STUDIES ON BLOOD GLYCOLYSIS

I. EFFECT OF ARSENATE

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Meyerhof (8) has shown that arsenate stimulates the decomposition of carbohydrate and of hexosephosphate esters by the glycolytic enzyme of muscles. This observation brings muscle glycolysis into close relationship with fermentation of sugar by yeast or yeast products. Harden and Young (6) who discovered the stimulating action of the phosphate ion on the process of alcoholic fermentation were led to investigate the effect of the related arsenate ion and found that this exerted a similar stimulating action. Neuberg and Kobel (10) later demonstrated that vanadate likewise stimulates yeast fermentation, a fact which Braunstein (3) has recently shown to hold also for blood glycolysis.

In the course of our studies on the effect of isotonic solutions of various salts on glycolysis of blood we found that NaCl, NaI, or NaBr either had no effect or caused only a slight decrease in the glycolytic activity, whereas an isotonic arsenate solution suppressed glycolysis almost as completely as does NaF. Inasmuch as we diluted blood with an equal volume of the salt solution, it means that m/16 arsenate can bring about almost total cessation of glycolysis. Prompted by this observation we extended the investigation to various concentrations of arsenate, studying the changes in sugar, lactic acid, and inorganic phosphate by a procedure discussed elsewhere (9) and found that the arsenate produced a definite though small inhibition of glycolysis even in a concentration of m/5120.

The results of these experiments are recorded graphically in Figs. 1 and 2 which show the effect of different arsenate concentrations, made up in physiological saline, on the disappearance of
sugar and formation of lactic acid and inorganic phosphate in glycolyzing dog blood. From these typical experiments it is clear that even \( \frac{m}{1280} \) sodium arsenate solution causes an unmistakable inhibition of the glycolytic process, which becomes progressively more marked as the arsenate concentration increases. The results in Fig. 1 represent the effect of 4 hours incubation on the glycolysis, while in Fig. 2 are recorded the results of experiments with smaller arsenate concentrations, ranging from \( \frac{m}{1280} \) to \( m/5120 \), and for a period of 2 and 4 hours of incubation. In this case the effect does not become apparent until after 4 hours of incubation. It will be noted that, while the glycolysis, i.e. disappearance of sugar and the formation of lactic acid, is inhibited, there is simultaneously a more or less extensive liberation of inorganic phosphate which seems to be progressive during the glycolysis experi-

![Fig. 1. Effect of various concentrations of arsenate on glycolysis of dog blood.](http://www.jbc.org/)

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**Fig. 1.** Effect of various concentrations of arsenate on glycolysis of dog blood.
ment. In our original experiments we were unable to determine the phosphate with any certainty in the presence of much arsenate, and our determinations were limited to those with the lowest arsenate concentrations. Nevertheless it was clear that an increase in inorganic phosphate was an invariable accompaniment of glycolysis in the presence of arsenate.

Since the above experiments were performed Barrenscheen and Hübner (2) reported that arsenate has a marked stimulating effect on glycolysis, and later Braunstein (3) also showed that the stimulation of the glycolysis was associated with an extensive liberation of inorganic phosphate. The fact that these reports corroborated our own findings so far as the changes in phosphates are concerned, but contradicted our results with regard to the rate of the disappearance of sugar, led us to reexamine the question with a view to finding the possible causes of such a discrepancy.

Two conditions which were different in our own experiments and those performed by Barrenscheen or Braunstein commanded
attention; namely, that we dissolved the arsenate in physiological saline while the other investigators used Locke's solution instead, and secondly, that we worked with dog blood while the others used rabbit blood. The glycolytic behavior of dog and rabbit blood does, of course, present certain distinct differences, as can be seen from Fig. 3 in which typical glycolysis curves are shown of dog and rabbit blood incubated at 37° for different lengths of time.

We performed, therefore, new experiments, using both dog and rabbit blood and dissolving the arsenate either in physiological saline or in Locke's solution. The results of these experiments are recorded graphically in Figs. 4 and 5. We also developed the phosphate determination so that it could be made in the presence of various concentrations of arsenate.

These experiments show strikingly the effect of arsenate on the hydrolysis of organic phosphates. Engelhardt and Braunstein (4) have also reported a similar observation. Our experiments show the relationship between the arsenate concentration and the liberation of phosphate. The progressive rise in inorganic phos-
phate, which we observed in glycolyzing dog blood, was accompanied by a gradually increasing suppression of glycolysis with increasing arsenate concentration. In this respect the experiments present nothing unusual, because it is now generally recognized by students of this problem that any factor which inhibits or checks

the glycolytic process conduces at the same time to the liberation of phosphoric acid from its esters (cf. Rona and Iwasaki (14) on the effect of checking glycolysis by the removal of sugar; Engelhardt and Braunstein (4) by fluoride; Engelhardt and Ljubimowa (5) by
hemolysis; Barrenscheen and Braun (1) by various agencies). In fact, when glycolysis is checked by removing sugar and especially by cytolyzing the cells, there is actually an explosive liberation of inorganic phosphate. But the results of Engelhardt and Braunstein (4) and of the subsequent experiments of Braunstein (3) are much more difficult to interpret, inasmuch as here the liberation of phosphoric acid is actually accompanied by an increase in glycolytic activity.

Fig. 5. Effect of arsenate on rabbit blood. The shaded boxes represent sugar; the solid boxes, inorganic P.
Braunstein (3) using the Fiske-Subbarow method for the phosphate determination depends upon rapid reading of the colors to avoid errors due to the presence of arsenate, the extra color development from arsenate presumably being a slower process. This, of course, is an uncertain and indeed a very uncritical procedure for quantitative estimations. Using the Kuttner method, we have worked out the correction for the arsenate, determining the latter as phosphate. Over a wide range of arsenate concentrations we found the equivalent phosphate values to be in a direct linear ratio. The proportionality of color development holds only for a limited range of colorimeter readings, and we adjusted the concentration of the unknown so that it would not deviate by more than 12.5 per cent from the standard (the readings being 17.5 to 22.5 mm. with the standard set at 20 mm.). After determining P according to total color development, the P value equivalent to the As concentration was deducted, and the difference represented, therefore, the true phosphate. This procedure checked up well with known mixtures.

The results of experiments with dog and rabbit blood in both Locke's or isotonic salt solution are recorded graphically in Figs. 4 and 5. It will be noted that there was invariably a marked increase in the inorganic phosphates, which becomes greater with the progressive rise in the arsenate concentration. It also seems that the increase in phosphate is greater in NaCl than in Locke's solution, this difference being perhaps more obvious in the glycolyzing dog blood than in the rabbit blood. The glycolysis of dog blood, judging by the amount of sugar disappearing, is definitely inhibited, the degree of inhibition varying with the arsenate concentration, as was also found in our earlier experiments. At the same time these experiments corroborate the findings of Engelhardt and Braunstein (4), Barrenscheen and Hübner (2), and of Braunstein (3), that arsenate greatly accelerates the glycolytic process in rabbit blood, this effect being more manifest in Locke's than in isotonic NaCl solution. In our experience, arsenate causes as great an increase in the inorganic phosphates of the glycolyzing system as does vanadate, the effect of which Braunstein considers rather extraordinary. It may also be observed that in the case of rabbit blood, both the liberation of phosphate and the glycolysis of sugar are much accelerated by arsenate. But in NaCl solution the
increase in inorganic phosphate is greater than in Locke's solution, while glycolysis is more accelerated in Locke's solution than in NaCl solution. We thus witness here a similar reciprocal relationship between the effect on the glycolytic process and on the hydrolysis of hexosephosphate esters (or perhaps the prevention of resynthesis of phosphate esters) as was already suggested by the experiments on dog blood.

Engelhardt and Braunstein (4) developed the hypothesis according to which the blood phosphate level during glycolysis is a balance between the $H_3PO_4$ set free from easily hydrolyzable esters and its resynthesis to more stable esters. The increase in inorganic phosphate is, therefore, due to a failure of glycolysis, as can be demonstrated by such means as NaF which suppresses the glycolytic process. The experiments with arsenate somehow do not fit properly into this scheme. In this case, as was found by Engelhardt and Braunstein (4), Barrenscheen and Braun (1), and later by Braunstein (3) and also by us, the phosphate invariably increases, sometimes even to a very marked degree, in the presence of arsenate. Furthermore, in the case of rabbit blood this great increase takes place while the glycolytic process is also greatly stimulated. It is, of course, entirely conceivable, as Braunstein actually argues (3), that hexosephosphate esters may be replaced by hexosearsenate esters, which are perhaps more labile, and this would also account for the setting free of phosphoric acid. Attractive as such an interpretation may seem at first sight, it meets with certain very serious objections. The replacement of phosphate by arsenate seems to us highly improbable because the color produced by As is more than 3 times as intense as that produced by an equivalent amount of P. In the event of an exchange of free $AsO_4^-$ and $PO_4^{3-}$ ions, one would naturally expect a gradual diminution in the total color evolution. Had such a thing actually happened, it could only have been interpreted as a diminution in the free phosphates. The analytical results, therefore, preclude any such assumption of an interchange of arsenate for phosphate in the hexose esters.

Braunstein furthermore suggests that the hexosearsenate esters might glycolyze more rapidly than hexosephosphates and thus account for the acceleration of the glycolytic process as is observed, for instance, in rabbit blood. This explanation, however, would
again fail to apply to dog blood, in which arsenate actually inhibits the glycolysis.

An examination of the results presented in Figs. 4 and 5 shows very clearly that arsenate has a definite effect, increasing the inorganic phosphates of the glycolyzing system proportionally to the arsenate concentration. Raymond (11) found that the phosphatase activity of yeast is markedly increased by 0.004 M arsenate. Unfortunately, this author, who also determined the phosphates by the Kuttner procedure, does not say whether he corrected for the color development due to the arsenate, which, of course, would materially affect the quantitative results. Macfarlane (7) found that arsenate stimulates phosphatase activity. One might thus be inclined to assume that the great liberation of phosphate noted in blood-glycolyzing systems in the presence of arsenate is likewise the outcome of the stimulation of the blood phosphatases. Braunstein denies the possibility of the phosphatase stimulation in blood. His contention is based on several arguments. First, he maintains that arsenate does not promote liberation of inorganic phosphate when glycolysis does not take place or is inhibited. In view of our experience with dog blood in which, in spite of the inhibition of the glycolysis, the inorganic phosphates do increase with rising arsenate concentration, this argument is deprived of its force. Roche and Roche (12) report that CaCl₂ causes increased liberation of phosphate without affecting the glycolysis of the blood. If the glycolysis is checked by NaF, the addition of the CaCl₂ within 30 minutes brings about reactivation of the glycolysis, but, if added after a lapse of 24 hours, there is no longer a reactivation of glycolysis but only an abundant liberation of phosphates. Obviously, therefore, the phosphatase and the glycolysis enzyme systems can be affected independently. The second argument, namely that in cytolyzed blood the addition of arsenate fails to affect the hydrolysis of phosphate esters and, therefore, cannot be effective in stimulating phosphatase activity, likewise has very little weight, inasmuch as in the hemolyzed blood the maximum liberation of phosphate has already taken place spontaneously. The third argument Braunstein bases upon experimental findings showing that the arsenate does not influence the blood phosphatase activity. Since the phosphatase activity is thus unaffected, Braunstein
argues that the liberation of phosphate in arsenate-containing glycolyzing systems must be due to a replacement of phosphate by arsenate esters. This last argument we tested by direct experimentation. As the source of phosphatase we used washed red blood cells which had been cytolysed with an equal volume of distilled water. The technique of these experiments was essentially the same as recommended by Roche (13), the reaction being carried out at 37° at pH about 6.5 for 4 hours. A solution of magnesium hexosediphosphate served as a substrate, the equivalent of 3 mg. of esterified P being used in each test-tube. The experiments were properly controlled. The arsenate concentrations employed were 0.004, 0.002, and 0.001 M. Correction was made for the free phosphate in the hexosediphosphate solution, for phosphate liberated by the cytolysed cells without any additional phosphate ester, as well as for the arsenate. The latter correction was made on the basis of direct determination on a corresponding arsenate concentration. The tests with arsenate have all shown an increased hydrolysis of the phosphate ester, as can be seen from the recorded data.

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<th></th>
<th>Control</th>
<th>Arsenate</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0.004M</td>
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<tr>
<td>P hydrolyzed, mg.</td>
<td>0.242</td>
<td>0.503</td>
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<tr>
<td>&quot; increase, mg.</td>
<td>0.261</td>
<td>0.163</td>
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<tr>
<td>Increase, per cent</td>
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<td>67.5</td>
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It is obvious, therefore, that arsenate does stimulate very markedly the phosphatase activity of the blood cells, this stimulation being directly related to the arsenate concentration, as was also the case in the blood glycolysis experiments.

We may conclude, therefore, that the great liberation of inorganic phosphate observed in blood-glycolyzing systems under the influence of arsenate is due to a stimulation of the blood phosphatases. The stimulation of the glycolyzing enzymes by arsenate, on the other hand, is not a general phenomenon. With rabbit blood this is very prominent, but with dog blood the arsenate acts as an inhibitor of glycolysis. Evidently the effect of arsenate on glycolysis depends upon the nature of the particular glycolyzing system. Just what part of this system is responsible for
either the stimulating or the inhibiting action of the arsenate must still wait for a solution.

SUMMARY

Arsenate may either accelerate or suppress blood glycolysis, the effect being more or less directly related to the concentration of the arsenate. Rabbit blood responds by increased glycolysis but dog blood responds by a diminished glycolysis. This is true whether the red blood cells are suspended in isotonic NaCl or in Locke’s solution. The inorganic phosphates of the glycolyzing blood, however, invariably increase under the influence of arsenate, and in direct relation to the arsenate concentration, owing to the stimulation of the blood phosphatases. The effect seems to be quantitatively greater in isotonic NaCl than in Locke’s solution.

BIBLIOGRAPHY

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