during 10 minutes of centrifugation at 3000 R.P.M. with a starting temperature of 40°C.

2. Batches of eggs from different worms showed different hatching characteristics depending on the treatment strength and time, and influenced by subsequent procedure.

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Effect of Insulin, Adrenal Cortical Hormones, Salt and dl-Alanine on Carbohydrate Metabolism in Scurvy.*

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Previous work[1,2] has shown that scorbutic guinea pigs display a disturbance of carbohydrate metabolism resulting in lowered glycogen stores in liver and body. Murray and Morgan[1] demonstrated that when glucose was fed the scorbutic animal failed to absorb it as readily as the normal animal. The blood sugar after this procedure was also abnormally high. Sigal and King[3] had previously demonstrated a lowered glucose tolerance in scorbutic animals.

Banerjee[4] has demonstrated in scorbutic guinea pigs an increase in adrenaline production. He attributed the disturbance in carbohydrate metabolism to an imbalance between adrenaline and insulin since the insulin content of the pancreas appeared to be markedly decreased in the scorbutic animal.[5]

Involvement of the adrenal gland in the changes incident to ascorbic acid deficiency has been suggested. Marked hypertrophy of this gland has been shown to accompany this deficiency. Sayers, Sayers, Liang and Long[6] found that the injection of adrenotrophic hormone lowered both the cholesterol and ascorbic acid content of the adrenals of rats and guinea pigs suggesting that these 2 compounds are involved in the synthesis of cortical hormones. This hormone also greatly increased the deposition of liver glycogen in both species.

Intestinal absorption has been shown to be impaired in adrenalectomized rats[7,8] and the improvement of this condition demonstrated by adding sodium chloride to the drinking water. There was a possibility that the decrease in absorption in the scorbutic animal could be improved by adding this compound to the drinking water.

MacKay and co-workers[9,10] have shown that dl-alanine when fed to fasting mice or rats results in the formation of a considerable amount of hepatic glycogen. The possibility existed therefore, that the scorbutic animal could convert compounds of 3 C atoms to glycogen normally, and this might be some index of the adrenal cortical function.

This study was undertaken to determine the effect of insulin, adrenal cortical extract and additions of sodium chloride in the

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1 Murray, H. C., and Morgan, A. F., *J. Biol. Chem.*, 1946, **163**, 401.

2 Hamne, B., *Acta Paediat.*, 1941, **28** Suppl.

3 Sigal, A., and King, C. G., *J. Biol. Chem.*, 1936, **116**, 489.

4 Banerjee, S., *J. Biol. Chem.*, 1945, **159**, 327.

5 Banerjee, S., and Ghosh, N. C., *J. Biol. Chem.*, 1947, **168**, 207.

6 Sayers, G., Sayers, M. A., Liang, T. Y., and Long, C. N. H., *Endocrinology*, 1946, **38**, 1.

7 Clark, W. G., and MacKay, E. M., *Am. J. Physiol.*, 1942, **137**, 104.

8 Anderson, E., Joseph, M., and Herring, V. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 477.

9 MacKay, E. M., Wick, A. N., and Carne, H. O., *J. Biol. Chem.*, 1940, **132**, 613.

10 MacKay, E. M., Wick, A. N., and Barnum, C. P., *J. Biol. Chem.*, 1941, **137**, 183.

drinking water on glucose absorption, blood sugar levels, glycogen content of liver and body in scorbutic guinea pigs. The effect of adrenaline on blood sugar, body and liver glycogen and the conversion of dl-alanine to glycogen were also investigated. The diverse roles of the adrenal and pancreatic hormones in ascorbic acid deficiency might thus be indicated.

Experimental. Young guinea pigs (550-600 g) of both sexes were used. They were paired as to sex and weight. Animals were pair-fed, the normal animal of each pair receiving the amount of basal diet that the deficient member had consumed the previous day. The basal diet used was a commercial rabbit food.† In addition to the diet all animals received a carotene in oil supplement sufficient to supply 1 mg of carotene daily. The normal animals were given a solution containing 10 mg of ascorbic acid 3 times weekly. All the animals were kept at a temperature of 75-80°F.

The animals kept on this diet without ascorbic acid supplements exhibited a decreased appetite in 19-24 days. At this stage scurvy was present as evidenced by extensive hemorrhages of the fascia of the musculature particularly of the legs. A rapid loss of weight accompanied the loss of appetite. A few days after inanition became apparent in the deficient member of the pair, the carbohydrate technic of Cori and Cori[11] was applied to both animals. They were allowed to fast for 24 hours and were then given a solution containing 2.5 g of glucose orally. One hour later each member of the pair was given an injection of 20 units of insulin intraperitoneally and sacrificed 6 hours later by the use of sodium amytal.

Animals used for the experiments with adrenal cortical extract were treated similarly to those in the insulin experiment with the exception that the deficient animal only received an intraperitoneal injection of ½ ml of adrenal cortical hormone‡ at hourly intervals 7 times in accordance with the assay procedure of Reinecke and Kendall.[12]

Animals used for the investigation of the effect of sodium chloride were treated as were the deficient animals in other groups except that a 0.5% solution of this compound was given for drinking water during the depletion period.

The effect of adrenaline was studied on animals prepared as were those in the above groups. After a 24 hour fast both the normal and deficient member of the group received an intraperitoneal injection of 0.15 ml of 1 to 1000 saline solution of adrenaline. They were sacrificed 3 hours later; blood sugar, liver and carcass glycogen determined.

In the investigation involving dl-alanine the deficient and normal animals were treated as were those in other groups. After a 24 hour fast a solution containing 1.4 g of dl-alanine was fed orally. Animals were sacrificed 12 hours later; liver and body glycogen determined.

The analytical procedure and methods as outlined by Murray and Morgan[1] were followed in all experiments.

Results and Discussion. The results of the analytical work are shown in Table I.

The injection of insulin failed to facilitate the storage of glycogen in the deficient animal; intestinal absorption was still impaired and blood sugar levels were high. Both the normal and deficient animals given insulin tended to show lower liver glycogen stores than animals not so treated; carcass glycogen did not seem to be affected. Cori and Cori[11] using the same technic with normal rats also found substantially lower amounts of liver glycogen in animals given insulin but body glycogen was comparable to that in the controls. Stetten and Klein[13] applying this same treatment to rats likewise found low liver glycogen levels, but large increases in carcass glycogen over

† Purina rabbit pellets.

11 Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1926, **70**, 557.

‡ Whole extract of the adrenal cortex—75 g/ml donated by Dr. E. C. Kendall, Mayo Clinic, Rochester, Minn.

12 Reinecke, R. M., and Kendall, E. C., *Endocrinology*, 1942, **31**, 573.

13 Stetten, D., Jr., and Klein, B., *J. Biol. Chem.*, 1945, **159**, 593.

TABLE I.
Effect of Insulin, Adrenal Cortical Extract, Adrenaline, Sodium Chloride and dl-Alanine on Carbohydrate Metabolism in Scorbutic and Normal Guinea Pigs.

Treatment	No. of animals	Status	Glucose in intestinal tract, mg	Blood sugar mg, %	Liver glycogen %	Body glycogen %
None	9*	Normal	58 ± 20†	92 ± 6†	4.07 ± 0.20†	.528 ± 0.14†
	9	Deficient	166 ± 41	162 ± 20	2.66 ± 0.33	.439 ± 0.19
			t = 2.34‡	t = 3.33§	t = 3.71§	t = 3.90§
Insulin 20 units	8	Normal	17 ± 6	54 ± 7	1.70 ± 0.15	.353 ± 0.03
	8	Deficient	157 ± 53	143 ± 48	0.52 ± 0.19	.307 ± 0.08
			t = 2.64‡	t = 1.85	t = 4.10§	t = 0.52
Adrenal cortical extr., 3.5 ml	6	Normal	8 ± 4	135 ± 9	3.18 ± 0.38	.372 ± 0.03
	6	Deficient	132 ± 60	230 ± 59	1.85 ± 0.34	.324 ± 0.02
			t = 2.07	t = 1.61	t = 2.60‡	t = 1.33
Adrenaline 0.15 ml 1-1000	6	Normal		185 ± 9	0.92 ± 0.11	.183 ± 0.02
	6	Deficient		260 ± 30	0.62 ± 0.11	.133 ± 0.02
				t = 2.40	t = 2.0	t = 1.80
Sodium chloride	8	Deficient given NaCl	138 ± 42	225 ± 49	1.34 ± 0.18	.321 ± 0.03
0.5% in drinking water	4	Deficient no NaCl	174 ± 50	293 ± 106	1.50 ± 0.29	.285 ± 0.02
			t = 0.55	t = 0.57	t = 0.47	t = 1.0
dl Alanine 1.4 g	8	Normal			0.79 ± 0.17	.309 ± 0.02
	8	Deficient			0.05 ± 0.02	.242 ± 0.03
					t = 4.0§	t = 1.60

* Figures taken from Table I, Murray, H. C., and Morgan, A. F., *J. Biol. Chem.*, 1946, **163**, 401.
† Standard error.
‡ Indicates significance at the 5% level.
§ Indicates significance at the 1% level.
Snedecor, G. W., Statistical Methods, Collegiate Press, Inc., Ames, Iowa, 1938.

fasting levels about 7-fold after feeding glucose and 3-fold after lactate.

Respiration trials run after feeding 2.5 g of glucose to 5 animals fasted 24 hours and injected with 4 units of insulin resulted in an average figure of 0.42 cc of oxygen per square centimeter of body surface per hour. When this same procedure was carried out on the same animals depleted of their ascorbic acid supply until scurvy appeared a figure of 0.43 cc was secured. The lower glycogen stores in the deficient animals given insulin can not then be due to increased oxidation.

Injections of adrenal cortical extract failed to improve absorption or promote glycogen storage. Kendall[14] noted that scorbutic animals given this extract survived longer than those not so treated but this did not cure the scurvy as the pathological lesions at death were the same whether animals received the treatment or not. Ratsimamanga[15] reported that scurvy produced an adrenal cortical deficiency and that injections of this hormone prolonged the survival period 1 to 3 weeks.

The addition of sodium chloride to the drinking water failed to improve any aspect of carbohydrate metabolism investigated in this study.

Adrenaline injections produced high blood sugar in both members of the pair; the level in the deficient animal was higher than that in the normal one. Lower glycogen levels were also found in the deficient animal, but the differences between the two groups were of no significance. Cori and Cori[16] observed that when rats were given adrenaline in the post absorptive state high blood sugar resulted, carcass glycogen was lowered and some increase in liver glycogen occurred.

The deficient animal was apparently not as competent in converting dl-alanine to liver

[14] Kendall, E. C., Mayo Clinic, Rochester, Minn., private communication.

[15] Ratsimamanga, A. R., *Compt. rendu. Soc. de Biol.*, 1944, **138**, 19.

[16] Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, 1928, **78**, lxii.

glycogen as was the normal.

Summary. The injection of insulin or adrenal cortical extract in guinea pigs deficient in ascorbic acid did not materially alter the disturbance in carbohydrate metabolism characteristic of scorbutic animals; glycogen levels were still low, intestinal absorption impaired and blood sugar levels high after feeding glucose. Oxygen consumption of animals fed glucose then injected with insulin before and after depletion was unchanged. The addition of sodium chloride to the drinking water of deficient animals failed to improve any aspect of carbohydrate metabolism studied. Adrenaline given to both members of the pair produced higher blood sugar levels in the normal than in deficient animals and slightly higher levels of liver glycogen. Normal animals demonstrated much greater ability to convert dl-alanine to liver glycogen than did the deficient.

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Secretion in Cow's Milk of Intravenously Injected Radioactive Phosphorus P^{32}.*

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Since phosphorus plays a major role in intermediary metabolism, especially in biological energy transformations, the measurement of phosphorus transfer and turnover rates in domestic animals is important in research on the basic factors of food utilization.

This report deals with the rate at which intravenously injected P^{32} is secreted in the milk, and its concentration in milk, as a function of time. These data are of particular interest for planning tracer experiments with large animals and for appraising cows as producers of labeled compounds, such as casein, for other trials. Comparing the secretion rate and concentration in milk of P^{32} with that of Sr^{89}, mentioned earlier by Erf and Pecher,[1] leads to the tentative conclusion that the phosphorus stored in the body is more mobile than the calcium.

The excretion of P^{32} in feces and urine, and specific activities in blood and other body constituents will be discussed in later papers in connection with the results of additional work still under way.

Method. Two lactating Jersey cows were used for the two trials on phosphate secretion reported in this paper. The same cows were, and still are, used in other trials with P^{32}. To simplify later comparisons, the cows will be

*This paper is the first report on a cooperative project, "Metabolic Research with Isotopes," started two years ago on the Davis campus among members of the Divisions of Chemistry and Animal Husbandry, and now including workers of other divisions. The authors take this opportunity to thank the colleagues who were instrumental for a promising start of this tracer work. Dr. H. Reiber of the Division of Chemistry provided us with the first sample of P^{32}. He also helped us with the analytical work during our first trial, as did Dr. J. K. Loosli, on sabbatical leave from Cornell University. Drs. M. E. Gardner and Charles G. Patten of the Division of Mathematics and Physics made many measurements of radioactivity for us and trained us in the use of the Geiger counter borrowed from Dr. W. B. Hewitt, Division of Plant Pathology. The generous help and advice of members of the Department of Medical Physics at Berkeley, especially Dr. H. Jones, has been most encouraging. Last but not least, we are glad to acknowledge the faithful and ever-ready help of Mr. Th. Chernikoff, technician in the Division of Animal Husbandry.

† At present at the Department of Medical Physics, Berkeley.

[1] Erf, L. A., and Pecher, Ch., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 762.