EFFECT OF DIPHTHERIA TOXIN ON RELATIONSHIP BETWEEN EXCESSIVE HEAT PRODUCTION RELATIVE TO GAS EXCHANGE AND DISSOCIATION OF RESPIRATION AND PHOSPHORYLATION IN LIVER CELLS

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UDC 612.35 [612.563+612.26] .014.46:615. 372:576.852.23

After Rubner [17] had established a relationship between the results of direct and indirect calorimetry in calculations of total heat production, indirect calorimetry quickly displaced direct in experimental and clinical practice for studying total energy metabolism. Modern information on the bioenergetics of pathological processes is entirely based on this method, and the universality of its application for the study of pathological states has so far remained almost undisputed.

Nevertheless, as long ago as in the 1890s, it was shown that in certain pathological processes a discrepancy between the results of direct and indirect calorimetry is a regular phenomenon, and calculation of heat production by Rubner's principle reflects its true dimensions insufficiently accurately [13, 14]. A. A. Studenskii [13] analyzed the possible reasons of this phenomenon and its importance in pathology on theoretical grounds. Unfortunately these observations did not receive proper attention at that time, and only in recent years has their importance for the study of general energetics in pathology received confirmation by the work of several Soviet investigators [1-4, 5, 9, 10, 15, 16].

Comparison of the results of direct and indirect calorimetry has confirmed that in some pathological processes heat formation in the course of energy conversions is reduced to a lesser degree than the intensity of gas exchange; in other states, conversely, heat production is increased (sometimes by as much as 20% or more) compared with the level of the gas exchange. In the first case calculation of heat production by Rubner's method gives results which are too high, in the second case, they are too low. These discrepancies may be observed not only within the limits of a few hours (as Rubner himself knew), but sometimes for many days.

To explain the development of excessive (compared with that calculated from the gas exchange) heat production in pathological states, in recent years resort has been made to S. A. Neifakh's hypothesis of an increase in the dissipation of energy of food substances entering the cell in the form of primary heat in connection with the dissociation of the processes of coupled respiration and phosphorylation in the cell, and the concept of the thermoregulatory importance of this phenomenon [6, 7, 12]. It has been shown that many factors causing predominance of "direct" heat production (2-4-dinitrophenol, thyroxin, staphylococcal and diphtheria toxins, staphylococcal infection) at the same time cause dissociation of respiration and phosphorylation; conversely, in the case of poisoning with bacterial products without a dissociating action (dysentery and paratyphoid toxins, lipopolysaccharides of bacteria, etc.), the results of direct and indirect calorimetry either coincide with the value obtained from the gas exchange, or they differ from it in the direction of a lower heat production [1-5].

As long as no accurate quantitative estimate of the thermal effect of dissociation has been established, it is difficult to judge to what extent the excess of heat formed, when determined for the organism as a whole, can be attributed to this effect. Nevertheless, if this parallel between the onset of dissociation and of excessive heat production during the action of certain agents exists constantly, this suggests that these phenomena are connected.

Calorimetric and biochemical investigations undertaken in the past have, however, been carried out at different times, on different animals (sometimes of different species of animals), by different workers, and in different conditions; this considerably reduces the reliability of their comparison. For instance, after administration of diphtheria toxin a decrease in the P:O ratio in the mitochondria of liver cells was

Department of General Pathology and Department of Biochemistry, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 64, No. 11, pp. 56-60, November, 1967. Original article submitted January 13, 1967.

TABLE 1. Respiration and Phosphorylation of Liver Mitochondria of Rabbits in Normal Conditions and After Subcutaneous, Intravenous, and Intraventricular Injection of DT (calculated per mg protein of mitochondria)

Experimental conditions	number of animals in group	O_2 absorption				Esterification of P				P:O	
		succinate		glutamate		succinate		glutamate		succinate	glutamate
		μatom	μl	μatom	μl	μatom	μl	μatom	μl		
Normal	15	3,36	6,67	1,46	2,93	4,41	24,00	3,67	20,22	1,34±0,02	2,61±0,05
Injection of DT subcutaneously	7	3,06	5,55	1,02	1,88	4,47	23,29	2,47	12,88	1,62±0,13 $P<0,1$	2,77±0,19 $P<0,5$
intravenously	8	5,26	9,77	1,58	2,91	1,91	9,34	1,77	8,82	0,37±0,04 $P<0,01$	1,24±0,20 $P<0,01$
into the brain	5	5,19	9,66	—	—	1,64	8,15	—	—	0,24±0,04 $P<0,01$	—

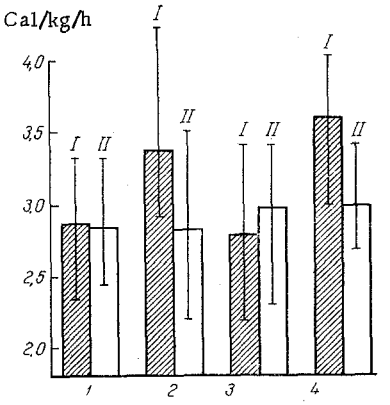

Fig. 1. Heat production (in Cal/kg/h) calculated calorimetrically (I) and from gas exchange (II), in control experiments (1) and in experiments with subcutaneous (2), intravenous (3), and intracerebral (4) injection of DT (mean results for groups of experiments). Vertical lines represent limits of variations.

established in experiments on guinea pigs [8], while an excess of actual heat production over that calculated from the gas exchange was found in calorimetric experiments on rabbits [4], and the severity of the poisoning and the periods of observation were not the same in these two series.

In the present investigation the total heat metabolism and energy metabolism in the liver were investigated during the action of diphtheria toxin (DT) in comparable conditions and on the same objects in order to establish more accurately the presence or absence of a parallel between the changes in these parameters.

EXPERIMENTAL METHOD

Experiments were carried out on 35 rabbits of both sexes weighing from 1.7 to 2.8 kg. DT (2 M.L.D. in a volume of 0.5 ml) was given by a single injection; subcutaneously into the animals of group 1, intravenously into those of group 2. On the day when the the liver was removed the rabbits were placed in a dynamic calorimeter (LITMO) for 3-4 h, and sacrificed (by decapitation) immediately after removal from the calorimeter. The liver was cooled and minced in 0.25 M sucrose solution. The mitochondria were isolated by a modification of Schneider's method —fractional centrifugation at 12,000 g. The oxygen consumption of the mitochondria and the decrease in organic phosphate were determined during incubation in media with succinate and glutamate in a Warburg's apparatus at 30° for 20 min, after which the coefficient of oxidative phosphorylation was calculated. Protein was determined by the biuret method. The results were analyzed statistically by Studenskii's method.

In the 3rd supplementary group of experiments, DT was injected directly into the lateral ventricle of the brain through a

cannula implanted by the method of Repin and Sorokin [11], in a volume of 0.1 ml: 4 rabbits received a dose of 0.04 M.L.D. toxin, and one rabbit 0.004 M.L.D.

EXPERIMENTAL RESULTS

In the control series, as the figure shows, the differences between results of direct and indirect calorimetry were very small, and in some cases did not exceed the limits of experimental error (± 5%), and on the average, Q_{cal} exceeded Q_{O_2} by 1.05%. The results for respiration and phosphorylation in the liver mitochondria of the healthy rabbits are given in the table.

In the experiments in which DT was injected subcutaneously, the general condition, body weight, and body temperature of the animals were not appreciably changed. The animals were killed 3-5 days after injection of DT. Before extraction of the liver, the value of Q_{cal} was significantly ($P = 0.05$) higher than Q_{O_2} for the animals of this group on the average by 19% (see Fig. 1). Heat production calculated from the gas exchange in this series remained within normal limits, and the difference between the results of direct and indirect calorimetry was entirely dependent on an increase in heat production not reflected in the oxygen consumption. However, no significant changes in energy metabolism were found in the liver mitochondria of these rabbits (see Table 1).

Intravenous injection of DT was accompanied by an increase of 1-2° in the body temperature, and on the 2nd-3rd day the temperature fell to its initial level or below, and during the animals' stay in the calorimeter with intensive ventilation, the temperature often fell by 1-2°, which was not observed in the control series or in the experiments with subcutaneous injection. Besides the marked disturbance of thermoregulation, the animals developed general apathy and their weight fell progressively. Two rabbits of this group were sacrificed at the end of the first day after injection, three on the second day, two on the third day, and only one rabbit (in a very serious, preagonal, state) on the fourth day. The rabbits of this group, although retaining an almost normal mean level of heat production (see Fig. 1), had a tendency to give values of indirect calorimetry higher than those of direct (on the average by 5.6% for the group). However, the difference from the control figure was not significant.

Meanwhile the energy metabolism of the liver mitochondria of all 8 rabbits of this group was severely disturbed (see Table 1); the decrease of P:O in these circumstances was due to marked inhibition of phosphorylation with no accompanying significant change in respiration.

One of the authors [8] has previously shown that DT has no effect in vitro on phosphorylation of liver mitochondria, but inhibits it in vivo. Taking into consideration the neurotropic properties of DT, it was interesting to discover whether it affects phosphorylation in the liver and the general heat metabolism if applied locally to act directly on the brain structures. Apart from a short initial period of severe excitation the animals' general condition remained unchanged in the experiments in which DT was injected intracerebrally. The body temperature fluctuated slightly around the initial level. In calorimetric experiments the heat metabolism was unchanged on the day of injection of DT; after the 2nd or 3rd days the results of direct and indirect calorimetry began to diverge appreciably. On the day the liver was taken (3rd-5th day), the "direct" heat production (by calorimetry) of 4 rabbits exceeded the figure calculated from the gas exchange, on the average, by 20% (see Fig. 1). In all the experiments the P:O ratio was sharply reduced (see Table 1). In the 5th rabbit, which lost about 1 ml of cerebrospinal fluid from the ventricular cannula before injection of toxin, the decrease of P:O was combined with an increase in oxygen absorption by the mitochondria, and in contrast to all the other animals, the total gas exchange of this rabbit was increased by comparison with the true heat production.

Injection of minimal doses of DT into the lateral ventricle thus caused marked (although not absolutely equivalent in all experiments) disturbances of energy metabolism in the liver and of the total energy metabolism.

Comparison of the results of the first two groups of experiments shows that there is no connection between the disturbance of energy metabolism in the liver and the excess of "direct" heat production in diphtheria poisoning. For unknown reasons, a marked divergence between the results of direct and indirect calorimetry developed in the rabbits only after subcutaneous injection of DT, and was not found in acute poisoning following intravenous injection. On the contrary, dissociation of respiration and phosphorylation the liver was well marked in the latter case, but was absent in the experiments with subcutaneous injection.

The results obtained do not rule out the possible participation of a thermal effect of dissociation between respiration and phosphorylation in the increase of "direct" heat production in pathological conditions. They do show, however, that a considerable inhibition of phosphorylation in the liver is not necessarily accompanied by a marked increase in total heat production, and, what is particularly important, that a considerable excess of "direct" heat production over that calculated from gas exchange may develop in the absence of dissociation of respiration and phosphorylation in the liver.

The results of preliminary experiments in which minimal doses of DT were injected into the lateral ventricle deserve special attention. In these experiments the peripheral action of the toxin on tissue metabolism and heat production was practically ruled out. Nevertheless, typical disturbances of general and local (in the liver cells) energy metabolism were well marked. It is too early to give a full interpretation of these findings. However, they show that the action of DT on the general heat metabolism and energy metabolism in the liver is mediated through neuro-humoral regulatory mechanisms. The fact that these disturbances of metabolism developed a considerable time after injection of the DT is in agreement with the slow development of pathological changes in the nervous system characteristic of the action of diptheria toxin.

The question of the sources of pathological heat production not corresponding to the oxygen consumption is thus one of considerable complexity. It clearly cannot be solved without analysis of the disturbances of the course and regulation of processes of energy transformation in the organism at different levels.

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