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An examination of Bardach's new protein test.

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In a recent paper, Bardach[1] drew attention to the fact that the formation of typical iodoform crystals from iodopotassium iodide and acetone in an alkaline solution may be prevented by albumin. In the presence of a sufficient proportion of albumin in such solutions, the production of characteristic hexagonal platelets or stellar masses of iodoform does not occur, but, instead, fine *yellow needles*, apparently of some other substance, gradually appear. Bardach found that the power of bringing about this interesting crystalline reaction is also exhibited in a general way by acidalbumin, protoalbumoses, peptones, phytovitellin, casein, yeast nuclein, hemoglobin, tendomucoid, gelatin, and by the following protein-containing materials: pancreatin, sperm, blood, sputum, normal urine and albuminous urine. He did not name any proteins that fail to give the reaction.

The best conditions and procedure for the test are stated by Bardach to be as follows: To 5 c.c. of the moderately concentrated albuminous liquid,[2] add at first 2 or 3 drops of a dilute aqueous acetone solution (0.5 per cent.), then sufficient Lugol reagent[3] to supply a moderate excess of iodine,[4] and lastly *considerable* ammonium hydroxid (usually about 3 c.c. of concentrated solution).

If iodine is employed in *moderate* excess, the ammonium hydroxid usually produces at once a black precipitate of iodonitro-compounds, upon which the yellow needles are gradually deposited. If just the right amount of iodine is present, the liquid soon becomes yellowish and the black precipitate formed at once by the

[1] Bardach: *Zeitschrift für physiologische Chemie*, 1908, liv, p. 355.

[2] The proportion of protein should not exceed 5 per cent.

[3] Iodine (4 grams) and potassium iodide (6 grams) in water (100 c.c.).

[4] Sufficient to impart a permanent reddish brown color to the shaken solution. The required amount of Lugol reagent varies from 1 drop to several c.c. according to the proportion of protein, sugar or other iodine-reacting materials present.

ammonium hydroxid gradually undergoes complete or nearly complete transformation into the yellow needles. Under both conditions the yellow needles form promptly, usually within an hour. In the presence of too large an excess of iodine the reaction may be prevented or hidden in the heavy black precipitate of iodonitro-compounds. Too little iodine or too much protein also prevents the reaction.[1]

We have confirmed practically all the statements made by Bardach regarding the conditions affecting the production, as well as the chemical and physical characters, of the yellow needles. Bardach is convinced that, in spite of their iodoform-like odor and color, the yellow needles do not consist of iodoform but of a hitherto undescribed iodo-compound, which he proposes to prepare in abundance for detailed investigation. In harmony with Bardach's statements we have found the crystals practically insoluble in cold dilute mineral acids and alkalies, but somewhat soluble in cold water. In alcohol, ether and chloroform the crystals apparently undergo gradual modification into iodoform and probably other products.

We have made no special effort to advance in this study in any direction beyond the limits reached by Bardach himself, except to ascertain whether other proteins than those named by Bardach respond to the test. Applying the test as detailed above, we have obtained strikingly positive results with the following *additional* proteins and protein-containing materials:

* myosin
* edestin
* alkali albuminate (from myosin)
* acidalbumin (from muscle stroma substance)
* heteromucoses
  deuteromucoses
  elastoses
  tendomucoid digestive products (peptic, soluble, mixed, including peptone)
* ligament mucoid
  tendon mucoid (non-precipitable by acid)
  bone mucoid (non-precipitable by acid)
* nucleoprotein (from ligament)
  gelatins (from ligament and bones)
† osseoalbumoid
† chondroalbumoid
† ossein
† collagen-elastin (mixed; from bones)

[1] When considerable protein and iodine are present, a *grayish green* precipitate is apt to form at once on adding ammonium hydroxid. If the proportions are favorable and the mixture is stirred continuously for a few minutes, the grayish green precipitate undergoes a gradual and very beautiful transformation into the glistening yellow needles.

* tendomucoid digestive residues (peptic)	† elastins (from ligament and tendon)
ligament mucoid digestive products (peptic, soluble, mixed, including peptone)	proteose-peptone (Witte's)
* osseomucoids	egg yolk (aqueous emulsion)
* chondromucoids	synovia
	serum (dog blood)
	lime water extract of tendon

* In dilute ammoniacal solution.

† Dilute solution, chiefly of *hydration products*, obtained by heating in dilute ammonium or potassium hydroxid.

It was found, on allowing the crystalline mixtures to stand for several days, that each precipitate diminished in amount very perceptibly and, also, on longer standing, that the crystals lost their characteristic canary-yellow color; they became opaque and when viewed through a microscope their outlines were less distinct than immediately after their formation.

The needle-like crystals obtained with the above named protein materials differed somewhat in character. The needles derived from some proteins were much finer and sharper than others. In some cases the needles were hair-like in appearance. As a rule the crystals were the same in each test with a given protein. Many of the crystals arranged themselves in rosettes or in bundle-like clusters; a few had a knobbed structure which gave them the appearance of nails. Some of the needles were branched like twigs of evergreen. It was a question whether the type of crystal was characteristic of the particular protein, or whether the differences in crystal-form were due to variations in the rapidity or other physical conditions of formation. It is probable, of course, that variations in the proportions of the reagents employed may have introduced chemical factors affecting the nature of the crystalline products. Iodoform crystals were mixed with the needle-like crystals whenever the proportion of protein was relatively low.

Whether Bardach's reaction is exhibited also by any non-protein substances has not been determined. It is probable, from the results of our own work, that without exception every soluble protein will give it. The test is quite delicate and promises to be useful in many ways, but its comparative value, and the disturbing influences in its application,[1] as a protein test await determination.

[1] Bardach has already noted a disturbing influence of earthy phosphate. Bardach: *loc. cit.*, p. 357.