Attempted Enzymatic Phosphorylation of Ribonucleic Acid. (20346)

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It has been suggested by many authors that the nucleic acids play some role in the mechanism of protein synthesis. Spiegelman and Kamen(1) early proposed that the nucleic acids acted as special mechanisms funneling energy into the synthetic process. More recently Pollister(2) made the suggestion that some of the nucleotides in a nucleic acid may be energy rich di- or triphosphates. The possible activity of such proposed phosphorylated compounds in acting as templates for peptide and nucleic acid synthesis has been described in detail by one of the authors(3). The presence of adenylic acid as one of the nucleotides in ribonucleic acid suggested the possibility that myokinase might act as a phosphorylating agent for ribonucleic acid when in the presence of adenosine triphosphate. Experiments have been carried out in this laboratory which have led to the tentative conclusion that the total phosphorus content of intact ribonucleic acid can be increased. This preliminary experimental work may prove to be of some interest in attempting to attack the problem of the role of nucleic acid in protein synthesis, but thus far the experiments are admittedly only suggestive.

Methods. For the experiments undertaken in this study, the ribonucleic acid used was prepared from calf pancreas and calf liver by the use of sodium dodecyl sulfate(4). The ATP used in the experiments was the sodium salt made from Ba_2 ATP previously prepared from rabbit muscle(5). The enzyme *myokinase* was prepared according to the method of Colowick and Kalckar(6) which is stated to yield a 14-fold purified material. The weight of ATP used in the experiments was equal in all cases to the weight of ribonucleic acid in the reaction vessel. Twenty-five mg of the sodium salt of ribonucleic acid were dissolved in 20 ml of water and warmed in a water bath to 30°C. Five ml of ATP solution were added and one ml of the enzyme solution was then added with stirring. The final solution thus obtained was incubated with shaking at 30° for varying periods of time. The pH of the reaction mixture was always between pH 7 and 7.4. At the end of the incubation period the solutions were quickly cooled to 0°C and sufficient 0.01 N HCl was added to lower the pH to 4.5. The solution was then centrifuged in the cold at 14,000 rpm for 30 minutes to remove the contaminating enzyme, together with a very small amount of RNA. The supernatant was decanted into a small beaker containing 1.4 g of NaCl, which was cooled in an ice bath and the pH was raised to 7 by cautious addition of 0.01 N NaOH. The sodium ribonucleate was precipitated by the addition of two volumes of 95% ethanol. The precipitate was washed with ethanol, acetone, and finally was air dried. The material was then dried *in vacuo* over P_2O_5 and was made up in water solution for analysis. An aliquot was digested and was subjected to analysis for total phosphorus (4).

Results. It was found in two experiments that the total ribonucleic acid phosphorus was increased by about 14% over that of the controls when calf liver ribonucleic acid was incubated with ATP in the presence of freshly prepared myokinase. This increase in phosphorus content is perhaps only suggestive of phosphorylation, but when calf pancreas ribonucleic acid was incubated under the same conditions, the total phosphorus was found to have increased above the control in increments ranging from 17 to 48% as shown in Table I. The possibility that inorganic phosphorus or ATP phosphorus was causing this increase was checked by adding excess orthophosphate and ATP to control solutions of calf liver RNA without the enzyme, and reisolating the ribonucleic acid as above. The reisolated RNA

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TABLE I. Phosphorylation of Ribonucleic Acid (Na-PNA Isolated from Calf Pancreas) with Myokinase and ATP.

Exp. No.	Reaction mixture	Time of incubation, min.	% phosphorus in untreated and reisolated PNA samples*
	mg		
1 (a)	25 Na-PNA 25 Na_4ATP No enzyme	60	7.5
1 (b)	25 Na-PNA 25 Na_4ATP 1 myokinase	60	9.9
	Control sample of Na-PNA, no treatment	—	7.8
2	25 Na-PNA 25 Na_4ATP 1 myokinase	15 60	11.2 12.0
3	25 Na-PNA 25 Na_4ATP 1 myokinase	60	9.1

* Values represent averages of duplicate colorimetric phosphate determinations.

which had been incubated with excess orthophosphate had an increased total phosphorus content of only 5% over the non-incubated controls. The sample incubated with ATP showed no increase. Thus the reisolation procedure appeared to remove nonnucleic acid phosphorus satisfactorily. When purified desoxyribonucleic acid was substituted for ribonucleic acid in a number of experiments, no phosphorus uptake was observed upon incubation with myokinase and ATP.

It became apparent during these preliminary experiments that the incorporation of extra phosphorus depended on the enzyme preparation used, since with an old enzyme preparation used with calf liver Na-PNA no incorporation of phosphorus was noted over that of the controls, and a variation was noted in the degree of activity of different active enzyme solutions. Since myokinase is thought to be an –SH enzyme and is rapidly inactivated by hydrogen peroxide at neutral pH(6), it is not surprising that an old enzyme preparation should lose activity. The drastic treatment used in preparing myokinase makes it unlikely that other enzymes are present in the preparation which could be affecting our results.

One of the interesting results of this work was the difference in the amount of phosphate added to the ribonucleic acids of two different tissues of the same species. This difference might reflect a difference in the adenylic nucleotide content of these two nucleic acids, but other interpretations are possible. The action of myokinase has been shown by Colowick and Kalckar to effect the following dismutation reaction: *2* adenosine triphosphate ⇋ adenylic acid + adenosine triphosphate. It is thus not impossible that in the presence of ATP the adenylic nucleotides of ribonucleic acid may be similarly acted upon. It cannot be stated at the present whether the guanylic nucleotide might also be phosphorylated.

The only work known to us of a nature similar to what is here reported is that of Bresler and Nidzyan(7) who found that rat liver homogenates affected a phosphorylation of ribonucleic acid in the presence of ATP. However, the added phosphorus was cleaved by phosphatase, and it is possible that a different type of phosphorylation was occurring.

It should be noted that in our experiments no adenylic deaminase or –SH compound such as cysteine were added. Since myokinase is supposed to be an –SH enzyme and is inhibited by adenylic acid(6), it is possible that better results could be achieved by adding cysteine and adenylic deaminase.

Summary. Tentative results have been obtained which indicate that the phosphorus content of ribonucleic acid can be increased by incubation with myokinase and ATP. An increase in phosphorus content of about 14% above the control values was obtained for calf liver PNA and increases ranging from 17 to 48% were obtained with calf pancreas ribonucleate.

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Effect of Mercurials on Thiamine Excretion in Patients with Congestive Heart Failure. (20347)

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(Introduced by Ernest Spiegel.)

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Much interest attaches to the question of thiamine excretion following mercurial diuresis. There are some references to thiamine losses due to diuretics used in the treatment of congestive heart failure; however, this question has not been systematically investigated. The opinion is held generally that the water-soluble vitamins are lost in greater quantities in the urine as the urinary volume increases. Actually, little is known of the mechanisms available in the kidney for regulation of thiamine excretion, or of the manner in which these mechanisms are affected by the action of mercurials upon the renal tubules.

The present study was undertaken to determine the effect of mercurials upon daily output of thiamine in the urine in patients under treatment for congestive heart failure. This work was conducted in conjunction with another study made upon a separate group of patients and control subjects to ascertain the status of thiamine nutrition in patients with chronic heart disease. The statistical analysis of the results obtained in this group of patients appeared to indicate that thiamine deficiency in association with chronic cardiac failure is more than a coincidence(1). The present group of patients was not included in that study because they had received supplemental vit. B complex in conjunction with their cardiac therapy prior to observations recorded in this communication.

Methods and materials. Patients under treatment for congestive heart failure were selected from the medical service of Temple University Hospital. In each instance the patient presented signs and symptoms of cardiac decompensation: orthopnea, edema, marked congestion of the lungs, engorgement of the liver. The causes leading to heart failure were hypertensive heart disease in 5 patients, arteriosclerotic heart disease in 3 patients, mitral stenosis in one patient, and amyloidosis in one patient. The cardiac failure was refractory to treatment with digitalis, mercurial diuretics, salt poor diet and vitamin supplements. Five of the 10 patients studied have succumbed to their disease since initiation of these studies. The food taken by each patient indicated a relatively low protein and caloric intake in most instances. No specific signs or symptoms of thiamine deficiency were elicited, possibly due to the reduced caloric intake as well as vitamin supplementation in the past. Vitamins and mercurials were discontinued for 5 days prior to initiation of the study. The urine was collected on the wards for 24-hour periods. During the period of observation, the patient was given a balanced diet providing approximately 1800-2000 calories and 1.0 to 1.2 mg of thiamine daily. The calculations were based on tables compiled by Bowes and Church(2). Urine collections during the first 3 days for each patient represented the control period. A mercurial diuretic, Thiomerin or Mercuhydrin, 2 cc, was given intramuscularly on the morning of the fourth day. The 24-hour urine collections continued during the day of mercurial injection, the day following, and for one or 2 more days of mercurial injections and days following. The urine was collected for an additional