of penicillin are put into the thoracic cavity and the chest closed in the usual manner. The post-operative course is uneventful.

Observation. No regurgitation through the anastomosis was ever observed. As long as the clamp at the base of the auricular appendage remains closed, no ventricular pulsations in the appendage and no hemorrhage are seen. After the clamp is opened, blood from the atrium streams through the anastomosis and pulsates in rhythm with the atrium. Bleeding may now occur around stitch openings accidentally torn in the thin appendage wall, but it can easily be controlled.

This operation has been performed on 13 dogs of various size. One death occurred during the operation. The reason for the relatively good survival incidence could be found in the fact that the heart during the whole period of surgical intervention remains fully active and its muscle supplied with fresh oxygenated blood. No attempt is made to operate in a bloodless field; rather the main consideration is given to reducing to a matter of seconds that phase of the operation when the ventricle is opened. This is achieved by means of the technic described above. The blood loss varies between 50 and 150 cc. After all, this technic deals with quickly taking care of a wound which has been produced in the heart before our very eyes, and that problem has long been solved in surgery.

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Experimental Hatching of the Eggs of *Ascaris suum*.*

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Yoshida and Toyoda[1] reported the *in vitro* hatching of the eggs of *Ascaris suum* by direct incubation of the eggs for 5 days in a variety of media. Fenwick[2] did not secure hatching in several of the media reported by Yoshida and Toyoda,[1] but he consistently produced hatching in dilutions of Milton solution, a commercial sodium hypochlorite preparation. Fenwick[3] secured living larvae by treating infective eggs with a 1:15 dilution of Milton solution for 12 hours at 38°C, expressing the larvae between a slide and coverslip, and then washing the liberated larvae with an experimental saline. Pick[4] reported the hatching of the eggs of *A. megalocephala* in a Ringer-horse serum solution 34 days after the liquid had evaporated, but during which time the residue had been kept moist. Pick[4] further observed spontaneous hatching among eggs 21 days after transferring them from a Ringer-horse serum solution to a glucose agar medium.

As a preliminary step in the study of the culture requirements of the larvae of *A. suum*, it was necessary to develop a method of hatching *Ascaris* eggs that would provide large numbers of living larvae in a short time.

Materials and methods. Fertile eggs were secured from the mature female *Ascaris* worms from the small intestines of hogs. The eggs from individual worms were incubated at 23° to 25°C in 40 ml of 2% formalin in separate 250 ml cotton-stoppered Ehrlenmeyer flasks.

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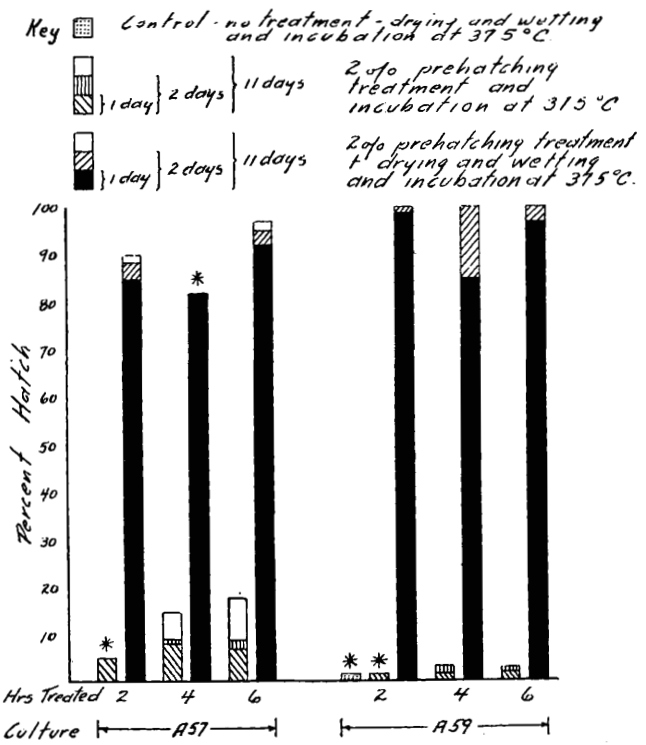

FIG. 1.
Bar graphs showing hatching results following treatment of *Ascaris* eggs in 2% "Clorox"-2% sodium hydroxide solution and subsequent drying, wetting and incubation.
* Per cent hatch did not increase after 1st day.

Under these conditions all eggs became embryonated in 24 days in most flasks. Cultures selected for hatching trials were at least 30 days old to allow for molting (Alicata[5]), had no eggs in stages earlier than the embryo, and showed at least 50% of the embryos motile.

Eggs to be used in hatching tests were treated at 37.5°C in a solution composed of 2% of the commercial sodium hypochlorite (5.25%) preparation "Clorox" and 2% sodium hydroxide. They were then washed five times in physiological saline. Eggs from individual worms were handled separately.

In the hatching tests 3 different methods were used:

1. Treated and washed eggs, in a few drops of physiological saline in shallow watch glasses, were incubated in moist chambers.

2. Suspensions of treated and washed eggs in shallow watch glasses were rapidly evaporated at 37.5°C with the aid of a fan. When salt crystals appeared throughout the suspension in each watch glass, the residue was rewet with saline and incubated in a moist chamber at 37.5°C.

3. Treated and washed eggs were centrifuged in physiological saline and the liberated larvae were available immediately after centrifuging.

Results. Some trials by method 1 resulted in all eggs hatching in 4 weeks of incubation at 40°C. However, most early-hatched larvae did not survive the length of time required to produce a high hatch.

By method 2 the liberation of larvae was more rapid, resulting in 100% hatches in 2 days in some trials. (Fig. 1). Up to 83.5% of the liberated larvae were alive in the hatching media at the end of 24 hours.

[5] Alicata, J. E., U.S.D.A., Tech. Bull. No. 489, 1935, 44.

TABLE I.
Results of Hatching Eggs of *A. suum* by 24 Hour Treatments in 2% "Clorox"-2% Sodium Hydroxide Solutions and Subsequent Centrifugation at 3000 R.P.M. for 10 Minutes. Starting Centrif. Temp. 40°C.

Orig. No.	Culture age (days)	% hatch
A76	37	77.1
A77	37	74.7
A78	37	65.8
A64	57	98.6
A61	57	99.1
A61	60	87.8
A76	67	74.2
A78	67	77.4
A77	104	70.5
A79	104	58.1

Method 3 produced hatching as high as 99.1%, and up to 87% of the liberated larvae were motile 24 hours after hatching. A treatment time of 24 hours in a 2% "Clorox"-2% sodium hydroxide solution followed by centrifugation at 3000 R.P.M.† for 10 minutes, gave consistently high hatches. (Table I). At the end of the 10-minute centrifuge period the temperature in the hatching tubes had dropped from 40°C to approximately 25°C.

Discussion. In a "Clorox"-sodium hydroxide solution the shells of *Ascaris* eggs are dissolved, so that after 24 hours many embryos are surrounded by thin elastic shell membranes only. At this stage motile embryos may produce prominent bulges in these elastic envelopes and may be liberated by their own activities. Later, a complete dissolving of the shells including the membranes may release the embryos in the treatment solution. The shells of eggs from the same worm do not dissolve at a uniform rate, and cultures of eggs from different worms show differences in the average speed at which the shells dissolve.

The hatching of treated eggs incubated in saline, as in method 1, appeared to be due to the penetration of the shell membrane by the active larvae and to some extent the result of osmotic pressure.

Hatching by drying and wetting treated eggs probably resulted from physical stresses, set up in the chitinous shells when the eggs were dried, which caused the shells to fracture when rewet, rupturing the shell membranes beneath and liberating the larvae.

The pressure of membrane covered eggs against each other and the pressure of larvae against the sides of their membranous envelopes is a probable explanation of the hatching of treated eggs during centrifugation. Significant increases in hatching that appeared when the starting centrifuge temperatures were increased from 20° to 40°C were probably due to the liberation of larvae by their own efforts, due to greatly stimulated activity of the embryos at the higher temperature.

The motility of the larvae after hatching varied with the treatment time and with hatching conditions.

A study of photomicrographs in the report of Yoshida and Toyoda[1] revealed that the eggs pictured had no typical mammillated layer, but were essentially the same in appearance as those treated in "Clorox"-sodium hydroxide solution. The eggs were photographed in a 0.05% peptone solution, which in the present work was found to have no effect on the shells. These workers caution that all things used in hatching must be disinfected, and, although this phase of their technic is not described, it is possible that they used a sodium hypochlorite disinfectant, or some other chemical which had the same effect on the egg shells. If *Ascaris* eggs were permitted to remain in contact with such a disinfectant, the shells would be dissolved as shown in the photomicrographs and would probably have hatched in a variety of media as reported.

Summary. 1. Infective *Ascaris* eggs treated in "Clorox"-sodium hydroxide solution, hatched and produced viable larvae when they were subsequently:

(a) incubated in physiological saline at 37.5° and 40°C. Hatching occurred up to 100% in 4 weeks.

(b) dried, rewet and incubated in saline at 37.5°C. Hatching occurred up to 100% in 2 days.

(c) centrifuged at speeds from 2000 to 3000 R.P.M. Hatching occurred up to 99.1%

† 1958 gravities.

during 10 minutes of centrifugation at 3000 R.P.M. with a starting temperature of 40°C.

2. Batches of eggs from different worms showed different hatching characteristics depending on the treatment strength and time, and influenced by subsequent procedure.

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Effect of Insulin, Adrenal Cortical Hormones, Salt and dl-Alanine on Carbohydrate Metabolism in Scurvy.*

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Previous work[1,2] has shown that scorbutic guinea pigs display a disturbance of carbohydrate metabolism resulting in lowered glycogen stores in liver and body. Murray and Morgan[1] demonstrated that when glucose was fed the scorbutic animal failed to absorb it as readily as the normal animal. The blood sugar after this procedure was also abnormally high. Sigal and King[3] had previously demonstrated a lowered glucose tolerance in scorbutic animals.

Banerjee[4] has demonstrated in scorbutic guinea pigs an increase in adrenaline production. He attributed the disturbance in carbohydrate metabolism to an imbalance between adrenaline and insulin since the insulin content of the pancreas appeared to be markedly decreased in the scorbutic animal.[5]

Involvement of the adrenal gland in the changes incident to ascorbic acid deficiency has been suggested. Marked hypertrophy of this gland has been shown to accompany this deficiency. Sayers, Sayers, Liang and Long[6] found that the injection of adrenotrophic hormone lowered both the cholesterol and ascorbic acid content of the adrenals of rats and guinea pigs suggesting that these 2 compounds are involved in the synthesis of cortical hormones. This hormone also greatly increased the deposition of liver glycogen in both species.

Intestinal absorption has been shown to be impaired in adrenalectomized rats[7,8] and the improvement of this condition demonstrated by adding sodium chloride to the drinking water. There was a possibility that the decrease in absorption in the scorbutic animal could be improved by adding this compound to the drinking water.

MacKay and co-workers[9,10] have shown that dl-alanine when fed to fasting mice or rats results in the formation of a considerable amount of hepatic glycogen. The possibility existed therefore, that the scorbutic animal could convert compounds of 3 C atoms to glycogen normally, and this might be some index of the adrenal cortical function.

This study was undertaken to determine the effect of insulin, adrenal cortical extract and additions of sodium chloride in the

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