PATHOGENESIS OF INFLAMMATION AND MECHANISM OF THE ANTI-INFLAMMATORY ACTION OF EXPERIMENTAL PELOTHERAPY

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The anti-inflammatory effect of pelotherapy was observed in rats with ovalbumin and formalin inflammation and to a lesser degree in rats with serotonin inflammation. It was not found in dextran or trypsin inflammation, and histamine edema was intensified. In dextran inflammation, synthesis of high-energy compounds was predominant, whereas in ovalbumin inflammation their breakdown was reduced. Breakdown of high-energy compounds was stimulated during pelotherapy, leading to a decrease in the energy metabolism in ovalbumin inflammation. This effect persisted when pituitary activity was depressed by chlorpromazine but it was not observed in adrenalectomized animals.

The anti-inflammatory activity of drugs is usually investigated on various models of inflammation. Certain drugs exhibit an anti-inflammatory effect on some models but not on others, probably because of differences in the action of the factors producing the inflammation. This makes it necessary to study the pathogenesis of different types of inflammation, and against this background to study the mechanism of action of drugs with anti-inflammatory activity.

The object of the investigation described below was to determine some aspects of the mechanism of the anti-inflammatory action of experimental pelotherapy by the use of six models of inflammation. In particular, the possible effect of this form of treatment on the mediators of inflammation, on preoteolysis, on energy metabolism, and on the role of the pituitary-adrenal system in this process was studied.

EXPERIMENTAL METHOD

Experiments were carried out on 204 albino rats weighing 150-220 g. Inflammation was induced by injecting 0.1 ml of a solution of 6% dextran, 0.01% serotonin, 0.1% histamine, 1.5% ovalbumin, 2% formaldehyde, or 0.5% trypsin into the hind limb beneath the plantar aponeurosis. The cause of the inflammation was determined from the increase in volume of the limb (oncometrically) over a period of 3 h, after which the animals were decapitated. High-energy compounds were estimated quantitatively by the method of Seits [4], and inorganic phosphorus by the method of Fiske and Subbarow in Uzbekov's modification [6]. Mitochrondria were isolated from liver homogenates for the investigation of ATPase (by Skulachev's method [5] with modifications by Grigorova et al. [2]) by differential centrifugation as described by Severin et al. [3]. NAD and NAD·H_2 were estimated quantitatively by Lewitas' method, lactic acid by the method of Mendel and Goldschider [11], and pyruvic acid in the blood by Natelson's method [12]. Experiments were carried out on 10-12 animals in each series of experiments with different types of inflammation (control) and in conjunction with pelotherapy, consisting of a course of eight applications of mud from the Kuyal'nitskii Liman lagoon (on alternate days, temperature 42°C, duration 20 min). In the experiments to study the role of the pituitary-adrenal system, 2.5% chlorpromazine solution (0.05 ml) was

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TABLE 1. Indices of Bioenergetics in Ovalbumin and Dextran Inflammation and During Pelotherapy (M±m)

Index	Intact animals	Ovalbumin inflammation			Dextran inflammation		
		in intact animals	during pelotherapy	P	in intact animals	during pelotherapy	P
Pyruvic acid (in mg %)	1,93±0,25	1,91±0,26	2,10±0,39	>0,6	1,06±0,07	2,29±0,39	<0,001
Lactic acid (in mg %)	19,9±2,1	37,3±3,83	16,8±1,32	<0,001	33,6±1,73	22,4±2,58	<0,001
Inorganic phosphorus (in μg/ml)	20,7±0,68	13,4±1,81	19,1±1,19	<0,02	21,5±3,0	16,1±2,94	>0,3
ATP; ADP (in μg/ml)	5,9±1,2	19,3±2,84	20,0±1,22	>0,8	32,7±1,41	20,6±3,10	<0,001
NAD (μg/g tissue)	272±60,7	479±77,9	326±41,9	>0,1	659±42,8	346±70,1	<0,001
NAD-H_2 (in μg/g tissue)	82±12,2	376±71,4	259±30,3	>0,2	615±38,5	225±31,1	<0,001
ATPase (in mg P/g tissue)	0,13±0,02	0,06±0,01	0,26±0,04	<0,001	0,39±0,03	0,20±0,04	<0,001

TABLE 2. Weight of Adrenals and Content of Ascorbic Acid in Them During Reproduction of Various Types of Inflammation in Conjunction with Pelotherapy (M±m)

Type of inflammation	Weight of adrenals (in mg/100 g body weight)		P	Content of ascorbic in adrenals (in mg %)		P
	inflammation	inflammation plus pelotherapy		inflammation	inflammation plus pelotherapy	
Ovalbumin	20,4±1,8	38,9±2,9	<0,001	345±33	240±13,6	<0,02
Formalin	19,7±1,3	28,4±1,4	<0,01	307±20	186±7,5	<0,001
Dextran	25,0±5,1	21,9±5,4	>0,5	271±8,6	302±15,8	>0,05
Serotonin	22,9±4,5	22,5±1,6	>0,5	442±28	293±39	<0,02
Histamine	19,7±1,5	26,8±3,0	>0,05	461±24	374±30	>0,05

Note. Data for intact animals (control): weight of adrenals 20.7±0.78 mg/100 g body weight; content of ascorbic acid in them 430±10.8 mg%.

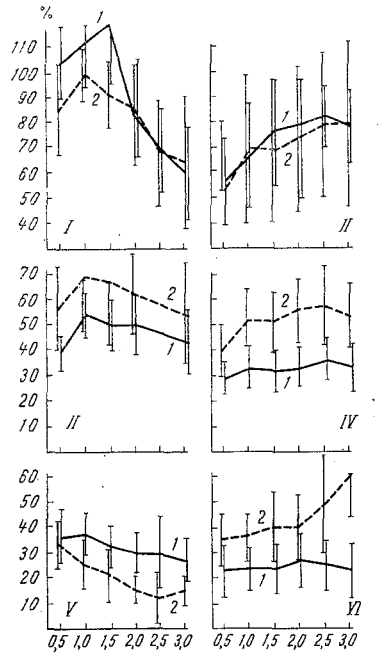

Fig. 1. Cause of inflammation produced by trypsin (I), dextran (II), serotonin (III), ovalbumin (IV), histamine (V), and formaldehyde (VI) in conjunction with experimental pelotherapy: 1) inflammation produced after a course of mud application; 2) model without application (control). Abscissa, time after injection of inflammatory agent (in h); ordinate, increase in volume of inflamed limb (in percent).

injected intramuscularly 40 min before each application or bilateral adrenalectomy was performed 3-4 days before the pelotherapy. The adrenals were weighed and their content of ascorbic acid determined by indophenol titration.

EXPERIMENTAL RESULTS

The course of mud applications had a marked anti-inflammatory action in ovalbumin inflammation. Treatment with mud had little effect at the beginning of the exedative phase in formalin inflammation, but an effect was manifested later (after 2.5-3 h). A tendency was observed for serotonin edema to diminish. Meanwhile, pelotherapy had no effect on the course of dextran or trypsin inflammation, and it produced a definite increase in the intensity of histamine edema (Fig. 1). Since the anti-inflammatory activity of the same therapeutic factor was exhibited in ovalbumin inflammation but not in dextran inflammation, which are very similar in the mediators concerned and in their anaphylactory character, differences in their pathogenesis had to be looked for.

The results showed (Table 1) that in both types of inflammation the intensity of anaerobic glycolysis and of the initial stages of oxidative phosphorylation was considerably increased. However, considerable accumulation of high-energy compounds and of both forms of nicotinamide coenzymes in ovalbumin inflammation was accompanied by a decrease in the inorganic phosphorus content and by a marked limitation of the ATPase activity of the mitochondria, which was not observed in dextran inflammation. Pelotherapy prevented the sharp increase in the intensity of glycolysis and stimulated the breakdown of high-energy compounds. This was clear from the increase in the content of inorganic phosphorus, the considerable increase in ATPase activity, and the tendency for the content of NAD and NAD $\cdot H_2$ to decrease in ovalbumin inflammation, and from the decrease in the total ATP plus ADP content in dextran inflammation. Meanwhile, during dextran inflammation the content of high-energy compounds remained high, while ATPase activity and the content of inorganic phosphorus were low.

Differences between the action of pelotherapy on the various types of inflammation were also associated with different reactions of the pituitary-adrenal system and differences in its role in the genesis of the various types of inflammation. The results (Table 2) showed that during pelotherapy the weight of the adrenals increased significantly in ovalbumin and formalin inflammation but showed little change in the other types of inflammation.

In ovalbumin, formalin, and serotonin inflammation in conjunction with pelotherapy there was a significant decrease in the ascorbic acid content in the adrenals which was not observed in dextran or histamine inflammation. Experiments on adrenalectomized animals showed that the anti-inflammatory effect of pelotherapy was not exhibited in ovalbumin inflammation: the increase in limb volume of the animals with intact adrenals was $36 \pm 5.6\%$ after 3 h compared with $86 \pm 6.9\%$ in the adrenalectomized animals. Administration of chlorpromazine, which inhibits pituitary hormonal activity [1, 15], to the animals with intact adrenals was followed by the appearance of an anti-inflammatory effect of pelotherapy (increase in limb volume $34 \pm 6.1\%$).

Pelotherapy had an anti-inflammatory action in ovalbumin inflammation but not in dextran inflammation, despite the important role of the mediators, histamine and serotonin, in their pathogenesis [9, 14]. The absence of anti-inflammatory action in dextran inflammation could be due to differences in the character of its effect on mediator action (diminution of serotonin and aggravation of histamine edema). However, in that case it would be difficult to explain the diminution of ovalbumin inflammation. Besides its action on one of the mediators (serotonin), in this case other features of the pathogenesis of this type of

inflammation, on which pelotherapy may act, could also play a significant role. From this standpoint it is interesting that pelotherapy reduces the development of formalin inflammation, in the genesis of which these mediators are not particularly important [13]. In necrotic inflammation, moreover, there is activation of proteolysis [10], which does not occur in dextran inflammation. It thus follows that pelotherapy may affect proteolysis. However, the results of the authors' experiments on the model of trypsin inflammation used for this purpose [7] showed that pelotherapy has no action on proteolysis.

Differences were found in the changes in energy metabolism, namely the accumulation of high-energy compounds as a result predominantly of their increased synthesis in dextran inflammation but of their reduced breakdown in ovalbumin inflammation. Under these conditions pelotherapy, intensifying the breakdown of high-energy compounds, when used in ovalbumin inflammation led to a marked decrease in the supply of energy for the inflammatory process, thus giving an anti-inflammatory effect. In dextran inflammation, on the other hand, the increase in the breakdown of high-energy compounds evidently took place concurrently with their intensive synthesis, so that a constant supply of energy was available to maintain the inflammation.

On the other hand, the anti-inflammatory effect of pelotherapy was exhibited in ovalbumin and formalin inflammation when adrenal function also was intensified. This effect was not found in dextran inflammation, on the course of which corticosteroids likewise have no action [2]. The view has been expressed [7] that substances active in formalin and inactive in dextran inflammation have a cortisone-like action. This can be applied to pelotherapy, more especially because its anti-inflammatory effect on the ovalbumin inflammation was not observed in adrenalectomized animals, although it was observed during pharmacological inhibition of pituitary function.

The various models of inflammation thus differ not only in their participating mediators, but also in certain biochemical changes and also in the response of pituitary-adrenal system. Important factors in the anti-inflammatory effect of pelotherapy revealed in these experiments are its limitation of the energy supply for the inflammatory changes, its activating action on the adrenals and, probably, a change in the response to one of the mediators – serotonin.

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