his skull who would have been willing to come with me on the Monte Rosa Expedition, but I was not successful in my search. Some other physiologist will, I hope, be luckier."

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PRODUCTION OF FIBRILLATION IN ISOLATED ATRIA OF RABBIT HEART

BY

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The atria of the hearts of small animals can be dissected from other tissue and suspended in a bath containing some modification of Ringer's solution, and in this bath they maintain their rhythmic contractions. A few observations have been made of the production of arrhythmias in such preparations. Dawes and Vane (1951) studied the guinea-pig atria at 37° C., stimulating them electrically at a rate slightly greater than the spontaneous rate. They observed that if a test-pulse was thrown in just outside the absolute refractory period, the atria often responded by repetitive discharges at a frequency much greater than the normal rate of beat. These discharges continued for periods up to one hour. DiPalma and Mascatello (1951) made similar observations in cat atria at 29° C., though the repetitive discharge was of shorter duration. West, Turner, and Loomis (1954), in the course of studies on excitability and conduction time, noted the occurrence of arrhythmias when the interval between two stimuli was near the absolute refractory period; these lasted from a few seconds to several minutes, and they said that the arrhythmias occurred regularly in the presence of acetylcholine.

Burn, Vaughan Williams, and Walker (1955) observed that during the infusion of acetylcholine into the heart-lung preparation of the dog stimulation of the right atrium resulted in fibrillation, which persisted when stimulation ceased. The fibrillation continued so long as acetylcholine was infused, and could be terminated at will by stopping the infusion or by injecting atropine. In a later paper

FIG. 1.—Broken lines show a glass tube, through which a pair of shielded and insulated wires run, terminating in platinum electrodes. The two limbs of the U are joined by a solid piece of glass, sloping as shown, to which the lower ends of the atria are attached. The electrodes pierce the atria.

Burn, Gunning, and Walker (1956) showed that the fibrillation was also stopped by a slow infusion of KCl.

We now describe the production of fibrillation in the isolated atria of the rabbit by stimulation in the presence of acetylcholine.

Method

Rabbits weighing 2–3 kg. were used. They were stunned by a blow on the head, and the hearts were removed and placed in Ringer's solution of the following composition: NaCl, 9 g.; KCl, 0.4 g.; CaCl₂, 0.24 g.; NaHCO₃, 0.5 g.; dextrose, 2 g.; distilled water, 1 litre. The atria were dissected free from fat and other tissue, attached to an electrode (see Fig. 1) designed by Mr. O. B. Saxby, of this department, and suspended in a bath of the solution described which was aerated with oxygen and maintained at 30–31° C. A mechanical record was taken on a kymograph. The atria were stimulated by square wave pulses of 0.75 msec. duration and of 6–10 volts strength. Surface electrograms were recorded from the stimulating electrodes on a Cossor machine (model 1314).

Production of Arrhythmia

In the initial experiments, atria, suspended in the bath, were stimulated at frequencies varying from 200 to 1,200 a minute for different periods. In all preparations the normal rhythm returned immediately on cessation of stimulation. Low concentrations of acetylcholine had very little effect on the sequence of events. With concentrations greater than 3×10^{-4} g. per ml. repetitive charges of brief duration, from 2 to 5 seconds, were occasionally observed after stopping the stimulation, but persistent arrhythmia was not seen.

In further experiments the concentration of KCl in the solution was reduced to one-quarter (100 mg. per litre). Stimulation still failed to produce arrhythmia in the presence of low concentrations of acetylcholine, but, when the concentration was greater than 3×10^{-4} g. per ml. and the frequency of stimulation was greater than 600 a minute, arrhythmia then persisted, as demonstrated in Fig. 2, which shows the electrogram record.

The electrogram in $\frac{1}{4}$ K solution is shown after stimulation in (c) and on addition of acetylcholine in (d). Stimulation in the presence of acetylcholine then caused fibrillation as in (e), continuing six minutes later as in (f). When the acetylcholine was removed by changing the bath fluid a normal rhythm returned as in (g).

Once the procedure which has been described was followed, flutter or

FIG. 2.—(c) E.C.G. in $\frac{1}{4}$ K solution after stimulation; (d) beats arrested by addition of acetylcholine; (e) fibrillation one minute after stimulation in presence of acetylcholine; (f) six minutes later; (g) after washing out acetylcholine.

fibrillation was observed in all atria tested. Flutter was observed in 43%, fibrillation in 40%, and a mixture of flutter and fibrillation in the rest. In one preparation fibrillation was observed for 45 minutes and was then terminated by removing the acetylcholine from the bath (see Fig. 3). Flutter or fibrillation was produced with regularity in either the right or the left atrium.

FIG. 3.—(a) E.C.G. in normal solution; (b) after changing to $\frac{1}{4}$ K solution; (c) one minute after stimulating in presence of acetylcholine 6×10^{-4} g. per ml.; (d) four minutes later; (e) 20 minutes later; (f) 35 minutes later; (g) 45 minutes later; (h) after removing acetylcholine.

Stimulus Frequency

The effect of stimulus frequency on the initiation of atrial fibrillation was carefully examined. The results are summarized in Fig. 4. Acetylcholine was added in a concentration of 3×10^{-4} g. per ml. in all trials, and atria were counted as fibrillating when the fibrillation persisted for three minutes after stimulation stopped. A low frequency was used first, and at the end of one minute's stimulation the stimulus was turned off for 10 seconds to see if an arrhythmia was persisting; if not, the frequency was increased and the stimulation continued. Fig. 4 shows that the commonest threshold frequency was between 500 and 600 a minute.

FIG. 4.—Effect of stimulus frequency on the initiation of fibrillation in isolated atria. Solution containing $\frac{1}{4}$ K, acetylcholine 3×10^{-4} g. per ml. On the abscissa is shown the rate of stimulation per minute. On the ordinate is the percentage of preparations in which fibrillation was produced. From 10 to 45 atria were used for each point.

Action of Atropine and Quinidine

Observations were made to test the effect of atropine and quinidine. By stimulation at 1,200 a minute in the presence of acetylcholine (3×10^{-4} g. per ml.) fibrillation was produced, and was observed for five minutes. It was then terminated by washing out the acetylcholine. Fifteen minutes later the fibrillation was induced again, and one minute after stimulation ceased atropine or quinidine was added to the bath. When atropine was added, fibrillation stopped within 30 seconds (see Fig. 5). When quinidine was added, fibrillation stopped after one to two minutes.

Discussion

The production of fibrillation in the isolated atria of the rabbit depends on three factors: the presence of a low concentration of potassium in the bath fluid, the presence of a high concentration of acetylcholine, and electrical stimulation at a high rate for a short period.

When the atria contract sodium enters the cells, and then, in order that they may become excitable once more, potassium leaves the cells. This potassium must then be replaced within the atrial cells by metabolic processes which occur in the pores of the cell membrane. If the external concentration of potassium is low the replacement of potassium within the cells becomes very difficult, and it is probably for this reason that the low potassium concentration favours fibrillation.

The presence of a high concentration of acetylcholine makes the cell membrane much more permeable to potassium (Harris and Hutter, 1956) and it greatly shortens the refractory period. Under normal conditions, when a contraction occurs there is a long interval (0.15 second) before excitability is restored and the fibres become re-excitable. In the presence of acetylcholine this interval is shortened, perhaps to 0.01 second (Hoffman and Suckling, 1953). If now stimulation is applied at a high rate (700 a minute) some of the fibres are probably unable to keep in phase, and some contract later than others. If these others have recovered their excitability very quickly (in the presence of acetylcholine) they may be stimulated by the ones contracting a little later. Thus fibrillation begins, and is perhaps a condition in which each fibre in contracting stimulates its neighbours. This process may go on indefinitely, until indeed the acetylcholine is removed and the time required for each fibre to recover excitability again becomes much longer.

The fibrillation process may, however, be related to a high net loss of potassium, for when the external potassium concentration is raised fibrillation is arrested (Burn, Gunning, and Walker, 1956).

Summary

Fibrillation can be produced in the isolated atria of the rabbit heart, using rabbits from 2–3 kg., by stimulation at frequencies from 500 to 800 a minute in the presence of acetylcholine (3 to 8×10^{-4} g. per ml.). The bath fluid should contain not more than 100 mg. of KCl per litre. The fibrillation continues after the

FIG. 5.—(c) E.C.G. in $\frac{1}{4}$ K solution after stimulation; (d) effect of adding acetylcholine 8×10^{-4} g. per ml.; (e) 30 seconds after stimulation stopped; (f) arrest of fibrillation by atropine (1×10^{-6} g. per ml.); (g) normal rhythm two minutes later.

stimulation stops. It can be arrested by removing acetylcholine from the bath or by adding atropine or quinidine.

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POLIOMYELITIS ANTIBODY STUDIES IN A GROUP OF LONDON CHILDREN

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In any virus disease in which subclinical infection takes place the distinction between those who have acquired natural immunity, and therefore have less pressing need for immunization, and those who are still susceptible is difficult. In this respect poliomyelitis stands in marked contrast to measles. It is known that recovery from paralytic poliomyelitis is followed by a prolonged state of immunity in that second attacks of paralysis are rarely encountered, but immunity also develops after non-paralytic and subclinical infection. It has now been clearly established that active immunity in all forms of poliomyelitis infection is closely associated with the presence of circulating antibody; moreover, the incidence of antibody in relation to different age groups has proved to be a reliable guide to the overall immune status of a population.

In the past, serological tests to determine this information were based almost exclusively on the incidence of type 2 antibody, as only one strain (Lansing, type 2 strain) could be handled with any convenience in the laboratory. Since the introduction of tissue-culture methods it is possible to examine sera for antibodies to all three types of poliomyelitis virus and thus gain an overall picture of the pattern of immunity, not only throughout the world but in different sections of a population. From the evidence so accumulated it is now apparent that the incidence of infection with each of the three types is much higher than would be assumed from the incidence of clinical disease, and varies greatly from one part of the world to another (Gear, 1955). For instance, 80% of children in Cairo have by the age of 3 years acquired antibodies to all three virus types. At the same time the evidence of clinical disease is low. In contrast, in an urban population in the United States the same figure of 80% is not reached until the 30–40 age group (Paul, 1955).

The present investigation was undertaken as a preliminary to the introduction of immunization with

poliomyelitis vaccine in the United Kingdom. The purpose of the study was first to determine the incidence of poliomyelitis antibodies (and therefore the inapparent infection rate amongst healthy children); and, secondly, to form some idea of the age at which natural infection begins to take place in such a group. A further purpose was to determine whether the complement-fixation test could be used as a screening test for evidence of past infection.

Materials and Methods

The Study Group.—Ninety children were included in the study during the period March–October, 1955. They were children who had been admitted to the Victoria Hospital for Children with minor medical or surgical complaints. There were approximately equal numbers in each yearly age group from 12 months to 10 years. In no case was there a history of contact with a known clinical case of poliomyelitis, nor of a febrile illness with signs of meningeal involvement. The majority of the children came from the Boroughs of Battersea, Chelsea, and Fulham and, except for three who had been abroad for a short time, had lived nowhere else. The socio-economic background of these children was assessed from the occupation of the father, the intelligence of the parents, and the care given to the child before admission, and was graded as poor, average, or good. The distribution of children from poor, average, and good homes was uniform throughout the age groups.

Sera.—A single specimen of blood was collected from each child. After separation from the clot, sera were divided into two parts; one part for neutralization tests was stored at -65°C ., the other for complement-fixation tests at 4°C .

Virus Strains.—The following strains were used both for complement-fixation and for neutralization tests: Mahoney (type 1), MEF-1 (type 2), Saukett (type 3).

Complement-fixation Test.—In order to economize in material the "drop method" on "perspex" sheets was employed as described for use with poliomyelitis viruses by Svedmyr, Enders, and Holloway (1953) and by Le Bouvier (1953).

Antigens.—These were prepared from monkey-kidney tissue culture and HeLa-cell cultures inoculated with each virus type. The fluids were harvested when cellular degeneration was complete. In the early stages of this investigation complement-fixing antigens were heated to destroy infectivity, but when it became clear that heating resulted in a loss of specificity, subsequent tests were carried out with untreated antigens, special precautions being taken to avoid risk of infection to those carrying out the tests. After inactivation serial twofold dilutions of serum were made in Ca-Mg saline; 4 units of antigen and 2, 2½, and 3 units of complement were used in each test. Fixation was allowed to proceed overnight in the refrigerator. The end-point was taken as the highest dilution of serum producing 50% fixation of antigen with 2½ units of complement.

Neutralization Test.—Preliminary tests were made with the HeLa-cell tissue culture method described by Syverton, Scherer, and Elwood (1954), but, as the results in our hands were at times variable, it was decided to carry out all subsequent tests in monolayer cultures of monkey-kidney epithelium grown in synthetic medium 199 containing 2% horse serum (Youngner, 1954). Monkey-kidney culture tubes prepared seven to eight days previously were examined before use to ensure that sheets of monolayer cells were present. The growth medium was replaced by fresh synthetic medium 199 without horse serum.

Virus.—Types 1, 2, and 3 poliomyelitis viruses from monkey-kidney tissue cultures were diluted so as to contain 100 tissue-culture infective doses previously determined by titration.

Serum Dilutions.—After inactivation serial dilutions of serum were made in medium 199. Screening tests were carried out at 1/4, 1/64, and 1/256; and final tests, if