other products, so that it may be regarded as being produced by a separate enzyme, but the other products of the fermentation, viz., succinic acid, acetic acid, formic acid, and alcohol, together with the gaseous products of the decomposition of formic acid, *i.e.*, carbon dioxide and hydrogen, all appeared to be grouped together and to form an alternative course for the decomposition of the glucose.

In the present communication it will be shown that this group of products must be subdivided into two groups, the one containing formic acid and its gaseous products carbon dioxide and hydrogen, and the other alcohol, succinic acid, and acetic acid.

The possibility of demonstrating the independence of formic acid on the one hand and acetic acid, alcohol, and succinic acid on the other, depends on finding a means of destroying the normal balance between these products. It is probable that this balance is conditioned by the hydrogen which arises through the decomposition of the formic acid, for this hydrogen might reduce acetaldehyde to alcohol, and in this way the formation of formic acid and alcohol would tend to keep pace with each other. However this may be, it is not difficult to upset this balance, and one method of so doing is to ferment the glucose in the presence of added formic acid in the form of calcium formate. How the added formate is able to upset the normal balance which exists between the products of this group, the writer is not prepared to say.

The present experiments were undertaken rather with the object of seeing whether the hydrogen nascent during the decomposition of formates could give rise to an increased yield of alcohol from the glucose. In one case such an increase actually occurred, but in most cases the reverse was the case. On the other hand, the results have been valuable in themselves for the reason stated above.

It must be confessed that the concentrations of calcium formate employed in these experiments appear to have been chosen somewhat at random. One of the reasons for this is the difficulty of deciding upon the use of varying concentrations of calcium formate, and at the same time keeping the concentration of salts within proper bounds. The criticism which may be raised on these grounds in regard to the order in which these experiments were carried out does not, however, in any way concern the conclusions which are drawn in the present communication. The results are considered alone in their relation to one another and not in regard to the cause, whatever it may be, which is responsible for them.

Outline of the Method Employed.

An emulsion of *B. coli communis* was prepared by the method described in Part II, and a quantity representing about 0.5 grm. of dried bacteria was added to a solution previously sterilised by heat, containing either glucose, or glucose together with calcium formate. The solution also contained potassium sulphate 0.6 per cent., and magnesium sulphate 0.1 per cent. as in Part III. Chalk was added to neutralise the acid which might arise during the fermentation.

In order to know the proportion of the products which arise from the glucose, it is necessary to subtract from the total products those which arise from the decomposition of the added formate. Accordingly it is necessary to determine at the end of each experiment how much formic acid remains, and to deduct from the gaseous products an amount of hydrogen and carbon dioxide equivalent to the formic acid which has been fermented.

In most cases the amount of formic acid present at the end of the experiment was less than at the start although, considering the large amount of gases evolved, it was surprising to find that the actual diminution in formic acid was not great. The formic acid was never exhausted, and in one case there was an actual increase in the formic acid over that present at the start.

The data for a series of experiments are given in Tables I and II.

Table I.

Data for experiments on the decomposition of glucose and mixtures of glucose and calcium formate, by *B. coli communis*.

	0.	1.	2.	3.	4.
Weight of glucose at the start	20·00	20·72	20·00	19·68	16·25
Weight of formic acid	1·320	1·325	3·312	3·750	5·244
Weight of glucose at the end	4·73	7·95	3·13	4·76	Nil
Weight of formic acid	0·4233	1·708	2·282	1·380	4·269
Weight of formic acid fermented...	—	—	1·030	2·370	0·975
Weight of glucose fermented	15·27	12·77	16·87	14·92	16·52
Weight of hydrogen produced	0·0495	0·1530	0·3234	0·2250	0·1260
Weight of hydrogen corresponding to added formate fermented	—	—	0·0453	0·1017	0·0417
Weight of hydrogen derived from the glucose fermented	0·0495	0·1530	0·2781	0·1233	0·0843
Weight of carbon dioxide evolved	5·901	7·003	9·953	8·448	4·950
Weight of carbon dioxide corre- sponding to the added formate fermented	—	—	0·986	2·266	0·933
Carbon dioxide from acids on chalk	3·495	3·080	2·160	0·726	1·243
Carbon dioxide from the glucose ...	2·497	3·922	6·807	5·460	2·770

Table II.

Products derived from glucose by the action of *B. coli communis* in the presence of calcium formate. Allowance has been made for the carbon dioxide and hydrogen which have resulted from the decomposition of the calcium formate added.

	Products from the glucose.				
	Glucose alone.	Glucose with calcium formate.			
		0.	1.	2.	3.
Hydrogen	0·050	0·153	0·278	0·123	0·084
Carbon dioxide	2·497	3·922	6·807	5·460	2·770
Formic acid	0·422	0·380	—	—	—
Acetic acid	2·952	2·400	4·212	2·149	2·505
Lactic acid	4·347	4·030	3·654	2·770	8·533
Succinic acid	3·103	1·534	0·549	1·770	1·823
Alcohol	1·686	0·640	0·244	2·560	0·466
Total products.....	15·06	13·06	15·74	14·83	16·18
Glucose fermented	15·27	12·77	16·87	14·92	16·52
Ratio CO ₂ /H ₂	2·04	1·17	1·11	2·01	1·50

In Table III the above results are calculated as percentages upon the sugar fermented.

Table III.

Products of the action of *B. coli communis* on glucose, calculated as percentages upon the glucose fermented. In the cases where the fermentation was carried out in the presence of calcium formate, allowance has been made for the hydrogen or carbon dioxide which has arisen from this source, so that the results tabulated represent products from the glucose itself.

	Products as percentages upon the glucose fermented.				
	Glucose alone.	Glucose fermented with calcium formate.			
	0.	1.	2.	3.	4.
Hydrogen	0·33	1·20	1·64	0·82	0·51
Carbon dioxide	16·37	30·71	45·41	36·60	16·77
Formic acid	2·76	2·98	—	—	—
Acetic acid	19·34	18·79	24·97	14·41	15·17
Lactic acid	28·47	31·56	21·61	18·57	51·65
Succinic acid	20·32	12·01	3·26	11·87	11·04
Alcohol	11·04	5·01	1·45	17·16	2·82
Total products.....	98·63	102·26	98·34	99·43	97·96

It will probably be admitted that these results are surprisingly accurate. Many analyses have to be carried out to obtain the proportions of all the products of such fermentations as will be seen for some of the data in Table I, and it may be fairly claimed that the present results are very satisfactory, and form a good basis for a consideration of the relations between the products concerned. It is essential to insist upon this fact, because, in the writer's opinion, the results recorded here are of fundamental significance in regard to the whole question of the manner of decomposition of the glucose molecule under the influence of bacteria. Such generalisations will, however, be reserved till Part VI of this series of communications, and in the present only the most obvious conclusions will be drawn.

It will assist in the consideration of these results to adopt the practice of Parts III and IV, and record the percentages of products in the form of curves. Such curves are obtained by plotting the percentages of the various products in columns corresponding to the individual experiments, the

columns being arranged from left to right in order of ascending values for some one product. Since lactic acid has been shown to be formed

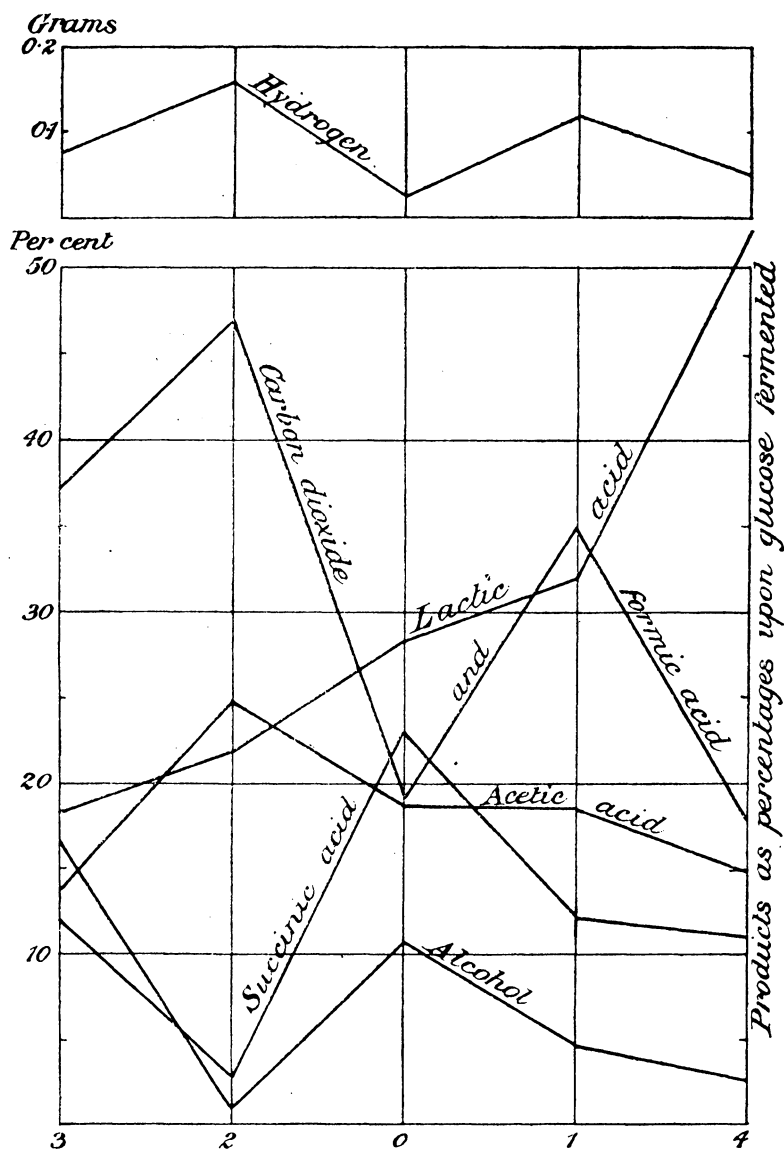


FIG. 1.—(Results arranged in order of increasing values of lactic acid.)

independently of the other products, it is convenient to arrange the columns in order of increasing values for this substance. This has been done in fig. 1.

If fig. 1 is compared with figs. 2 and 3 of Part II of this series,* it will be

* See 'Roy. Soc. Proc.,' B, vol. 87, p. 87 (1917).

seen that there is a very general resemblance between the curves, and especially in the respect that they all show the formation of lactic acid to be independent of the formation of the other products. On the other hand, there is a greater divergence of the curve for formic acid and its gaseous products from the other curves in fig. 1 of this present communication than there is in any of the previous figures given. It will also be noticed that the curve for formic acid, including carbon dioxide and hydrogen, reaches a much higher level here than it did previously.

In the upper part of fig. 1 the hydrogen curve is seen to oscillate in a manner which is complementary to the curve for alcohol below. This constitutes the first absolute proof that alcohol is formed from glucose by reduction *in vivo*.

The formation of alcohol by reduction of acetaldehyde by means of intramolecular hydrogen was first postulated by Kostycheff for alcoholic fermentation by yeast, and later by the writer for bacterial fermentation. Still later this idea was incorporated by Neuberg in his theory of alcoholic fermentation, but, up to the present, the proof of such reduction has never been given either for alcoholic fermentation by bacteria or by yeast, although Neuberg's recent researches on phytochemical reduction have lent considerable support to the view.

The proof that alcohol is in fact formed by reduction is given in fig. 1. Moreover, it will be observed that the highest point in the alcohol curve is in the column for Experiment 3. In this case almost twice as much alcohol has been produced as in the normal experiment without added formate, so that it is clear that when formic acid is added the hydrogen which is liberated by its decomposition may also act as a reducing agent and thus augment the yield of alcohol.

As was noted in the introductory remarks to this communication, only in one out of the four experiments with formate added to the glucose was there any increase in the yield of alcohol. In the other cases there is a very marked decrease. Future experiments must decide what the conditions are which enable formic acid to act as a reducing agent in the fermentation of glucose.

Next to the proof of the participation of nascent hydrogen formed by the fermentation in the production of alcohol, the most remarkable result is the demonstration of the relation of added formic acid to the production of formic acid from glucose. The effect of the formic acid added in the form of calcium formate has been to enormously increase the production of hydrogen and carbon dioxide from the glucose.

We are accustomed to think that when one of the products of a fermentation

is added at the beginning of such a fermentation the rate of production of this product should be diminished, and at the end of the experiment we expect to find a lessened accumulation of the substance in question. We should accordingly anticipate that formic acid added at the beginning of the fermentation would lessen the production of formic acid from the glucose. Now, as a matter of fact, the analysis of the solution at the end of all the experiments, except No. 1, showed that there was no excess of formic acid over that added at the beginning; but, for obvious reasons, we are not in a position to say how much of the formic acid which remained at the end of the experiment, if any, had been produced from the glucose and how much more merely represented unaltered formic acid added. The striking fact, however, is the liberation of such an increased amount of carbon dioxide and hydrogen from the glucose, for we have hitherto held the view that these gaseous products come from pre-existing formic acid. Now, in these experiments, in which glucose was fermented in the presence of calcium formate, not only the equivalent of all the formic acid which normally is formed by the decomposition of the glucose appears as carbon dioxide and hydrogen, but the total yield of these gaseous products is greatly enhanced, reaching, as will be seen in the figure in the case of Experiment 2, to nearly 50 per cent. of the glucose, a value more than double that obtained in the case of the fermentation of glucose in the absence of added formate. *

How then are we to explain the fact that formic acid which itself is decomposed by *B. coli* into carbon dioxide and hydrogen, increases the production of these same gases from glucose when the glucose and formic acid are fermented together.

An attempt will be made to answer this question in Part V of this series. It seems to the writer that the answering of the question will necessitate the giving up of the prevalent idea as to the manner in which glucose undergoes decomposition in fermentation, and that it will be best to reserve the discussion until certain other facts are pointed out.

It is possible to see several important relationships from fig. 1, but it will considerably simplify the consideration of such relationships if all the curves can be considered apart from the influence of the lactic acid; for since the course of the lactic acid production runs independently of that of the other products we shall be justified in setting aside that portion of the sugar which has been converted into lactic acid, and calculating the remaining products as percentages upon the remaining portion of the glucose. This calculation has been made and the results are tabulated in Table IV. These results are further plotted in the form of curve in fig. 2 in a manner similar to that adopted in the construction of fig. 1.

The results set out in fig. 2 are arranged from left to right in order of increasing values for carbon dioxide and formic acid which after setting aside

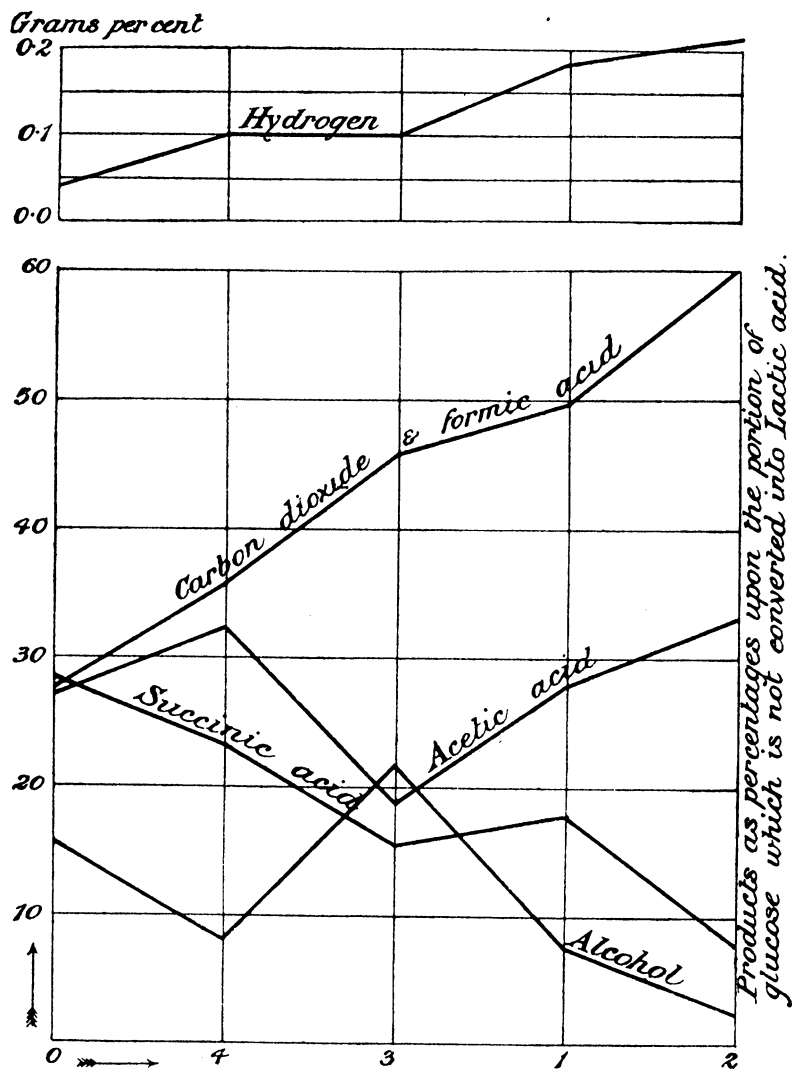


FIG. 2.—(Results arranged in order of increasing values of carbon dioxide and formic acid.)

the lactic acid is now seen to be the most variable of the remaining products from the glucose.

The immediate effect of replotting the curves after ignoring the portion of the glucose which has been converted into lactic acid is to make very clear the relationships which exist between the other products. It will be seen

Table IV.

Products of the action of *B. coli communis* on glucose fermented in the presence of formic acid. The products are calculated as percentages upon that portion of the fermented sugar which is not converted into lactic acid.

	Products derived from the glucose itself.				
	Glucose fermented alone.	Glucose fermented with formic acid.			
	0.	1.	2.	3.	4.
Hydrogen	0·46	1·75	2·10	1·01	1·05
Carbon dioxide	22·89	44·86	59·94	44·98	34·69
Formic acid	3·86	4·35	—	—	—
Acetic acid	27·03	27·48	31·86	17·70	31·37
Succinic acid	28·41	17·55	4·15	14·58	22·53
Alcohol	15·44	7·32	1·85	21·09	5·84
Total products	98·09	103·30	99·90	99·66	95·68

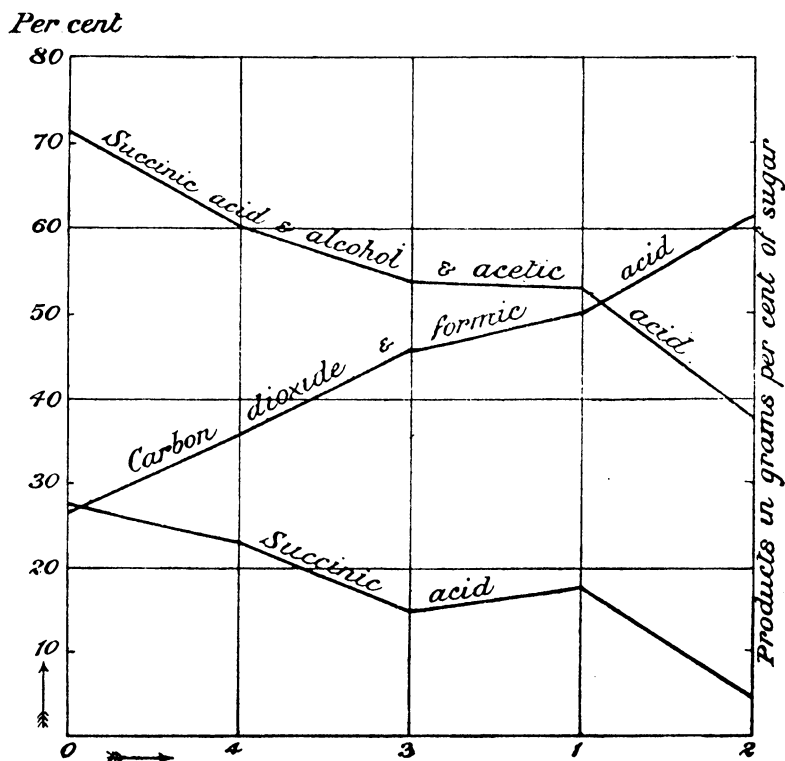


FIG. 3.—(Results arranged in order of increasing values of carbon dioxide and formic acid.)

that these products divide themselves into two groups each represented by two curves which stand in complementary relationship to one another. The acetic acid curve is the mirror image of the curve for alcohol, and the succinic acid curve is roughly the reflection of the curve representing total carbon dioxide and formic acid. In fig. 2 the succinic acid is seen to rise somewhat, opposite column of Experiment 1, whereas it should fall in correspondence to the rise in the carbonic acid curve, but reference to the figures for the total products in this experiment (Table IV) will show that in this one case they totalled to over a hundred per cent., due probably to a small amount of acid derived from the nitrogenous material of the culture medium. The important fact, however, is that the succinic acid belongs to a group of substances all consisting of two or a multiple of two carbon atoms, viz., succinic acid, acetic acid and alcohol, and that it is this group which stands in complementary relationship to the group containing formic acid and carbon dioxide. This is well seen from fig. 3.

Summary and Conclusions of Part IV.

(1) Glucose has been shown to break down under the influence of *Bacterium coli* into three main groups of products :—

- (1) Lactic acid.
- (2) Acetic acid, alcohol, and succinic acid.
- (3) Carbon dioxide, hydrogen, and formic acid.

There is a closer relationship between the products of Groups 2 and 3 than exists between either group and Group 1. The reason for this may be that the rate at which the substances of Group 2 are formed is conditioned by the availability of the hydrogen of Group 3. If the mother substance of alcohol and acetic acid is acetaldehyde, it is easy to understand that the rate of formation of this substance would be influenced by the rate at which part of it was reduced to alcohol by the hydrogen nascent in Group 3, so that the formation of the products of the last two groups would tend to keep pace with one another, whereas the first reaction, of which the end product is lactic acid, being independent of the co-operation of hydrogen for its formation, would run a course quite independent of the other reactions, which, as a matter of fact, it is found to do.

It was shown in Parts II and III that succinic and acetic acid were closely related in origin, and this view is confirmed by the present result. Moreover, it is also clear that, in regard to the interchangeability of these two products of the fermentation, the availability of hydrogen is a critical factor. If there is no available hydrogen, succinic acid would be formed,

with more hydrogen available, acetic acid, and with still more, alcohol. The proportions in which the products of Group 2 appear depends therefore on the intimacy with which the reactions of this group co-operate with the reactions of Group 1, and this further explains the fact that, in many normal fermentations, there is a tendency for Groups 2 and 3 to appear as constituting one group.

(2) It has been demonstrated for the first time in this communication that hydrogen, nascent during the fermentation, does take part in the production of alcohol. This has been shown to be true, not only for the hydrogen which arises from the decomposition of the glucose itself, but also when hydrogen is supplied in the nascent condition by the simultaneous fermentation of formic acid added to the system in the form of calcium formate. Such a proof has never been obtained before, either for bacterial or for yeast fermentation. In the case of alcoholic fermentation by yeast, although the vague expression intramolecular hydrogen was employed to indicate the idea of the participation of hydrogen in the formation of alcohol, the idea was not substantiated by any facts. More recently, some indirect evidence has been obtained by Neuberg that alcohol may be produced by the reduction by yeast of added acetaldehyde, but there is an absence of any direct proof that it is hydrogen itself which effects this reduction. Indeed, in the case of yeast fermentation, it is difficult to see how the participation of hydrogen in the process could be demonstrated, since the whole of the hydrogen of the sugar appears bound up in the final products of the reaction, whether these are alcohol, aldehyde, or glycerine. On the other hand, fermentation by means of *Bacterium coli* is admirably adapted to settle the question, for hydrogen is evolved during the fermentation, and this evolved hydrogen acts as a gauge of the amount of hydrogen which has been absorbed in the formation of alcohol.

(3) In the fermentation of glucose by *B. coli communis*, it has been shown that the effect of the presence of calcium formate is peculiar.

If carbon dioxide and hydrogen arise in the fermentation of glucose through the intermediate formation of formic acid between glucose and these products, as has hitherto been thought, then it is to be expected that the addition of formic acid to the system would tend to depress the formation of that substance, and consequently lead to a diminished yield of its gaseous products, carbon dioxide and hydrogen. In these experiments, however, the opposite has occurred. This seems to mean that either the action of calcium formate has been, by virtue of properties independent of the formic acid to which it gives rise, or else carbon dioxide and hydrogen do not normally arise by the decomposition of preformed formic acid. The

writer proposes to reserve the consideration of these alternatives to Part V of this series.

(4) The method described in the present communication of carrying out the fermentation of substances in the presence of one or other of the products of the reactions added at the outset gives promise, in the writer's opinion, of lending valuable aid in the solution of the problems of fermentation.

In conclusion, I wish to express my thanks to Prof. F. Gowland Hopkins, F.R.S., for his kind help and criticism during the course of this research. I would also like to acknowledge my debt to Prof. Arthur Harden, F.R.S., for his valuable criticism.

Studies on Synapsis. II.—Parallel Conjugation and the Prophase Complex in Periplaneta with Special Reference to the Pre-meiotic Telophase.

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(Communicated by Prof. E. W. MacBride, F.R.S.—Received May 15, 1920.)

[PLATES 10-12.]

The reality of synapsis, or the pairing of chromosomes of biparental origin preparatory to their segregation during the maturation divisions of the germ cells, provides at present the most fruitful basis for any attempt to correlate genetic phenomena with cell structure; and it rests upon data derived from sources that are strictly independent of the behaviour of the chromosomes during the remarkable series of events intercalated in the prophase of the heterotype mitosis. It is, however, only possible to gain a knowledge regarding the manner in which homologous chromosomes are brought into association with one another by a study of the meiotic phase itself. To the earlier workers the reality of synapsis was the all-absorbing problem, while the means by which it is effected formed a secondary consideration. It is possible, given the conjugation of chromosomes derived from alternate parents, to explain the independent segregation of allelomorphic pairs on the assumption that their material basis resides in different bivalents; while