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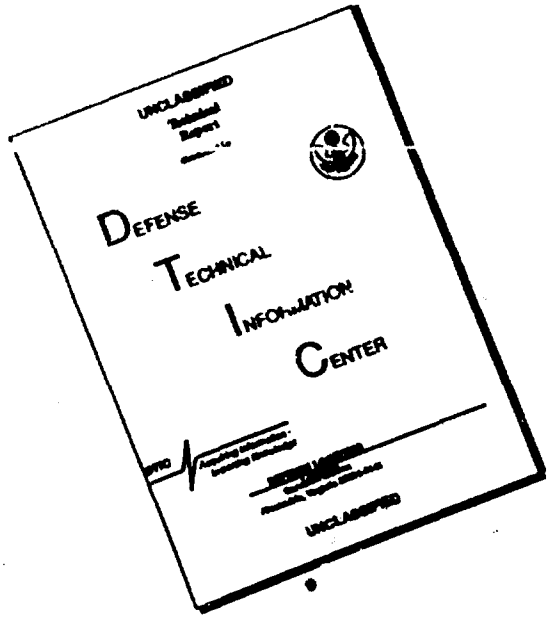

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MECHANISM OF THE PASTEUR EFFECT

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When nucleated pigeon erythrocytes are treated with nitrite or hydrogen peroxide the Pasteur effect is lost or greatly diminished: the respiration remains unchanged, but aerobic lactic acid formation sets in, attaining the level of anaerobic glycolysis. The effect does not depend on methemoglobin formation: in nitrited cells incubation with excess lactate brings about a complete reduction of methemoglobin, but the inhibition of the Pasteur effect persists. The suppresion of the Pasteur effect produced by H_2O_2 was found to be reversible and disappears on subsequent treatment with sodium hydrosulphite, though this has no effect on nitrited cells.

The conclusion is drawn that the Pasteur mechanism is independent of tne respiratory mechanism of the cell.

The nucleated erythrocytes of birds have an intensive respiration and a clearly expressed Pasteur effect: under aerobic conditions only traces of lactic acid are formed, while under anaerobic conditions an intensive glycolysis takes place.

In research on the processes of enzymatic reduction of methemoglobin [1] it was noticed that treatment of pigeon erythrocytes with nitrite (0.5% --- in future these will be designated as nitrited erythrocytes) causes, along with the formation of methemoglobin, the development of a strong aerobic glycolysis, almost attaining the level of anaerobic glycolysis.

TABLE 1

Aerobic Glycolysis in Nitrited Erythrocytes

Experiment	Increase of lactic acid of erythrocytes, in mg/ml, per 4 hours		
	I	II	III
Erythrocytes:			
Normal	0.09	0.12	0.07
Normal + KCN	1.92	1.80	2.27
Nitrited	1.80	1.74	2.15

One might think that this fact is the simple consequence of a depression of respiration resulting from the treatment. This idea, however, must be discarded, because measurement of O_2 absorption in the Warburg apparatus showed that the intensity of respiration in the nitrited erythrocytes remains unchanged as compared with normal, unaltered erythrocytes (see Table 2).

TABLE 2

Absorption of Oxygen by Pigeon Erythrocytes
(The figures represent μl O_2 per ml of erythrocytes)

Cells		Time in minutes 15	30	45	60	90
Normal erythrocytes without substrate	a	-	89	-	150	190
	b	63	110	168	188	-
Normal erythrocytes + glucose		-	90	-	148	184
Nitrited erythrocytes without substrate	a	-	92	-	150	188
	b	61	112	165	190	-
Nitrited erythrocytes + glucose		-	90	-	146	188

Undoubtedly, then, there takes place here a blocking of the Pasteur effect: respiration, though fully maintained, nevertheless is seen to be incapable of suppressing aerobic glycolysis. Study of the causes of such inhibition of the Pasteur effect, together with thorough analysis, may yield material for the deciphering and understanding the mechanism of the Pasteur effect, just as the method of using specific enzyme inhibitors has helped to sort out in all their complexity the enzyme systems of glycolysis, even in the numerous intermediate stages thereof. In the present work we took it upon ourselves to carry out such an analysis.

The development of aerobic glycolysis in nitrited erythrocytes coincides with the formation of methemoglobin. Hence it was natural to suspect a causal connection between the two. But special experiments showed that the complete biological reduction of methemoglobin, effected by a 2.5-hour incubation with lactate at 37°C, does not lead to the disappearance or diminishment of aerobic glycolysis (see Table 3).

TABLE 3

Increase of Lactic Acid in 3-Hour Period

Experiment	Lactic acid per ml of erythrocytes in mg
Before reduction of methemoglobin	1.00
After reduction of methemoglobin	0.96

Consequently the formation and presence of methemoglobin are unrelated to the suppression of the Pasteur effect.

The above-described phenomenon of the experimental suppression of the Pasteur effect is not an isolated case. In the literature there are described numerous cases of blocking of the Pasteur effect, that is, the development of aerobic glycolysis (or fermentation) without disturbance of respiration in different tissues under certain experimental influences. Some of these data from the literature may be tabulated as follows:

Author	Agent	Tissue	Remark
Warburg [2]	Ethyl cyanide	Sarcoma	
Genevois [3]	Cyanide	Barley sprouts and water plants	
Lasser [4]	Cyanide	Retina	In bicarbonated Ringer solution
Lasser [5]	CO	Liver, kidney	
Lasser [6]	10% O_2	Liver, kidney	

The authors of the papers here enumerated left these important facts unexplained, aside from Warburg's quite important remark in connection with the experiments: namely, that the sensitivity of the Pasteur reaction to ethyl cyanide indicates a metallic catalysis to be involved in the mechanism of this reaction.

All these facts, indicating that in many cases it is possible to inhibit the Pasteur effect without affecting respiration, lead us to an idea that at first glance contradicts prevailing views, namely to the idea that the Pasteur effect is autonomous --- independent of respiration.

Against this conclusion one may immediately object that in such experimental derangements of the Pasteur effect there may be hidden, behind the invariance of the total oxygen absorption, internal changes in the catalytic respiratory systems such as to make respiration incapable of suppressing enzyme activity. In order to clear up this question, we tested the sensitivity to cyanide and sensitivity to malonate of the respiration of nitrited erythrocytes with the Pasteur effect inhibited.

Malonate, as a specific succinic-dehydrogenase poison, was tested in connection with the fact that Semenov in our laboratory had demonstrated the participation of the C_4-dicarboxylic acids in the respiratory system of nucleated erythrocytes (unpublished data). Tests with cyanide and malonate might be expected to put us in a position to judge the state of the Warburg-Keilin system and dehydrogenase mechanisms. To bring out more clearly the inhibiting role of malonate, we carried out the experiments in Figure 1, using hemolysed nucleated erythrocytes, in which succinate stimulates and increases by many times the small respiration proper to them.

From Figures 1 and 2 it is seen that treatment of erythrocytes with nitrite does not cause any change in the respiratory catalytic systems, because cyanide and malonate were found to inhibit to the same degree the respiration of nitrited cells and normal cells.

Besides this it was discovered that the nitrited cells also preserve the particular features characteristic of normal pigeon erythrocytes in the utilization of the substrate: sugar does not stimulate their respiration, and is not required in the respiratory process (see Tables 2 and 4). Thus it is possible to state with some confidence that in this case the Pasteur effect is suppressed without disturbing respiration either in its quantitative or its qualitative aspects.

TABLE 4

Details of Utilization of Substrate by Pigeon Erythrocytes

Erythrocytes	Decrease of sugar (mg/ml)	Increase of lactic acid (mg/ml)	Time (hrs)
Nitrited	2.05	2.15	5
	1.80	1.84	5
	1.17	1.05	4
Normal	0.15	0.11	4
	0.13	0.11	4
	0.03	0.05	4

The thesis that the Pasteur effect is independent of respiration may also be supported by certain data in the literature. Thus there are indications that it is possible to differentiate the Pasteur effect and respiration in two further ways: 1) suppressing respiration without touching the Pasteur effect, as was the case in Mendl's [7] experiments on the action of dioxyacetone on sarcoma; 2) suppressing respiration and reinforcing the Pasteur effect (that is, reducing aerobic glycolysis, as was the case in Krah's experiments [8] on muscular sarcoma as affected by saccharic acid) without affecting anaerobic glycolysis.

Finally, as still another argument in favor of the Pasteur effect's independence of respiration we may cite the above-mentioned fact that in pigeon erythrocytes, where the Pasteur effect is clearly manifested, it is impossible to detect the utilization of sugar as the substrate of respiration, while in anaerobiosis and in the experimental suppression of the Pasteur effect the amount of added sugar is reduced in proportion to the amount of lactic acid formed (see Table 4). Thus we here observe a natural differentiation of the respiration and glycolysis processes with respect to their substrates. Most often, however, our ideas of the mechanism of the Pasteur reaction proceed from the assumption of a common route, or a partly common route, of carbohydrate decomposition in its oxidative (respiratory) and glycolytic conversions. The above fact permits us to doubt the universality of this assumption, and we have grounds for asserting that not only is a common route of respiratory and glycolytic sugar decomposition unnecessary, but even the presence of a common respiratory and glycolytic substrate is unnecessary.

If the aerobic inhibition of glycolysis is not caused by respiration, then how are we to visualize its mechanism? On the basis of indirect data and analogy (sensitivity of the Pasteur effect to cyanide and to agents acting on hemines), I put forward the suggestion * that the depression of glycolysis under aerobic conditions is accomplished by a special system containing iron, possibly of heminic nature, and that the nitrite treatment, in the case of nucleated erythrocytes, puts this system out of order by way of a crude oxydation thereof, in the same way that hemoglobin is oxidized to methemoglobin. On this basis we could look for a restoration of the Pasteur effect upon treatment of the cell with energetic reducing agents, such as

* Paper read at Leningrad University, 1938 (unpublished).

sodium hydrosulfite for instance. But attempts to restore the Pasteur effect in nitrited cells by this means gave no positive result. It appears that nitrite irreversibly destroys the Pasteur system.

In further experiments on the suppression of the Pasteur effect in nucleated erythrocytes, we tested the action of an oxidizing reagent other than nitrite, namely hydrogen peroxide. It was found that in the majority of cases the treatment of the erythrocytes with 0.3-0.5% H_2O_2 was able, without affecting respiration, to evoke aerobic glycolysis, though not in fact as strong a glycolysis as in the case of nitrite.

It should be noted that pigeon erythrocytes show an individual sensitivity to H_2O_2; H_2O_2 concentrations that in one case do not cause hemolysis will cause hemolysis in another lot of erythrocytes. The threshold concentration of H_2O_2 is that which just fails to evoke hemolysis but already inhibits to some degree the Pasteur effect.

In erythrocytes where the Pasteur effect has been suppressed by the action of H_2O_2 subsequent incubation with 1% $Na_2S_2O_4$ is enough to inhibit aerobic glycolysis again, that is, to restore the Pasteur effect (see Table 5).

TABLE 5

Restoration of Pasteur Effect After Treatment with Hydrogen Peroxide

Pigeon erythrocytes	Increase of lactic acid in mg per ml of erythrocytes (aerobic glycolysis)			
	After 4.5 hrs	After 4 hrs	After 4 hrs	After 4.5 hrs
Normal	0.24	0.15	0.1	0.12
Treated with H_2O_2	2.25	1.81	1.74	1.91
Normal + KCN	2.51	2.63	2.30	2.50
Treated with H_2O_2, then with $Na_2S_2O_4$	0.30	0.16	0.35	0.6
Treated with $HaNO_2$, then with $Na_2S_2O_4$	2.41	2.36	-	-

From the experiments with nitrited cells it appears that hydrosulfite does not have any effect on the glycolysis itself, and does not inhibit it. My suggestion about the existence of a special hemin system, connected with the implementation of the Pasteur reaction, has received important confirmation in the work of Stern and Melnick [9], who were able to suppress the spectrum of the Pasteur system, which they call the Pasteur enzyme. This spectrum was found to be extremely close to the spectra of the theohemins. Consequently the existence of a special Pasteur system or enzyme becomes quite likely.

The fact that by lowering the partial pressure or by selection of a certain CO concentration [5;6] we may evoke aerobic glycolysis without depressing respiration is indubitable testimony that the Pasteur system reacts directly with the oxygen of the air, and does not enter as a side

reaction subsequent to the Warburg-Keilin system. Thus respiration and aerobic inhibition of fermentation are processes independent one of the other, processes taking place simultaneously and requiring for their establishment the same identical condition --- the presence of atmospheric oxygen.

This point of view is not contradicted by the well-known fact of the simultaneous depression of the Pasteur effect (that is, increase of aerobic glycolysis to the anaerobic level) and of respiration by cyanide, for the ordinarly used concentrations of cyanide are sufficient to poison both the Warburg-Keilin system and the Pasteur system (which is even more sensitive to cyanide than is the respiratory system [4]). The views set forth here are in some sense the development of the general line of interpretation of the Pasteur effect advocated in his time by Lipmann [10], who affirmed, against Meyerhof's widely accepted resynthesis theory, that under aerobic conditions there occurs a true and not an apparent inhibition of fermentation due to oxidative inactivation of the enzyme systems involved. This principle of Lipmann's has, in the main, correctly seized the actual relationships; numerous and diversified data accumulating of late in the literature and a careful analysis of the factual material set forth in the works of Meyerhof's own school make it possible to reject entirely the so-called Pasteur-Meyerhof reaction as an explanation of the mechanism of the Pasteur effect.

It is appropriate here to bring forward still another observation regarding the blocking of the Pasteur effect in nucleated erythrocytes.

After treatment of pigeon erythrocytes with nitrite, quinone or methylene blue there commences, along with development of aerobic glycolysis plus full preservation of respiration (that is, along with the suppression of the Pasteur effect), a rapid breakdown of the pyrophosphate fraction, almost reaching the anaerobic level. For instance, after two hours there is as much as 215-220 μg of inorganic orthophosphate formed per ml of nitrited erythrocytes, and in normal erythrocytes poisoned with cyanide, as much as 230 μg; in normal unpoisoned erythrocytes under aerobic conditions, no breakdown whatever of the pyrophosphate fraction is observed.

The phenomenon of breakdown of adenosine triphosphoric acid (ATP) in pigeon erythrocytes in anaerobiosis and its resynthesis in aerobiosis was, as we know, discovered by Engel'hardt, and it led into the idea of the coenzymatic role of ATP not only in the process of glycolysis, but also in the respiratory process.

This fact compels one to think that the question has more complications still, and that the stabilization of ATP is connected not only with respiration but also with the normal working of the Pasteur effect.

In conclusion, we must attempt, even in the most general and provisional outline, to form ourselves a picture of the aerobic inhibition of enzymatic activity. It seems that as a result of interaction with atmospheric oxygen the Pasteur system acquires the ability to oxidize certain intermediate compounds, which in their turn oxidatively inactivate a definite part of the glycolytic enzyme system, thereby stopping the whole process of glycolysis. At a time when the present work had been finished (its experimental part and basic formulation date from the year 1941) it became known to me, from a personal communication of V.A. Engel'hardt and from a subsequently published paper, that the above ideas in many respects agree with experimental data obtained by Engel'hardt and Sakov [11].

The views here set forth, of course, do not constitute any complete theory of the mechanism of the Pasteur effect. In the present paper we have attempted only to put forward certain general principles which could be of assistance in the further deciphering of this mechanism.

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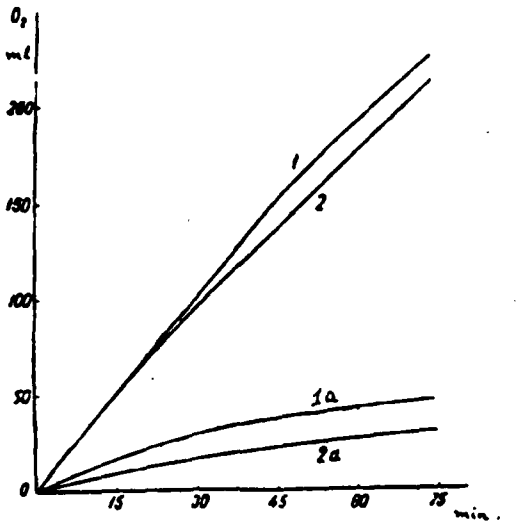

Fig. 1. Absorption of O_2 by hemolysates of erythrocytes in the presence of respiratory poisons.

1 - hemolysate of normal erythrocytes + succinate;
1a - the same, in the presence of malonate;
2 - hemolysate of nitrited erythrocytes + succinate;
2a - the same, in the presence of malonate.

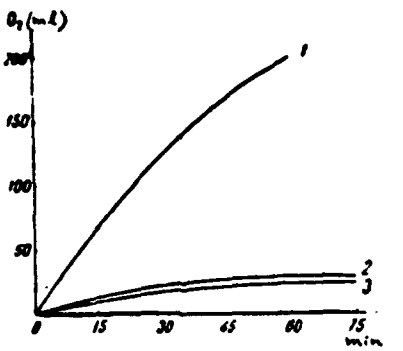

Fig. 2. Effect of cyanide: 1 - nitrited erythrocytes; 2 - normal erythrocytes + KCN; 3 - nitrited erythrocytes + KCN.