impulses; and (c) liberation of certain hormones such as epinephrine or sympathin. Paresis of the vagal inhibitory mechanism is directly antagonistic to one of the actions of digitalis. Decreased vagal stimulation of the SA and AV nodes would act to shift the pacemaker upward by virtue of the resultant greater automaticity of the nodal tissue. In 2 dogs, however, a slower rate was seen during anesthesia than was present in the unanesthetized but over-digitalized heart. An effect upon the relative irritability of the auricles and ventricles must be concluded, since the rhythm was SA with this slower rate.

With thiopental sodium 4 of the 10 animals had no change of the arrhythmia or conduction defect caused by over-digitalization; 2 were worse; and 4 were improved. In 3 of the latter 4 the short acting glycoside ouabaine had been used. Since electrocardiograms taken upon recovery from anesthesia did not show reversion to the abnormalities present before anesthesia, there is some doubt as to whether the effect was due to the anesthetic agent or to the decrease in activity of the digitalis preparation.

Thiopental sodium was found to produce either no effect or an increase in responsiveness of the heart to vagal stimulation by Gruber *et al.*(8). In the present study this anesthetic agent was seen to exert no beneficial effect upon the severity of the arrhythmias induced by digitalis preparations.

Summary. Dogs, digitalized to cardiac toxicity with ouabaine (0.07 or 0.08 mg/kg) or with digitoxin (0.3 mg/kg) were anesthetized with cyclopropane, ether and thiopental sodium.

Cyclopropane improved or abolished the arrhythmias produced by digitalis preparations. Ether caused a reversion to normal rhythm. Thiopental sodium exerted no effect upon the electrocardiographic abnormalities of over-digitalized dogs.

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Hydroxyproline Content of the Developing Chick Embryo. (18091)

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Previously reported investigations have shown that in the developing chick embryo the percentage of arginine and lysine remain quite constant, tyrosine and histidine decrease slightly, and tryptophan and cystine increase somewhat(1-4). Hydroxyproline, separated as the reineckate according to the procedure of Kapfhammer and Eck(5), has been reported to occur in a constant amount in the egg during the period of incubation(6).

In the present study the colorimetric procedure of Neuman and Logan(7) for determining hydroxyproline shows it to be absent from egg white and yolk, and to accumulate in the embryo and embryonic membranes as development proceeds. The presence of hydroxyproline in the egg-shell membranes of the chicken, duck, and turkey is also reported.

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TABLE I. Hydroxyproline Content of the Egg.

Species	Hydroxyproline content of membranes—% of dry wt		
	Inner (egg)	Outer (shell)	Whole
Duck, White Pekin	1.12	0.66	0.89
Turkey, Mammoth Bronze	0.87	0.63	0.66
Chicken,* White Leghorn	1.12	0.89	1.00

* Egg white and yolk and chalaza and vitelline membrane contained no hydroxyproline.

Procedure. White leghorn eggs were obtained from a commercial hatchery and incubated at 37.5 to 38.5°C at a humidity of 50 to 60%. They were turned once a day. The hydroxyproline content of white and yolk, chalaza and vitelline membrane, and egg-shell membranes of the fresh egg was determined by the colorimetric procedure of Neuman and Logan(7) with correction for color due to tyrosine. At intervals as the incubation proceeded, eggs were withdrawn and the embryos and embryonic membranes were removed, separated, blotted lightly with filter paper, and weighed. They were suspended in 20 to 50 times their weight of acetone for 3 hours, and overnight with the same quantity of fresh acetone, then removed and dried to constant weight at 108°C for analysis. To determine the extractable hydroxyproline, embryos and serosa were subjected to autoclaving in distilled water for two 3-hour periods at 15 lb pressure. The solutions and washings of the residues were transferred to test tubes. The contents were dried by directing a stream of air into the tube placed in a boiling water bath. The hydroxyproline content of the material removed by extraction was determined.

Results. As shown in Table I the whole egg-shell membranes from different species contained 0.66 to 1.00% hydroxyproline. The inner membranes of all species contained a higher content of hydroxyproline than did the outer membranes. The hydroxyproline was solubilized to an extent of 22% by autoclaving 6 hours at 15 lb. Pepsin and trypsin did not significantly digest the hydroxyproline-containing substances. Other keratinous materials analyzed by the present procedure did not contain hydroxyproline(7). The egg white and yolk, and chalaza and vitelline membrane contained no detectable amounts of hydroxyproline.

As shown in Table II hydroxyproline was not detectable in the chick embryo during the first 4 days, but appeared in traces on the fifth day, and increased throughout the incubation period (19 days) to 1.16% of the dry weight. The logarithmic values of the total hydroxyproline of the embryo bear an approximately straight line relationship to the logarithmic values for the dry weight of the embryo. As recorded in Table II hydroxyproline of the serosa was not detectable until the sixth day. The content increased to a maximum of 2% on the fourteenth day. On the nineteenth day the total hydroxyproline of the extra-embryonic membrances was 0.92 mg and the total hydroxyproline of the embryo was 54.0 mg. The egg-shell membranes did not contribute significantly to the accumulated hydroxyproline for they did not de-

TABLE II. Hydroxyproline Content of Developing Chick Embryo and Extra-Embryonic Membranes.

Age of embryo, days	Dry wt of embryo, mg	Hydroxyproline content —Embryo—		Serosa, μg/mg
		μg/mg	Total mg	
1–4	5.6	0	0	0
5	5.8	.08	.0005	0
	7.1	.15	.0011	
6	7.3	.25	.0018	0.21
	11.3	.44	.0050	
7	39.6	1.14	.045	2.10
	53.0	1.46	.077	
8	55.2	1.77	.097	2.67
	61.9	1.69	.105	
9	63.9	1.95	.125	2.14
	112.8	3.01	.34	
10	134.6	3.78	.508	3.39
	150.4	3.33	.501	
11	163.1	3.67	.598	4.68
	250.0	4.80	1.20	
12	328.2	5.93	1.95	5.49
13	675.0	7.22	5.07	13.1
	700.4	6.42	4.52	
14	748.9	6.80	5.09	21.0
15	1645	7.45	12.02	16.5
16	2183	8.42	18.02	14.9
	2337	8.78	20.10	
17	2363	10.41	24.6	12.1
18	4552	11.15	50.8	12.0
19	4672	11.55	54.0	14.9
				12.0*

* Amnion.

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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