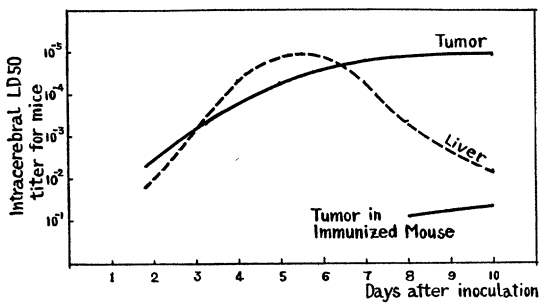

FIG. 2. Experiment with the ornithosis virus.

was measured each 2 to 3 days. One of the typical experiments is shown in Fig. 1. Infective titer of the tumor was higher than that of liver at the first stage of infection, and then both became almost equal at the eighth day after inoculation. Infected tumors developed almost equally with the normal one, probably because it takes some time for propagation of rickettsiae. Bioassay of these heavily infected tumors was performed, and they were found to have minimal ability to develop in 2 weeks. In some instances no growth of the tumor occurred after transplantation. Mice that received transplants infected with *Rickettsia tsutsugamushi* died from infection about 14 days later. Further transplantation of tumors was not successful. Similar results were obtained with fructose-induced sarcoma.

Experiments with ornithosis virus. Brain suspensions from mice infected with ornithosis virus (10^3 LD_{50}, measured by the intracerebral route) were inoculated into carcinomas in a similar way. In this instance, although virus in the tumor reached $10^{-4.5}$ LD_{50} on the fifth day after inoculation and persisted longer than 10 days without decrease, virus in the liver began to decrease after 7 days (Fig. 2). Bioassay of virus-infected tumors revealed the occurrence of partial interference, though it was less marked than in the experiment with rickettsia. However, serial transplantation of virus-infected tumors failed to continue beyond the fourth generation. Dissociation in infective titer of liver and tumor tissues infected with ornithosis virus may be interpreted from the viewpoint that immunity develops in liver, but not in tumor cells (*14*). When mice were immunized intraperitoneally with the active virus before tumor transplantation, the challenged virus did not propagate in tumors, despite few if any, neutralizing antibodies in serum. Details of this aspect of the problem will be published separately.

Comparative studies of the behavior of various viruses toward neoplasms may throw light on the biological nature of viruses on the one hand and on that of tumors on the other.

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The Control of Pyruvate Oxidation in a Cell-free Rat Heart Preparation by Phosphate Acceptors[1]

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The "creatine effect" observed by Belitzer (*1*) was a recognition of the role of secondary phosphate acceptors[3] in the control of biological oxidations. This effect was manifested by a marked increase in the respiration of muscle minces when creatine was added to the incubation medium. The increase, however, could not be observed when the tissue was reduced to a fine dispersion. Since that time, numerous studies have been made on enzyme systems capable of coupling phosphorylation with oxidation, but only recently have preliminary reports appeared showing actual control of biological oxidations by phosphate acceptors in heart (*2*) and liver tissue (*3, 4*). In this paper data are presented showing the requirement of hexokinase and glucose for the maximal rate of oxidation of pyruvate by a washed rat heart preparation.

TABLE 1

STIMULATION OF PYRUVATE AND OXALACETATE OXIDATION BY YEAST HEXOKINASE*

Substrate	Solution of yeast hexokinase (μl)	Oxygen uptake (μl/10 min)
Pyruvate $5 \times 10^{-3}M$	0	27
	5	52
	25	77
		μl/40 min
Oxalacetate $1.7 \times 10^{-3}M$	0	66
	25	135

* The procedure used to obtain the enzyme preparation from rat heart, concentrations of various added substances, and conditions of assay have been previously described (*2*).

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[3] Creatine and glucose may be considered as secondary phosphate acceptors, whereas adenosine diphosphate (ADP) is a primary acceptor.

Thus, in addition to fumarate, magnesium, and adenosine triphosphate (ATP) (*5*), an enzyme capable of removing phosphate from the ATP is required for the maximum rate of pyruvate oxidation.

As shown by representative data given in Table 1, hexokinase added to a rat heart preparation in the presence of pyruvate, fumarate, ATP, Mg^{++}, phosphate, and glucose increased the rate of oxidation of pyruvate nearly threefold. When oxalacetate was the stubstrate, the stimulation was more than twofold. Moreover, as shown in Fig. 1, when the addition of hexokinase was made from a side arm to an already functioning pyruvate oxidase system in presence of fluoride, the rate of oxidation was nearly doubled. Also of interest is the marked stimulation even in the absence of fluoride. Since the terminal phosphate of ATP is transferred by hexokinase to glucose, the regeneration of ADP and the resulting increase in pyruvate oxidation should depend upon the presence of glucose as the final phosphate acceptor. In harmony with this, addition of glucose to the otherwise complete system gave a stimulation of oxidation of pyruvate similar to that obtained with hexokinase. Hexokinase additions in the absence of glucose did not increase the oxygen uptake.

When pyruvate was omitted the oxygen uptake was very low (7% of the complete system), thus indicating that the glucose added served as a phosphate acceptor and not as a source of oxidizable substrate. Although depletion of inorganic phosphate as well as phosphate acceptors may be important in the control of oxidation, as suggested by Johnson (*6*) and Lynen (*7*), clearly the effect reported herein depends on the concentration of a phosphate acceptor.

The highest values of the P : O ratios[4] consistently obtained for pyruvate oxidation by rat or rabbit heart preparations (*2*) have been in the range 2.4 to 2.6 in the presence of 0.02 *M* fluoride. Even in the absence of fluoride P : O ratios above 2 have been obtained. Without phosphate acceptors present, the rate of oxidation is limited by the rate of formation of ADP from ATP through action of enzymes in the heart preparation. Such action should be minimal in the presence of fluoride and of glucose and hexokinase added in excess; hence the observed P : O ratios probably represent the maximum obtainable from the oxidations occurring. The probable theoretical maximum P : O ratio for the different steps of the Krebs cycle may be deduced from kinetic and thermodynamic considerations, on the basis of phosphate esterification occurring only with oxidations below the cytochrome level (*8*). These considerations suggest the possibility of uptake of 1 mole of inorganic phosphate with transfer of 2 electrons to cytochrome *c* from flavoproteins or succinic dehydrogenase, uptake of 1 mole of phosphate with interaction of reduced DPN or TPN with oxidized flavoproteins for all substrates of the citric acid cycle except succinate, and uptake of

[4] The P : O ratio equals the number of molecules of inorganic phosphate taken up divided by the atoms of oxygen consumed.

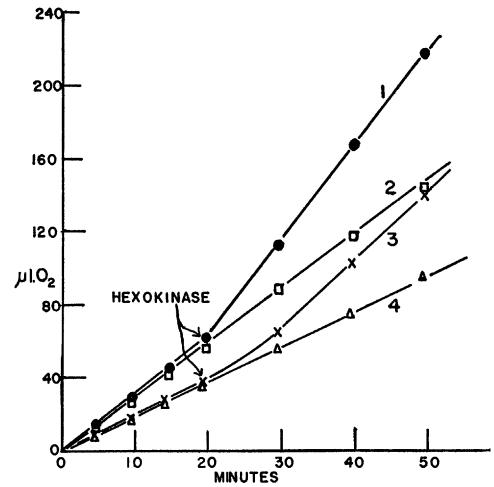

FIG. 1. The increase in rate of pyruvate oxidation after a delayed addition of yeast hexokinase. All flasks contained initially the complete reaction system (Table 1) except hexokinase. 1—without fluoride, hexokinase added as shown ; 2—without fluoride, without hexokinase ; 3—with 0.02 *M* fluoride, hexokinase added as shown ; 4—with 0.02 *M* fluoride, without hexokinase.

1 or, less likely, 2 moles of phosphate with the oxidative decarboxylation of pyruvate and of α-ketoglutarate. This would lead to P : O ratios of 2.2 to 2.6 for the complete oxidation of pyruvate. If the oxidation proceeded only to the citrate stage, the probable P : O ratio would be 3.0. Electron transport through the cytochrome system, even though not directly coupled with phosphorylation, could drive the coupled phosphorylation reactions until high ATP/ADP ratios are obtained.

The phenomenon reported here serves to indicate not only the compulsory nature of the phosphorylation coupled with the oxidation of pyruvate, but also the mutual regulation of these two phenomena through the coupled enzymic reactions. It supports Lennerstrand's (*9*) speculation on the cause of the Pasteur effect. This he attributed to an inhibition of glycolysis, which was brought about by the decreased concentration of required phosphate acceptors. If, as shown here, decreased concentration of the latter can inhibit pyruvate oxidation, it may also be expected to inhibit triose phosphate oxidation. The relative requirements of various oxidative phosphorylation reactions for particular steady-state concentrations of primary phosphate acceptors would be determined by the free energy changes of the coupled oxidative reactions.

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