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RESPIRATION AND FUNCTIONAL ACTIVITY

By W. DEUTSCH and H. S. RAPER

(*From the Department of Physiology, University of Manchester*)

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It has long been established that in some organs an increase in functional activity is accompanied by an increase in oxygen consumption. On the other hand, we have as yet no precise knowledge as to how this increased respiratory activity is controlled. Investigations up to the present have been carried out almost exclusively on organs *in vivo* by measurements of the blood flow and analysis of blood gases, although a few experiments have been done on isolated frog muscle which show that the respiratory activity after stimulation is greater than that of resting muscle.

During recent years the use of tissue slices introduced originally by Warburg has found increasing use in studying the respiratory activity of tissues, and it seemed to us worth while to attempt to make use of this method for investigating the increased respiration associated with functional activity. It has the great advantage that the chemical environment of the tissue can be modified more readily than with an organ *in vivo*. It is also possible to measure the respiration independently of changes in blood flow and reflex nervous stimulation.

The question naturally arises, to what extent is the respiration of tissue slices comparable in a quantitative sense with that of the organ itself *in vivo*? Not many comparative observations on this point have been made, but Minami [1923] found the respiration of liver slices of cats near the upper limit of the values obtained by Barcroft and Shore [1912] on perfusing the liver of living cats.

With the submaxillary gland our own observations show that the resting respiration *in vitro* of tissue slices is certainly not less and usually somewhat greater than that obtained by the blood gas-blood flow method. The resting oxygen consumption of the submaxillary gland of dogs *in vivo* as shown in the paper by Barcroft and Kato [1916] is 7·8, 8·1, 3

and 6μl. per hour and mg. dry weight of the gland when given in Warburg's units. Our figures for dogs lie between 6·3 and 10·6μl. For cats the average value taken from a great number of experiments was still higher, namely 10·2μl.

We have chosen the salivary glands for this study in the first instance because of the large number of observations that have been made with them *in vivo* by others and which therefore serve as a basis for comparison. It has also been established by Babkin, Gibbs and Wolff [1932] and by Babkin, Alley and Stavraky [1932] that the influence on the submaxillary gland of stimulation of its parasympathetic nerve is to liberate a chemical transmitter, in all probability, acetylcholine, and that this transmitter by itself will cause increased secretion. As the increase in functional activity thus appears to be a purely chemical effect, it was of interest to find out the effect of acetylcholine on slices of the gland *in vitro*. The parallel effect of stimulation of the sympathetic nerves which may also be presumed to act by the liberation of a transmitter—in this case adrenaline or some similar substance—has been studied by the investigation of the action of adrenaline on the respiration of tissue slices of salivary glands. The observations which follow are essentially preliminary and are intended to demonstrate the possibilities of this method for the particular object in view.

Methods

The measurement of the oxygen uptake was carried out according to Warburg's manometric method [1926] in manometers with cone-shaped vessels with side bulb and inner container, the total volume of which was about 14 ml. The temperature in all experiments was 37·5° C. The gas phase was, if not stated otherwise, oxygen from a cylinder, as a precautionary measure, although control experiments did not show any difference when air was used instead of oxygen, thanks to an improved method of preparing the tissue slices. The solution used was Ringer with 0·2 p.c. glucose and $3{\cdot}0 \times 10^{-3}$ *M* $NaHCO_3$ per litre. The inner cup contained 0·1 ml. 8 p.c. KOH. The various solutions the effect of which on the tissue respiration was to be examined were placed in the side bulb and tilted into the main vessel after the respiration of the resting gland had been examined for 20–30 min. (according to the amount of tissue used). The ensuing rate of the oxygen uptake was again observed after an interval of 5–10 min. (for restoring the equilibrium) and the percentage increase in respiration over that of the resting tissue determined. Usually a control was carried out with tissue of the same gland without addition

of drugs. The results are given in Warburg's units Q_{O_2} = μl. O_2 per mg. dry weight hour. Cats were used for providing the tissue if not otherwise stated.

Preparation of the tissue slices. This was carried out by a modification of the original Warburg method which has already been described by one of us [1936].

Anæsthesia. The best results were obtained if a short and quickly performed anæsthesia with ether taken from a bomb was employed before the gland was taken out. In a few of our earlier experiments the glands failed to respond for no obvious reason.

Results

In Table I the results of a typical experiment are given in which pilocarpine was added to tissue slices of a cat's submaxillary gland.

Table I. Submaxillary gland of a cat. Gas phase: air. 1·33 mg. pilocarpine nitrate in 100 ml. Slices of the same gland in two manometers

					Control	
	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase
During 30 min. before adding pilocarpine	9·9	—	10·4	—	11·3	—
During first 30 min. after	14·6	+47	16·5	+59	11·8	+4·5
During second 30 min. after	12·4	+25	13·7	+30	10·3	−9·1

The increase in respiration produced by pilocarpine is clearly demonstrated. Several experiments of this kind have shown that the magnitude of the effect varies from gland to gland and sometimes even between various slices of the same gland since the tissue of the gland is composed of various elements, but the qualitative effect remains constant. The percentage increase in oxygen uptake varied in several experiments between 20 and 100.

Table II gives examples of the results obtained by adding acetylcholine with and without eserine, eserine alone or pilocarpine alone to gland slices. Cats, dogs and rabbits were used in these experiments and both the submaxillary and the parotid glands were investigated.

The figures show that acetylcholine has a definite positive effect in stimulating the respiration of the gland slices, and this effect is markedly increased by eserine. Eserine alone has an action similar to that of pilocarpine. The greatest increases in respiration were always obtained by a combination of acetylcholine and eserine. Parotid and submaxillary glands behave alike in their response to acetylcholine and eserine, and this is true of all three types of animals used.

The figures for the experiments with acetylcholine alone given in Table II are taken from a comparative experiment and the values are calculated in the same way as in Exps. 3–10 of the same table, that is to

TABLE II. The effect of various drugs on submaxillary gland and parotid gland of various animals

No.	Animal	Gland	Drug	mg. per 100 ml.	Q_{O_2} Before addition	Q_{O_2} After addition	p.c. increase	Q_{O_2} Control	Q_{O_2} Control	p.c. increase	Gas
1	Cat	Submaxillary	Acetylcholine	0·066	9·3	12·1	+30	8·8	7·9	− 10	Air
2	Cat	Submaxillary	Acetylcholine	0·198	11·2	15	+34	8·8	7·9	− 10	Air
3	Cat	Submaxillary	Eserine	4·0	13·6	22·6	+66	14·3	15·2	+6	O_2
4	Cat	Submaxillary	Acetylcholine +eserine	0·066+4·0	10·7	22·7	+120	8·3	7·7	− 7	O_2
5	Cat	Parotid	Acetylcholine +eserine	0·066+4·0	4·9	9·1	+86	—	—	—	O_2
6	Cat	Parotid	Pilocarpine	2·0	7·3	15·2	+108	6·4	6·7	+5	O_2
7	Dog	Submaxillary	Acetylcholine +eserine	0·066+4·0	10·6	19·6	+85	—	—	—	O_2
8	Dog	Parotid	Acetylcholine +eserine	0·066+4·0	10·6	19·7	+86	—	—	—	O_2
9	Rabbit	Submaxillary	Acetylcholine +eserine	0·066+4·0	13·4	22·0	+64	—	—	—	O
10	Rabbit	Parotid	Acetylcholine +eserine	0·066+4·0	7·8	9·4	+21	—	—	—	O_2

say by taking the oxygen uptake during 30 min. after addition of the drug and comparing it with the resting value. The special features of the oxygen uptake in experiments in which acetylcholine alone is added do not become as evident as when the following more detailed description is given.

In Table III a comparative experiment is recorded in which the effect of different amounts of acetylcholine with and without eserine on tissue

TABLE III

	Manometer 1		Manometer 2		Manometer 3		Manometer 4	
	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase
Before tilting	9·3	—	11·2	—	10·8	—	8·8	—
During first 10 min. after	13·8	+48	16·9	+51	16·4	+52	8·4	−5
During second 10 min. after	11·8	+27	14	+25	17·2	+59	8·4	−5
During third 10 min. after	10·8	+15	14	+25	15·4	+43	7	−20
During next 15 min. after	8·6	−7	12·2	+9	15·2	+39	7·5	−15
During next 15 min. after	8·6	−7	12·2	+9	15·2	+39	7·5	−15
During next 20 min. after	8·4	−9	11·2	0	14·6	+35	8·1	−8

slices was examined. The concentration of acetylcholine in manometer 1 after tilting was 0·066 mg. per 100 ml.; in manometer 2, 0·198 mg. per 100 ml., *i.e.* three times as much; and in manometer 3, 0·132 mg. per

100 ml., *i.e.* twice as much as in manometer 1. Manometer 3 contained, in addition to the acetylcholine, eserine of the concentration 2·67 mg. per 100 ml. Manometer 4 served as control as usual.

It is evident from this experiment:

(1) That the smaller quantity of acetylcholine combined with eserine (manometer 3) has a greater effect than the greater quantity of acetylcholine (manometer 2).

(2) That the percentage increase figures in manometers 1, 2 and 3 are very much alike during the first 10 min., but the oxygen uptake passes off as time goes on most quickly in manometer 1, less in manometer 2 and still less in 3. The effects of acetylcholine and eserine on the activity of the slices are overlapping in manometer 3. In order to differentiate clearly the interaction of the drugs it is necessary to lower the concentration of the eserine appreciably as was done in the following comparative experiment (Table IV).

The concentration of the acetylcholine after tilting was 0·1 mg. per 100 ml., and the concentration of the eserine 0·0083 mg. per 100 ml. (=1 : 12,000,000).

Manometer 1 contained only acetylcholine.

Manometer 2 contained only eserine.

Manometer 3 contained acetylcholine + eserine.

TABLE IV

	Manometer 1 (ACh.)		Manometer 2 (Es.)		Manometer 3 (ACh. + Es.)	
		p.c. increase		p.c. increase		p.c. increase
During 20 min. resting	14·5	—	13·6	—	13·5	—
After tilting						
During 10 min.	27·9	92·5	13·3	0	31·2	133
During next 10 min.	19·5	34·5	15	11	28·5	115
During next 10 min.	14·1	0	15·5	14	27	104

The increase in oxygen uptake passes off quickly as time goes on, when acetylcholine alone is applied (manometer 1). The oxygen uptake caused by the very low concentration of eserine is just noticeable (manometer 2), whereas the oxygen uptake induced by the mixture of acetylcholine and eserine (manometer 3) is a potentiated one and passes off slower than in manometer 1.

The inhibitory effect of atropine on the response to acetylcholine-eserine

Since atropine inhibits the secretory effect of the chorda tympani in the cat's submaxillary gland its effect on the increase in respiration induced by acetylcholine and eserine was studied. The results of such an experiment are given in Table V.

TABLE V. Gase phase: oxygen

Manometer	1	2	3	4	5
The side bulb contained:					
0·8 p.c. eserine solution (ml.)	0·15	0·15	—	0·15	—
0·002 p.c. acetylcholine solution (ml.)	0·1	0·1	—	—	—
0·1 p.c. atropine solution (ml.)	0·1	—	0·1	—	—
In the main vessel:					
Ringer bicarbonate solution (ml.)	2·65	2·75	2·9	2·85	3
Q_{O_2} during 20 min. before (μl.)	9·2	12·6	9	9·4	9·4
Q_{O_2} during 30 min. after tilting (μl.)	8·3	25·2	8·8	14·2	9·3
Increase (p.c.)	−10	+100	−2	+51	−1

This experiment shows the complete inhibition of the acetylcholine effect by atropine (manometer 1). The respiration after addition of the drug remains at the resting value within the limit of the experimental error. Atropine itself (manometer 3) does not act on the resting respiration.

The effect of adrenaline

In order to examine the effect of adrenaline on the oxygen uptake it was necessary to prevent the adrenaline from being oxidized before it came in contact with the tissue. The time during which it had to be kept in the side bulb is about 40 min. (time for reaching the equilibrium of the temperature plus time for the observation of the resting respiration). Preliminary experiments were carried out to prevent oxidation by mixing the adrenaline with phosphate buffer of *p*H 5·9 since adrenaline is not readily oxidized at that *p*H. The solution in the main vessel was phosphate Ringer instead of bicarbonate Ringer solution. But the results obtained were unsatisfactory, for the substitution of phosphate for bicarbonate hampers the effect of the stimulation of the oxygen uptake. When the method was changed by keeping the adrenaline in a very dilute hydrochloric acid solution in the side bulb the results were satisfactory.

Immediately before starting the experiment an adrenaline solution of the strength 1 in 40,000 was made up with Ringer solution of the usual composition but without bicarbonate, containing in addition $3{\cdot}1 \times 10^{-4}M$ HCl in 1000 ml. 0·25 ml. of this solution was placed in the side bulb, and when tilted into the 2·75 ml. Ringer bicarbonate solution contained in the

main vessel made an adrenaline concentration of 1 in 480,000. A premature oxidation of adrenaline was so prevented. It may be worth mentioning that the measurement of the oxygen uptake could not be disturbed if an oxidation of the adrenaline occurred after adding it to the main vessel, since the amount of adrenaline added takes up only 1·03 μl. O_2 at the maximum. The amount of hydrochloric acid added could not interfere either, for the equivalent of carbon dioxide driven out by it is only 1·7 μl. which were readily absorbed by the KOH before the determination of the effect began. Two experiments of this kind are shown in Table VI.

TABLE VI Submaxillary gland of two different cats. Gas phase: air

	Q_{O_2}			Q_{O_2} control		Adrenaline mg. per 100 ml.
	Before	After	p.c. increase	Before	After	
Cat 1	8·2	12·9	58	8	8	0·28
Cat 2	8·2	12·8	56	8·5	8·5	0·14

Experiments of the same kind with cat's parotid gland failed to show any increase in respiration on the addition of adrenaline. Furthermore, neither the submaxillary nor parotid glands of dogs and rabbits gave an increased respiration with adrenaline.

The effect of secretin on slices of pancreatic tissue

The experiments so far dealt with were concerned with chemical reagents in the proper sense known to cause secretion from the salivary glands. It was of interest to test a hormone such as secretin causing pancreatic secretion in regard to the effect on the oxygen uptake of slices of pancreatic tissue. An experiment of this kind is recorded below.

The secretin was prepared by grinding up the mucous membrane of the duodenum of a recently killed pig with sand, extracting one part of the pulp with two parts Ringer's solution and subsequently centrifuging and filtering.

This extract was immediately used for the respiration experiment and later on when tested for its activity in a second cat (chloralose anæsthesia) proved to be active. The secretion of pancreatic juice started a few seconds after intravenous injection of 10 ml. secretin solution at a rate of 1 drop per minute.

It was observed that the secretin solution reacted slightly acid (pH = 6·4). Since the reaction of the secretin solution is of importance not only for its activity but also for the possible interference with the manometric measurement of the oxygen uptake the secretin solution was divided into three parts, A, B and C, which were made up as follows:

A, 10 ml. original secretin solution, 0·5 ml. $NaHCO_3$ solution (0·155 ml.), 0·5 ml. water; B, 10 ml. original secretin solution, 1 ml. $NaHCO_3$ solution (0·155 ml.); C, 10 ml. original secretin solution, 1 ml. water. The resulting *p*H values were: A, 7·7; B, 8·7; C, 6·4. The side bulb of manometer 1 contained 0·7 ml. of solution A; manometer 2, 0·7 ml. of B; and manometer 3, 0·7 ml. of C; manometer 4 served as control with tissue without secretin. Three other manometers made up as manometers 1–3 but without tissue controlled the possible manometric change which might occur when the secretin solution was tilted into the main vessel. Gas disappeared in each of these three manometers at the same rate of 1·7 μl. per 10 min., which must be subtracted in calculating the experiment. The result is recorded in Table VII, which includes also the action of acetylcholine plus eserine on pancreatic tissue.

TABLE VII

	A		B		C		Control with normal tissue	ACh. + Es.	
	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	Q_{O_2}	p.c. increase
During 20 min. resting	5·5	—	6·5	—	5·8	—	6	4·7	—
Working:									
During first 10 min.	6·7	22	7·2	11	9·1	57	5·4	6·5	38
During next 10 min.	6·5	18	7·8	20	7·3	26	5·5	6·1	31
During next 10 min.	6·1	11	7·6	17	7	21	5·9	6·2	34

The greatest increase in oxygen uptake is produced by the original secretin solution C in accordance with the known destructibility of secretin in alkaline solutions.

In the subsequent experiment with pancreatic slices of a second cat the same three solutions A, B and C were kept for half an hour in a boiling water bath and the oxygen uptake again observed in the same way as in the previous experiment. The result is shown in Table VIII.

TABLE VIII

	A		B		C		Control
	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}	p.c. increase	Q_{O_2}
During 20 min. resting	3·9	—	2·8	—	3·7	—	3·2
Working:							
During first 10 min.	5	28	2·8	0	5·9	59	3·3
During next 10 min.	4·8	23	2·8	0	4·7	27	3·3
During next 10 min.	4·5	16	2·6	−7	4·3	18	3·3

In the most alkaline solution B the activity of the secretin has disappeared.

Experiments with saliva

On the assumption that a substance in saliva may occur which could produce an effect on the metabolism of the gland, an experiment with a concentrated saliva was undertaken which showed no effect. The respiration remained at its resting value for the first 45 min. after addition of the saliva and then decreased rapidly, showing some damaging effect to the tissue. The procedure was as follows: 20 ml. human saliva were collected, 80 ml. absolute alcohol added and the mixture centrifuged. The supernatant liquid was concentrated *in vacuo* at 37° to about 1·3 ml., and 1 ml. of this liquid placed into the side bulb of the manometer.

Discussion

The intention of these preliminary experiments was to survey the possibilities of this method of attack on the problem of how the respiration associated with increased functional activity is controlled. It is therefore of interest that they show that the respiratory effects which accompany secretion are also observed when chemical agents which will cause secretion are applied to tissue slices *in vitro*. An analysis of the factors concerned in the increase of respiration observed, whether for instance it is due to alterations in the respiratory catalysts or to the supply of the necessary substrates, will take a considerable time, and further work in this direction is proceeding. It should be mentioned that Barcroft and Müller [1912] have already shown that increased blood flow alone is not a cause of increased respiratory activity, thus ruling out oxygen supply as a limiting factor. Our own results with tissue slices confirm this fully. As regards the magnitude of the increase in respiration, it is already possible to make comparisons with work of others on the whole organ *in vivo*. Barcroft and Kato [1916] have published detailed figures of the respiration of the dog's submaxillary gland before and after administration of pilocarpine. These converted into Warburg's units (Q_{O_2} = μl. O_2 per mg. dry weight hour) are as shown in the table on p. 284.

The maximum of the oxygen uptake is reached in Exps. 2 and 4, 5 min. after injection, in Exp. 1 15 min. after. Only in Exp. 3 does it take appreciably more time, *i.e.* 55 min. The maximum percentage increase in respiration with respect to the resting respiration is, for Exp. 1, 108 p.c.; for Exp. 2, 123 p.c.; for Exp. 3, 680 p.c.; and for Exp. 4, 310 p.c.

Exp. 3 is outstanding again, for the percentage increase is exceptionally great, less, however, on account of the absolute Q_{O_2} value than of its very low resting respiration. Barcroft and Kato's figures for the

oxygen uptake represent in each instance an instantaneous picture of the metabolism, whereas our figures represent an average value during 30 min. Nevertheless, the Q_{O_2} values after stimulation were of the same order as in Barcroft and Kato's Exps. 1 and 2, but less than in Exps. 3 and 4 in regard to the maximum increase. The reason is partly that our resting values were generally greater as has been mentioned in the introductory note. The return to the resting value in our experiments was just as irregular as in Barcroft and Kato's experiments, but the effect was always less lasting.

	Before pilocarpine injection	After pilocarpine injection			
1	15 min. Q_{O_2}=7·8	5 min. 7·8	15 min. 16·2	40 min. 13·3	1 hr. 10 min. 13·0
2	10 min. Q_{O_2}=8·1	5 min. 18·9	16 min. 18·9	50 min. 15·0	1 hr. 20 min. 9·9
3	8 min. Q_{O_2}=3·0	— —	18 min. 14·1	36 min. 13·5	55 min. 23·0
4	25 min. Q_{O_2}=6·0	6 min. 24·6	27 min. 19·0	— —	56 min. 19·0
1	15 min. Q_{O_2}=7·8	1 hr. 40 min. 15·6	— —	3 hr. 25 min. 12·6	4 hr. 49 min. 11·4
2	10 min. Q_{O_2}=8·1	2 hr. 12 min. 11·7	2 hr. 50 min. 4·5	— —	— —
3	8 min. Q_{O_2}=3·0	1 hr. 18 min. 12·0	1 hr. 43 min. 6·0	— —	— —
4	25 min. Q_{O_2}=6·0	1 hr. 20 min. 19·0	2 hr. 20 min. 17·4	3 hr. 1 min. 12·9	4 hr. 38 min. 10·8

In another paper Barcroft and Piper [1912] examined the influence of adrenaline on the metabolism of cat's submaxillary glands. There are three experiments in which successive blood samples were taken. The first before the injection of adrenaline, the second during the injection of adrenaline, the third and fourth whilst the saliva was still flowing, the fifth immediately following. The table given below is calculated in Warburg's units (Q_{O_2}).

	Resting	Stimulated					
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
Exp. 3	4·8	10·2	25·2	18·0	13·8	9·3	—
Exp. 4	6·3	7·5	14·1	23·1	8·4	—	—
Exp. 5	6·6	6·0	21·0	20·7	15·0	9·9	6·6

The corresponding percentage increase of oxygen uptake amounts to:

Exp. 3	112	425	275	208	94	—
Exp. 4	19	124	267	49	—	—
Exp. 5	−9	218	214	128	50	0

The percentage increase in our experiments never exceeded 100 p.c. and varied between 40 and 100 p.c. The circumstances in the living animal appear to be more suitable for experiments with adrenaline. As regards the adrenaline experiments with glands from other animals than cats, there are unfortunately no experiments with living animals of the same kind as Barcroft and Piper's available for comparison. It is not possible therefore to decide whether the lack of response of tissue slices of dog's and rabbit's submaxillary and parotid gland and of cat's parotid gland to adrenaline is due to technical reasons or to some intrinsic difference between these glands and the submaxillary gland of the cat. Investigators who have studied the response of the dog's parotid to sympathetic stimulation have obtained very varied results. On the other hand, the following statement by Starling [1933] suggests that the dog's parotid does not secrete on sympathetic stimulation.

"The effects of exciting the sympathetic nerve supply differ according to the gland and the animal which is the subject of experiment. In the dog excitation of the cervical sympathetic causes the secretion of a few drops of thick viscid saliva from the submaxillary gland. In this animal no secretion is obtained at all from the parotid gland on exciting the sympathetic but the influence is shown by the occurrence of histological changes in the gland cells. In the cat the submaxillary saliva obtained on sympathetic excitation may be as copious as, and even more watery than, the saliva obtained from the same gland on stimulation of the chorda tympani."

The experiments with acetylcholine do not need discussion, since all phenomena could be reproduced in accordance with facts already known in regard to this substance.

Summary

In a series of experiments it is demonstrated that the problem of how the respiration of glands associated with increased functional activity is controlled, can be attacked by using Warburg's *in vitro* method with tissue slices. The increase in respiration of salivary glands can be reproduced when chemical agents which will cause secretion are applied. In the case of pancreatic tissue also the hormone secretin causes an increase in respiration and loses this property when inactivated. The influence of acetylcholine, eserine and pilocarpine on the oxygen uptake of submaxillary and parotid gland were examined.

The increase in respiration caused by these drugs can be inhibited by atropine which itself is without effect on the resting respiration of these

glands. The potentiating effect of a combination of eserine with acetylcholine can be shown under suitable conditions.

Adrenaline causes an increase in respiration only in cat's submaxillary gland and not in cat's parotid gland. Adrenaline is without effect on both the parotid and submaxillary gland of dogs and rabbits.

Experiments with an alcoholic extract of human saliva failed to show the presence of a substance causing increased respiration of the submaxillary gland.

The results obtained with the tissue slice method are compared with the *in vivo* method of earlier investigators.

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