CCLXXXIV. THE EFFECTS OF OXIDATION-REDUCTION POTENTIAL INDICATOR DYES ON THE METABOLISM OF TUMOUR AND NORMAL TISSUES.

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IN most of the studies made in recent years on the effects of dyes on the respiration and glycolysis of tissues, very low concentrations of dyes have been used. Using a relatively high concentration of 2:6-dichlorophenolindophenol, $1.3 \times 10^{-3} M$, Elliott [1934] found a peculiar difference between the behaviours of tumour and kidney tissues. The respiration of kidney tissue was always found to be almost completely inhibited by the dye; the respiration of tumour tissue in the absence of glucose was also completely inhibited, but, when glucose was present, tumour tissue continued to respire. It was of interest, therefore, to see whether this difference of behaviour in the presence and absence of glucose was a unique property of tumour tissue, and whether the behaviour with 2:6-dichlorophenolindophenol was a property shared by other reversible oxidation-reduction systems. Both these questions are answered in the affirmative, and various other points of interest are brought out in the following paper.

EXPERIMENTAL.

The metabolism of thin slices of various tissues was studied in the presence and absence of dyes in the apparatus of Dixon and Keilin [1933], following the details of technique described by Elliott and Schroeder [1934]. The bicarbonate-containing medium of Krebs [1932] was used, and when glucose was added, this was made up to a concentration of 0.24%.

2:6-Dichlorophenolindophenol.

A stock $1.3 \times 10^{-3} M$ solution of the dye was made up in glucose-free Krebs medium and standardised by titration against ascorbic acid by the method of Birch *et al.* [1933]. For experiments with glucose present, the necessary amount of a strong glucose solution was added to a portion of the dye solution shortly before use. Stock solutions containing the dye and glucose slowly lose bicarbonate; it is probable that the dye catalyses an oxidative breakdown of glucose with the formation of acid products.

In a concentration of $1.3 \times 10^{-3} M$, 2:6-dichlorophenolindophenol produced 80–100% inhibition of respiration, whether glucose was present or not, with all the following tissues: rabbit and rat kidney cortex, rabbit and rat brain cortex, rat testis, rat retina and 4-day chick embryo. With rat liver, the inhibition varied between 30 and 89%, the mean being 56%, and in every case the R.Q. of the residual respiration was lowered, the mean effect being a drop in R.Q. from 0.95 to 0.60 (averages of 20 experiments). The presence or absence

of glucose caused no difference in the effects with liver, the average values for 10 experiments under each condition being the same. Tumour tissue (Philadelphia No. 1 sarcoma [Waldschmidt-Leitz, McDonald *et al.*, 1933] and Walker No. 256 carcinoma) alone showed a difference in the effect of the dye depending on the presence of glucose. In the absence of glucose, the respiration was always inhibited about 90 % whilst with glucose present there was only a small, variable inhibition of respiration and glycolysis. The R.Q. was always lowered from about 0.86 to about 0.70 (averages of 7 experiments). Experiments showed that even in the presence of glucose, the dye at this concentration produced a progressive inhibition of the metabolism of tumour slices. In the first 20 min. there may have been acceleration, but after 45 min. there was very considerable inhibition. With tumour in the absence of glucose, and with other tissues with or without glucose, the inhibition must have set in almost immediately.

Other oxidation-reduction dyes in high concentration.

The dyes used for these experiments were the products of various firms¹ and, with the exception of prune, were not specially purified. Prune was purified by the salting-out method of Melville and Richardson [1934]. Since the initial experiments with 2:6-dichlorophenolindophenol were made at a concentration of $1.3 \times 10^{-3} M$, roughly this concentration was used for all the dyes. Usually a solution or suspension of 12 times this strength was prepared in Krebs medium, and 0.25 ml. was pipetted into 2.75 ml. of medium in the manometer vessels. In many cases, the dyes were not very soluble, or, as with Bindschedler's green and cresyl violet, they precipitated out in strong solution in the bicarbonate-containing medium. In such cases, the dye was added as a fine suspension, which usually became clear on dilution and at 37°. In nearly all cases, the tissue slices absorbed the dye so that they became more deeply stained than the medium; it was thus impossible to say what was the effective concentration of the dyes within the tissue. For experiments in the previous section, the 2:6-dichlorophenolindophenol solution was adjusted to $1.3 \times 10^{-3} M$ after titration with ascorbic acid. In preparing solutions of this dye, much sparingly soluble material was removed by filtration so that the solution used, being fairly pure, actually may have been of a higher effective concentration than the unpurified suspensions or solutions of the other dyes used. The small inhibition of tumour respiration with glucose and of liver respiration mentioned in the previous section seems to have been determined by the higher dye concentration, and in the experiments shown in this section where one-third of this concentration is used, the results are entirely similar to those obtained with the other dyes.

¹ The dyes used were the products of the following firms:

Eastman Kodak Company: 2:6-dibromophenolindo-*m*-bromophenol (sodium 2:6-dibromobenzenone-indo-2'-bromophenol); *o*-chlorophenolindophenol (sodium benzenone-indo-3'-chlorophenol); 2:6-dichlorophenolindophenol (sodium 2:6-dichlorobenzenone-indophenol); 4:6-dinitro-*o*-cresol (3:5-dinitro-*o*-cresol).

British Drug Houses: Bindschedler's green; *m*-toluylenediamine-indophenol (*m*-tolylene-diamine-indophenol).

La Motte: 1-naphthol-2-sulfonate-indophenol, K₄ indigotetrasulphonate, K₂ indigodisulphonate (Indigo carmine).

Coleman, Bell and Co.: Thionine.

Ciba and Co.: Prune.

E. Merck (Darmstadt): Methylene blue (chemically pure, zinc chloride-free).

National Aniline and Chemical Co.: Nile blue A, cresyl violet.

In Tables I–III the effects of the various dyes in a concentration of approximately $10^{-3} M$ on tumour, brain, testis, retina, kidney and liver tissues in the presence and absence of glucose are shown. The dye solutions were always freshly prepared before use. The experimental period was in all cases one hour.

Table I. *Effects of dyes in approximately $10^{-3} M$ concentration on the metabolism of tumours.*

Dye	E_0' at p_H 7 volts	Glucose present			Glucose absent. Effect on O_2 uptake %	
		R.Q.		Effect on aerobic glycolysis %		
		Normal	With dye			
1. Philadelphia No. 1 rat sarcoma.						
Ferricyanide	+0.43†	0.82	0.90	-10	-13	-23
Quinone	+0.29	0.89	0.58	0	-36	-51
2:6-Dibromophenol- <i>m</i> -bromo-indophenol	+0.252‡	0.81	0.73	-25	-51	-70
<i>o</i> -Chlorophenolindophenol	+0.233†	0.90	0.72	-10	-40	—
		0.93	0.89	+15	-1	-55
		0.97	0.87	+38	+22	—
Bindschedler's green	+0.224†	0.81	0.79	-18	+44	-71
2:6-Dichlorophenolindophenol*	+0.217	0.95	0.87	+8	+43	-73
<i>m</i> -Toluylenediamine-indophenol	+0.125†	0.81	0.79	-1	+85	-56
		0.97	0.80	+4	+92	—
1-Naphthol-2-sulphonate-indophenol	+0.123†	0.93	0.90	-3	+55	-59
		0.99	0.93	-17	+50	-69
Thionine (Lauth's violet)	+0.06†	0.97	0.79	+69	+91	-37
		0.97	0.79	+45	+87	—
Prune	+0.053§	0.97	0.71	+31	+51	-61
Methylene blue	+0.011†	0.97	0.73	+79	+63	-66
						-52
Cresyl violet	-0.167	0.93	0.88	+32	+51	-1
		0.99	0.89	+44	+60	-1
4:6-Dinitro- <i>o</i> -cresol	—	0.78	0.93	+40	-27	-75
		0.77	0.96	+13	-46	-72
2. Walker No. 256 carcinoma.						
<i>m</i> -Toluylenediamine-indophenol	+0.125	0.86	0.81	+12	+82	-61
Methylene blue	+0.011	0.86	0.73	+67	+45	-57
Cresyl violet	-0.167	0.86	0.83	+11	+12	-17

* Standardised and used at a concentration of $4.3 \times 10^{-4} M$. See text. † Cohen [1933].

‡ Cohen *et al.* [1924]. § Melville and Richardson [1934]. || Wurmser [1930].

The one effect that is common to all the dyes tried with tumour is that when a dye is in the medium the respiration is greater in the presence of glucose than in its absence, although normally the presence of glucose has little effect on the oxygen uptake of tumours [see Elliott and Baker, 1935]. With the series from *o*-chlorophenolindophenol to cresyl violet this contrast is strong, since all these dyes accelerate the respiration in the presence of glucose, and with the exception of cresyl violet which has the lowest E_0' , inhibit the respiration in the absence of glucose. There is often considerable variation between the results of similar experiments with slices from different tumours. Nevertheless, the qualitative effects of this series of dyes on the O_2 uptake are fairly uniform. In their effects on glycolysis the results are very variable, but an increased glycolysis is often observed, and this was consistently found with the thiazine and oxazine dyes, namely, thionine, prune, methylene blue and cresyl violet. Elliott, Benoy and Baker [1935] noted some variation in glycolysis even between slices of the same tumour in normal conditions. The effects of the dyes on the

respiratory quotient in the presence of glucose are also variable but, in general, the R.Q. is lowered. The values for the R.Q. in the absence of glucose are not given since the inhibition then occurring leaves the residual respiratory exchange too small for accurate measurement.

Experiments with three other dyes, K_4 indigotetrasulphonate ($E_0' = -0.046$), K_2 indigodisulphonate ($E_0' = -0.125$), and Nile blue A ($E_0' = -0.122$) were made, since their potentials [Cohen, 1933] lie between those of methylene blue and cresyl violet. None of these three substances, however, had any appreciable effect on the respiration or glycolysis in the presence or absence of glucose. The ineffectiveness of the two highly sulphonated indigo dyes is probably due to their failure to penetrate into the cells [Chambers *et al.*, 1931]. (It was noticed that the tissues did not become noticeably stained in the presence of these dyes.) Although Nile blue penetrates well and stains the tissues, the colloidal nature of this dye perhaps prevents its access to the active centres reached by other dyes.

There is an indication of a grading of the effects of the dyes on the respiration of tumour dependent on their oxidation-reduction potentials. Dyes more positive than *o*-chlorophenolindophenol inhibit the respiration somewhat even in the presence of glucose; dyes in the main group accelerate the respiration in the presence of glucose and inhibit it in its absence, and cresyl violet, at the negative end of the series, accelerates in the presence of glucose but does not inhibit the respiration appreciably in the absence of glucose.

Results of Friedheim [1934] with pyocyanine are in quite good agreement with those in Tables I and II. Friedheim, using a $2 \times 10^{-3} M$ solution of pyocyanine in Ringer solution, found that there was a large increase in the respiration of various tumours in the presence of glucose and very little effect in its absence, and that the aerobic glycolysis was considerably inhibited. In its effect on respiration, then, pyocyanine behaves like cresyl violet towards tumour and this is in accord with its low oxidation-reduction potential ($E_0' = -0.035 \pm$) [Michaelis *et al.*, 1932]. In its inhibitory effect on the glycolysis, it behaves differently from cresyl violet and the azine dyes.

The inhibitory effect of the dyes on the respiration in the absence of glucose appears to be largely reversible. An experiment with *m*-toluylenediamine-indophenol showed a 69% acceleration of respiration when glucose was present from the start; when samples of tissue from the same tumour were kept without glucose at 37° in the presence of the dye for 6 min. and for 20 min., and then glucose added, the subsequent respiration rates showed accelerations of 43 and 30% respectively. A similar result was obtained in a corresponding experiment with 1-naphthol-2-sulphonate-indophenol.

Included in Table I are results of experiments with 4:6-dinitro-*o*-cresol. As with the other dyes, the respiration is greater in the presence of glucose than in its absence. However, dinitrocresol does not behave as an ordinary reversible dye [Greville and Stern, 1935], and it does not fall into any of the above groups. There is some inhibition of respiration, even with glucose, accompanied by increased glycolysis, and the R.Q. is raised.

Table II shows the effects of various dyes at a concentration of $10^{-3} M$ on the metabolism of brain, testis, retina and kidney. The most obvious effect is the large inhibition of the respiration of all these tissues even in the presence of glucose. These tissues, in fact, behave as does tumour tissue in the absence of glucose. With cresyl violet there is in several cases little effect, and this result was also obtained with tumour in the absence of glucose. Friedheim [1934] found that pyocyanine, which, like cresyl violet, has a negative E_0' , also had

Table II. *Effects of dyes in approximately 10^{-3} M concentration on the metabolism of various rat tissues.*

Tissue	Dye	Glucose present			Glucose absent.
		$Q_A^{O_2}$		Effect on O_2 uptake	Effect on O_2 uptake
		Normal	With dye	%	%
Brain cortex	Bindschedler's green	1.9	2.5	-72	—
	Methylene blue	2.0	2.8	-75	—
	Cresyl violet	2.0	7.2	-15	—
	4:6-Dinitro- <i>o</i> -cresol	2.0	18.9	-64	-78
Testis	Bindschedler's green	4.9	6.0	-72	—
	Methylene blue	6.0	9.4	-65	—
	Cresyl violet	4.9	7.8	-38	-68
		6.0	7.1	-51	—
	4:6-Dinitro- <i>o</i> -cresol	4.9	14.5	-64	-40
		4.6	12.2	-64	—
Retina*	Methylene blue	30.0	21.0	-85	—
Kidney	2:6-Dibromophenol- <i>m</i> -bromo-indophenol	0.6	1.6	-93	-100
	Bindschedler's green	1.3	2.1	-83	—
	Methylene blue	-0.6	2.5	-62	-83
	Cresyl violet	-0.6	-0.1	+ 8	- 9
	4:6-Dinitro- <i>o</i> -cresol	1.3	4.8	-68	-87

The term $Q_A^{O_2}$ represents the aerobic acid formation or glycolysis in terms of μ l. of CO_2 liberated from the bicarbonate in the medium per hour, per mg. dry weight of tissue.

* Experimental period 2 hours.

little effect on kidney tissue in carbonate-Ringer solution (although in the presence of phosphate there was some acceleration); however, his finding that pyocyanine in 2×10^{-3} M concentration increased the respiration of testis in carbonate-Ringer solution indicates that the action of this pigment is not quite similar to that of cresyl violet. It will be noticed that cresyl violet and especially 4:6-dinitro-*o*-cresol increase the aerobic glycolysis of brain and testis considerably, the increases being relatively greater than those obtained with tumour. Indigodisulphonate was tried with kidney tissue, but, as with tumour, this dye neither stains the tissue nor affects its metabolism appreciably.

Table III shows the effects of a few dyes on liver metabolism. It is seen that an increase in respiration was obtained both in the presence and the absence of

Table III. *Effects of dyes in approximately 10^{-3} M concentration.*

Rat liver.						
Dye	Glucose present	Q_A		R.Q.		Effect on O_2 uptake %
		Normal	With dye	Normal	With dye	
Bindschedler's green	+	1.2	2.6	0.99	0.87	+29
	-	1.8	0.3	0.73	0.81	+24
Methylene blue	+	1.2	7.4	0.99	0.85	+51
	-	1.8	1.4	0.73	0.85	+33
	-	0.9	3.0	1.07	0.90	+46
Cresyl violet	+	1.2	3.3	0.99	0.95	+46
	-	1.8	1.9	0.73	0.82	+42
4:6-Dinitro- <i>o</i> -cresol	-	0.9	8.1	1.07	0.74	-40

glucose with the reversible dyes, but that there was inhibition with the dinitro-cresol. Liver thus behaves in the presence or absence of glucose as does tumour tissue in the presence of glucose.

Effects of dyes in low concentrations.

Most of the work done by other authors on the effects of dyes on tissue respiration has been with very low concentrations of the dyes. Barron [1930, 1] found no effect or a decrease in the respiration of kidney, testis and liver, and an increase with brain and tumours, using methylene blue at a concentration of 0.005–0.0005 % (about 10^{-4} to $10^{-5} M$) in phosphate-Ringer solution. Himwich *et al.* [1933] found a decrease in respiration of brain tissue under similar conditions. Jares [1935], with 0.01 % ($3 \times 10^{-4} M$) methylene blue in phosphate-Ringer solution, found that the O_2 uptake of a number of tissues was accelerated during the first 20 min., but that after 80 min. an inhibition had usually set in, while with tumour tissue the acceleration continued for a longer time. In Table IV, the results of a few experiments with dyes at $10^{-5} M$ concentration

Table IV. *Effect of dyes in $10^{-5} M$ concentration on the metabolism of various tissues.*

		Glucose present						Glucose absent. Effect on O ₂ uptake %
Tissue	Dye	Q _A ^{O₂}		Effect on O ₂ uptake %	R.Q.		Extra O ₂ uptake	
		Normal	Dye		Normal	Dye		
Phila. No. 1 sarcoma	Methylene blue	12.7	12.1	– 1	0.99	1.04	—	—
	Thionine	12.4	12.4	– 2	0.85	0.85	—	—
		12.1	10.2	– 1	0.95	1.00	—	– 3
	Cresyl violet	13.5	10.2	+ 10	0.77	0.93	—	– 2
	4:6-Dinitro- o-cresol	12.1	17.0	+ 65	0.95	0.99	1.05	+ 12
		13.5	12.2	+ 37	0.77	0.96	1.48	+ 19
		14.1	15.3	+ 57	0.90	1.04	1.28	—
Brain cortex	Methylene blue	2.8	6.0	+ 14	0.80	0.84	1.14	– 47*
	Cresyl violet	2.8	3.7	+ 12	0.80	0.85	1.33	– 40*
	4:6-Dinitro- o-cresol	2.8	2.9	+ 30	0.80	0.93	1.38	– 18*
Kidney cortex	Methylene blue	– 0.1	– 0.9	+ 5	0.83	0.83	—	– 27
	Cresyl violet	– 0.1	– 0.4	+ 7	0.83	0.84	—	– 29
	4:6-Dinitro- o-cresol	– 0.1	– 0.8	0	0.83	0.90	—	– 13
		– 0.1	– 0.2	+ 7	0.81	0.89	—	—
Glucose absent								
Liver	Methylene blue	+ 0.4	– 0.3	+ 6	0.90	0.90	—	—
	Cresyl violet	+ 0.4	+ 0.8	+ 6	0.90	0.85	—	—
	4:6-Dinitro- o-cresol	+ 0.9	+ 1.4	+ 25	1.07	0.98	—	—

* The respiration of brain in the absence of glucose is normally very low.

(0.0003 % $\pm$) are shown. These figures represent the average effects over the 60-min. experimental period, and with respect to methylene blue effects, they are probably about what might be expected from Jares's observations. In general, the effects of the dyes are less marked in the low concentrations than in the high, and there are no marked or constant effects on the glycolysis; often a small acceleration of O_2 uptake is found instead of the inhibition found at the higher dye concentration. 4:6-Dinitro-o-cresol, however, increased the respiration and glycolysis of tumour, brain and liver tissue quite distinctly, and raised the R.Q.; this result is in agreement with the findings of Dodds and Greville [1934] with Jensen rat sarcoma. Dodds and Greville [1933] found the respiration of kidney tissue to be greatly increased by the dinitrocresol in lactate-containing phosphate medium, and they also reported an acceleration in the presence of glucose in

bicarbonate-containing medium but not in phosphate solution. In one experiment with glucose, shown in Table IV, no effect on the respiration rate of kidney tissue was observed, the medium containing bicarbonate as usual. The varying results of Dodds and Greville in the two media may have been due to chance variations in the tissues used.

Dickens [1934], who showed that thionine in $5 \times 10^{-5} M$ concentration produced a large acceleration of kidney respiration in lactate-containing medium, also mentioned an increased respiration of kidney and Jensen rat sarcoma in glucose-containing medium. This observation with thionine on kidney is similar to the result in Table IV with methylene blue; Philadelphia No. 1 sarcoma tissue, however, in the presence of glucose with thionine or methylene blue ($10^{-5} M$) did not show such acceleration. This disagreement may be due to the different tumours used or possibly to the somewhat higher dye concentration used by Dickens.

DISCUSSION.

The chief point of interest in the above results is the difference in the effects of dyes on the O_2 uptake of tumour tissue depending on the presence or absence of glucose. The effects on the respiration caused by dyes in the main group in Table I in $10^{-3} M$ concentration seem to divide the tissues studied into the following three groups:

(1) *Brain, testis, kidney, retina.* O_2 uptake inhibited in the presence or absence of glucose.

(2) *Tumour.* O_2 uptake inhibited in the absence of glucose and accelerated in its presence.

(3) *Liver.* O_2 uptake accelerated in the presence or absence of glucose.

Judging from results obtained with dyes in low concentration and from those given by Jares [1935] for methylene blue, it would seem that all these reversibly oxidised substances are capable, under suitable conditions, of accelerating the respiration of tissues in general. As has been suggested by Barron [1930, 2], this is probably due to their ability to be reduced by various mechanisms in the cell and re-oxidised by atmospheric oxygen, so that they provide an alternative oxygen-activating mechanism. Besides this catalytic property, the dyes also seem to have a progressive poisoning effect as was made clear for methylene blue by Jares, and as is also shown even by tumour tissue *plus* glucose with strong 2:6-dichlorophenolindophenol. Such a combination of catalytic acceleration and progressive toxic inhibition of oxidative processes by dyes has been observed by Quastel and Wheatley [1931] in minced muscle.

When the concentration of the dyes is increased to $10^{-3} M$, the poisoning effect on tumour without glucose, and on brain, testis, kidney and retina with or without glucose, appears to be so rapid that the respiration is inhibited almost immediately. The various tables show that with all the tissues examined except liver, there is a tendency for the respiration to be more strongly inhibited by the dyes in the absence of glucose than in its presence, but the effect is much more marked with tumour, the respiration of which is, in the presence of glucose, actually accelerated by most of the dyes. It is not possible at this stage to explain this unique acceleration of tumour respiration. It may be that intermediate products of glycolysis in tumour are able to counteract the dye reactions which ordinarily inhibit respiration. More probably, such intermediate products are oxidised under the catalysis of the dyes; Barron and Harrop [1928] offered a similar explanation for results obtained with mammalian red blood cells and dilute methylene blue. The reason for the special position of liver is quite

obscure, though it might be expected that the behaviour of this tissue would be independent of glucose, since its normal O_2 uptake, R.Q. and glycolysis are not affected by the presence or absence of glucose [Dickens and Greville, 1933; Elliott and Baker, 1935].

The effects of the dyes on glycolysis are also interesting, but again difficult to explain. Bumm *et al.* [1934] have brought forward and summarised evidence that respiration and glycolysis are not directly connected with each other, and they maintain that the principle of the Pasteur reaction, coupling increased O_2 uptake with decreased fermentation, does not apply to most tissues. The effects shown in Table I provide further evidence against this principle since large increases in respiration are accompanied in some cases by no change in glycolysis and in some cases by considerable increases in glycolysis. According to the Embden-Meyerhof scheme [Meyerhof, 1935], there are stages in the glycolytic process which involve the reduction of one compound at the expense of the oxidation of another. At steps such as this, it is possible that certain reversibly oxidisable substances could act as catalysts, provided that their oxidation-reduction potentials and their chemical nature were suitable. In this way, the accelerating effects of methylene blue, thionine, prune and cresyl violet on tumour glycolysis might be explained. Lipmann showed that the glycolytic mechanism of muscle extract [1933] and the fermentation by yeast juice [1934] can be inhibited by oxidising agents or by dyes with high oxidation-reduction potential in the presence of oxygen. The converse effect, namely, acceleration of glycolysis by dyes of low oxidation-reduction potential in the presence of the reducing systems of the tissues, might then be expected. This seems to be realised in the marked increase in aerobic glycolysis of tumour and brain tissues produced by $10^{-5}M$ phenosafranine ($E'_0 = -0.25$) [Cohen, 1933] which was observed by Dickens [1935], and by the fact that cresyl violet ($E'_0 = -0.167$), especially in the higher concentration, and also methylene blue, increase the glycolysis of tumour, brain and testis.

Though 4:6-dinitro-*o*-cresol in low concentration markedly accelerates the respiration of tumour, brain and liver, its toxic effect in the higher concentration used is so great that the oxygen uptakes of liver and of tumour even with glucose are inhibited. As with other dyes, however, the inhibition is greater with other tissues and with tumour in the absence of glucose. The most interesting effect shown here with the dinitrophenol in high concentration is the very large acceleration it produces in the glycolysis of brain, testis and liver as well as of tumour; from this and the fact that in general its presence causes a rise in the R.Q. toward the level required for carbohydrate oxidation, it seems that 4:6-dinitro-*o*-cresol in particular exerts its main catalytic effect on the carbohydrate metabolism.

SUMMARY.

1. 2:6-Dichlorophenolindophenol in $1.3 \times 10^{-3}M$ concentration produces almost complete inhibition of the respiration of tumour tissue in the absence of glucose, and of kidney, brain, testis, retina and chick embryo in the presence or absence of glucose. Liver respiration is also considerably inhibited in either case, and its R.Q. is lowered. Tumour tissue alone, in the presence of glucose, continues to respire, although there is a progressive inhibition.

2. 2:6-Dichlorophenolindophenol in one-third of the above concentration, and a number of other oxidation-reduction potential indicator dyes in approximately $10^{-3}M$ concentration, accelerate the respiration of tumour tissue in the presence of glucose and inhibit it in the absence of glucose. In this concen-

tration, these dyes produce considerable inhibition of respiration with brain, testis, kidney and retina, even in the presence of glucose. The respiration of liver tissue is accelerated in the presence or absence of glucose.

3. Thionine, prune, methylene blue and cresyl violet in $10^{-3}M$ concentration, besides accelerating the respiration of tumour slices in glucose medium, also increase the aerobic glycolysis. Cresyl violet also increases the glycolysis of brain and testis somewhat.

4. In $10^{-5}M$ concentration the effects of the dyes are less marked. With all the tissues tried there is usually a slight increase in the average rate of respiration in the presence of glucose over a 60-minute experimental period and some inhibition in the absence of glucose.

5. In $10^{-3}M$ concentration, 4:6-dinitro-*o*-cresol increases the glycolysis of tumour tissue and of brain, liver and testis. It inhibits the respiration of liver and of tumour tissue somewhat even in the presence of glucose. As with the other dyes, there is a large inhibition of the respiration of tumour tissue in the absence of glucose and of other tissues in the presence or absence of glucose. In $10^{-5}M$ concentration, 4:6-dinitro-*o*-cresol accelerates the respiration of tumour, brain and liver tissues considerably.

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