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.

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

identified by their herd number, and the trials by Roman figures.

Cow 798 was 7 years old, weighed 400 kg, and was in the 5th month of lactation when, for the trial III, she was injected intravenously with 50 ml of a phosphate solution isotonic with blood and containing 15 millicuries of P^{32}. The same amount of a similar solution, but containing 30 millicuries of P^{32}, was injected into cow 890 which was 3 years old, weighed 370 kg, and had calved 5½ months before the trial started. Cow 798 produced daily 9.5 liters of milk; cow 890 8.7 liters. These production rates are means for the second to the seventh day after injection. The radioactive solution was injected into the jugular vein through plastic tubes otherwise used to insulate wires and known to electricians as "spaghetti". This method, which will be described in detail later, causes a minimum of excitement, and provides therefore a good chance that no "hot" solution is spilled or leaks into the tissues around the vein. The cows were milked just before injection, at 1, 2, 6 and 12 hours after injection, and later twice a day. The radioactivity of the milk was measured in 0.1 ml samples pipetted on 1" diameter disks of lens cleaning paper, dried on 1¼" diameter aluminum disks and then fixed with a spray of an alcoholic shellac dispersion.

Results. The concentration of P^{32} in milk reached a maximum during the period of 3 to 8 hours after injection. This maximum amounted to 1.21% of the injected dose per

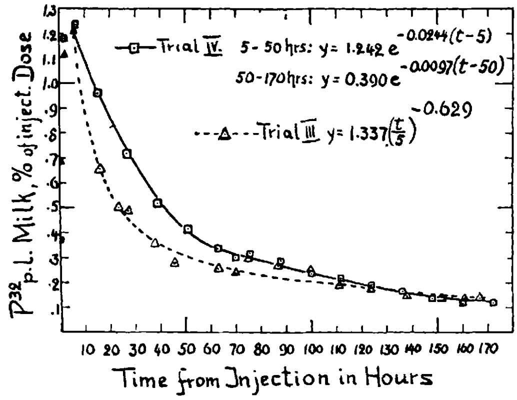

FIG. 1.
Radioactivity in milk.

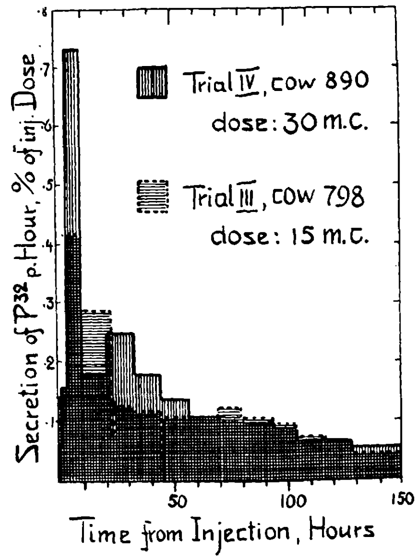

FIG. 2.
P^{32} secretion.

liter milk of cow 798 in Trial III and to 1.24% per liter milk of cow 890 in Trial IV.

Fig. 1 shows the P^{32} concentration in milk as a function of time from injection.

The relative rates of secretion of P^{32} in milk are illustrated by Fig. 2 The histogram gives the results of the direct measurements in each period.

The average daily secretion of P^{32} in milk as percentage of the injected dose for the first to the 7th day after injection amounted to 7.4, 4.9, 2.8, 2.1, 1.6 and 1.4% respectively.

In a week, cow 798 (Trial III) secreted, in 66 liters of milk, 20% of the injected P^{32}; cow 890 secreted, in 58 liters of milk, 23% of the injected dose.

Discussion. Cows injected intravenously with 15 and 30 millicuries of P^{32} respectively produced "hot" milk almost immediately. The radioactivity of the milk of both cows reached the same peak at about 5 hours after injection when 1 liter of milk contained 1.2% of the injected P^{32}. Doubling the injected dose of P^{32} thus changed neither the time at which the maximum activity in milk was reached nor the relative height of the maximum P^{32} concentration in milk as percentage of the injected dose.

At the time of maximum activity in the milk, the inorganic P (trichloracetic acid filtrate) was relatively more active than the organic P. This conclusion is drawn from the observation that even 10 hours after injection, the organic P^{32} in milk amounted to less than 20% of the total P^{32}, whereas during the 5th to 7th week of lactation, according to Van Slyke and Bosworth[2] 20% of the milk P is present in organic form, practically all in casein. Aten and Hevesy[3] have reported such a lag in the specific activity of casein P as compared to inorganic P in goat milk. However, 5 hours after injection, the specific activities were already equalized. Goats may have higher turnover rates than cows. Experiments are under way to get more definite information on the partition of P^{32} among the various constituents of the milk. Despite the lag mentioned, cows are good producers of labeled casein. From milk collected for 3 days after injecting 40 millicuries of P^{32} per cow, we have prepared casein with a radioactivity of 2 microcuries per gram.

The peak of P^{32} activity in the milk of our cows was reached at nearly the same time after intravenous injection as the peak of Sr^{98} in milk of two Holstein cows in the earlier trials of Erf and Pecher (l.c.). Sr^{98}, however, reached a higher maximum relative concentration, namely 2.2% of the injected dose per liter milk, and then decreased faster than the P^{32} in our trials. For 4½ days after injection of the Sr^{98}, the two cows secreted 8% and 11% respectively of the injected isotope in the milk, whereas in 4 days after injection of P^{32} 15.1 and 17.9% respectively were secreted by our 2 cows.

The different results with Sr (representing Ca) and P might be expected if cows had a greater storage capacity for P than for Ca in terms of the corresponding amounts secreted in milk. Such an explanation, however, would be unsatisfactory, in view of the relative concentrations of these elements in milk and body. According to Van Slyke and Bosworth (l.c.), milk contains 0.103% P and 0.143% Ca. Using figures of Hogan and Nierman[4] for steers, one may estimate that the body of cattle contains 0.72% P and 1.36% Ca. If this estimate is applicable to our cows, then the ratio (P in body : P in milk) is only ¾ of the ratio (Ca in body : Ca in milk).

The different behavior of Sr (substituting for Ca) and P in lactating cows may be explained by the assumption that the P stored in the body is on the average more mobile than the Ca stored. A faster storage of P would explain the lower peak in milk, and a faster exchange of the stored P would account for the subsequent relatively higher level of P^{32} in the milk.

Summary. Intravenous injection of 15 and 30 millicuries of P^{32} respectively into 2 lactating cows produced a maximum relative activity per liter of milk of 1.2% of the injected dose at about 5 hours after injection. The relative activity per liter milk is plotted against time after injection and regression equations are presented for interpolating the data. A week after injection, the milk still contains slightly over 0.1% of the injected P^{32} per liter.

Casein with a radioactivity of 2 microcuries per gram was prepared out of milk collected during 3 days after injection of 40 millicuries of P^{32}.

A histogram shows the relative rate of P^{32} secretion in milk. In a week, the 2 cows secreted in their milk 20 and 23% respectively of the injected P^{32}.

2 Van Slyke, D. D., and Bosworth, A. W., *J. Biol. Chem.*, 1915, **20**, 141.

3 Aten, A. H. W., Jr., and Hevesy, G., *Nature*, 1938, **142**, 111.

4 Hogan, A. G., and Nierman, J. L., *Univ. Mo. Res. Bull.*, 1927, **107**, 45.