Concentration or Exclusion of Some Heterocyclic Compounds by Brain Cortex, in Relation to their Effects on Carbohydrate Metabolism

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Some pyridine and phenazine derivatives which disturb the normal balance of carbohydrate metabolism in brain-cortex slices inhibit the degradation of cozymase (McIlwain, 1949). To assess the relation between the two actions of such compounds, it was decided to determine the concentrations at which a number of them acted on each process. The two processes have however been studied under different conditions. Degradation of cozymase has been followed in homogenates and extracts of tissues from the central nervous system, and the actions of the various compounds upon it are described elsewhere (McIlwain, 1950). The reaction is slower and of less constant rate in brain slices, which cozymase does not appear to enter (Gore, Ibbott & McIlwain, 1950).

The balance of carbohydrate metabolism, on the other hand, has been studied in brain slices. Physiological experiments with resting men or dogs, breathing air, show that *in vivo* the brain converts to lactic acid about 10–15 % of the glucose which it utilizes; almost all the remainder is oxidized completely (Gibbs, Lennox, Nims & Gibbs, 1942; Himwich & Himwich, 1946). The proportion converted to lactic acid increases in hypo-oxia and in certain other conditions. A similar balance between glycolysis and oxidation is maintained in brain slices, and, in particular, their glycolysis is high anaerobically but suppressed in air; they thus exhibit the Pasteur effect (see, for example, the review of Burk, 1939). In homogenates, however, this is not necessarily the case (Reiner, 1947).

During this study it became evident that in comparing the action of substances in homogenates and in slices, the interaction of slice and added substance must be examined in detail. One of the added substances was found to reach concentrations in the slices a hundred times those in the surrounding medium, while in another case, the ratio was 0.2. To assist in the interpretation of such findings, factors conditioning the entry of substances to the slices were studied. The concentrations of the added substances in the slices were then determined during experiments in which their effects on respiration and aerobic glycolysis were being studied.

METHODS

Tissue slices. Guinea pigs were killed by a blow on the neck, sometimes bled, and the brain removed and sliced, while moistened with the saline used in the latter part of the experiment. Slices of cortex were cut with a recessed microscope slide as guide and washed into a dish of the appropriate saline. When sufficient slices for an experiment had been cut, they were drained free from saline, weighed on a torsion balance, and dropped into Warburg vessels which already contained all other reagents. Slices weighing 50–60 mg. were commonly used and the outer ones were rejected. The dry weight was found to be 0.13 of the moist weight of slices handled in this way; weights of tissue quoted in the text are moist weights.

Salines. Phosphate saline contained 134 mM-NaCl; 5.4 mM-KCl; 1.34 mM-KH₂PO₄, MgSO₄, and CaCl₂; 13 mM-glucose; 10.4 mM-Na₂HPO₄ brought to pH 7.4 by HCl; it was saturated with O₂. Bicarbonate saline contained 124 mM-NaCl; 5 mM-KCl, 1.24 mM-KH₂PO₄, and CaCl₂; 12 mM-glucose and 26.1 mM-NaHCO₃, and was equilibrated with 5 % CO₂ in O₂.

Respiration and aerobic glycolysis. Respiration was determined with Warburg monometers and vessels with a centre well containing KOH paper. The total lactic acid formation was obtained at the end of experiments by Barker & Summerson's (1941) method, and the course of its formation was determined manometrically by calculation from the respiration and the net gas exchange in vessels with bicarbonate saline and 5 % CO₂-O₂. The choice of this method and its applicability to the present experiments are assessed below.

Determination of the heterocyclic compounds studied. Several of these were coloured and were determined in a photoelectric absorptiometer, both in the solution and in the tissue after metabolism. The solution was centrifuged if necessary to free it from tissue debris and compared with a solution of the compound concerned, in buffer of the same pH as that from the metabolic mixture. The slices were drained from liquid and rubbed in a tube and glass rod, with five times their weight of anhydrous Na₂SO₄. The added compound was then extracted from the dried mixture with 2–5 portions of 0.6–1.5 ml. of CHCl₃, according to the nature and amount of pigment. The total extract was weighed, or made up to a standard volume, and compared photoelectrically with standard solutions of the pigment in CHCl₃. Slices without pigment were extracted similarly as controls. In the photoelectric comparisons, Ilford spectrum filter

no. 603 was used for α -aminophenazine methosulphate and neutral red; no. 604 for phenosafranine; no. 605 for ethyl red; no. 607 for diazine green and pyocyanine; and no. 608 for brilliant cresyl blue and thionine blue.

Nicotine was determined by Trim's (1948) method. Estimation of nicotinamide followed previous practice (McIlwain & Rodnight, 1949); it was extracted from slices by grinding in the ZnSO_4 used for deproteinizing, after which NaOH was added to neutrality, the whole diluted to a known

gradual, and to take between 30 and 80 min. to reach an approximately stable value. Such observations are illustrated in Fig. 1 with respect to phenosafranine, neutral red, diazine green, and ethyl red at concentrations about those at which they exert their metabolic effects. The quantity in the tissue was found in each case to be stable between 60 and 120 or 150 min.

Fig. 1. Course of absorption of some heterocyclic compounds by guinea pig-brain cortex. Aerobically, with glucose: ●—●. Anaerobically, no glucose: ○—○. Slice wt./ml. of saline: 15–25 mg. Concentrations quoted are those in solution at the beginning of the experiment.

volume, centrifuged, and samples of the supernatant taken for estimation. The pyridine derivatives, in the concentrations in which they were studied, were found to be partly excluded from slices. In determining their concentration in slices, it was especially necessary to examine the conditions chosen for the separation of slice and solution. Results obtained by simple draining and by one or by two washes of 1 and 3 sec. in saline were therefore compared. Differences between them were small.

RESULTS

Entry of heterocyclic compounds into slices

Course of entry aerobically

This was first examined under normal metabolic conditions: aerobically, with glucose, in a balanced physiological saline. The entry was found to be

The experiments of Fig. 1 showed that when equilibrium was reached, some 60–90 % of the added substance had left the medium for the tissue slice. As the slice was only about one-fiftieth of the volume of the medium, this indicated a very considerable concentration of the substance in the tissue. The manner in which the added substances were distributed between medium and tissue was therefore assessed quantitatively at different concentrations; these results are given in Table 1. From this it is seen that the concentration of most of the added substances in the tissue reached 50 to 300 times that of the substance remaining in solution. The ratios of the two concentrations in some cases tend to vary with change in the initial amount of added substance, and in others to be independent of this.

Table 1. *Concentration of substances in saline and tissue when equilibrium is reached or approached*

(Experiments were aerobic, lasting 80–100 min., and with about 20 mg. tissue/ml. solution. Diazine green not attached to tissue was found to be partly precipitated. The values quoted for the solution were obtained after removing such precipitated material.)

Initial solution			Final concentrations of added compounds (M)		Ratio of concentrations: tissue/solution
Added compound (M)	Saline (B, bicarbonate; P, phosphate)		Solution	Tissue	
Phenosafranine	10^{-5}	B	0.38×10^{-5}	3.16×10^{-4}	83
	1.08×10^{-5}	P	0.49×10^{-5}	3.4×10^{-4}	70
	5×10^{-5}	B	1.7×10^{-5}	9.55×10^{-4}	56
	5×10^{-5}	P	1.6×10^{-5}	9.65×10^{-4}	60
Diazine green	2.5×10^{-5}	P	0.95×10^{-5}	3.7×10^{-4}	40
	2.5×10^{-5}	B	0.90×10^{-5}	4.3×10^{-4}	48
	7.5×10^{-5}	P	1.1×10^{-5}	12×10^{-4}	109
	7.5×10^{-5}	B	1.05×10^{-5}	15×10^{-4}	145
Neutral red	5×10^{-5}	B	0.5×10^{-5}	7.65×10^{-4}	153
	10^{-4}	B	1.2×10^{-5}	1.53×10^{-3}	130
	3×10^{-4}	B	2.55×10^{-5}	3.13×10^{-3}	120
Ethyl red	10^{-6}	B	$<10^{-7}$	7.5×10^{-5}	>750
	3.3×10^{-6}	B	10^{-6}	1.5×10^{-4}	150
	3.3×10^{-6}	P	10^{-6}	1.38×10^{-4}	138
	10^{-5}	B	1.12×10^{-6}	3.4×10^{-4}	300
Nicotinamide	2×10^{-2}	P	2×10^{-2}	1.37×10^{-2}	0.69
	2×10^{-2}	B	2×10^{-2}	1.40×10^{-2}	0.70
	5×10^{-2}	P	5×10^{-2}	3.80×10^{-2}	0.76
Nicotine	3.3×10^{-2}	P	3.3×10^{-2}	0.73×10^{-2}	0.22
	8.3×10^{-2}	P	8.4×10^{-2}	1.26×10^{-2}	0.15

Table 2. *Treatment of slices, and their absorption of phenosafranine*

(Slices with and without treatment were exposed for 60 min. in the glucose-saline medium with 10^{-5} M-phenosafranine, aerobically. Respiration was followed manometrically, and the phenosafranine of slice and solution was determined at the end of the experiment. Slice wt./ml. medium, 20–22 mg.)

Treatment	Ratio: $\frac{\text{Concn. phenosafranine in slice}}{\text{Concn. phenosafranine in medium}}$	Respiration (% control)
None	90	(100)
None: medium contains 0.1 M-NaF	33	30
Slice placed in ethanol for 5 min. and rinsed in saline before experiment	24	2
Slice in saline put in bath at 50° for 5 min. before experiment	24	40
Slice in saline put in bath at 60° for 5 min. before experiment	27	8

In marked contrast to these results were those obtained with nicotine and nicotinamide, in which the concentration of substance in the slices was only 0.2 of that in the medium. It must, however, be noted that this result concerns much higher concentrations of added compounds, and that the difference may not be specific for the compounds concerned. The concentrations chosen for study were those at which the different substances disturbed carbohydrate metabolism.

Effect of changed conditions on the entry of heterocyclic compounds to tissue slices

Table 1 showed that there is no large or consistent difference in the distribution of the compounds examined, when bicarbonate replaced phosphate as the main buffer.

It was, however, noticed that the compounds tended to leak from the slices if the experimental vessels were left without shaking or if the slices were removed from saline at the end of an experiment. Further experiments showed that the general metabolism of the tissue conditioned the concentration of added compounds in it. This is shown in Fig. 1 with respect to three compounds. Phenosafranine, neutral red, and ethyl red are absorbed less rapidly by the tissue anaerobically in the absence of substrate, than aerobically with glucose. The content of, for example, phenosafranine in the tissue is anaerobically about half that aerobically. This corresponds to a large difference in the ratios of substance in tissue/medium, under the two conditions.

Other circumstances which disturbed the metabolic activities of the slices also greatly affected their absorption of added substances (Table 2).

Nature of the compounds after assimilation by slices

No chemical change after assimilation was found in the majority of cases. In many experiments with phenosafranine, neutral red, diazine green, ethyl red, and nicotine, the data permitted comparison of the amount of substance added with that found in the slice and medium. Recovery was normally between 95 and 105 %. In addition, phenosafranine was examined in detail and the absorption spectrum of material recovered from slices was determined between 400 and 800 m μ . The only absorption observed was between 450 and 570 m μ . with a maximum at 515, and close correspondence over that range with the absorption of phenosafranine itself. Any association between phenosafranine and tissue constituents was therefore broken by the method of extraction used. This employed only sodium sulphate and chloroform at room temperature.

Fig. 2. Loss of neutral red and ethyl red from guinea pig-brain cortex slices. The substances were absorbed initially, aerobically, from glucose saline. Slices were then transferred: (a) to glucose saline and incubated aerobically, and (b) to saline without glucose, incubated anaerobically. The quantities of substances remaining in the slices are given above: (a) ●-●; (b) ○-○.

Histological localization of assimilated substances

Several of the substances examined are coloured. Portions of the guinea pig-brain cortex slices which had assimilated them during manometric experiments were examined promptly after mounting under a coverslip in the saline of the experiment, and the following observations made. The concentrations of dyestuffs examined were about those whose metabolic effects were under examination.

Phenosafranine. Guinea pig-brain cortex slices were examined after respiring in air for 0.5 and 2 hr. in glucose saline containing initially 10^{-5} M- and 5×10^{-5} M-phenosafranine (3 ml./slice of about 60 mg.). The general picture was of a uniformly pink tissue in which outlines of cells and intracellular

structures could be seen. Against the background of very uniform colour the nuclei of nerve cells appeared somewhat less coloured, and some endothelial cells around capillaries, more coloured. The granules at which neutral red and diazine green became concentrated were not differentially stained.

2-Aminophenazine methosulphate (10^{-4} M) was also evenly distributed.

Neutral red. General staining was observed, together with the localized staining of granules which probably corresponded with those described by others (cf. Covel & Scott, 1929; Cowdry, 1944) as being stained by neutral red by normal histological techniques (see also Cobb, Cohen & Ney, 1938).

Diazine green. General staining was observed, with nuclei and nerve fibres less coloured than other structures. Small intracellular granules were more deeply stained; these appeared to be mitochondria which are differentially stained by the related

diazine green S (or Janus green B) after tissues have been fixed and dehydrated (cf. Cowdry, 1944).

Ethyl red. Staining was to a large extent general, and did not appear to be localized at any of the intracellular structures in the manner described above for other compounds. The first few outer layers of the cells of the cortex appeared more deeply stained.

Brilliant cresyl blue. This was localized in the fashion described for ethyl red, except that red blood cells when present were very strongly stained.

Loss of compounds from slices

In assessing the manner in which the added substances are held in the tissues, slices which had already assimilated them were transferred to fresh

media under various experimental conditions. The loss of added substances varied according to the presence or absence of glucose and oxygen (Fig. 2).

Actions of heterocyclic compounds on respiration and glycolysis

Choice of methods. The compounds examined included several which were absorbed by tissue slices over a period of an hour until a large proportion of added substance was within the slice. Comparison of the effects of different substances therefore required observations at frequent intervals such as those obtainable by manometric techniques. Determination of respiration in this manner was straightforward. In determining glycolysis, the applicability of the following considerations was examined. Gas exchange in a bicarbonate-buffered medium would be expected to be the summation of oxygen uptake, carbon dioxide formation and carbon dioxide displacement by acid. The respiratory quotient of brain cortex is almost exactly unity (Dickens, 1936*a*; cf. Elliott, Scott & Libet, 1942), so that determinations of respiration and total carbon dioxide evolution would enable the carbon dioxide displaced by acid to be found. Of methods for determining exchange in both carbon dioxide and oxygen, that employing two widely different volumes of bicarbonate-containing media presented the following difficulties. If, in determining the effect of a compound, equal concentrations of it were present in the fluid, with similar slices in the vessels, the two vessels would contain markedly different ratios of quantities of compound to tissue. As a large part of many of the compounds is finally concentrated in the slices, such procedure would give different final concentrations of the substances at their point of action. If, on the other hand, the ratio of the quantities of tissue and added substance is made equal in the two vessels, the concentration of dye in the tissues will be equal only if all added substance is absorbed by them. This was not the case. It was therefore decided to employ the method in which slices of similar weight are used in two vessels containing equal volumes of saline with the same concentrations of added compound. In one vessel respiration was measured in phosphate saline. In the other the net result of oxygen uptake and carbon dioxide output from bicarbonate saline was determined. Respiration in the two vessels was assumed to be equal and calculation of the carbon dioxide evolved followed Dixon (1943).

Experiments in which the applicability of the method was assessed are quoted in Table 3. In a series of experiments without added substances, lactic acid formation was determined in the bicarbonate-containing vessel colorimetrically, after periods of metabolism between 105 and 130 min. Manometric observations were begun in these experi-

ments between 9 and 15 min. after mixing. Acid formation manometrically was then assessed by extrapolation to zero time of the rate observed between, for example, 10 and 120 min. The values obtained manometrically are lower than those observed chemically by a mean value of 13 %. This is considered to represent a reasonable agreement. The discrepancy is attributed to a small retention of carbon dioxide in the bicarbonate solution, and to the defect inherent in the method of allowing for the first few minutes' metabolism when no manometric observations could be made. This is known to extrapolate to a period of rapid lactic acid formation, the lower rate of the remaining experimental period (cf. Fig. 3 and Elliott *et al.* 1942).

Table 3. *Acid formation by slices; comparison of chemical and manometric methods*

Additions to media (M)	Products ($\mu\text{mol./g. wet wt.}$) determined after incubation (in different exp.) for 105–130 min.	
	Lactic acid (colori- metrically)	CO ₂ displaced (mano- metrically)
None (8 exp.)	40, 39, 52, 63 51, 44, 53, 49	43, 34, 40, 50 46, 42, 44, 40
Phenosafranine 10 ⁻⁵	106	105
Phenosafranine 5 $\times$ 10 ⁻⁵	126	142
Ethyl red 5 $\times$ 10 ⁻⁷	111	102
Ethyl red 1.6 $\times$ 10 ⁻⁶	140	157
Diazine green 2.5 $\times$ 10 ⁻⁵	75	90
Thionine blue 10 ⁻⁴	40	42

Comparison of the colorimetric and manometric determinations of lactic acid resulting from glycolysis in the presence of added compounds is also included in Table 3. The manometric results here are 6 % higher than the colorimetric ones. These experiments include many in which the total level of lactic acid formation is raised, so that the two factors to which the lowered manometric results were attributed above, would be expected to have less effect. Moreover, extrapolation is now from a period of higher to one of lower lactic acid formation.

Lactic acid formation and absorption of added compounds in bicarbonate and phosphate salines. In a series of experiments without added heterocyclic compounds, lactic acid was determined colorimetrically after metabolism in both the bicarbonate and phosphate salines. It was almost invariably found to be greater in the bicarbonate saline (Table 4). In eleven experiments in which the total quantity formed in phosphate saline lay between 25 and 55 $\mu\text{mol./g.}$, that in bicarbonate was (mean value) 45 % higher. This difference was reduced in sixteen experiments with added substances, to a mean value of +5 %. The total lactic acid formation in the experiments with added compounds was much greater.

Table 4. Acid formation by slices in phosphate and bicarbonate salines

(Experiments were aerobic, lasting 80–150 min., and with about 20 mg. tissue/ml.)

Additions to media (M)	Lactic acid ($\mu\text{mol./g.}$)	
	In phosphate	In bicarbonate
None (11 exp.)	22, 24, 40, 35, 32, 41, 39, 40, 45, 45, 55	40, 39, 52, 63, 51, 54, 72, 44, 53, 77, 49
Phenosafranine 5×10^{-5}	126	126
Ethyl red 5×10^{-7}	84	101
Ethyl red 1.6×10^{-6}	117	128
Ethyl red 3×10^{-6}	117	140
Ethyl red 10^{-5}	143	163
Diazine green 2.5×10^{-5}	73	75
Diazine green 5×10^{-5}	60	62
Diazine green 7.5×10^{-5}	88	80
Diazine green 1.7×10^{-4}	50	34

Uptake of added compounds by the tissue was also determined in phosphate and bicarbonate salines. Any differences between the two values were small. Some comparative values are included in Table 1.

Respiration. The rate of respiration of guinea pig-brain cortex slices during the present experiments was 60–66 $\mu\text{mol./g. moist wt./hr.}$, or 0.46–0.51 $\mu\text{mol./mg. dry wt./hr.}$, and it remained at this value during the 2 hr. of typical experiments. The effects of added substances on respiratory rates are included in Table 5, which is discussed more fully below. It will be seen that many of the substances examined affected respiration, a common finding being a small acceleration at lower concentrations and inhibition at higher ones (compare Dickens, 1936b).

The course of aerobic glycolysis. Manometric results from a typical experiment with three concentrations of phenosafranine are shown in Fig. 3. Phenosafranine, as shown by Dickens (1936c), greatly increases glycolysis. The $\mu\text{mol.}$ of lactic acid formed can become numerically about equal to the $\mu\text{mol.}$ of oxygen absorbed in respiration. This implies an increase in the rate of glucose breakdown to four times its normal value. Glycolysis in Fig. 3, in the absence of added substance, has fallen after 30–40 min. to a steady rate of about 10 $\mu\text{mol./g. tissue/hr.}$ In the presence of the two higher concentrations of phenosafranine, glycolysis was accelerated during the first 50 min., after which it remained steady. The lower concentration of phenosafranine showed a combination of these two effects. The period during which glycolysis is accelerating corresponds to the period during which phenosafranine is being absorbed by the slices (Fig. 1); after 50 min., steady concentrations had been reached. On the basis of this result, and of similar ones with other heterocyclic compounds, it was decided that the values during the second hour of metabolism would give the most suitable basis for comparison of metabolic

effects. The concentrations of phenosafranine in these slices were therefore determined 2 hr. after commencing incubation.

Fig. 3. Course of action of phenosafranine on aerobic glycolysis of guinea pig cerebral cortex slices, determined manometrically. Tissue weight: 25–28 mg./ml. Numbers by curves give the initial concentrations (M) of phenosafranine in the media.

The action of phenosafranine was then compared with the concentration in the slices, as shown in Fig. 4. Results with other compounds are also included in Fig. 4 and have been derived from other experiments of the type shown in Fig. 3. It is seen that all the substances under discussion, except possibly ethyl red, raise lactic acid formation at concentrations at which they do not inhibit, but may even accelerate, respiration. At the higher concentrations, inhibition of respiration is also observed in almost every case.

A given increase in rate of glycolysis is associated with the presence in the slices of widely different concentrations of the various substances. The numerical values for such concentrations, derived from Fig. 4, are the main outcome of this study and are discussed below in relation to other properties of the substances concerned.

Related compounds, and compounds with little or no effect on aerobic glycolysis. The substances in Fig. 4 are some only of the phenazine and pyridine derivatives whose action on glycolysis and respiration have been studied. Results with others are summarized in Table 5, where values for phenosafranine and nicotinamide are also quoted for comparison. The majority of the other compounds had much less action on aerobic glycolysis, and their concentrations in the slices were not determined.

Fig. 4. Effects of added substances on respiration (○), and aerobic glycolysis (●), of guinea pig brain-cortex slices, during their second hour of metabolism.

Table 5. Action of heterocyclic compounds on respiration and glycolysis of guinea pig-brain cortex

(The values are derived from experiments of the type illustrated in Fig. 3.)

Substance (M)	Respiration (% of control)		Aerobic glycolysis (% of control)	
	1st hr.	2nd hr.	1st hr.	2nd hr.
Phenosafranine: 2×10^{-6}	105	103	117	230
10^{-5}	100	105	220	470
5×10^{-5}	100	78	310	690
2-Aminophenazine methosulphate:				
10^{-5}	139	133	73	90
10^{-4}	123	124	70	130
2×10^{-3}	124	42	110	60
Pyrocyanine: 10^{-3}	125	123	71	158
3×10^{-4}	129	128	55	90
Brilliant cresyl blue: 10^{-4}	155	100	100	105
10^{-3}	60	15	95	56
Thionine blue: 10^{-4}	130	70	92	105
10^{-3}	70	14	100	90
Nicotine: 3.3×10^{-2}	100	105	128	145
8.3×10^{-2}	98	88	210	450
Pyridylglyoxaline: 3.3×10^{-3}	94	90	156	275
Nicotinamide: 2×10^{-2}	98	95	108	150
5×10^{-2}	94	91	132	230
Nicotinamide methiodide: 10^{-2}	72	72	80	30
Trigonelline chloride: 10^{-2}	100	100	105	100
Coramine: 10^{-2}	89	89	100	130
Pyridine-3-sulphonamide: 10^{-2}	105	105	95	95
3-Acetylpyridine: 10^{-2}	71	71	80	50
10^{-3}	100	100	90	100
Quinine: 5×10^{-3}	5	5	78	15
10^{-3}	5	3	91	100

The magnitude of the difference can be seen by comparing the result with phenosafranine with those given by the aminophenazine methosulphate, pyrocyanine, brilliant cresyl blue, or thionine blue. Only the first two of these showed any tendency to increase aerobic glycolysis, and this was relatively small. Our observations with pyrocyanine agree with those of Young (1937). The effects of the pyridine derivatives are to be compared with that of nicotine and again are small except in the case of the pyridylglyoxaline whose action is noteworthy.

DISCUSSION

Absorption and exclusion by slices. The results emphasize how important it is in tissue-slice experiments to study the assimilation into the tissue of substances whose metabolic effects are under investigation. The phenazine derivatives reached concentrations in respiring brain-cortex slices, of the order of 100-fold those in the surrounding media. These concentrations were not reached only by a process of directly combining with tissue constituents, or staining the tissue, but were dependent on metabolic activities in the slice. Thus, the concentrations fell to about one-third of their value in the absence of glucose oxidation. The degree of concentration of these compounds in the tissue is thus an instance of the active transport of materials across cell barriers, which has been the subject of many studies in other systems (cf. Hoeber, 1945; Davson & Danielli, 1943). Some staining probably takes place also, as the tissue absorbs a certain

quantity of, for example, phenosafranine, when glucose is not being oxidized. Also when a slice which has assimilated phenosafranine aerobically is placed in anaerobic conditions without glucose, much but not all of the phenosafranine is released.

An implication of these findings in metabolic studies with slices is that a given concentration of phenosafranine in a medium could be expected to have different effects aerobically and anaerobically, on the basis only of the different concentrations reached in the slice. The substances studied showed a range of over 1000-fold in the ratio of their concentrations in tissue and in medium. The physiological significance of the systems which assimilate, e.g. phenosafranine to cortex slices is unknown, but they may represent the means by which heterocyclic compounds of importance to the tissue are assimilated from the blood stream. The uptake by the slices of ethyl red, neutral red and phenosafranine was initially at rates of some 0.3–1 $\mu\text{mol./g. moist wt./hr.}$ (Fig. 1). In the case of phenosafranine, the tissue slices appeared to take up the compound uniformly, but the results with diazine green and neutral red raise questions of the distribution of the added substances intracellularly.

Action of glycolysis. Certain substances only, of those examined, increased aerobic glycolysis without marked inhibition of respiration. Among pyridine derivatives nicotine, nicotinamide, and the pyridylglyoxaline were active. These all have an unsubstituted pyridine N, and in the 3-position a substituent which includes the grouping $\text{R}_1\text{R}_2\text{C-NHR}_3$. The inactive compounds trigonelline chloride, coramine, pyridine-3-sulphonamide, and 3-acetylpyridine differ in one or more ways from this structure. The most active phenazine derivatives have basic substituents in positions 3 and 6, and have an *N*-phenyl group (for structures see McIlwain, 1950). Neutral red, without the *N*-phenyl group, is, however, active to some extent; the oxazine and thiazine, brilliant cresyl blue and thiazine blue, were inactive.

Comparison with the results of the preceding paper shows that the compounds which increase

aerobic glycolysis are those which inhibit cozymase degradation, while those not increasing glycolysis have little or no action on cozymase degradation. A compound such as quinine as well as those named above, is included within this generalization.

The six compounds with largest effects on cozymase degradation and glycolysis were selected for a quantitative comparison of their effects on the two processes. These were the phenazine derivatives, phenosafranine, diazine green, neutral red; ethyl red, which is a quinoline derivative; and the pyridine derivatives nicotine and nicotinamide. The comparison is shown in Table 6. In this comparison, questions of the intracellular distribution of the different substances have not been taken into consideration. This appears justified because comparison has been between (i) the concentrations of substances added to suspensions of ground or homogenized brain in isotonic saline in which subcellular particles still exist as formed elements, and (ii) the amount of substance found to be reached within a given weight of the intact tissue, and expressed as an average concentration. Any selective absorption would be expected to be similar in the two cases, although an element of uncertainty remains.

In Table 6, columns A give the concentrations of the substances which are needed to raise aerobic glycolysis to arbitrarily chosen values. These concentrations were obtained from Fig. 4. The effects which these concentrations have on cozymase degradation were then found from concentration-action curves derived from data such as those of Tables 3, 5 and 6 of McIlwain (1950). These values are quoted in columns B, Table 6. There is a marked uniformity in the actions of four if not five of these compounds. Phenosafranine, diazine green, neutral red, and nicotine all raise aerobic glycolysis to 15 $\mu\text{mol./g./hr.}$ in concentrations which inhibit cozymase degradation by 52–65 %; they raise it to 30 $\mu\text{mol./g./hr.}$ in concentrations inhibiting degradation by 72–79 %. The actual concentrations of substances in the slices which are needed to produce a given effect on glycolysis or on cozymase differ in the different

Table 6. *Inhibition of cozymase degradation which is associated with given effects of different substances on aerobic glycolysis*

(Derivation of the values is described in the text.)

Added substance	Columns A, concentration (M) raising the rate of aerobic lactic acid formation to ($\mu\text{mol./g./hr.}$):			Columns B, inhibition of cozymase degradation caused by the concentration of substance which raises the rate of acid formation (col. A) to ($\mu\text{mol./g./hr.}$):		
	15	30	60	15	30	60
Phenosafranine	6×10^{-5}	1.6×10^{-4}	7×10^{-4}	58	79	approx. 95
Diazine green	7×10^{-5}	2.4×10^{-4}	—	52	75	—
Neutral red	1.4×10^{-3}	5.5×10^{-3}	—	58	72	—
Ethyl red	10^{-5}	1.5×10^{-5}	3×10^{-5}	<5	<5	<5
Nicotinamide	5×10^{-3}	—	—	82	—	—
Nicotine	6×10^{-3}	1.1×10^{-2}	—	65	73	—

compounds by a factor of 100. The concentrations which are required of them in solution to produce comparable effects, differ by over 10,000-fold. The approximation of the values in columns B with respect to these four compounds is therefore noteworthy. In the case of nicotinamide, a given increase in glycolysis was found to be associated with a somewhat greater inhibition of cozymase degradation. The difference is not, however, great (and nicotinamide, unlike the other compounds, could conceivably yield cozymase). The result with ethyl red, however, is radically different from that with the other compounds. Inhibition of cozymase degradation is not apparent until a concentration is reached which is 10–30 times that at which glycolysis is affected. The substance thus belongs to those discussed earlier (McIlwain, 1950) which increase aerobic glycolysis without affecting cozymase degradation. The relation between cozymase degradation and the disturbance of glycolysis is being examined further.

SUMMARY

1. Slices of guinea pig-brain cortex shaken aerobically in glucose saline absorbed a number of phenazine and quinoline derivatives (initially at 10^{-7} to 10^{-4} M) until their concentrations in the slices were 50–300 times those in the solutions. Phenosafranine, diazine green, neutral red, and ethyl red were among the substances studied quantitatively. Nicotine, and nicotinamide, initially at 10^{-3} to 8×10^{-2} M, were largely excluded from the slices.

2. Absorption from solutions, with 20–30 mg.

moist weight of slice/ml., took place gradually over a period of about an hour and at a maximum rate of about 1 μ mol./g. moist wt./hr.

3. The absorption was greater aerobically with glucose than anaerobically without glucose; continued metabolism of glucose was necessary for the phenazine and quinoline derivatives to be held in the tissue to their maximum extent.

4. The substances named above have the common property of increasing aerobic glycolysis in brain-cortex slices, when present in concentrations which have little or no effect on respiration. The course of their action on aerobic glycolysis has been followed. Observations have been made on the structural requirements in such compounds, for increase of aerobic glycolysis, by examining a number of related substances.

5. The actions of many of the substances on carbohydrate metabolism have been evaluated in terms of the concentrations of substances actually present in the slices. The relative potencies so obtained for the compounds differed by up to 100-fold from their relative potencies expressed in terms of the concentrations of the compounds present in the initial reaction mixtures.

6. The concentrations of substances in the slices have been used as the basis for comparing the actions of the substances on carbohydrate and on cozymase metabolism.

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