

XXXV. ON THE CHEMICAL NATURE OF SUBSTANCES FROM ALCOHOLIC EXTRACTS OF VARIOUS FOODSTUFFS WHICH GIVE A COLOUR REACTION WITH PHOSPHOTUNGSTIC AND PHOSPHOMOLYBDIC ACIDS. (PRELIMINARY COMMUNICATION.)

By CASIMIR FUNK, *Beit Memorial Research Fellow*, AND ARCHIBALD BRUCE MACALLUM, *Beit Memorial Research Fellow*.

From the Biochemical Department, Lister Institute.

(Received May 22nd, 1913.)

Two colour reactions have recently been described, one with phosphotungstic acid for uric acid [Folin and Macallum, 1912], the other with phosphotungstic and phosphomolybdic acids for polyphenols [Folin and Denis, 1912], which they recommend for the determination of these substances. One of us previously observed during the fractionation of yeast and rice-polishings [Funk, 1913] that the vitamine-fraction constantly gave a blue coloration with both reagents, that the reaction gradually disappeared during the further purification of the products and at the same time that the curative power for polyneuritis in birds slightly diminished. As these reacting substances might be of some importance for the process of curing, we have tested a large number of compounds with both reagents, in order to obtain some information as to the chemical nature of these substances, and we have found that a certain number of them which occur in nature show the colour reactions.

With the uric acid reagent certain purine bodies and some tyrosine derivatives give a very strong reaction. The alcoholic extracts of different foodstuffs, especially those which are known to be rich in vitamines, give in general both reactions very markedly. The substances giving the reaction seem to pass into the phosphotungstic acid filtrate and we are proceeding to isolate them.

The reactions with purine and pyrimidine derivatives.

Substance	Uric acid reagent	Phenol reagent
Xanthine	-	++
Hypoxanthine	-	+
Paraxanthine	-	-
Guanine	-	++
Alloxantin	+++	+++
Alloxan	-	-
Thymine	-	-
Theophylline	-	-
3-Methyl-uric acid ..	+	++
7- " "	trace	++
Hydantoin	-	-
Uracil	-	-
Guanidine carbonate	-	-
Hydantoic acid	-	-
Allantoin	-	-
Adenine	-	-
Uridine	-	-
Guanosine	-	trace
Adenosine	-	-
Cytidine nitrate	-	-
Yeast nucleic acid ...	trace	trace
Thymonucleic acid ...	-	-

Tyrosine derivatives.

<i>l</i> -Tyrosine	-	+++
Nitro-tyrosine	-	-
3-4-Dihydroxyphenylalanine	+++	+++
2-Aminotyrosine	+++	+++
3-Aminotyrosine	++	+++
<i>l</i> -Tyrosine anhydride	-	-?
Glycyl- <i>l</i> -tyrosine	trace	++

We have also investigated a number of amino-acids, polypeptides and diketopiperazines, not including tryptophane, oxy-tryptophane and oxy-proline, all of which were entirely negative to both reagents.

Foodstuffs.

Ceridin (alcoholic extract of yeast) ...	trace	+
Zymin	trace	+
Alcoholic extract of rice-polishings ...	+	++
Subs. C ₂₄ H ₁₉ O ₉ N ₅ from vitamine-fraction of yeast	-	-
Subst. C ₂₉ H ₂₃ O ₉ N ₅ from the same fraction ...	-	+
Nicotinic acid from yeast and rice ...	-	-
C ₂₆ H ₂₀ O ₉ N ₄ from vitamine-fraction of rice ...	-	-
Alcoholic extract from caseinogen (crude) ...	+	+
Alcoholic extract of milk	+	+
Whey powder from milk	+	+
Filtrate from milk precipitated by acid ...	trace	+
Alcoholic extract of whey powder	trace	+
Cod liver oil and the aqueous extract ...	+	++
Alcoholic and aqueous extract of Rous' chicken sarcoma	trace	++

We see from the table that the reactions are very specific for purine derivatives and polyphenols and they may therefore serve as a guide as to what groups the substances giving the reactions may belong. As the reactions are very sensitive, it seems to us that they might be used to ascertain the purity of phosphatides and other substances like caseinogen which are prepared from foodstuffs, and for which up to the present we possess no standard of purity. This test has already been found very useful in the investigation of the vitamine-fraction.

The table for purine derivatives shows that a substitution of one hydrogen atom in the purine ring lessens or destroys the power to give the uric acid reaction. In the case of the phenol reagent this is also brought about when two hydrogen atoms are substituted. The colour reactions with tyrosine derivatives and alloxantin were remarkably stable as compared with the others.

REFERENCES.

- Folin and Macallum junr. (1912), *J. Biol. Chem.* **11**, 265; **13**, 363.
Folin and Denis (1912), *J. Biol. Chem.* **12**, 239.
Casimir Funk (1913), *Brit. Med. J.* April 12th; *J. Physiol.* **46**, 173.