XXI. THE EFFECT OF POTASSIUM ON THE GLUCOLYSIS OF BRAIN TISSUE WITH REFERENCE TO THE PASTEUR EFFECT.

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VARIOUS explanations have hitherto been put forward as to the mechanism of the Pasteur effect. Meyerhof *et al.* [1925] and others have suggested that the lactic acid formed in anaerobiosis is also formed under aerobic conditions, but here removed by oxidation and perhaps resynthesis. Another explanation has been given by Lipmann [1933], who considers that oxygen interferes with the activity of the fermentative or glucolytic enzyme and thus spares glucolysis. It seemed also possible that one of the reactants in the lactic acid-forming reaction (as demonstrated by the work of Embden *et al.* [1933] and Meyerhof and Kieseling [1933] in the case of muscle) might be removed by oxygen. Thus, assuming glucose to split into two molecules a and b which react together to give lactic acid, we have glucose $= a + b = 2$ lactic acid. If either a or b is removed by oxygen, lactic acid formation is lessened, and furthermore one atom of oxygen could prevent the formation of 2 molecules of lactic acid. That is, one molecule of oxygen absorbed prevents 4 molecules of lactic acid from accumulating, and in this way a maximum Meyerhof quotient of 4 could be explained.

In the original project, it was decided in the first place to investigate the effect of adding α-glycerophosphate and pyruvate (which was already known [Bumm *et al.*, 1933; Mendel *et al.*, 1931] to increase anaerobic glucolysis) to tissues in which oxygen was exercising its sparing action on glucolysis. If either of these substances occurred in the normal glucolysis and was removed by oxygen, addition of this substance during aerobiosis might restore and increase the aerobic glucolytic rate.

It was necessary to use for this purpose a tissue with low aerobic glucolysis, and for this purpose brain slices were selected for a start, although it is not believed [Ashford and Holmes, 1929; Ashford, 1933; 1934] that the Embden-Meyerhof scheme for glucolysis obtains in brain tissue. The work reported here is the outcome of these experiments.

METHODS.

The tissue employed was the cortex of rabbit's brain. Slices of this were cut immediately after the rabbit was killed. These slices were immersed in 10 ml. of liquid which contained Ringer in normal concentration and 0·025M sodium bicarbonate. In this solution glucose was present in a concentration of 0·2 %, also any substance whose effect on glucolysis was to be determined. This volume of fluid containing the slices was contained in flasks provided with glass stoppers (as described in a previous paper [Ashford, 1934]). Oxygen or nitrogen containing 5 % CO_2 was bubbled through the liquid in which the slices were immersed for

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about 20 mins. Then the taps were closed and the flasks with their contained slices under physiological conditions were shaken in a bath at 37° for periods of 1, 2 or 3 hours. At the end of the period of shaking the flasks were removed from the bath, and 3 ml. of 20 % trichloroacetic acid were added to the contents of each which were quantitatively filtered and washed into a 50 ml. volumetric flask after standing about 10 mins. The slices contained in each shaking flask were transferred from the filter-paper into weighing bottles and their dry weight determined.

The filtrates were made up to 50 ml. with water, and their lactic acid contents were determined, after treatment with copper lime, by Friedemann, Cotonio and Shaffer's [1927] method.

Experimental.

The effect of potassium α-glycerophosphate on the aerobic and anaerobic glucolysis was first studied. This salt was prepared in approximately 4 % solution by treatment of Merck's "Calcium glycerophosphoricum neutrale" with potassium oxalate at 0°. The calcium oxalate was centrifuged off, and the resulting filtrate was tested for freedom from both calcium and oxalate ions by the addition of small quantities of potassium oxalate or calcium chloride. The results of a number of experiments are shown in Table I. It will be seen that the

Table I. *Effect of potassium glycerophosphates on anaerobic and aerobic glucolysis.*

All values in mg. lactic acid per g. dry tissue per hour.
The salts used had a final concentration of about 0·7 %.

		Nitrogen		Oxygen		
Exp. No.	Time	Glucose alone	Glucose + glycerophosphate	Glucose alone	Glucose + glycerophosphate	Remarks
1	3 hours	41·0	17·0	15·0	57·0	α-salt
2	3 hours	57·0	25·0	15·5	45·0	α-salt
3	2 hours	66·5	26·0	—	—	α-salt
4	2 hours	59·5	22·0	—	—	β-salt
5	2 hours 10 mins.	62·0	25·6	23·1	57·6	α-salt

addition of potassium α-glycerophosphate has markedly increased the aerobic glucolysis while at the same time the anaerobic glucolysis is so depressed as to be below the level of the aerobic in the presence of the potassium α-glycerophosphate.

The next step was to try the effect of potassium β-glycerophosphate and also the effects of the sodium salts (Tables I and II). In Table I it is shown that

Table II. *Effect of sodium glycerophosphates on anaerobic and aerobic glucolysis.*

All values in mg. lactic acid per g. dry tissue per hour.

Exp. No.	Time hours	Gas	Glucose alone	Glucose + α-glycero-phosphate	Glucose + β-glycero-phosphate	Remarks
1	3	N_2	58·0	85·0	81·0	0·4 % salts
		O_2	26·0	27·0	21·0	„ „
2	3	N_2	65·0	61·0	54(?)	0·6 % salts
		O_2	17·0	27·0	24·0	„ „
3	3	O_2	12(?)	27·7	21·7	0·9 % salts
		O_2	15·4	16·5	—	0·3 % „
4	2	N_2	53·5	—	61·0	0·3 % salts

potassium β-glycerophosphate has the same inhibitory effect on the anaerobic glucolysis as the α-salt. The sodium salts, however, have no effect, except in one case perhaps causing a slight rise in aerobic glucolysis, but not by any means of the same order as that caused by the potassium salt (Table II).

A further step was to see how much of this effect was due to the potassium ion or whether the glycerophosphate was the determining factor. To test this parallel experiments were carried out using potassium α-glycerophosphate and a solution of potassium choride against one another. Table III shows the result of

Table III. *Comparison of effects of potassium α-glycerophosphate and potassium chloride on aerobic and anaerobic glucolysis.*

All values in mg. lactic acid per g. dry tissue per hour.
Duration of experiment—2 hours.

Gas	Glucose alone	Glucose + 1·4 % K α-glycero-phosphate	Glucose + 0·9 % KCl
N_2	50	16	9
O_2	13	75	51

the experiment and makes it clear that this ability to stimulate aerobic glucolysis and to inhibit anaerobic glucolysis in brain is a property of the potassium ion. It has already been noted [Ashford, 1934] that the presence of a potassium phosphate buffer reduces the anaerobic glucolytic rate in brain tissue.

Experiments were next performed to determine whether sodium chloride had any similar effect to potassium chloride. It is shown however in Table IV that sodium chloride has no significant effect on glucolysis, while experiments with the same concentration of potassium chloride show the effect in a marked manner.

Table IV. *Comparison of the effects of potassium and sodium chlorides on aerobic and anaerobic glucolysis.*

All values in mg. lactic acid per g. dry weight per hour.
Duration of experiment—2 hours.

Gas	Glucose alone	Glucose + 0·1*M* NaCl	Glucose + 0·1*M* KCl
N_2	75	66	23
O_2	18	22	61

The effect of variation in concentration of potassium chloride was next studied. In Table V, the results of four experiments with 5 different concentrations of potassium chloride are shown. (It was unfortunately not possible to perform aerobic and anaerobic experiments on the same brain owing to the im-

Table V. *Effects of different concentrations of potassium chloride on aerobic and anaerobic glucolysis.*

All values in mg. lactic acid per g. dry tissue per hour.
Duration of experiments—2 hours.

Gas	Glucose + no KCl	Glucose + 0·005*M*	Glucose + 0·02*M*	Glucose + 0·1*M*	Glucose + 0·3*M*	Glucose + 0·5*M*	Date
N_2	54	67	41	14	13	8	31. vii. 34
N_2	53	55	30	10	9	14	8. viii. 34
O_2	17	17	30	49	35	19	30. vii. 34
O_2	19	16	25	58	32	24	3. viii. 34

possibility of obtaining sufficient tissue.) The results are shown in Fig. 1, where the glucolysis is plotted against the logarithm of the potassium chloride concentration, each experiment being plotted as a separate curve. It brings out clearly that the effect of potassium is greatest at a concentration of about 0·1*M*. The aerobic glucolysis has been increased 200 % while the anaerobic has been reduced 75 %, and it is worthy of note that at this concentration the aerobic glucolysis is 4 or 5 times as large as the anaerobic.

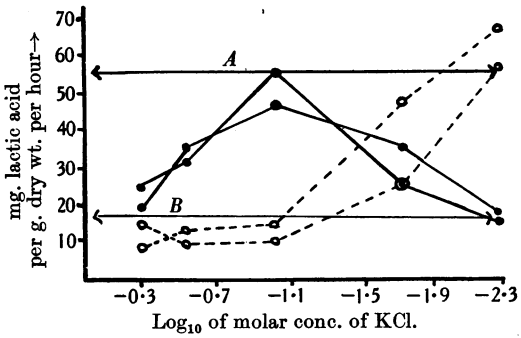

Fig. 1. Relation between anaerobic and aerobic glucolysis and potassium chloride. *A*, average level anaerobic, no KCl. *B*, average level aerobic, no KCl.

•—•—• Aerobic glucolysis. o--o--o Anaerobic glucolysis.

It would appear probable that at the concentration of normal Ringer, potassium does not exert this effect. Lasnitzki [1933] has shown that the absence of potassium and to some extent of calcium from the incubation fluid markedly reduces anaerobic glycolysis in tumour, but he did not study the aerobic effect. Beebe [1904] and Clowes and Frisbie [1905] have reported high values for potassium content of malignant tumours and these were often associated with low calcium content. Since high aerobic glucolysis is shown by tumours it was decided to ascertain whether glucolysis in brain was affected by the absence of calcium from the Ringer (Table VI). It will be seen that the marked effect of high potassium concentration is not shown by lack of calcium.

Table VI. *Effect of calcium-free Ringer on glucolysis.*

Gas	Medium (containing 0·2 % glucose)	mg. lactic acid per g. dry wt. per hour
N_2	Ringer	65
N_2	Ca-free Ringer	53
O_2	Ringer	13
O_2	Ca-free Ringer	20

It seemed of importance to ascertain whether the addition of pyruvate to brain slices under anaerobic or aerobic conditions would increase the glucolysis and also if the addition of co-ferment T would in any way modify the inhibitory effect of potassium salts on the anaerobic glucolysis, since it has been shown by the work of Mendel *et al.* [1931] and by that of Bumm *et al.* [1933] that in the phenomenon of inhibition by glyceraldehyde, in the case of tumour slices, this inhibition is removed by pyruvate and by co-ferment T. It will be seen from Table VII that in these experiments pyruvate does not increase the aerobic or anaerobic glucolysis. The absence of effect on the latter process is readily ex-

Table VII. *Effects of pyruvate and of pyruvate in the presence of KCl on glucolysis.*

Exp.	Medium	mg. lactic acid per g. dry tissue per hour: Anaerobic	Aerobic
1	Normal Ringer + glucose	83	22
	" " + 0·01*M* pyruvate	81	24
	" " + 0·001*M* "	—	26
2	Normal Ringer + glucose	70	—
	" " + 0·1*M* KCl	18	—
	" " + 0·002*M* pyruvate	69	—
	" " + " " + 0·1*M* KCl	20	—

plained by the fact that the glucolytic rate is already very high and is not further increased by addition of co-ferment T. The result is not therefore out of harmony with the work of the authors just cited, since in their experiments with brain tissue the anaerobic rates without pyruvate were usually of a somewhat low order. The absence of effect on the aerobic process is in agreement with Bumm *et al.* [1933]. Evidently oxidative removal of pyruvate does not influence lactic acid formation, since the addition of pyruvate has not increased the aerobic glucolysis. Table VII also shows that pyruvate has no significant effect on the inhibition of anaerobic glucolysis by potassium.

It has previously been shown that *dl*-glyceraldehyde markedly inhibits the anaerobic glucolysis of tumour [Mendel, 1929]. It was decided to see if the large aerobic glucolysis of brain slices in the presence of added potassium chloride was affected by glyceraldehyde. It was considered that if another mechanism of lactic acid formation in aerobiosis were operative here, this mechanism might not be inhibited by glyceraldehyde, as is the normal anaerobic mechanism. The results of experiments to test this point are shown in Table VIII together with the

Table VIII. *Effects of* dl-*glyceraldehyde and of glyceraldehyde with KCl on glucolysis.*

Exp.	Medium	mg. lactic acid per g. dry tissue per hour: Anaerobic	Aerobic
1	Normal Ringer + glucose	55	11
	" " + 0·03 % glyceraldehyde	4	6
2	Normal Ringer + glucose	—	15
	" " + 0·03 % glyceraldehyde	—	6
	" " + 0·1*M* KCl	—	40
	" " + " " + 0·03 % glyceraldehyde	—	6

results of an experiment on the effect of glyceraldehyde on both normal anaerobic and aerobic glucolysis. It is apparent that the high aerobic glucolysis in the presence of potassium salts is inhibited by glyceraldehyde as is the normal anaerobic glucolysis. Further it appears that the aerobic glucolysis in the absence of potassium is also inhibited, though, owing to the small absolute value of the aerobic glucolysis, this can hardly be said to be proved.

Reversibility of the potassium effect.

The question as to whether these effects of addition of potassium are reversible has been investigated. The experiments are recorded in tabular form in

Table IX. *Experiment to test the reversibility of the potassium effect.*

No. of flask	Gas	All fluids contained 0·2 % glucose. Media and procedure	mg. lactic acid per g. dry tissue per hour
1	N_2	Ringer + 0·1*M* KCl, stopped after one hour	19
2	O_2	As flask 1	60
3	N_2 then O_2	Ringer + 0·1*M* KCl, for one hour in N_2, then O_2 bubbled through and incubated for one hour further	17 (in 2nd hour)
4	N_2	Ringer + 0·1*M* KCl for one hour, then slices to flask 5	19
5	N_2	Ringer (no KCl) + slices from 4, for one more hour	4
6	O_2	Ringer + 0·1*M* KCl, for one hour, then slices transferred to flask 7	44
7	O_2	Ringer (no KCl) + slices from 6 for one more hour	20

Table IX, the numbers of the glass flasks in which the slices were incubated and their contents, gaseous and fluid are given, and the glucolysis is also recorded. From the value of the glucolysis in flask No. 3, we see that admitting O_2 to slices which have been in N_2 and KCl for 1 hour fails now to establish a high glucolytic rate, since only 36 mg. of lactic acid per g. dry weight are formed in No. 3, and 19 mg. of this have been formed in the first hour as in No. 1, 17 mg. having been formed in the second hour in O_2 and KCl. Also the high normal anaerobic glucolysis is not re-established in normal Ringer after the tissues have been incubated anaerobically for 1 hour with added KCl; this is seen from Nos. 4 and 5. On transferring tissues from Ringer with KCl, in which they have been for 1 hour, to normal Ringer, all under aerobic conditions, we see that the aerobic glucolysis now falls approximately to the normal level; this is shown by the figures for Nos. 6 and 7.

It appeared necessary to ascertain if the glucolysis in the second hour when tissues are transferred to another medium, is as high as in the first hour in the original medium, accordingly the experiment recorded in Table X was performed.

Table X. *Effect on glucolytic rates of transferring tissues from one medium to another.*

No. of flask	Gas	All fluids contained 0·2 % glucose. Media and procedure	mg. lactic acid per g. dry tissue per hour
1	N_2	Ringer, then tissues to flask 2 after one hour	73
2	N_2	Ringer, slices from 1 for another hour	56
3	N_2	Ringer + 0·1*M* KCl for one hour	31
4	N_2	Ringer + 0·1*M* KCl + slices from flask 3 for a further hour	13
5	N_2	Ringer + 0·1*M* KCl for one hour	28
6	O_2	Ringer + 0·1*M* KCl + slices from flask 5 for a further hour	10

Nos. 1 and 2 show that lactic acid formation in normal Ringer falls off to some extent on transferring the tissues from their original fluid. Nos. 3 and 4 demonstrate a falling off in KCl under anaerobic conditions, while Nos. 5 and 6 show again that admission of O_2 to slices which have been an hour in KCl and N_2 cannot restore the high glucolytic rate seen in KCl and O_2.

From these experiments it is seen that the inhibition caused by KCl and N_2 is associated, at any rate after exposure for 1 hour to 0·1*M* KCl, with an irre-

versible change in the cells, so that they cannot regain a high glucolysis. Also it appears that tissues placed in normal Ringer after having been incubated in O_2 and KCl can now only show their normal low aerobic glucolysis (Nos. 5 and 6). It has been shown that the glucolysis in the second hour in O_2 and KCl is of the same order as in the first, this is seen from figures showing the glucolytic rate calculated from experiments of 1 hour's duration, which give similar figures to the earlier experiments of 2 hours' duration. This is also seen from Table XI where two experiments of 1 hour's duration are recorded.

Table XI. *Glucolytic rates in experiments of one hour's duration only.*

All fluids contained 0·2 % glucose.

Gas	Medium	mg. lactic acid per g. tissue per hour	
		Exp. 1	Exp. 2
N_2	Ringer	48	64
N_2	„ +0·1*M* KCl	18	25
O_2	Ringer	23	31
O_2	„ +0·1*M* KCl	47	75

Since very high glucolysis is observed in O_2 and KCl, and it has previously been suggested that the lactic acid formed in N_2 is removed in O_2 [Holmes, 1930; Krebs, 1931], it seemed of importance to see if the aerobic removal of lactic acid were inhibited by KCl. Accordingly the rate of disappearance of 0·05 % lithium lactate was measured both in presence and absence of potassium. The figures recorded (Table XII) show that the aerobic removal under conditions similar to those of the former experiments is of a very low order but is not markedly affected by potassium.

Table XII. *Oxidative removal of lactic acid in presence and absence of 0·1* M *potassium chloride.*

Exp. No.	Duration hours	mg. lactic acid removed per g. dry tissue	
		Ringer alone	Ringer +0·1*M* KCl
1	2·5	17	21
2	2·0	25	32
3	2·0	36	36

It was attempted to ascertain if any intermediaries accumulated in the presence of potassium salts both in O_2 and N_2. The trichloroacetic acid extract when tested for pyruvic acid by the nitroprusside reaction was negative, nor did potassium influence the bisulphite-binding capacity of the extract, which was in any case small.

One further point has been investigated. Dodds and Pope [1933] showed that 2 : 4-dinitro-*o*-cresol increased the metabolic rate of mice and Dodds and Greville [1934] have shown that this substance increases both respiration and aerobic glucolysis in tumour tissue. They did not study the effect on anaerobic glucolysis. It would seem possible that potassium salts might also affect in some way the respiration of brain slices. An earlier preliminary experiment in the Barcroft manometer and in phosphate buffer had suggested that this was not the case, and the point was not pursued, except to show that the rate of lactic acid

oxidation was not affected in the presence of potassium, at any rate in bicarbonate buffer. Some manometric experiments have now been made using the Warburg indirect method and simultaneous measurements of oxygen consumption and aerobic glucolysis and also anaerobic glucolysis have been carried out. The results shown in Table XIII amply bear out our results obtained by the chemical method, namely that anaerobic glucolysis is inhibited, while aerobic glucolysis is markedly increased, by 0·1*M* KCl. Further the very interesting fact emerges that the respiration is also increased by values which range from 50 to 200 % of the normal respiration. Except in Exp. 1, there is no apparent correlation between the increase in respiration and the increase in aerobic glucolysis, which might suggest that in the presence of potassium there is a general stimulation of the aerobic metabolism of the cell and that this increased aerobic glucolysis is one method of manifestation of this phenomenon. The significance of these results is discussed again later. It will be noticed (Table XIII) that the

Table XIII. *The effect of* M/*10 potassium chloride on the respiration and glucolysis of brain slices.*

Exp. No.	(I) Ringer + *M*/10 KCl					(II) Ringer alone						Remarks
	Q_{O_2}	Q_{S,CO_2}	$Q_L^{O_2}$	$Q_L^{O_2}$*	$Q_L^{N_2}$	Q_{O_2}	Q_{S,CO_2}	$Q_L^{O_2}$	$Q_L^{O_2}$*	$Q_L^{N_2}$	MQ	
1	19·0	30·5	11·5	—	4·5	6·5	6·5	0	—	15·5	2·4	
2	10·5	21·0	10·5	—	—	4·0	4·5	0·5	—	—	—	
3	11·5	22·5	11·0	—	—	7·5	8·5	1·0	—	—	—	
4†	16·5	33·5	17·0	15·5	→	11·0	15·5	4·5	3·0	—	—	In this exp. the tissues were transferred from (I) to (II) after one hour
5†	24·5	42·0 (1st hour)	17·5	15·5	→	13·0	19·0 (2nd hour)	6·0	4·5	—	→	Ditto, but after 2nd hour tissue returned to medium (I)
	19·0	31·0 (3rd hour)	12·0	10·5	—	—	—	—	—	—	—	
6	21·5	42·0 (1st hour)	20·5	18·5	→	—	— (2nd hour)	—	4·5	—	→	Exp. as exp. 5
	—	— (3rd hour)	—	9·5	—	—	—	—	—	—	—	

* Indicates that the results in this column were obtained by the simplified method. All other results by the indirect method (*i.e.* $Q_L^{O_2} = Q_{S,CO_2} - Q_{O_2}$).

† Indicates 0·2*M* bicarbonate. All others in 0·25*M* bicarbonate.

→ Indicates transfer of tissue from one medium to another.

aerobic glucolysis in the absence of potassium chloride is practically zero, whereas our chemical estimations of $Q_L^{O_2}$ vary from 2·5 to 5·0. Such figures correspond to 10–20 mg. lactic acid per g. of dry weight. This discrepancy is almost certainly due to the fact that some lactic acid remains even in the washed tissue slices and of course is estimated in the chemical determinations, whereas the manometric method is unaffected by initial lactic acid in the tissue.

These results also show that the addition of *M*/10 KCl inhibits the "Pasteur reaction" in brain tissue, if we accept as a criterion of this inhibition a rise in aerobic glucolysis without any fall in respiration (see Dickens [1934] for further discussion on this point). Moreover, this inhibition is largely reversible as is seen from Exps. 4, 5 and 6 in Table XIII. In each of these the same tissue slices were used throughout. After a period of one hour in the Warburg pots containing Ringer and KCl, the slices were transferred to a second pair of pots containing Ringer without excess KCl, after, of course, washing in normal Ringer. In Exps. 5 and 6, a second transfer back to Ringer and KCl was effected. The manometric

results obtained during these periods in different media show that the high aerobic glucolysis and oxygen uptake, characteristic of the slices in KCl, fall to lower and normal values when KCl is no longer in the medium and return again to the high values when $M/10$ KCl is again present. The effect of KCl on the aerobic process is therefore reversible.

One point should be mentioned in connection with these experiments. In Exp. 6 only pots containing a small volume of fluid were employed after the first transfer, so that O_2 uptake was not determined. However, a value for aerobic glucolysis ($Q_L^{O_2}$) can be obtained since the volume used was only 1·5 ml. and it is assumed that none of the CO_2 liberated was retained. How far this assumption is warrantable is shown by comparing the value of $Q_L^{O_2}$ calculated by this simple method with the values obtained by the complete indirect method with pots containing both large (5·5 ml.) and small (1·5 ml.) fluid volumes as shown in Exps. 4, 5 and 6. The values obtained with the simplified method are indicated with an asterisk.

Discussion.

The main result of these experiments, namely, that the anaerobic glucolysis is markedly decreased by the addition of K salts while the aerobic glucolysis is raised to be several times as large as the anaerobic, is hard to explain. It would seem possible that the potassium would merely inhibit the Pasteur reaction and so raise the aerobic glucolysis. At the same time this concentration of potassium might also inhibit the anaerobic glucolytic system. The explanation would only be feasible if the aerobic glucolysis were raised and the anaerobic glucolysis lowered until they attained one and the same level. It cannot possibly be put forward, however, in view of the fact that so far from attaining the same level the aerobic glucolysis is several times as large as the anaerobic in the presence of added potassium.

A possible explanation is that the large formation of lactic acid in the presence of KCl is due to a different system coming into play from that occurring in normal anaerobic glucolysis. One could assume that owing to the presence of potassium some substance in the normal glucolytic chain accumulates slightly, in that its further reaction is inhibited. If, however, this substance could be oxidised directly to lactic acid, then formation of lactic acid would occur in oxygen. Lactic aldehyde is a substance which on oxidation would give lactic acid; it would be interesting to ascertain the effect of this substance on brain slices in view of the fact that it is known to disappear in liver "brei" as does methylglyoxal [Neuberg, 1913]. An argument which militates against a second method of lactic acid formation occurring in O_2 and KCl is that this glucolytic system like the normal anaerobic mechanism is inhibited markedly by glyceraldehyde. However, glyceraldehyde may act at some earlier point common to both mechanisms.

Another possibility, which Dr H. A. Krebs has put before us, is that KCl acts by injury to the slices, thus damaging the glucolytic mechanism, and that this damage is greater in N_2 than in O_2 where only the Pasteur effect is eliminated. This would explain the non-recovery of high glucolysis in N_2 on placing slices again in normal Ringer after they had been incubated in N_2 and KCl for 1 hour, and also the failure to obtain high glucolysis on bubbling O_2 through the medium in which slices were suspended which had been previously incubated anaerobically in the presence of potassium. However, since normally glucolysis occurs to such a high extent under anaerobic conditions, and also since anaerobiosis alone does not seem to kill the brain cells quickly in the presence of glucose,

it is difficult to see how the mere presence of O_2 should impede the damage to the cells by potassium when KCl in O_2 does not cause this damage.

It would seem probable that the damage is connected with the inhibition of glucolysis by potassium. Under aerobic conditions there is still a mechanism of lactic acid formation, and this, or oxidation of other substances, may give the cells a necessary metabolic reaction. In N_2, however, glucolysis can no longer proceed, and so the cells are injured irreversibly, perhaps as they have no longer a means of obtaining the energy necessary for life. In this regard it is interesting that Dickens and Greville [1933] have shown that incubating brain slices at 38° for as short a time as 20 mins. anaerobically and without glucose destroys all but 12 % of their power of glucolysis when glucose is added. From these considerations the irreversibility of the effect of addition of KCl in N_2 does not seem contrary to the above theory of two different glucolytic mechanisms, in nitrogen and in oxygen.

From the results of our experiments (Table XIII) in which both respiration and glucolysis were determined, it may be said that KCl definitely inhibits the Pasteur reaction (and further that this inhibition is reversible) provided that the Pasteur reaction is regarded strictly as the inhibition of lactic acid formation by oxygen. It must be emphasised, however, that the mechanism of formation of lactic acid in oxygen when KCl is added may not necessarily be the same as that occurring normally in nitrogen; in fact this appears unlikely in view of the inhibitory effect of potassium chloride on anaerobic glucolysis. Secondly, as well as increasing aerobic glucolysis, the addition of potassium markedly increases the respiration. The significance of this is uncertain; it may be connected with general stimulation of metabolism or perhaps oxidation of some intermediary to lactic acid. It is possibly due to the increasing concentration of lactic acid, but we have shown that the rate of removal of lactic acid is not affected. There is apparently no correlation between increase in oxygen uptake and aerobic glucolysis, though it appears that a relation exists between the total oxygen uptake and the aerobic glucolysis in experiments in which $M/10$ KCl was added. Further the question asked by Dickens [1934], as to whether aerobic glucolysis can be increased in tissues where carbohydrate oxidation is not diminished, can now be answered. These conditions are satisfied in brain slices glucolysing aerobically in the presence of KCl, since presumably here the high respiration is due to an oxidation of the carbohydrate type [see Dickens and Sîmer, 1930]. Thus we can say that KCl does not behave in the same way as Dickens [1934] has suggested for phenylhydrazine, since this substance does not inhibit anaerobic glycolysis.

As regards the original purpose of these experiments, that is to see if oxygen interferes with lactic acid production by removing some reactant in the glucolytic chain, we have not succeeded in demonstrating any positive evidence for this theory by addition of possible substances and so are thrown back on either the explanation of Meyerhof or that of Lipmann as to the mechanism of the Pasteur effect.

From our results, however, the former explanation, that is that lactic acid formed as in N_2 is merely removed by the oxygen, becomes unlikely, at any rate in brain, since if this were the case it would be hard to explain how potassium so markedly increases the aerobic glucolysis when it apparently does not interfere with the removal of lactic acid from brain slices under aerobic conditions. It is possible, however, in view of the increased O_2 uptake, that the general metabolism might be so stimulated as to increase the rate of lactic acid formation in the presence of O_2 and KCl. However, it seems unlikely that increased O_2 uptake

would be associated with increased lactic acid production unless the lactic acid production were caused by some direct oxidation. This is, in fact, the same as postulating another mechanism of lactic acid formation coming into play in O_2 and KCl. Another argument against the view that lactic acid is formed as under anaerobic conditions but removed in O_2 is the slow rate of removal of added lactate in brain (Ashford and Holmes [1931] and our more recent observations on brain slices) compared with the high rate of lactic acid formation by similar tissue under anaerobic conditions.

From this we may conclude that the theory of Lipmann is not, as the others are, at variance with these observations. The mechanism of the action of potassium salts is at present completely obscure, and the existence of another mechanism for glucolysis occurring in tissues in O_2 and KCl is still a possibility. An even more disturbing possibility is that under artificial conditions a cell may actually develop a new and different mechanism. If this were so it would throw into doubt much work done on excised tissues and extracts. At present it is simpler to postulate that there are different mechanisms for lactic acid production in oxygen and in nitrogen and that the former is activated by potassium and the latter inhibited.

Summary.

1. The addition of potassium α-glycerophosphate to glucolysing brain slices was found to increase the aerobic and inhibit the anaerobic glucolysis, so that the aerobic glucolysis became several times greater than the anaerobic. This effect is not shown by the sodium salt.

2. Potassium chloride, but not sodium chloride, was found to have the same effect in 0·1*M* concentration, the aerobic glucolysis assuming values from 3 to 6 times as large as the much diminished anaerobic glucolysis. The phenomenon is due entirely to the potassium ion.

3. The effect of varying concentrations of potassium chloride on both aerobic and anaerobic glucolysis in brain slices was studied.

4. Potassium does not affect the oxidative removal of lactic acid.

5. *dl*-Glyceraldehyde was shown to inhibit the raised aerobic glucolysis occurring in the presence of *M*/10 KCl.

6. The addition of pyruvate did not increase the aerobic glucolysis or remove the inhibitory effect of potassium on the anaerobic phase.

7. The oxygen uptake was shown to be increased in the presence of *M*/10 KCl.

8. The reversibility of these effects has been investigated. The inhibition of anaerobic glucolysis by KCl in N_2 was shown to be irreversible, whereas the increase in aerobic glucolysis and oxygen uptake by addition of KCl during aerobiosis is reversible.

9. The bearing of these results on the significance of glucolysis and on the Pasteur effect is discussed.

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