LXXXIX. THE EFFECT OF METALLIC SALTS ON THE GLYCOLYSIS AND RESPIRATION OF TISSUES[1].

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The importance of glycolysis in the metabolism of malignant tissues has been established by the work of Warburg and his collaborators [1924–1927]. Warburg has at the same time developed satisfactory methods of measuring *in vitro* the respiration and glycolysis occurring in tissues. By glycolysis is meant the splitting of glucose to lactic acid.

Warburg's findings may be summarised in the statements: (*a*) glycolysis under *anaerobic* conditions takes place to any considerable extent only in growing tissues, and (*b*) glycolysis under *aerobic* conditions takes place to any considerable extent only in malignant tissues. Glycolysis under aerobic conditions is always less than under anaerobic, respiration having the power of lessening glycolysis by an amount in proportion to its own magnitude, this effect being termed the Pasteur reaction.

These *in vitro* findings have been confirmed by the work of Rona and Deutsch [1926] and of Murphy and Hawkins [1925]. Confirmatory work *in vivo* has been carried out by Tadenuma, Hotta and Homma [1924], Cori and Cori [1925], and by Warburg, Wind and Negelein [1926].

The importance of these results for the problems of malignant disease suggested that an investigation of the effects of some heavy metal salts on glycolysis and respiration in tissues would be of interest, especially in view of the use of lead compounds as therapeutic agents in cases of malignant disease by Blair Bell *et al.* [1926].

The present paper is an account of such an investigation. At least one result of importance has been obtained, namely that several metallic salts have a poisoning action on glycolysis and respiration which is progressive with time, the poisoning proceeding as a unimolecular reaction. The rate of the metabolic process thus decreases as an exponential function of the time. Such an exponential effect, so far as we are aware, has not hitherto been

[1] This investigation was undertaken on behalf of the Liverpool Medical Research Organisation (Director: Prof. W. Blair Bell, University of Liverpool).

described for the action of poisons on any enzyme process, whether intracellular or extracellular.

Rahn [1916], in discussing theoretically the effect of poisons on enzyme processes, suggested that poisons would have a dual action, accelerating both the rate of enzyme action and the rate of enzyme decomposition, so that an initial acceleration of the process would be followed by retardation owing to inactivation of the enzyme. It was suggested that the enzyme concentration would decrease exponentially with time, but no quantitative evidence was brought forward to support this hypothesis. It was also suggested that the accelerative phase would be greater in dilute solutions of the poison. These views form a quantitative expression of the so-called Arndt-Schulz law, to which such work as that of Neuberg and Sandberg [1920] on yeast fermentation appears to lend support. Doubt has been cast on the reality of the accelerative phase in the action of poisons on the aerobic fermentation of yeast by the work of Meier [1926], who showed that the apparent acceleration by mercuric chloride is due to the neglected effect of the poison on respiration. Schoeller and Gehrke [1926] have however found alkaloids to exert at low concentrations a stimulating effect on the anaerobic glycolysis of yeast. While in the present work we have not found accelerations by metallic salts in most cases, lead appears able to produce accelerations.

Other workers on the action of heavy metals on enzyme processes, whether extracellular [Ollson, 1921] or intracellular [Meier, 1926], have found the effect of metallic poisons to be progressive with time, but the effects do not appear to be an exponential function of time.

Cook [1926] studied the effect of salts of heavy metals on the respiration of *Aspergillus niger*. His results show a time-effect of poisons on the rate which follows a formula for the concentration of an intermediary product in consecutive reactions, but his theoretical considerations appear to be unsound. A latent period was observed, and sometimes an initial acceleration.

The most serious attempt to analyse the effect of heavy metals on enzyme processes appears to be that of Euler and Svanberg [1919] and Euler and Walles [1924], who found the action of heavy metal salts on saccharase, whether isolated or within the cell, to be very different from that found by us for the processes studied in this paper. An initial poisoning was observed, which disappeared with the passage of time, the enzyme being gradually regenerated.

The glycolysis and respiration of some tissues.

Although the main object of the present work has been to study the effect of metallic salts on glycolysis and respiration, some measurements of the absolute values of these metabolic rates have been made. These are summarised in Table I, as they may be of interest in connection with similar data obtained by other observers.

Table I. *Metabolism of various tissues.*

Tissue	Q_{O_2}	$Q_{CO_2}^{O_2}$	$Q_{CO_2}^{N_2}$	R.Q.	U
Rat sarcoma R 10	11	—	42	—	+20
Flexner rat carc.	6	18	28	—	+16
Carc. omentum (hum.)	—	—	13	—	—
Carc. mammae (hum.)	8	10	20	—	+ 4
Rat liver	9·7	0	1·4	0·96	−18
Rat spleen	—	—	10·0	—	—
Rat testis	—	—	9·9	—	—
Rabbit spleen	12	5	—	—	—

Q_{O_2} (Respiration) = mm.³ oxygen consumed per hr. per mg. tissue (dry weight).

$Q_{CO_2}^{O_2}$ (Aerobic glycolysis) = mm.³ carbon dioxide evolved per hr. per mg. tissue (dry weight) in presence of oxygen, beyond the evolution due to respiration.

$Q_{CO_2}^{N_2}$ (Anaerobic glycolysis) = mm.³ carbon dioxide evolved per hr. per mg. tissue (dry weight) in absence of oxygen.

The carbon dioxide evolved in glycolysis is derived from neutralisation by bicarbonate of the lactic acid formed. Warburg assumes [1927] that under limiting conditions of efficiency of the Pasteur reaction, such as may be found *in vivo*, the aerobic glycolysis will be equal to the "excess fermentation," U, which is defined by the relation

$$U = Q_{CO_2}^{N_2} - 2Q_{O_2}.$$

A large positive value of U is a characteristic of malignant tissue.

Returning now to a consideration of Table I, we should state that the values given there represent in most cases the mean values from a number of experiments. They are in the main very similar to those recorded by other workers [Cannan, 1927].

A number of experiments on the anaerobic glycolysis of rat tumours showed that the value of $Q_{CO_2}^{N_2}$ is not altogether constant[1]. Thus in a young, rapidly growing tumour (R 10) $Q_{CO_2}^{N_2}$ showed a value of 60, as against the average value of 42.

The data given for the Flexner rat carcinoma are very similar to those of other workers, those in the table being a mean from four experiments carried out in duplicate. Nine experiments on the anaerobic glycolysis of the same tumour, four or five slices being taken in each experiment, gave a mean value for $Q_{CO_2}^{N_2}$ of 32, which is practically identical with the value of other workers. Here again, however, one experiment showed a value of 53.

The human tumours reported on were diluted with non-malignant tissue.

Other workers have reported a slight aerobic glycolysis in the case of liver, but in two out of three experiments we have found none, the values of the respiratory quotient observed being 0·885, 0·955 and 1·040. The measurements refer to a one-hour period.

In the case of spleen, our results are confirmatory of those of Murphy and Hawkins [1925], who found aerobic glycolysis. This we find apparently to increase with time, which may be explained as due to a progressive damaging of the mechanism of Pasteur's reaction.

[1] For an example see Protocol 1, p. 736.

Experimental methods.

In the present work, the apparatus and methods of Warburg have been used for the measurement of respiration and glycolysis. The experimental details are fully described in Warburg's papers [1924, 1926]. The method is a manometric one[1]. A slice of the tissue under investigation, thin enough (less than 0·5 mm. in thickness) to allow adequate diffusion of the metabolites throughout, is shaken in a suitable medium, at a temperature of 37·5° and the gas exchange is observed.

Glycolysis. The medium used was either Ringer's solution or horse serum to which 0·2 % glucose had been added. The Ringer's solution was prepared by mixing isotonic solutions (*i.e.* solutions of freezing point − 0·56°) of the various salts and unless otherwise stated had the following composition:

Salt	Moles per litre
NaCl	0·139
KCl	0·0029
$CaCl_2$	0·0020
$NaHCO_3$	0·0191

The gas-mixtures used for saturating the media (and as the gas-phase in the experimental vessels) were 5 % carbon dioxide in oxygen for the measurement of aerobic glycolysis, and for anaerobic glycolysis 5 % carbon dioxide in nitrogen. The p_H of the medium is then given by the Henderson-Hasselbalch equation [Hasselbalch, 1917]. In the present case, the p_H at 37·5° was 7·35.

The quantities measured are the amounts of oxygen consumed by the tissue and the amount of carbon dioxide evolved. The latter may arise from respiration and from glycolysis (lactic acid formed liberates carbon dioxide from the bicarbonate of the medium).

The anaerobic glycolysis can be determined from an experiment with one tissue slice. For the aerobic glycolysis, it is necessary to carry out an experiment with two slices of tissue, each in its own vessel with a different volume of medium. When serum is used as medium additional experiments are required, as the formation of a given amount of lactic acid does not liberate an equivalent amount of carbon dioxide [Warburg, 1925].

Respiration. The medium used was a Locke's solution containing 0·2 % glucose, and having the following composition:

Salt	Moles per litre
NaCl	0·158
KCl	0·0033
$CaCl_2$	0·0023

The solution was saturated with oxygen, which also formed the gas-phase in the experimental vessels. The vessels have an additional compartment containing a concentrated solution of NaOH which absorbs the carbon dioxide evolved by the tissue. The quantity measured is therefore the amount of oxygen consumed by the tissue.

[1] For a method of calibration of the Barcroft manometer which was found useful, see previous paper by Jowett [1928].

In view of the sensitivity of the Pasteur reaction to experimental injury the experiments on the action of metallic salts have been confined to the effects exerted on glycolysis under anaerobic conditions, and on respiration under conditions where no glycolysis takes place (*i.e.* in Locke's solution).

Over the periods of time covered by the majority of the experiments, the tissue slices did not usually maintain a constant rate of glycolysis or respiration. A slow falling off in rate was usually observed after 30–60 minutes. For this reason the following procedure was adopted.

The rate of glycolysis or respiration of a number of slices of the tissue under investigation in different vessels was observed over a period of 30 mins. The substance to be tested was then added to several of the vessels, no addition being made to two of the vessels, which then acted as "controls." The rates were then followed for all the vessels until slices similarly treated began to show divergent behaviour or the "control" slices fell off considerably in rate.

It was assumed that the effect of the added substance on the rate of glycolysis or respiration was an effect superimposed on any spontaneous falling off in rate observed in the case of the two "control" slices, and the effect was calculated on this basis. The results were only accepted when the behaviour of duplicates was in good agreement.

The observed effects on the rate of glycolysis or respiration are considered to be accurate within 2–5 %.

The mode of addition of the substance under investigation to the vessels containing tissue slices was such that no appreciable change was brought about in the bicarbonate content or isotonicity of the medium (with the exception of the experiments where lead acetate solution was added directly to vessels containing Ringer's solution). The volume of added solution or suspension was always small, except in some experiments with calcium chloride. In those cases where a slightly soluble salt (*e.g.* $PbCO_3$) was used the solid was added directly or in the form of a suspension in the medium, while soluble salts (*e.g.*, $ZnCl_2$ or $CdCl_2$) were added in the form of a solution in the medium.

The majority of the experiments were carried out with rat tissues. Two transplantable rat tumours have been used, the Flexner-Jobling rat carcinoma and a rat sarcoma R 10. In a few cases human malignant tissues have been studied.

Experiments with mercuric halides.

Under the conditions of concentration employed in the present work, mercuric halides have proved to have the most rapid action of any of the salts studied. As experiments *in vitro* have been limited to a few hours' duration, the action of mercury salts has thus been the most amenable to quantitative investigation and analysis.

In particular, the exponential law already referred to has been most readily established for mercuric halides.

One experiment will be given in some detail to illustrate the nature of our observations in this connection. The rates of glycolysis given in Table II are stated as percentages of the initial rate (*i.e.* the rate before mercuric chloride was added to the medium)[1]. The average rate over a period is referred to the mid-point in time of the period, and the times stated are the number of minutes that have elapsed since the addition of the mercury salt. In each case a slice of rat spleen of about 5–6 mg. dry weight was shaken in about 5 cc. of Ringer's solution containing initially the stated concentration of mercuric chloride. (The concentration decreases considerably and rapidly owing to adsorption by the tissue: evidence of this will be given later.)

Table II. *Exp.* 57. *Effect of mercuric chloride on glycolysis of rat spleen.*

	M/5000 $HgCl_2$		*M*/24,000 $HgCl_2$		*M*/97,000 $HgCl_2$	
Time (mins.)	Rate observed	Rate calculated	Rate observed	Rate calculated	Rate observed	Rate calculated
25	100	84	95	93	97	97
70	53	62	78	82	93	92
115	41	45	72	73	85	88
145	37	39	67	67	86	85
208	25	24	57	56	79	79
275	21	15	49	47	79	73

The rates of glycolysis calculated and given in Table II, which are in fair agreement with the observed values, are obtained from the relation

$$V = V_0 . e^{-\alpha t},$$

where V_0 is the initial rate, V is the rate at time t (t being measured in hours), and α is a coefficient giving a measure of the effect of the poison on the rate of the process. The values of α used in the above table were 0·414, 0·166, 0·07.

In all our other experiments the effect of mercuric halides on glycolysis and respiration of tissues was found, within the experimental error, to follow this law, the values of α naturally varying from case to case. The simplest explanation of the exponential law is that the rate of action of the enzyme is unaffected by mercury except in so far as the enzyme is inactivated by the mercury, and on this assumption the inactivation is (stoicheiometrically) a unimolecular reaction.

For further analysis of the action of mercury, it must be decided which is the slower, and therefore the controlling process, (*a*) the diffusion of mercuric chloride into the tissue, or (*b*) the reaction of mercuric chloride present in the tissue with the glycolytic enzyme.

Experiments were made in which tissue-slices (of Flexner rat carcinoma) were placed for 20–30 minutes in Locke's solution at room temperature containing mercuric chloride, and, after washing, their rate of glycolysis again

[1] For fuller details see Protocol 2, p. 737.

measured in Ringer's solution containing no mercury. The rate was lower than that prior to the mercury treatment, but remained at a steady value, instead of falling off as it would have done if mercuric chloride had been present throughout.

The experiments show that the action of mercury is irreversible under our experimental conditions, but do not decide between the possibilities (*a*) and (*b*) above. A steady lower rate is consistent with either possibility.

Experiments such as the following were therefore carried out. After measuring the glycolysis of several slices of rat testis in Ringer's solution a solution of mercuric chloride in Ringer's solution was added to some of the slices (from the additional compartment with which the vessels were fitted). After 4 and 12 minutes' further shaking respectively in the thermostat, two of the slices were removed from their vessels, washed in Ringer's solution, and placed in vessels containing Ringer's solution alone, and their glycolysis was again measured. Another slice was left in contact with the Ringer's solution containing $HgCl_2$ throughout the experiment. The results obtained are given in Table III.

Table III. *Exp.* 114. *The effect of varying times of immersion in M/5000 $HgCl_2$.*

Time after addition of $HgCl_2$ (mins.)	Rates (relative to controls = 100)		
	Tissue slice in contact with $HgCl_2$ throughout	Tissue slice immersed for 4 mins.	Tissue slice immersed for 12 mins.
10–25	37	—	—
25–40	29	33	33
40–70	20	30	28
70–85	16	32	34

If a slow diffusion of mercuric chloride into the tissue were the controlling process in the inactivation, the effect of 12 minutes' immersion in $HgCl_2$ on the glycolytic rate should have been much greater than that of 4 minutes' immersion. This is evidently not the case.

The alternative view must therefore be correct, namely that diffusion of mercuric chloride into the tissue is rapid compared with the subsequent inactivation of the glycolytic enzyme.

Evidently in 4 minutes or less diffusion equilibrium is more or less set up between tissue and medium. Diffusion into thin plates has been treated by McBain [1910], and on the basis of his treatment, if we assume that mercuric chloride diffuses through tissues at the same rate as through water, it can be calculated that equilibrium will be set up in 1–2 minutes. It thus appears that mercuric chloride does diffuse through tissues about as readily as through water.

We must suppose that on removing the tissue from the mercuric chloride solution the $HgCl_2$ which is free or reversibly bound by the tissue is redistributed between the tissue-slices and the new medium, the concentration in

the tissue being very much lowered by this process, so that the glycolytic enzyme suffers little further inactivation beyond that which it has already, irreversibly, undergone.

It was mentioned earlier that a considerable proportion of the mercuric chloride added to the Ringer's solution is removed by the tissue. This is shown by such experiments as that given in Table IV, in which α, the poisoning-coefficient, is shown to be dependent on the volume of mercuric chloride solution on which the tissue-slice can draw.

Table IV. *Exp.* 123. $M/5000$ $HgCl_2$. *Glycolysis of rat spleen.*

Cc. of solution	1·9	3·8	7·6
Weight of tissue (mg.)	7·9	8·2	6·9
Cc. of solution per mg. of tissue	0·24	0·46	1·1
α	0·26	0·36	0·55

A similar experiment with rat testis showed a similar result, namely, that α increases with the volume of $M/5000$ mercuric chloride from which unit weight of tissue can take up the salt. The explanation is that after the rapid taking up of mercury the concentration in the medium is no longer $M/5000$ but lies considerably below this value, the decrease being less the larger the volume the tissue can draw from, and α being reasonably taken to depend on the *equilibrium* concentration of mercury in the medium. That is, we take it that for mercuric chloride an adsorption equilibrium is set up rapidly between tissue and medium.

The considerable effect of the amount of adsorption on α makes it necessary to be cautious in making deductions from values of α obtained for different tissues even if the same volume of $M/5000$ mercuric chloride is available per mg. of tissue, as the specific adsorption may vary from tissue to tissue. Estimates of the "sensitivity" of glycolysis in various tissues to mercury can evidently not be made in this way, except in an uncertain qualitative manner.

We do not therefore propose to report a number of experiments of this kind that we have made, but pass on to a type of experiment in which the difficulty does not arise.

In the experiments to be reported, solid mercuric iodide was added to the Ringer's solution in place of mercuric chloride, and the effect of saturated HgI_2 solution on glycolysis and respiration measured. In this way we are studying the effect of a constant concentration (or more strictly, constant thermodynamic activity) of mercuric iodide on the processes.

Mercuric iodide apparently dissolves rapidly enough to keep the media sufficiently near to saturation. This was shown in an experiment where slices of tissue placed in Ringer's solution already saturated with mercuric iodide (solid also being present), showed the same decrease in glycolysis as slices placed in Ringer's solution to which solid mercuric iodide was then added.

It should be mentioned that neither mercuric chloride nor mercuric iodide is appreciably ionised. Abegg [1903] has studied the various equilibria

concerned at 25°. On the basis of his work the figures in Table V have been calculated roughly, and they will be applicable at least in point of order of magnitude to the temperature 37·5°.

Table V. *Concentrations in moles per litre.*

	M/5000 $HgCl_2$ Locke	Saturated HgI_2 Locke	Saturated HgI_2 Ringer
$HgCl_2$	$0{\cdot}6 \times 10^{-4}$	$1{\cdot}2 \times 10^{-6}$	$1{\cdot}3 \times 10^{-6}$
$HgCl_4''$	$1{\cdot}4 \times 10^{-4}$	$2{\cdot}9 \times 10^{-6}$	$2{\cdot}4 \times 10^{-6}$
$HgCl'$	$1{\cdot}1 \times 10^{-10}$	$2{\cdot}0 \times 10^{-12}$	$2{\cdot}4 \times 10^{-12}$
$Hg^{\cdot\cdot}$	$2{\cdot}0 \times 10^{-17}$	$5{\cdot}0 \times 10^{-19}$	$6{\cdot}0 \times 10^{-19}$
HgI_2	—	$1{\cdot}3 \times 10^{-4}$	$1{\cdot}3 \times 10^{-4}$
HgI_4''	—	$4{\cdot}2 \times 10^{-9}$	$3{\cdot}4 \times 10^{-9}$
HgI'	—	$1{\cdot}0 \times 10^{-10}$	$1{\cdot}1 \times 10^{-10}$
I'	—	$8{\cdot}2 \times 10^{-6}$	$7{\cdot}4 \times 10^{-6}$

Evidently for the action of mercuric chloride the chemical species $HgCl_2$ and $HgCl_4''$ are important. One or both of these, perhaps rather $HgCl_2$, will be the principal form in which mercury penetrates tissues. Which form is actually responsible for the effect on the glycolytic enzyme must be left undecided, but it appears very improbable that the mercuric ion plays a part at such low concentrations.

Similarly with mercuric iodide it is the un-ionised HgI_2 which predominates in amount, and it will probably be the principal form in which mercury will penetrate tissues in our experiments. It is reasonable to suppose that adsorption equilibrium with the tissue is rapidly set up in the present case. This supposition was confirmed by an experiment of a similar nature to those given earlier for mercuric chloride. Slices of tissue were immersed for varying periods of a few minutes in a large volume of Ringer's solution saturated with HgI_2 but containing none of the solid salt. Their subsequent rate of glycolysis was fairly similar and constant when compared with that of a tissue-slice shaken all the time in Ringer's solution saturated with HgI_2.

Experiments were undertaken in which various concentrations of sodium iodide were incorporated in the Ringer's or Locke's solution (sodium iodide replacing sodium chloride). The iodide was found to have no effect on the rate of glycolysis. It has however a considerable effect on the action of mercuric iodide on glycolysis and respiration. The results are given in Table VI, in which α has the same significance as before[1].

Table VI. *Effect of mercuric iodide in presence of sodium iodide.*

Exp. No.	Process	Values of α: Without NaI	*M*/1000 NaI	*M*/100 NaI	*M*/10 NaI
133	Anaerobic glycolysis of rat spleen	0·8	—	1·7	—
134	Anaerobic glycolysis of rat spleen	—	1·2	1·6	2·1
137	Anaerobic glycolysis of rat testis	1·0	1·7	2·0	2·4
139	Respiration of rat testis	2·1	2·2	2·4	3·4

The effect of NaI may be explained on the basis of its effect on the concentration of HgI_4'' in the medium. The concentration of HgI_2 (calculated for

[1] See also Protocol 3 (p. 737).

25°) is throughout $1{\cdot}3 \times 10^{-4}$ molar, while as we pass from the Ringer's solution containing no added sodium iodide to that containing $M/10$ sodium iodide, the concentration of HgI_4'' increases as shown in Table VII, calculated roughly on the basis of Abegg's figures for 25°.

Table VII. *Effect of iodide ion on HgI_4''.*

Added NaI	I′	HgI_4''	$Hg^{••}$	Total concentration of Hg compounds
0	7×10^{-6}	3×10^{-9}	6×10^{-19}	$1{\cdot}3 \times 10^{-4}$
0·001	9×10^{-4}	5×10^{-5}	4×10^{-23}	$1{\cdot}8 \times 10^{-4}$
0·01	6×10^{-3}	$2{\cdot}1 \times 10^{-3}$	1×10^{-24}	$2{\cdot}2 \times 10^{-3}$
0·1	$2{\cdot}5 \times 10^{-2}$	$3{\cdot}8 \times 10^{-2}$	5×10^{-26}	$3{\cdot}8 \times 10^{-2}$

In the presence of added sodium iodide the only mercury compounds present in any appreciable amount are HgI_2 and HgI_4''. The concentration of mercuric ion decreases from a very low value to a vanishingly small one.

If we now suppose that α is a function of the total concentration of mercury compounds in the medium (HgI_2 and HgI_4'' being assumed for this purpose to be equally effective), the values of α can be approximately reproduced as a linear function of this concentration raised to the power 0·1 (the last three experiments in the table above giving values for the exponent of 0·11, 0·08 and 0·10). This exponent is a not unlikely one for the adsorption of ($HgI_2 + HgI_4''$) by tissues or by some important tissue constituent.

It is thus not improbable that α is proportional to the amount of mercury adsorbed by the tissue as a whole, by the enzyme itself, or by some structural constituent with which the enzyme is associated. Minami [1923] has made it probable, by a consideration of experiments with narcotics, that the glycolytic enzyme acts at the surface of cell-structures.

We can therefore with some justification advance a theory of the mechanism of the inactivation of the glycolytic enzyme by mercury halides. The halide is rapidly adsorbed, the adsorption with which we are concerned being either (*a*) adsorption by the enzyme itself, or (*b*) adsorption by structures lying near the enzyme. The process of inactivation then consists in either (*a*) a comparatively slow change of the mercury-enzyme adsorption compound into an inactive form of the enzyme in which the mercury is bound by an essential chemical group of the enzyme, or (*b*) a similar combination of mercury and enzyme, the mercury passing over to the enzyme from structures nearby where it is adsorbed. In either case, the rate of inactivation would be proportional to the concentration of enzyme still active, and also probably to the amount of mercury adsorbed.

This relation finds its mathematical formulation in the equation

$$-\frac{dE}{dt} = k.E.c^{1/n},$$

where E is the active mass of the enzyme at the time t, and c the concentration of mercury compounds (HgI_2 and HgI_4'') in the medium (and also, it must be assumed, in the tissue fluids), and $1/n$ is the adsorption exponent.

When integrated, this relation becomes

$$E = E_0 . e^{-kc^{1/n} t},$$

where E_0 is the active mass of enzyme when $t = 0$, *i.e.* at the time of addition of the poison. If we now take V, the rate of the process, as proportional to the active mass of the enzyme, this equation is identical with that deduced experimentally earlier in this section, provided that $\alpha = kc^{1/n}$. For ($HgI_2 + HgI_4''$) the experimental value of $1/n$ was found to be 0·1. We take it that k will be approximately constant for any one tissue.

Values of α for media saturated with mercuric iodide have been determined, and are given in Table VIII. The anaerobic glycolysis was studied in Ringer's solution, and the respiration in Locke's solution (containing no bicarbonate). That the medium has no effect on the value of α was shown by two experiments in which α for $M/5000$ mercuric chloride was measured for the respiration of rat liver both in Locke's solution and in Ringer's solution, care being taken that the weight of tissue per unit volume was kept approximately constant in each experiment. In one experiment α (Locke) was 0·45, α (Ringer) 0·44; in the other α (Locke) was 0·37, α (Ringer) 0·38. The sensitivity of the process is thus the same in the two media.

Table VIII. *Values of α for saturated HgI_2.*

Tissue	α (glycolysis)	α (respiration)
Rat sarcoma	2·8	1·3
„ testis	1·9, 1·0	2·6, 2·1
„ spleen	1·15, 0·84	1·15
„ kidney	0·9	1·1

The glycolysis of malignant tissue is evidently more sensitive to HgI_2 than that of normal tissues. Furthermore, while in the case of normal tissues respiration is a more sensitive process than glycolysis, the reverse is the case for the rat sarcoma. The experiments with mercuric chloride show the same order for the various tissues in respect of glycolysis, the tumours being the Flexner rat carcinoma and a human cancer of the breast; they also show that the respiration of testis is more sensitive than its glycolysis. In solutions of (initially) $M/100{,}000$ $HgCl_2$ slight retardation of glycolysis could be observed. With $M/1{,}000{,}000$ $HgCl_2$ no effect on the glycolysis of Flexner rat carcinoma was observed.

The effects of copper, zinc, cadmium and calcium salts on glycolysis.

The effect of several metallic salts on glycolysis has been investigated in less detail than in the case of mercury.

Copper salts. The salts used were cupric chloride and sulphate. An $M/100$ solution was diluted several times with Ringer's solution and the resulting suspension added to the experimental vessels containing tissue slices whose rate of glycolysis had previously been observed. The amounts added were such as to make the concentration of copper in the medium initially $M/5000$

if all the added precipitate had dissolved, of which we are not certain. In the experiments given in Table IX where values of α are given to two significant figures, the resulting decrease in rate certainly followed the exponential relation previously discussed. Some evidence was obtained that the values of α were not so dependent on the relative amounts of tissue and medium as in the cases where mercuric chloride was used, and hence are more comparable for different tissues.

Table IX. *Effect of M/5000 $CuCl_2$ on glycolysis.*

Tissue	α
Flexner rat carcinoma	0·3, 0·6
Carc. omentum (human)	0·58
Rat spleen	0·23, 0·22
Rat kidney	0 — 0·1

Lower concentrations of copper salts had a smaller effect on glycolysis. From these results it seems probable that copper has a greater effect on the glycolysis of malignant tissues than on that of normal tissues.

In one experiment on the respiration of rat kidney in Locke's solution α for $M/5000$ $CuCl_2$ was found to be 0·45. This suggests that, as in the case of mercury, respiration of normal tissues is more sensitive than glycolysis.

Rat testis behaves anomalously to copper salts; the exponential law is definitely not followed. After a rapid fall in the rate of glycolysis, the rate remains fairly constant, as is illustrated in Table X.

Table X. *Exp.* 136. *Effect of M/5000 $CuCl_2$ on glycolysis of rat testis.*

Time (mins.)	% rate	Time (mins.)	% rate
19	57	24	49
39	48	54	45
69	46	84	41
124	46	129	42

The effect has not been further investigated. It is the only case of those studied in which, for copper or mercury salts, the exponential law definitely does not hold. For the other tissues the more accurate the experiment appears to be, the more accurately is the law followed; in some the agreement is only moderate, but no consistent divergences have been found, such as would suggest that some other law should be preferentially adopted.

Zinc, cadmium and calcium salts. The action of the chlorides of zinc, cadmium and calcium on the anaerobic glycolysis of Flexner rat carcinoma is given in Table XI. The effects, being small, have not been studied in detail.

Table XI. *Glycolysis of Flexner rat carcinoma.*

Exp. No.	Salt	Initial concentration Moles/litre	Percentage effect on rate 0–30 mins.	30–120 mins.
39	$ZnCl_2$	0·00018	+6	−13
40	,,	,,	−1	−15
50	$CdCl_2$	0·0017	+4	−14
54	,,	0·0002	−1	− 6
42	$CaCl_2$	0·0065	−3	0
53	,,	0·0205	+2	− 2
60	,,	0·054	−1	− 4

It can be seen that zinc ion, and cadmium ion to a smaller extent, depress somewhat the rate of glycolysis. The effect becomes more considerable as time passes, and possibly follows an exponential law.

Calcium ion has no appreciable effect over a wide range of concentration (the concentration already present in the Ringer's solution is 0·0022 *M*). Our result differs from that of Waterman [1924], who found that calcium salts depressed tumour glycolysis considerably. In our experiments we maintained the bicarbonate concentration of the medium at a constant value when any appreciable volume of isotonic calcium chloride was added. Neglect of this or other measures might give false results.

The effect of lead salts on glycolysis.

The effects of lead salts on glycolysis (and respiration) have been found to be small over the short periods of time necessarily employed. For this reason it was impossible to study in any detail the change in effect with time.

A series of experiments was carried out in which solutions of lead acetate were added to the experimental vessels containing slices of tumour tissue (suspended in Ringer's solution) whose glycolysis had previously been measured. With Ringer's solution as medium the p_H and bicarbonate concentration necessarily limit the concentration of lead ion attainable in the experimental vessels to a very small value.

The amount of lead acetate added was always in excess of the solubility of lead carbonate, which was precipitated. The p_H and bicarbonate concentration of the medium were thereby decreased, which leads *per se* to a decrease in the rate of glycolysis. The effect of varying these two factors on the anaerobic glycolysis of Flexner rat carcinoma has been determined quantitatively by Warburg, Posener and Negelein [1924]. Corrections calculated from their data were applied to the observed rates for (*a*) lowering of p_H and [$NaHCO_3$] due to addition of lead acetate; (*b*) lowering of p_H and [$NaHCO_3$] due to the production of lactic acid by glycolysis (a small correction).

Table XII. *Exp.* 18. *Flexner rat carcinoma.*

Volume of medium 5·0 cc. To each vessel was added 0·55 cc. of the following solutions after 30 mins.

	Lead acetate			
	M/50	*M*/500	*M*/2000	Water
Observed $Q_{CO_2}^{N_2}$ before addition	23·0	22·8	30·8	23·7
Observed $Q_{CO_2}^{N_2}$ after addition	17·7	19·2	27·0	20·0
Corrected $Q_{CO_2}^{N_2}$ before addition	23·2	23·0	31·1	23·8
Corrected $Q_{CO_2}^{N_2}$ after addition	22·0	20·1	27·9	20·4
Percentage change in $Q_{CO_2}^{N_2}$ (calculated from corrected values)	− 5	−12·5	−10	−14
Percentage change in $Q_{CO_2}^{N_2}$ relative to "control"	+11	+ 2	+ 5	—

The corrected rates of glycolysis refer therefore to the initial conditions of p_H and bicarbonate concentration, in these cases 7·23 and 0·0143 M respectively, and any alteration observed can be attributed to the specific effect of lead. The results of a typical experiment are given in Table XII.

The effect of lead is taken to be given by the percentage change in the corrected rates relative to the control. It will be seen that the rate of glycolysis is slightly accelerated by the addition of lead acetate. This result has been confirmed by other experiments which are summarised in Table XIII. The amounts of lead acetate added were similar to those given in the experiment quoted above. The experiments in the table are arranged in order of the p_H and calculated lead ion concentration[1] obtained after addition of lead acetate. The change in rate refers to the total change observed over the period investigated after the addition, which in no case exceeded 90 minutes.

Table XIII.

Exp. No.	p_H	Concentration of lead ion Moles/litre × 10^7	Tumour	Percentage effect on the rate of anaerobic glycolysis
18	7·20	1·0	F.R.C.	+ 5
18	7·19	1·0	F.R.C.	+ 2
23	7·18	1·1	R 10	+24
21	7·14	1·3	R 10	− 5
21	7·13	1·4	R 10	+ 9
18	7·06	1·9	F.R.C.	+11
23	7·01	2·4	R 10	+15
24	7·00	2·5	R 10	+27
16	6·96	3·1	F.R.C.	+33
21	6·93	3·6	R 10	+16

It will be seen that, although the observed accelerations are somewhat irregular, they are roughly proportional to the lead ion concentration.

These results are open to objection on the grounds that the corrections applied were often large and may have been somewhat in error. Experiments were therefore undertaken which should be free from this objection, the lead being added in the form of a suspension of lead carbonate in Ringer's solution.

The $PbCO_3$ suspension was prepared by adding 1 cc. $M/100$ lead acetate to 4 cc. Ringer's solution and a sufficient volume of this suspension was added to the experimental vessel to make the total amount of lead present equal to that in a $M/5000$ solution. In these experiments a Ringer's solution with a bicarbonate concentration of 0·0102 M was used, of which the p_H was 7·08. The resulting lead ion concentration in the experimental vessels would therefore be approximately $1{\cdot}7 \times 10^{-7}$ M.

In three cases horse serum was used as medium, and to the experimental vessels was added a mixture prepared by adding 1 cc. $M/100$ lead acetate to 4 cc. serum. The lead ion concentration would be rather less than the value in Ringer's solution, corresponding to the smaller solubility of lead phosphate

[1] These values are calculated on the basis of the data on the solubility of lead carbonate of Pleissner [1907].

in serum. The total amount of lead present was equivalent to $M/5000$ in each case in the experimental vessels.

The results of these experiments are summarised in Table XIV. The percentage change observed in the rate of glycolysis is given for two consecutive periods after the addition of lead carbonate (with the exception of Exp. 31).

Table XIV. *Effect of lead carbonate on anaerobic glycolysis.*

For Ringer's solution $[NaHCO_3] = 0{\cdot}0102\ M.$, $p_H = 7{\cdot}08$, $[Pb^{\cdot\cdot}] = ca.\ 1{\cdot}7 \times 10^{-7}\ M.$

Exp. No.	Tissue	Medium	% change in rate after addition of $PbCO_3$	
			0–30 mins.	30–120 mins.
31	F.R.C.	Ringer	—	+28 (0–120 mins.)
37	,,	,,	+ 8	0
38	,,	,,	+13	—
65	,,	,,	+ 4	+2
34	,,	Serum	+26	—
43	,,	,,	+35	+7
47	,,	,,	+ 7	−8
58	Rat spleen	Ringer	− 2	−7
66	,,	,,	+ 8	−4

The results are in agreement with those previously obtained. Lead causes an acceleration of the glycolysis of Flexner rat carcinoma in both Ringer's solution and serum. This acceleration becomes smaller as time passes and in one case (Exp. 47) changes to a retardation. The experiments with spleen show smaller effects of lead but the general behaviour is similar, the acceleration passing over more quickly to a retardation.

These results are of interest in connection with the view previously referred to that an enzyme poison at low concentrations acting over a short period of time may produce acceleration of the process, the effect becoming a retardation as time or concentration (or both) are increased.

In attempting to obtain further information on this point the impossibility was encountered of raising the lead ion concentration in the Ringer's solution to a much higher value and still maintaining conditions (of p_H and $[NaHCO_3]$) under which glycolysis could take place. The following procedure was therefore adopted. The anaerobic glycolysis of a number of tissue slices was measured in the usual way over a 30-minute period. Some of these slices were removed from the experimental vessels and, after washing in Locke's solution, immersed for a given period in 10 cc. of Locke's solution (at room temperature) containing various concentrations of lead acetate. After washing in Locke's solution and Ringer's solution their glycolysis was again measured. At least two control slices were treated in a similar way, the Locke's solution in which they were immersed containing no lead acetate. In the experiments given in Table XV the time of immersion was 30 minutes in each case. The times subsequent to immersion over which the glycolysis was measured varied from 45 to 75 minutes.

Table XV. *Effect of immersion in lead acetate (in Locke's solution) on subsequent anaerobic glycolysis.*

Exp. No.	Tissue	Concentration of lead acetate	Percentage effect on rate
87	F.R.C.	*M*/1000	−25
96	,,	*M*/2000	−14
83	,,	*M*/5000	−13
87	Rat spleen	*M*/1000	+ 5
86	,,	*M*/2000	− 5
85	,,	*M*/2000	+ 6
103	Rat testis	*M*/1000, *M*/3000, *M*/10,000	− 2, +1, −1
118	,,	*M*/1000, *M*/3000, *M*/10,000	− 4, −8, 0

It will be seen that relatively large concentrations of lead ion bring about an appreciable retardation in the subsequent rate of glycolysis of tumour tissue. Rat spleen and rat testis are evidently less sensitive to the action of lead ion, the effects lying within the experimental error.

These results are consistent with the view discussed in the introduction, that an enzyme poison which in small concentrations causes an acceleration of the process may, when its concentration is greatly increased, give rise to a retardation.

THE EFFECT OF LEAD SALTS ON RESPIRATION.

The effect of lead acetate on the respiration of various tissues has been studied. With Locke's solution as medium, it was possible to obtain relatively high concentrations of lead ion. The p_H of the Locke's solution used (*ca.* 7·0) was slightly decreased by the addition of the more concentrated solutions of lead acetate.

The results obtained are summarised in Table XVI. The percentage change in rate refers to the total change observed over the period investigated after the addition of lead acetate (40 to 100 mins.).

Table XVI. *Effect of lead acetate on the respiration of various tissues in Locke's solution.*

Exp. No.	Tissue	Initial concentration of lead acetate in Locke's solution	Percentage change in rate
93	Carc. mammae (human)	*M*/5000	+10
97	Sarc. shoulder (human)	*M*/5000	0
98	Sarc. groin (human)	*M*/5000	+ 4
94	Rat spleen	*M*/2500, *M*/5000	− 6, + 5
99	,, liver	*M*/1000, *M*/2000, *M*/4000	+20, +14, +6
101	,, ,,	*M*/1000, *M*/2000, *M*/4000	+20, +13, +5
102	,, testis	*M*/1000, *M*/2000, *M*/4000	−16, − 8, −6
104	,, ,,	*M*/1000, *M*/2000, *M*/4000	−25, −15, −6
105	,, ,,	*M*/10,000, *M*/100,000	− 2, 0

The presence of lead ion under these conditions accelerates slightly the respiration of human malignant tissue and also of rat liver. In the latter case, the effect increases with the concentration of lead acetate used. Rat spleen is little affected, while the respiration of rat testis is retarded, the effect increasing with increasing concentration. It is of interest that the effects on normal tissues should vary to this extent.

If the so-called Arndt-Schulz law, previously referred to in connection with the action of lead on glycolysis, is applicable to the case of respiration, it would be concluded as regards malignant tissue that respiration is much less sensitive to the action of lead than is glycolysis. In the case of spleen the effect of lead on both respiration and glycolysis appears to be very small, while with testis the effect is greater and respiration appears to be somewhat more sensitive than glycolysis. These conclusions, as regards relative sensitivity of normal and malignant tissue, are similar to those obtained with mercury.

Application to vital conditions.

It was found by Yabusoe [1926] that the effect of dye-stuffs on tumour glycolysis is much smaller with serum as medium than with Ringer's solution. It would seem probable that the effect of metallic salts at the same ionic concentration would be alike in the two media, since Yabusoe's result is attributed to adsorption of the dyes by serum proteins.

The experiments in which conditions of salt concentration are nearest to those possible *in vivo* are those on the action of lead on glycolysis. If the action of lead could be studied over longer periods of time, such as are available to the organism, it might show itself to be more toxic; the work of Reznikoff [1926] is suggestive in this connection.

Protocols.

Details of several of the experiments referred to previously are given below in order to indicate the degree of accuracy obtained, and the methods of calculation of results which have been used.

Protocol 1.

Exp. 20. *Anaerobic glycolysis of Flexner rat carcinoma.*

Ringer $NaHCO_3 = 0{\cdot}0191\ M$, glucose 0·2 %. $p_H = 7{\cdot}35$.

Gas mixture 5 % CO_2 in N_2.

Vessel	4	5	7	8	10
Cc. Ringer's solution	5·0	5·0	5·0	5·0	5·0
Dry weight of tissue	3·69 mg.	1·73 mg.	1·52 mg.	2·07 mg.	2·10 mg.
k_{CO_2}	1·078	1·043	1·040	1·009	0·982
Manometer change per 15 mins.:					
0–15′	39·1	16·0	11·4	18·3	20·7
15–30′	35·5	13·8	9·3	15·4	18·6
30–45′	36·2	14·5	11·6	15·4	19·5
$Q^{N_2}_{CO_2}$ (0–45′)	43·2	35·6	29·5	31·9	41·3

Protocol 2.

Exp. 57. *Effect of different amounts of $HgCl_2$ on the anaerobic glycolysis of rat spleen in Ringer's solution.*

Vessel	4	5	7	8	10
Cc. Ringer's solution ...	5·0	5·0	5·0	5·0	5·0

After measuring $Q_{CO_2}^{N_2}$ for 30 mins. the following additions of $HgCl_2$ in Ringer's solution were made to the experimental vessels.

Added	—	0·17 cc. *M*/3200 $HgCl_2$	0·17 cc. *M*/800 $HgCl_2$	0·95 cc. *M*/800 $HgCl_2$	—

Rates after addition of $HgCl_2$ expressed as per cent. of initial rates:

Time	Mean of controls 4 and 10	*M*/97,000 $HgCl_2$	*M*/24,000 $HgCl_2$	*M*/5000 $HgCl_2$
10– 40′	101	99	96	89
40–100′	102	95	80	55
100–130′	100	85	79	42
130–160′	100	85	67	38
163–253′	95	76	54	25
253–297′	88	70	43	19
Rates relative to mean of controls = 100:				
25′	—	97	95	100
70′	—	93	78	53
115′	—	85	72	41
145′	—	86	67	37
208′	—	79	57	25
275′	—	79	49	21

The calculated rates obtained from the relation $V = V_0 . e^{-at}$ are given in Table III.

Protocol 3.

Exp. 134. *Effect of HgI_2 in Ringer's solution containing various concentrations of sodium iodide on the anaerobic glycolysis of rat spleen.*

Rates after addition of HgI_2 expressed in per cent. of initial rates (relative to controls = 100) together with the calculated values of *a*.

Concentration of I′ in Ringer								
M/1000			*M*/100			*M*/10		
t	*V*	*a*	*t*	*V*	*a*	*t*	*V*	*a*
15·5	(116)	—	14·5	69·6	1·50	15·5	63·2	1·78
30·5	56	1·13	29·5	45·7	1·59	30·5	31·9	2·25
45·5	39·6	1·21	44·5	29·1	1·67	45·5	22·1	1·99
60·5	28·5	1·24	59·5	14·9	1·92	60·5	7·8	2·53
102	17·6	1·02	101	12·4	1·24	102	4·0	1·89

SUMMARY.

1. Measurements made on the glycolysis and respiration of various tissues agree in the most part with those of other workers. No aerobic glycolysis of liver was found.

2. The toxicity of metallic salts to glycolysis is in the order HgI_2, $HgCl_2 > Cu^{\cdot\cdot} > Zn^{\cdot\cdot} > Cd^{\cdot\cdot} > Ca^{\cdot\cdot}$.

3. Lead ion at low concentrations accelerates slightly the glycolysis of malignant tissues, and at much higher concentrations their respiration. With normal tissues its effects are smaller.

4. The glycolysis of malignant tissue is more sensitive to metallic poisons than that of normal tissues.

5. Glycolysis appears to be more sensitive than respiration to heavy metals in the case of malignant tissue. For normal tissues the converse is true.

6. A mechanism of the action of mercuric halides on glycolysis and respiration is suggested, in which the halide penetrates tissues rapidly, and the inactivation of the enzyme follows a unimolecular law, mercury passing from an adsorption compound to a combination with the enzyme which is inactive.

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