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NUCLEIC ACID DERIVATIVES AND THE HEART BEAT.

By A. N. DRURY[1].

(*From the Department of Pathology, University of Cambridge.*)

In a previous paper in which the physiological reactions of various nucleic acid derivatives were compared [Bennet and Drury, 1931], it was stated that the perfused rabbit's heart was improved, as a preparation, when adenosine or adenylic acid was added to the perfusate. Drury and Szent-Györgyi [1929] had found that adenosine did not influence the mechanogram of the heart in the intact dog, and Wedd [1931] obtained the same result for the perfused rabbit's heart. On the other hand Rothman [1930] and Lindner and Rigler [1931] found that both adenylic acid and adenosine strengthen the heart beat.

Both adenosine and adenylic acid increase the flow through the heart [Wedd, 1931], and it is reasonable to conclude that this will, under certain circumstances, be accompanied by an increased amplitude of contraction. For this reason Bennet and Drury [1931] made no observations upon the mechanogram as they felt they could not distinguish between the effects due to the greater flow of perfusate through the heart and those associated with a specific action of the substances upon the musculature. More recently the general physiological properties of guanylic acid have been under investigation, and as this substance decreases the flow through the perfused rabbit's heart, observations have been made upon the mechanogram, and the results allow a much more definite idea to be formed of the manner in which nucleic acid derivatives influence the heart beat.

The rabbits' hearts have been perfused through a cannula inserted into the aorta in the usual manner, Locke-Ringer solution at a pressure of 30–40 cm. being used. The perfusate has been oxygenated by bubbling oxygen through it. In certain experiments the animal has been bled, under ether anæsthesia, prior to removing the heart, and after defibrination the blood has been added to the Ringer solution. The whole preparation has been housed in a moist warm chamber at 37° C., so that

[1] Working on behalf of the Medical Research Council.

the temperature of the heart remains constant throughout the observations. The coronary outflow has been recorded by collecting the fluid leaving the heart in a tipping bucket, while the substances have been injected into the tubing carrying the perfusate to the heart. The injections, usually 1 c.c., have been made slowly, and have been brought if necessary to a *p*H of 7·4–7·6 by the addition of sodium bicarbonate. The mechanogram has been recorded by attaching a thread to the right ventricle midway between the base and apex of the heart, and leading it to a lever whose movements have been damped by a rubber band. The apex of the heart has been firmly fixed to a rigid bar, the base being fixed by the aortic cannula, so that the lever records the movement of the right ventricular wall, free from swing of the heart. The rate of the heart has been maintained constant by passing rhythmic shocks through fish-hook electrodes, embedded in the right or left ventricle. The importance of maintaining a constant rate of beating, when observations are being made upon the mechanogram, has been clearly shown by Dale [1930]. It is of direct importance in these observations, as certain of the substances have a definite influence upon the rate of beating.

The influence of guanylic acid upon the coronary outflow.

The influence of adenylic acid, both yeast and muscle, adenosine, and guanosine upon the coronary outflow has already been reported [Wedd, 1931; Bennet and Drury, 1931]. The first three substances increase the outflow, while the last has no influence. Guanylic acid, on the other hand, decreases the outflow, though the doses necessary to bring this about are considerably higher than those required for the dilator substances to exert their effect. The result of injecting a series of increasing doses of guanylic acid is shown in Fig. 1, and an injection of 1–2 mg. of the substance invariably leads to a definite decrease in the outflow. Guanylic acid therefore differs essentially in this respect from the other substances already studied, and it can be safely assumed that any change in the mechanogram of the heart consequent upon the introduction of this substance cannot be ascribed to an increase in the coronary flow.

The influence of nucleic acid derivatives upon the mechanogram.

Four substances have been studied, namely, guanylic acid[1], guanosine[1], yeast adenylic acid[1] and adenosine[1]. Muscle adenylic acid[2] has also

[1] Prepared by British Drug Houses, London.

[2] Prepared from ox-heart muscle [Drury and Szent-Györgyi, 1929].

been tested upon one or two occasions. The experiments have consistently shown that in hearts which are beating well after perfusion has been commenced and in which the coronary flow is above 10 c.c. per min., guanylic acid and yeast adenylic acid increase definitely the amplitude of the mechanogram (Fig. 2). The increase in the amplitude is preceded by a decrease, which is of brief duration, but is consistently present. The increased amplitude persists for a considerable time,

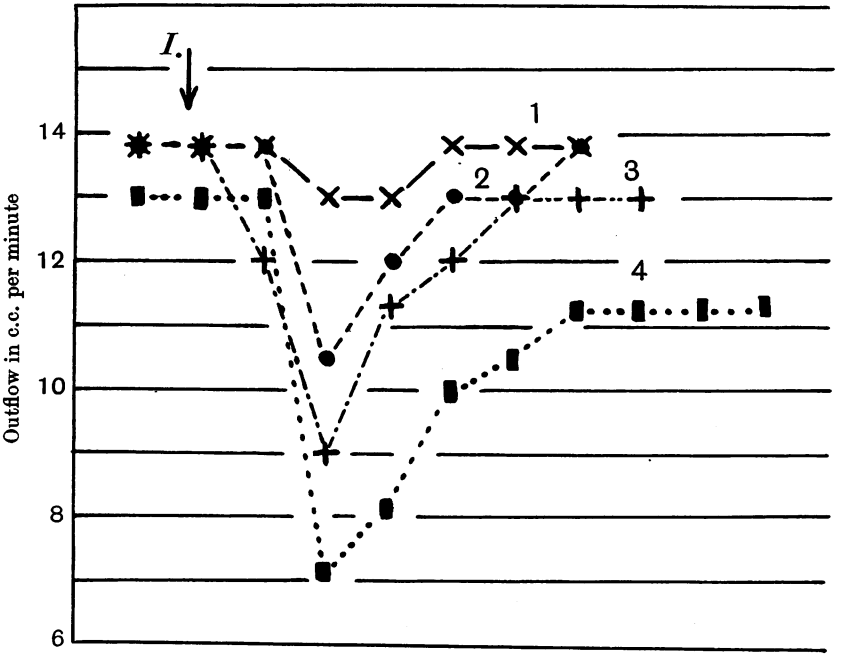

Fig. 1 (1. vii. 16). Influence of guanylic acid upon coronary outflow. Injection of 0·1, 0·5, 1·0 and 2·0 mg. guanylic acid in 1 c.c. of saline at 1, 2, 3 and 4 respectively. Outflow calculated in c.c. per min. from time taken for each successive 5 c.c. to flow through. *I* = injection.

usually 5–10 min., and may persist longer. If further injections are given they lead to a further increase, so that the mechanogram may be 4–5 times as large as it was at the beginning of the experiment, after several injections have been made over the period of half an hour. When guanosine and adenosine are injected no change is seen (Fig. 2). On rare occasions adenosine leads to a slight increase in the mechanogram, which usually occurs when the heart is in poor condition and the coronary outflow is considerably increased by the injection. The absence of change in good preparations is in agreement with the findings

of Wedd [1931]. On a few occasions also a very slight increase has been noted after the injection of guanosine. It is to be noted that the occasional increases seen with both adenosine and guanosine are never preceded by a decreased amplitude. In all these experiments the rate of the heart

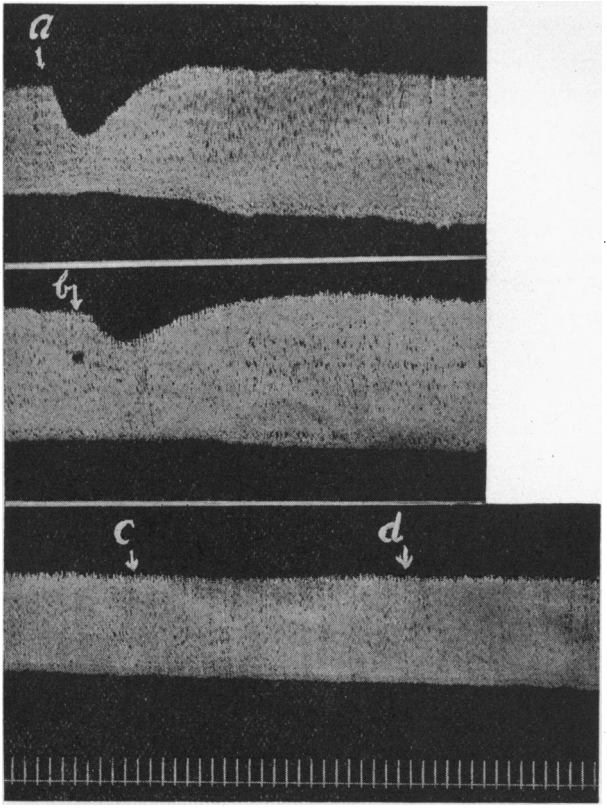

Fig. 2 (1. ix. 13). Influence of nucleic acid derivatives upon amplitude of mechanogram. Injection of 2·0 mg. of yeast adenylic acid, guanylic acid, adenosine and guanosine at *a*, *b*, *c* and *d* respectively. Rate of rhythmic beating = 144 per min. Time marker = 5 sec.

has been maintained constant. If this condition is not fulfilled the rate effect upon the size of the mechanogram is introduced, and this may dominate the result [Dale, 1930]. For instance, adenosine slows the natural rate of beating, and the mechanogram is consequently decreased; guanosine, on the other hand, often enhances the rate of beating, and the mechanogram is thus increased.

In comparing adenylic acid obtained from yeast with the same acid obtained from muscle, no difference has been noted in the general reaction, both producing an initial decrease followed by an increase in amplitude. The results in general suggest that muscle adenylic acid is more effective than yeast adenylic acid, while guanylic acid is the least potent of the three substances.

The substances divide themselves according to these experiments into two very definite groups, those which give rise to a brief decrease, followed by a prolonged increase in amplitude, namely, muscle and yeast adenylic acid, and guanylic acid, and those which are without such influence, namely, adenosine and guanosine. The activity of the first group cannot be due to an influence upon the coronary flow, for while the adenylic acids increase, guanylic acid decreases the flow. In addition adenosine, which is the most efficient dilator, is without influence upon the mechanogram. The changes seen must be associated with some action upon the musculature. In this it is impossible to consider that the ease with which the substances are deaminated comes into play [Drury and Szent-Györgyi, 1929], for adenosine is much more easily deaminated than yeast adenylic acid [Schmidt, 1928]. Chemically the two groups differ from one another in a definite particular. The active substances are composed of a purine base, a sugar and phosphoric acid; the inactive still contain the purine base and the sugar, but the phosphoric acid group has been split off. It would appear that the changes seen in the amplitude of the mechanogram may be due to an effect of this phosphoric group upon the muscle.

To test this point the influence of sodium orthophosphate (di-sodium salt) and pyrophosphate has been examined. It is hardly to be expected that the addition of these substances to the perfusate will produce identical changes with those seen when the phosphoric acid grouping is liberated intracellularly, which occurs when guanylic or adenylic acid is introduced, but it can be hoped that the differences will be in degree only.

Both the substances decrease, in considerable measure, the coronary outflow. Moreover, successive doses appear to have an increasing influence, so that the coronary flow is quickly reduced to such proportions that the preparation fails. This has been obviated in great measure by adding adenosine in concentrations of 1 in 1,000,000 to the perfusate which gives a maximal dilatation of the coronary vessels [Bennet and Drury, 1931] and by using a high perfusion pressure. In these circumstances the first injections produce a decrease in coronary flow which has no detrimental effect upon the preparation. Sooner or

later, however, the coronary flow is greatly reduced, and cannot be increased by heavy doses of either adenosine or nitrites.

Sodium orthophosphate in doses of 0·5–2 mg. leads to an increase in the amplitude (Fig. 3); on rare occasions it has no influence. The curve always lacks, however, the initial decrease which is so characteristic of that produced by adenylic and guanylic acids.

Sodium pyrophosphate, in similar doses, gives rise to an initial brief decrease followed by a prolonged increase in the amplitude. The curve is indistinguishable from that produced by adenylic acid and guanylic

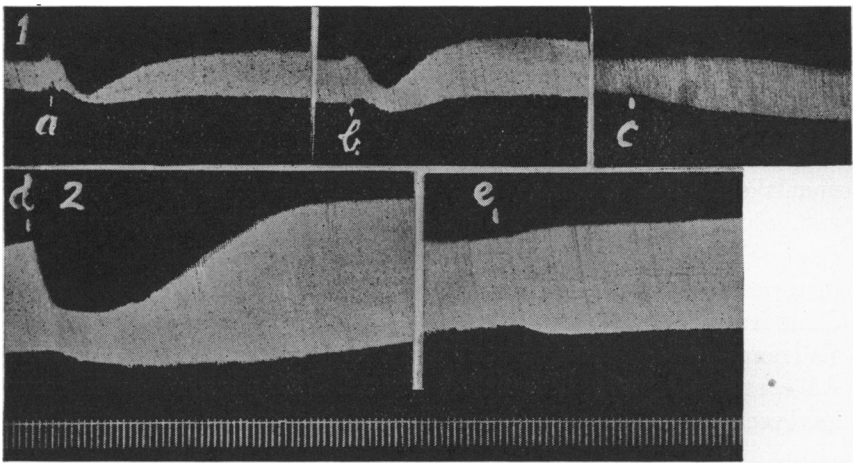

Fig. 3. Influence of sodium orthophosphate (di-sodium salt) and pyrophosphate upon the amplitude of the mechanogram. (1) (1. ix. 26). Rhythmically beating heart. 130 per min. Injection of 2·0 mg. sodium pyrophosphate, 2·0 mg. yeast adenylic acid and 1·0 mg. sodium orthophosphate at *a*, *b* and *c* respectively. (2) (1. ix. 28). Naturally beating heart. Injection of 1·0 mg. sodium pyrophosphate and orthophosphate at *d* and *e* respectively. Time marker 5 sec.

acid (Fig. 3). It would seem, therefore, that the dual effect is associated with a pyrophosphoric acid grouping. The molecular weight of adenylic and guanylic acid is consistent only with the presence of an orthophosphoric acid group, so that it must be assumed that in the intracellular breakdown of these substances either pyrophosphoric acid is formed or that adenylic acid pyrophosphate (and presumably guanylic acid pyrophosphate) is synthesized. This may actually account for the primary brief decrease in the mechanogram. The secondary prolonged increase may be associated with the breakdown of the pyrophosphoric acid or hydrolysis of the adenylic or guanylic acid and the production of

orthophosphoric acid, for this increase is seen when sodium orthophosphate is added.

Parnas and Ostern [1931] have come to the conclusion that adenylic acid and adenine nucleotide pyrophosphate act as "potential poisons" [Loewes, 1928], and that neither deamination with production of ammonia nor other chemical change underlies the reactions. Their observations have been made upon the rhythm of the frog's heart and concern therefore the action of these substances upon the rhythmicity of specialized tissue such as the sinus. The results reported in this paper deal with the influence of derivatives upon the strength of contraction of the muscle of the warm-blooded heart, so that it is not surprising that different conclusions have been arrived at as to their mode of action[1]. The derivatives have many physiological properties; they influence cardiac rhythm, the beat of the heart, the calibre of arteries, intestinal movement, etc. [Drury and Szent-Györgyi, 1929; Bennet and Drury, 1931], and it is unlikely that any one explanation will be sufficient. As further work is undertaken upon the mode of action of these substances the conclusions drawn may appear extremely contradictory unless the varied reactions are clearly appreciated.

It has been noted that adenylic acid is, weight for weight, a less powerful coronary dilator than adenosine [Wedd, 1931], the higher molecular weight of the former being offered as the explanation. The fact that both ortho- and pyrophosphoric acid reduce the coronary flow must be taken into consideration, and the liberation of these substances from adenylic acid, which the described experiments suggest, may decrease the dilator effect of the contained adenosine.

In considering therefore the influence of the nucleic acid derivatives on the beat of the perfused rabbit's heart, two aspects must be considered. Those substances which dilate the coronary system will improve the beat by a more efficient nourishment of the musculature of the heart; those which contain a phosphoric acid group will exercise a beneficial influence through a specific action on the musculature itself.

Influence of various factors upon the action of adenylic and guanylic acid.

Several experiments have been performed in which various changes have been made. On several occasions defibrinated blood from the same animal has been added during the experiment. The amount added has

[1] Whether the conclusions of Parnas and Ostern are applicable to the rhythm changes seen in the warm-blooded heart, it would be out of place to discuss here.

usually been about 10 p.c., and has usually increased the amplitude of the mechanogram. The usual reactions, namely an initial decrease followed by an increase in the mechanogram, have always been observed subsequently upon injecting the two substances. Reducing the calcium content of the Ringer-Locke solution to 0·12 and increasing it to 0·48 p.c. has no effect upon the reaction. After cutting off the supply of oxygen for half an hour, which leads to a definite decrease in the mechanogram, the same general reactions are observed. More complete oxygenation, as provided by the addition of defibrinated blood, has already been noted to leave the reaction unchanged. Atropine added to the perfusate in doses of up to $\frac{1}{2}$ c.c. of a 1 p.c. solution of atropine to the litre is without effect. After the addition of quinine to the perfusate sufficient to reduce the mechanogram from 16 mm. to 5 mm., the same curve is seen when the two substances are introduced. The same result is obtained when the amplitude has been reduced by 50 p.c. by the addition of chloroform. If the substances are added to hearts which have been perfused for 2–3 hours, the usual reaction is obtained and differs little in degree from the reactions obtained in hearts which have been perfused for a short time only.

The whole animal.

In the intact dog, anæsthetized with morphia and chloralose, the mechanogram of the right ventricle has been recorded in the manner already described [Drury, 1923]. Injections of guanylic acid and yeast adenylic acid doses up to 100 mg. have no influence upon the mechanogram. These observations support the conclusions already arrived at for muscle adenylic acid [Drury and Szent-Györgyi, 1929].

Some general reactions of guanylic acid.

Guanylic acid has no influence upon the rhythm of the intact guinea-pig's heart comparable to that produced by adenosine [Drury and Szent-Györgyi, 1929]. Doses up to 5 mg. have no influence upon the rate, but the T wave of the electrocardiogram is inverted when high doses are used. The blood-pressure of the intact anæsthetized rabbit is not affected by intravenous injections of 2 mg. of the substance, while doses of 10 mg. give an indefinite rise of pressure.

The virgin guinea-pig's uterus is definitely relaxed when the concentration of the substance in the saline bath reaches 1 in 500,000.

When two drops of a 10 p.c. solution, brought to a pH of 7·4 with sodium bicarbonate, are instilled into the rabbit's eye every 10 min. over

a period of 3 hours, no pus can be detected at the inner canthus [Bennet and Drury, 1931].

SUMMARY.

1. In the perfused rabbit's heart, the addition of either guanylic or adenylic acid leads to a brief primary decrease followed by a prolonged increase in the amplitude of the mechanogram; adenosine and guanosine have no such influence.

2. The reaction is not due to an increase in coronary flow as guanylic acid decreases the outflow.

3. Nucleic acid derivatives which contain a phosphoric acid grouping are alone capable of producing the change, and observations with sodium orthophosphate and pyrophosphate suggest that both pyrophosphoric acid and orthophosphoric acid take part in the reaction.

4. In doses up to 100 mg., introduced intravenously, the derivatives have no influence upon the mechanogram of the intact dog's heart.

5. Guanylic acid is without influence upon the rhythm of the intact guinea-pig's heart, and upon the blood-pressure of the intact rabbit. It relaxes the virgin guinea-pig's uterus.

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