J. Physiol. (1953) 120, 122–128

SOME OBSERVATIONS UPON ISOLATED PERFUSED HUMAN FOETAL HEARTS

By J. B. E. BAKER

From the Department of Physiology, Charing Cross Hospital Medical School, London

(*Received* 29 *September* 1952)

There appears to be little previous record of direct physiological experiment upon human foetal hearts. Lloyd (1929) investigated the effect of calcium concentration upon two hearts perfused by Langendorff's method, and Garrey & Townsend (1948) observed the action of adrenaline, acetylcholine, eserine and atropine upon the isolated chambers of one heart. Patten (1949), even in a review article on the early heart beat in vertebrate embryos, mentions no work on human material.

In the present series of observations nine hearts were used from human foetuses of 16–24 weeks, obtained from operations for the termination of pregnancy in women with advanced tuberculosis or cardiac disease. Only one foetus had any obvious abnormality in that its aortic valves were so incompetent that satisfactory perfusion by Langendorff's method was impossible.

Experiments were made upon whole perfused hearts to determine (i) the potency of noradrenaline relative to that of adrenaline in causing increased amplitude of ventricular beat, (ii) the qualitative effects of acetylcholine, adrenaline, and noradrenaline upon coronary flow, and (iii) whether ouabain enhances the action of acetylcholine in slowing the heart. Isolated auricle preparations were used to determine (iv) whether acetylcholine or adrenaline would start the beat of isolated auricles allowed to beat until they stopped spontaneously.

METHODS

Foetuses either still *in utero* or at least with membranes intact were transported immersed in NaCl 0·9% (w/v). Upon opening the chest some spontaneous cardiac activity was still visible in every instance, varying from feeble slow auricular movements when transport took $2\frac{1}{2}$ hr to fairly vigorous beating with 3:1 auriculo-ventricular block when transport took only $\frac{3}{4}$ hr. Excision of the hearts and perfusion by the Langendorff method resulted in regular and forceful beats always within 20 min and usually within 5 min, the time for recovery not being obviously related to time since the operation. Perfusions were maintained at a pressure of 30 cm H_2O with Locke's solution at 37° C and equilibrated with 97% O_2 and 3% CO_2, except in the case of three hearts which were

perfused with Tyrode's solution equilibrated with 95% O_2 and 5% CO_2. These three were used later for the isolated auricle experiments for which it was desired to duplicate the solution used by Bülbring & Burn (1949).

The apparatus used has already been described (Baker, 1951), essential features being that the heart is kept jacketed at the same temperature as the perfusion fluid. Coronary flow changes cannot alter the temperature at which the heart receives fluid, and are measured by drip chamber between heart and reservoir of Locke's solution. Recording of ventricular beats is by spring-loaded lever on a kymograph. After regular beats were established all hearts were left for 45 min during which the amplitude of beat increased steadily and then reached a constant level. Drug injections were made in 0·2 ml. NaCl 0·9% (w/v). Doses of adrenaline and noradrenaline refer to the L-isomer base, the salts used being the hydrochloride and the bitartrate monohydrate respectively.

RESULTS

Relative effect of adrenaline and of noradrenaline upon amplitude of beat

Since hearts did not give a constant response to repeated injection of the same dose after perfusion for about 2 hr, and since even the smallest dose could exert effects lasting for 10 min, it was decided not to compare adrenaline and noradrenaline by attempts to obtain closely matched responses but by constructing dose-response curves, with the emphasis on that for adrenaline. Amplitude of contraction was measured just before drug injection and again at the time of maximal response, and the effect tabulated as percentage change in amplitude. The results in Table 1 are from comparisons on four

TABLE 1. Mean increase (%) in amplitude of ventricular beat. Bracketed figures indicate number of hearts

Dose (μg) ...	0·005	0·01	0·02	0·05	0·1	0·5	1·0
Adrenaline	15 (3)	25 (3)	33 (3)	59 (5)	65 (5)	—	—
Noradrenaline	—	6 (1)	—	27 (2)	30 (1)	46 (1)	78 (1)

hearts, together with results from two more hearts included for the adrenaline curve only, since there was no noradrenaline available when they were obtained. Too few readings were obtained with doses of noradrenaline to provide a satisfactory dose-response curve, but by plotting the curve for adrenaline and interpolating the responses for the noradrenaline doses it was found that noradrenaline was from 3 to 16 times less active than adrenaline upon amplitude of contraction. Results obtained on one further heart are excluded from Table 1 as the sensitivity of the heart was four times that of the others; even 5 mμg adrenaline increased its amplitude by 115%. Noradrenaline was 28 times less active than adrenaline on amplitude in this heart.

As regards relative effect of these agents in increasing rate of beat, data are not being presented. Under these conditions of experiment the increases of rate were so transient that there was no interval of constant rate long enough to yield a reliable count. Even by choosing a set interval, namely the half minute immediately following the onset of effect, results were so inconsistent that counting was abandoned.

Since the human foetal hearts gave measurable responses with injections of as little as 5 mμg of adrenaline the question arises whether this great sensitivity is something peculiar to the foetus or whether the adult heart would behave similarly. It was thought that suggestive evidence might be obtained from comparing foetal and adult heart responses of other species, since suitable adult human hearts were unlikely to be obtained. Upon the hearts of two pregnant cats and those of their six foetuses (a week before the expected end of gestation) adrenaline was equally effective upon rate and amplitude and the coronary vessels were dilated. However, in the only experiment so far upon

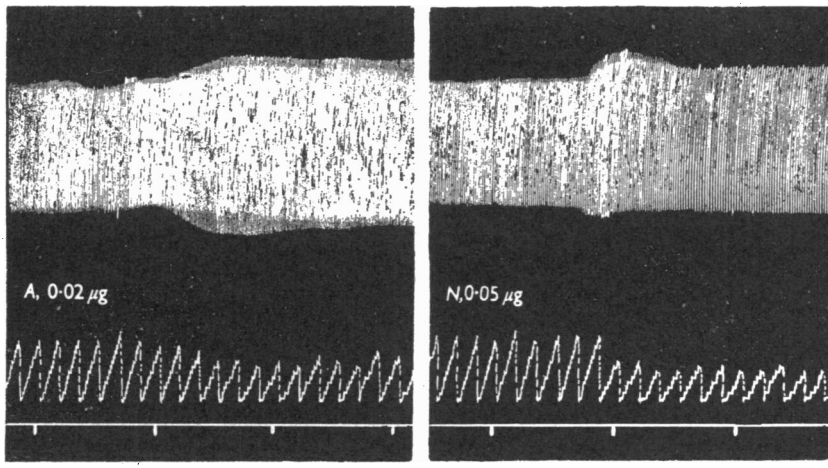

Fig. 1. 18 week-human foetal heart. Langendorff perfusion. Systole downwards. Coronary flow in drops/10 sec. Time marker in minutes. Injection of adrenaline 0·02 μg (*A*) and noradrenaline 0·05 μg (*N*) both caused marked diminution of coronary flow. The noradrenaline was the less effective upon amplitude of beat.

the hearts of a pregnant guinea-pig and its two foetuses (about 10 days before gestation would have been completed) adrenaline was equally effective upon amplitude of beat but was less effective in the foetuses upon rate of beat, and the coronary vessels were constricted in both. It was possible to observe accurately effects upon rate of beat in these experiments because the adrenaline effect was prolonged by adding the drug to the reservoirs of perfusion fluid, concentrations of 5 and 40 mμg/ml. being used.

Coronary flow

Both adrenaline and noradrenaline diminished coronary flow in seven of eight human foetal hearts and usually appeared equipotent in this respect though noradrenaline was sometimes the weaker. A typical result is shown in Fig. 1. In the remaining one they were either without effect or very slightly increased the flow. Acetylcholine 0·1–1·0 μg was injected into four hearts, and in every instance it diminished the flow.

Influence of ouabain upon acetylcholine-induced slowing

In all three hearts tested acetylcholine 0·1–0·4 μg produced progressively greater degrees of slowing whilst the perfusion fluid contained ouabain 0·2 μg/ml. A typical result is shown in Fig. 2. It is interesting to note that the

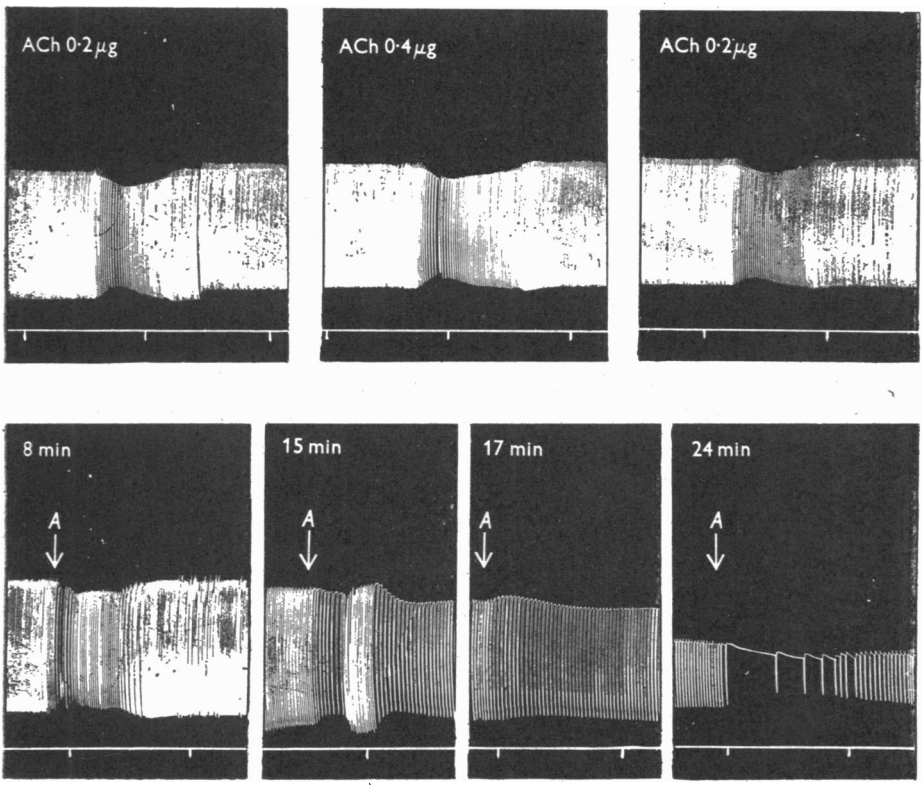

Fig. 2. 24 week human foetal heart. Langendorff perfusion. Systole downwards. Time marker in minutes. Upper row, sensitivity to injected acetylcholine. Lower row, increasing effect upon rate by injections of 0·2 μg acetylcholine (*A*) repeated at the minutes as indicated after commencing perfusion with ouabain 0·2 μg/ml. At 15 min the rhythm became idioventricular after a brief recovery from the acetylcholine. The idioventricular rate was little affected by the acetylcholine at 17 min yet profoundly affected by injection at 24 min.

effect was quickly apparent whilst the ventricular rate was still under auricular control, but that when complete auriculo-ventricular block supervened the subsequent injection of acetylcholine hardly affected ventricular rates at first. Then, within about 15 min, acetylcholine in the same dose as before caused ventricular slowing which was more pronounced upon each repetition.

Isolated auricles

After revival by Langendorff perfusion, the auricles from three hearts were suspended in baths of oxygenated Tyrode's solution at 29° C. Acetylcholine and adrenaline doses had the same effect as upon isolated rabbit auricles. Overnight the baths were left at room temperature and during the night the auricles stopped beating. When the temperature was raised to 29° C again next morning the auricles failed to start beating, even when left for an hour. Nor did they start beating when acetylcholine 0·1–1·0 mg was added, either when the drug was left in contact for up to 30 min or when the bath was washed out and a similar time allowed to elapse. However, two of the auricles started beating, feebly but regularly, when adrenaline 0·5 and 1·0 mg was added to the baths after acetylcholine had failed to start them and had been washed out. Such revived auricles responded to acetylcholine by the usual depression and slowing when it was added, and in the absence of adrenaline, they soon stopped again.

DISCUSSION

Cardiac tissue is more resistant to asphyxia than is generally supposed, and it seems to be largely forgotten that Kuliabko (1903) and Ossinowsky (1940) claimed to have revived children's hearts up to 30 and 24 hr after death respectively. With the above facts in mind, as well as the well-known resistance of foetal tissue to asphyxia, it does not seem remarkable that these human foetal hearts were easily revived by perfusion only 2½ hr after loss of maternal oxygenation. However, these hearts when perfused with Locke's or Tyrode's solution by Langendorff's method did become obviously and rather rapidly oedematous, and this possibly accounts for their responses to drugs becoming inconsistent within an hour or two.

The scatter of the results of adrenaline and noradrenaline upon amplitude of beat is wide. It is possibly partly because the foetuses were of varying ages, and results upon far more hearts are needed to settle the interesting point that sensitivity to noradrenaline may change during development. The dose-response curves for adrenaline and noradrenaline are also probably not parallel.

The most surprising effect of adrenaline and noradrenaline on the human foetal hearts was that they reduced the coronary flow instead of increasing it. From work upon perfused human hearts and isolated rings of human coronary vessels (Cruickshank & Rau, 1927; Kountz, 1932; Kountz, Pearson & Koenig, 1935; Anrep, 1936) it seems fairly well established that adrenaline usually dilates the coronary vessels of the child and adult, though not necessarily markedly, and Barbour (1912) found that adrenaline caused only constriction of isolated rings of human coronary arteries. Possibly the overall effect changes at some stage of foetal life, for, although in the foetal cats the coronary

vessels reacted to adrenaline as in the adult cat, by dilatation, these cat foetuses were within a week of birth, whereas the oldest human foetuses were only within 16 weeks of birth. It might be argued that changes in rate of beat affected the flow, yet late in experiments the flow was sometimes the only function which altered in response to injection. Further, Hammouda & Kinosita (1926) showed that, in the rabbit heart at least, wide alterations in rate of beat did not affect coronary flow.

The effect of cardiac glycosides in increasing the slowing of the heart induced by acetylcholine or by vagal stimulation has long been known to occur in lower animals (Cushny, 1925) and is generally accepted, though Wells, Dragstedt, Rall & Ruge (1943) deny that it occurs. It is of particular interest because it has never been proved to be due to an effect upon cholinesterase.

The failure to start any of the three isolated auricles with acetylcholine and the success on two of them with adrenaline after their spontaneous cessation of beat is not surprising. Bülbring & Burn (1949) found that acetylcholine started the beat in fourteen of twenty-two isolated rabbit auricles, but they also found that some auricles which were started with adrenaline and then stopped by washing it out could not be revived by acetylcholine.

SUMMARY

1. The hearts of nine human foetuses of 16–24 weeks have been revived by Langendorff perfusion.

2. In four hearts L-noradrenaline appeared to be from 3 to 16 times less active than L-adrenaline upon amplitude of beat.

3. Both L-adrenaline and L-noradrenaline constricted the coronary vessels in seven of eight hearts, and acetylcholine constricted them in all four tested.

4. Oubain 0·2 μg/ml. in the perfusion fluid enhanced the slowing effect of injections of acetylcholine.

5. Of three isolated auricle preparations allowed to beat until they stopped spontaneously acetylcholine started none and adrenaline started two.

I should like to thank Prof. W. J. Hamilton for giving me the hearts from human foetuses. The noradrenaline used was kindly given by Bayer Products Ltd.

REFERENCES

ANREP, G. V. (1936). Lane medical lectures: studies in cardiovascular regulation. *Stanf. Univ. Publ. med. Sci.* **3**, 267–288.

BAKER, J. B. E. (1951). An improved apparatus for mammalian heart perfusion. *J. Physiol.* **115**, 30–32*P*.

BARBOUR, H. G. (1912). The constricting influence of adrenalin upon human coronary arteries. *J. exp. Med.* **15**, 404–414.

BÜLBRING, E. & BURN, J. H. (1949). Action of acetylcholine on rabbit auricles in relation to acetylcholine synthesis. *J. Physiol.* **108**, 508–524.

CRUICKSHANK, E. W. H. & RAU, A. S. (1927). Reactions of isolated systemic and coronary arteries. *J. Physiol.* **64**, 65–77.

CUSHNY, A. R. (1925). *Digitalis and its Allies*, pp. 107–111. London: Longmans, Green and Co.

GARREY, W. E. & TOWNSEND, S. E. (1948). Neural responses and reactions of the heart of a human embryo. *Amer. J. Physiol.* **152**, 219–224.

HAMMOUDA, M. & KINOSITA, R. (1926). The coronary circulation in the isolated heart. *J. Physiol.* **61**, 615–628.

KOUNTZ, W. B. (1932). Studies on the coronary arteries of the human heart. *J. Pharmacol.* **45**, 65–76.

KOUNTZ, W. B., PEARSON, E. F. & KOENIG, K. F. (1935). Observations on the effect of vagus and sympathetic stimulation on the coronary flow of the revived human heart. *J. clin. Invest.* **13**, 1065–1078.

KULIABKO, M. A. (1903). Sur la reviviscence du cœur. Rappel des battements du cœur humain trente heures après la mort. *C.R. Acad. Sci., Paris*, **136**, 63–64.

LLOYD, W. D. M. (1929). The action of calcium on the isolated human foetal heart. *J. Pharmacol.* **36**, 185–193.

OSSINOWSKY, N. I. (1940). Versuche einer Wiederbelebung des isolierten Kinderherzens. *Acta paediat. Stockh.*, **27**, 233–244.

PATTEN, B. M. (1949). Initiation and early changes in the character of the heart beat in vertebrate embryos. *Physiol. Rev.* **29**, 31–47.

WELLS, J. A., DRAGSTEDT, C. A., RALL, J. E. & RUGE, D. A. (1943). Inhibition of the cardio-inhibitory action of acetylcholine by digitalis. *Fed. Proc.* **2**, 93–94.