CXCI. THE ACTION OF ARSENITE ON TISSUE RESPIRATION.

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THE action of arsenious acid on respiration was studied first by Onaka [1911]. This author found that arsenite, like cyanide, strongly inhibits the oxygen uptake of avian red blood corpuscles. More recently Dresel [1926] found the oxygen uptake of rat liver to be inhibited by arsenite, while the formation of lactic acid by Jensen's sarcoma was but little affected by this poison. Both the fermentation and oxygen uptake of yeast were found to be greatly inhibited by arsenite. No attempts were made to determine more closely the part of the oxidation system on which arsenite exerts its influence. Onaka's results suggested that the mode of action was identical with that of cyanide which is known to inactivate the indophenol oxidase (Atmungsferment, cytochrome oxidase).

Rabbit's liver was used as material in the present investigation. The oxygen uptake was measured in the Barcroft apparatus. The rates of respiration with and without arsenite or cyanide respectively are given in Table I. As the table shows the respiration is inhibited to a similar (large) extent by both poisons.

Table I.

	O ₂ uptake in 10 min. (mm. ³)			Mean
	240	293	218	
Liver (1 g.)	86	88	106	93
„ + 0.01 M KCN	99	112	67	93
„ + 0.002 M As ₂ O ₃	64	70	67	67
% inhibition by KCN	59	62	79	67
„ As ₂ O ₃				

Table II.

As ₂ O ₃ (M)	% inhibition of O ₂ uptake by liver pulp	
	1st 10 min.	2nd 10 min.
0.01	80	86
0.0045	76	85
0.002	77	80
0.0009	75	80
0.0004	71	79
0.00013	50	68
0.00004	16	25

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Table II gives the inhibition by different concentrations of arsenite. It will be seen that even very high dilutions have a strong depressing action and that the inhibition tends to reach a maximal value at a very small concentration and then to remain roughly constant over a wide range of dilutions. In both respects arsenite resembles cyanide [Dixon and Elliot, 1929].

The difference of the mechanism of action of cyanide and arsenite can however be demonstrated very clearly by the behaviour of the indophenol oxidase. If the minced liver is washed with water and then suspended in phosphate in presence of *p*-phenylenediamine the system shows a strong oxygen uptake owing to the oxidation of the phenylenediamine by the indophenol oxidase. This enzymic action, which represents the so-called oxygen activation, is entirely inhibited by small concentrations of cyanide, but is quite insensitive even to the highest (0.01 *M*) concentrations of arsenite (Table III).

Seeing that the arsenite had no effect on the oxygen activation it seemed probable that it poisoned the hydrogen activation. This latter mechanism can easily be observed in the oxidation of succinic acid. As shown by Fleisch [1924] and also by myself [1924] activation of both hydrogen and oxygen is required for the oxidation of this substance. If either of these activating mechanisms is impaired the oxygen uptake stops. As shown in Table II the oxidation of succinic acid is relatively insensitive to high concentrations of arsenite¹.

These experiments led therefore to the conclusion that arsenite does not inhibit respiration by poisoning the activation either of hydrogen or of oxygen. According to our present knowledge of the mechanism of oxidations in tissues these observations suggest that the arsenite interferes with some reaction or reactions which take place prior to the hydrogen activation, in which the metabolites are prepared by anaerobic changes for their oxidation. It is generally held by biologists that oxidations are always preceded by anaerobic changes, which are usually regarded as fermentation. It seems probable that arsenite interferes with one or more of these reactions, and inhibits the respiration by inhibiting the elaboration of dehydrogenisable, *i.e.* combustible, substances.

The behaviour of fresh unwashed liver tissue towards methylene blue under anaerobic conditions is in good agreement with this conclusion. If the freshly minced liver is suspended in phosphate and methylene blue is added *in vacuo*, the dye is rapidly decolorised owing to the oxidation of metabolites accumulated during the manipulations or formed at the time of the experiment. If the suspension is kept in contact with arsenite prior to its incubation with methylene blue, the reduction time will be found to be 25 % longer, owing apparently to the slower production of dehydrogenisable substances during the experiment. If prior to the addition of arsenite the suspension is

¹ Citric and lactic acid were used in an attempt to get additional evidence, but it was found that both substances were oxidised by washed liver too slowly to give reliable results.

incubated anaerobically, time being given for metabolites to accumulate, the reduction time is found to be but slightly increased by arsenite. This shows that the latter has but little effect on the rate of dehydrogenation of natural hydrogen donors. If on the other hand the suspension is aerated, time being given for the metabolites already formed to oxidise, the increase in reduction time in presence of arsenite becomes considerable (280 %).

It seemed interesting to compare these results obtained on liver with the action of arsenite on the respiration of yeast. It was found that the oxygen uptake of yeast can also be greatly inhibited by arsenite, but much higher concentrations of the poison are required¹ (Table IV).

The alcoholic fermentation (as measured by the CO₂ production) is practically unaffected by 0.057 *M* arsenite, which concentration almost completely inhibits the oxygen uptake (Table IV).

Table III.

	O ₂ uptake in 10 min. (mm. ³)	
[Liver (0.8 g.) + succinate	368	346
" " + As ₂ O ₃	356	324
" + <i>p</i> -phenylenediamine	90	175
" " + As ₂ O ₃	87	182

Table IV.

Yeast (mg.)	As ₂ O ₃ (<i>M</i>)	% inhibition	
		O ₂ uptake	CO ₂ output
100	0.06	90	4
100	0.03	49	0
100	0.003	44	0
100	0.0003	0	0

Arsenite thus gives a further method for the separation of respiration from alcoholic fermentation in yeast. The results are at variance with those obtained by Dresel [1926]. They are in accordance with the view of Lundsgaard [1930], who was led, by the study of the action of iodoacetic acid, to the conclusion that the processes involved in respiration and alcoholic fermentation are at least partly different. While iodoacetic acid separates respiration from alcoholic fermentation by inhibiting the latter, arsenite makes a separation possible by inhibiting the former.

EXPERIMENTAL.

The liver of the freshly killed rabbit was freed from the big veins and the gall bladder and minced in a machine to a fine pulp, which was kept at 0°. Portions of 1 g. were weighed out on watch glasses. The pulp was washed with 1 cc. of phosphate into the flask of the Barcroft apparatus of the usual type as described by Dixon and Elliott [1930]. 0.04 *M* phosphate was

¹ This difference might be due to a smaller permeability, though identical results were obtained with "plasmolysed" yeast.

used, prepared by mixing 2 parts of 0.04 M Na_2HPO_4 with 1 part of 0.04 M KH_2PO_4 . 0.5 cc. of 0.05 M As_2O_3 or 0.1 M KCN was added. The middle tube was filled with 0.5 cc. of strong potassium hydroxide solution absorbed on filter paper [Dixon and Elliott, 1930]. In the compensating vessel the liver was replaced by 1 cc. of water.

The flasks were connected to the manometer and immersed in a water-bath at 40°. Shaking was immediately begun. After 10 min. the taps were closed, and readings were taken after 10, 20 and 30 min. The results are given in Table I.

In the experiments with *p*-phenylenediamine and succinic acid the liver pulp was suspended in 10 times its own volume of distilled water, the suspension was gently stirred for 10 min., and then centrifuged. The centrifugate was reduced in the mortar with sand to a macroscopically homogeneous pulp. This was suspended in phosphate buffer, 2 cc. being used per g. of washed liver pulp. The suspension was filtered through fine muslin, and 2 cc. of the filtrate were introduced into the Barcroft apparatus together with 0.5 cc. of 0.05 M As_2O_3 and 1 cc. of 3 % neutralised *p*-phenylenediamine or 5 % sodium succinate. In the compensating vessel the liver suspension was replaced by phosphate. Without succinate or phenylenediamine the suspension showed practically no O_2 uptake.

In the experiments with methylene blue the freshly minced liver was suspended in phosphate buffer as before. To one part of this suspension 1/20 vol. of 0.056 M As_2O_3 was added and to the other part 1/20 vol. of water. Both suspensions were left at 0° for 10 min., to give time for the arsenite to diffuse into the cells. 3 cc. of the liver suspension were introduced into Thunberg tubes, and there mixed with 1 cc. of 1:5000 methylene blue; the tubes were evacuated and incubated. Reduction time with arsenite 132 sec. and 145 sec.; without arsenite 105 sec. and 115 sec.

Part of the suspension was incubated without arsenite *in vacuo* for 30 min. Then the suspension was divided into two parts. To one part 1/20 vol. 0.056 M As_2O_3 was added and to the other part an equal volume of water was added. Both were left for 10 min. at 0°, and then samples of 3 cc. were taken and introduced into Thunberg tubes with methylene blue as above. Time of decoloration of sample with As_2O_3 195 sec. and 205 sec.; without As_2O_3 180 sec. and 180 sec.

If prior to their incubation the suspensions were aerated by gently shaking them in the water-bath for 10 min. in big open flasks the reduction times were the following; aerated without As_2O_3 2 min. 55 sec. and 2 min. 45 sec.; with As_2O_3 6 min. 30 sec. and 6 min. 30 sec.

In experiments with baker's yeast 10 g. of yeast were suspended in 100 cc. of 0.01 M buffer containing Na_2HPO_4 and KH_2PO_4 in the ratio 1:4. 1 cc. of this suspension was introduced into the Barcroft apparatus together with 1 cc. of 5 % glucose and 1 cc. of As_2O_3 solution was added. The compensating vessel contained in place of yeast only buffer solution. If O_2 uptake was

measured the middle tube was filled with paper soaked in strong potassium hydroxide. The Barcroft apparatus was immersed in the water-bath at room temperature (18°). Taps were closed 10 min. later. Readings were taken after 10 min. In experiments in which the CO₂ production was measured no potash was placed in the middle tube. 0.3 cc. of 0.1 *M* KCN was added to the suspension in order to exclude the Pasteur reaction. Corrections were made for the oxygen uptake. The results are given in Table IV.

SUMMARY.

Arsenite strongly inhibits the respiration of minced liver tissue, but has no effect on oxygen activation, and but little effect on hydrogen activation. In yeast arsenite in higher concentrations inhibits respiration but has no effect on alcoholic fermentation.

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