

# Carbohydrate Metabolism in Ascorbic Acid Deficiency

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SEVERAL studies have demonstrated that there exists a disturbance of carbohydrate metabolism in ascorbic acid deficiency and in scurvy (1-3). Banerjee *et al.* (2, 3) have shown that the diminished glucose tolerance of scorbutic guinea pigs is probably not related to the increased epinephrine content of the adrenals of such animals. The diminished glucose tolerance was ascribed to the decrease in the insulin content of the pancreatic tissue occurring in such animals (2, 4). Several other workers have also shown that a diminished glucose tolerance occurs in scurvy (5, 6). On this basis it has been suggested also that adrenal cortical hyperactivity may be responsible for the disturbance in carbohydrate metabolism in scurvy (5, 7).

This paper reports data suggesting that the disturbance in carbohydrate metabolism in ascorbic acid deficiency is probably independent of the insulin deficiency, and of adrenal hyperactivity.

## MATERIALS AND METHODS

Insulin tolerance and glucose-insulin tolerance tests were conducted on ascorbic acid-deficient, and in paired control, guinea pigs. The dietary fares used here have been discussed in a previous paper (8). All animals were fed with Purina Rabbit Chow and tap water. In all experiments the animals were divided into two groups, *A* and *B*. *Group A* animals were supplied no green vegetables or other sources of ascorbic acid. *Group B* guinea pigs received in addition to the Chow and water, liberal amounts of fresh cabbage or lettuce. The Purina Rabbit Chow diet, unsupplemented by sources of ascorbic acid, is known to be highly scorbutigenic in guinea pigs (8, 6). The ad libitum supply of greens was found to be as successful as the daily injection of ascorbate employed earlier (8). The food intake of the control group was regulated on the basis of the intake of the deficient group.

In all cases blood for glucose determinations was obtained in 0.1-ml amounts from the toes of the animals while they were under Nembutal anesthesia. The method has been described previously (9). Blood glucose levels were determined by the micro-method of Folin and Malmros (10).

## EXPERIMENTS AND RESULTS

**Experiment 1.** The first experiment was conducted on ascorbic acid-deficient, and control, intact female guinea pigs weighing 250-300 gm at the beginning of the experiment. Twelve animals were used for insulin tolerance tests at the beginning of the experiment. Following these tests one group of six animals (*group A*) was placed on the scorbutigenic diet (i.e., no greens), while the remaining six animals (*group B*) were placed on the complete diet. Insulin tolerance tests were again conducted on both groups on the 17th, and on the 24th days on the dietary fares. The insulin tolerance test was conducted as follows. A baseline (0 hr.) blood glucose level was determined following an 18-hour fast, then each animal was given a single subcutaneous injection of regular insulin (Lilly, Iletin) at the dose of 0.1 U/100 gm body weight. This amount of insulin is 10 times that used in the conventional tolerance test. The reason for the employment of such a high dose will be discussed later. Following the injection of insulin blood samples were taken at hourly intervals for 4 hours. The blood glucose levels at these instances were compared to the baseline (0 hr.) levels, and the percentage decrease  $\pm$  S.E. were calculated. The data from this experiment are presented in figure 1 in which the mean percentage decrease  $\pm$  S.E. are presented. Statistical analysis involved the comparison of the changes (from 0 hr.) in the groups. Since the responses of the control animals in the various tests were similar to those of the entire group of 12 animals at the beginning of the experiment, the control curve was constructed as a composite of these responses. The data indicate that the hypoglycemic response in the guinea pigs on the deficient diet for 24 days is significantly less at the first and second hours of the test than that of the control group at these instants. In addition the maximum

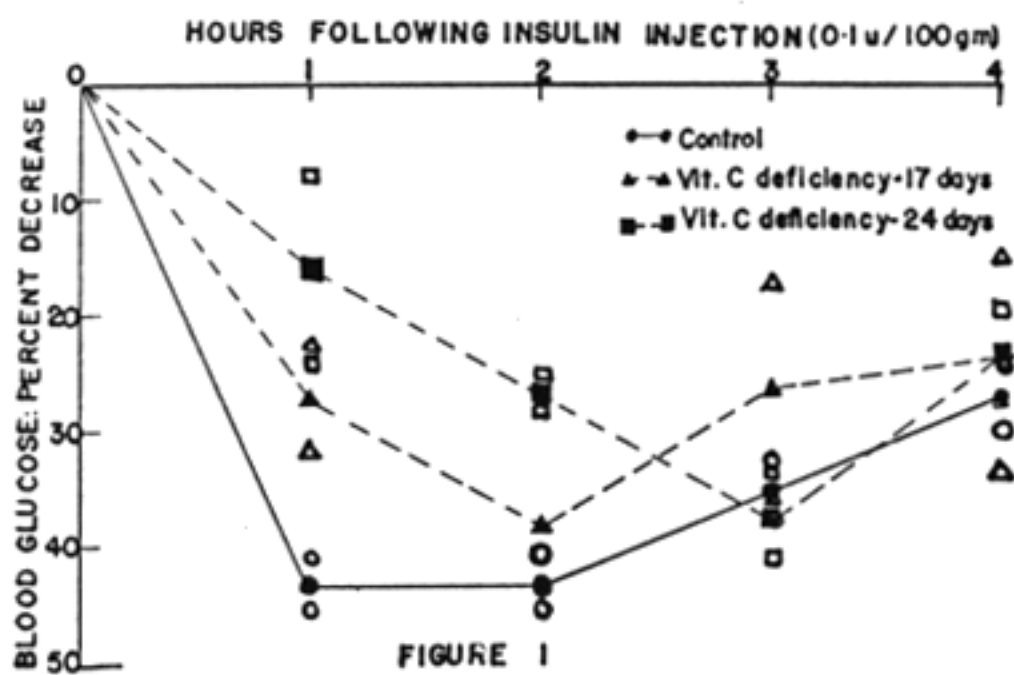


FIG. 1. Insulin tolerance tests in ascorbic acid-deficient and control female guinea pigs. Mean percentage decreases of blood glucose from fasting level at 0 hr. are presented. Control curve is a composite of the responses of all animals before submission to the dietary fares, and of the responses of the control (*group B*) animals on the 17th and 24th days. Six animals in each group. Open symbols indicate standard errors of mean percentage changes.

hypoglycemia is delayed by 1–2 hours in the deficient group. The animals on the deficient diet for 17 days show a similar pattern as those on the diet for 24 days, but the effect is not as marked. The data indicate that the ascorbic acid-deficient guinea pig exhibits a marked resistance to insulin, even in these high doses. It may be noted that, in agreement with Banerjee *et al.*, we have not observed any significant difference between the fasting blood glucose level of the control animal, and that of the deficient animal (3).

**Experiment 2.** Glucose-insulin tolerance tests were conducted on two similar groups of animals as above. Each group consisted of six guinea pigs. The tests were conducted on the 8–9th days of the dietary fares. The test was conducted as follows. The baseline (0 hr.) blood glucose level of each animal following an 18-hour fast was determined. Following this each animal was given 80 mg of glucose/100 gm body weight by stomach tube. The glucose solution contained 80 mg/1.0 ml. The animals were immediately given single subcutaneous injections of regular insulin at the dose of 0.01 U/100 gm. Blood glucose levels were determined at 30, 60, 90, 120, 180, and occasionally, 240 minutes, after the administration of glucose and insulin. The data are presented in figure 2 in which the mean percentage change of blood glucose from baseline is presented. It is seen that the blood glucose level of the control group does

not significantly or markedly differ from the level at 0 hr. The blood glucose levels in the deficient group, on the other hand, are significantly greater than the baseline levels throughout the 3 hours of observations. The data suggest that the guinea pig on the ascorbic acid-deficient diet is relatively more resistant to the administered insulin than is the control animal.

**Experiment 2a.** Three animals of *group A* and three of *group B*, were used for glucose-insulin tolerance tests on the 11–12th day of the dietary fares. In this experiment the glucose was given *per os* as above, but the insulin (0.01 U/100 gm) was given by intravenous injection. This experiment was conducted in order to rule out any possible differences in subcutaneous absorption being responsible for the differences in the responses observed in the two previous experiments. It was not necessary to give the glucose by the intravenous route. It has been observed that the ascorbic acid-deficient animal may absorb glucose at a slower rate than normal (5, 6). This factor did not assume great importance here since the blood glucose levels of the deficient group were resistant to insulin (i.e., higher than in the control group at the 30-minute intervals). In these experiments, also, we failed to observe any rise in the fasting glucose level in the deficient animals. The data are presented in figure 2. A similar pattern as in *experiment 2* is observed.

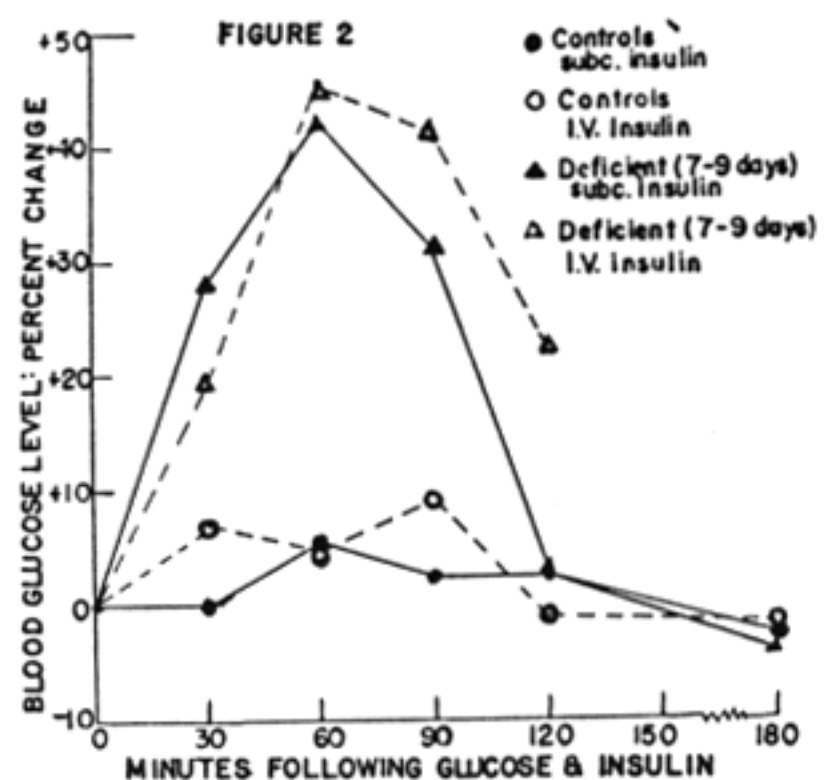


FIG. 2. Glucose-insulin tolerance tests in ascorbic acid-deficient and control female guinea pigs. Mean percentage changes of blood glucose levels from fasting levels at 0 hr. are presented. Each animal was given 80 mg glucose/100 gm *per os*, together with 0.01 U insulin/100 gm by subcutaneous or intravenous injection.

**Experiment 3.** Glucose-insulin tolerance tests were conducted on ascorbic acid-deficient, and control, adrenalectomized guinea pigs. The preparation of adrenalectomized animals for this study was particularly difficult. The animals were bilaterally adrenalectomized under Nembutal anesthesia. The lumbar approach was employed. In several cases, following successful operative procedures, the animals died by the 4-5th day postoperatively, despite supportive therapy with 0.9% NaCl and small amounts of cortisone acetate. Three adrenalectomized guinea pigs on the complete diet with 0.9% NaCl, and three on the deficient diet (with 0.9% NaCl) were finally used in the experiment. Two of the latter group were adrenalectomized prior to exposure to the deficient diet, the third was adrenalectomized on the 7th day on the deficient fare, and used on the 9th day. The glucose-insulin tolerance tests were conducted on all animals on the 9th day on the dietary fares. Glucose was given as in *experiment 2*, and insulin was given by subcutaneous injection. The control curve consisted of the observations on the three control animals on the ninth day on the dietary fare, and on one of the experimental (*group A*) animals before submission to the deficient diet. The data are presented in figure 3. It is seen that the blood-glucose level of the animals on the deficient diet is significantly higher than that of the control group up to the 4th hour of observations. These data are similar to those derived from intact animals (i.e., non-adrenalectomized).

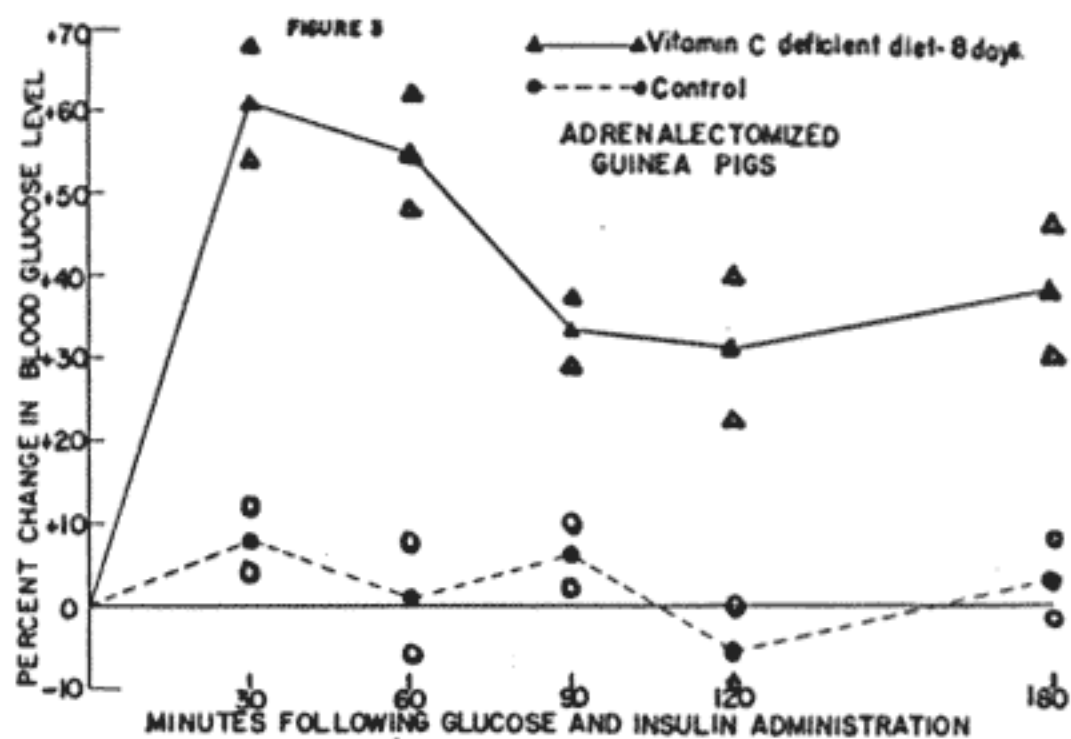


FIG. 3. Glucose-insulin tolerance tests in ascorbic acid-deficient and pair-fed control adrenalectomized female guinea pigs. Mean percentage changes of blood glucose levels are indicated by block symbols, with the standard errors indicated by the open symbols. Insulin and glucose dosage as in fig. 2.

**Experiment 4.** Two animals on the deficient diet, and two on the control diet were employed for the carbohydrate 'utilization' procedure on the 10th day of the dietary fare. The body weights of both groups of animals were similar at this stage of the dietary fares. A baseline blood glucose level was taken following an 18-hour fast. Each animal was then given 2,400 mg of glucose in a 35% solution by stomach tube, and immediately afterwards was given 0.30 U of insulin by subcutaneous injection. All animals were anesthetized with Nembutal  $3\frac{1}{2}$ -4 hours after the above treatments. Blood was obtained for glucose determinations, and following laparotomy the entire liver of each animal was plunged into hot 30% KOH in a tared Erlenmeyer flask. After complete hydrolysis and cooling, the flasks were weighed, and the weight of the tissue ascertained. This is essentially the method employed by Cori and Cori (11). The entire carcass was plunged into hot 50% KOH. At the completion of hydrolysis aliquots of the cooled liver extract and of the cooled carcass extract were employed for glycogen determinations after the method of Good, Kramer, and Somogyi (12). The data are presented in table 1. It is seen that the liver and body glycogen values of the control animals are significantly greater than those of the animals on the deficient diet ( $P < 0.05$ ). The differences in the blood glucose responses were not marked. This experiment confirms the data of Murray (6) who concluded that insulin fails to correct the defect in glycogenesis in ascorbic acid-deficient animals. That author employed very large doses of insulin, sufficient to increase glucose oxidation (11), hence the difference in the body glycogen values was not as apparent as in the present study.

#### DISCUSSION

The first experiment demonstrated that the ascorbic acid-deficient guinea pig is more resistant to the hypoglycemic action of insulin than is the pair-fed control partner. Similar data have been presented by Stewart *et al.* (7) in monkeys. Those authors employed one-tenth of the dose of insulin used in the present study. It was thought desirable to determine the extent of resistance exerted by the deficient animal to massive doses of insulin. The diminished glucose tolerance of scorbutic

guinea pigs was ascribed by Banerjee *et al.* (3) to the diminished insulin content of the pancreas of such animals. The observation that the deficient animal is relatively resistant to both small, and large doses of insulin suggests that the diminished glucose tolerance in ascorbic acid deficiency is probably ascribable to some factor other than a diminished insulin content of the pancreas.

In addition to the above reasons, the data from *experiments 2* and *3* in the present study may be cited as suggestive evidence that certain aspects of the carbohydrate disturbance in ascorbic acid-deficiency are not directly related to the decreased insulin content of the pancreas. In the glucose-insulin tolerance test a measured amount of glucose is given to the animal. The test is so arranged that the insulin administered balances the glucose, and hence, in the absence of a disturbance in carbohydrate metabolism, the blood glucose level should remain approximately similar to the baseline (13). This was observed in all animals on the control diet; intact and adrenalectomized responded similarly. The fact that the administered insulin failed to prevent the hyperglycemia following the infusion of glucose suggests that the ascorbic acid-deficient animal is resistant to insulin. It is unlikely, therefore, that the disturbance in carbohydrate metabolism in ascorbic acid-deficiency is solely ascribable to a decreased insulin content of the pancreas.

On the basis of similar observations on the insulin resistance of ascorbic acid-deficient animals, Stewart *et al.* (7) have ascribed the disturbance of carbohydrate metabolism to adrenal cortical hyperactivity. Such a conclusion has apparently been warranted by the data of several past studies. The data of the present study, and of Stewart *et al.* (7) clearly suggest that adrenal cortical hyperactivity is not responsible for the disturbance in ascorbic acid deficiency. Stewart *et al.* (7) observed their single scorbutic-adrenalectomized monkey to be insulin-resistant in comparison to the adrenalectomized animal on the complete diet. This insulin resistance in the animal was ascribed to 'adaptability'—independent of the adrenal cortex. In the present study the insulin resistance observed in the groups of intact (non-adrenalectomized) ascorbic acid-deficient animals was also observed in our

TABLE I. LIVER AND CARCASS GLYCOGEN CONTENTS IN ASCORBIC ACID-DEFICIENT AND CONTROL GUINEA PIGS GIVEN GLUCOSE AND INSULIN

Body Wt., gm	Blood Glucose, mg/100 ml	Liver Glycogen, gm/100 gm	Carcass Glycogen, gm/100 gm
<i>Ascorbic acid-deficient—10 days</i>			
310	260	2.76 ± 1.47* (3)†	0.247 ± 0.054 (97)
<i>Pair-fed controls</i>			
305	230	25.10 ± 5.50 (6)	0.586 ± 0.030 (94)
		<i>P</i> < 0.05	<i>P</i> < 0.05

\* Standard Error =  $\sqrt{\sum(x - \bar{x})^2/n(n-1)}$ . † Figures in parenthesis indicate complement of glycogen in the organ as percentage of total body glycogen.

group of adrenalectomized animals on the deficient diet. In view of the above consideration the insulin resistance of the deficient animals cannot be reasonably ascribed to adrenal cortical hyperfunction (see ADDENDUM).

The nature of the disturbance in carbohydrate metabolism responsible for the observed insulin resistance in ascorbic acid deficiency cannot be completely ascertained from our data. Convincing data have been provided by other workers that glycogenesis may be diminished in ascorbic acid-deficiency (5, 6). This diminution of glycogenesis is probably not due to insulin deficiency as indicated by the data of Murray (6), and of the present study. Murray (6) provided data indicating that the diminished glycogenesis is not secondary to an increased aerobic oxidation in such animals. It has been shown that the absorption of glucose by the deficient guinea pig is less than normal. That this diminished absorption is not responsible for the decreased deposition of glycogen has been proved (5, 6). It may be noted also, that by 4 hours after the administration of glucose, there is almost complete absorption in both groups.

In addition to the diminution in glycogenesis discussed above, it is also apparent that the gluconeogenic process is disturbed in ascorbic acid deficiency. Murray (6) and McKee *et al.* (14) demonstrated that the gluconeogenic potency of exogenous cortical hormones was diminished in ascorbic acid-deficient animals. In our own studies it was observed that the gluconeogenic potency of exogenous cortical hormone is enhanced in the presence of ascorbic acid excess (15). Whether these processes are related cannot be ascertained at present.

## SUMMARY

The nature of the disturbance of carbohydrate metabolism in ascorbic acid deficiency was studied in guinea pigs. Insulin tolerance tests on ascorbic acid-deficient, and in control guinea pigs indicated that a progressive resistance to the hypoglycemic action of insulin accompanies ascorbic acid deficiency. Glucose-insulin tolerance tests confirmed the above findings. Glucose-insulin tolerance tests were conducted in ascorbic acid-deficient, and control, adrenalectomized guinea pigs in order to ascertain whether the disturbance is ascribable to adrenal hyperactivity. The data demonstrate that the ascorbic acid-deficient adrenalectomized, like the intact (i.e., non-adrenalectomized) deficient guinea pig, is resistant to the hypoglycemic action of insulin. The carbohydrate-utilization technique of Cori and Cori (11) was employed in a small series of animals. The data indicated that glycogenesis is disturbed in ascorbic acid deficiency.

The data are interpreted to indicate that the disturbance of carbohydrate metabolism occurring in ascorbic acid deficiency is *a*) probably not due to diminished insulin secretion, since exogenous insulin failed to correct the disturbance, *b*) is probably not ascribable to adrenal hyperactivity since the insulin resistance is observed even in ascorbic acid-deficient adrenalectomized guinea pigs. Whether there is an associated diminution of glycolysis occurring in ascorbic acid deficiency remains to be ascertained.

## ADDENDUM

Stewart *et al.* (7) support their suggestion of increased adrenal cortical hormones in scurvy by reference to their observation of an 'increased' excretion of 17-ketosteroids (17-KS) in scorbutic monkeys (16). The following points should be considered in evaluating the results. *a*) Those workers compared the urinary excretion of 17-KS—mg/kg of body weight per day, of normal and scorbutic monkeys. The pair-feeding technique was not employed, and the authors reported weight loss and wasting in the animals on the scorbutigenic diet. Since the 'control' animals did not suffer similar weight changes, the urinary excretion of 17-KS/kg of body weight does not constitute a valid control for the animals on the ascorbic acid-deficient diet which weighed less than the normal controls. *b*) The urinary 17-KS may not be a completely reliable index of adrenal cortical activity, especially in the absence of additional

data. We have obtained data (17, 18) indicating that liver tissues of ascorbic acid-deficient animals metabolize cortical hormones to 17-KS and other metabolites at an increased rate. In addition, other studies (8, 19, 20) indicated that in late ascorbic acid deficiency there occurs a decreased urinary 17-KS excretion. These data are in agreement with our observations (8) that in late ascorbic acid deficiency there is a decreased excretion of corticosteroids and of 17-KS. In early deficiency (3–5 days in the guinea pig), on the other hand, there was observed an increase in the 17-KS excretion associated with a diminished corticosteroid excretion. Our data suggest that in ascorbic acid deficiency there occurs an increase in the breakdown of cortical hormones to 17-KS, and also, that in late deficiency there occurs a diminished production of corticosteroids (8). Stepto *et al.* (21) demonstrated morphological evidence of adrenal damage and hypofunction in ascorbic acid deficiency and scurvy. These data are in agreement with our observations of diminished adrenal and plasma contents of 17-hydroxycorticosteroids in ascorbic acid-deficient animals (18).

## REFERENCES

1. SIGAL, A. AND C. G. KING. *J. Biol. Chem.* 116: 489, 1936.
2. BANERJEE, S. *J. Biol. Chem.* 166: 25, 1946.
3. BANERJEE, S. AND N. C. GHOSH. *J. Biol. Chem.* 207, 1949.
4. BANERJEE, S. *Nature* 152: 329, 1943.
5. MURRAY, H. C. AND A. F. MORGAN. *J. Biol. Chem.* 163: 401, 1946.
6. MURRAY, H. C. *Proc. Soc. Exper. Biol. & Med.* 69: 351, 1948.
7. STEWART, C. T., R. J. SALMON AND C. D. MAY. *Am. J. Dis Child.* 84: 677, 1952.
8. BACCHUS, H. AND M. H. HEIFFER. *Am. J. Physiol.* 174: 243, 1953.
9. BACCHUS, H. AND M. H. HEIFFER. *Endocrinology* 51: 94, 1952.
10. FOLIN, O. AND H. MALMROS. *J. Biol. Chem.* 83: 115, 1929.
11. CORI, C. F. AND G. T. CORI. *J. Biol. Chem.* 60: 557, 1926.
12. GOOD, C. A., H. KRAMER AND M. SOMOGYI. *J. Biol. Chem.* 100: 485, 1933.
13. FRASER, P., F. ALBRIGHT AND P. SMITH. *J. Clin. Endocrinol.* 1: 297, 1941.
14. MCKEE, R. W., T. S. COBBEY AND Q. GEIMAN. *Endocrinology* 45: 21, 1949.
15. BACCHUS, H. AND M. H. HEIFFER. *Am. J. Physiol.* 172: 276, 1953.
16. MAY, C. D., R. J. SALMON AND C. T. STEWART. *J. Lab. & Clin. Med.* 40: 657, 1952.
17. BACCHUS, H. *Endocrinology* 53: 441, 1953.
18. BACCHUS, H. AND M. H. HEIFFER. *Abstr. XIX Int. Physiol. Congr.* p. 182, 1953.
19. BANERJEE, S. AND C. DEB. *J. Biol. Chem.* 195: 575, 1952.
20. TARANTINO, C. *Folia endocrinol.* iii: 49, 1950.
21. STEPTO, R. C., C. L. PIRANI, C. F. CONSOLAZIO AND H. BELL. *Endocrinology* 49: 755, 1951.