J. Physiol. (1953) 120, 160–170

A QUANTITATIVE STUDY OF THE RESPONSE TO ACETYLCHOLINE AND HISTAMINE OF THE BLOOD VESSELS OF THE HUMAN HAND AND FOREARM

By F. DUFF, A. D. M. GREENFIELD, J. T. SHEPHERD and I. D. THOMPSON

From the Department of Physiology, The Queen's University of Belfast

(*Received* 20 *October* 1952)

Many investigators have injected acetylcholine and histamine into man both intra-arterially and intravenously. The effects of intra-arterial injection of acetylcholine on the circulation in the legs were studied by Carmichael & Fraser (1933) who observed skin colour, and by Ellis & Weiss (1932) who measured skin temperature and arterio-venous oxygen differences. We have been unable, however, to find detailed quantitative measurements of their effects on the vessels. Since such observations were required for another purpose we have made them, and this paper describes the results.

METHODS

Observations have been made on twenty-six men and three women between the ages of 19 and 34. All were in good health, and none suffered from peripheral vascular disease. They arrived at the laboratory at least 1 hr before observations started and were recumbent during, and for half an hour before, the observations. They wore normal indoor clothing, and the trunk and legs and as much as possible of the arms were wrapped in blankets. The laboratory temperature was 20–23° C.

Blood flow

The blood flow through the hands and forearms was measured every 15 sec, or more frequently, by venous occlusion plethysmography. In most experiments the plethysmographs were filled with stirred water at 32° C for the hand and at 34° C for the forearm. They were fitted with loose gloves for the hands or loose sleeves for the forearms. In some experiments, in which it was desired to measure simultaneously the hand and forearm flow on the same side, a light celluloid air-filled plethysmograph was used on the forearm.

The venous occlusion cuffs, at the wrist for the hand, and above the elbow (proximal to the needle in the brachial artery) for the forearm, were, unless otherwise stated, inflated to 70 mm Hg during the periods of collection. A wrist cuff was inflated to 200 mm Hg for 1 min before, and during, observations of forearm blood flow. When, however, the hand and forearm blood flows

were measured simultaneously the wrist and arm cuffs were inflated to the collecting pressure from a common air reservoir. Care was taken in the interpretation of the records of these experiments to avoid errors due to the spill-over of blood from the hand into the forearm; this may occur at high hand flows after a few heart beats, and lead to a steepening of the forearm inflow curve coinciding with a flattening of the hand inflow curve.

Both acetylcholine and histamine injected into the brachial artery in small doses powerfully dilate the forearm blood vessels (Fig. 2). It was thought that this might sufficiently reduce the pressure in the arteries at the wrist for the collecting cuff at 70 mm Hg partially or wholly to occlude them, and so invalidate the measurements of the hand flows. In some experiments therefore (which are noted in the legends to the figures) a collecting pressure of 40 mm Hg was employed, but this did not appear to alter the results.

Drug injections

All injections were made intra-arterially through 21 s.w.g. needles. In the majority of experiments the subject became aware of the drug injections only when the dose was sufficient to cause subjective sensations. Most injections were into the brachial artery at the elbow, but some were into the radial artery at the wrist, or the subclavian artery as it crosses the first rib. The skin was anaesthetized with about 0·5 ml. of 1% procaine with adrenaline. As soon as the needle was in place an infusion of normal saline was started at about 1 ml./min from a motor driven syringe. The latter was connected by about 1 m of 1 mm bore polythene tubing to a 'Record' fitting T tap, which was in turn connected by about 25 cm of polythene tubing to the arterial needle. Drug injections were delivered from a separate syringe attached to the T tap; this syringe could be changed or manipulated without the subject's knowledge. The syringe was emptied by hand, the amount delivered being checked against the clock every 12 sec. The dead space from the drug syringe to needle tip was 0·4–0·5 ml. Allowance has been made for this whenever necessary in timing the events following injections.

In each experiment flows were recorded before and after insertion of the needle, and on several occasions the forearm flows were also measured during the insertion. There was never any diminution in flow or in the size of the pulse waves on the plethysmogram or in the volume of the radial pulse as judged by palpation. There was therefore no evidence of spasm of the brachial artery as a result of the needling.

The doses were prepared immediately before the experiment from acetylcholine hydrochloride ampoules (100 mg of crystalline substance, Roche) or histamine acid phosphate ampoules (1 mg in 1 ml., B.D.H.). All doses are expressed as weights of these salts. Dilutions were made in sterile saline, and unless otherwise mentioned, the dose was made up in 5 ml. for injection in 1 min. Control injections of 5 ml. of saline in 1 min were without detectable effect on hand and forearm blood flow. Dilutions for the smaller doses (16 μg or less) were made immediately before administration. The doses were usually given in ascending order of magnitude. The following evidence suggests that even the dilute solutions of acetylcholine and histamine were stable for the duration of the experiment. A stock solution was prepared containing 16 μg acetylcholine in each 5 ml. Doses of 16 μg were injected over 1 min with intervals of 5 min. The plateau levels of forearm blood flow were respectively 13·1, 15·9, 14·5 and 14·7 ml./100 ml./min. A similar experiment with doses of 4 μg histamine gave values of 16·3, 16·8, 17·9 and 16·8 ml./100 ml./min. Thus, unless the blood vessels were becoming more sensitive as time went on, there is no reason to suppose that the drugs were undergoing destruction.

Presentation of results

It is not easy to decide on the best method of presenting results so that fair comparison can be made between the effect of different drugs or dosages. The following were considered as indices of the effect on the blood flow: (1) The highest of a series of observations made at definite intervals during and after the administration of the drug. (2) The average of the n highest of the same

series of observations. (3) The amount by which (1) or (2) exceeds the resting level. (4) The ratio of (1) or (2) to the resting level.

A chance technical error or misinterpretation might seriously upset (1), but this danger is reduced in (2). Apart from this, the objection to both (1) and (2) is that the values observed probably depend, although in no simple way, on the resting level of flow and that the latter is disregarded. Account is taken of the resting level in (3) and (4), but these are both open to the objection that a dose of drug which dilates the blood vessels fully may appear by these indices to have a smaller effect when the resting level is high than when it is low. It is also uncertain and even unlikely that, for example, a change from 3 to 9 ml. represents the same degree of drug effect as a change from 12 to 18 (as would appear to be the case by (3)) or from 8 to 24 (as would appear to be the case by (4)).

As none of these indices is entirely satisfactory it was decided to combine (2) (the average of the four highest observations of a series made at intervals of 15 sec during and after the administration of the drug) with a statement of the resting level (average of four observations) before any drugs were administered.

RESULTS

Sensations

Acetylcholine injected into the brachial artery in doses of 1000–4000 μg caused mild tingling and in some subjects a sensation of fullness or bursting in the arm; there were no general symptoms. Histamine in doses of 16 μg or more caused mild sensations of heat and tingling in the arm. Doses of 32 μg occasionally, and of 64 μg usually, caused mild throbbing in the head and flushing of the face. Subjective sensations with either drug were never more than uncomfortable.

Appearance

In order to observe the colour changes in the skin, plethysmograph measurements were omitted in a few experiments. All but the smaller doses of both acetylcholine and histamine caused flushing of the skin. After injections into the brachial artery the upper limit of the flush at the elbow followed an irregular but clearly defined line. Our impression is that the forearm flush after the smaller doses of acetylcholine was patchy and after histamine was confluent. The flush appeared gradually, and it was difficult to say whether it appeared in the forearm before the hand. The onset of the flush appeared to be less abrupt than the rise in blood flow (Fig. 4).

Blood flow

Typical results of injection of acetylcholine and histamine into the brachial artery are shown in Fig. 1. Acetylcholine caused an increase in forearm blood flow in a dose of 1 μg but had no effect on hand flow in a dose of 256 μg. Histamine, however, in a dose of 1 μg caused an increase in blood flow in both the forearm and hand. The results of a number of experiments of the type shown in Fig. 1 are summarized in Fig. 2. In all experiments, acetylcholine when injected into the brachial artery had much less effect on the blood flow through the hand than on that through the forearm. Thus, to raise the flow

appreciably above the resting level, $\frac{1}{4}$–1 μg is needed for the forearm, but 256–4000 μg for the hand. In contrast, histamine in doses of 1 μg invariably increased both the hand and forearm flow. This discrepancy might be accounted for in the following ways: (1) The drug, in passing to the hand vessels,

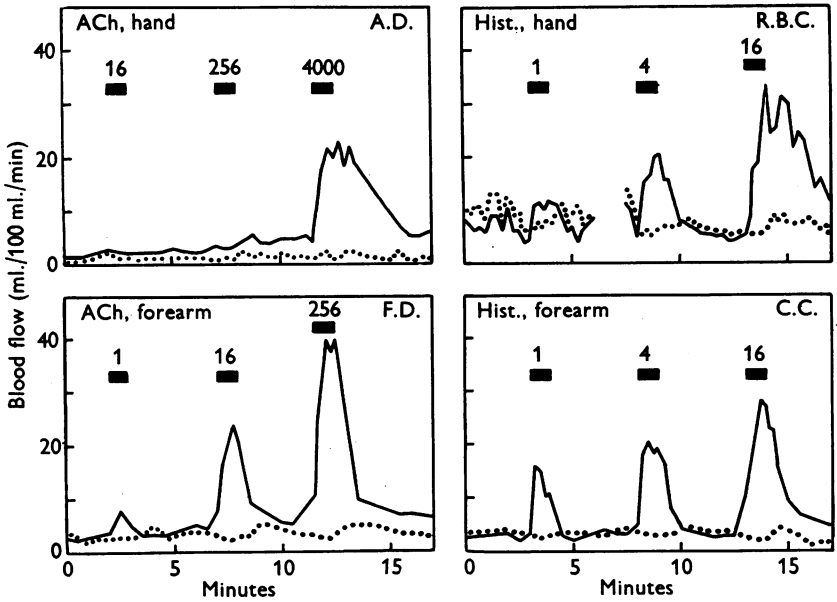

Fig. 1. The effect on forearm and hand blood flow of various doses of acetylcholine and histamine injected over 1 min periods into the brachial artery of four normal subjects. Continuous line: injected side; dotted line: control side. Doses are in μg.

is exposed to the action of cholinesterase for a longer time than in passing to the forearm vessels. The difference in time of exposure may allow a larger proportion of the acetylcholine to be hydrolysed. (2) The hand vessels may be very much less sensitive to acetylcholine than the forearm vessels. (3) The first part of the drug injected into the brachial artery dilates the vessels in the forearm and most of the remainder may circulate through these dilated vessels. (4) The dilatation of the forearm vessels may so reduce the perfusion pressure at the hand that the hand flow is unaltered although the vessels are dilated. The fourth possibility is rendered unlikely because following injection of histamine into the brachial artery, or during reactive hyperaemia after arrest of the circulation above the elbow, a large hand blood flow is present at the same time as a large forearm flow.

To distinguish between the remaining possibilities, the following experiments were devised:

(1) *Injection into the radial artery.* Acetylcholine was injected into the radial artery of I.D.T. in doses of 16, 256 and 1000 μg and produced flows of 13·4,

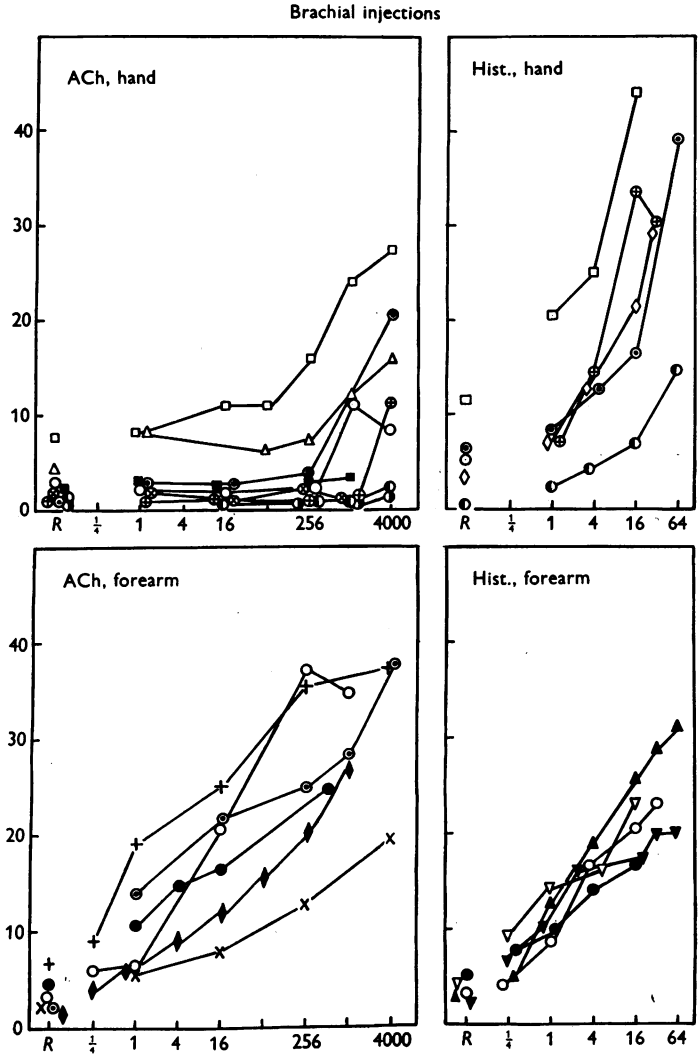

Fig. 2. The response of the hand and forearm vessels to graded doses of acetylcholine and histamine injected into the brachial artery. Ordinate: blood flow in ml./100 ml./min, Abscissa: dose in μg injected over 1 min. *R*, average resting flow. Each symbol in this and the following figure represents a different subject. Collecting pressure was 40 mm Hg during the following observations: acetylcholine hand, Δ; histamine hand, ⊕◇; acetylcholine forearm, ⊙; histamine forearm, ○●▼.

20·1 and 33·2 ml./100 ml./min respectively, the resting flow being 3·4. One μg of acetylcholine injected into the radial artery of F.D. raised the hand blood flow to 6·8 from a resting value of 3·7 ml./100 ml./min. In contrast to this, in only one of the nine subjects in whom acetylcholine was injected into the

brachial artery (Fig. 2) did a dose of 256 μg of acetylcholine produce an appreciable effect on the hand flow, and in five subjects, 1000 μg was without effect. An injection into the radial artery may be distributed to a very limited region of the hand, which will receive a concentrated dose, while the injection into the brachial artery is probably distributed more uniformly. Nevertheless, when reasonable allowance is made for this, these results strongly suggest that the hand blood vessels are not peculiarly insensitive to acetylcholine.

(2) *Injection into the subclavian artery.* In order to increase the forearm blood flow, a dose of 1000–4000 μg of acetylcholine (Fig. 3) must be injected

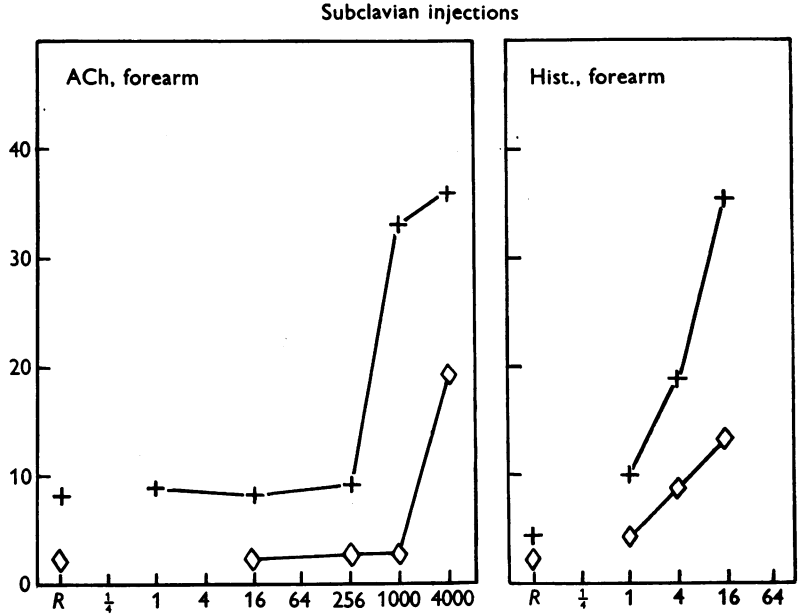

Fig. 3. The response of the forearm blood vessels in two of the subjects shown in Fig. 2 to graded doses of acetylcholine and histamine injected into the subclavian artery. Conventions as in Fig. 2. +, collecting pressure 70 mm Hg; ◇, collecting pressure 40 mm Hg.

into the subclavian artery, although in every subject tested an increase was obtained with ¼ μg injected into the brachial artery. Even when generous allowance is made for the difference in volume of the receiving territory in the two cases, it seems clear that much of the subclavian dose must be diverted or destroyed and fail to reach the forearm. The corresponding observations with histamine, which has very similar effects on the forearm blood flow whether injected into the subclavian or brachial artery (Fig. 3), seem to exclude diversion or altered haemodynamics as an explanation.

(3) *Exposure of the acetylcholine to arterial blood* in vitro. It was repeatedly observed that the delay between starting the injection and the onset of vaso-

dilatation was much greater in the hand than in the forearm. The delay appeared to depend to some extent on the resting level of the blood flow at the time of the injection, and also on the dose injected. Nevertheless, the difference in delay at the hand and forearm is clear cut. In twenty-four observations on five subjects the average delay in the hand was 17·9 sec (S.E. 1·9) and in thirty-five observations on seven subjects the average delay in the forearm was 6·2 sec (S.E. 0·4). These figures are from experiments in which histamine was used, and in which there was no likelihood of drug destruction in the blood stream (Anrep, Barsoum & Ibrahim, 1947). The injections were made over ½–1 min. When histamine was made up in 2 ml. saline and injected in 2–3 sec there was still a substantial difference between the delay before vaso-

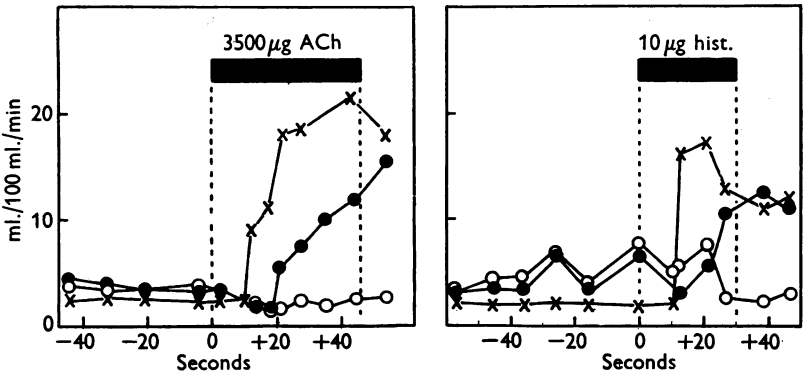

Fig. 4. The time of onset of dilatation in the forearm and hand vessels following injections into the brachial artery of 3500 μg acetylcholine and 10 μg histamine. ×, forearm flow, injected side; ●, hand flow, injected side; ○, hand flow, control side.

dilatation in the hand (17·0 sec, S.E. 2·0) and in the forearm (7·5 sec, S.E. 0·9). In some experiments, of which examples are shown in Fig. 4, the time of onset of vasodilatation in the hand and forearm of the same limb was determined by making simultaneous observations, and the results fully confirm those already quoted.

There was, therefore, in general a difference of about 10 sec in the time of onset of vasodilatation in the hand and in the forearm, and experiments were designed to test the effect of exposure of acetylcholine to the action of blood for about this length of time. For these experiments the syringe containing the drug and as much as possible of the connecting system was immersed in a water-bath at 37° C. The dead space from syringe to needle tip was less than 1 ml. The response to varying doses of acetylcholine in 5 ml. saline injected in about 5 sec was first determined. Control injections of saline usually doubled the forearm flow for a few seconds, but the flow returned to normal within 18 sec. Acetylcholine, even in the smallest effective doses, caused a much more persistent rise in flow.

Acetylcholine was then made up in 1 ml. saline and together with 1 ml. air, was contained in a syringe attached to the infusion system. About 3 ml. blood was rapidly withdrawn into the syringe, the contents of which were mixed by swirling all the time. This usually took 5–7 sec. The mixture of blood and acetylcholine was then injected as rapidly as possible, usually in about 5 sec. No very precise time limits can be laid down for the exposure of the drug to the action of the blood, but the total time (10–12 sec) was of the same order as the difference in the time of onset of dilatation in the forearm and hand following injection of histamine into the brachial artery. Control injections of 3 ml. blood plus 1 ml. saline usually caused a slightly larger and

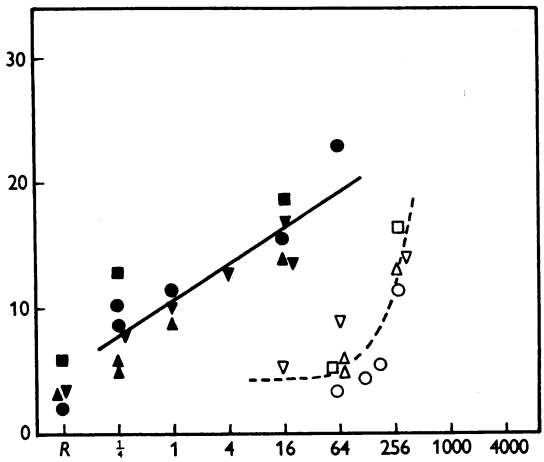

Fig. 5. A comparison in three subjects of the effect of injection into the brachial artery of acetylcholine in saline (solid symbols) with that of acetylcholine mixed with blood as described in the text (open symbols). Ordinate: blood flow in ml./100 ml./min. Abscissa: dose in μg. The continuous and dotted lines were drawn by inspection.

more prolonged increase than the controls with 5 ml. of saline only. Experiments were carried out in four subjects and the results in three of them are shown in Fig. 5. These results were obtained by disregarding flows during the first 18 sec after the injection and averaging the two highest of the following flows. This allows fully for the transient increase in flow due to saline, but tends to give values which are too high for the effects of the mixture of acetylcholine and blood, owing to the longer period of vasodilatation following the re-injection of blood alone.

It is clear that 64–192 μg of acetylcholine, after exposure to arterial blood in the way described, produced a smaller change in forearm blood flow than $\frac{1}{4}$ μg of acetylcholine injected at the same rate, but without preliminary exposure to blood. This shows that 3 ml. or less of arterial blood reduced the activity of 192 μg of acetylcholine by more than 99·7%.

From these experiments it seemed that the most likely cause of the failure of acetylcholine to dilate the hand vessels when injected into the brachial artery in doses of 256 μg or less was its inactivation on the way to the hand vessels.

DISCUSSION

The following factors, among others, may influence the response of a particular blood vessel to a substance, S, in the blood passing through it: (1) the concentration of S in the blood; (2) the rate of flow of blood, and hence the quantity of S passing through in unit time; (3) the duration of the passage of S; (4) the reactivity of the musculature of the vessel wall, which may depend, among other things, on its initial state of contraction or relaxation, and on the level of vasomotor tone.

When a substance is injected into a main limb artery and the blood flow response in the limb is observed, the following considerations must also be borne in mind: (1) In any region, such as a limb, provided with a collateral circulation, the territory supplied by one artery, even the main artery, may vary greatly with local conditions and is difficult or impossible to define. Doses cannot, therefore, be accurately or usefully calculated in terms of the weight or volume of the tissue receiving them. (2) The distribution of blood, and hence of substance S mixed with it, may well be uneven and changing within the territory of supply of the artery. (3) The injected material may be incompletely mixed with the arterial blood at the origin of the first branch of the artery so that the distribution of S may not follow that of the blood. It is never certain, for example, that the ulnar and radial arteries each receive their proper share of material injected into the brachial artery. (4) The time course of delivery of S into the artery can be accurately controlled, but the time course of its arrival at the peripheral resistance vessels is different and cannot be measured. (5) The blood flow in the whole limb is the sum of that in all the small vessels, in each of which the flow varies approximately with the 4th power of the diameter, and so approximately with the 4th power of the length of the muscle cells in the vessel wall.

In spite of these complex considerations, there is a linear relationship between the log dose of substance injected into the brachial artery and the resulting rate of blood flow in the forearm. This type of dose response relationship is, of course, usual, but in this instance the relationship holds only if the index of the response is taken to be the rate of blood flow, and not, for example, the mean calibre of the resistance vessels, which according to Poiseuille's theorem varies as the 4th root of the rate of flow. The log dose/blood flow relationship is linear for doses of acetylcholine ranging from $\frac{1}{4}$ to 4000 μg and for doses of histamine ranging from $\frac{1}{4}$ to 64 μg. In Fig. 6, results from all subjects have been averaged, and although the points in this graph are not all equally weighted, the straightness of the line along which

they lie is remarkable. When the log of the dose of histamine injected into the brachial artery is plotted against the resulting rate of hand blood flow, however, the points for each individual (Fig. 2) fall on a curve with an upward concavity, but the averaged points (Fig. 6) lie close to those of the effect of histamine on the forearm. The response of the hand vessels to acetylcholine, however, deviates markedly from all the other slopes. We attribute this difference to the rapid destruction of acetylcholine by the blood cholinesterase. If the dose of histamine is plotted on an arithmetic scale against the hand blood flow, a linear relationship is found for each individual. Our data is insufficient to allow us to assess the significance of this relationship, but it appears to be regular.

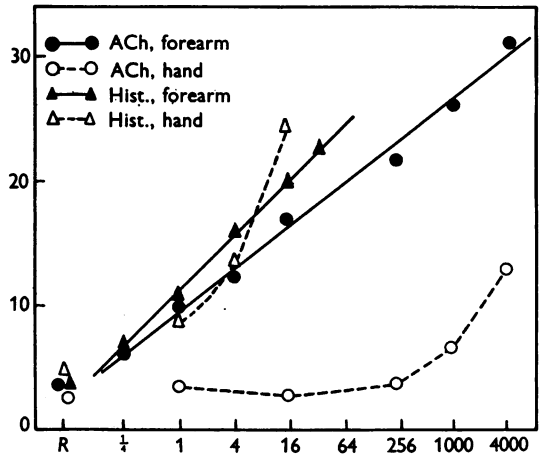

Fig. 6. The relationship between the forearm flows and the logarithm of the dose of acetylcholine and histamine. Points were obtained by averaging the flows of the individual subjects illustrated in Fig. 2. Lines were drawn by inspection. Ordinate: blood flow in ml./100 ml./min. Abscissa: dose in μg on log scale.

The difference of about 10 sec in time of onset of vasodilatation in the hand and forearm when histamine is injected into the brachial artery suggests that it takes about 10 sec longer to reach the hand than the forearm vessels. This is a surprisingly long time, but the following considerations suggest that it is within the bounds of possibility. When histamine is injected into the subclavian artery, the delay before the start of vasodilatation in the forearm was, in five experiments on two subjects, 18, 17, 16, 17 and 21 sec, although when injected into the brachial artery the average delay was 6·5 sec. This difference is clearly due to a difference in transport time to the vessels, and not to reaction time of the vessels themselves. This suggests that the hand and forearm delays after brachial injections of histamine may also be explained by a difference in transport time. It seems fair to assume, therefore, that

acetylcholine is exposed to the action of cholinesterase for 10 sec longer in travelling to the hand than to the forearm vessels.

The view that the acetylcholine is largely destroyed in the blood stream before reaching the more distant parts of the limb rests mainly on a comparison of the response to the substance (*a*) of the hand vessels after brachial injection (Fig. 6), (*b*) of the forearm vessels after subclavian injection (Fig. 3) and (*c*) of the forearm vessels after *in vitro* exposure to blood for about 10 sec followed by brachial injection (Fig. 5). These dose-response curves are very similar. They suggest that up to a dose of at least 250 μg the acetylcholine was virtually all destroyed. With larger doses, presumably because of the saturation of the blood cholinesterase, a substantial part of the dose reached the vessels unchanged.

Since nearly the whole of a small dose of acetylcholine is destroyed before reaching the hand, we must suppose that a great deal is destroyed before reaching the forearm. The interval between injection and the onset of vasodilatation in the forearm is of the order of 6·5 sec, and in this interval between 90 and 99% of the injected dose may well be hydrolysed. If this is so, the dose actually reaching the vessels when $\frac{1}{4}$ μg is injected may be of the order of 0·025–0·0025 μg.

SUMMARY

1. Injections of acetylcholine and histamine have been made into the radial, brachial and subclavian arteries, and the effects on the circulation of the hand and forearm studied.

2. Injection into the brachial artery of $\frac{1}{4}$ μg of either substance over 1 min causes a definite increase in the rate of blood flow through the forearm.

3. Injection of 1 μg of histamine over 1 min causes a definite increase in hand flow, but doses of acetylcholine of less than 250–4000 μg are without effect.

4. Evidence is presented which shows that the relative insensitivity of the hand vessels to acetylcholine injected into the brachial artery is due to destruction of the drug in the blood stream.

5. The flows in the forearm after injection into the brachial artery of $\frac{1}{4}$–4000 μg acetylcholine or $\frac{1}{4}$–64 μg of histamine are proportional to the logarithm of the dose.

We wish to thank our colleagues and students at the Queen's University of Belfast who have acted as subjects for many of these experiments.

REFERENCES

ANREP, G. V., BARSOUM, G. S. & IBRAHIM, A. (1947). The histaminolytic action of blood during pregnancy. *J. Physiol.* **106**, 379–393.

CARMICHAEL, E. A. & FRASER, F. R. (1933). The effects of acetylcholine in man. *Heart*, **16**, 263–274.

ELLIS, L. B. & WEISS, S. (1932). A study of the cardiovascular responses in man to the intravenous and intra-arterial injection of acetylcholine. *J. Pharmacol.* **44**, 235–251.