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PROCESS FOR THE PREPARATION OF A MIXTURE OF NUCLEOTIDES CONTAINING PRE-DOMINANTLY ADENOSINTRIPHOSPHATE

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This invention relates to a process for the preparation of a mixture of nucleotides containing predominantly adenosintriphosphate, that is the triphosphate of the carbohydrate derivative of 6-aminopurine (adenine) which adenosintriphosphate is present as such in muscle tissue.

Adenosintriphosphate and related nucleotides are suitable for use in physiological research and in human therapy mainly for curing disturbances of the vascular system or of organs mainly consisting of smooth muscles, such as the uterus.

Such nucleotides were hitherto prepared from animal or vegetable tissues or cells by direct extraction, the extracts obtained being subjected to various methods of purification to isolate the nucleotides. Such methods have a very low yield since the nucleotides are present in the extracts in very dilute state and are accompanied by a large number of other substances from which said nucleotides can be separated only with considerable loss and by laborious methods.

The present invention is based on the discovery that the nucleotides hereinbefore referred to are not present in free condition in animal or vegetable tissues or cells but are linked to protein. It has furthermore been ascertained that only natural proteins are capable of such linkage and that such proteins, if denatured, are adapted to release the nucleotides, however only in the presence of an aqueous medium. Thus for instance, in the case of muscle tissue it has been found that adenosintriphosphate is linked with myosin and can be extracted from insolubilised myosin by water.

On the basis of the foregoing discoveries, the present invention provides a process for the preparation of a mixture of nucleotides containing predominantly adenosintriphosphate, which comprises comminuting animal or vegetable tissue or cells containing both protein and adenosintriphosphate, subjecting the comminuted tissue or cells to a treatment with a dehydrating agent adapted to insolubilise and precipitate protein, separating said dehydrating agent from the insoluble residue and treating said insoluble residue with water to produce an extract containing a substantial concentration of adenosintriphosphate.

The nucleotide can be isolated with ease from the aforesaid aqueous extract by various well known methods.

Suitable dehydrating agents for the purposes of the present invention are ethyl alcohol, methyl alcohol and acetone, ethyl alcohol being preferred.

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The invention will be illustrated by the following example:

In order to produce adenosintriphosphate from horse muscle, the following procedure is adopted.

The animal is killed, the muscles are cut out and minced in a suitable mincing machine. The minced muscle tissue drops from the mincing machine into a vessel containing ethyl alcohol of 96% concentration, 4 litres of ethyl alcohol being used per kg. of muscle tissue. The minced muscle tissue is allowed to stand for about an hour in the alcohol, whereupon the alcohol together with water and other extracted substances is separated from the insoluble mass by filter-pressing or centrifuging. The separated alcohol is rejected.

The muscle residue is next suspended in a further quantity of ethyl alcohol of 96% concentration, 1 litre of alcohol being employed for every kg. of muscle tissue originally used.

The mixture of muscle tissue and alcohol is thereupon heated to boiling point in order to precipitate the proteins and render them insoluble.

After cooling, the alcohol and substances dissolved therein are again separated from the insoluble residue either by filtration or centrifuging.

The two treatments with alcohol remove from the muscle tissue not only water but also the bulk of the water-soluble substances present therein, as well as fats and lipins.

The sole water soluble substances retained in the muscle tissue are low molecular substances which are linked to the insolubilised proteins. Of these substances adenosintriphosphate is one which can be readily extracted from the muscle tissue residue by water.

Since many of the other low-molecular substances are more strongly adsorbed to the protein than adenosintriphosphate, the aqueous extract obtained will contain adenosintriphosphate of a relatively high degree of purity and in relatively high concentration.

The adenosintriphosphate can be purified by precipitating the aqueous extract with a solution of mercuric acetate acidified with acetic acid. The adenosintriphosphate can be liberated from the precipitate by treatment with sulphuretted hydrogen, the product dissolved in alcohol and re-precipitated as acid calcium salt by addition of calcium chloride to the alcoholic solution.

I claim:

1. In a process for extracting a natural animal tissue containing adenosintriphosphate and related substances, a first step of treating said tissue with a liquid dehydrating agent insolubiliz-

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ing protein, and a second step of preparing an aqueous extract of said tissue after separating the liquid dehydrating agent from the tissue.

2. In a process for the recovery of a mixture of nucleotides containing predominantly adenosin-
triphosphate, from an animal tissue containing
both protein and adenosintriphosphate, a first
step of subjecting said animal tissue to a treat-
ment with ethyl alcohol and a second step of
preparing an aqueous extract of said tissue after
separation of the ethyl alcohol from the tissue.

3. In a process for the recovery of a mixture of nucleotides containing predominantly adenosin-
triphosphate, from an animal tissue containing
both protein and adenosintriphosphate, a first
step of subjecting said animal tissue to a treat-
ment with ethyl alcohol as a dehydrating and
protein-insolubilizing agent, part of said treat-
ment being carried out at the boiling temperature
of ethyl alcohol, and a subsequent step of ex-
tracting the tissue with water.

4. A process for the preparation of a mixture of nucleotides containing predominantly adenosin-
triphosphate which comprises the step of sub-
jecting the comminuted tissue to a treatment with
a dehydrating agent adapted to insolubilize and
precipitate protein, the step of separating said
dehydrating agent from the insoluble residue and
extracting said insoluble residue with water and
purifying the extract formed by precipitation with
mercuric acetate in the presence of acetic acid,
subsequent treatment with hydrogen sulfide, dis-
solution of the product in alcohol and reprecipi-
tation by calcium chloride.

5. Process for the preparation of adenosintri-
phosphate comprising immersing minced horse

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muscle in ethyl alcohol of 96%, allowing the mix-
ture to stand, separating the alcohol from the
muscle, suspending the muscle tissue in a further
quantity of ethyl alcohol of 96%, bringing the
mixture of muscle tissue and alcohol to boiling
point, allowing the mixture to cool, separating
the alcohol from the muscle tissue, and extracting
the latter with water.

6. Process according to claim 5, in which the
resulting aqueous extract is purified by precipi-
tation with a solution of mercuric acetate acidi-
fied with acetic acid, treating the precipitate with
hydrogen sulfide, dissolution of the product in
alcohol and reprecipitation of calcium chloride
from the alcoholic solution.

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