January 26, 1893.

Sir JOHN EVANS, K.C.B., D.C.L., LL.D., Vice-President and Treasurer, in the Chair.

A List of the Presents received was laid on the table, and thanks ordered for them.

The following Papers were read:—

I. "On the Physiology of the Embryonic Heart (Preliminary Communication)." By J. W. PICKERING, B.Sc., Assistant Demonstrator in Biology at St. Bartholomew's Medical School. Communicated by Professor HALLIBURTON, F.R.S. Received November 25, 1892.

(From the Physiological Laboratory of King's College, London.)

The object of the following experiments has been to study the effect of varying conditions on the heart previous to the development of a nervous mechanism, and thus to throw some light on the discussion as to the relative importance of the two factors in the heart's action, viz., the contractile tissue and the nervous elements. The heart I have used is that of the chick* at a period of incubation of seventy-two hours at a temperature of 38° C. In some cases the embryos have been a few hours older or younger. The embryo is not removed from the egg, but a window is cut 3 cm. square through the shell and shell membrane, exposing the albumen and blastoderm, which remain undisturbed; the egg and embryo is fixed in a small chamber surrounded on five sides by a water-jacket. The uppermost side is covered with glass, while the air of the chamber is kept moist by the evaporation of water from a small bowl placed inside it. The temperature of this chamber can be kept constant or varied at pleasure. My experiments have fallen under three main heads: 1. The results of varying the temperature. 2. The introduction of drugs. 3. Electrical stimulation. In my full paper the results will be shown in tables giving the number of heart beats per minute, the peculiarities in the beat, when such exist, being duly noted. At present, however, I am only prepared to give an abstract of the results obtained, in so far as temperature and drugs are concerned. The electrical experiments are not yet completed.

* Observations are being carried on upon the mammalian embryo *in situ*.

1. *Temperature.*

Each embryo has an individual rhythm of its own, which, if the conditions are constant, remains unaltered, but different embryos, even of the same age, may have different rhythms, so that it is necessary to determine for each embryo its normal rhythm before variations can be studied. An embryo's heart, aged seventy-two hours, at a temperature of 31° C., was beating with a regular rhythm of 84 per minute. The temperature of the air of the chamber was rapidly raised to 42° C., when the rhythm rose to 91 per minute. A further rise to 50° C. increased the rhythm to 128, it still remaining regular. The temperature was then rapidly lowered to 26° C., when the rhythm fell to 114 per minute. A further fall to 16° C. reduced the rhythm to 34 per minute. The temperature was then raised to 46° C., when the rhythm rose to 117° per minute. On again letting the temperature fall to 25° C., the rhythm fell to 36° per minute.

The above experiment, taken as an instance from several, shows that, other factors being constant, the rhythm of the embryonic heart varies directly with the temperature of the surrounding medium.

Extremes of temperature stop the heart; thus exposure to a temperature of 10° C. causes the beats to become weaker and slower, and finally to stop in diastole. If the air of the incubator be raised above 50° C., the beats become so rapid as to be uncountable. They are feeble, and the heart is pale, due to the passage of less blood through it than in the normal state. Violent systolic spasms alternate with periods of quiescence. It stops in an expanded condition when the surrounding temperature is about 55° C. Lowering the temperature restores the beating, but the heart is enfeebled. If the temperature is raised much above this limit the heart is killed. Mechanical stimulation of the heart in standstill, due to either extreme of temperature, if applied at the ventricular end, gives rise to one or more waves of contraction, commencing from the auricular end, and showing the direct conduction through the fibres of the heart. The heart will respond to auricular stimulation when irresponsive to ventricular stimulation. Small variations of temperature, such as one or two degrees, occurring over a long period of time, as in an hour, do not affect the rhythm.

2. *The Introduction of Drugs.*

The drugs employed were applied directly to the heart substance at the temperature of the embryo, and dissolved in normal saline (0·65 per cent. sodium chloride) solution.

a. *Caffeine.*—An embryo, aged sixty-eight hours, at 33° C. had a rhythm of 88 per minute. To its heart 0·00015 gram* of caffeine was

* All weights of drugs used are expressed in grams.

administered, and in two minutes the rhythm rose to 100 per minute, and remained constant for two and a half minutes, when it fell to 96 per minute. A second dose of 0·00015 gram raised the rate to 102 per minute. The beats were also of greater force, since more blood was seen passing through the heart. A dose of 0·0025 gram was fatal. When given to an embryo, aged seventy-five hours, at 37° C., beating with a rhythm of 116 per minute, it reduced the rhythm, after one minute's action, to 100 per minute. The beats, however, remained very strong. After one minute forty-five seconds' action the heart stopped in strong systole, but started again and gave a few powerful beats. After the drug had acted nine minutes thirty seconds the heart stopped permanently in powerful contraction. Caffeine, therefore, acts directly on the cells of the embryonic heart.

b. *Strychnine* was given to a seventy hours' embryo in a dose of 0·000017 gram, and depressed the rhythm of the heart from 112 per minute to 52 per minute. There was no spasm. In an eighty hour embryo, at 39° C., a dose of 0·00002 gram temporarily increased the rhythm, both in force and number of beats; then the systole rapidly became weakened and the rhythm irregular. A further dose of 0·00002 gram still more rapidly reduced both force and frequency of beating, till death in diastole occurred.

c. *Morphine acetate*, if given in doses of 0·0001 gram, is a powerful depressant. With a dose of 0·0002 gram, after one minute's action on an eighty-five hours' embryo at 40° C., irregularities and slowing were obtained; after two minutes' action the beating stopped, but went on again, the waves of contraction sometimes passing from ventricle to auricle, and at others in the normal direction. Periods of rest alternated with violent bouts of rapid beating.

d. *Veratrine.*—Doses of 0·0001 gram increase the number of beats per minute. Larger doses may cause, temporarily, an increase of rhythm, but soon depress the heart by greatly lengthening the systole, which becomes very weak while the diastole is complete. The heart stops in an expanded condition. The heart of a seventy-two hours' embryo that had stopped in diastole, after a dose of 0·0005 gram, was restored by the application of 0·01 gram of potassium chloride almost to its normal rhythm. This agrees with Ringer's observation on the frog's heart.

e. *Potassium chloride*, when applied in a dose of 0·005 gram to an embryo aged seventy-two hours, reduced the normal rhythm of 76 per minute to 60 per minute. A further dose of 0·01 gram reduced the rhythm to 64 per minute. After the administration of a total amount of 0·07 gram of the substance, the heart stopped in diastole.

f. *Nicotine*, in very minute doses, stimulated the embryonic heart; $\frac{1}{4}$ c.c. of a solution containing $\frac{1}{2}$ c.c. of nicotine to 100 c.c. of normal

saline was a stimulant; with $\frac{1}{2}$ c.c. the frequency and force of the heart diminished, systole becoming almost absent, while the heart was finally paralysed in diastole. The addition of 0·03 gram of potassium chloride restored the heart to almost its normal rhythm, the beats at the same time becoming strong, both as regards systole and diastole. A further dose of nicotine depressed the heart, and again brought it into diastolic stoppage, the systoles having become weaker and weaker. There was no spasm.

g. *Atropine.*—Doses of 0·001 gram had, in a sixty hours' embryo, a slightly depressant effect, and even after 0·006 gram had been administered, the rhythm of the heart had only fallen from 96 to 72 per minute. In a seventy-two hours' embryo, with a heart beating at 116 per minute, 0·012 gram, after three minutes' action, had depressed the rhythm to 80 per minute, while even after the administration of 0·275 gram the rhythm was strongly maintained at 64 per minute.

h. *Muscarine Nitrate.*—To the heart of a seventy-two hours' embryo at 35° C., which was beating with a rhythm of 90 per minute, 3 drops of half saturated solution of muscarine nitrate were applied; the rhythm remained constant for two minutes, after which period 2 more drops were added, and the rhythm kept constant at 94 per minute during the next three minutes, after which period 4 more drops were added, and the ensuing rhythm was 93 per minute; 2 drops of saturated solution were then added, which was so concentrated as to stain the embryo brown. During the following five minutes the rhythm was constant at 84 per minute, each beat remaining normal in direction and force. Two more drops of saturated solution caused slight irregularities, but the rhythm during the next seven minutes averaged 72 beats per minute. Finally 2 more drops of saturated solution were added, and during the following seven minutes the heart's rhythm was 75 per minute. The whole experiment lasted thirty minutes, and 10 drops of half saturated *plus* 9 drops of saturated solution of muscarine nitrate were administered. A control experiment with the hearts of two frogs showed that the muscarine used stopped their beats, which were typically restored by atropine. In a similar experiment, witnessed by Professor Halliburton, with both embryonic and frogs' hearts, the rhythm of the former was maintained at 136 per minute, while the latter was stopped and subsequently restored by atropine. Identical results were obtained with a ninety-six hours' embryo. In an embryo aged seventy hours at a temperature of 30° C., which is subnormal in the chick, a rhythm of 92 beats was obtained after the application of 1 c.c. of half saturated solution for the following nine minutes, after which 1 c.c. of saturated solution was applied. This was fatal to the heart, almost instantly coagulating the tissues. There were no typical phenomena

of muscarine poisoning, and the application of atropine failed to restore the rhythm. Probably any strongly alkaloidal body in such a concentrated solution would produce a similar effect.

i. *Schmiedeberg's Digitalin.*—An embryo aged seventy-two hours at 30° C. had a heart rhythm of 132 per minute. To it 1 c.c. of normal saline containing 0·000022 gram of digitalin was applied. During the next eleven minutes the rhythm remained constant, after which time 1 c.c. containing 0·00005 gram was added, which produced no change in the rhythm; then 0·0001 gram was put in, and after one minute's action the frequency of the rhythm had fallen to 92 per minute, but both the systole and diastole were strong. The rhythm after six minutes' action rose to 104 per minute. After this another 0·0001 gram was added, and the rhythm fell after two minutes' action to 50 per minute. The systole was typically perfect, but the diastole was incomplete. The whole heart after two minutes' more action of the drug became very pale and in a state of tonic contraction with very feeble fluttering diastoles, which faded away, leaving the heart stopped in a contracted condition.

j. *Strophanthin* (of Merck's manufacture).—A seventy-two hours' embryo at a temperature of 32° C. had a heart rhythm of 132 per minute. A dose of 0·00006 gram did not alter the rhythm. A second dose of the same amount after twenty minutes' action reduced the rhythm to 54 per minute; both systole and diastole were regular and complete. Five minutes after this the diastole became irregular, and the systole was more marked than in the normal condition. After another minute had elapsed the ventricle passed into a state of tonic contraction with a few feeble beats, in which the diastole was very weak. The auricles had a marked diastole and a weak systole, and were engorged with blood. During the next five minutes the auricle had a rhythm averaging 24 beats per minute, while the ventricle remained in tonic contraction. Finally, forty-one minutes after the administration of the dose the auricle stopped in diastole, the ventricle remaining in tonic contraction. The auricles responded by 10 beats to a mechanical stimulus; the beats did not extend to the ventricle. Six minutes after this the auricle responded to mechanical stimuli, the wave of contraction passing either from the ventricular end to the auricle or *vice versâ*, according to which end of the auricle the stimulus was applied.

In larger doses of 0·0002 gram the rhythm in a seventy hour embryo at 33° C. was depressed from 120 to 102 per minute, the systole becoming very strong and the diastole imperfect. After four minutes' action the rhythm returned to the normal both in frequency and force. To the same embryo 0·00025 gram was then added, when after one minute's action the auricle dilated, giving small twitch-like contractions, while the ventricle passed into tonic contraction. The

auricle remained for six minutes feebly responsive to mechanical stimuli.

k. *Nitrite of Amyl.*—A ninety-six hours' embryo kept at 35° C. was subjected to the influence of the vapour of 5 minims of nitrite of amyl. After one minute's action the rhythm rose from 96 to 124, and after another minute fell to 112. After another minute it had fallen to 104, and six minutes afterwards was at the normal. In a seventy-two hour embryo at a temperature of 47° the rhythm was 124 per minute. A dose of 1 c.c. of solution of amyl nitrite dissolved in olive oil (strength being 1·5 c.c. of the drug to 10 c.c. of olive oil) was given, and the frequency of the rhythm fell in one minute to 112, but the beats were strong. Six minutes afterwards another c.c. of the solution was introduced, and the rhythm fell to 104, but was strong. Three minutes later another c.c. was put in, and the rhythm rose to 112, but was very weak and irregular, and finally before death the rhythm was reversed.

Concluding Remarks.

The observations here recorded show that the embryonic heart when kept under favourable conditions reacts in a very delicate manner to all those classes of stimuli which influence the adult heart. The experiments on temperature show that its variations act directly on the cardiac muscle, and thus confirm the opinion of Newell Martin* and others who have arrived at the same conclusion from experiments on the adult heart.

The action of caffeine, morphine acetate, potassium chloride, veratrine, nicotine, digitalin, strophanthin, and amyl nitrite is direct on the contractile tissue of the embryonic heart. This greatly favours the view that they act direct on the adult cardiac muscle. It will be noted that many of the actions here described on the embryonic heart are almost identical to those observed by others on the adult heart. Notoriously so is the antagonism between veratrine and potassium chloride, where my observations are identical with those of Ringer† on the frog's heart. A similar antagonism exists between nicotine and potassium chloride. The remarkable correspondence of my results with strophanthin on the embryonic heart with those of Professor Fraser‡ on the frog's heart greatly supports the view of that observer as to the direct action of strophanthin on cardiac muscle without the intervention of any nervous mechanism, and, further, the absence of diastolic stoppages in my experiments also supports Fraser's view that that condition in the frog's heart is due to the

* Newell Martin, 'Phil. Trans.,' 1883, p. 663.

† Ringer, 'Practitioner,' vol. 30 (1883), p. 17.

‡ Fraser, 'Edinburgh Roy. Soc. Trans.,' vol. 36 (1890-91), Part II, p. 388, *et seq.*

action of small doses of strophanthin on the cardiac nervous mechanism of that animal.

The lengthening out of the systole in veratrine poisoning corresponds to the same well-known lengthening of the systole in the frog's heart under veratrine. The reversing of rhythm observed in morphine poisoning is similar to that mentioned by Ludwig* as occurring in the mammalian ventricle when under the influence of opium, for then the auricular beats follow instead of precede the ventricular beats, the rhythm being reversed. The same occurs in amyl nitrite poisoning.

Krukenberg† has stated that neither atropine nor muscarine affects the heart of Ascidians.

My observations on the action of atropine and muscarine, which have been made on a large number of embryos, show that in the absence of a nervous mechanism they do not influence the heart. This will probably modify the current views on the action of these drugs, and my results show that the method I have adopted is a valuable one for differentiating the functions of cardiac muscle from those of the nerves which supply it.

II. "Further Researches in Connexion with the Metallurgy of Bismuth." By EDWARD MATTHEY, F.S.A., F.C.S., Assoc. Roy. Sch. Mines. Communicated by Sir G. G. STOKES, Bart., F.R.S. Received November 21, 1892.

In 1886–87 and in 1890 I submitted papers to the Royal Society bringing under notice facts which had come to my knowledge whilst engaged upon the practical extraction of this beautiful metal from its ores, and in its separation from impurities which are always associated with it when in a crude or unrefined state.

IV. *Bismuth, its Separation from Arsenic.*

In a paper dated February 10, 1887,‡ allusion is made to the fact that arsenic is often one of these impurities, and at the same time a method is given by which the separation of this metal from bismuth was then successfully effected.

The process adopted when that paper was read, and for a considerable period subsequently, when working upon bismuth containing arsenic, consisted in removing the arsenic by fusing the arsenical

* Ludwig, 'Lehrbuch der Physiol. des Menschen,' Bd. 2 (1861), p. 38.

† Krukenberg, quoted in Brunton's 'Text-Book of Pharmacology,' &c. (3rd edition, p. 114).

‡ 'Proc. Roy. Soc.,' vol. 49.