water and a mixture of n-butyl alcohol, acetic acid, and water (approximately 11:3:4).

The results are illustrated in Fig. 1. Spots 1, 9 and 10 in *Rana* (Fig. 1A-B) and marks 2 and 12 in *Hyla* (Fig. 1C-D) are assumed to be peptides since they disappeared upon acid hydrolysis (refluxed with 6N HCl for 20 hours). They will be analyzed in a later study.

Rana and *Hyla* chromatograms are remarkably similar. *Rana,* but not *Hyla,* shows a spot tentatively designated methionine sulfoxide (Fig. 1A, No. 7). Methionine added to the extract of *Rana* zygotes intensifies this spot, presumably by the oxidized form and a new spot (unoxidized methionine) appears to the right and in the position of valine. Valine and methionine have similar R_f values, but the mark designated valine on chromatograms of older material probably is not methionine. The methionine sulfoxide spot is absent or very faint in tailbud papers whereas the valine spot has by that stage become very definite. Alanine, leucine (or isoleucine), and valine present in *Rana* after the beginning of gastrulation, can be detected in the youngest *Hyla* material. Unfortunately because of the difficulty of procuring an adequate quantity of *Hyla* zygotes, early cleavage stages were also included so that the early *Rana* and *Hyla* extracts are not strictly comparable.

There is no marked change in the picture as development proceeds. In both species the large peptide spots decrease in intensity; phenylalanine first appears in neurulae in *Rana* and in gastrulae in *Hyla;* and in *Rana* leucine (or isoleucine) and valine are first detectable in gastrular extracts. In addition, the spot on *Rana* papers tentatively identified as oxidized methionine is so faint on stage 18 chromatograms that it was not circled in the photograph (Fig. 1B).

Finally we report one trial experiment in which extracts of dorsal lip explants in *Hyla* were compared chromatographically with those of explants of ventral ectoderm. For this one run approximately 300 explants of each type were needed. The explants corresponded to those used by Friedberg and Eakin(8) in their study of glycine uptake. The two chromatograms were essentially alike in the number and intensity of the spots and agreed with the picture for the whole gastrula.

Summary. Several free amino acids and one amide were identified by paper chromatography in alcoholic extracts of eggs and early developmental stages of *Rana pipiens* and *Hyla regilla.* Other ninhydrin-positive substances, including several peptides, will be studied later. One experiment on *Hyla* revealed no major chromatographic difference between explants of dorsal lip and ventral ectoderm.

8. Friedberg, F., and Eakin, R. M., *J. Exp. Zool.,* 1949, v110, 33.

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The Effect of Cyclopropane, Ether, and Thiopental Sodium upon the Over-Digitalized Heart.* (18090)

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Since cyclopropane tends to shift upward the pacemaker which has been displaced by digitalis preparations(1), it seemed of interest to investigate the effect of certain other anesthetics. This report deals with a comparison of the effects of cyclopropane, ether

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1. Stutzman, J. W., Allen, C. R., and Meek, W. J., *Anesthesiology,* 1942, v3, 644.

and thiopental sodium† upon the cardiac arrhythmias caused by over-digitalization.

Procedure. Twelve adult mongrel dogs, weighing between 5 and 13 kg, were used in this study. Four dogs were digitalized by the intravenous injection of digitoxin,† 0.3 mg/kg. Eight dogs were digitalized by the intravenous injection of Strophanthin G (Arnaud's Ouabaine),‡ 0.07 or 0.08 mg/kg each day that they were used. Criteria for over-digitalization were (a) the production of arrhythmias, (b) the prolongation of the PR interval, or (c) alterations of the ST segment from the position seen in the control electrocardiogram. All dogs vomited following the administration of the digitalis preparations, and in each dog at least one of the signs of cardiac toxicity was seen. It was found that the abnormalities induced by digitoxin persisted for days. The maximum effect of ouabaine was seen in one hour and arrhythmias persisted for 5 to 7 hours. Dogs were not digitalized with ouabaine more often than once in 7 days.

An electrocardiogram, Standard Lead II, was taken before and after digitalization, during anesthesia after 30 minutes equilibration, and upon recovery from anesthesia. Recovery was deemed to be the time the animal could stand and walk, and was at least 30 minutes after the administration of the anesthetic was discontinued.

Each anesthetic was given to 10 dogs. Eight of the dogs received all 3 anesthetics. Cyclopropane and thiopental sodium were administered the same day to each dog, with an interval of at least 2 hours between recovery from the first and induction with the second anesthetic. The order of administration of the two agents was varied. Ether was never given within 24 hours of any other anesthetic and was always the last agent tested.

Dogs were anesthetized with cyclopropane by rebreathing a cyclopropane-oxygen mixture via mask from a 5 liter bag. An endotracheal tube with an inflatable cuff was then inserted to insure a closed system and an open airway. The system was connected through a soda-lime carbon dioxide absorber to a large rubberized bag containing a 28 to 31 per cent mixture of cyclopropane in oxygen. Ether anesthesia was induced by drop on a gauze mask. An endotracheal tube with an inflatable cuff was inserted and connected with a Wolff bottle adjusted for maintenance. Thiopental sodium, 30 mg/cc, was injected intravenously in average total dosage of 25 to 30 mg/kg. An effort was made with each agent to maintain a comparable depth of surgical anesthesia with partial intercostal paralysis. Animals were maintained at this level for 30 minutes and then allowed to recover.

Results and discussion. In Table I are summarized the effects of each of the 3 anesthetics on the over-digitalized heart. Under cyclopropane anesthesia 8 of the 10 dogs showed improvement in the digitalis-induced arrhythmias. Upward displacement of the pacemaker to the SA node was seen in 4 dogs; upward displacement of the pacemaker to the AV node from ventricular foci occurred in 1 dog; and reduction to control levels of the PR interval, with consequent disappearance of the partial AV block, was shown in 3 dogs. Two had no improvement. All dogs demonstrated a return to or maintenance of pre-anesthetic abnormalities upon recovery from anesthesia.

Digitalis has been reported to produce cardiac arrhythmias by two mechanisms. In smaller dosage, reflex vagal stimulation(2) and direct depression of the conduction time account for the observed irregularities(3). In higher dosage the effect upon the myocardium becomes predominant, causing an increase in irritability which is manifested by the appearance of ectopic beats and rhythms (4).

† Thiopental sodium and Digitoxin used in this study generously supplied by Abbott Laboratories, North Chicago, Ill.

‡ Arnaud's Ouabaine generously supplied by E. Fougera Company, New York City.

2. Heymans, C., Bouckaert, J. J. and Regniers, P., *Compt. rend. soc. de biol.*, 1932, v110, 572.

3. Gold, H., Kwit, N. T., Otto, H., and Fox, T., *J. Pharm. and Exp. Therap.*, 1939, v67, 224.

4. Cushny, A. R., Digitalis and Its Allies, London, Longmans, Green and Co., 1925.

TABLE I. Effect of Cyclopropane, Ether, and Thiopental Sodium on the Electrocardiogram of the Over-digitalized Animal.

	Cyclopropane series		Ether series		Thiopental sod. series	
	No. of animals	Rate avg (range)	No. of animals	Rate avg (range)	No. of animals	Rate avg (range)
Control						
SA	10	116 (93-130)	10	122 (100-158)	10	116 (93-130)
Digitalized						
SA	2	135 (130-140)	2	108 (90-125)	2	125 (110-140)
SA c̄ partial AV block	2	123 (112-135)	5	92 (66-114)	5	93 (73-110)
SA c̄ V Exs	1	65	0		0	
AV	0		0		1	180
Shifting pacemaker	2	146 (132-160)	1	93	0	
V	3	225 (210-240)	2	202 (184-220)	2	225 (210-240)
Anesthetized						
SA	7	176 (115-205)	10	166 (120-230)	4	149 (80-220)
SA c̄ partial AV block	0		0		2	79 (70- 88)
SA c̄ V Exs	2	153 (146-160)	0		1	150
AV	1	194	0		1	170
V	0		0		2	188 (185-190)
Recovered						
SA	2	127 (125-130)	4	139 (120-155)	4	129 (110-140)
SA c̄ partial AV block	4	112 (100-125)	5	120 (92-140)	3	129 (112-140)
AV	1	152	0		2	180
Shifting pacemaker	1	195	1	140	0	
V	2	215 (190-240)	0		1	190

SA = Sino-auricular; AV = Auriculo-ventricular; V = Ventricular; V Exs = Ventricular extrasystoles; Shifting pacemaker = AV to V.

The increase of heart rate with cyclopropane in all dogs except No. 8 suggests a decreased vagal tone as postulated by Meek (5).§ Such a reduction of vagal tone combined with an increased irritability of supraventricular tissues might be adequate to explain the counteraction of digitalis arrhythmias by cyclopropane.

All electrocardiograms taken during ether anesthesia showed either a return to normal conduction time or an upward displacement of the pacemaker of the over-digitalized heart. In only one dog was there failure to demonstrate an abnormal electrocardiogram upon recovery from anesthesia. Eight of the dogs had heart rate increases which ranged from 25 to 180%. One had no change and one a decrease of 18%.

The mechanisms responsible for the increased heart rate seen in ether anesthesia have been reported by Samaan(7) to be (a) paresis of the vagal inhibitory mechanism; (b) augmentation of the cardio-sympathetic

5. Meek, W. J., Harvey Lectures, 1940-1941, Series XXXVI:188.

§ These results are in contrast to those observed by Rovenstine *et al.*(6) in isolated turtle hearts exposed to cyclopropane.

6. Rovenstine, E. A. and Adriani, *J. Anesth. and Analg.*, 1942, v21, 111.

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.

7. Samaan, A., *Arch. internat. de pharmacodyn. et de therap.*, 1934, v50, 101.

8. Gruber, C. M., Haury, V. G., and Drake, M. E., *Am. Heart J.*, 1940, v20, 329.

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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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3. Calvery, H. O., *J. Biol. Chem.*, 1930, v87, 691.

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5. Kapfhammer, J., and Eck, R., *Z. physiol. Chem.*, 1927, v170, 294.

6. Ido, R., *Ber. ges. Physiol. u. exp. Pharmakol.*, 1931, v63, 426.

7. Neuman, R. E., and Logan, M. A., *J. Biol. Chem.*, 1950, v184, 299.