are without myelin sheaths. A single intact nerve of the dog held 3487 nerve fibers, which were divided into 2299 myelinated and 1188 unmyelinated axons.

An average of 597 sensory nerve fibers remains in the greater superficial petrosal nerve of the cat after its motor axons have been eliminated. Of this average number of sensory nerve fibers, 461 are myelinated and 136 are unmyelinated. The deefferented petrosal nerve of one dog contained 960 sensory axons and 664 of these were myelinated while 296 were without myelin sheaths.

It is estimated that the greater superficial petrosal nerve of the cat has an average of approximately 1596 motor nerve fibers and these are divided into 1164 myelinated and 432 unmyelinated nerve fibers. The greater superficial petrosal nerve of the dog contained 2527 motor axons and 1635 of these were myelinated and 892 were unmyelinated.

It is evident, therefore, that the majority of the axons of the greater superficial petrosal nerve are motor nerve fibers, there being an average of 2 plus motor fibers for each sensory axon.

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Employment of the Embryonic Duck Heart for the Detection of Minute Amounts of a Digitalis Glycoside* (Lanatoside C).

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Exact quantitative information concerning the absorption and excretion (or destruction) of digitalis in the human body is still to be obtained. Such knowledge has not been gained primarily because of the absence of any test sufficiently sensitive to detect the relatively minute amounts of digitalis which must be present in the tissues of patients receiving digitalis. The use of the frog, cat and pigeon for the assay of digitalis has been limited to the quantitation of relatively large quantities of the drug and cannot be utilized for the demonstration of digitalis if the latter is present in but fractions of a microgram.

Pickering[1] was the first to employ the embryonic chick heart for the detection of digitalis. Using embryos between 60-75 hours of age, he demonstrated the striking similarity of such hearts to that of the adult mammal, especially in regard to their response to digitalin and strophanthin. He found that the embryonic chick heart would cease beating after the injection of 0.012 mg of digitalis *in situ*. Lagen and Sampson[2] applied digifoline directly upon the embryonic heart of the chick and were able to demonstrate an electrocardiographic effect after such a procedure. Hall[3] also employed the embryonic chick heart for the assay of crude digitalis. Paff[4-6] dissected the heart from the chick embryo and immersed it in the solution containing the drug to be tested. Using the occurrence of an arrhythmia as a test for the presence of both ouabain and

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digitoxin, he believed he could detect the presence of as little as 1/100,000 of ouabain and 0.001 mg per cc of digitoxin. He found however, that there was a wide fluctuation in the time of appearance of the arrhythmia at any given concentration of the drug tested.

Although the embryonic chick heart appeared to be suitable for employment in the detection of digitalis, it still was not sufficiently sensitive to give much promise as an aid in the quantitation of the concentration of digitalis in the human body. For this reason, the embryonic duck heart was studied and compared to the chick heart in particular respect to their sensitivity to the digitalis glycoside, Lanatoside C. The results of this study are given in this communication.

Methods. A. The Embryonic Duck Heart. Supposedly fertile duck eggs (White Peking China) were incubated for 88-92 hours at 39°C. They then were transilluminated and the position of the embryo marked on the shell. The shells were then partially removed until the embryo and its vascular sinus were in full view. After the diameter of the sinus had been measured, the total embryo was removed with a sharp scissors and placed in a slide well containing Tyrode's solution. Under a dissecting microscope (12×), the heart was removed from the embryo by means of dissecting, cataract knives. At this stage of development, the heart bulges from the body, just caudal to the head and easily can be identified. It also apparently represents one of the most adhesive, solid tissues of the embryo for it may be separated in its entirety by simple teasing of embryonic tissue lying adjacent to it. After separation, the heart is floated gently upon a small spatula and transferred to a second slide well containing 0.1 cc of the solution to be tested. This second slide rests on the stage of a compound microscope which is in a box automatically maintained at 33°C, by means of an electric lamp of 30 watts in circuit with a mercury thermostat and vacuum tube relay switch. After this second transfer, the heart is observed through the microscope (30×) and (1) strength and rate of contraction, (2) occurrence of an arrhythmia and (3) duration of beating are noted.

It was found after preliminary studies that those hearts of embryos contained in vascular sinuses measuring from 20-30 mm in diameter were most suitable for the detection of the digitalis glycoside employed.

Lanatoside C, the glycoside of digitalis lanata, was used in all experiments. An ampule containing 0.2 mg per cc was opened for each day's experiments. Dilutions of this drug varying from 0.001 to 0.00001 mg per cc were studied, after a control series of embryonic hearts were immersed in simple Tyrode's solution and studied.

B. *The Embryonic Chick Heart.* Embryonic chick hearts were obtained and studied in the same manner except that (1) they were incubated only 66 hours at 38.5°C and (2) no embryo having a sinus diameter greater than 30 mm was used.

Results. A. *The Embryonic Duck Heart.* The embryonic duck heart was found to be extraordinarily sensitive to the digitalis glycoside employed. The first indication of this sensitivity was an acceleration and an intensification in the vigor of cardiac contractions. Then, just prior to the occurrence of auriculoventricular block (which was used as the actual indicator for the presence of digitalis) the heart was observed to contract irregularly, very rapidly, jerkily and weakly. The auriculoventricular block always began as partial A-V block and progressed to complete block. Concomitant with the progression of the block, the irregularity of the rate of beating became more manifest. Finally, if sufficient concentrations of digitalis were used, the heart stopped beating in systole.

Thus it was found (Table I A) that when 23 embryonic duck hearts were placed in 0.1 cc of a solution containing 0.001 mg of the glycoside per cc, each began to accelerate and all began to demonstrate auriculoventricular block after an average period of 12 minutes (range: 6 to 28 minutes). They ceased to beat after an average period of 37 minutes (range: 29 to 62 minutes). Approximately the same phenomena were observed when 15 hearts were placed in 0.1 cc of a

TABLE I.
Effect of Digitalis on the Embryonic Duck and Chick Heart.

Digitalis (mg/cc)	Avg sinus diameter (mm)	Hearts (No.)	A-V block (No.)	Onset-A-V block (min.)	Duration of beating (min.)	Avg rate	Type of Contractions
			A. Embryonic Duck Heart.				
0 (control)	30	15	0	—	>60	59	Moderate
.001	31	23	23	12	37	85	Vigorous
.0005	33	15	15	15	42	82	"
.0001	29	15	15	35	54	76	"
.00005	32	18	14	39	>60	73	"
.00001	28	16	0	—	>60	61	Moderate
			B. Embryonic Chick Heart.				
0 (control)	27	10	0	—	>60	76	"
.001	21	25	21	15	41	96	Vigorous
.0005	23	14	10	18	>60	96	"
.0001	17	10	0	—	>60	82	Moderate

solution containing 0.0005 mg of glycoside per cc (Table I A) except that onset of auriculoventricular block occurred somewhat later (average: 15 minutes) and the hearts continued to beat somewhat longer (average: 42 minutes). When 15 hearts were placed in a solution containing 0.0001 mg of glycoside per cc, auriculoventricular block occurred in all of them (Table I A) but the average time of onset was 35 minutes. These latter hearts also continued to contract for an average period of 54 minutes (range: 43 to 71 minutes). Auriculoventricular block occurred in 14 of 18 hearts (78%) placed in a solution containing 0.00005 mg of glycoside per cc after an average period of 39 minutes (range: 24 to 68 minutes). The longevity of these hearts was not affected however by the concentration of glycoside employed. No auriculoventricular block occurred in any of 16 embryonic hearts immersed in a solution containing 0.00001 mg of glycoside per cc (Table I A).

B. *The Embryonic Chick Heart.* As can be seen by inspection of Table I B, the action of the digitalis glycoside on the embryonic chick heart was similar to its effect upon the duck heart except that the former type of heart was much less sensitive to the drug. Thus (Table I B) whereas a concentration of as little as 0.00005 mg of glycoside per cc could be detected by the use of the duck heart preparation, only 0.0005 mg per cc could be detected by the chick heart. Furthermore, even as much of the digitalis glycoside as 0.001 mg per cc could not be invariably detected by using the chick heart method.

Discussion. The above results indicated that the embryonic duck heart was far more sensitive to the digitalis glycoside employed than the embryonic chick heart. By the former's employment, 0.1 cc of a one to 20 millionth dilution of Lanatoside C. (0.000005 mg) could be detected. It is believed that this represents the most sensitive indicator for the presence of a digitalis glycoside yet described.

The embryonic heart of the duck also has certain advantages for its use in the study of cardiac physiology. Pickering[1] earlier stressed the similarity of the chick heart to the mammalian heart and it is most likely that the duck heart also has the same similarities. There are no nervous elements in the embryonic heart at this stage,[1] a state of affairs which allows the study of cardiac musculature alone. Furthermore, the ability of this type of heart to contract regularly over a period of at least 60 minutes in very small quantities of fluid, allows the possibility of testing samples, meager in amount. Finally, because of the relative paucity of tissue composing the heart, more opportunity is allowed for changing the ionic milieu of the cardiac cells (when desired) than might be possible were an adult mammalian heart the subject of experimentation.

Conclusion. The embryonic duck heart, similar to the embryonic chick heart was found to exhibit sensitivity to the action of a digitalis glycoside as characterized by alteration in rate, rhythm and force of contraction. Moreover the embryonic duck heart was able to be used to detect as little as one two-hundredth of a microgram of the digitalis glycoside (Lanatoside C.) This is thought to represent the most sensitive indicator now available for the presence of a digitalis glycoside.

15733 P

Isolation of Pleuropneumonia-like Organisms from Pathological Specimens with the Aid of Penicillin.*

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Pleuropneumonia-like organisms (P.P.L.O.) were isolated from the genito-urinary tract of human patients and in rare cases from other locations.[1-5] Such organisms are often present in the vagina or cervix without apparent connection with any disease. In males they occur only in pathological conditions, and, according to observations collected in the last few years, they play a noticeable role as causative agents in urethritis, prostatitis and cystitis. They have been isolated from cases of severe cystitis which were repeatedly negative for the usual bacteria. Infection of the genito-urinary tract was complicated in about 30% of the cases with acute polyarthritis. It is essential in studying the role of these organisms in human disease that the methods used for their culture and identification be free from error.

The presence of P.P.L.O. can be proven only by cultivation. They grow on media enriched with human or animal serum or with ascitic fluid. Large colonies (0.1-0.3 mm) can be identified with the low power of the microscope. If the colonies remain small (.01-0.1 mm) as often happens in cultures from pathological specimens, they cannot be identified by this method. The agar fixation method which Klieneberger[4] and Salaman[5] used utilizes the impression left by the culture on a coverslip. Small colonies do not adhere to the glass and can not be recognized. This method is subject to error even in the case of large colonies. They are identified by the round bodies which develop on the surface layer and it is not unusual for bacteria to produce similar large bodies. The most reliable method for the identification of the P.P.L.O. is the use of stained agar preparations.[6] A square of the agar culture is cut out and is then covered with a coverslip carrying the stain. The colonies are present in their entirety in the preparations and can be identified with certainty.

Demonstration of P.P.L.O. is usually not possible in the presence of abundant bacterial growth. The P.P.L.O. are resistant to sulfonamides and to penicillin both of which were recommended to suppress bacterial growth. Salaman used penicillin cups for this purpose.[5] The author can confirm the

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