rats in a limited number of experiments the secretion of aldosterone was depressed as compared to hypophysectomized rats receiving only corticotropin. This finding indicates that suppression of aldosterone secretion with DCA is not mediated by the pituitary.

A preliminary report has appeared of the studies of J. H. Laragh and H. C. Stoerk (Proc. Am. Soc. Clin. Invest., J. Clin. Invest. 34, 918 (1955)) on the mechanism of secretion of aldosterone in dogs. As in the rat experiments of Singer and Stack-Dunne (loc. cit.), restriction of dietary sodium produced only slight aldosterone activity in the urine in the absence of potassium. The administration of excess amounts of potassium caused a marked increase in the excretion of aldosterone. These authors have suggested the hypothesis that the level of potassium may be an important regulator of aldosterone release.

From the investigations which have been reviewed and others in the literature, it is becoming increasingly apparent that the levels of dietary sodium and perhaps more importantly potassium influence the excretion of aldosterone, and that this hormone has an important role in influencing the excretion of sodium relative to potassium. With the possible exception of the dependence of thyroid function on iodine intake, there is no better example of the direct interplay of endocrine function and nutrition. The experiments with ACTH and hypophysectomy indicate that this hormone has some influence on the secretion of aldosterone. However, the bulk of the evidence shows that under most circumstances the release of aldosterone is independent of the release of other corticosteroids.

Singer and Stack-Dunne have made an important contribution to this field in demonstrating the presence of aldosterone in adrenal vein blood in the rat and using this species for physiologic studies of aldosterone release. Their results must be accepted with reservation, however, because they were obtained during the stress of anesthesia and a major surgical procedure.

**ISOLATION OF A VITAMIN B₁₂-PROTEIN COMPLEX FROM MILK**

In the study of the reaction between vitamin B₁₂ and intrinsic factor to produce the active erythrocyte maturation principle interest has centered in the mechanism of binding of the vitamin (Nutrition Reviews 10, 229 (1952); 12, 164 (1954)). Indications are that only by such binding is the vitamin made available and physiologically active. It has been determined by M. E. Gregory and E. S. Holdsworth (Biochem. J. 55, 330 (1953)) that a substance exists in sow’s milk which binds vitamin B₁₂ in much the same way as does an intrinsic factor concentrate prepared from pig stomach. The original observations of these authors have been extended (Gregory and Holdsworth, Ibid. 59, 329 (1955)). The findings contribute importantly to an understanding of the mechanisms of binding of the vitamin.

In these studies quantitative assays for vitamin B₁₂ were done utilizing a microbiologic procedure and the organism, *Lactobacillus leichmannii*. After addition of an excess of the vitamin to a milk sample or concentrate, the free or unbound vitamin was determined in an aliquot of an ultrafiltrate. Bound vitamin B₁₂ was determined after enzymatic digestion of the protein complex. Papain was utilized for this purpose.

The milk of a number of different species was tested for its capacity to bind added vitamin B₁₂. Included were samples of milk from the cow, goat, pig, rat and woman and of colostrum from the cow and goat. The
binding property was greatest in the milk of the pig and the rat. The quantity of vitamin $\text{B}_{12}$ bound by sow's milk averaged 0.24 micrograms per milliliter, while that bound by human milk was only 0.08 micrograms per milliliter. The binding capacity of cow's milk was much less than that for human milk. The two samples of colostrum examined bound more of the vitamin than did milk of the same species. The evidence presented indicates that milk of all of the species tested establishes a relatively stable bond with added vitamin $\text{B}_{12}$, but that there is quantitatively greater binding capacity in the milk of some species than in others.

The next step taken in these investigations was to separate the protein fractions contained in the whey of the milk samples by means of electrophoresis and to determine the amount of vitamin $\text{B}_{12}$ bound by proteins of different electrophoretic mobility. Two procedures were utilized to identify the bound vitamin. One was to extract the protein from segments of the paper strip and then to apply the microbiologic assay to the extract. The second procedure was to add vitamin $\text{B}_{12}$ containing radioactive cobalt to the whey prior to electrophoresis and locate the bound vitamin by placing the dried paper strip on x-ray film. Description of the results of these experiments is of significant interest because the protein patterns of whey from the various species show little similarity. However, vitamin $\text{B}_{12}$ is bound and associated with a protein which has the same mobility in all preparations. Moreover, the naturally occurring vitamin in these milks is bound by the same protein, which is considered by the authors to be a glycoprotein. For purposes of comparison the proteins of an intrinsic factor concentrate prepared from pig stomach were separated by the same procedure and it was determined that vitamin $\text{B}_{12}$ was bound by a fraction with electrophoretic mobility identical with that of the vitamin-binding protein in the milk samples.

The investigators proceeded to isolate a highly purified form of the protein-bound vitamin $\text{B}_{12}$ from sow's milk. This was done by saturating the protein with the cobalt-labeled vitamin and identifying the complex by its radioactivity. Purification was achieved by successive application of precipitation with ammonium sulfate, electrophoretic separation and precipitation with isopropanol in the cold. The final product was a pink powder containing 23.6 micrograms of vitamin $\text{B}_{12}$ per milligram of complex. That a high degree of purification had been achieved was indicated by homogeneity with electrophoresis and by the ratio of vitamin to protein which was unchanged by further steps of precipitation with isopropanol.

In a second paper the authors describe experiments which were devised to determine some of the constituents and chemical properties of the purified complex and to examine the type of linkage between the vitamin and the protein (M. E. Gregory and E. S. Holdsworth, *Biochem. J.* 59, 336 (1965)). Evidence was derived supporting the contention that the binding substance is a glycoprotein. It contained 16 per cent nitrogen and had an amino acid composition typical of proteins, except for a relatively great content of tyrosine. In addition, the material contained 7 per cent carbohydrate and 9 per cent hexosamine. The absorption spectrum is recorded and corroborates the presence of vitamin $\text{B}_{12}$ and a protein. Assuming that a single molecule of vitamin $\text{B}_{12}$ is bound by one molecule of protein, the authors deduce a molecular weight of 55,000 for the protein. It is of interest that other investigators have isolated a similar bound form of the vitamin from pig gastric mucosa, which has an identical absorption spectrum but which is estimated to have a molecular weight of 100,000 (H. G. Wijmenga, K. W. Thompson, K. G. Stern, and D. J. O'Connell, *Biochim. et biophys. acta* 13, 144 (1964)).

In the effort to determine which structural
Characteristics of the protein molecule are concerned in its combination with the vitamin B12. A variety of specific groups were blocked by chemical procedures. The information derived indicates that the bond is formed either by a phenolic group or by an amino group. Other observations demonstrate that neither nucleotide groups nor primary amide configurations of the vitamin B12 molecule are involved in the combination with the protein. However, hydrolysis of the hexa- and hepta-acids in the structure of the vitamin prevented formation of the complex.

These observations of Gregory and Holdsworth (loc. cit.) afford additional information concerning the binding of vitamin B12. All indications are that the process involving milk protein is similar and wholly analogous to the binding of the vitamin by intrinsic factor. Extension of these investigations and use of the purified protein-vitamin B12 complex in studies of the physiologic activity of the vitamin should permit important new advances in the elucidation of mechanisms of action.

**ROLE OF FOLACIN IN NIACIN METABOLISM**

The inclusion of relatively large amounts of niacinamide in the diets of certain experimental animals has been shown to deplete methyl group reserves. In consequence growth may be depressed, and excess fat accumulates in the liver. This is presumably brought about because of the obligatory methylation of niacinamide to form the urinary excretory product, N-methylniacinamide. Since folacin is involved in methyl group metabolism, the role of this vitamin in the methylation of niacinamide has been investigated (P. Fatterpaker, U. Marfatia, and A. Sreenivasan, Biochem. J. 59, 470 (1955)).

In one experiment folacin deficiency was induced in rats by feeding a purified ration containing iodinated casein but no added folacin. In a second experiment the deficiency was induced by injecting aminopterin into rats receiving a laboratory stock ration. In the first experiment various combinations of niacinamide, choline and folacin were administered to some animals, while in the second experiment niacinamide was given orally. Feeding niacinamide to moderately folacin-deficient rats (receiving the iodinated casein ration) increased the liver weight through the deposition of extra fat. The addition of choline as a methyl donor counteracted these effects of niacinamide. Folacin exerted an action almost identical with that of choline. Methylation of the administered niacinamide was markedly enhanced (as indicated by urinary levels of N-methylniacinamide) by the administration of either choline or folacin. The combination of choline and folacin was no more effective than either substance alone in decreasing liver size and fat content and increasing the methylation of the niacin compound.

The choline oxidase level of liver was diminished by folacin deficiency. The level was depressed further by the administration of niacinamide. Dietary choline counteracted the deleterious effect of niacinamide but not that of folacin deficiency. The effect of folacin deficiency was corrected only by folacin administration. Xanthine oxidase activity, pantothenic acid and coenzyme A content of the liver were depressed by niacinamide. This was counteracted by choline but not by folacin. Folacin deficiency had no effect on xanthine oxidase levels. Folacin deficiency resulted in enlargement of the adrenals, particularly in the presence of niacinamide. The enlargement was accompanied by a decrease in the concentration of epinephrine.

The experiments with severe folacin deficiency induced by aminopterin showed that niacinamide aggravates the fat accumu-