TABLE III. Extractable Hydroxyproline of Chick Embryo and Serosa.

Age of embryo, days	Dry wt of embryo, mg	Hydroxyproline — Embryo — Calculated,* μg/mg	Embryo — Extracted, μg/mg	Serosa — Determined,† μg/mg	Serosa — Extracted, μg/mg
4	1.7	0	0		
10	100.0	3.00	3.05		
12	255.3	5.17	5.48		
13	701.3	7.06	7.50	10.8	10.6
15				16.5	15.4
16				8.6	9.2
17	3852	10.43	9.46	12.1	14.6
18				10.4	9.7
19				14.9	13.9

* From best curve for log total hydroxyproline versus log dry weight of embryo.

† Serosa from the same egg as extracted serosa.

crease in weight or hydroxyproline content during incubation of the egg. The egg-shell membranes did not contain more than 1.7 mg of hydroxyproline.

Essentially the entire quantity of hydroxyproline-containing substances of the chick embryo and serosa at different ages is extractable by autoclaving (Table III). Apparently the hydroxyproline of these structures is principally in the form of collagen or collagen-like substances which are converted to gelatin by the autoclaving.

Summary. The egg-shell membranes of the chicken, duck, and turkey contain 0.66 to 1.00% hydroxyproline depending upon species. The remainder of the contents of the egg contains no detectable amount of hydroxyproline. Hydroxyproline appears in detectable amounts in the chick embryo after the fourth day and increases to 1.16% of the dry weight on the nineteenth day. The serosa contains hydroxyproline after the fifth day which increases to a maximum of 2.1% on the fourteenth day. Most of the hydroxyproline of the embryo and serosa is present in a form extractable by autoclaving, probably as collagen.

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Inactivation of Microbiological Activity of Crystalline Vitamin B_{12} by Reducing Agents.* (18092)

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When crystalline vitamin B_{12} is hydrogenated with platinum as catalyst, its red color disappears but can be restored upon shaking with air. In spite of the restoration of the original color, the product possesses only a small fraction of the original microbiological activity. Hence, it is biologically different and chemically distinct from vit. B_{12} and is given the name vit. B_{12a}(1). Since hydrogenation of vit. B_{12} may involve a simple reduction (that is, gain of electrons) or addition of hydrogen atoms to double bonds, it is of interest to ascertain whether reducing agents can likewise abolish the microbiological activity of this vitamin. The results of such a study are presented in this communication.

Experimental procedure and results. A. *Inactivation of the microbiological activity of vitamin B_{12} with various reducing agents.* Attempts were made to inactivate vit. B_{12} with different reducing agents. The procedure of a typical experiment is given as follows: 400 mg of several reducing agents, such as cysteine hydrochloride, ascorbic acid, thiamine hydrochloride, and hydroquinone, were dissolved in 10 ml of water. One ml aliquots of these solutions were titrated with

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1. Kaczka, E., Wolfe, D. E., and Folkers, K., *J.A.C.S.*, 1949, v71, 1514.

TABLE I. Inactivation of Microbiological Activity of Vitamin B_{12} by Various Reducing Agents at Different pH's.

Reducing agent added	pH			
	7.3	5.8	4.5	3.0
None (control)	0	0	0	0
Cysteine HCl	96	75	45	70
Thiamine HCl	91	25	28	0
Ascorbic acid	52	40	35	25
Hydroquinone	9	22	25	0

Inactivation is expressed as % of the original activity destroyed.

approximately 0.5 N NaOH to pH 7.3 in order to determine the amount of alkali necessary for neutralization. One-half ml of each of the solutions of reducing agents was pipetted into a series of tubes and neutralized with the requisite amount of sodium hydroxide. In order to stabilize the solution at the desired pH, 1 ml of 1.0 M phosphate buffer at pH 7.3 was added and the total volume of liquid in each tube was brought to 3.0 ml with water. Into each tube was pipetted 1.0 ml of a crystalline vitamin B_{12} solution (200 μg/ml). For a control on the stability of this vitamin at pH 7.3, 1.0 ml of the vitamin solution was pipetted into a tube containing only 1.0 ml of phosphate buffer and 2 ml of water. To all tubes were added a few drops of toluene as a preservative. They were sealed and incubated at 37°C for 7 days, at the end of which the contents of the tube were diluted tenfold with water and assayed for the microbiological activity of vit. B_{12}. The results are given in Table I. The data demonstrate that in the absence of a reducing agent there was no loss of microbiological activity at this pH, whereas in its presence the destruction was marked.

B. *Effect of pH on inactivation.* In order to ascertain whether hydrogen ion concentration plays a role in the inactivation of vitamin B_{12} this vitamin was allowed to react with the same series of reducing agents at different pH's ranging from 4 to 7.5. Our other experiments showed that beyond this limit of hydrogen ion concentration, there was some appreciable loss of activity on incubation. The ratio of reducing agents to vit. B_{12} remained 1 to 100 by weight. The results of this typical experiment (Table I) demonstrate that under our experimental conditions there was no loss of microbiological activity in the control solutions kept at pH's between 7 and 4, whereas the destruction of microbiological activity by ascorbic acid, thiamine, and cysteine was most effective at the high pH (7.3). The inactivation by hydroquinone at pH 7.3 was less than that at pH 4.5 or 5.8. However, it should be pointed out that a considerable portion of the added reducing agent was destroyed at the neutral pH as evidenced by the appearance of the brownish color in the reaction mixture. The above data demonstrate that when vit. B_{12} solution was allowed to react with a series of reducing agents, there was a destruction of microbiological activity. However, it was not accompanied by a concomitant decrease in the intensity of the red color. It is unlikely that the reduction of the microbiological activity is correlated to the disappearance of the red color. It is our belief that the inactivation is probably due to the reduction of the part of the vit. B_{12} molecule which does not contribute to the color of the vitamin. The hypothesis that inactivation is due to simple reduction is further substantiated by the fact that methionine does not appreciably reduce the microbiological activity of vit. B_{12}. This amino acid is similar to homocysteine except that the hydrogen of the SH group is replaced with a methyl group, thus making this compound incapable of reduction.

The animal protein factor activity of the treated substance was also tested by the use of vit. B_{12} deficient rats. The procedure used was essentially that described by Register, *et al.*(2). The rats were born and raised by mothers on a soybean protein diet. A solution of vit. B_{12} whose microbiological activity was destroyed by cysteine hydrochloride to the extent of 95% was tested for its APF activity at levels of 0.025 μg/day and 0.15 μg/day. The lower level was chosen because it represented the minimum dosage which will stimulate a definite growth response by our rats. The rats receiving the lower dosage gave as good a growth response

2. Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, v177, 129.

as the rats in the control group which received an equal amount of untreated vitamin. Our data therefore indicate that if there were a simultaneous destruction of APF activity, the degree of destruction could not be as extensive as that of the microbiological activity.

Summary and conclusion. When a solution of crystalline vit. B_{12} was subjected to a series of reducing agents between pH 4 and 7, at which range this vitamin is stable, a marked loss of the microbiological activity occurred. This loss of microbiological activity can be attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color. Our preliminary data also demonstrate that the destruction of the microbiological activity was not accompanied by the destruction to an equal extent of the APF activity.

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Failure to Isolate Brucella from Prostatic Tissue of Individuals Living in an Endemic Area.* (18093)

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Brucella have been recovered from the fallopian tubes, appendix, gall bladder, ovaries, serous surfaces of the intestines, joint fluid, feces and urine, and from extirpated human tonsils(1-4). Parsons and Poston(5) cultured brucella from the lymph nodes of 10 out of 19 cases of Hodgkins' disease, whereas brucella were recovered from the nodes of only one of 67 apparently normal individuals. Forbus and Gunter(6) reported that in 5 cases of Hodgkins' disease, *Brucella suis* or *Brucella melitensis* was isolated from one or more of the following: lymph nodes, liver, spleen, testis, bile, kidneys, and rib marrow. More recently, McVay(7) cultured prostatic tissue obtained from 34 apparently healthy individuals. *Brucella abortus* was isolated from the glands of 2 men, and *Br. melitensis* from a third. This report has prompted a similar study at the University of Minnesota Hospitals. The majority of the patients entering the hospitals are referred from rural areas, where brucellosis is endemic. In a recent epidemiologic survey of bacteriologic proved brucellosis in Minnesota, it was observed that the disease not only occurred more frequently in a rural population, but also, that brucellosis was recognized most frequently in adult males(8). Through the cooperation and help of Dr. C. D. Creevy, Director of the Division of Urological Surgery at the University of Minnesota Hospitals, patients presenting themselves for resection of the prostate gland were selected for study.

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