THE EFFECT OF IODOACETATE ON THE ELECTRICAL POTENTIAL AND ON THE OXYGEN UPTAKE OF FROG SKIN

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The relationship between the maintenance of the electrical potential difference across frog skin and the oxygen consumption of the skin has been investigated by Mansfield (1910), Lund (1925, 1926, 1928), Lund & Moorman (1931), Francis & Pumphrey (1933), Francis (1933, 1934), Taylor (1935), Boell & Taylor (1933), and Ponder & Macleod (1937). These workers investigated the effects of varying temperatures and varying concentrations of cyanide, oxygen, sulphide, carbon monoxide, butyl alcohol, amyl alcohol, and of various carbamates. The objective of the present work has been to use the somewhat more specific respiratory inhibitor iodoacetate to trace further the oxidative biochemistry of frog skin in so far as it is concerned with the maintenance of the electrical potential difference and to be able to cover all statements made by adequate tests of statistical significance and thereby to eliminate troubles arising from the variability of the skins.

The electrical potentials and electrical polarization data were obtained by methods similar to those of Francis & Pumphrey (1933) and Pumphrey (1934); oxygen uptake was determined by Barcroft differential manometers. The experiments were conducted throughout in accordance with all obvious precautions excepting only for three factors. First, the *p*H of the solutions was adjusted only to $\pm 0{\cdot}1$ unit and by means of indicators instead of being adjusted by using a glass or a hydrogen electrode[1]; secondly, the solutions were not analysed and were made up by weight from standard "Analar" B.D.H. salts which had not been recrystallized or dried to constant weight; thirdly, the skins were thermostated only in the case of measurements of oxygen uptake. The errors introduced by the first two factors, however, were shown to be negligible by the work of Dean (unpublished results), and the errors arising from the third should also be negligible, using either the temperature coefficient of potential obtained by Lund & Moorman (1931) for *Rana pipiens* or that given by Francis (1933) for *R. temporaria*. The most important source of error is the variability of the skins. This can be eliminated by working with controls off the same frog and using adequate statistical tests of significance. Seasonal and sexual variations of potential were detected by these tests and have been given due consideration. Effects arising from the movement of the skin or from the existence of small pressure gradients across the skin have been taken into

[1] A glass electrode was used for permanganate solutions.

consideration and also effects due to the need of continually renewing the solutions in contact with the skin. None of these effects alter the provisional conclusions obtained from the results discussed in this paper. Potentials, resistances, and mm^3 of O_2 at N.T.P. consumed were all observed as functions of time. Neglect of the time factor reduces the significance of some of the previously published work on skin potentials.

Table I

Time	*M*/100 iodoacetate	*M*/500 iodoacetate	*t* for *n*=4 5 comparisons
−30	21·2	22·5	−0·22
−20	23·2	22·8	+0·05
−10	25·3	23·5	+0·21
0	24·9	24·6	+0·04
+10	31·6	31·7	−0·02
20	39·0	33·7	+1·71
30	44·9	39·7	+0·86
40	34·9	38·7	−0·88
50	24·7***	38·3	−4·95
60	17·0*	33·0	−3·06
70	11·6*	26·9	−3·68
80	9·2*	22·6	−2·88
90	8·1	18·6	−2·28
100	7·9	16·2	−2·40
110	7·6	13·8	−1·16
120	6·9	12·1	−2·44
130	5·7	10·0	−2·58

Table II

Time	*M*/500 iodoacetate	*v.*	*M*/1000 iodoacetate	*t* for *n*=4	*M*/1000 iodoacetate	*v.*	*M*/5000 iodoacetate	*t* for *n*=7
−30	56·0		56·6	−0·09	41·1		41·8	−0·33
−15	57·5		57·7	−0·06	43·4		45·7	−0·93
± 0	56·7		57·0	−0·08	43·4		45·3	−0·66
+15	60·2		65·1	−0·99	42·6		52·3	−2·31
+30	60·1		69·0	−2·23	42·8		46·75	−0·88
+45	58·0		63·0	−1·55	41·1		43·75	−1·04
+60	50·6		63·0	−1·53	39·25		41·2	−0·44
+75	40·4		58·0	−1·54	35·6		39·9	−0·88
+90	31·6		50·8	−1·73	30·1		39·2	−2·12
+105	24·5		43·7	−1·85	25·75		39·9***	−5·84
+120	18·7		38·4	−2·05	21·6		38·25**	−3·19
+135	14·5		33·2	−2·16	18·25		31·6	−2·03
+150	11·0		28·3	−2·32	15·75		27·75	−1·95
+165	7·6		23·2	−2·26	13·3		24·75	−1·93
+180	5·5		18·7	−2·32	10·75		21·75	−2·16
+195	—		—	—	9·3		18·0	−2·07

TYPICAL RESULTS

The effect of iodoacetate on skin potential is shown in Tables I and II and on skin Q_{O_2} (in mm^3 O_2 at N.T.P. $min.^{-1}$ $g.^{-1}$ of dried skin) in Table III. The antagonizing effect of *dl*-lactate, pyruvate, and acetate on the action of iodoacetate is shown in Table IV. The action of benzoate in inhibiting the antagonizing effect of propionate

and *n*-butyrate on the effect of iodoacetate on the potential is shown in Table V. All these results refer to the same solution on both sides of the skin. At negative times this was frog's Ringer solution, dextrose free, and at *p*H 8. At subsequent times the solutions were frog's Ringer with chloride substituted at *p*H 8 by the given organic anions to the stated concentrations. Each comparison refers to different halves of the belly skin of the same frog. The first column, after that giving the time in minutes, refers in all tables except Table III to the mean potential in millivolts of one set of half skins at interpolated intervals of time, the second column refers to the corresponding mean potential in the other solution, and the third column to values for "Student's" *t* (Fisher, 1931) calculated to test at each time whether the mean difference of potential between half skins and their controls differs from zero. Differences lying between the 5 and 2 per cent levels of significance are marked with one asterisk, between the 2 and 1 per cent levels with two

Table III

Time	Potential	Q_{O_2}
0	100	—
45	100	100
75	72·2	65 7
105	49 6	50 8
135	30 4	41 6
165	21 0 (extrap.)	33·2

asterisks, and beyond the 1 per cent level by three asterisks. Potentials are reckoned as positive when the calomel electrode in the Ringer in contact with the morphologically inner side of the skin is the more positive. Time potential and time Q_{O_2} curves at interpolated intervals of time have been obtained and also corresponding values for *t* for every result obtained during the work described in this paper. Full details of the work have been deposited under the title "The effect of iodoacetate on frog skin potential" by W. L. Francis and O. Gatty at the Natural History Museum, South Kensington, London.

The mean potential of eighty-eight skins in *M*/500 iodoacetate gives a time potential curve which can be compared on a percentage basis to the mean Q_{O_2} time curve for fourteen skins also expressed on a percentage basis. This is done in Table III. It will be seen that there is a certain similarity in the two curves. The mean potential was 39 mV. at time 0 and the mean Q_{O_2} was 10·00 mm.^{3} O_2 g.$^{-1}$ dry skin min.$^{-1}$ At 135 min. the standard deviation per observation of Q_{O_2} is 0·922 so that to the 5 per cent level of significance Q_{O_2} lies between 3·625 and 4·695. On the percentage basis 36·25 is seen to be considerably greater than 30·4. The fact that the oxygen uptake falls away to a significantly lesser extent than does the potential in solutions of *M*/500 iodoacetate Ringer corresponds to the observations of Taylor (1935) on oxygen lack, and on CO, of Taylor (1935) and Boell & Taylor (1933) on carbamates, and of Ponder & Macleod (1937) on carbamates.

The value of Q_{O_2} in *M*/500 iodoacetate Ringer at time 45 does not differ

significantly from the mean value of Q_{O_2} for twelve skins in ordinary Ringer at 65 min. which was 9·33 with a standard deviation per observation of 3·42. The decrease in Q_{O_2} with time in iodoacetate Ringer is significant beyond the 1 per cent level, but in plain Ringer the same twelve skins gave a mean Q_{O_2} at 165 min. of 10·03.

Table IV

Time	Lact 20	IAc. 500	*v.* IAc. 500	*t* for $n=7$	Pyr 20	IAc 500	*v* IAc. 500	*t* for $n=7$	Ac 20	IAc. 500	*v* IAc. 500	*t* for $n=8$
−30	32·6		34 4	−0·43	33 3		34 6	−0 32	36 7		41·2	−1 19
−20	34 3		34·2	+0 03	32 9		35 3	−0·57	39·2		41·9	−1·06
−10	35·2		34·2	+0·29	32 4		33 25	−0·20	37 9		42 7	−1·59
± 0	34·5		33·6	+0·26	31 4		32 7	−0·37	41 8		43·1	−0·68
+10	36·1		36·6	−0·14	38 9		37 25	+0 35	51·0		52·0	−0 20
+20	32·7		33 1	−0·09	41 75		36·5	+1 06	52 6		49 9	+0·59
30	31·9		31 1	+0·16	40 6		36·25	+0 84	51·2		48·7	+0 86
40	32·4		31 2	+0 21	42·1		37·75	+0·80	50·4		48·9	+0 51
50	32·9		28 3	+0 84	43·5		40 4	+0·67	55·2		50·6	+1 22
60	32·5		24 4	+1 47	46·4		39 9	+1·98	61·6		53·05	+2·01
70	31·3		21·1	+1 86	48·3***		36 2	+3 76	63·9**		50·6	+3 12
80	29 1		17 9	+2 28	48·6***		29 9	+12·31	63·8**		44·05	+7·55
90	26 2*		15·1	+2·77	47·3***		23 75	+8·00	62·2***		38·8	+5·89
100	21·7**		12·4	+3·17	44 25***		20·0	+5·36	60·9***		33·1	+6·30
110	20 4**		10 6	+5 0	38 6***		16 9	+6·25	54·3***		27 4	+5·94
120	—		—	—	32·3***		14·6	+5·47	46 9***		22·2	+5·84
130	—		—	—	26·2***		12 7	+4·75	37 7***		19 2	+5·49
140	—		—	—	23·1***		11 6	+4·89	29·1***		17 2	+3·94
150	—		—	—	—		—	—	24·3**		15·4	+3 00

Table V

Time	Benz 50	3 Prop. 100	IAc. 500	*v*	3 Prop 100 IAc. 500	*t* for $n=4$ 5 comparisons	Benz 50	3 But. 100	IAc. 500	*v.*	3 But. 100 IAc. 500	*t* for $n=3$ 4 comparisons
−30		21 8			27 7	−1·77		42·1			36·4	+1·8
−20		21·6			27·7	−1·90		41 25			38·25	+0·84
−10		21·4			27 9	−1·96		40 0			40·0	±0
0		21 2			28·2	−2 04		38·6			41·75	−0·61
10		24 5			33·2	−1 51		42 75			41·0	+0·39
20		26 8			36 8	−1·35		41 6			39 25	+0·51
30		27 3			37·8	−1·52		38 75			37 4	+0·42
40		30·3			41·3	−1·35		38·75			37 75	+0·36
50		33·1			49·5	−1·95		40·5			40·6	−0·03
60		34 4			55·6	−2·28		43·0			45·5	−0·47
70		34·9			60 2	−2·53		44·6			51·25	−1·23
80		34·2			63·6*	−2·82		43·75*			55·75	−3 2
90		31 8			65 1*	−3 33		41·0**			56 5	−5·8
100		32 6			63·2	−2·50		35·9***			53·75	−6·3
110		24 5			59·1**	−4·48		30·9**			47 75	−4·75
120		20·1			51·0***	−5·16		27·9*			40 75	−3·66
130		16·2			43·8***	−5 64		—			—	—
140		12·9			37·2***	−5·30		—			—	—
150		10·5			32·4***	−5·54		—			—	—
160		8·9			28·6***	−5·51		—			—	—
170		7 3			25·4***	−5·50		—			—	—
180		6 0			21·9***	−5·35		—			—	—

THE INTERPRETATION OF THE RESULTS

It has not been possible as yet to decide whether the primary function of respiration in maintaining the potential is to supply diffusible ions of a specific nature or whether it is to preserve certain essential features of the structure of the cell surface or whether both factors are involved. It seems probable, however, that the respiratory processes which lead to the formation of substances which affect the potential have been traced a short way and that these methods could be used to trace them further. Owing to the number of different possible ways of interpreting the effect of any single substance on the potential the conclusions mentioned in this section must be regarded as provisional only; their main strength being the number of observations with different substances that fall into the general scheme discussed below.[1]

It seems that oxygen uptake is necessary to maintain the potential. Lund (1926), Francis (1934) and Taylor (1935) all agree that reduced partial pressures of oxygen reduce the potential and the oxygen uptake. The same is true for a large number of respiratory inhibitors and at present no single substance has been found to lower the oxygen uptake over a period of 1 hour or more and not to reduce the potential. (A temporary rise in potential appears with iodoacetate but after 45 min. the potential is falling in $M/500$ solutions.) For instance the potential is reduced (Francis, 1934) by cyanide, sulphide, and carbon monoxide; it seems probable therefore that those respiratory processes which lead to the formation of precursors of the electrical potential involve the working of an indophenol oxidase-cytochrome system. Dehydrogenase inhibitors like urethane (Boell & Taylor, 1933), amyl alcohol, and butyl alcohol were found by Francis (1934) to reduce the potential; these facts also favour the cytochrome hypothesis. A similar result has been obtained with acetaldehyde.[2] A reduction of potential also occurs with the respiratory inhibitors arsenite,[2] Ouabaine, fluoride and iodoacetate.

The correlation between potential and oxygen uptake is not complete, however, since the potential is affected by the nature and concentration of the ions present in the solution even when the oxygen uptake is unaltered as, for instance, in the work of Dean (unpublished results) on nitrate Ringer (Dean & Gatty, 1937). Correlations between potential and oxygen uptake from data in the literature are difficult to interpret and of little significance owing to neglect of the time factor. In the case of iodoacetate the potential falls to a significantly lower percentage of its initial value than the value to which the oxygen uptake falls. Taylor (1935) finds similar results with low p_{O_2}, with CO, and with Boell (1933) finds similar results with urethane.[3] The action of Ouabaine is similar except that it is relatively more lethal to the potential and less so to the oxygen uptake. Permanganate increases the oxygen uptake but has been found (Dean & Gatty, unpublished results) to depress the potential to zero. Again in the presence of iodoacetate acrylate lowers the potential but raises the oxygen uptake. These observations are explicable by

[1] For the full facts as opposed to tentative conclusions see W. L. Francis & O. Gatty, *loc cit.*
[2] Provisional results only; not more than four experiments.
[3] Confirmed by Ponder & Macleod (1937).

regarding respiratory processes as merely modifying existing diffusion and adsorption potentials so that the connexion between respiration and potential is not one of direct proportionality nor does it admit of any simple mathematical expression, and also by further assuming that only some respiratory reactions can supply precursors of the electrical potential. In addition complete correspondence between time potential curves and time Q_{O_2} curves is not to be expected owing to the presence of different quantities of varyingly accessible internal supplies of oxidizable material.

The assumption that precursors of potential are supplied only by specific respiratory processes seems reasonable on general grounds. Moreover the 60 per cent oxygen uptake with Ouabaine and $<7{\cdot}2$ per cent potential and the effects of acrylate in iodoacetate Ringer are both difficult to reconcile with the hypothesis that the electrical role of respiration is mere evolution of CO_2 or the mere production of concentration gradients of $[HCO_3]^-$. Permanganate, however, destroys the cell surface since it lowers the electrical resistance of the skin as well as the potential. The effect of permanganate does not therefore exclude the "CO_2 or bicarbonate" hypothesis. Ouabaine, however, might affect the potential because it changed the properties of the cell surface in a similar or a different way. Ouabaine in fact lowers the electrical resistance (Dean & Gatty, unpublished results) but the substances that it attacks are not known or even known to be likely to possess known chemical groups. Therefore the "CO_2 or bicarbonate" hypothesis cannot definitely be discarded and it is interesting to note that carbonic anhydrase is cyanide sensitive.

The role of respiration in supporting the potential might be supposed to be the maintenance, against diffusion, of concentration gradients of certain ions. Adequate testing of this hypothesis for a given ion involves topochemical considerations that are outside the scope of the present paper. Concentration falls from $M/20$ to zero in either direction across frog skin do not affect the potential in the cases of propionate, acetate or *dl*-lactate. The fact that pyruvate and succinate have no effect when applied to both sides of the skin in the absence of iodoacetate at once suggests that they would not have much effect when applied to one side only quite apart from the direct evidence with three similar anions, the reason being that an exact cancelling out of two one-side-only effects is neither to be expected on general grounds nor has it yet been observed with any substance so far tried on frog skin.[1] In addition Dean (unpublished results) finds that concentration gradients of hydrogen ion from *p*H 6 to 8 across the skin lower the potential in a somewhat similar fashion irrespective of which side of the skin is the more acid. Concentration gradients of bicarbonate (Dean & Gatty, 1937 and unpublished results) and of citrate (Dean & Gatty, 1937 and unpublished results), however, may turn out to have a bearing on the origin of the potential. It may be concluded, however, that concentration gradients or acetate, propionate, *dl*-lactate, succinate and hydrogen ion are not to be regarded as being directly responsible for the potential.

The theory that the potential depends on the maintenance, against continuous decomposition, of a sufficient concentration of a phospho-creatine-like substance

[1] The effects of concentration gradients of $[HCO_3]^-$ across the skin are largely cancelled, however, by having the same bicarbonate rich Ringer on both sides of the skin instead of on one side only.

in the skin can also be rejected since $M/347$ yeast-adenylate has very little effect, if any at all, upon the potential and adenylate should cause almost complete breakdown of phospho-creatine.

The normal precursors of potential probably arise from the oxidative breakdown of internal supplies of carbohydrate. Iodoacetate poisoning cuts off the supply of these precursors as soon as it has diffused into the skin to concentrations sufficient to prevent glycolysis. For a short period of time after glycolysis has been inhibited by $M/500$ iodoacetate the glycolytic products lactate and pyruvate which are still present in the skin can be oxidized and so form precursors of the potential. During this period the skin also forms precursors of potential by oxidizing internal supplies of fatty acids. The latter process is inhibited by acrylate and by benzoate. The former process is not inhibited by benzoate but probably is by high enough concentration ratios of external acrylate to internal (pyruvate + lactate). The inhibition of pyruvate oxidation by acrylate would only become important when the supplies of internal pyruvate begin to get low as, for example, in the case of $M/500$ iodoacetate poisoning. The iodoacetate concentration inside the skin rises, with $M/500$ iodoacetate in the external Ringer that is in contact with the inside of the skin, and eventually it becomes great enough to stop the oxidations of pyruvate, lactate, and the appropriate fatty acids. With the external iodoacetate at $M/500$ these relatively high internal concentrations of iodoacetate are only reached *after* the skin has used up its own internal supplies of lactate, pyruvate, and fatty acid anions. For a certain time after the exhaustion of the internal supplies of these anions it is still possible to maintain the potential by letting the skin oxidize external supplies of the same anions, but once the internal concentration of iodoacetate begins to reach a critical value in the neighbourhood of centres of respiratory activity the oxidation of the appropriate external supplies is inhibited and the potential falls to zero. In the absence of external supplies of substrates that can be oxidized to precursors of potential iodoacetate takes less time to initiate a fall in potential, since it begins in this case as soon as the internal supplies of these substrates have been used up.

The evidence in support of the views of the preceding paragraph is best outlined by considering what external substrates can support the potential in the presence of $M/500$ iodoacetate for appreciable times after the potentials of the controls have begun to fall. The substances that have been found to support the potential in $M/20$ solutions are *dl*-lactate, pyruvate, acetate, propionate, *n*-butyrate, isobutyrate, and in all probability a slight effect is shown by crotonate. The effects in $M/500$ iodoacetate Ringer due to acetate, propionate, and *n*-butyrate are antagonized with these substances at $3M/100$ by $M/50$ benzoate, but that due to $3M/100$ pyruvate is not so antagonized. In $M/20$ solutions *dl*-lactate, acetate, and *n*-butyrate all increase the oxygen uptake of frog skin both in plain Ringer and in $M/500$ iodoacetate Ringer solutions.

Boyland & Meyerhoff (1931) find that in muscle pyruvate, as well as lactate, can increase the oxygen uptake in the presence of iodoacetate, while Jowett & Quastel (1935) find that rat and guinea-pig liver slices, and slices of several other tissues

show increased oxygen uptake in iodoacetate solutions when the latter contain acetate, propionate, *n*-butyrate, iso-butyrate or crotonate. The oxidations of fatty acids investigated by Jowett & Quastel (1935) were found to be inhibited by benzoate. It seems possible therefore that the oxidation of fatty acids to form precursors of potential are processes somewhat similar to the reactions investigated by these workers. Benzoate is a relatively inactive substance that was not even oxidized while it was inhibiting the reactions investigated by Jowett & Quastel (1935) and its effect may well be supposed to be fairly specific. It therefore seems a reasonable hypothesis to assume that propionate and iso-butyrate also increase the oxygen uptake of frog skin in the presence of iodoacetate. The lesser effect due to crotonate finds a parallel in that Jowett & Quastel (1935) found that crotonate was oxidized to acetoacetate more slowly than *n*-butyrate. If the above hypotheses are true it follows that all substances which have yet been found to produce precursors of potential in the presence of $M/500$ iodoacetate can be said also to raise the oxygen uptake in these solutions. Acrylate shows that the converse is not true. Nevertheless the hypothesis may well be extended to say that the precursors of the potential can be formed by oxidizing the former anionic substrates.

The potential of frog skin is irreversibly reduced by iodoacetate even in solutions as dilute as $M/5000$ when applied to the inside of the skin which is also the side which is most sensitive to the respiratory inhibitors, Ouabaine, and arsenite and fluoride (the rapid effect of fluoride Ringer on the outside of the skin is a chloride-free effect (Dean, unpublished results and Dean & Gatty, 1937) and raises rather than depresses the potential). Jowett & Quastel (1935) found that the fatty acid oxidations they investigated were just inhibited by $M/5000$ iodoacetate but the iodoacetate must take some time to reach this concentration inside the skin when the external solution is only $M/5000$ in iodoacetate. In more dilute solutions iodoacetate is not likely to inhibit a great variety of reactions other than the glycolytic breakdown of carbohydrate to lactate and pyruvate. It is argued therefore that the effects of $M/5000$ iodoacetate and the antagonism of lactate and pyruvate to the effects of $M/500$ iodoacetate point to oxidation of carbohydrates through lactate and pyruvate as being a process that leads to the formation of precursors of the potential. The source of carbohydrate is internal because the skin can maintain its potential in dextrose-free Ringer as well as in $M/90$ dextrose Ringer and no significant difference appears during the 50 hours or so that the potentials take to fall to negligible values. The absence of effect of dextrose has been reported by Taylor (1935) and is contrary to the observations of Francis (1934). These data do not prove that external carbohydrate cannot be used as a source of precursors of potential but merely that it is not a necessary source and that the skins do not die of carbohydrate starvation if external carbohydrate can supply these precursors.

If the $M/500$ iodoacetate is used along with $M/50$ benzoate a slight increase in the toxicity of the solution appears and this probably implies that a certain amount of fatty acids are oxidized to form precursors of the potential in iodoacetate poisoned skins. Jowett & Quastel (1935) found that acrylate competed for the fatty acid oxidizing enzymes with other fatty acids and so inhibited the oxidation of these

acids to acetoacetate. Edson (1936) infers that fatty acid anions compete with pyruvate for Quastel's enzymes on the grounds that pyruvate when added reduces the formation of acetoacetate from fatty acids. Since *M*/20 acrylate in the presence of *M*/500 iodoacetate lowers the potential and to a greater extent than *M*/50 benzoate it seems probable that it inhibits not only the fatty acid oxidations but also those of pyruvate as well. Neither *M*/50 benzoate nor *M*/20 acrylate has any effect on the potential in plain Ringer without iodoacetate. The former fact is explained by saying that the normal reactions leading to the formation of precursors of potential proceed through pyruvate but not subsequently through non-activated molecules or anions of fatty acids. The latter fact can perhaps be explained with acrylate pyruvate antagonism by saying that the concentration ratio external acrylate/internal pyruvate has to exceed a certain figure for acrylate inhibition to be appreciable; acrylate inhibition only appearing therefore when the supplies of internal pyruvate are reduced by iodoacetate poisoning. Further support for the competition between acrylate and other substrates for the oxidizing enzymes is that *M*/20 acrylate increases the oxygen uptake both in Ringer and in *M*/500 iodoacetate Ringer even though it depresses the potential in the latter case.

A number of other substrates have been tried with a view to tracing further the oxidative reactions that produce precursors of potential and have shown no signs of being capable of forming them in the presence of *M*/500 iodoacetate. These substances include *M*/20 formate, *M*/20 glycollate, and *M*/40 succinate which are possible products of the oxidation of acetate. *M*/40 succinate did not increase the oxygen uptake of the skins either in Ringer or in iodoacetate Ringer nor that of the skin brei in Ringer. In addition Thunberg tube experiments with skin brei and methylene blue failed to show any succinic dehydrogenase. If acetate is converted to succinate in the skin it is inferred that the subsequent oxidation of succinate must be slow and cannot therefore lead to the formation of precursors of potential. The fate of acetate might be oxalate or citrate or some other substance or an activated molecule or radical. The effect of the two former ions on the potential will be reported in a subsequent paper from this laboratory.

Other substances failing to produce precursors of the potential include *M*/20 acrylate and *M*/40 malonate which are possible oxidation products of propionate. Malonate is a succinic dehydrogenase inhibitor but did not depress the potential below the control in plain *M*/500 iodoacetate Ringer. The fact that *M*/20 acrylate increases the oxygen uptake and does not support the potential suggests that acrylate and propionate are oxidized to different substances, the latter perhaps to pyruvate which is known to form precursors of the potential.

Other substances failing to produce precursors of the potential are *M*/20 *dl*-β-hydroxybutyrate and *M*/20 acetoacetate. Since Jowett & Quastel (1935) using several different tissues could recover from $\frac{1}{2}$ to $\frac{1}{3}$ of their *n*-butyrate after oxidation in the form of acetoacetate and since *M*/20 crotonate produces precursors of potential less rapidly than *M*/20 *n*-butyrate (probable result as judged by results on different sets of frogs but no direct comparison made yet with the same lot of frogs) it seems that the actual oxidation of *n*-butyrate to acetoacetate produces precursors

of the potential which are not crotonate, β-hydroxybutyrate, nor acetoacetate itself. Since Jowett & Quastel (1935) found acetoacetate production as well as the oxygen uptake in the presence of butyrate to be benzoate sensitive the precursors are formed from a process which has at least one inhibitor in common with the reaction leading to the formation of acetoacetate. The precursor may well be some organic radical or else an activated molecule of acetoacetate itself.

Iso-butyrate might perhaps lose its methyl side chain group during oxidation Dakin (1912) and so form propionate. The possibility of higher fatty acids forming precursors of potential is restricted by the failure of caproate to do so.

The story has not yet been traced any further. If it should turn out that respiration has primarily to supply a specific respiratory ion the most likely guess seems to be citrate. Bicarbonate as an additional non-specific respiratory ion cannot be excluded. The effect of respiration on the maintenance of the structure of the cell membrane cannot be judged on existing data which should be extended to polarization curves and to other and more specific inhibitors.

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NOTE ADDED IN PROOF

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Additional results on the relationship between oxygen uptake and potential are discussed below.

(1) *Skin oxidases.* The potential is reduced by $M/500$ N_3^- at *p*H 7·5. This is too alkaline for most N_3^- inhibitions (Keilin, 1936) except that of indophenol oxidase, but the N_3^- may only be acting as an inhibitor after diffusion to more acid regions. Some indophenol oxidase was detected in skin brei, but preliminary spectroscopic tests show it to have very little cytochrome. Adding cytochrome *c* and lactate or succinate only showed faint lines corresponding to reduced cytochrome. This is to be related to preliminary work quoted above showing that brei is succinic dehydrogenase free and also to the increased skin Q_{O_2} in presence of lactate. Another similarity in action on skin of CN^- and N_3^- is that they both increase the electrical resistance of skin; observations for longer periods have only been made with CN^- which eventually depresses skin resistance. Skin potential, like oxidase activity, is only CO sensitive in the dark (Francis, 1934). The great rapidity with which potential is affected by changes in oxygen tension and by oxidase inhibitors suggests that the primary cause of the potential is the reaction

$$\text{Oxidase—}Fe^{++}\text{—complex} + O_2 \rightarrow \text{Oxidase—}Fe^{+++}\text{—complex} + O_2^-,$$

and that other reactions are necessary to prevent complete oxidation of the oxidase. This view is supported by the rapid effect of CN^- and N_3^- on resistance and the rapid recovery of potential after oxygen shortage. The initial rapid rise of potential with oxygen lack is not explained by this theory.

(2) HCO_3^- *effect.* Dean finds that HCO_3^- for Cl^- in the Ringer on the outer side of the skin depresses the potential but raises skin resistance; subsequently replacing the chloride by bicarbonate in the Ringer on the inner side tends to restore the potential. The potential results suggest that HCO_3^- diffusion maintains potential. If CO_2 is buffled through dilute but weakly buffered solutions the point of CO_2 entry becomes electrically positive; if acid is added instead the point of entry becomes negative. The acid effect is observed in frog Ringer but the CO_2 effect is less than 1 mV. If, therefore, diffusion of HCO_3^- originates the potential the diffusion gradient must contain a region other than Ringer and where HCO_3^- has an abnormally high transport number. The resistance result quoted above apparently refutes the

existence of such a region. Thus a diffusion of HCO_3^- controlled by normal and by pseudo-acid buffering cannot be the sole cause of the potential. The HCO_3^- effect on potential might be due to the relative amounts and positions of oxidized and reduced oxidase being affected by HCO_3^-. Thus oxy-haemoglobin is a stronger acid than haemoglobin, whose NH_2—groups may also unite with CO_2 to form carbamino groups.

(3) *Iodoacetate effect.* It appears that external supplies of aceto-pyruvate cannot be oxidized to precursors of potential in the presence of $M/500$ iodoacetate; also that concentration gradients of $M/50$ to zero of aceto-pyruvate across the skin do not affect the potential. Acetopyruvate is a possible intermediary between pyruvate and acetoacetate (Krebs & Johnson, 1937) so that it is possible that precursors of potential are found during the formation both of aceto-pyruvate from pyruvate and of aceto-acetate from butyrate. Both products are pseudo-acids capable of forming chelated compounds with metal ions and are to be composed with Osterhout's guaiacol.

(4) *Other cyanide effects and respiratory ions.* If a dilute Na^+ free solution is on the outer side of the skin the potential is negative. Replacing the inner Ringer by cyanide Ringer causes the potential to become still more negative showing that the CN^- sensitive electrical asymmetry does not change sign, like the potential, with dilution of the outer solution. Also during the first few minutes of passage of depolarizing currents (Gatty, 1937) there is an increase in skin resistance. This may be due to concentration polarization of an ion with an abnormally high transport number in some region of the skin. The effect is antagonized both by higher concentrations of K^+ and by CN^- but not by *p*H 6. The cyanide antagonism suggests that the concentration polarization may be of a respiratory ion or of oxygen.

SUMMARY

The oxidative biochemistry of living frog skin in so far as it is concerned with the maintenance of the electrical potential across the skin has been investigated by the use of the respiratory inhibitor iodoacetate. It appears that internal supplies of carbohydrate and external supplies of *dl*-lactate, pyruvate, acetate, propionate, *n*-butyrate, iso-butyrate and possibly crotonate can all be oxidized to precursors of the potential. This is not so for external supplies of formate, glycollate, succinate, acrylate, malonate, *dl*-β-hydroxybutyrate, and acetoacetate.

REFERENCES

BOELL, E. J & TAYLOR, A. B. (1933). *J. cell comp Physiol.* **3**, 355.
BOYLAND, E. & MEYERHOFF, O (1931) *J Soc. chem. Ind.*, Lond, **50**, 156
DAKIN, H D. (1912). *Oxidations and Reductions in the Animal Body*, pp. 41–7. Longmans.
DEAN, R. B. Unpublished results.
DEAN, R. B & GATTY, O (1937). *Trans. Faraday Soc* **33**, 1040, and also 946, 965, 981, 1017, 1053, 1056
—— —— Unpublished results.
EDSON, N. L. (1936) *Biochem. J.* **30**, 1862.
FISHER, R. A. (1931). *Statistical Methods for Research Workers.* Edinburgh: Oliver and Boyd.
FRANCIS, W. L. (1933). *Nature*, Lond., **131**, 805.
—— (1934). *J exp Biol.* **11**, 35
FRANCIS, W. L & PUMPHREY, R J. (1933). *J exp. Biol.* **10**, 379.
GATTY (1937) *Trans Faraday Soc* **33**, 1017.
JOWETT, M. & QUASTEL, J. H. (1935) *Biochem J.* **29**, 2143, 2159, 2181.
KEILIN (1936). *Proc. Roy Soc* B, **121**, 165
KREBS & JOHNSON (1937). *Biochem J* **31**, 772.
LUND, E. J. (1925) *J. exp. Zool.* **41**, 155
—— (1926). *J. exp. Zool.* **44**, 383.
—— (1928) *J. exp. Zool* **51**, 265, 291, 327.
LUND, E. J. & MOORMAN, J. B. (1931). *J. exp. Zool* **60**, 249.
MANSFIELD, G. (1910). *Pflüg. Arch. ges Physiol.* **131**, 457.
PONDER, E & MACLEOD, J. (1937). *J. gen. Physiol* **20**, 433.
PUMPHREY, R. J (1934). *J. exp. Biol.* **11**, 423, 429.
TAYLOR, A. S (1935). *J. cell. comp. Physiol.* **7**, 1.