THE FUNCTIONAL INTERACTION OF VITAMINS C, P, AND B_1

E. F. Shamrai and T. V. Fetisova

Department of Biochemistry (Head – Prof. E. F. Shamrai) of the Kiev Medical Institute

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

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The isolation of the individual vitamins and the study of their specific action on animals were the main tasks of research workers in the early period of development of vitaminology. At the present time, when vitamins are widely used in medical practice for therapeutic purposes, the question of the functional interrelationship between the members of this group of biologically active substances is one of particular urgency.

There are reports in the literature of metabolic synergism and metabolic antagonism between certain vitamins, and in particular, metabolic synergism has been established between vitamins C and P [1-4, 9, 10, 12, 16, 20].

In our laboratory we have synthesized the preparation "galascorbin", a complex compound of the sodium salts of ascorbic and gallic acids. Because of the presence of tannins in the composition of galascorbin, it possesses vitamin P as well as vitamin C activity. This preparation diminishes the permeability of capillaries, increases the resistance of animals to a rarefied atmosphere, and promotes the accumulation of ascorbic acid in the organs and tissues of guinea pigs under treatment for scurvy [9, 11, 17].

A series of investigations [1, 9, 13, 14, 15] has demonstrated the beneficial effect of galascorbin on wound healing. When injected parenterally, it stimulates repair processes in damaged tissues [8, 18, 19]. Since vitamin B_1 is directly concerned in the carbohydrate metabolism of tissues, some metabolic synergism between it and galascorbin was to be expected.

In order to test this hypothesis, we studied the influence of simultaneous injections of galascorbin and thiamin on the indices of the carbohydrate-phosphorus metabolism of traumatized tissues. We also tested the action of each preparation separately under the same conditions.

The object on which we carried out our comparative investigations was the muscle of a limb after the prolonged (for 6 hours) application of a tourniquet to arrest the blood flow.

As we have previously shown, application of a tourniquet to the limb of a rabbit for 6 hours is accompanied by the development of a severe atrophy of the muscles and by profound disturbances of their carbohydrate-phosphorus metabolism, the maximum changes being observed one month after trauma [6, 7].

METHOD

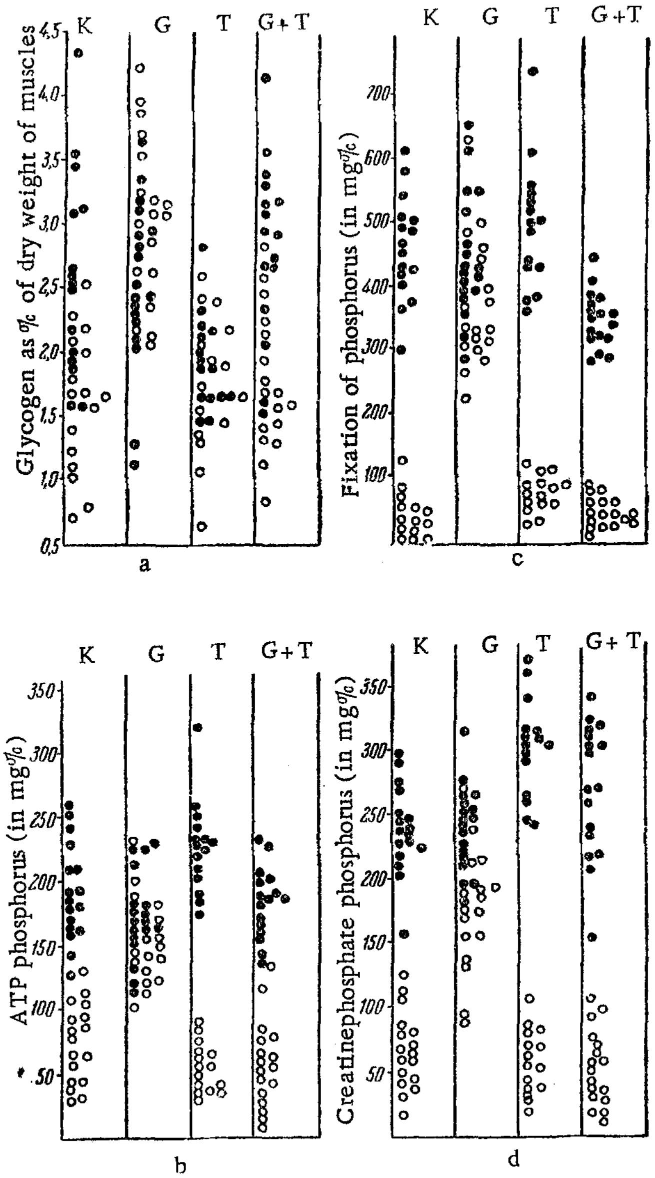
A tourniquet was applied to the middle third of the thigh of 80 rabbits, weighing 2.0-2.5 kg, for 6 hours, after which the animals were subdivided into 4 equal groups.

The first group acted as a control, and the animals in this group received a subcutaneous injection of 5 ml of physiological saline daily for one month. The animals of the second group received a subcutaneous injection of galascorbin, in a dose of 50 mg/kg body weight, made up in 5 ml of physiological saline, every day. The animals of the third group received daily subcutaneous injections of thiamin in a dose of 5 mg/kg body weight. Finally, the rabbits of the fourth group were injected with galascorbin and thiamin in the above doses, simultaneously.

One month after trauma, the gastrocnemius muscles from the injured and uninjured limbs were excised from the animals, under ether anesthesia. The muscles of the uninjured limb were used as a relative control. The content of glycogen, ATP, and creatinephosphate and the phosphorylase activity in the muscles were determined by the generally accepted methods [5]. The results obtained were calculated per dry weight of muscle. In order to judge the degree of atrophy present, homonymous muscles of the two limbs were weighed (extensor digitorum longus).

RESULTS

In the animals of the control group, immediately after removal of the tourniquet, marked edema and paralysis of the traumatized limb were observed. After 4-5 days, the edema, as estimated by palpation, had disappeared, and on the 10th-12th day an appreciable diminution in the volume of the muscles of the injured limb was observed, by comparison with the muscles of



Biochemical indices of phosphorus metabolism in the muscles of rabbits. a) Glycogen; b) ATP; c) phosphorylase activity; d) creatine phosphate. Conventional signs: K) control; G) galascorbin; T) thiamin; G + T) galascorbin and thiamin. The black circles indicate the results for the uninjured limb, the clear rings those for the injured limb.

the intact limb. The atrophic changes progressed until the end of the observation. The motor function of the limb also remained disturbed one month after trauma, and in approximately one half of the cases, the paralysis of the limb was maintained.

An analogous clinical picture was observed in the animals receiving vitamin B_1 and also, contrary to our expectations, in the rabbits receiving galascorbin and thiamin simultaneously. Meanwhile, in the rabbits receiving galascorbin alone, the edema and subsequent reduction in volume of the traumatized muscles were much less pronounced than in the remaining groups, and the motor function of the limb was almost completely restored on the 15th–20th day after trauma.

Preliminary clinical observations alone were thus sufficient to suggest that vitamin B_1, in the doses which we used, not only did not stimulate the repair processes in the muscles undergoing compression ischemia, but completely abolished the therapeutic effect of galascorbin, so clearly demonstrated under these condtions.

Not to confine ourselves to clinical observations on the animals, we determined the main biochemical indices of carbohydrate – phosphorus metabolism in the muscles of the injured and uninjured limbs of the animals. The results of these investigations are shown in the form of a graph in the figure (the results of each examination of the muscles of the uninjured limb are indicated by black circles and those of the injured limb, by clear rings).

Despite the wide range of individual variations, in general characteristic of the components of muscle tissue which we were studying, the data in the figure illustrate the normalizing action of galascorbin on the carbohydrate – phosphorus metabolism of traumatized muscles and the absence of such action after administration of thiamin alone or thiamin in conjuction with galascorbin.

Since the individual variations in the levels of the components under study masked, to a certain extent, the results of the experiments, in each series of experiments we give the mean values and also the ratio between the indices of carbohydrate – phosphorus metabolism, as a percentage, in the injured and uninjured limbs (see Table).

It will be seen from the results of the investigation that the values obtained from biochemical analysis of the muscles fully agreed with the clinical observations.

In the animals of the control group one month after trauma, the weight of the muscles of the injured limb fell almost by one half, and this, together with the marked proliferation of connective tissue, was evidence of severe atrophy. The glycogen content fell by $\frac{1}{3}$, the ATP by $\frac{2}{3}$, the creatinephosphate by $\frac{3}{4}$, and the phosphorylase activity amounted to only 10% of the activity of the enzyme in the muscles of the uninjured limb. Similar results were obtained from investigations in animals receiving thiamin alone or thiamin and galascorbin simultaneously.

In contrast to this, in animals receiving galascorbin alone, the atrophy and the changes in carbohydrate-phosphorus metabolism of the injured muscles were less pronounced. The glycogen content, for example, even exceeded slightly that in the muscles of the uninjured limb. The ATP content reached 89%, whereas in the animals of the other groups it amounted to 24–38% of the ATP content in the muscles of the uninjured limb. The creatinephosphate content in the injured muscles was almost twice that in the animals not receiving galascorbin. The results of the determination of phos-

Weight and Indices of Carbohydrate–Phosphorus Metabolism of Gastrocnemius Muscles of Rabbits One Month After Application of Tourniquet (Mean Values)

Preparation injected	Weight of extensor digitorum longus, g			Glycogen, %			Phosphorylase activity, mg % of phosphorus combined during 2 hr incubation			ATP phosphorus, mg%			Creatinephosphate phosphorus		
	injured limb A	uninjured limb B	$\frac{A}{B}$ · %	injured limb A	uninjured limb B	$\frac{A}{B}$ · %	injured limb A	uninjured limb B	$\frac{A}{B}$ · %	injured limb A	uninjured limb B	$\frac{A}{B}$ · %	injured limb A	uninjured limb B	$\frac{A}{B}$ · %
Physiological saline	1,22	2,16	56	1,67	2,55	65	51,2	459,8	11	68,5	176,7	38	62,2	238,0	26
Galascorbin	1,65	2,04	81	3,06	2,54	132	361,3	434,0	83	153,9	172,0	89	171,4	238,0	72
Thiamin	1,48	2,55	58	1,77	1,97	90	79,9	498,0	16	58,8	226,0	24	58,8	302,2	19
Thiamin and galascorbin	1,59	2,70	59	1,78	2,94	60	43,9	349,8	12	58,4	184,1	31	53,5	268,0	20

phorylase activity are particularly illuminating. Whereas in the animals of the other groups, the activity of the enzyme amounted to only 10-16% of that in the control muscles, in the group of rabbits receiving galascorbin, it reached 83%, i.e., it was much closer to normal.

It is a noteworthy fact that administration of galascorbin had no effect on the magnitude of the indices studied, in the muscles of the uninjured limb. In every case these values remained within normal limits. The preparation apparently had a selective action on injured tissue, in which it promoted the restoration of normal energy metabolism. It must be mentioned that, as we have repeatedly observed, the biological activity of vitamin P is clearly demonstrated when given to a patient with scurvy, but it cannot always be discovered when vitamin P preparations are given to healthy animals.

SUMMARY

We may thus conclude that the preparation "galascorbin", which possesses properties of vitamins C and P, exerts a definite stimulatory effect on repair processes in injured tissues, which gives prospects for the future clinical use of the drug on a wide scale.

Administration of vitamin B_1, in these experimental conditions, has no appreciable influence on the course of the atrophic process and on the biochemical indices of the energy metabolism of the injured muscles.

Vitamin B_1, injected simultaneously with galascorbin in proportions of 1 : 10 (5 mg thiamin and 50 mg galascorbin per kg body weight), abolishes the stimulating action of the galascorbin on the repair processes in the injured muscles.

This observation provides evidence of the necessity for further experimental investigation of the principles governing the use of vitamins in medical practice.

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