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THE EFFECT OF CORTICOSTEROIDS ON THE ISOLATED MAMMALIAN HEART AND ITS RESPONSE TO ADRENALINE

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During the last few years there have been several reports of the effects of steroid substances on heart muscle. These effects have been obtained on a variety of preparations with widely differing doses.

Cornman (1950) used tissue cultures of foetal heart fragments from chicks or mice. The beat of these fragments was first increased and then depressed by deoxycorticosterone. Nahum, Geller, Levine & Sikand (1951) and Emele & Bonnycastle (1956) used the electrically driven papillary muscle of the cat. Nahum *et al.* found that progesterone, at a concentration of 10^{-6} g/ml. in 80% homologous serum, initially increased the force of the contraction and subsequently depressed it. The effects of pregnenolone were not different from those of the propylene glycol used to dissolve it. Emele & Bonnycastle found that corticosterone increased the force of the contraction at concentrations of 10^{-7} to 5×10^{-7} g/ml., but at concentrations of 5×10^{-6} and 10^{-5} g/ml. the force was decreased. Hydrocortisone depressed the muscle at a concentration of 10^{-7} g/ml., stimulated it at $2{\cdot}5 \times 10^{-7}$ and depressed it at 5×10^{-7} g/ml. At a concentration of 5×10^{-6} g/ml. there was depression followed by stimulation. Deoxycorticosterone was ineffective at low concentrations and depressant at high concentrations.

In 1954 Hoffman reported the effects of various corticoids on isolated frog and guinea-pig hearts. The frog hearts were perfused by Straub's method and the guinea-pig hearts by Langendorf's method. In these experiments concentrations of deoxycorticosterone and cortisone of the order 10^{-6} to 10^{-5} g/ml. caused an initial stimulation followed by depression. There was a diminution of the coronary flow, which was considered to be the cause of the negative inotropic effect ultimately obtained.

In view of the diversity of the preparations used, the high concentrations

of steroids employed in most cases, and the mixed results obtained in these earlier works, it seemed desirable to re-investigate the actions on the mammalian heart.

METHODS

Heart perfusion. Guinea-pig and rat hearts were perfused by the Langendorf method, using the apparatus described by Hancock & Nasmyth (1956). In the experiments using homologous plasma, defibrinated blood or heparinized blood, a re-circulation apparatus was used. This simply consisted of substitution of the usual Marriot bottle by a small glass funnel containing a cotton gauze filter. The outflow from the heart was collected into a rubber tube and conveyed back to the funnel by means of an 'air' (95% O_2 + 5% CO_2) lift, which also served to oxygenate the solution. Frothing in these solutions was prevented by the use of a sufficient quantity of 'Antifoam A' (Midland Silicones) which did not affect the heart beat in any way.

Perfusion fluid. Krebs-Henseleit solution containing NaH_2PO_4 instead of KH_2PO_4 as a buffer was used at a temperature of 37° C unless otherwise stated. This solution had the following composition: NaCl 0·69, KCl 0·035, $CaCl_2$ 0·028, $MgSO_4$ 0·0294, $NaHCO_3$ 0·21, NaH_2PO_4 0·0162, glucose 0·2, glass-distilled water to 100%, and is referred to as Krebs's solution in the text. The same solution was also used to dilute the blood and plasma preparations.

Defibrinated blood. This blood was obtained by heart puncture in guinea-pigs anaesthetized with ether, and was defibrinated by shaking it with glass beads in a beaker. The defibrinated blood was mixed with Krebs's solution to give a final concentration of 30% of blood.

Heparinized blood. This was also obtained by heart puncture, as described for defibrinated blood, except that heparin was included in the syringe and its final concentration was adjusted to 10 u./ml. Heparinized blood also was used at a concentration of 30% in Krebs's solution.

Plasma. Blood was collected and heparinized as described above, and centrifuged at 2000 rev/min for 1 hr. The plasma was then pipetted off, and Krebs's solution was added until the plasma concentration in the mixture was 30%.

Amplitude of heart beat. This was measured in terms of the magnitude of the excursion of the Brodie Universal Lever used to record the heart movements.

Heart rate was recorded by adding a 1 sec time trace to the kymograph records and suitably increasing the drum speed. For quantitative measurements the rate was counted for 10 sec.

Coronary flow was measured by collecting the outflow in a measuring cylinder for periods of 5 min after the introduction of corticoids and recording the volume every minute. Qualitative records were made with an outflow recorder (Stephenson, 1948).

Adrenalectomy. Rats weighing about 250–300 g were given 1 mg of pentobarbitone intraperitoneally and anaesthesia was completed with ether. The adrenals were then removed through a mid-line incision in the skin of the back.

Drugs

Hydrocortisone (Cpd. F). Hydrocortistab (Boots Pure Drug Co.) or 'Efcortelan' (Glaxo Ltd.) was used. These were solutions of hydrocortisone (free alcohol) 5 mg/ml. in 50% ethyl alcohol. Appropriate dilutions of these solutions were made in Krebs's solution to give the concentration required. Corresponding amounts of ethyl alcohol were added to the control Krebs's solution.

Corticosterone (Cpd.B). Stock solutions of corticosterone were made containing 1 mg/ml. in 50% ethyl alcohol. Dilutions for use were made in Krebs's solution and corresponding amounts of alcohol were added to the control Krebs's solution.

Adrenaline. A stock solution was made by dissolving 10 mg synthetic L-adrenaline in 1 ml. 0·1 N-HCl and diluting to 10 ml. with distilled water. This solution was stored in a refrigerator and any remaining 14 days after its preparation was discarded. Solutions for injection into the cannula were made by diluting the stock solution with Krebs's solution to give a final concentration of 1 μg/ml.

RESULTS

Guinea-pig heart

Effect of hydrocortisone (Cpd.F) 10^{-7} g/ml. on amplitude, rate and coronary flow. In all the following experiments the heart was perfused for 10 min before any records were taken, in order that the preparation should have reached a steady state. After this period of stabilization records of the amplitude and rate of the heart beat were taken, and the coronary flow was measured. When the Kreb's solution was changed for one containing hydrocortisone at a concentration of 10^{-7} g/ml., there was a gradual reduction in the amplitude of the beat in all the experiments. The reduction began as soon as the dead space in the cannula had been cleared and was usually maximal within 5 min. In seven experiments the mean amplitude of the beat was 30 mm during the control period. Five minutes after perfusion with hydrocortisone the mean amplitude of the beat was 21 mm, representing a 30% reduction.

The mean heart rate before the introduction of hydrocortisone was 258/min. Five minutes after perfusion with hydrocortisone the mean rate had been reduced to 223 beats/min, representing a 12% reduction. The effect of the hydrocortisone on the heart rate was less in magnitude than its effect on the amplitude of the beat. However, in no instance was the heart rate increased or unchanged by the corticoid, and the reduction was significant.

The effect of reverting to the control perfusion fluid 5 min after perfusion with hydrocortisone was to cause some recovery in the amplitude and rate of the beat in some cases. Recovery, when it occurred, was always incomplete and was not maintained.

The coronary flow was measured continuously for 3 min before the introduction of hydrocortisone, readings being taken every minute. Following the commencement of perfusion with hydrocortisone, measurements of the coronary flow were continued in a similar manner for 5 min. There was a gradual increase in the flow rate after the introduction of hydrocortisone until after 5 min it was 20% greater than it was during the control period. This increase in the coronary flow is depicted in Fig. 1 as also are the effects of hydrocortisone on the amplitude and rate of the heart beat. The mean figures are recorded in Table 1.

Effects of hydrocortisone at a concentration of 10^{-6} g/ml. At this concentration the same pattern of events was observed. The difference lay in the wider variation in the results at this dose level. For example, the mean reduction in the amplitude of the beat was greater with this higher dose of the steroid, but there was a wide variation in the extent of the effect in different experiments. Despite the wide variation in degree, the effect of the steroid at this higher dose level was always to reduce the amplitude of the beat.

The effect of this concentration of hydrocortisone on the heart rate was inconsistent, being decreased in five experiments, increased in two, and in two remaining unchanged. The mean figures given in Table 1 show the heart rate to be reduced at this concentration but the reduction is not significant, since the change is not consistent. A record of the effects is presented in Fig. 1.

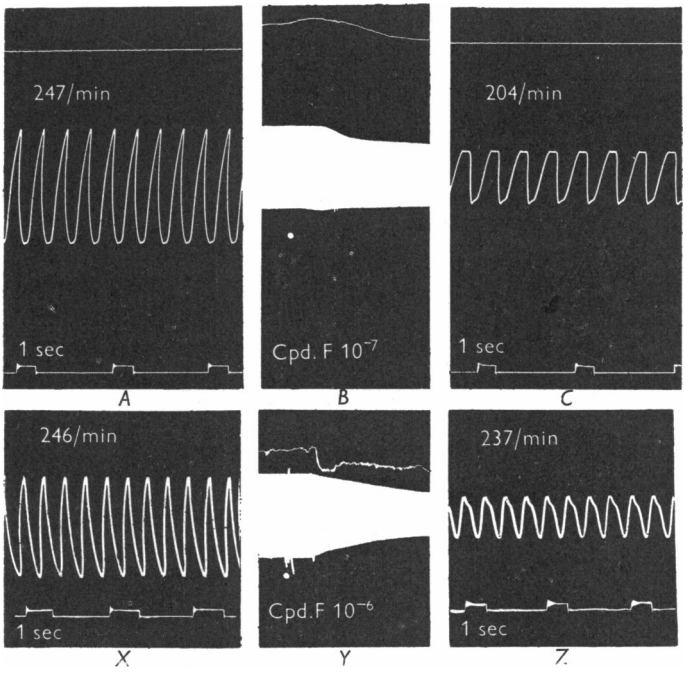

Fig. 1. The effect of hydrocortisone (Cpd.F) on the rate, amplitude and coronary flow of the isolated guinea-pig heart perfused with Krebs's solution. Tracing *A* shows the normal heart rate and amplitude; *B* the effect of adding hydrocortisone at a concentration 10^{-7} g/ml. to the perfusion fluid (upper tracing is the coronary flow recorded with a Stephenson outflow recorder, downward movement of the lever indicates dilatation of the vessels); *C*, the heart rate 5 min after perfusion with hydrocortisone. Tracings *X*, *Y*, and *Z* were obtained from another heart and show the effect of using a concentration of 10^{-6} g/ml. hydrocortisone.

The effect of lower concentrations of hydrocortisone. Two experiments were performed to obtain some idea of the lower limits of concentration at which the effects described above could be obtained. In one experiment well marked effects, identical with those described for hydrocortisone at a concentration of 10^{-7} g/ml., were obtained when using a concentration of 10^{-8} g/ml. At a concentration of 10^{-9} g/ml., hydrocortisone was without effect on the one occasion on which it was tried.

TABLE 1. The effects of hydrocortisone and corticosterone on the isolated perfused heart of the guinea-pig and rat respectively

Species	Observation	Control soln. (Krebs's only)	Krebs's soln. + hydrocortisone 10^{-7} g/ml.	Mean difference ± S.E.	Percentage change	Probability (P)	No. of expts.
Guinea-pig	Mean amplitude* (mm)	30	21	9·0	−30		7
	Mean heart rate (beats/min)	258	223	31 ± 6·66	−12	<0·01	7
	Coronary flow (ml./min)	10	12·0	2·0 ± 0·155	+20	<0·001	7
			Krebs's soln. + hydrocortisone 10^{-6} g/ml.				
Guinea-pig	Mean amplitude* (mm)	25	13	12	−48		9
	Mean heart rate (beats/min)	255	238	17 ± 17	− 6·7	<0·40	9
			Krebs's soln. + corticosterone 10^{-7} g/ml.				
Rat	Mean amplitude* (mm)	10·8	8·4	2·4	−22		6
	Mean heart rate (beats/min)	222	146	76 ± 25·8	−34	<0·05	6
	Coronary flow (ml./min)	3·5	2·8	0·7 ± 0·25	−20	<0·05	6

* Statistical treatment has not been applied to the amplitude of the beat since the degree, but not the direction, of the effect would be affected by the characteristics of the lever in relation to the heart rate.

Rat heart

Effect of corticosterone (Cpd.B) 10^{-7} g/ml. The experiments described above were repeated using rat hearts and corticosterone instead of hydrocortisone. Hydrocortisone is the normal secretion of the adrenal cortex in guinea-pigs, while corticosterone is the normal secretion in rats.

In four experiments out of six the amplitude of the heart beat was reduced by the corticosterone, in one it was unchanged and in one it was slightly increased. The mean effect reported in Table 1 was a 22% reduction in the amplitude of the beat, but it was doubtful whether it was significant because it did not appear consistently.

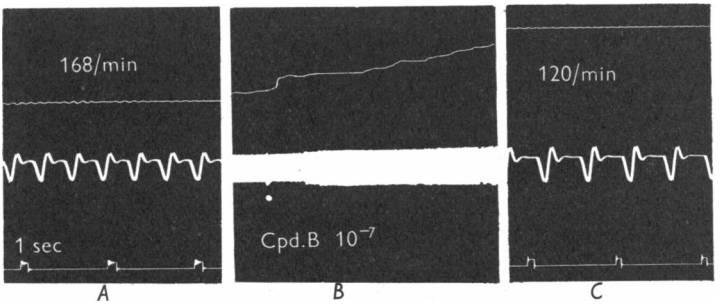

Fig. 2. The effect of corticosterone (Cpd.B) 10^{-7} g/ml. on the rate, amplitude and coronary flow of the isolated rat heart perfused with Krebs's solution. Tracing *A* shows the heart rate and amplitude during the control period; in this instance the corticoid caused a slight increase in the amplitude of the beat. The upper tracing is the coronary flow recorded with a Stephenson outflow recorder; upward movement of the lever indicates constriction. *B* shows the effect of adding corticosterone. *C* shows the heart rate 5 min after continuous perfusion with corticosterone.

The mean heart rate was reduced by 34% 5 min after perfusion with corticosterone was started. The significance of this large difference reported in Table 1, is, however, not as great as might have been expected. The reason for the rather low value of less than 0·05 for the probability is that in one experiment the heart rate increased.

The coronary flow was decreased by corticosterone in all but one experiment, in which it remained unchanged. However, the mean reduction of 20% in the coronary flow was significant, and the figure is reported in Table 1. The effects of corticosterone on the rat heart are depicted in Fig. 2.

Effects of corticosterone (Cpd.B) 10^{-7} g/ml. on hearts from adrenalectomized rats. It is easier to remove the adrenals from a rat than to perform the same operation in the guinea-pig. Accordingly, rats were used in these experiments to determine the effect of corticoids on hearts taken from animals suffering from cortical deficiency.

Four hearts were used; two were from animals operated on 24 hr previously and two were taken 72 hr after adrenalectomy. The first heart, taken 24 hr after operation, had a beat which was irregular in rate and amplitude, and perfusion with corticosterone did not appear to affect it in any way. The second heart had a regular beat and behaved exactly like the normal heart when perfused with corticosterone, showing a reduction in both force and rate. In both these hearts the coronary flow was decreased by corticosterone. Of the hearts taken 72 hr after adrenalectomy, one behaved like the normal hearts, while the other showed excessive sensitivity to adrenaline and the amplitude of the beat was slightly increased by corticosterone. The effects of the corticosterone on the coronary flow and on the heart rate were the same in this preparation as they were on normal hearts. In view of the great irregularities present in these hearts and the difficulty of assessing any changes further work with them was abandoned.

The effect of corticosteroids on the response of the heart muscle to adrenaline

Guinea-pigs. Adrenaline normally causes an increase in the rate and amplitude of beat of the isolated heart. Control experiments showed that provided the doses of 0·1 μg adrenaline were injected into the cannula at regular intervals of not less than 5 min there was only a very slight diminution in the response with successive doses. This diminution in the response was practically imperceptible between adjacent doses, and the changes produced by hydrocortisone were therefore unmistakably due to the corticoid and not to any spontaneous change in the sensitivity of the heart muscle to adrenaline.

In each experiment designed to assess the effect of hydrocortisone 10^{-7} g/ml. on the response of the heart to adrenaline, three doses of 0·1 μg were given at 5 min intervals during the control period. The perfusion fluid was then changed for one containing the hydrocortisone, and 5 min later the first of two doses of 0·1 μg adrenaline was injected. In thirteen such experiments the mean increase in amplitude produced by adrenaline during the control period was 26 mm. Five minutes after the introduction of hydrocortisone it was only 6·3 mm. Since the corticoid itself produced a reduction in the amplitude of the beat, it seemed undesirable to compare these two figures directly. By converting them to percentage increases the amplitude of the beat immediately before the dose of adrenaline was taken into account. On this basis the mean increase produced by adrenaline during the control period was 83% and 5 min after perfusion with hydrocortisone it was 35%. The figures are presented in detail in Table 2 and the effect is depicted in Fig. 3.

The effect of 0·1 μg adrenaline on the heart rate was determined in six experiments and the percentage increase before and after hydrocortisone (10^{-7} g/ml.) was estimated. The mean heart rate during the control period was increased by 26·2% by adrenaline. Five minutes after perfusion with hydro-

cortisone adrenaline increased the rate by 24·6%. These two figures are not significantly different from one another, nor are the actual figures for the increase in rate caused by adrenaline. These figures are presented in Table 2.

The perfusion fluid containing hydrocortisone was changed for the control solution 5 min after recording the last response to adrenaline. There was very slight recovery after 5 min, but it was not maintained, even though perfusion with normal Krebs's solution was continued. This effect is also shown in Fig. 3.

Rats. The effects of corticosterone 10^{-7} g/ml. on the response of the rat's isolated perfused heart to 0·1 μg doses of adrenaline were also determined. Only the increase in amplitude produced by adrenaline was measured and the

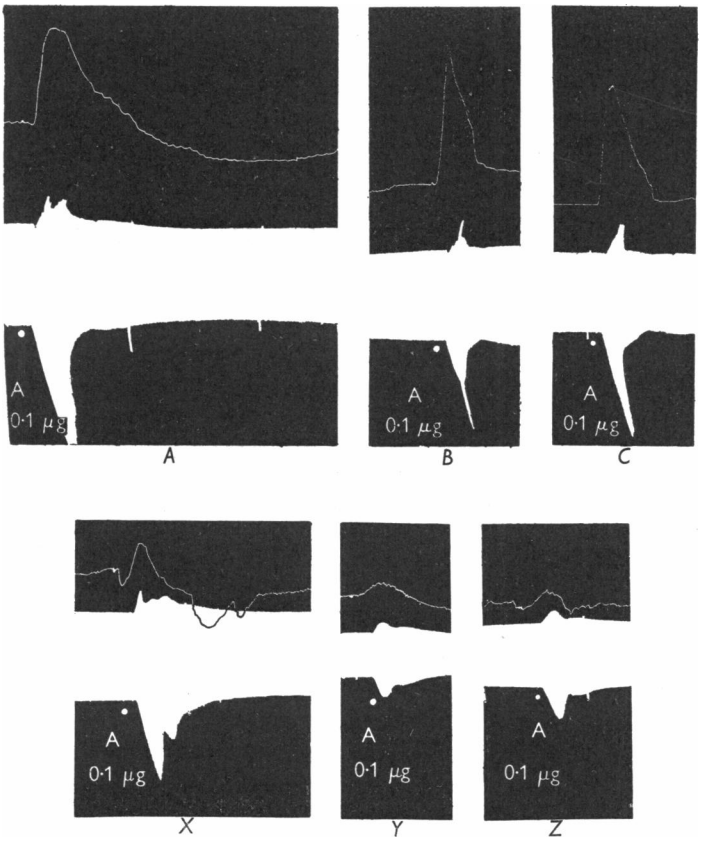

Fig. 3. The effect of hydrocortisone 10^{-7} g/ml. on the response of the isolated perfused guinea-pig heart to adrenaline. Upper record, coronary flow, upward movement indicates coronary constriction, lower record, amplitude of heart beat. Tracing *A* shows the normal response to adrenaline (A); note that the coronary response is constriction followed by a small but prolonged dilatation. Tracing *B* was obtained 5 min after perfusing with hydrocortisone 10^{-7} g/ml.; the coronary response is now purely constrictor. Tracing *C* shows the response to adrenaline 5 min after reverting to normal Krebs's solution. *X*, *Y* and *Z* show the effects of hydrocortisone 10^{-6} g/ml. on the response to adrenaline.

TABLE 2. The effect of adrenaline on the amplitude and rate of the isolated perfused heart in the guinea-pig and the rat before and after hydrocortisone and corticosterone respectively

		Krebs's solution				5 min after perfusion with Krebs's soln. + hydrocortisone 10^{-7} g/ml.					
Species	Observation	Control	Adrenaline	Actual increase ±S.E.	Percentage increase ±S.E.	Control	Adrenaline	Actual increase ±S.E.	Percentage increase ±S.E.	Probability (P)	No. of expts.
Guinea-pig	Mean amplitude* (mm)	28·5	54·5	26	83	15·7	22·0	6·3	35		13
	Mean heart rate (beats/min)	260	324	64±15·1	26·2±7·4	230	286	56±6·8	24±3·7	<0·40	6
		Krebs's solution				5 min after perfusion with Krebs's soln. + corticosterone 10^{-7} g/ml.					
Rat	Mean amplitude* (mm)	18	62·0	44	244	18	19	1·0	5·5	—	5

* Statistical treatment has not been applied to the amplitude of the beat since the degree, but not the direction, of the effect would be affected by the characteristics of the lever in relation to the heart rate.

figures are presented in Table 2. The adrenaline produced a greater increase in amplitude in the rat heart than it did in the guinea-pig heart and there was a correspondingly greater reduction in the response in the presence of corticosterone.

As with the guinea-pig heart a return to normal Krebs's solution after 5 min perfusion with corticosterone caused some recovery in the adrenaline response after a further 5 min. However, it was not maintained even though perfusion with normal Krebs's solution was continued. The effect is shown in Fig. 4.

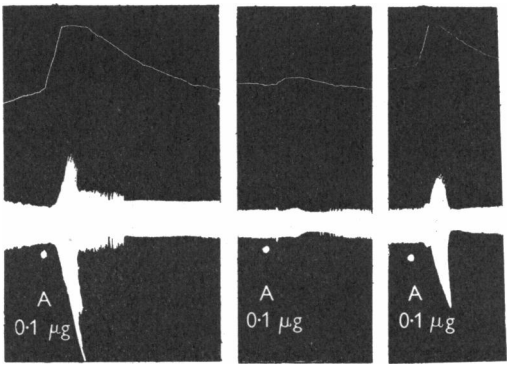

Fig. 4. The effect of corticosterone (Cpd.B) 10^{-7} g/ml. on the response of the isolated perfused rat heart to adrenaline. Upper records, coronary flow, upward movement indicates constriction: Tracing A, normal: B, 5 min after perfusion with corticosterone; C, 5 min after reverting to normal Krebs's solution.

The influence of hydrocortisone on the concentration of potassium in the perfusate from the guinea-pig heart

The phenomenon described above could have been caused by interference with the potassium flux in and out of the cells in the heart muscle. In an attempt to test this possibility fourteen guinea-pig hearts were perfused with Krebs's solution and the concentration of potassium in the perfusate was measured with a direct-reading flame photometer, capable of distinguishing differences of 0·05 m-equiv/l. Two samples were taken during the control period in each experiment and the mean figure for the potassium concentration was calculated after they had been assessed. The perfusion fluid was then changed for one containing hydrocortisone at a concentration of 10^{-6} g/ml. Samples of the perfusate were then collected every minute for 5 min and their potassium content was estimated. The results are reported in Table 3 and it is clear that the introduction of corticoid did not produce a detectable change in the potassium concentration of the perfusate.

In a further series of experiments it was shown that when 0·1 μg adrenaline was injected the potassium concentration in the perfusate was significantly

increased. This increase in the potassium concentration still occurred when adrenaline was injected, 5 min after perfusion with hydrocortisone had commenced. Thus it was evident that the reduced response of the heart muscle to adrenaline, after perfusion with a solution containing hydrocortisone, was not due to prevention of potassium efflux.

Clearly the increased potassium content of the perfusate after adrenaline must have originated from the cells in the heart muscle. It might be expected that, following the effect of adrenaline, the heart would reabsorb the potassium which it had lost. Continuing observation on the concentration of potassium in the perfusate after the peak effect showed only a gradual return to the control value.

TABLE 3. The effect of hydrocortisone 10^{-6} g/ml. on the concentration (m-equiv/l.) of potassium in the perfusate from an isolated guinea-pig heart perfused by Langendorf's method before and after adrenaline.

Control	Adrenaline	Difference	Probability	No. of expts.
	Krebs's solution			
$4{\cdot}43 \pm 0{\cdot}026$	$4{\cdot}69 \pm 0{\cdot}056$	0·26	<0·01	14
	Krebs's solution and hydrocortisone 10^{-6} g/ml.			
$4{\cdot}41 \pm 0{\cdot}026$	$4{\cdot}68 \pm 0{\cdot}072$	0·27	<0·01	14

The influence of blood and plasma on the response of the guinea-pig heart to corticoids. The effects of the corticoids on the isolated heart perfused with Krebs's solution appeared to be entirely depressant. This was surprising, and it seemed to be of some interest to determine whether or not the presence of blood or plasma in the perfusion fluid would modify the effects.

In the first experiment 30% defibrinated blood was used. The heart beat, using this solution, was very much more vigorous than when Krebs's solution alone was used. However, the response to a dose of 0·1 μg adrenaline was almost negligible. This reduced sensitivity to adrenaline was observed in all the experiments employing plasma or whole blood. A reasonable response could be obtained with a dose of 1·0 μg adrenaline, but since the perfusion fluid was re-circulated in these experiments it was deemed unwise repeatedly to inject relatively large doses of adrenaline. Observations were therefore confined to determining the effect of hydrocortisone on the rate and amplitude of the heart beat. When sufficient hydrocortisone was introduced into the reservoir to produce a final concentration of 10^{-7} g/ml. there was no effect of any consequence on either the amplitude or the rate of the heart beat.

In the second experiment 30% heparinized blood was used and again a concentration of 10^{-7} g/ml. of hydrocortisone was without effect. In both the experiment using defibrinated blood and that employing heparinized blood the concentration of hydrocortisone was increased stepwise, and it was not until a concentration of 4×10^{-5} g/ml. had been reached that an effect was

seen, comparable with that obtained in hearts perfused with Krebs's solution containing hydrocortisone at a concentration of 10^{-7} g/ml.

It seemed possible that the corticoids were being absorbed by the cellular elements. To test this, four experiments were performed using 30% of homologous plasma in Krebs's solution as the perfusion fluid. The results obtained were identical with those employing defibrinated or heparinized blood. The mean amplitude of the beat in these four experiments during the control period was 55 mm. Five minutes after the corticoid had commenced to circulate through the heart it was 54 mm. The mean heart rate was 254/min during the control period and 250/min after perfusion of the corticoid.

DISCUSSION

When the isolated guinea-pig heart was perfused with Krebs's solution, the introduction of hydrocortisone in concentrations which might normally be expected in the plasma caused a reduction in the amplitude and rate of the heart beat. The coronary flow was increased by the corticoid and the effect on the myocardium cannot therefore be explained in terms of a restriction in the supply of nutrient solution. Similar effects were observed in the rat, but in this species the coronary vessels were constricted. It is unlikely that the effect in the rat heart can be interpreted entirely in terms of coronary constriction, though this probably contributes to it.

In both the guinea-pig and the rat the increase in amplitude of the heart beat produced by adrenaline was diminished by the corticoids. The increase in heart rate produced by adrenaline was measured only in the guinea-pig and was not significantly affected by the corticoid. The diminution in the amplitude of the response to adrenaline caused by the corticoid agrees with the observation of Hoffman (1954) that cortisone abolishes the effect of threshold doses of adrenaline in the frog heart. The fact that the corticoid influenced only the increase in the amplitude of the beat caused by adrenaline and not the increase in heart rate is interesting in view of the suggestion of Lands & Howard (1952) that there are different receptors for the rate and amplitude changes wrought by adrenaline.

The attempt to explain the effects in terms of changes in the potassium flux was not entirely successful. It was shown that when corticoid was introduced the potassium concentration in the perfusate was unchanged, so far as could be determined by flame photometry. However, when a dose of adrenaline was given, the concentration of potassium in the perfusate was increased significantly at the peak of the effect on the myocardium, and it was constant whether corticoid was present or not. This extra potassium must clearly have come from the cells, and the efflux must have been rapid for the concentration in the Krebs's solution to have risen measurably. Continuation of measurements of the potassium concentration in the perfusate during the period of

recovery from the dose of adrenaline showed only a relatively slow return to the normal concentration. This also occurred whether corticoid was present or not. Normally the potassium discharged during the response to adrenaline would be reabsorbed by the cells during the recovery period, but this process is evidently much slower than the discharge, since if it had been as rapid the concentration of potassium in the perfusate must have fallen below the normal levels during the recovery period. It is conceivable that the corticoids do not produce their effects on the myocardium by inhibiting potassium efflux, but by preventing potassium influx. This possibility is supported by the observation of Cornman (1950) that foetal hearts stopped by high concentrations of potassium could be restarted with deoxycorticosterone.

Concentrations of corticoid which produced a marked effect when the heart was perfused with Krebs's solution were without effect when 30% defibrinated blood was used. It seemed that this might have been due to antagonism of the effect by substances such as 5-HT, which would be liberated from platelets by the process of defibrination. However, this possibility was eliminated by the experiment using 30% heparinized blood, since with this solution the corticoids were still without effect. Another possibility was that the corticoid was taken up by the cellular elements. This possibility was also eliminated, since it was shown that the corticoids were without effect when 30% plasma was used. Other possibilities are that the blood or plasma already contains some endogenous corticoid, which has produced its effect before the exogenous corticoid is added. This seems unlikely, since the effect could be produced when the concentration was high enough. Daughaday & Bremer (1955) showed that at physiological concentrations corticosteroids are largely bound to plasma proteins. From the results of later work Daughaday (1956) concluded that human plasma contained two hydrocortisone binding systems. One, probably associated with albumin, has a low affinity for hydrocortisone but is not readily saturated. The other system, predominant under physiological conditions, has high affinity but is saturated at low hydrocortisone concentrations. It seems more likely, therefore, that the added corticoid is bound to the plasma proteins; and when so bound is unable to produce the effects which have been described. If this explanation is correct, then it is of importance to discover how the corticoid is made available to the tissues. Experiments are now being performed to elucidate this problem.

SUMMARY

1. Concentrations of 10^{-7} and 10^{-6} g/ml. of hydrocortisone cause coronary dilatation and depression of the amplitude and rate of beating of the isolated guinea-pig heart perfused with Krebs's solution.

2. The isolated perfused rat heart is similarly but less certainly depressed by corticosterone. The coronary vessels are usually constricted by the corticoid.

3. The increase in the amplitude, but not the rate of the heart beat, caused by adrenaline, is depressed by corticoids in both the guinea-pig and the rat.

4. The inhibition of the heart beat caused by hydrocortisone is not accompanied by any change in the potassium concentration in the perfusion fluid. When adrenaline is injected, however, the potassium concentration increases whether corticoid is present or not.

5. When 30% of defibrinated blood, heparinized blood or plasma is used as the perfusion fluid, the corticoids are without effect unless relatively high concentrations are used. This is thought to be due to their adsorption.

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