BULL. PHIL. BIOCHEM. SOC. 1(2): 24-40 (1978)

STUDIES ON THE EFFECTS OF NUTRITIONAL IRON-DEFICIENCY ANEMIA ON GLUCOSE METABOLISM IN RATS+

Francisco M. Strebel, Jr. * and Manuel P. Macapinlac **

ABSTRACT

The effects of iron-deficiency anemia on glucose metabolism in albino rats of the Sprague-Dawley strain were examined. Fasting of animals followed by an oral dose of glucose and measurement of the rate of glucose absorption showed no notable differences among groups. Glucose tolerance test was done on anemic, pair-fed and control rats using Nelson-Somogyi method of glucose analysis. The glucose tolerance patterns of anemic rats definitely showed abnormally higher glucose levels. Glucosuria was not observed. However, there was more glycogen in the livers of anemic rats.

The incorporation of intraperitoneally injected ^{14}C-glucose into hepatic glycogen expressed per 100 grams fresh liver indicated no significant differences among groups suggesting comparable rates of glycogen synthesis from labelled glucose. However, in the anemic group the specific activity of liver glycogen was significantly lower suggesting dilution of the ^{14}C-glucose content of glycogen probably by enhanced gluconeogenesis.

Measurement of $^{14}CO_2$ output following intraperitoneal ^{14}C-glucose injection demonstrated that the peak of oxidation of ^{14}C-glucose to $^{14}CO_2$ and H_2O occurred essentially between the 2nd and 3rd hours in all animals. Surprisingly in the iron-deficient rats, the cummulative $^{14}CO_2$ output (expressed as percent oxidation per hour) clearly showed that the rate of ^{14}C-glucose oxidation to $^{14}CO_2 + H_2O$ was relatively faster. This might be due to compensatory mechanisms designed to increase O_2 supply to the peripheral tissues.

From the present study, nutritional iron-deficiency anemia clearly produces abnormalities in carbohydrate metabolism. More basic studies are needed to better understand and elucidate these metabolic abnormalities.

INTRODUCTION

While studies on iron metabolism are numerous, particularly investigations on its absorption (1-3), little is known about the effect of iron-deficiency anemia on carbohydrate metabolism. The only study so far on the effects of

---

+M.S. thesis of F.M.S. presented to the Dept. of Biochemistry, University of the Philippines, College of Medicine.

*Fellow at the Dept. of Medicine, University of the Philippines, Philippine General Hospital.

**Prof. of Biochemistry, Dept. of Biochemistry, University of the Philippines, College of Medicine.

iron-deficiency anemia on glucose metabolism appears to be that of Macdougall's (4) who reported increased uptake of glucose by red cells, as well as an increase in the enzymes of the glycolytic and hexosemonophosphate pathways of these cells. All other investigations on iron-deficiency anemia were studies on mitochondrial and microsomal cytochromes in different organs (5-9) as well as effects of 2,3-diphosphoglycerate on oxygen binding of hemoglobin (10-14). A study of the effect of iron-deficiency anemia on carbohydrate metabolism is particularly relevant considering that a relatively large proportion of the calories in the Filipino diet comes from carbohydrates (15-18). Moreover, iron-deficiency anemia is one of the major nutritional problems in the Philippines. The incidence remains high (19-20). In fact, iron fortification of food items especially those of babies has been advocated (21).

In this study, initially the effect of iron-deficiency on the rate of glucose absorption in the intestines was measured by determining the Cori-Cori coefficient. Glucose tolerance test was done to get an overview of the effects of iron-deficiency on carbohydrate metabolism. Possible alterations in glucose storage was investigated by determining hepatic glycogen following an oral dose of glucose. Finally, the rate of D-[U- ^{14}C]-glucose incorporation into liver glycogen and oxidation to $^{14}CO_2 + H_2O$ were measured to clarify the observations made.

EXPERIMENTAL PROCEDURES

Animals – Weanling rats (21 days old) of the Sprague-Dawley strain were housed individually in stainless steel cages throughout the experiment. Rats were divided into 3 groups: experimental, pair-fed and control, with 7 rats to a group. De-ionized drinking water was given *ad libitum*. The experimental group was given an iron-deficient diet used by Bailey-Wood, et al. (22). The diet of the control and pair-fed rats was supplemented with 300 mg $FeSO_4 \cdot 7H_2O$ per Kg of diet. Pair-fed rats were given only the amount of food consumed daily by the corresponding experimental rats. All animals were fed for 3 months the respective synthetic diets.

Blood Hemoglobin – Hemoglobin and hematocrit levels were taken after the 6th and 10th weeks of feeding using the cyanmethemoglobin method (23,24) for hemoglobin and the micromethod using a Clay-Adams hematocrit centrifuge and Spiracrit reading device for hematocrit.

Glucose Absorption – The coefficient of glucose absorption was determined by the procedure outlined by D'Amour and Blood (25). However, intestinal glucose was measured spectrophotometrically rather than by titration procedures.

Blood Glucose – Blood glucose was determined by the method of Nelson-and Somogyi (26,27) from 0.04 ml whole blood obtained from the tail vein at 0, ½, 1, 2, and 3 hours after an intraperitoneal dose of 1 gram glucose per Kg body weight. Following color development, absorbances were read at 420 nm using spectronic 20 Spectrophotometer.

Urine Sugar – Benedict's method (28) modified by the teaching staff of the Department of Biochemistry, College of Medicine, U.P. (29) was adapted for urine sugar determination.

Liver Glycogen – Liver glycogen was isolated and purified using the procedures described by Clark (30) and Seifter, et al. (31) which were essentially a modification of the classical Pfluger method (32) and quantitated by the method developed by Jermyn (33). Radioactivity of liver glycogen was measured by transfering 1.0 ml sample into 10 ml Beckman Ready Solv Solution VI cocktail and counted using Beckman LS-100. CPM (counts per minute)/100 grams fresh liver was divided by the total milligrams of glycogen per 100 grams fresh liver and specific activity expressed as CPM/mg liver glycogen.

Glucose Oxidation – $^{14}CO_2$ output was measured from D-[U ^{14}C]-glucose injected intraperitoneally at a dose of 1 ml (3.1×10^6 CPM)/100 gram body weight as described by Cowgill and Pardee (34).

Statistical Analysis – From the data on the glucose tolerance test, the elimination, absorption and distribution rate constants for open two compartment model – extravascular were computed. This afforded the fitting of the necessary curves to show significant differences between groups graphically as well as the measuring of the half life of blood glucose in each group of animals (35). The same statistical procedure was applied to the data obtained on the $^{14}CO_2$ output. Analysis of Variance (Anova) and Least Significant Difference (LSD) analyses were further done to determine the significance of the values obtained numerically. Data on liver glycogen and glucose absorption were analyzed by Anova and LSD analyses and for more specific comparisons between groups, Duncan's test (36) was used.

RESULTS

After six weeks of feeding, the hemoglobin and hematocrit values in experimental rats were low compared to pair-fed and control animals such that severe anemia was evident after 10 weeks (Tables I and II) and was accompanied by slower rate of growth (Fig. I). The rates of growth of the experimental and the pair-fed were almost comparable.

Table I. Hemoglobin levels. Each value represents mean ± S.D. of seven rats.

Experimental rats		Pair-fed rats		Control rats	
6 weeks	10 weeks	6 weeks	10 weeks	6 weeks	10 weeks
4.90	3.70	14.28	16.64	14.65	16.50
± 0.9388	±0.414	± 1.164	± 1.544	± 1.466	± 1.061

Table II. Hematocrit values after 6 weeks of feeding. Each value represents the mean ± S.D. of seven rats.

Experimental rats	Pair-fed rats	Control rats
23.19 ± 4.488	48.57 ± 2.760	50.43 ± 1.9023

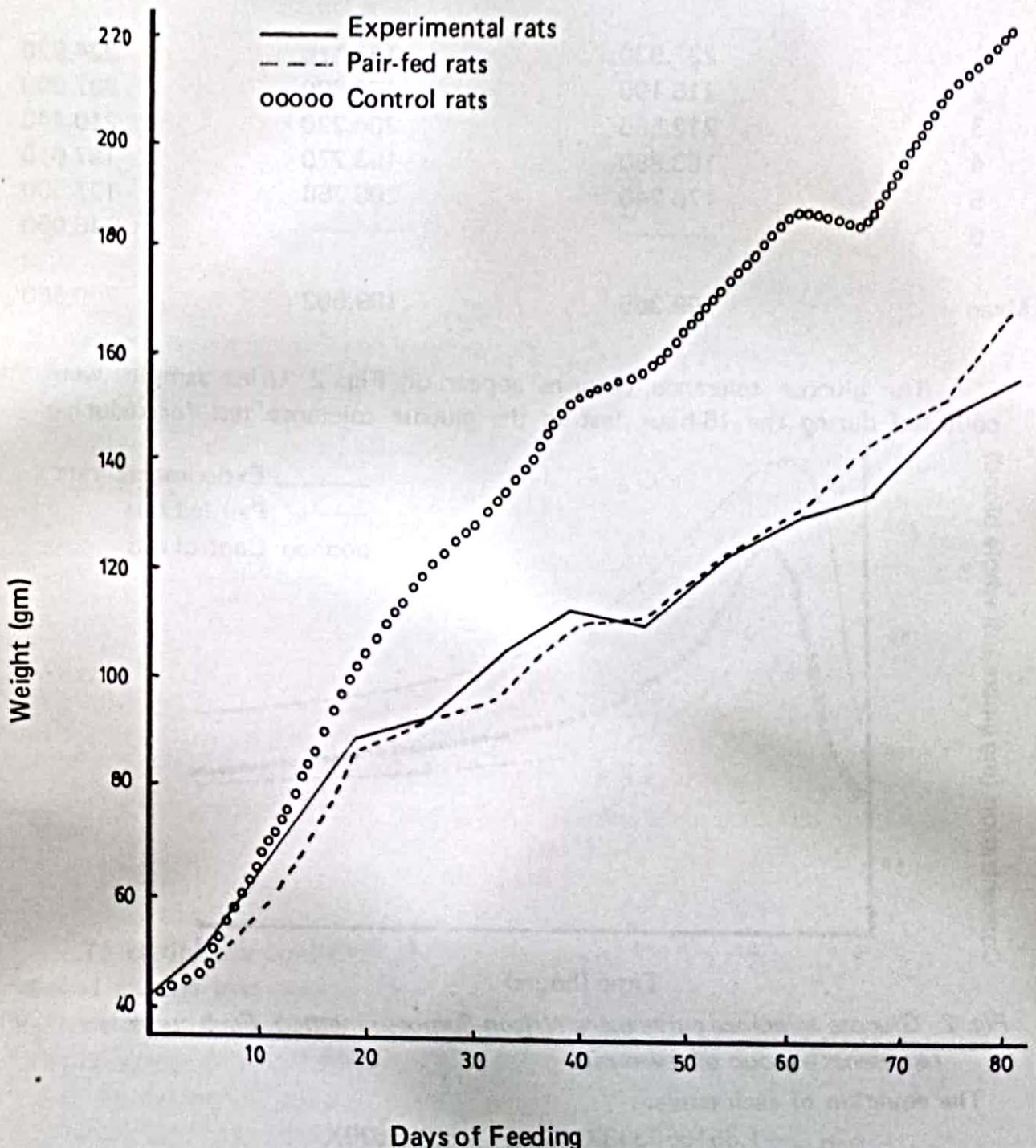

Fig. 1. Growth curve. Each line represents the mean of seven rats.

Table III shows the rates of glucose absorption which indicates no statistically significant differences among groups suggesting normal absorption of glucose from the intestines during iron-deficiency anemia.

Table III. Cori-Cori coefficient of glucose absorption.

Rat Number	Experimental rats	Pair-fed rats	Control rats
	(mg glucose absorbed per 100 grams body weight per hour)		
1	227.930	161.170	224.820
2	216.190	180.600	207.900
3	212.585	206.220	210.140
4	163.880	193.770	187.610
5	176.240	205.750	127.500
6	----------	----------	246.050
Mean	199.365	189.502	200.650

The glucose tolerance patterns appear on Fig. 2. Urine samples were collected during the 16-hour fast in the glucose tolerance test for reducing

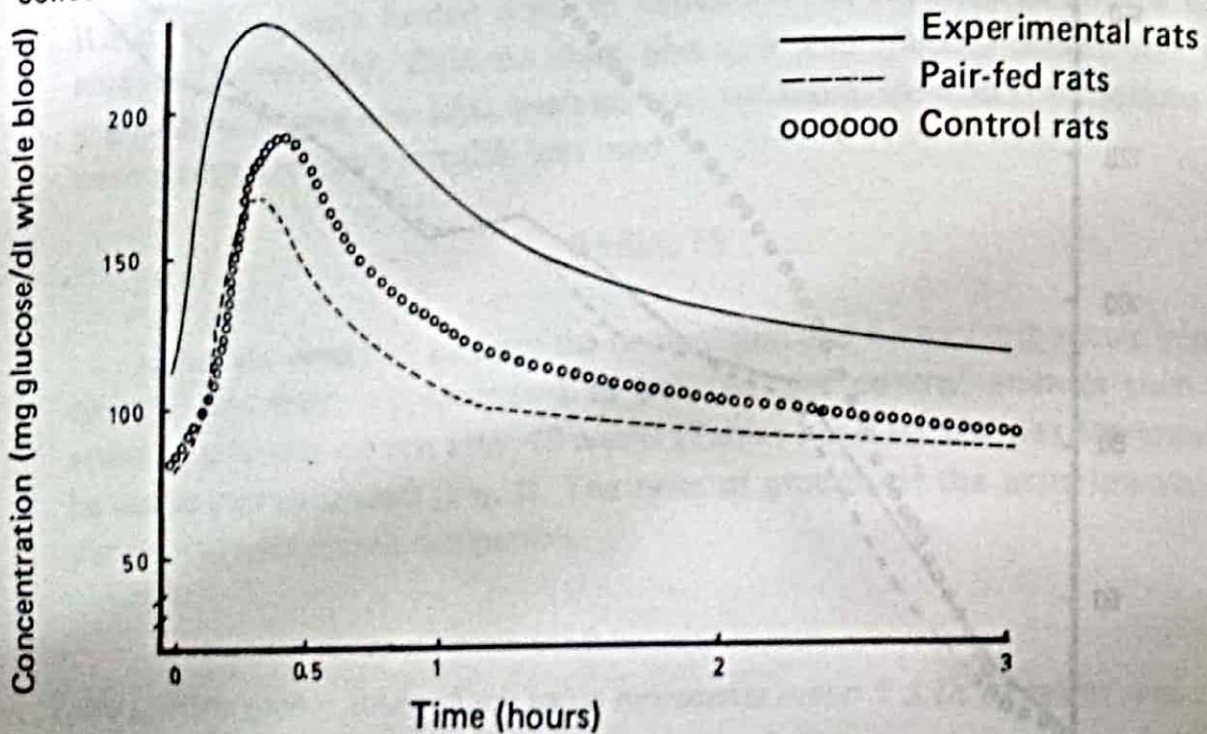

Fig. 2. Glucose tolerance curve using Nelson-Somogyi method. Each curve represents a group of seven rats.

The equation of each curve:

——— $y = 240\ e^{-1.351550333X} - 240\ e^{-5.878629X}$

– – – – $y = 37\ e^{-.4247475X} + 250\ e^{-3.71346375X} - 286\ e^{-1.0X - 25.9487X^2}$

ooooo $y = 78\ e^{-.6557X} + 31\ e^{-3.6314197X} - 387\ e^{-.377424X - 17.6688X^2}$

sugar. The samples were slightly positive in two anemic rats and traces only in four and completely negative in one rat as shown on Table IV. Interestingly, when the urine samples were tested with Tes Tape (Eli Lilly and Co.) which makes use of paper impregnated with glucose oxidase to detect glucose specifically, all the urine samples gave negative tests for glucose.

Table IV. Benedicts test of urine samples of experimental (anemic) rats.

Rat Number	8 drops	16 drops	24 drops	32 drops
1	–	–	traces	nt
2	+	nt*		
3	–	–	–	–
4	–	–	traces	nt
5	–	+	nt	
6	–	traces	nt	
7	+	nt		

* not tested

Data on liver glycogen depots appear on Table V. The glycogen concentrations in the liver of anemic rats were significantly greater compared to pair-fed and control groups.

Table V. Whole liver glycogen 2 hours after an oral dose of glucose.

Rat Number	Experimental rats	Pair-fed rats	Control rats
	(mg glycogen / 100 gm fresh liver)		
1	1776.27	1380.15	1296.10
2	1583.65	863.965	1343.65
3	1727.90	1272.05	1296.26
4	1872.30	1259.95	1248.06
Mean	1740.015	1194.03	1295.0175

To clarify the observations made, the rate of incorporation of uniformly labelled glucose into hepatic glycogen and its complete oxidation to $^{14}CO_2$ + H_2O were measured. Table VI shows liver glycogen concentration to be significantly greater in iron-deficient rats when compared to pair-fed rats. However, Table VII shows the radioactivity expressed as CPM of hepatic glycogen per 100 grams liver and statistically indicates no significant differences between any two groups. Table VIII gives the specific activity of liver glycogen. Significantly lower specific activity in the anemic group is indicated compared to pair-fed and control groups.

Table VI. Liver glycogen 6 hours after intraperitoneal dosing with glucose.

Rat Number	Experimental rats	Pair-fed rats	Control rats
	(mg glycogen / 100 gm fresh liver)		
1	1,092.00	502.86	1,043.00
2	190.40	34.50	114.80
3	1,050.00	16.15	851.00
4	1,470.00	163.80	----------
5	1,512.00	273.00	295.40
Mean	1,062.88	208.06	526.05

Table VII. CPM (counts per minute) of liver glycogen per 100 grams of fresh liver (for statistical purposes, the logarithm of the raw values to the base 10 were used).

Rat Number	Experimental rats	Pair-fed rats	Control rats
1	6.83×10^5	1.70×10^6	5.53×10^6
2	2.14×10^5	1.31×10^5	4.64×10^5
3	5.31×10^4	1.64×10^5	2.46×10^5
4	6.58×10^5	2.53×10^5	3.12×10^6
5	2.34×10^6	6.96×10^5	----------
6	4.35×10^6	1.42×10^6	1.97×10^6
Mean	1.38×10^6	1.06×10^6	2.27×10^6

*Table VIII. Specific activity of liver glycogen.**

Rat Number	Experimental rats	Pair-fed rats	Control rats
	(CPM (counts per minute) / mg glycogen)		
1	626	3,387	5,303
2	1,123	3,785	4,045
3	3,790	4,986	3,193
4	627	3,824	4,794
5	1,590	4,251	——
6	2,880	5,204	6,668
Mean	1,776	4,243	4,803

* For statistical purposes, the logarithm of the raw values to the base 10 were used.

Measurement of $^{14}CO_2$ output demonstrated that the peak of oxidation of ^{14}C-glucose to $^{14}CO_2 + H_2O$ occurred essentially between the 2nd and 3rd hours in all animals. However, anemic rats evidently exhibited a characteristically higher peak (Fig. 3). Plot of the cummulative $^{14}CO_2$ output clearly showed that the rate of glucose oxidation is definitely faster (Fig. 4) although not markedly in the iron-deficient rats.

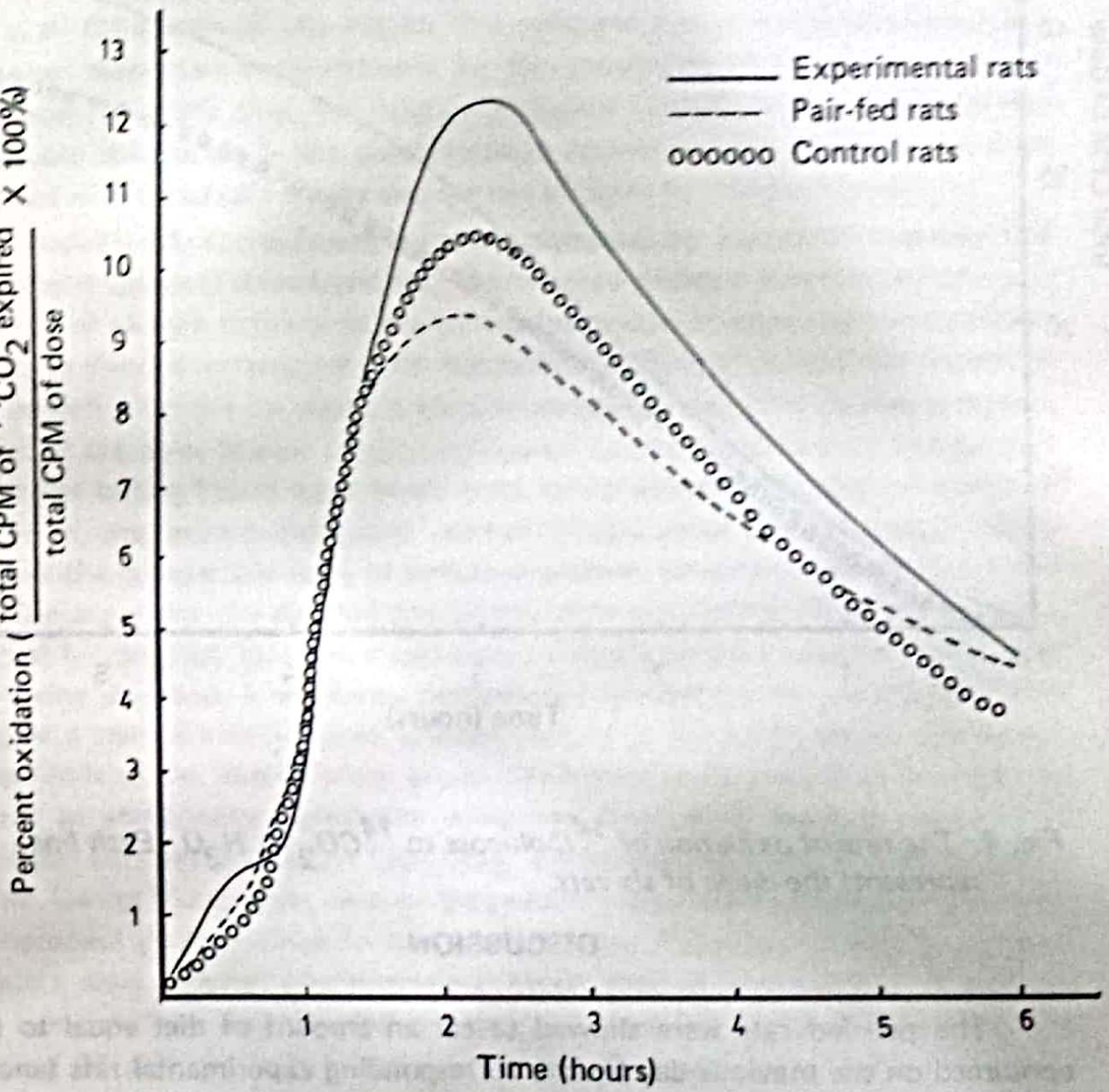

Fig. 3. Oxidation pattern of ^{14}C-glucose to $^{14}CO_2 + H_2O$. Each curve represents a group of six rats.

The regression equation of each curve:

——— $Ye = 20\ e^{-.2760X} + 8.6\ e^{-.62031X} - 27.1\ e^{-27.1\ e^{-.212495X} - .58431X^2}$

– – – – – $Ye = 13.5\ e^{-20585X} + 46\ e^{-1.568616X} - 58.305\ e^{-1.2081X} - 0.2739X^2$

ooooo $Ye = 15\ e^{-.2657X} + 11.5\ e^{-.6286X} - 25.737\ e^{-.43678X} - .4612X^2$

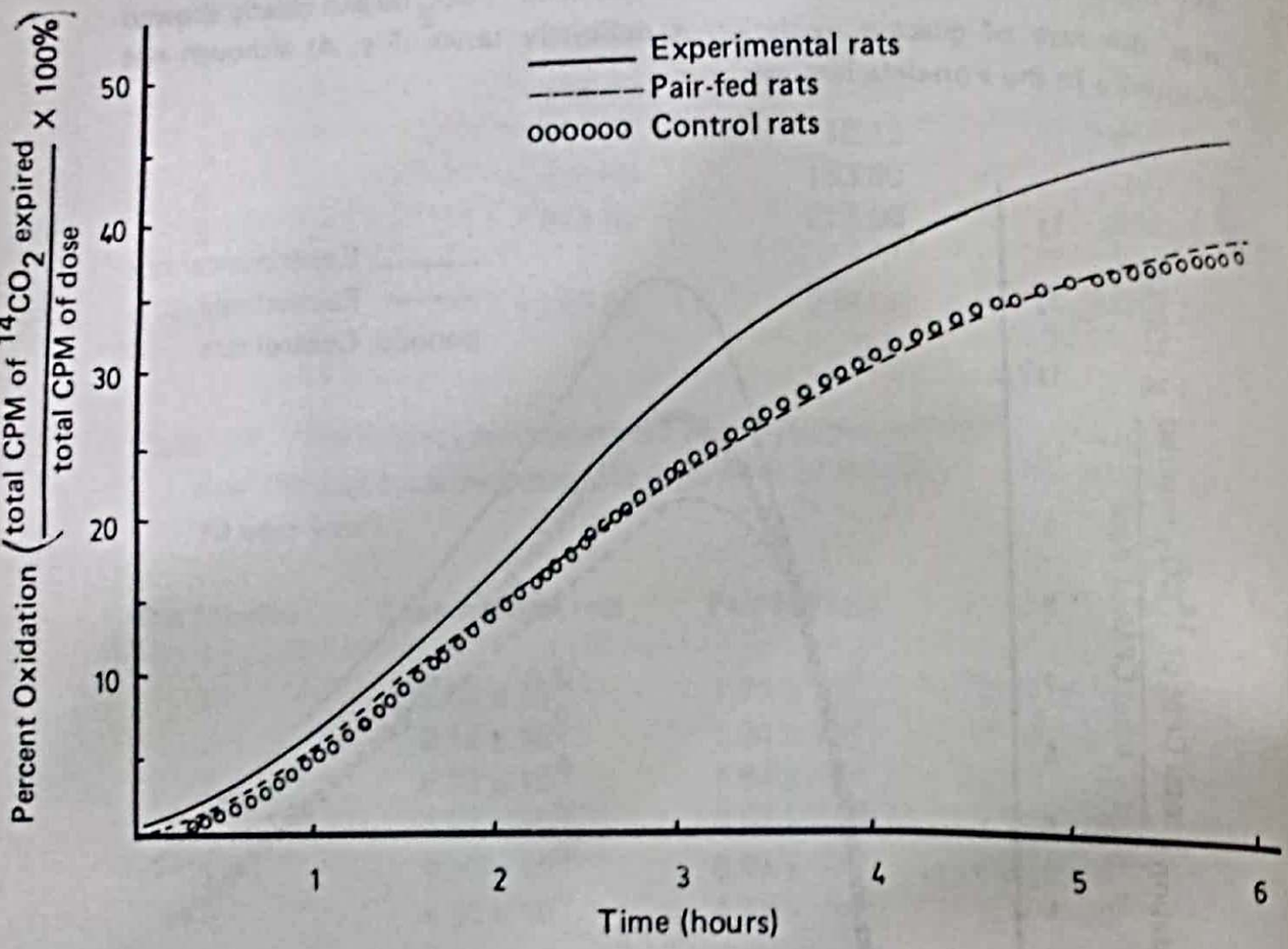

Fig. 4. The rate of oxidation of ^{14}C-glucose to $^{14}CO_2 + H_2O$. Each line represents the mean of six rats.

DISCUSSION

The pair-fed rats were allowed to eat an amount of diet equal to that consumed on the previous day by the corresponding experimental rats (anemic rats). The experimental rats frequently exhibited loss of appetite during the feeding period such that they were meal nibblers. On the contrary, pair-fed rats finished their food rations quickly such that they were meal eaters, indicating that these rats could have eaten much more had they been fed as much as they can, i.e., *ad libitum*. However, control rats ate much more per day compared to the two groups such that the growth was faster during the period of observation. Therefore, the growth curve (Fig. I) suggests that the rate of growth was largely dependent on daily food intake since the synthetic diets were nutritionally complete, except for the experimental diet which was not supplemented with iron. It seems that absence of dietary iron induces loss of appetite

such that less food was consumed. The slow growth in anemia may be compensatory in nature to prevent further dilution of body iron as a consequence of an increase in weight. This shows necessity of pair-fed controls.

Glucose is absorbed by an active transport process that has been postulated to involve ATP expenditure, and in particular the $Na^+ + K^+$-dependent ATPase (37). ATP is mainly produced via the electron transport chain where the majority of the respiratory enzymes either contain or require iron for optimal activities. The comparable rates of intestinal glucose absorption (Table III) in all the groups of rats suggest that adequate energy is still supplied to the intestinal absorptive compartments for the absorption of glucose even during iron lack. It seems that the separate intestinal compartment systems involve in glucose absorption – the glucose carrier system and the ATPase system are supplied with metabolic energy and are not affected by iron-deficiency.

Nutritional iron-deficiency anemia significantly lowers hemoglobin and hematocrit values (Tables I and II). This marked decrease in red blood cells and possibly of oxygen delivery to the tissues for oxidation may result in an abnormality in glucose metabolism. The metabolic handling of glucose was examined in iron-deficient rats by doing a glucose tolerance test. The glucose tolerance curves of the three groups of animals showed normal patterns (Fig. 2) such that if the pre-fasting blood sugar levels were not considered the glucose tolerance curves of the experimental and control groups were superimposable which support the comparable rates of intestinal glucose absorption observed in Table III. The curve for the pair-fed group was somewhat lower. This could be explained by the fact that these rats were relatively starved such that as glucose was being absorbed, it was being immediately utilized by the peripheral tissues to give a comparatively lower glucose, tolerance curve. However, the blood sugar levels of the iron-deficient group was higher at all times (Fig. 2). This was shown as statistically significant using the Regression method, Analysis of Variance (Anova) and Least Significant Difference analyses. The significantly higher fasting blood sugar level in the anemic group may be due to comparatively increased glycogenolysis in the anemic livers. Referring to Fig. 2, eighteen minutes after glucose administration, blood glucose level reached its peak in the iron-deficient and pair-fed groups. The peak of blood glucose level was reached 24 minutes later in the control rats. Conventionally, glucose tolerance test is done orally and the glucose peak is attained in ½-1 hour after ingestion. However, since the rate of enteric glucose absorption was found to be comparable in all three groups of animals, glucose was given intraperitoneally. Since this glucose route by-passes the intestinal absorptive compartments, it therefore accounts for the much earlier occurrence of the peaks of the blood glucose observed in the animals. After the peaks have been attained, the rate of decrease of blood glucose levels were essentially comparable in all groups.

The overall half life of blood glucose was also calculated in all groups of rats (Fig. 2). After measurement of rate constants for open two compartment model – extravascular, at exactly one (1) hour after glucose administration,

50% of the blood glucose was removed from circulation in the anemic group. At exactly 42 minutes, 50% of the glucose in the circulation remained in the pair-fed group, while at exactly 55 minutes,, 50% of the glucose in the blood remained in the control group. From these results, it was evident that the net removal of blood glucose was slower in the iron-deficient rats considering that it took longer time (1 hour) to remove 50% of the glucose in blood compared to 42 minutes in the pair-fed and 55 minutes in the control groups. It is obvious that the net removal of glucose from the circulation was fastest in the pair-fed rats perhaps owing to the fact that the peripheral and hepatic tissues of the rats in this group were relatively starved for glucose. The results therefore suggested a comparative delay in the net removal of glucose in blood in the iron-deficient rats so that they had significantly higher blood glucose levels.

The liver has been shown to sensitively regulate glycogen synthesis and breakdown in response to circulating glucose concentrations using perfused liver of rat (38). Pines, et al. (39) in the brief summary of their work, reported that adult normal rats showed an increase in glycogen synthesis in the liver following glucose injection due to activation of glycogen synthetase with concomittant inactivation of glycogen phosphorylase. It appears that high blood glucose level serves as a stimulus for the deposition into liver glycogen to maintain the blood sugar level. Hyperglycemia, which is evident in the anemic rats, may therefore suggest abnormal hepatic glycogen metabolism. The glycogen concentrations in the liver of anemic rats were significantly greater compared to pair-fed and control groups (Table V) suggesting either enhanced glycogen synthesis or decreased glycogenolysis or both during anemia. The rate of incorporation of uniformly labeled glucose into hepatic glygogen was measured to get a better insight of glycogen metabolism in the liver during iron-deficiency.

Liver glycogen concentration was significantly greater in iron-deficient rats when compared to pair-fed rats (Table VI). However, radioactivity expressed as counts per minute (CPM) of hepatic glycogen per 100 grams liver essentially showed no significant differences among groups (Table VII) indicating comparable rates of glucose incorporation into liver glycogen for all animals. When the specific activity of the liver glycogen was measured, the anemic rats showed significantly lower specific activity compared to both pair-fed and control rats (Table VIII). This suggests dilution of the ^{14}C-glucose content of liver glycogen by other glucose sources. Such glucose may be supplied by gluconeogenesis which may be enhanced in iron-deficiency. In rats, extrahepatic (40) and hepatic (41) gluconeogenesis involves mediation of glucagon. The stimulatory effect of glucagon on gluconeogenesis has been observed in isolated perfused liver (42). The synthesis of phosphoenol pyruvate carboxykinase in rat liver (43-45) is stimulated by the pancreatic hormone and perhaps the other gluconeogenetic enzymes as well – glucose-6-phosphatase and pyruvate carboxylase (46,47). This hormone enhances ^{14}C-lactate incorporation in plasma glucose (40) and increase incorporation of [3-^{14}C]-pyruvate into hepatic glycogen (42) in rats.

Also, glucagon enhances uptake of amino acids in liver (48) and glycogen has been shown to appear after a protein meal (49). Thus, glucogenic amino acids give rise to glucose synthesis. In the face of adequate supply of dietary proteins, the glucose formed from amino acids is stored as glycogen. In iron-deficiency, antibody production (50,51) is reduced resulting in increased susceptibility to infection (52). This together with the retardation of growth (Fig. I) observed in rodent iron-deficiency anemia may suggest impaired utilization of amino acids for protein synthesis. Thus, it is possible that glucogenic amino acids are channeled to glucose synthesis along with glucose formation from trioses which may stimulate the rate of synthesis of glycogen which in turn will dilute the ^{14}C-glucose content of hepatic glycogen to account for the statistically very low specific activity of liver glycogen (Table VIII) in the anemic rats. This can be examined experimentally by injecting isotopically labeled amino acids like alanine, serine, threonine and glycine since these have been shown to give rise to significant amounts of glucose in perfused liver (53).

Although anemic rats consistently had higher glycogen reserves comparatively, the glycogen stores apparently diminished from 2 hours to 6 hours in all groups of animals if data from Table V is compared with data in Table VI implicating glycogenolysis. Considering that hepatic glycogen stores in anemic rats significantly differed from those of pair-fed and control rats at 2 hours (Table V) but did not differ statistically from those of control rats at 6 hours after glucose dosing (Table VI) suggests that glycogenolysis appears to be increased in iron-deficiency. Enhanced hepatic glycogenolysis may actually be due primarily to increased levels of glucagon (54). In liver, glucagon activates adenyl cyclase which leads to increase in cyclic AMP levels (55). Cyclic -AMP activates the enzyme dephosphophosphorylase kinase that results in an increase of hepatic phosphorylase, the rate limiting enzyme in glycogen degradation. Activation of glycogen phosphorylase obviously results in rapid glycogenolysis and therefore, hepatic output of glucose. Enhanced glycogenolysis in iron-deficient livers would indeed necessitate intense glucose formation from both trioses and amino acids to explain the significantly low specific activity of liver glycogen as caused by dilution of label from these endogenous sources. Since liver glycogenolysis is primarily controlled by glucagon, measurement of blood glucagon levels during severe iron-deficiency anemia may be of interest.

In diabetic rats, hepatic glycogen content is markedly decreased from 15% to 25% of normal (56,57). However, upon insulin therapy, liver glycogen abnormally accumulates to 300% of normal levels within 24 hours (58,59). Insulin presumably allows entry of glucose into cells – mainly, hepatic cells, for storage into glycogen thereby preventing glucose accumulation in blood characteristic of diabetes. Therefore, the comparable rates of ^{14}C-glucose incorporation into liver glycogen in all groups of rats indicate normal insulin secretion in iron-deficiency inferred previously from the shape of the glucose tolerance curves. Thus, in spite of adequate insulin secretion, high glucose levels in blood persists which may very well be due to intense gluconeogenesis together with enhanced

hepatic glycogenolysis that continuously pour glucose into the circulation.

The lack of iron by the respiratory enzymes in the cytochrome system may depress their function thereby preventing adequate oxidation which may lead to slower removal of glucose from the circulation. This may further contribute to the abnormally high glucose levels in blood observed during iron-deficiency anemia in rats. However, comparison of the rate of glucose oxidation between groups showed that the oxidation in anemic rats is about 7.1% higher than the pair-fed group and about 7.9% higher than the control group (Fig. 4). Apparently, nutritional iron-deficiency anemia promotes utilization of glucose for energy. The enhanced rate of glucose oxidation indicates that the function of the enzymes of the respiratory chain remains relatively unaltered. This finding in the present study agrees with reports that the enzyme activities when tested *in vitro* remain normal during iron-deficiency (56). However, other *in vitro* studies have reported decreased activity (7,8). It is obvious that compensatory mechanisms are operative to enhance oxidation of ^{14}C-glucose to $^{14}CO_2 + H_2O$.

Compensatory mechanisms are indeed known to operate in iron-deficiency anemia. The physiological compensatory signs are increased cardiac output and rate (60). Clements (61) reported that cardiac output apparently increases only at hemoglobin levels below 7 or 8 grams/100 ml. The biochemical compensatory mechanisms appear to be at the red cell level where the concentration of 2,3-diphosphoglycerate (DPG) has been shown consistently to increase markedly in anemia from 16.45 um/gram hemoglobin for normal patients to 27.35 um/gram hemoglobin for the nutritionally iron-deficient patient (60). Compensatory mechanisms appear to efficiently aid oxidation such that enhanced supply of both glucose and oxygen to various peripheral tissues for oxidation is ensured. The relatively faster ^{14}C-glucose breakdown to $^{14}CO_2$ suggests its adequate removal from circulation. However, its rate removal may may not be fast enough so that hyperglycemia persists.

Macdougall (4) reported increased glucose uptake by red blood cells in iron-deficiency with marked increased in the enzymes of the Embden-Meyerhoff and hexose monophosphate pathways. Increased red cell glycolysis due to levels of deoxygenated hemoglobin that raises the pH (10) elevates 2,3 -DPG levels in iron-deficiency anemia which have been repeatedly shown to shift the hemoglobin-oxygen dissociation curve to the right (11,60,62) to favor release of oxygen from hemoglobin to tissues for oxidation. Benesch and Benesch (11,63) have previously reported that dialyzed hemoglobin has high affinity for oxygen and that 2,3 -DPG reduces the affinity of oxygen to purified hemoglobin, which were also shown by Chanutin and Curnish (10). This increases the oxygen unloading potential which affords anemic patients to extract more oxygen from circulating hemoglobin (14) aided further by the increased cardiac output and rate (60).

Although anemia should suggest inadequate oxygen delivery to tissues for oxidation because of the low hemoglobin circulating, it appears not to be the

case as far as data on the $^{14}CO_2$ output are concerned. Moreover, it is a biochemical impression that the activities of the respiratory enzymes as well as aconitase in the Kreb's cycle must be depressed since some of them are either iron containing or requiring for optimal function. However, mechanisms are resorted to by the system to prevent serious consequences. It appears that during iron-deficiency anemia, the increased cardiac output and rate would necessarily increase delivery of substrate, e.g., glucose to tissues while increased levels of 2,3-DPG in the red blood cells during this condition of increased rate of circulation ensures enough oxygen supply and release to tissues for oxidation.

Although the data tend to demonstrate an "overcompensation", it could just be more apparent than real considering that the level of the circulating hemoglobin was too low (severe) in the first place. Had it been possible to administer ^{14}C-glucose dose in terms of per unit square cm. of body surface (64) rather than on body weight basis, oxidation with compensation in anemia may "trim-down" to normal or little below normal $^{14}CO_2$ output.

Should the increased rate of glycolysis following glucose uptake operate in other cells as well, such as hepatic cells, this may bring about increased levels of other metabolites. Increased glycolysis may generate substantial amounts of dihydroxyacetone phosphate which may stimulate the synthesis of triglycerides. Although fatty livers are never observed in iron-deficiency anemia triglyceridemia is frequently noted (65,68). However, Guthrie and others (69) found that the level of lipoprotein lipase is decreased in rodent iron-deficiency anemia and this may possibly explain the significantly high triglyceride levels in the blood.

Acknowledgements – This study was supported by NSDB-PHCA grant no. Md 7502, UP-NSDB Project 2.28.1 and F.N.R.I. Grateful acknowledgments are made to all the people who in one way or another extended cooperation.

REFERENCES

1. Bothwell, T. *Br. J. Haematol. 14:* 453 (1968).
2. Forth, W. et al. *Physiol. Rev. 53:* 724-792 (1973).
3. Van Campen, D. *Fed. Proc. 33:* 100-105 (1974).
4. Macdougall, Y. G. *J. Pediat. 72:* 303 (1968).
5. Dallman, P. and J. Goodman. *J. Cell Biol. 48:* 79-90 (1971).
6. Goodman, J., J. Warshaw and P. Dallman. *Pediat. Res. 4:* 244-256 (1970).
7. Salmon, H. A. *J. Physiol. 164:* 17(1962).
8. Jacobs, A. *Br. J. Haematol. 28*(1): 140 (1974).

9. Beutler, E. *Am. J. Med Sci. 234:* 517 (1957).

10. Chanutin, A. and R. Curnisch. *Arch. Biochem. Biophy. 121:* 96 (1967).

11. Benesch, R. and R. E. Benesch. *Fed. Proc. 26:* 673 (1967).

12. Bellingham, A. and A. Grimes. *Br. J. Haematol. 25*(5): 555-562 (1973).

13. Valeri, C. and N. Fortier. *New Engl. J. Med. 281:* 1452 (1969).

14. Pollock, A. and K. Cotter. *Br. J. Haematol. 25:* 631-636 (1973).

15. Quiogue, E., et al. *Phil. J. Nutr. 22*(2):61-78 (1969).

16. Food Consumption Survey in Luzon. FNRI-NSDB, Manila, Phil., 1974.

17. Food Consumption Survey in Visayas. FNRI-NSDB, Manila, Phil., 1975.

18. Bulatao-Jayme, J. et al. Unpublished nutrition survey FNRI-NSDB, Manila, Phil., 1975.

19. Bailey, K. V. *Phil. J. Nutr. 25*(2): 161 (1972).

20. World Health Organization (WHO). Nutritional anemias. Technical Report Series No. 405 (1968).

21. *Nutr. Rev. 34*(10): 298-300 (1976).

22. Bailey-Wood, R., et al. *Br. J. Expt. Pathol. 56*(3): 193-198 (1975).

23. Hain, A. Hemoglobin. In: Standard Method of Clinical Chemistry Vol. II, pp. 49-60, Academic Press, New York, 1958.

24. Cannan, R. K. *Science 127:* 1376-1378 (1958).

25. D' Amour, F. and F. Blood. *Manual for laboratory Work in Mammalian Physiology.* The University of Chicago Press, Chicago. Ill., 1953.

26. Nelson, *J. Biol. Chem. 153:* 375 (1944).

27. Somogyi, *J. Biol. Chem. 160:* 62 (1945).

28. Benedict, *J. Am. Med. Assoc. 57:* 1193 (1911).

29. Experiments in Biochemistry: Basic Concepts and Selected Techniques, Dept. of Biochemistry, College of Medicine, University of the Philippines, 1973.

30. Clark, John, Jr., ed. *Experimental Biochemistry.* W. H. Freeman and Co. San Francisco and London, 1964.

31. Seifter, S., S. Dayton, B. Novic and E. Muntwryler. *Arch. Biochem. 25*(1): 191 (1950).

32. Pfluger, E. W. Das Glykogen., p. 53 Bonn, Germany, (1905).

33. Jermyn, M. A. *Anal. Biochem. 68:* 332-335 (1975).

34. Cowgill and Pardee. Biochemical Research Techniques. John Wiley and Sons. Inc., New York. 1957.

35. Ritschel, Wolfgang, A. *Handbook of Pharmakokinetics,* Chapter XVII: Determination of Rate Constants, pp. 185-188. Drug Intelligence Publications, Inc., 1976. Printed by: Hamilton Press Inc. Hamilton, Illinois 62341, U.S.A.

36. Steel, G. and J. Torrie. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., N.Y. 107-114, 1960.

37. Kimmich, G. *Biochem. 9*(19): 3669-3677, 1970.

38. Sparks, J., A. Lynch and W. Glinsman. *Metabolism 25*(1): 47-55 (1976).

39. Pines, M., N. Bashan and S. Moses. *Isrl. J. Med. Sci. 12*(1): 80-81 (1976).

40. Snell, K and D. Walker. *Biochem, J. 134*(4): 899-906 (1973).

41. Philippidis, H. and F. Ballard. *Biochem. J. 120:* 385-392 (1970).

42. Exton, J., L. Jefferson, R. Butcher and C. Park. *Am. J. Med. 40:* 709 (1966).

43. Garcia, A., R. Williamson and G. Cahill. *Diabetes 15:* 188 (1969).

44. Hanson, R., L. Reshef and F. Ballard. *Fed. Proc. 34:* 166 (1975).

45. Jost, J. and H. Rickenberg. Cyclic AMP. *Ann. Rev. Biochem. 40:* 741 (1976).

46. Ballard, F. and R. Hanson. *Biochem. J. 104:* 866-877 (1967).

47. Yeung, D., R. Stanley and I. Oliver. *Biochem. J. 105:* 1219-1227 (1967).

48. Marliss, E., T. Aoki, R. Unger, J. Soelduer and G. Cahill. *J. Clin. Invest. 49:* 2256 (1976).

49. Elwyn, D., H. Parikh and W. Shoemaker. *Amer. J. Physiol. 215:* 1260 (1968).

50. Baggs, R. and S. Miller. *J. Nutr. 103*(11): 1554-1560 (1973).

51. Nalder, B., A. Mahoney, R. Ramakrishnan and D. Hendricks. *J. Nutr. 102:* 535-542 (1972).

52. Chandra, R. *Nutr. Rev. 34*(5): 129-132 (1976).

53. Exton, J. H. *Metabolism 21:* 945-990 (1972).

54. Unger, R., E. Aguilar-Parada, W. Muller and A. Eisentraut. *Clin. Invest. 49:* 837 (1970).

55. Jefferson, L., J. Exton, R. Butcher, E. Sutherland and C. Park. *J. Biol. Chem. 243:* 1031 (1968).

56. Renold, A., A. Hastings and F. Nesbett. *J. Biol. Chem. 209:* 687 (1954).

57. Anderson, J. W. *Am. J. Clin. Nutr. 27*(7): 746-755 (1974).

58. Steiner, D. and K. King. *J. Biol. Chem. 239:* 1292 (1964).

59. Spiro, R., J. Asharnore and A. Hastings. *J. Biol. Chem. 230:* 761 (1958).

60. Lovric, V., P. Beal and A. Lammi. *J. Pediat. 86*(2): 194-197 (1975).

61. Clements, F. W. *Med. J. Aust. 1*(21): 654-656 (1975).

62. Oski, F. A. *J. Pediat. 83:* 353 (1973).

63. Benesch, R. and R. E. Benesch. *Biochem. Biophys. Res. Commun. 26:* 162 (1967).

64. Oser, L. B. (ed): Hawk's Physiological Chemistry. Chapter on energy metabolism. McGraw-Hill Book Co., New York, 1955.

65. Boggs, T. and R. Morris. *J. Exp. Med. 11:* 553 (1909).

66. Lewis, M. and R. Iammarino. *J. Lab. Clin. Med. 78:* 546 (1971).

67. Amine, E. and D. Hegsted. *J. Nutr. 101:* 1575 (1971).

68. Nosanchuck, J., M. Roark and C. Wanser. *Am. J. Clin. Pathol. 62:* 838-839 (1974).

69. Guthrie, H., et al. *J. Nutr. 104:* 1273 (1974).