XXXIX. THE EFFECT OF A YEAST EXTRACT ON THE OXYGEN CONSUMPTION OF WASHED FROG MUSCLE.

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IN 1911 Batelli and Stern [1911] showed that if some finely divided mammalian muscle was either allowed to respire until the velocity of its respiration was somewhat diminished or was washed with a little cold water, which produced a similar effect, the addition of an extract of various mammalian tissues made with cold water increased the velocity of the respiration and in some cases restored it to its initial value.

They showed that the effect was temporary, but that a second addition of such a tissue extract produced a second, temporary, increase in the velocity of respiration. They assumed that a substance which they called "Pnein" was present in these extracts. They found that by a short period, 45 minutes, of respiration, the "Pnein" was not destroyed to any great extent.

In 1918 and 1919 Meyerhof working on "acetone-yeast" [1918] and frog muscle [1919, 1] showed that in the case of each of these substances the velocity of respiration could be very considerably reduced by exhaustive washing and could afterwards be restored by the addition of an extract of yeast or of animal tissues made with boiling water ("Koch-saft").

He assumed the presence in these extracts of a substance or a mixture of substances, which accelerated the velocity of respiration, and named it the "Respiration-substance" (Atmungs-körper). He advanced the hypothesis that the "respiration-substance" probably consists of a "co-enzyme to respiration" which is an oxygen-carrier [Meyerhof, 1919, 2] and is identical with the co-enzyme to alcoholic fermentation [Harden and Young, 1906], together with a supply of oxidisable material. He observed that, on warming such an extract for some hours in the air [Meyerhof 1918], a portion of the "respiration-substance" was destroyed.

The experiments described in this paper were undertaken in an attempt to obtain evidence as to the actual existence of a "co-enzyme to respiration" of the type suggested by Meyerhof.

Some finely chopped frog's muscle was washed several times with distilled water. It was then aerated for several hours at the ordinary temperature of

the laboratory whilst suspended in a yeast "Koch-saft." The liquid was then filtered off from the muscle, and used as the suspension fluid in which more freshly washed frog's muscle was allowed to respire in a Barcroft micro-respirometer, by means of which its oxygen uptake was measured. Some more of the same "Koch-saft" was stored at 0° out of contact with the air for the same period of time and its effect on the respiration of washed frog's muscle was compared with that of the aerated "Koch-saft."

If the "respiration-substance" were a true co-enzyme and the oxidisable substances were contained in the washed muscle the velocity of respiration in the two cases should not differ considerably. If, however, the oxidisable substances were contained in the "Koch-saft" the unused one should be the more active. In the latter case the existence of a respiration co-enzyme would not be disproved. The assumption of its existence would, however, seem to be unnecessary.

EXPERIMENTAL DETAILS.

The yeast extracts were made by boiling some baker's yeast with about twice its weight of distilled water for a few minutes and then filtering as quickly as possible. They were adjusted with caustic soda to p_H 7.6, and divided into two portions. One was passed through a Berkefeld filter and stored in small evacuated tubes in an ice-chest. The other portion was aerated at room temperature in contact with some frog's muscle which had been washed several times with distilled water. The exact figures for each case are given in the table of results. It was then filtered from the muscle, passed through a Berkefeld filter and stored in an ice-chest until it was used. In order to determine the effects of the two portions on the respiration of washed frog's muscle some more of the latter was taken and half a gram weighed out into each of three respirometers. In them were placed 3 cc. respectively of each of the two samples of "Koch-saft" and of a 1 % solution of KH_2PO_4 , each being adjusted with caustic soda to p_H 7.6. They were placed in a bath maintained at a known temperature and shaken by means of an electric motor. Soda was placed in the cups to absorb the carbon dioxide evolved.

In experiments 7 and 8, to the yeast extract was added an excess of saturated lead acetate solution and enough of a suspension of lead hydroxide to keep the liquid slightly alkaline. This removes a large number of substances, among them glutathione and any inorganic phosphates. The precipitate was filtered off and the excess lead was removed from the filtrate with sulphuretted hydrogen and the solution concentrated *in vacuo*. The acid potassium phosphate was added in experiment 8 in accordance with the discovery by Meyerhof that it makes the respiration velocity more nearly constant during the experiment.

It can be seen from the table of results that during respiration a great part of the "respiration-substance" is destroyed.

No.	Extract	1st respiration					2nd respiration					
		Vol. cc.	Wt. of frog's muscle grams	Times washed	Vols. of H ₂ O each washing	Time of aeration hours	Temp. °C.	cmm. O ₂ absorbed per gram of muscle in one hour			Frog's muscle	
								(1) In phosphate	(2) In previously respired extract	(3) In stored extract	Times washed	Vols. of H ₂ O each washing
1	Yeast	30	2.5	6	50	18	23	36	98	424	7	50
2	"	45	4.5	8	50	18	22	42	88	254	8	75
3	"	30	3	6	60	18	23	20	20	296	8	50
4	"	26	2.6	8	50	21	22	30	33	296	8	50
5	"	50	5	6	50	23	22	33	147	420	8	80
6	"	50	5	6	50	45	21.5	37	116	337	8	80
7	Yeast filtrate after basic lead acetate	50	5	6	50	44	23.5	43	58	558	8	60
8	As 7 but with 1 % KH ₂ PO ₄ added	40	4	6	50	20	21.5	28	105	449	8	75

DISCUSSION OF RESULTS.

Meyerhof observed that the addition of yeast or of tissue extracts to washed tissue increases the velocity of its respiration. He attributed this effect to the presence in such extracts of a co-enzyme which, working in conjunction with the washed tissue, increased the velocity of the oxidation of the oxidisable substances present. It seemed to him that the known oxidisable substances were present in too small a concentration to account for the effects observed.

Research, subsequent to the publication of Meyerhof's papers, has extended our knowledge of the number and concentration of oxidisable substances contained in such extracts. This knowledge, in conjunction with the results described in this paper, suggests that the stimulation of the respiration of washed tissues, observed on the addition of such extracts, may reasonably be attributed to the united effects of the different oxidisable substances therein contained, rather than to the presence of a co-enzyme, the necessity for which whether as an oxygen-carrier or in any other capacity has not yet been clearly demonstrated.

SUMMARY.

If an extract of yeast, made with boiling water, be aerated for some hours at room temperature in contact with some washed frog's muscle it loses its power to increase the velocity of oxygen uptake of another preparation of fresh washed muscle. This suggests that the effects attributed to a "respiration-substance" capable of providing a general stimulus to intracellular oxidative processes are really to be attributed to the collection of oxidisable substances contained in the yeast extract. When these have been oxidised the extract has no further power to stimulate the respiration of washed frog's muscle.

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