CXCIX. DEHYDROGENATIONS BY BRAIN TISSUE. THE EFFECTS OF NARCOTICS.

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It has recently been shown [Quastel and Wheatley, 1932, 2] that exposure of fresh brain tissue to narcotics at low concentrations results in specific inhibitory effects on the oxidations of the brain. The narcotics exert a profound inhibitory action on the oxidation of substances important in carbohydrate metabolism, *viz.* glucose and lactic and pyruvic acids, this action being accomplished at concentrations of narcotics which are of the same order as those required to produce deep narcosis in animals. The narcotics, however, at these concentrations, do not interfere with the oxidation by the brain of sodium succinate or *p*-phenylenediamine. This indicates that their behaviour is not due to a disturbance of the brain-cell whereby its mechanisms for accomplishing oxidations as a whole are affected. Their behaviour appears to be due to an effect, possibly at cell interfaces, whereby the mechanisms which result in an activation of the molecules of lactic and pyruvic acids are greatly disturbed. It has also been shown that among narcotics belonging to the same chemical type those with the greater hypnotic activity have the greater inhibitory action on the oxidation by brain of glucose or lactic acid. The conclusion was drawn that an adsorption of the narcotic took place at a specific area or centre of the nervous system. This resulted in an inhibition of the oxidation of glucose, and of lactic and pyruvic acids by the cells constituting this nervous centre. The access or activation of oxygen was unimpaired, but the diminished ability of the cells to oxidise glucose or lactic acid resulted in a lowering of the amount of energy available for the cells to accomplish their functional activities. Narcosis—or a depression of the normal functional activity of the nervous centre in question—might then ensue.

It follows, if this view is correct, that the action of the narcotic will be reflected not only in diminished oxygen uptake by lactic acid or glucose but in a diminished power of the brain-cell to effect the anaerobic oxidation of glucose or lactic acid, the fundamental phenomenon being a disturbance of the mechanism involving the activation of glucose, lactic acid or pyruvic acid.

This paper is concerned with the dehydrogenating powers of brain tissue and of the effects of a number of narcotics on these. It will be shown, in full

confirmation of earlier results, that narcotics at low concentration share the general property of markedly inhibiting dehydrogenations by the brain of glucose, lactic acid or pyruvic acid.

TECHNIQUE.

Brain tissue was obtained as soon as possible after death of the animal. In the case of large animals such as sheep or bullocks a maximum period of 3 hours elapsed between death of the animal and examination of the brain. Storage where circumstances made this necessary was always at 0°. A serious difficulty in working with human material was to obtain brain tissue less than 6 hours old; nevertheless results with human tissue have been such as to confirm fully results with the tissues of other animals, and no evidence has been secured as yet to indicate that human material differs more than quantitatively, so far as dehydrogenating mechanisms are concerned, from that of the animals studied in this investigation.

The *pia* was removed from the whole brain, which, after being freed from all membrane and possible blood clots, was well minced under ice-cold conditions and made into a homogeneous mixture by pressing through muslin. A portion was then mixed with three times its weight of saline, shaken in a machine and finally filtered through muslin. A creamy homogeneous suspension was obtained which proved to be satisfactory for investigations of dehydrogenating power. The suspension must be kept at 0° and used as freshly as possible. The effect of age on the activity of the suspension will be discussed later.

Grey and white matter have been compared, using mostly human material for this purpose. Suspensions of grey and white matter were made up in saline as indicated for whole brain.

Dehydrogenase activity was investigated by the Thunberg methylene blue technique. Each vacuum tube contained 2 cc. *M*/15 phosphate buffer solution p_H 7.4, 2 cc. of the suspension prepared as above, 1 cc. 1/5000 methylene blue solution and 1 cc. of a solution containing the substance under investigation. The tube was evacuated and the reduction time of the methylene blue at 37° was noted. Whenever a suspension gave a reduction time, in the absence of added hydrogen donors, of less than 20 minutes, it was diluted with saline until the suspension gave a reduction time of approximately 1 hour.

DEHYDROGENATIONS BY THE BRAIN.

Thunberg [1923] has already shown that nervous tissue possesses the power of activating a number of substrates as hydrogen donors. Such substances are glucose, sodium lactate, sodium succinate, sodium citrate. Ashford and Holmes [1931] have stated that lactate, succinate, glycerophosphate and glucose have approximately the same activity. Actually, although these sub-

stances are, unquestionably, active donators of hydrogen in presence of brain tissue, there are considerable differences between them so far as their activities are concerned.

A point of some importance in carrying out this work is the effect of relatively high concentrations of salt upon brain dehydrogenations. If a suspension of brain tissue be made up in distilled water, it has relatively little reducing power when examined under the conditions specified above. If, however, physiological saline instead of water be added to the tissue there is a marked increase in reducing power. In a typical experiment a reduction time of 120 mins. was changed to 14 mins. It seems that the change in osmotic concentration in the solution surrounding the brain-cells results in the withdrawal from the tissue of certain substances which effect the reduction of methylene blue adsorbed at the cell surfaces, or possibly the change of salt concentration affects the permeability of brain-cells towards methylene blue. In view of this result it has been necessary in studying dehydrogenating action to use tissue and substrates made up in saline solution and to carry out adequate controls in order to form an estimate of the effects of changes in osmotic concentration.

Table I gives a typical series of results obtained with bullock (whole) brain.

Table I. *Reduction times due to various substrates in presence of bullock (whole) brain.*

Substrate	Reduction time (in minutes)
Saline (physiological)	100
Saline (0.2 <i>M</i>)	51
Glucose (0.16 <i>M</i>)	25
Fructose (0.16 <i>M</i>)	40
Galactose (0.16 <i>M</i>)	22
Mannitol (0.16 <i>M</i>)	27
Sodium lactate (0.13 <i>M</i>)	14
Sodium citrate (0.03 <i>M</i>)	40
Sodium glutamate (0.13 <i>M</i>)	28
Sodium succinate (0.13 <i>M</i>)	21
Sodium glycerophosphate (0.13 <i>M</i>)	16

(a) *The sugars and polyhydric alcohols.* Glucose, fructose, galactose and mannitol, at the concentrations quoted, are all activated as hydrogen donators. It is easy to explain the reducing activity of glucose on the basis of rapid glycolysis to lactic acid which is an efficient hydrogen donator with brain. It is much more difficult to explain the activities of the other sugars on these lines. Löbel [1925] states that fructose gives rise to little or no lactic acid in the presence of brain, yet it is oxidised freely, and its oxidation, like that of glucose, is inhibited by preliminary treatment of brain tissue with very dilute iodoacetic acid [Quastel and Wheatley, 1932, 1]. Fructose, therefore, is either oxidised by brain in a different manner from glucose (whose oxidation is known to proceed largely *via* lactic acid) or it gives rise to a small quantity of lactic acid just sufficient to account for its oxidation by oxygen or methylene blue. Mannitol and galactose, however, which are

hydrogen donators, do not take up oxygen in presence of brain at an appreciable rate [Quastel and Wheatley, 1932, 1]. Presumably these substances are sufficiently activated by the brain to reduce methylene blue, but insufficiently so to reduce at any marked rate the natural respiratory carrier (? cytochrome) of the brain.

(b) *Succinic, lactic, pyruvic, citric, glutamic and glycerophosphoric acids.* These substances, used as their sodium salts, reduce methylene blue in presence of brain tissue. They are all capable, too, of being oxidised by this tissue, their presence resulting in a marked oxygen uptake by brain [Quastel and Wheatley, 1931; 1932, 1; Ashford and Holmes, 1931].

The activities of a number of these substances, as well as those of glucose and fructose, are shown in Figs. 1 and 2 where the velocity of reduction is plotted against the logarithm of the concentration.

Fig. 1.

Fig. 2.

The velocity of reduction of the hydrogen donator has been calculated on the basis that the observed velocity of reduction is the sum of that due to the added donator and that of the tissue itself. This must be considered correct only to a first approximation.

It will be noted that velocity — log conc. curves show in general the same phenomenon: an increase in velocity with concentration to a maximum after which there is a decrease in velocity as the concentration is increased. It is important therefore in estimating relative activities of hydrogen donators to determine the rates of reduction with varying concentrations of the substrates.

Such a general phenomenon probably has a common explanation in all cases. It is similar to that described by Dixon and Thurlow [1924], who, working with the xanthine oxidase of milk, showed that increase of concentration of the hydrogen donator led, after the attainment of a peak, to a considerable drop in reduction velocity. This was ascribed to a blocking of the enzyme by excess hypoxanthine which in this way hindered the access of methylene blue and retarded the velocity of reduction. This view seems to be confirmed by the recent results of Harrison [1932]. It is probable that the

results incorporated in Figs. 1 and 2 have a similar explanation. Bernheim [1928] noted the same phenomenon in respect to the dehydrogenation of citric acid using an enzyme preparation from liver.

In Table II are shown the concentrations of a number of hydrogen donors at which half the maximum velocity of reduction in presence of brain tissue is attained. The very high activities of succinic, lactic and pyruvic acids compared with those of glucose and fructose are noteworthy.

Table II.

Substrate	Concentration at which half maximum velocity is reached (<i>M</i>)
Glucose	0.0135
Fructose	0.021
Sodium lactate	0.0045
Sodium pyruvate	0.0052
Sodium citrate	0.018
Sodium succinate	0.004

Comparison of the activities of grey and white matter.

A comparison of the dehydrogenating abilities of grey and white matter shows that the former is three to four times as active as the latter in respect of all hydrogen donors investigated. This refers to bullock, sheep and human brain. Table III gives a representative series of results. It is to be noted that this difference in activity is precisely that found between the rates of oxygen uptake of grey and white matter in the presence of various substrates [Quastel and Wheatley, 1932, 1].

Table III. *Reduction times in minutes of hydrogen donors in presence of human grey or white matter (equal weights).*

Substrate	Grey matter	White matter
Saline	65	120
Glucose (0.18 <i>M</i>)	18	54
Fructose (0.18 <i>M</i>)	25	75
Galactose (0.18 <i>M</i>)	15	60
Sodium glycerophosphate (0.13 <i>M</i>)	16	51
Sodium succinate (0.13 <i>M</i>)	9	27

Effects of storage at 0° on the lability of brain dehydrogenases.

Storage of a brain suspension at 0° results in a slow inactivation of the dehydrogenases. The activities due to the sugars disappear more quickly than those of succinic and glycerophosphoric acids. Table IV, which incorporates representative results, shows that the lability of the mechanisms concerned with the sugars is very considerable, that with mannitol being still more marked. Brain tissue which has not been made into a suspension but allowed to stand intact at 0° shows the same phenomenon, though the effects are slower than with a brain suspension. With intact brain tissue the velocity of reduction of methylene blue due to added glucose does not decrease at a rate

Table IV. *Effects of storage of brain suspension (bullock) at 0° on the activity of dehydrogenases.*

Substrate	Reduction times in minutes.		
	Period of storage at 0° (hrs.)		
	3	49	74
Saline	18	35.0	49
Glucose (0.18 M)	7.4	22	25
Fructose (0.18 M)	9.0	19	27
Galactose (0.18 M)	6.4	18	22
Mannitol (0.18 M)	8.6	27	46
Sodium succinate (0.13 M)	6.0	13	14
Sodium glycerophosphate (0.13 M)	6.8	12	14
Sodium citrate (0.03 M)	7.0	24	24

linearly proportional to the time of storage, but remains fairly steady for 20 to 40 hours, after which there is a rapid decrease in the velocity of reduction, which becomes more marked as the time of storage is prolonged.

There is some evidence to show that if a tissue suspension be kept anaerobically (in nitrogen or hydrogen) at 0° the dehydrogenases retain their activity for a longer period than when the tissue is stored at 0° in the presence of oxygen (see Table V).

Table V. *Effects of storage of brain suspensions (sheep) for 18 hours at 0° in oxygen and in hydrogen.*

Substrate	Reduction time in minutes.	
	Storage in oxygen	Storage in hydrogen
Saline	>100	>100
Glucose (0.18 M)	>100	33
Mannitol (0.18 M)	>100	31

Lability of brain dehydrogenases at 37°.

The activities of a brain tissue suspension towards the sugars fall off very rapidly on prolonged incubation of the suspension at 37°. The citric acid dehydrogenase is also very labile whilst those of succinic, lactic and glycerophosphoric acids are relatively more stable (see Table VI).

Table VI. *Effect of incubation of brain tissue (sheep) suspensions at 37° on dehydrogenations.*

Substrate	Reduction times in minutes.	
	Time of incubation at 37° (mins.)	
	0	60
Glucose (0.16 M)	56	110
Fructose (0.16 M)	40	84
Sodium succinate (0.12 M)	22	25
Sodium lactate (0.6 M)	8	10
Sodium citrate (0.03 M)	6	14
Sodium glycerophosphate (0.13 M)	12	12

These facts are of importance in investigating the action of narcotics and it is essential to realise when considering the effects of inhibitors on brain dehydrogenases that prolonged exposure at 37° under our experimental conditions is in itself detrimental to some of the mechanisms involved in bringing about dehydrogenations. Doubtless this phenomenon is responsible to some extent for the relatively high values found for the concentrations of sugars which are necessary to achieve half the maximum velocity (Table II). It cannot account entirely for the high values found, owing to the fact that the reduction times obtained with the sugars (used in compiling Table II) were smaller than those required to effect any appreciable destruction of the relevant dehydrogenases by incubation at 37° alone.

The action of somnifaine on brain dehydrogenations.

Somnifaine¹ is a drug composed of equal parts of veronal and allylisopropylbarbituric acid and is commonly used in narcosis treatment of psychotic subjects. It has been shown [Quastel and Wheatley, 1932, 2] that exposure of brain tissue to the drug results in a considerable lowering in the rate of oxygen uptake, this amounting to a decrease of more than 50 % in the velocity of oxidation and taking place with concentrations of the narcotic which are of the same order as those required to induce narcosis. Moreover, the inhibitory action of the drug was found to be restricted to the oxidations of glucose, lactic and pyruvic acids.

Exposure of brain tissue to somnifaine at a concentration of 0.4 % resulted in a complete inhibition of the tissue's own reducing ability and of the power of the tissue to activate the sugars. Using a concentration of 0.1 % there is a marked increase in the reduction time of the tissue alone, and the power to activate glucose is reduced by 50 %. The activations, however, of succinic acid and glycerophosphoric acid are much less disturbed. Representative results are shown in Table VII.

Table VII. *Reduction times in minutes of various substrates in the presence of brain tissue (bullock) alone and together with somnifaine (0.1 %).*

Both the tissue alone and the tissue and somnifaine were incubated for 30 mins. at 37° prior to addition of substrate and methylene blue solution.

Substrate	Reduction time alone	Reduction time in presence of somniafaine
Glucose (0.16 M)	23	54
Fructose (0.16 M)	26	74
Galactose (0.16 M)	37	> 100
Mannitol (0.16 M)	30	95
Sodium succinate (0.11 M)	21	26
Sodium glycerophosphate (0.11 M)	12	14

¹ Somnifaine, as used for injection purposes, is an alcohol-glycerol-water solution of a mixture of 10 % veronal and 10 % allylisopropylbarbituric acid.

These results fully confirm earlier work [Quastel and Wheatley, 1932, 2] and show the specificity of inhibition evinced by the narcotic towards the activation of glucose.

Effects of incubating brain tissue with somnifaine.

If a brain suspension be incubated with somnifaine (0.1 %) for various periods at 37° it is found that there is progressively more and more inhibition of glucose activation as the time of incubation increases, the inhibitions being much greater than in the absence of the narcotic. Representative results are noted graphically in Fig. 3, where it will be seen that whereas the glucose activation decreases with length of incubation of the brain tissue with somnifaine, that of succinic acid or glycerophosphoric acid is but little affected.

Fig. 3. Effects of incubation of brain tissue with somnifaine (0.1 %) on reduction times of substrates.

Fig. 4.

The activations of fructose, mannitol and galactose are influenced in a similar manner to that of glucose. These effects have been found to occur with the brains of guinea-pig, rabbit, sheep, bullock and man.

The action of luminal and veronal on brain dehydrogenations.

Luminal, phenylethylbarbituric acid, behaves qualitatively in precisely the same way as somnifaine so far as brain dehydrogenations are affected. Veronal, which has much less hypnotic power than luminal or somnifaine, has much less inhibiting activity. These facts are in accordance with those

found in the study of the effects of narcotics on the oxygen uptake of brain. There exists a definite parallelism between hypnotic activity of the narcotic and its power to inhibit the oxidation by brain of glucose, lactic acid or pyruvic acid [Quastel and Wheatley, 1932, 2].

Results illustrating the effects of luminal and veronal on the activity of brain tissue towards the sugars are shown in Table VIII. These narcotics have little or no action on the dehydrogenation of succinic and glycerophosphoric acids.

Table VIII. *Effects of incubating brain tissue (sheep) for 30 mins. at 37° alone and in the presence of luminal (0.2 %) and veronal (0.2 %) on the activity of the dehydrogenases.*

Substrate	Reduction times in minutes.		
	Tissue alone	Tissue with luminal	Tissue with veronal
Glucose (0.16 <i>M</i>)	22	51	26
Fructose (0.16 <i>M</i>)	22	54	26
Galactose (0.16 <i>M</i>)	22	78	26
Mannitol (0.16 <i>M</i>)	32	78	40
Sodium succinate (0.11 <i>M</i>)	24	33	25
Sodium glycerophosphate (0.11 <i>M</i>)	24	24	24

The effect of luminal on the activation of lactic acid.

Neither somnifaine (0.1 %) nor luminal (0.2 %) appears to have an appreciable retarding effect on the reduction of methylene blue by brain tissue in presence of high concentrations of sodium lactate. In fact somnifaine seems to have as little effect on the activation of 0.3 *M* or 0.5 *M* sodium lactate as upon the activation of sodium succinate. When however the lactic acid concentration is decreased to limits which are those found in brain tissue itself somnifaine and luminal effect very considerable inactivations. Typical results with luminal are noted in Table IX.

Table IX. *Reduction times in minutes of varying concentrations of sodium lactate in presence of brain tissue (bullock) alone and with luminal (0.2 %).*

The saline controls gave reduction times >100 minutes.

Sodium lactate conc. (<i>M</i>)	Tissue alone	Tissue with luminal
0.03	39	37
0.024	40	47
0.015	45	73
0.003	90	>100

Luminal also brings about an elimination of the activation of sodium pyruvate when this is present at low concentrations (a normal reduction time of 51 mins. was increased to 144 mins. using pyruvate at a concentration of 0.003 *M*). This inhibiting effect of luminal (0.2 %) on the activation of lactate or pyruvate at low concentration does not take place to the same marked extent with low concentration of succinate or glycerophosphate. Here reduction times were increased (at 0.003 *M* concentration) from 15 to 16 mins. in

the case of succinate and were unchanged in the case of glycerophosphate (26 mins.).

It appears that the narcotic (sominifaine or luminal) is only effective as an inhibitor of lactic acid activation when this is present at low concentrations. This fact is of some importance as it shows that the narcotic does not inactivate a portion of the enzyme irreversibly; if this occurred the reduction times of high concentrations of lactate would be affected to the same extent as those of low. The facts indicate that the inhibiting action of the narcotic is inversely proportional to the concentration of lactic acid present and point to a competition between the drug and the lactic acid for the dehydrogenase concerned.

The action of chloretone.

Chloretone, a hypnotic of widely different chemical constitution from the dialkylbarbituric acid series, shows the same qualitative effects as somnifaine or luminal on brain dehydrogenases. Its action on the activation of lactic acid at varying concentrations of this donator is shown in Fig. 4 (p. 1679). It is found that the curve (curve *A*, Fig. 4) representing the action of chloretone inhibition on lactic acid oxidation is identical with that mathematically derived on the assumption that chloretone and lactic acid compete for the same activating centre or dehydrogenase.

The equations involved are

Let e be the total molar concentration of enzyme,
 c " " " " chloretone,
 l " " " " lactic acid.

Let K_l = dissociation constant of EL and K_c = dissociation constant of EC .
 Let x = the concentration of EL molecules and y = the concentration of EC molecules. Then the concentration of E molecules is $(e - x - y)$.

$$\text{We have} \quad K_l x = l (e - x - y) \quad \dots\dots(i),$$

$$K_c y = c (e - x - y) \quad \dots\dots(ii),$$

e being small compared with c or l .

Then we have [*cf.* Haldane, 1930]

$$\frac{v'}{v} = \frac{1}{1 + \frac{K_l c}{K_c(K_l + l)}},$$

where v' = velocity of reduction in presence of chloretone (for constant quantity of methylene blue)

and v = velocity of reduction in absence of chloretone.

Table X gives reduction times calculated from the above equation taking

$$K_i = 0.0045 \text{ (Michaelis constant),}$$

$$K_c = 0.0038.$$

K_c was calculated from the following pair of points:

reduction time in presence of chloretone = 25 mins.

„ absence „ = 14 mins.

K_i , the Michaelis constant, was derived from an entirely different set of readings (see Table II).

It will be noted that there is a striking agreement between the calculated and observed results for the reduction times in presence of chloretone. The starred figure was taken from the curve Fig. 4. The results confirm the hypothesis that a competition exists between chloretone and sodium lactate for the dehydrogenase. Similar results were obtained with luminal.

Table X. *Reduction times in minutes of sheep brain tissue in presence of sodium lactate with and without chloretone (0.023 M).*

Controls with saline gave reduction times > 120 minutes.

Molar concentration of sodium lactate	Reduction time in absence of chloretone	Reduction time with chloretone (observed)	Reduction time with chloretone (calculated)
0.12	11	14	13.4
0.06	13	18	18
0.03	14	25	25
0.024	15	26	29
0.015	18	41	43
0.003	21	100*	97

The concentration of lactate required to give half the maximum velocity of reduction is raised from 0.0045 *M* to 0.065 *M* by subjection of brain (bullock) tissue to 0.4 % chloretone.

Reversibility of narcotic action.

On adding a mixture of glucose and somnifaine (or luminal) to brain tissue and incubating this for varying intervals of time at 37°, reduction times of methylene blue added at the end of these periods are much smaller than those obtained if glucose is added after the tissue has been incubated in the presence of the narcotic. The addition of glucose at the same time as the narcotic results apparently in a protective action. The following are typical results:

Reduction time by brain tissue (bullock) incubated with somnifaine (0.1 %) and glucose (0.18 *M*) for 30 mins. ... 12 mins.

Reduction time by the same tissue incubated with somnifaine (0.1 %) for 30 mins. glucose (0.18 *M*) then being added 24 mins.

Löbel [1925] has shown that narcotics at low concentrations do not interfere greatly with glycolysis by brain and it would seem, in view of the well-known

high glycolytic power of brain tissue, that the protective action described is simply due to lactic acid being produced from the glucose at a concentration sufficiently high to overcome the inhibitory action of the narcotic. This idea seems reasonable in view of the facts already given indicating the existence of competition between the narcotic and lactic acid for the dehydrogenase concerned. On the other hand there still remains the possibility that a true protection against the narcotic is afforded by glucose itself. In support of this there is evidence that both fructose and galactose show "protective" effects against somnifaine which are of the same order as that demonstrated by glucose. It is therefore clear that the problem is not yet settled and must remain in abeyance until more is known of the details of metabolism of the various sugars in presence of brain.

SUMMARY.

1. The dehydrogenating powers of brain tissue of various animals have been examined and the Michaelis constants have been determined for a number of substrates. Determinations of velocity-concentration curves have been made. The curves all exhibit maxima, above which increase of concentration leads to decreased reduction velocities.

2. Grey matter is three to four times as active as white matter.

3. Glucose, fructose, galactose and mannitol are activated as hydrogen donors by fresh brain tissue. The activities of glucose and fructose are less than those of sodium succinate, sodium lactate and sodium pyruvate.

4. The activations of the sugars disappear more quickly on storage of the brain at 0° or on incubation at 37° than those of sodium succinate and glycerophosphate.

5. Exposure of brain tissue to narcotics (somnifaine, luminal, chloretone) results in an elimination of the activation of the sugars, those of sodium succinate and glycerophosphate being relatively undisturbed.

6. The activations of sodium lactate and sodium pyruvate at low concentration are inhibited by the narcotics.

7. It is shown that the narcotic and sodium lactate compete for the same activating enzyme or centre. The narcotic does not inactivate the lactic dehydrogenase irreversibly but its inhibiting action is dependent on the concentration of lactic acid present.

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REFERENCES.

- Ashford and Holmes (1931). *Biochem. J.* **25**, 2028.
Bernheim (1928). *Biochem. J.* **22**, 1182.
Dixon and Thurlow (1924). *Biochem. J.* **18**, 980.
Haldane (1930). *Enzymes*, p. 46. (Longmans, Green and Co., London.)
Harrison (1932). *Biochem. J.* **26**, 472.
Löbel (1925). *Biochem. Z.* **161**, 219.
Quastel and Wheatley (1931). *Biochem. J.* **25**, 117.
—— — (1932, 1). *Biochem. J.* **26**, 725.
—— — (1932, 2). *Proc. Roy. Soc. Lond. B* (in the press).
Thunberg (1923). *Skand. Arch. Physiol.* **43**, 375.