ION-EXCHANGE CHROMATOGRAPHIC INVESTIGATION OF THE NUCLEOTIDE CONTENT OF LIVER AND SKELETAL MUSCLE OF GUINEA PIGS TREATED BY FEVER-PRODUCING SUBSTANCES

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SUMMARY

The compcsition of several nucleotides in both liver and skeletal muscle has been studied by ion-exchange chromatography in normal guinea pigs, as well as in guinea pigs treated either with 2,4-dinitrophenol or with the lipopolysaccharide of *Salmonella abortus equi*. A decrease in the fraction containing ATP and ADX (an unknown adenosine compound) has been recorded in both tissues of the treated animals. This change also occurred in treated animals which were anaesthetized by magnesium sulfate 15 min before death.

INTRODUCTION

The hypothesis that the increased production of heat occurring during fever is related to the decreased efficiency of oxidative phosphorylation was first put forward by LARDY AND ELVEHJEM[1]. This hypothesis was based on the fact that well-known fever-producing substances, such as 2,4-dinitrophenol (DNP)* inhibit oxidative phosphorylation and stimulate adenosine triphosphatase *in vitro*. The hypothesis was also supported by the results of RONZONI AND EHRENFEST[2] and of LARDY AND PHILLIPS[3], who described a decrease of phosphocreatine, as well as an increase of glycolysis, in the tissues of animals treated by DNP. Recently, PASQUINELLI AND CALZOLARI[4] and PASQUINELLI, CALZOLARI AND D'ALESSANDRO[5] have shown that the phosphorylation of injected glucose is decreased in rabbits treated with a fever-producing yeast hydrolysate. ATPase activity increases in both the liver and skeletal muscle. DIANZANI AND SCURO[6] and DIANZANI AND DIANZANI MOR[7] have found a decreased P/O ratio in some tissues of rats treated by DNP. ATPase activity of mitochondria was found to be increased. Similar results were obtained by VORBRODT[8] and by ALDRIDGE AND STONER[9]. The latter authors used 3,5-dinitro-orthocresol in their investigations. ATPase activity of mitochondria increases also after treatment with other fever-producing substances,

* The following abbreviations were used throughout this paper: DNP, 2,4-dinitrophenol; LPS, lipopolysaccharide of *Salmonella abortus equi*; PCA, perchloric acid; DPN, diphosphopyridine nucleotide; AMP, adenosine monophosphoric acid; TPN, triphosphopyridine nucleotide; IMP, inosine monophosphoric acid; ADP, adenosine diphosphoric acid; FAD, flavine adenosine dinucleotide; GDP, guanosine diphosphoric acid; ATP, adenosine triphosphoric acid; ITP, inosine triphosphoric acid.

such as the lipopolysaccharide of *Salmonella abortus equi* (LPS) and lysergic acid diethylamide[10]. The mitochondria contained in the tissues of animals treated with these substances appear to be considerably swollen[11].

The acid-labile phosphorus content of the tissues of rats treated with 3,5-dinitro-*o*-cresol, another fever-producing substance structurally related to DNP, was found by STONER, THRELFALL AND GREEN[12] to be decreased.

Similar results were obtained also by PARKER[13] in rats treated with lethal amounts of this substance. The extent of the decrease was higher in rats which survived for a longer time. A decrease in the acid-labile phosphorus, as well as in the phosphocreatine, has been found also by DIANZANI[14] in several organs of guinea pigs treated either with DNP or with LPS. The decrease of ATP in the liver of animals treated by DNP was shown also by an enzymic method of determination.

The present paper describes the results of an investigation on the nucleotide content of both liver and skeletal muscle of guinea pigs, as detected by the ion-exchange chromatographic method of SIEKEVITZ AND POTTER[15]. The determination was made in normal animals, as well as in guinea pigs previously injected with DNP or with LPS.

MATERIALS AND METHODS

Guinea pigs weighing 300–350 g, fed on a vegetable diet were used. The temperature of the room in which they lived was 22 ± 2°. The thermal stability of the animals was checked for 7–10 days before the treatment. Colon temperature measurements were made with an electric apparatus (Hartmann and Braun).

DNP was given intraperitoneally in amounts of 4 mg/100 g of body weight, as the sodium salt (Merck). LPS was administered in a similar way, in amounts of 0.05 μg/100 g of body weight. This was kindly supplied by the firm of Wander of Bern.

The animals were killed by decapitation. Those which had been previously injected with the fever-producing substances were killed 2 h after the injection, when the body temperature had increased by about 2°. Immediately after the removal of the head, the liver (about 13 g) and the muscles of the legs (also about 13 g) were dissected in a cold room at 2° and placed in a small blendor (MSE type) containing 26 ml of 1.5 *M* perchloric acid (PCA). The weight of the homogenizer containing the PCA had previously been determined. The exact weight of the amount of tissue used was calculated by weighing the container again. The time between the death of the animal and the immersion of the tissue in PCA was 16–20 sec in the case of the liver, 50–70 sec in that of the muscle. The homogenization was started within 2 min after the death of the animals in both cases.

The homogenates were then centrifuged at 1000 *g* for 10 min. The sediment was resuspended in 26 ml of 0.6 *M* PCA, homogenized and submitted to a new centrifugation at the same speed for the same time. The combined supernatant fluids were cooled in chopped ice and neutralized with 1 *N* KOH. The neutralized fluid was then placed onto the chromatographic column. This was 15 cm high and 1 cm wide. It was filled with Dowex 1-X4 resin. The chloride form of this had been previously converted into the formate form by washing with formic acid. Other washings were made with deionized water until the acid reaction disappeared.

Elution of the nucleotides from the column was carried out with the formic acid–

ammonium formate procedure described by SIEKEVITZ AND POTTER[15]. The eluates were collected in a Shandon fraction collector.

The optical density of each fraction was read at 260 mμ in a Beckman spectrophotometer Mod. D.U., after convenient dilution.

The values of the optical density were taken as a measure of the concentration of the nucleotides. The average values ± the standard deviation are reported in Table I.

TABLE I

NUCLEOTIDE CONTENT IN LIVER AND SKELETAL MUSCLE OF NORMAL GUINEA PIGS, AND OF GUINEA PIGS TREATED EITHER WITH DNP OR WITH LPS

The data represent the optical density at 260 mμ of nucleotides corresponding to 1 g of fresh tissue. The values whose difference from the normal is statistically significant ("P" < 0.05) are indicated with *. A factor of 1000 was used in representing the data.

Fraction	*Normal*	*Anaesthetized by $MgSO_4$*	*Treated with DNP*	*Treated with LPS*
A. Liver				
DPN	366 ± 19	400 ± 20	422 ± 146	371 ± 168
AMP	2364 ± 540	1295 ± 212	1998 ± 665	1952 ± 436
TPN	242 ± 172	96 ± 54	137 ± 99	171 ± 97
IMP	409 ± 67	256 ± 212	507 ± 180	498 ± 77
"AD"	496 ± 127	682 ± 210	607 ± 105	566 ± 115
ADP	959 ± 249	1494 ± 227	1128 ± 237	1011 ± 130
FAD	152 ± 34	128 ± 58	212 ± 149	205 ± 191
GDP	300 ± 112	313 ± 53	274 ± 135	404 ± 143
ATP–ADX	1733 ± 184	2332 ± 162*	1365 ± 75*	1315 ± 161*
ITP	111 ± 29	234 ± 5*	105 ± 36	105 ± 49
B. Muscle				
DPN	72 ± 37	117 ± 13	78 ± 7	152 ± 51
AMP	615 ± 36	730 ± 101	711 ± 143	663 ± 314
TPN	200 ± 80	92 ± 10	257 ± 65	255 ± 59
IMP	175 ± 57	120 ± 77	238 ± 55	66 ± 8*
"AD"	638 ± 114	272 ± 104	1118 ± 299*	666 ± 102
ADP	1836 ± 245	1041 ± 195*	1498 ± 220	1294 ± 357
FAD	333 ± 136	138 ± 87	76 ± 38*	200 ± 27
GDP	173 ± 110	143 ± 116	175 ± 45	171 ± 110
ATP–ADX	5934 ± 162	6284 ± 891	3176 ± 458*	5494 ± 55*
ITP	221 ± 34	159 ± 132	61 ± 21*	126 ± 14*

The significance of the differences between two averages was estimated by calculating the "t" value of FISHER. Only the differences with a "t" value corresponding to a probability $P < 0.05$ were retained as significant.

In some experiments, the animals were anaesthetized by intraperitoneal injection of magnesium sulfate, according to the method of DUBOIS, ALBAUM AND POTTER[16], 15 min before to be killed. This treatment was applied in order to decrease the dephosphorylation of ATP probably occurring during the time between the death of the animal and homogenization.

RESULTS

The results obtained are shown in the figures. Their statistical evaluation is given in Table I. In the figures, the number of the tube, containing a fractions of 10 g of the eluate, is reported under the abscissa, while the optical density is given under the

ordinata. The values are referred to 13 g of fresh tissue and represent the average of at least 5 determinations.

Thirteen absorption peaks were observed for both liver and skeletal muscle. They are indicated on the diagrams with arabic numerals. The identification of the substances corresponding to each peak was made by comparison with a chromatogram

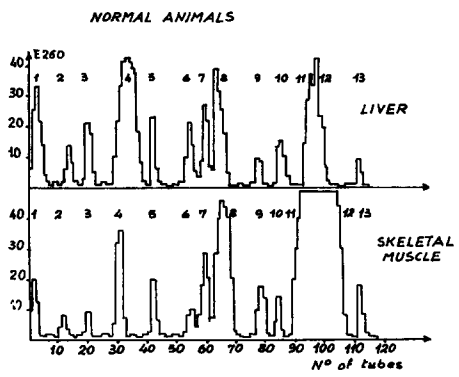

Fig. 1. Nucleotide content of both liver and skeletal muscle of normal guinea pigs.

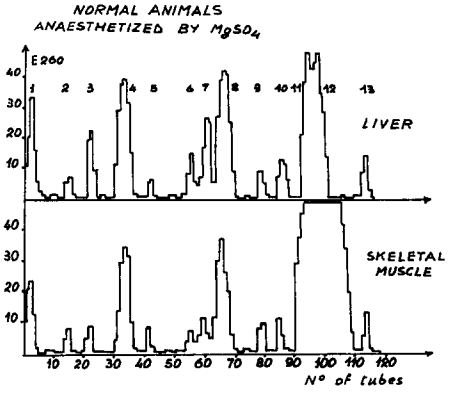

Fig. 2. Nucleotide content of both liver and skeletal muscle of normal guinea pigs anaesthetized by $MgSO_4$.

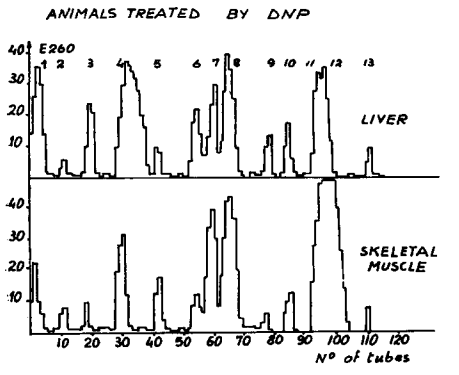

Fig. 3. Nucleotide content of both liver and skeletal muscle of guinea pigs treated with DNP.

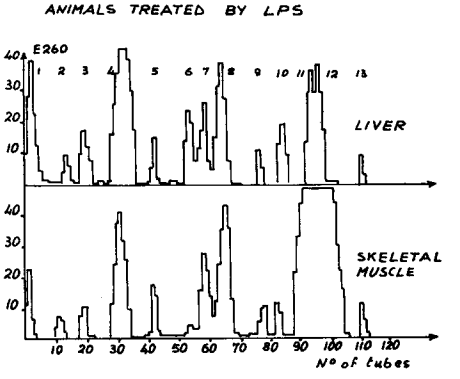

Fig. 4. Nucleotide content of both liver and skeletal muscle of guinea pigs treated with LPS.

run of known substances. It was possible to identify in this way only 9 substances: peak No. 3 was due to diphosphopyridine nucleotide; peak No. 4 to adenosine monophosphate; peak No. 5 to triphosphopyridine nucleotide; peak No. 6 to inosine monophosphate; peak No. 8 to adenosine diphosphate; peak No. 9 to flavine adenyldinucleotide; peak No. 10 to guanosine diphosphate; peak No. 12 to adenosine triphosphate and peak No. 13 to inosine triphosphate. According to SIEKEVITZ AND POTTER[15], peak No. 9 contains, in addition to flavine adenyldinucleotide, also an unidentified uridine compound (UDX); peak No. 11, which is indicated by these authors as corresponding to an unidentified adenosine compound (ADX), was not

completely separable from adenosine triphosphate. The substances responsible for peaks Nos. 1, 2 and 7 were not identified. According to SIEKEVITZ AND POTTER[15], the last peak corresponds to an unknown adenosine compound, designated as "AD".

The heights of the peaks show some differences between the liver and skeletal muscle. Peaks Nos. 1, 3, 4 and 9 are taller for the liver than for the skeletal muscle. On the contrary, peaks No. 8, 11, 12 and 13 are taller for the latter tissue.

In normal animals treated with $MgSO_4$, the amount of ADX + ATP, as well as that of ITP, present in the liver was slightly higher than in untreated guinea pigs. No difference was found between anaesthetized and unanaesthetized animals in the case of skeletal muscle.

In guinea pigs treated with 2,4-dinitrophenol the only fraction which was found significantly different from the normal in both liver and muscle was that containing ADX + ATP, which was strongly decreased. The extent of the decrease was higher in the latter tissue. In skeletal muscle significant decreases of FAD and of ITP were also observed, as well as an increase in the height of peak No. 7 ("AD"). Very similar results were obtained in guinea pigs treated with DNP followed by anaesthetization with $MgSO_4$. A large decrease of the fraction containing ADX + ATP was recorded also in the animals treated with LPS. The decrease was significant in both liver and skeletal muscle, but its extent was less than that observed in the case of DNP-treated guinea pigs.

In the latter, ITP was found to be decreased in the muscle, but remained unchanged in the liver. As opposed to the guinea pigs treated with DNP, IMP was found to be decreased in skeletal muscle, while FAD + UDX and "AD" remained unchanged.

The results obtained with guinea pigs anaesthetized with $MgSO_4$ did not differ from those observed in unaesthetized animals, also in the case of LPS treatment.

DISCUSSION

Parenteral treatment with LPS or with DNP produces a noticeable decrease in the nucleotide fraction containing ADX + ATP in both liver and skeletal muscle of guinea pigs. As these components were not separated from each other, it is impossible to know with certainty if both, or only one of them, is responsible for the decreased height of the peak.

A decrease of ATP in both liver and skeletal muscle has been found by DIANZANI[14] by an enzymic method of determination. It seems probable then that the results obtained in this paper may be interpreted in the sense that the ATP concentration is decreased. In the skeletal muscle, another triphosphate (ITP) is also decreased.

The fact that the DPN concentration remains unchanged agrees with previous results obtained by another method of determination[17]. It seems possible that the cause for the decrease in the height of peaks Nos. 11, 12 and 13 is related to the impairment of oxidative phosphorylation, as well as to the activation of ATPase[5–10].

It seems, however, improbable that this decrease is connected with an eventual increase of preagonic contractions. In fact, a decrease of ATP was also recorded in guinea pigs anaesthetized by $MgSO_4$, whose muscles were relaxed. The fact that no change in the ATPase activity of myofibrils occurs in guinea pigs treated either by DNP or by LPS[10] favours this interpretation.

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DETERMINATION OF THE DIFFUSION CONSTANT OF POLIOVIRUS BY THE GEL PRECIPITIN TECHNIQUE

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SUMMARY

A gel diffusion technique is recommended for the determination of the diffusion constants of materials of which only small quantities are available. In the present work this technique has been applied to the investigation of the diffusion rate of a type II poliovirus strain. A diffusion constant of $1.29 \cdot 10^{-7}$ cm^2/sec at 20° was obtained for this virus. This figure is probably within $\pm 5\%$ of the true value, an assumption based on previous data obtained when the method was applied to the study of the diffusion constants of a variety of proteins.

INTRODUCTION

In a previous communication, VAN REGENMORTEL[1], reported on the determination of the diffusion constant of TYMV by the double gel diffusion technique of POLSON[2]. The value found by him agreed very well with the figure obtained by MARKHAM[3], who used the method of free diffusion in conjunction with the well known Lamm scale method.

Abbreviation: TYMV, turnip yellow Mosaic virus.