The anatomical phase of this investigation was supported by research grant *H*-1907 (*C* 3, 4), National Heart Institute, National Institutes of Health, United States Public Health Service.

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July 6.

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Blood Keto-acids in Kwashiorkor

During desalting in an electrolytic desalter (Shandon Scientific Co. Ltd., London) of urines from kwashiorkor patients, it was observed that a considerable amount of a black mercury amalgam was invariably formed. Estimation of the concentration of ammonia in these urines confirmed that the amalgam formation was due to a high ammonia content. This was in agreement with a report made earlier by Platt and Heard that ammonia excretion was increased in protein malnutrition. It was suspected at the time that this increased ammonia output may be the result of an acidification defect due to reduced hydrogen ion excretion by the renal tubules or else to the excretion of increased amounts of organic acids. Afterwards, while measuring serum transaminase-levels by the spectrophotometric method[1] it was noted that on the addition of malic or lactic dehydrogenase and reduced diphosphopyridine nucleotide to the buffered serum, the specimens from cases of kwashiorkor consumed more reduced diphosphopydrine nuleotide than normal serum. In many instances more than 30 min. were required to produce equilibrium conditions and in most cases extra reduced diphosphopyridine nucleotide would have to be added in order to produce a steady state and a high enough initial spectrophotometric reading. With normal serum on the other hand, equilibrium was usually attained in less than 10 min. and it is unusual for extra reduced diphosphopyridine nucleotide to be required. This observation pointed to the probability that ketoacids which are substrates for malic dehydrogenase and lactic dehydrogenase must accumulate in the blood in kwashiorkor.

Paper chromatography of ketoacid hydrazones according to the procedure of McArdle[2] confirmed that pyruvate mainly, and in some case α-ketoglutaric acid were present in increased concentration in the blood in kwashiorkor. Blood pyruvate was then determined by the enzyme spectrophotometric method of Segal *et al.*[3]. All the normal children and adults examined by this method had fasting blood pyruvate concentrations of 0·40–0·85 mgm./100 ml. The twenty-five kwashiorkor patients examined had fasting blood pyruvate levels ranging from 0·50 mgm./100 ml. to 2·8 mgm./100 ml.; the value was more than 1·00 mgm./100 ml. in 14 of the 25 patients. There was no correlation between blood pyruvate concentration and the clinical assessment of the severity of the case.

α-Ketoglutaric acid was determined in the perchloric acid extract used for pyruvate extimation by measuring the yellow colour of the hydrazone after pyruvate had been destroyed with lactic dehydrogenase. Normal values ranged from 0·08 to 0·22 mgm./100 ml. The kwashiorkor cases showed much variation but in only five of the twenty-five cases was α-ketoglutarate clearly above the normal range.

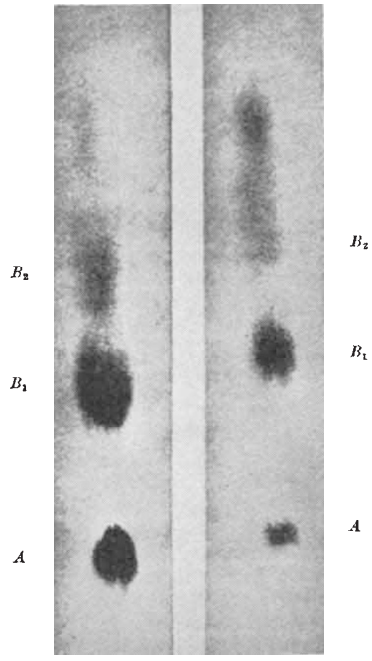

Fig. 1. (1) Chromatogram of blood ketoacids on admission. The equivalent of 0·8 ml. blood was applied. (2) Chromatogram of blood ketoacids after patient had been given the methionine supplemented, high carbohydrate diet. Same quantity of blood as in (1).
A, ketoglutaric acid spot; B_1 and B_2 pyruvate spots.

When patients are successfully treated with milk and vitamin supplements the blood pyruvate returned to normal level. When they were fed for three days with a standard diet of high carbohydrate and low protein content supplemented with 25 mgm. thiamine daily by intramuscular injection as well as other vitamins by mouth the blood pyruvate did not show any significant change. In two cases fed for three days with the same standard diet to which was added 3 gm. DL-methionine daily, the blood pyruvate showed dramatic reduction. The chromatograms of one of the cases is shown in the accompanying photographs (Fig. 1), which were taken under ultra-violet light after the papers were treated with 2 per cent. sodium hydroxide in 90 per cent ethanol. The blood pyruvate in this case was 2·6 mgm./100 ml. on admission. After three days on the diet supplement with methionine, the value had fallen to 1·2 mgm./100 ml. There is thus evidence that the accumulation of pyruvate may be due, at least in part, to deficiency of sulphydryl groups. The matter is being further investigated in this laboratory.

I am indebted to Dr. W. R. F. Collis, head of the Department of Child Health in the College, for clinical facilities to carry out this investigation.

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