PATHOLOGY

An Unidentified Compound in the Serum of Children with Kwashiorkor (Protein–Calorie Malnutrition)

When using the rapid chromatographic method recently developed for measuring the balance of amino-acids in the serum of children with kwashiorkor[1] we have found an unidentifiable compound with an R_F value slightly less than that of valine. The solvent used was *A* in Table 1. This communication describes various properties of the compound and the conditions under which it can be demonstrated.

Table 1. R_F VALUES ON NO. 20 WHATMAN PAPER OF THE UNIDENTIFIED SERUM COMPOUND IN KWASHIORKOR AND OF PIPERIDINE-2-CARBOXYLIC ACID

	Solvent				
	A	*B*	*C*	*D*	*E*
Piperidine-2-carboxylic acid (authentic)	0·44	0·88	0·45	0·41	0·51
Unidentified compound	0·45	0·86	0·45	0·40	0·50
Co-chromatography	0·45	0·86	0·44	0·40	0·50
Piperidine-2-carboxylic acid (ref. 3)	0·44	0·93	0·45	0·41	—

The solvents were: *A*, butan-1-ol–acetic acid–water (12 : 3 : 5, by vol.); *B*, phenol–water (500 g, 125 ml.); *C*, butan-1-ol–pyridine–water (1 : 1 : 1, by vol.); *D*, ethanol–ammonia–water (18 : 1 : 1, by vol.); *E*, butan-1-ol–water–formic acid (7 : 2 : 1, by vol.).

Unlike the amino-acids that are eluted and estimated for the balance method, the compound did not react with ninhydrin in the cold. On heating to 120° C, however, a purple colour was produced that was more blue than the colour usually formed by amino-acids. The ninhydrin derivative had two unusual properties. In ultra-violet light it had a cherry-red fluorescence, unlike the derivatives of amino-acids, which absorb ultra-violet light and appear black. When the chromatogram was dipped through a solution of ethanolic copper nitrate the colour did not change to the typical pinkish-orange but remained blue-purple. A compound with these properties has been described; it is piperidine-2-carboxylic acid (pipecolic acid)[2,3], a derivative of lysine. The unknown compound gave a strong blue-green reaction with isatin, indicating a relationship to pyrrolidine or piperidine derivatives[4].

Deproteinized samples of serum were prepared and applied without de-salting to Whatman No. 20 paper as previously described[1]. The chromatographic properties of the compound were compared in five solvents with those of a sample of authentic piperidine-2-carboxylic acid obtained from the California Corporation for Biochemical Research, Los Angeles. The results are recorded in Table 1. Colour reactions of the unknown and the known substances agreed closely, and co-chromatography in each of the solvents failed to separate them. The solvent systems used will separate piperidine-2-carboxylic acid from piperidine-3-carboxylic acid, piperidine-4-carboxylic acid, piperidine-2,6-dicarboxylic acid and 5-hydroxy-piperidine-2-carboxylic acid[5].

The unidentified compound is not found in all samples of serum taken from children admitted for the treatment of kwashiorkor. There appears to be no correlation between the presence or absence of the compound and the severity of the kwashiorkor on admission. After a meal containing protein or after a dose of *l*-lysine, it is very often demonstrable. When treatment is proceeding favourably, the compound slowly disappears from the serum and none can be detected after about 10 days. In less favourable circumstances, especially when the concentration of the total serum proteins does not rise satisfactorily, the amino-acid balance ratio rises and the weight gain is poor, the amount of the compound appears to increase even beyond the level found on admission.

Disturbances in the metabolism of histidine, phenylalanine and tyrosine in kwashiorkor have already been described[6,7]. The present finding demonstrates the abnormal metabolism of an additional nitrogenous compound. Confirmation of our tentative identification of the compound as piperidine-2-carboxylic acid would indicate that the metabolism of lysine is at fault.

We have at present no definite information about the source of the unknown compound. It is unlikely to be merely a component of the diet, since it is found in samples of blood taken after an overnight fast, and does not increase in amount towards the end of successful treatment in pace with the improvement of the child's appetite and food intake. The rise in serum concentration after milk protein or lysine has been given suggests, but does not prove, that the source is an amino-acid.

The structure of piperidine-2-carboxylic acid closely resembles that of the amino-acid proline and the vitamin nicotinic acid, and it is possible that the raised concentration of piperidine-2-carboxylic acid in the serum may affect the metabolism of these two substances.

Samples of serum containing the unknown compound are being collected for an elementary analysis and the determination of molecular weight. The collection will take a very long time, because only a small amount of blood can justifiably be taken from the severely ill children in whose serum the compound appears. It is hoped that the publication of this report will encourage other workers to look for the compound. It may have been confused with β-aminoisobutyric acid or ethanolamine, both of which have been reported in the serum in kwashiorkor. The ninhydrin derivatives of these compounds do not fluoresce in ultra-violet light; they give the normal orange colour in ethanolic copper nitrate.

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Influence of Diet and Iodine-131 Injections on the Survival of Dystrophic Mice

Diet has been shown to influence the survival of mice with hereditary muscular dystrophy, since an increased lifespan and an extended period of growth have been recorded when a semi-synthetic diet containing high-quality protein and lipid was used, and the further addition of glycine to the diet increased the growth rate still more[1]. Apparently, the vitamin content of the diet did not produce the effect, since other investigations using massive doses of vitamin mixtures did not prolong the survival of dystrophic mice[2]. Suppression of the thyroid gland activity by ^{131}I (ref. 3), or surgical removal of the gland[4], delayed muscular degeneration in vitamin E-deficient rabbits, indicating an influence of the thyroid hormone on this type of muscle pathology.

Mice with hereditary muscular dystrophy (*dydy*) were obtained by mating *Jax*/129 (*Dydy*) mice from the Jackson Memorial Laboratory, Bar Harbor, Maine, and were maintained in temperature-controlled quarters. Food and water were available *ad libitum*. Mice were weaned at 3–4 weeks, experimental groups were formed by dividing litters, and treatments were initiated at weaning. During the first trial, the diet consisted of 'Purina Laboratory Chow', and treated mice were given a single intraperitoneal injection of 200 mc. ^{131}I as sodium iodide in a small amount of physiological saline at three weeks of age.