Brit. J. Pharmacol. (1961), 17, 380–391.

THE RESERPINE-TREATED CAT

BY

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(*Received September* 4, 1961)

In cats, 24 hr after the administration of 1 mg/kg of reserpine, it was found that (*a*) the heart is in failure; (*b*) the sensitivity of the peripheral vessels to adrenaline, noradrenaline and isoprenaline, administered intravenously or close-arterially, is decreased; (*c*) any blood pressure changes are, as a rule, secondary to changes in heart contraction; and (*d*) the peripheral blood flow passively follows the blood pressure changes. Furthermore, any improvement of the circulation at this stage was found to be almost exclusively the result of an amelioration in the force of cardiac contraction.

Reserpine is known to reduce or deplete the various tissues of their catecholamine content, and its pharmacological actions on circulation have been, as a rule, attributed to this depletion (Burn & Rand, 1958; Axelrod & Tomchick, 1960). Because of this, during the last few years the cardiovascular system of animals pretreated with reserpine has been extensively used for the pharmacological analysis of the action of a multitude of drugs. In most studies the cat has been a favourite experimental animal, and, in order to " reserpinize " it, doses of at least 1 mg/kg of the drug have usually been used. The severe clinical condition, however, in which the cats are found 24 hr after the administration of such a dose of reserpine and the marked degenerative changes of the heart muscle revealed by histological examination (Zaimis, 1961) were considered unlikely to be the result only of a depletion, however complete, of the animal's catecholamines. Therefore, experiments were undertaken in which the various components of the cardiovascular system could be studied simultaneously *in vivo*.

METHODS

Both the control and the reserpine-treated cats were anaesthetized with a mixture of chloralose (80 mg/kg) and pentobarbitone (6 mg/kg) injected into the cephalic vein of the fore limb or the saphenous vein of the hind limb. The addition of pentobarbitone prevented the initial stage of excitement which normally follows the intravenous injection of chloralose alone. Because of their poor condition, the reserpine-treated animals had to be anaesthetized very slowly.

Recording of the venous outflow from the hind limb. An incision was made in the skin of the medial surface of the thigh, and all branches of both femoral artery and vein down to the popliteal space, including the saphenous vein, were ligated and cut, with the exception of the artery chosen for the intra-arterial administration of the drugs. In order to maintain a regular blood-flow through the limb, the muscles were stimulated indirectly by the application of electrical shocks to the sciatic nerve at a frequency of 6 stimuli/min. Heparin

(1,000 units/kg) was administered intravenously 10 min before any vessels were opened. The method described by Hilton (1952, 1953) was used for recording the venous outflow. According to this method the femoral vein is cannulated, and the blood is allowed to pass through a drop chamber and then straight back into the animal. The drugs were injected *close-arterially* by means of a cannula made from a no. 18 hypodermic needle tied into the cut end of a branch of the femoral artery, usually the small artery supplying the gracilis muscle.

Direct measurement of changes in cardiac contractile force. The chest was opened and the pericardium incised and secured to the chest wall. The lungs were ventilated throughout by means of a Starling pump. The force of ventricular contraction was measured with a strain gauge arch and the signals from the arch were fed into an ultra-violet polygraph (New Electronics Products), and recorded on moving paper. The strain gauge arch was attached directly to the muscle of either the right or the left ventricle by cotton sutures so placed as to avoid occlusion of any major branch of a coronary vessel. The strain gauge technique is a safe and practical method for assessing the inotropic effects of various drugs (Cotten & Bay, 1956). As a rule, no blood pressure irregularities appeared after the arch had been attached to the heart, and, furthermore, in control experiments it was found that both force of heart contraction and blood pressure remained steady for periods as long as 24 hr.

Drugs. Adrenaline acid tartrate, noradrenaline acid tartrate, isoprenaline hydrochloride and tyramine hydrochloride were used throughout these experiments, and are referred to as adrenaline, noradrenaline, isoprenaline and tyramine, respectively. Phenoxybenzamine (dibenyline hydrochloride) was dissolved in propylene glycol (1%), and immediately before administration a 1:10 dilution in physiological saline was made. A 2.5 mg/ml. solution of reserpine (Serpasil; Ciba) was used as such or further diluted with physiological saline. The drug was administered either subcutaneously or intraperitoneally. Most animals were given a single dose of 1 mg/kg on the day before the experiment. A few received the same amount of the drug spread over two or three days.

RESULTS

Clinical picture

Twenty-four hours after the administration of 1 mg/kg of reserpine the cats, although not paralysed, lay immobile, unresponsive to sound, and appeared almost lifeless. When handled and gently caressed, however, they opened their eyes and with great difficulty succeeded in making some movements, while purring and yet crying simultaneously. The temperature of the room in which the cats were kept was about 20° C, and physiological saline and 5% dextrose were administered orally and intravenously twice daily. In these animals, despite all precautions, body temperature tended to fall, respiration was depressed, blood pressure low and the heart rate markedly reduced. There was a loss of body weight, possibly because the animals neither ate nor drank and, in addition, suffered from marked diarrhoea. In other words, a cat treated in this way is a very sick animal and presents a picture of great misery very similar in many ways to that of a patient in advanced shock (for example, late stages of acute circulatory failure) and definitely very much unlike the picture of an animal under the influence of an ordinary central depressant drug.

Cardiovascular studies

Peripheral vessels. In the first group of experiments the simultaneous recording of blood pressure and peripheral blood flow revealed that in the animals treated with reserpine the sensitivity of the peripheral vessels to adrenaline, noradrenaline and isoprenaline, administered either intravenously or close-arterially, was decreased.

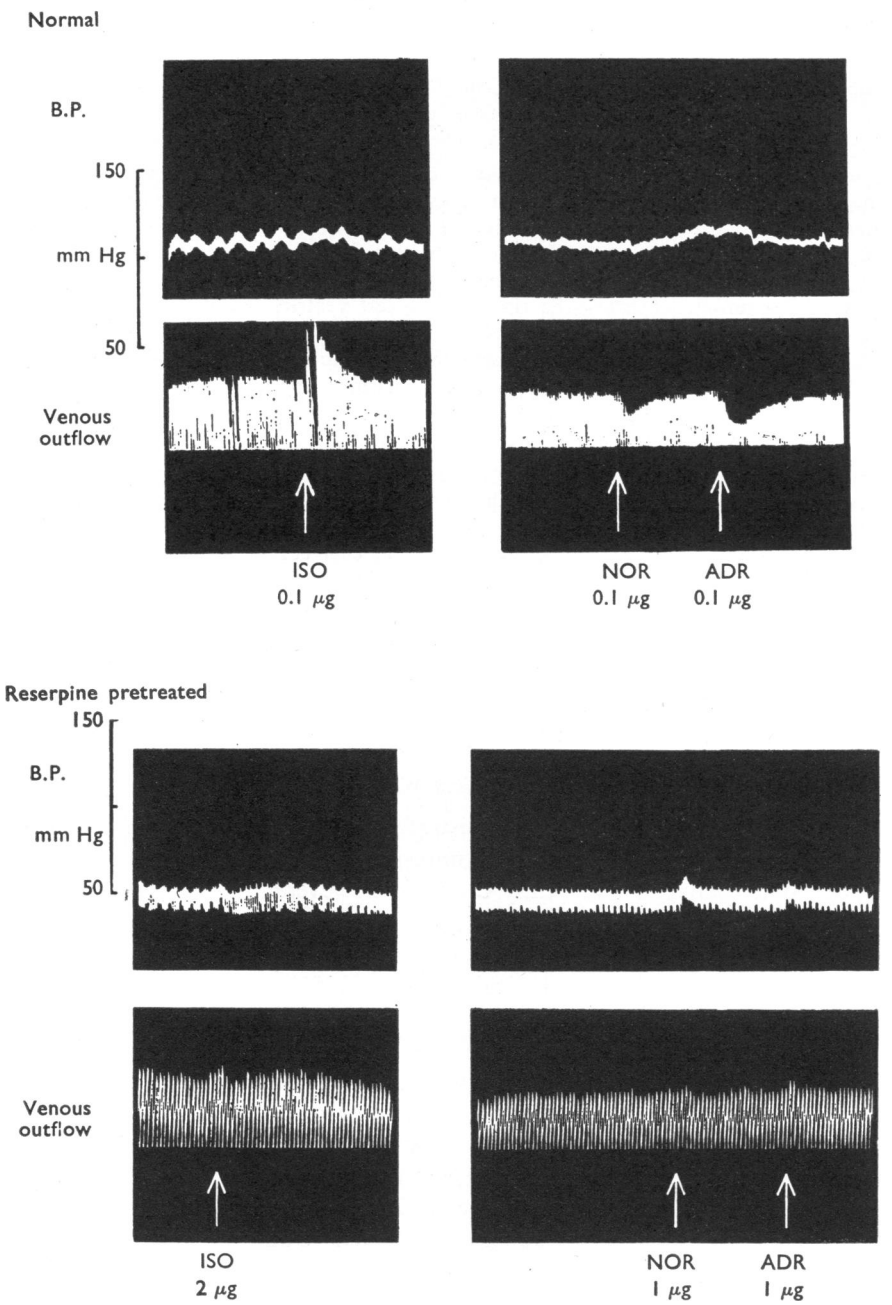

Fig. 1. Blood pressure and venous outflow from a hind limb recorded simultaneously. Isoprenaline (ISO), noradrenaline (NOR) and adrenaline (ADR) were administered by close-arterial injection. Upper tracing: control cat. Lower tracing: reserpine-treated, 1 mg/kg, on the day before the experiment.

As a rule in the normal cat after a close-arterial injection of 0.1 μg of isoprenaline, noradrenaline or adrenaline there is no change in general blood pressure. However, as Fig. 1 shows, this dose of isoprenaline produced a local vasodilatation accompanied by an increase in blood flow, while after noradrenaline or adrenaline, because of the local vasoconstriction, the blood flow was decreased. In contrast, in animals pretreated with 1 mg/kg of reserpine similar or even larger doses of the catecholamines appeared ineffective or gave rise to very reduced responses (Fig. 1). This decreased sensitivity of the peripheral vessels was found also in experiments in which larger doses of the same drugs were administered intravenously. Fig. 2 illustrates typical differences between a normal and a reserpine-treated animal. In the normal animal, an intravenous dose of 10 μg of noradrenaline was followed by a large increase in blood pressure and a simultaneous decrease in the peripheral blood flow. A similar effect was produced by tyramine. In the same preparation, 10 μg of isoprenaline produced the usual fall in blood pressure and a simultaneous increase in the peripheral blood flow. The picture in the reserpine-treated animal was quite different: the peripheral blood flow appeared to follow passively the blood pressure changes, as if the vessels had lost their ability to participate actively in the control of the circulation. There was no sign of either vasoconstriction following the administration of noradrenaline, adrenaline or tyramine, or of vasodilatation following that of isoprenaline. These results suggested that, in the animals treated with reserpine, vasoconstriction could play only a very small rôle, if any, in the rise of blood pressure elicited by noradrenaline or adrenaline.

Heart. In order to elucidate the mechanism of the pressor response to catecholamines in the reserpine-treated cats, experiments were performed in which the force of the cardiac contraction and changes in blood pressure and blood flow could be recorded simultaneously. When the chest was opened, the heart was found to be enlarged, flabby and soft, and the superior and inferior venae cavae and their tributaries engorged. At this stage the right side of the heart was, as a rule, more affected than the left. Moreover, in several experiments in which absolute calibration of the force of cardiac contraction (in grams) had been done, it was found that in the reserpine-treated animals the force of contraction was about one-half or one-third of that measured in the normal cat. Thus the weak contraction of the myocardium together with the macroscopic picture suggested strongly that these hearts were in failure.

The results obtained from the simultaneous recording of blood pressure, force of cardiac contraction, and peripheral blood flow showed that in the reserpine-treated animal any blood pressure changes were, as a rule, secondary to changes in cardiac contraction. Fig. 3 illustrates an experiment in which noradrenaline was injected before and after the administration of 4 mg/kg of dibenyline—a dose large enough to abolish in the normal animal the vasoconstrictor action of noradrenaline. In cats treated with reserpine, 2 hr following the administration of dibenyline, noradrenaline produced the same changes in both the force of cardiac contraction and in blood pressure. Obviously in this animal the rise in blood pressure elicited by noradrenaline was secondary to the former effect. In other experiments, after dibenyline the rise in blood pressure elicited by noradrenaline appeared to be

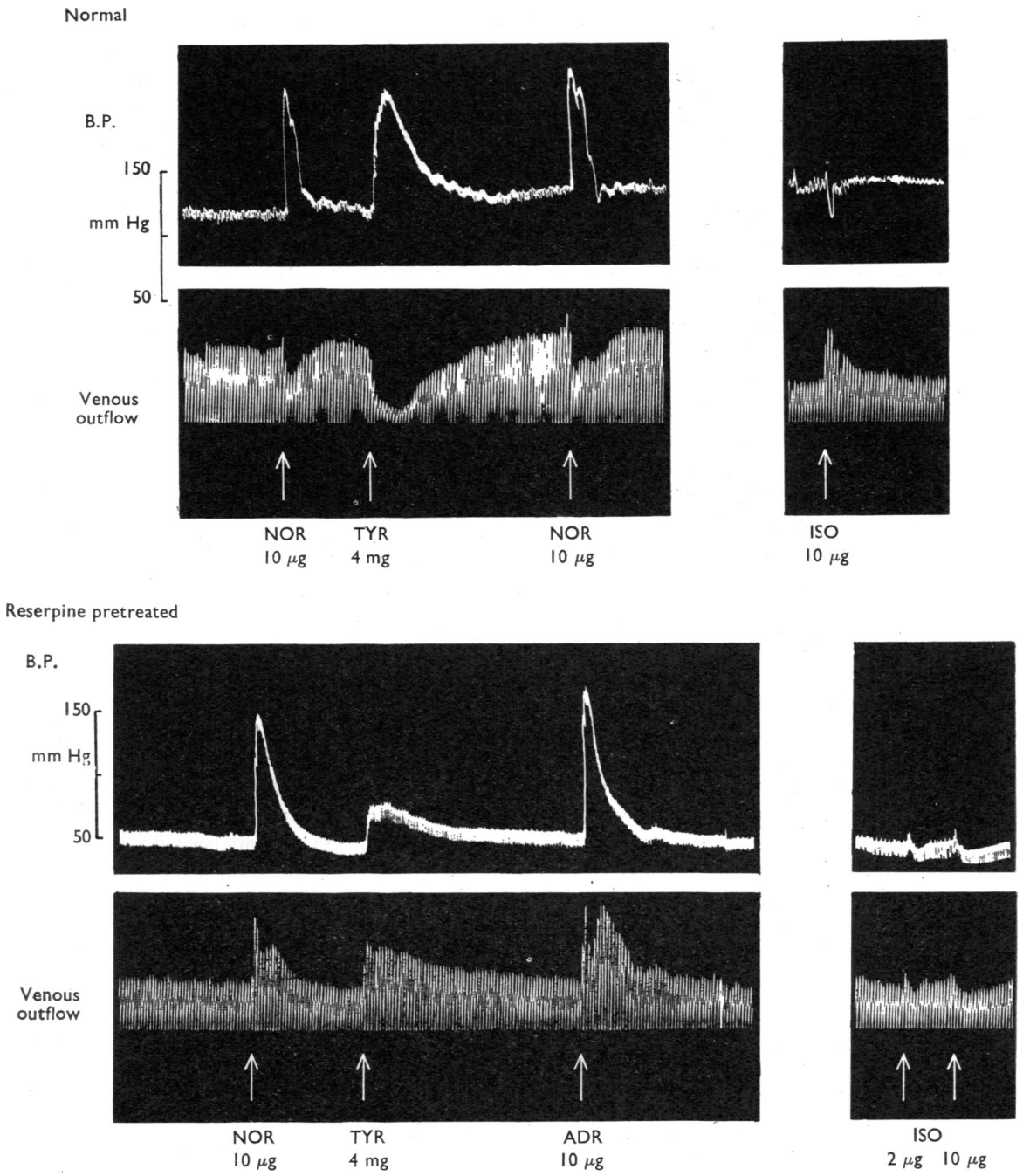

Fig. 2. Blood pressure and venous outflow from a hind limb recorded simultaneously. Noradrenaline (NOR), tyramine (TYR), isoprenaline (ISO) and adrenaline (ADR) were administered intravenously. Upper tracing: control cat. Lower tracing: reserpine-treated, 1 mg/kg, on the day before the experiment.

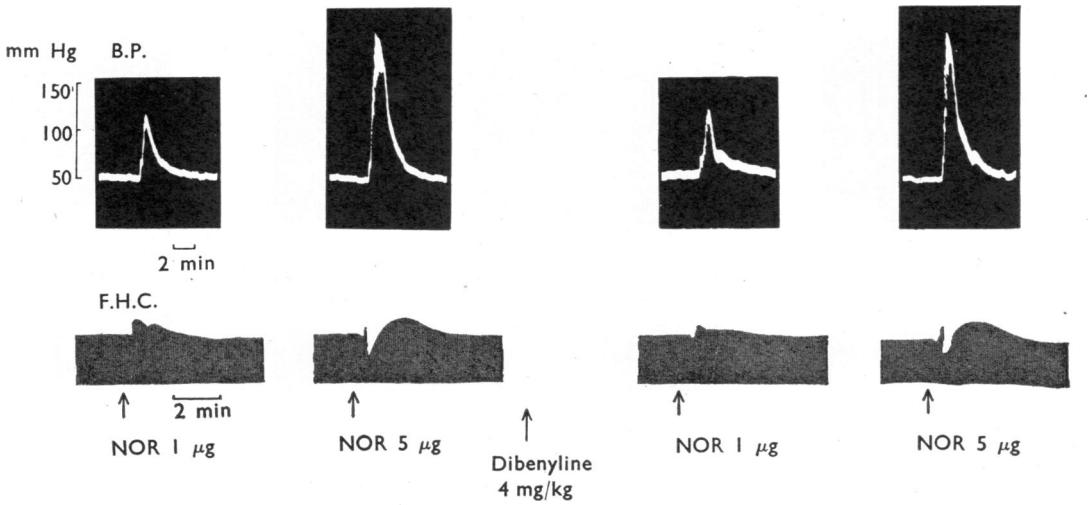

Fig. 3. Cat, 2.9 kg. Reserpine-treated, 0.5 mg/kg, for 2 days before the experiment. Upper tracing: blood pressure. Lower tracing: precise reproduction of actual recordings of ventricular contractile force. Force of heart contraction (F.H.C.). The last 2 doses of noradrenaline (NOR) were injected 2 hr after the administration of dibenyline.

reduced. However, the blood pressure reduction was always accompanied by a reduced effect of noradrenaline on the force of cardiac contraction. Fig. 4 illustrates such an experiment. After dibenyline, noradrenaline produced a smaller positive inotropic effect and at the same time a smaller rise in blood pressure. Once more the peripheral blood flow passively followed the blood pressure changes.

Further proof that the hearts of animals treated with reserpine were in failure was obtained from experiments in which the effect of ouabain was tested. In control cats a dose of 50 to 100 μg of ouabain produced very little or no change at all in either the force of cardiac contraction or in the blood pressure. In contrast, in the reserpine-treated animals after ouabain a gradual increase in the amplitude of cardiac contraction occurred, accompanied by a simultaneous rise in blood pressure. Furthermore, in the presence of ouabain the positive inotropic effect of various drugs, which in the animals treated with reserpine became less effective or depressant to the heart, was partially restored. For example, in the experiment just described and illustrated in Fig. 4, after 50 μg of ouabain the positive inotropic effect of noradrenaline increased, and as a result a greater rise in blood pressure was produced. This beneficial effect of ouabain was seen also in experiments in which the effect of tyramine was studied. In control animals, the administration of tyramine was accompanied by a large rise in blood pressure and a marked increase in the force of cardiac contraction. In the cats treated with reserpine, tyramine depressed cardiac contraction and the rise in blood pressure was markedly reduced. After ouabain, however, the depression of cardiac contraction produced by tyramine was prevented and the rise in blood pressure increased. Fig. 5 illustrates these effects.

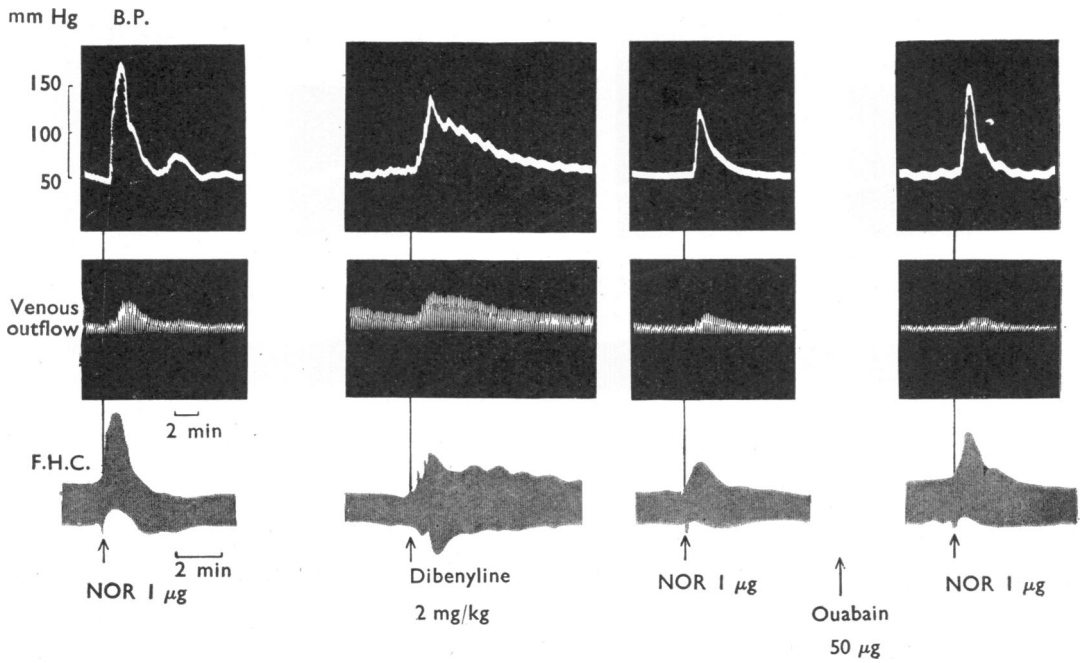

Fig. 4. Cat, 1.8 kg. Reserpine-treated, 1 mg/kg, the day before the experiment. Upper tracing: blood pressure. Middle tracing: venous outflow from a hind limb. Lower tracing: precise reproduction of actual recordings of ventricular contractile force. The second dose of noradrenaline (NOR) was injected 1 hr after the administration of dibenyline. The third dose of noradrenaline was injected 20 min after the administration of ouabain.

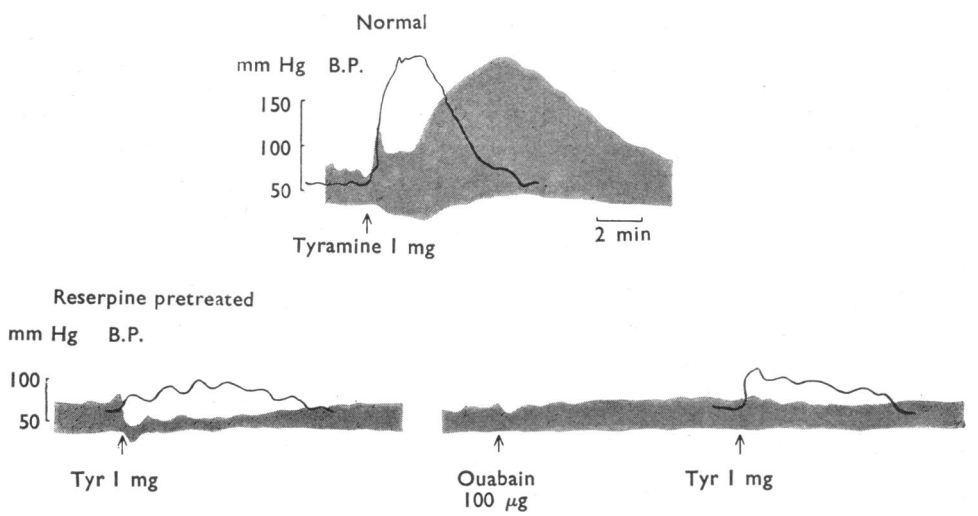

Fig. 5. Upper tracing: cat, 1.6 kg.; control. Lower tracing: cat, 2.7 kg.; reserpine-treated, 1 mg/kg, the day before the experiment. Precise reproductions of blood pressure changes and of actual recordings of ventricular contractile force.

It is well known that in the normal cat adrenaline, noradrenaline and isoprenaline increase the force of cardiac contraction, isoprenaline being the most powerful of the three (Fig. 6). In the reserpine-treated animal, however, the picture was completely different. Noradrenaline then produced, as a rule, the greatest inotropic effect, adrenaline was second, and isoprenaline, according to the condition of the heart, produced an increase, a depression or no effect. Fig. 6 illustrates an experiment in which 1 μg of isoprenaline, injected intravenously into an animal which had received the day before the experiment 1 mg/kg of reserpine, produced a marked depression of cardiac contraction.

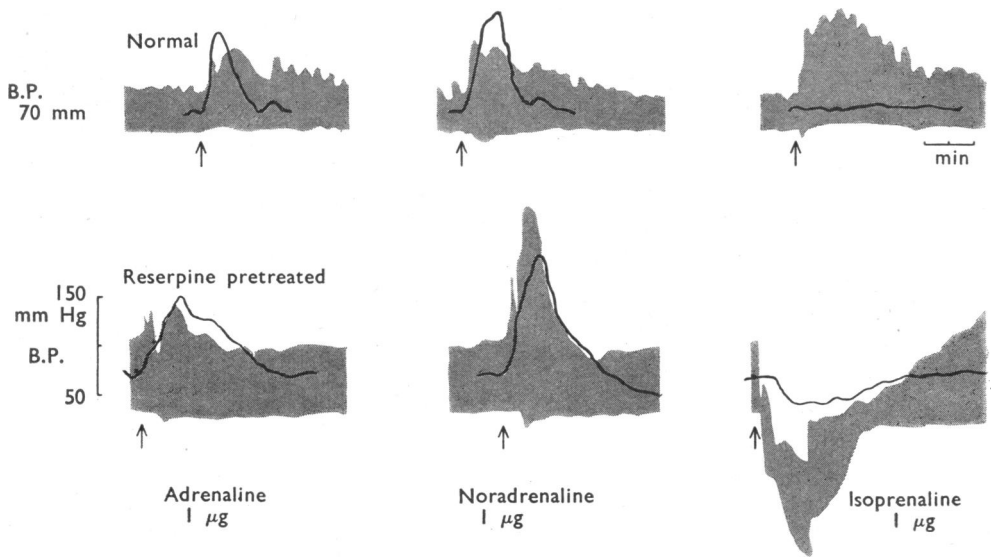

Fig. 6. Upper tracing: cat, 2.1 kg.; control. Lower tracing: cat, 2.7 kg.; reserpine-treated, 1 mg/kg, the day before the experiment. Precise reproductions of blood pressure changes and of actual recordings of ventricular contractile force.

Ouabain once more improved the response of the heart to isoprenaline. Fig. 7 illustrates an experiment in which isoprenaline produced very little change in the force of the heart contraction. Following 100 μg of ouabain, however, its positive inotropic effect improved, and there was a simultaneous rise in blood pressure.

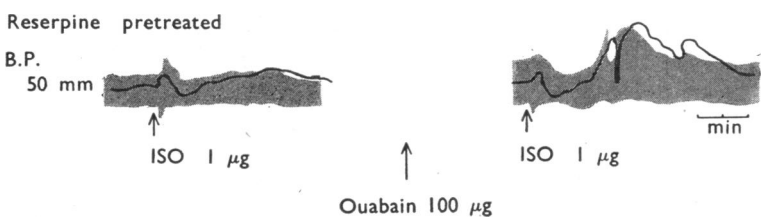

Fig. 7. Cat, 1.8 kg. Reserpine-treated, 1 mg/kg, the day before the experiment. Precise reproductions of blood pressure changes and of actual recordings of ventricular contractile force. The second dose of isoprenaline (ISO) was administered 30 min after the injection of ouabain.

Such a rise in blood pressure always occurred when isoprenaline increased the force of cardiac contraction in a reserpine-treated animal. Again, this is an abnormal response, since the powerful inotropic effect which isoprenaline elicits in the normal cat is not usually accompanied by a rise in blood pressure. In contrast, in the animal treated with reserpine the blood pressure changes faithfully followed the effects of isoprenaline on the force of cardiac contraction, probably because in these animals the direct effect of the drug on the peripheral blood vessels had been almost abolished. Thus isoprenaline, when positively inotropic, produced a clear rise in blood pressure, lasting as long as its effect on the heart persisted. If there was no positive inotropic effect or if the drug depressed the heart muscle, the blood pressure was unaffected or actually lowered.

The fact that the heart is possibly one of the first targets in the activity of reserpine is clearly illustrated by an experiment performed on a cat which received for a period of two months a daily dose of 10 μg/kg of reserpine. At the end of the two months the general condition of this animal, its body temperature and its blood pressure appeared to be fairly normal. The cat was then anaesthetized and prepared for the simultaneous recording of blood pressure, blood flow and force of cardiac contraction. The results showed that, although the force of cardiac contraction appeared fairly normal, the sensitivity of the myocardium to both catecholamines and ouabain was very high. For example, all three catecholamines, particularly isoprenaline, produced good positive inotropic effects in a total dose of only 0.1 μg. Moreover, as Fig. 8 shows, the positive inotropic effect of isoprenaline was accompanied by a diphasic response of the blood pressure, a small fall followed by a

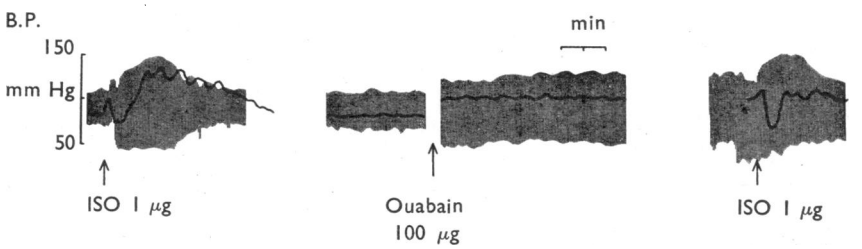

Fig. 8. Cat, 2.4 kg. Reserpine-treated, 10 μg/kg, for two months. Precise reproductions of blood pressure changes and of actual recordings of ventricular contractile force. The third section of the record was taken 20 min after the administration of ouabain and the fourth 15 min later.

rather pronounced and prolonged rise. Following the administration of 100 μg of ouabain, the force of cardiac contraction nearly doubled and there was a simultaneous substantial rise in blood pressure. The ouabain effect in this animal was far larger than that recorded previously in cats which had been treated with a dose of 1 mg/kg of reserpine and which at the time of the experiment were in obvious heart failure. Furthermore, following the administration of ouabain the features of cardiac insufficiency disappeared, the isoprenaline effect became normal, and its inotropic effect was accompanied once more by a fall and not a rise in blood pressure. Both these points are illustrated by Fig. 8. Thus, the results obtained

from this experiment, when put together, indicate (*a*) that the heart can be affected by even very small doses of reserpine administered over long periods of time; (*b*) that insufficiency of the myocardium may be present at a stage when obvious signs of heart failure are absent; and, finally, (*c*) that at this stage both catecholamines and ouabain exhibit powerful positive inotropic effects.

Heart rate. The reason for the rather puzzling behaviour of the catecholamines in animals treated with reserpine became apparent when heart rate changes were recorded at the same time as the other parameters. It was found that there was a close relationship between the inotropic effect of the drug and the ability of the failing heart to sustain an increased activity. In the treated as in the control animals, isoprenaline produced the most marked increase in heart rate; adrenaline came second, producing a smaller but still sustained tachycardia, while noradrenaline was the least effective in both magnitude and duration of the effect. The failing heart of the animal treated with reserpine, however, in contrast to the control one, proved unable to sustain an increased activity. Therefore the response to isoprenaline varied according to the condition of the heart at the moment of the drug administration. If very poor, the heart faced the abrupt increase in heart rate with a clear-cut depression in the force of its contraction. On the other hand, when failure was not very advanced, the cardiac contraction could still improve, but even then the response was always much smaller than that usually seen in the control heart. A similar variation, although less pronounced, was found with adrenaline. In the majority of experiments adrenaline did produce a positive inotropic effect. Occasionally, however, when heart failure was very advanced, the effect of adrenaline on the heart was depressant. Only the administration of noradrenaline was always followed by a positive inotropic effect.

DISCUSSION

The results just described show that, in cats, 24 hr after the administration of reserpine in a dose of about 1 mg/kg, the heart is in failure and the sensitivity of the peripheral vessels to adrenaline, noradrenaline and isoprenaline, administered intravenously or close-arterially, decreased. Furthermore, any blood pressure changes are, as a rule, secondary to changes in cardiac contraction and the peripheral blood flow passively follows the blood pressure changes. Moreover, any improvement of the circulation at this stage was found to be almost exclusively the result of an amelioration in the force of cardiac contraction. For example, ouabain increased the amplitude of the cardiac contraction and partially restored the positive inotropic effect of various drugs which in the animals treated with reserpine became ineffective or depressant to the heart. This was the case with both tyramine and isoprenaline. In other words, in a very short period of time, the cat treated with reserpine exhibited a most spectacular circulatory failure.

In the present experiments the doses of reserpine have been much larger than those used in therapeutics. However, there are several clinical reports of oedema and congestive failure related to the administration of relatively small doses of reserpine. Perera (1955) reported 5 cases which within a week after the daily administration of 0.4 mg of reserpine or 200 mg of raudixin developed oedema,

especially of the ankles, occasionally accompanied by moderate exertional dyspnoea. Within a week after the drug had been discontinued all manifestations of abnormal fluid retention disappeared, and during a period of observation of from 1 to 3 months after treatment no patient developed any recurrent oedema or cardiac symptoms. Later on reserpine was re-administered to one patient for one week and the same symptoms appeared again. Marley & Pare (1956) presented two cases of cardiac failure which occurred following the administration of reserpine, and very recently the *British Medical Journal* (1961, i, 1022) through an editorial article urged the clinicians to consider patients on reserpine as potentially "poor risks" when their cardiovascular system had to cope "with an increased load of work—for example, during labour, electro-shock therapy or anaesthesia and surgery."

Histological examination of the heart and other organs removed from animals pretreated for one or several days with a total dose of reserpine of 1 to 1.25 mg/kg revealed pronounced changes (Zaimis, 1961). The heart muscle showed extremely severe degenerative changes, the adrenal cortex a picture characteristic of severe stress, and the liver centrilobular (fatty) changes indicative of heart failure and increased venous pressure.

All these results put together indicate the remarkable toxicity of reserpine on the myocardium. With large doses, of the order of 1 mg/kg, the heart goes, in a few hours, into a most spectacular failure. Even with very small doses administered over a long period of time the myocardium is affected, but it appears that the changes become obvious only when a special demand is put upon the heart.

There is a striking similarity between the behaviour of a heart poisoned with a large dose of reserpine (1 mg/kg) and that developing in conditions in which the oxygen supply or the oxygen utilization of the myocardium is substantially decreased. In both instances the cardiac capacity for work declines rapidly and the heart slows down in order to do a given amount of work per unit of time more economically. However, as the heart tires and the functional capacity decreases its mechanical efficiency is diminished. To do the same amount of work the heart dilates continuously and finally failure of function sets in.

The mechanism by which reserpine gives rise to these toxic changes remains to be elucidated; probably the drug produces either a defect in the myocardial metabolism of various substrates or an abnormality of the contractile proteins. Meantime, one definite conclusion can be drawn: cats, pretreated with 1 mg/kg of reserpine, because of the acute circulatory failure, must be considered as most unreliable experimental tools.

Our thanks are due to the Medical Research Council (E.Z.) for a grant for technical assistance, and to the National Institutes of Health, U.S. Public Health Service [Grant B-2474 (CI)], for partially financing this research.

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